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KARINE RAMIRES LIMA

MECANISMOS NEUROQUÍMICOS ENVOLVIDOS NA MODULAÇÃO DA PERSISTÊNCIA DA MEMÓRIA INDUZIDA PELO EXERCÍCIO FÍSICO AGUDO

Uruguaiana, RS
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Tese de doutorado apresentada Programa de Pós-graduação em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Doutora em Bioquímica.

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“Segue o teu destino,
Rega as tuas plantas,
Ama as tuas rosas.
O resto é a sombra
De árvores alheias.”

Fernando Pessoa

RESUMO

A modulação da memória pelo exercício físico (EF) agudo visa melhorar sua consolidação e persistência. Sabe-se que uma única sessão de EF, como a corrida em esteira de intensidade moderada, promove esses efeitos. Os mecanismos envolvidos incluem a liberação neurotransmissores como noradrenalina e dopamina. Contudo, persistem lacunas significativas sobre os efeitos e mecanismos do EF na modulação da memória. O objetivo desta tese é explorar como o EF agudo influencia o aprendizado e a persistência da memória. Utilizando ratos Wistar machos adultos como modelo experimental, investigamos três questões essenciais: i) o papel das regiões cerebrais área tegmental ventral (ATV) e *locus coeruleus* (LC) nos efeitos do EF agudo na memória; ii) o requerimento da síntese proteica hipocampal para os efeitos do EF agudo na persistência da memória; e iii) o impacto e os mecanismos do EF agudo quando aplicado durante a janela de consolidação tardia, 11 horas após o aprendizado. Nossos resultados indicam que o LC desempenha um papel crucial nos efeitos do EF agudo na consolidação e persistência da memória, enquanto a ATV parece não ser necessária para esse processo. Além disso, evidenciamos que, embora a síntese proteica hipocampal seja vital para a consolidação da memória, o EF agudo supera a inibição da síntese proteica ribossomal e da via mTOR, promovendo a consolidação, mas não a persistência da memória. Ainda, demonstramos que o EF agudo não eleva os níveis do fator neurotrófico derivado do cérebro (BDNF, do inglês *brain-derived neurotrophic factor*) no hipocampo nas primeiras horas após sua prática. Por fim, o EF agudo aplicado durante a janela de consolidação tardia mostrou promover a persistência da memória por meio da ativação de receptores beta-adrenérgicos, dopaminérgicos do tipo D1/D5 e síntese proteica no hipocampo. Essas descobertas não apenas expandem nossa compreensão acerca dos mecanismos subjacentes aos efeitos do EF agudo na memória, mas também oferecem conhecimentos valiosos para o desenvolvimento de estratégias clínicas e terapêuticas baseadas nesse tipo de EF, com potencial de aprimorar a memória e o aprendizado.

Palavras-Chave: neuroquímica; consolidação; aprendizado.

ABSTRACT

The modulation of memory by acute physical exercise (PE) aims to enhance memory consolidation and persistence. It is known that a single session of PE, such as moderate-intensity treadmill running, promotes this effect. The mechanisms involved include the release of neurotransmitters such as noradrenaline and dopamine. However, significant gaps persist regarding the effects and mechanisms of acute PE in memory modulation. The objective of this thesis is to explore how acute PE influences learning and memory persistence. Using adult male Wistar rats as an experimental model, we investigated three essential questions: i) the role of brain regions ventral tegmental area (VTA) and locus coeruleus (LC) in the effects of acute PE on memory; ii) the requirement of hippocampal protein synthesis for acute PE effects on memory persistence; and iii) the impact and mechanisms of acute PE when applied during the late consolidation window, 11 hours after learning. Our results indicate that LC plays a crucial role in the effects of acute PE on memory consolidation and persistence, while VTA does not seem necessary for this process. Furthermore, we found that although hippocampal protein synthesis is vital for memory consolidation, acute PE overcomes inhibition of ribosomal protein synthesis and the mTOR pathway, promoting consolidation but not memory persistence. Moreover, we demonstrated that acute exercise does not elevate the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus during the initial hours following its practice. Lastly, acute PE applied during the late consolidation window was shown to promote memory persistence through activation of beta-adrenergic and dopamine D1/D5 receptors and hippocampal protein synthesis. These findings not only expand our understanding of the mechanisms underlying the effects of acute PE on memory but also provide valuable insights for the development of clinical and therapeutic strategies based on this type of PE, with the potential to enhance memory and learning.

Keywords: neurochemistry; consolidation; learning.

LISTA DE FIGURAS

LISTA DE SIGLAS

AMPA – alfa-amino-3-hidroxi-5-metil-4- isoxazolpropiônico

AMPc – adenosina 3',5'-monofosfato cíclico

ATV – Área tegmental ventral

BDNF – Fator neurotrófico derivado do cérebro; do inglês, *brain-derived neurotrophic factor*

cAMP – adenosina 3',5'-monofosfato cíclico; do inglês *cyclic adenosine monophosphate*

CEUA – Comissão de Ética para Uso de Animais

CREB – proteína de ligação responsiva ao AMPc; do inglês *response element-binding protein*

DNA – ácido desoxirribonucleico

EF – Exercício físico

E-LTP – LTP inicial; do inglês *early* LTP

L-LTP – LTP tardia; do inglês *late* LTP

GD – Giro denteado

LC – *Locus coeruleus*

LTP – Potenciação de longa duração; do inglês, *long-term potentiation*

MCD – Memória de curta duração

MLD – Memória de longa duração

mGlu-R – Receptor metabotrópico de glutamato

RNA – ácido ribonucleico

mRNA – RNA mensageiro

mTOR – Alvo da rapamicina em mamíferos; do inglês, *mammalian target of rapamycin*

NMDA – N-metil D-Aspartato

PKA - Proteínas quinase A

PRPs – Proteínas relacionadas à plasticidade

PSD-95 – Proteína de densidade pós-sináptica 95; do inglês, *post synaptic density protein 95*

RO – Reconhecimento de objetos

TH^+ – tirosina hidroxilase

TrkB – receptor de tropomiosina-relacionada à quinase B

tRNA – RNA transportador

UNIPAMPA – Universidade Federal do Pampa

SUMÁRIO

APRESENTAÇÃO

Nessa tese, o objetivo é aprofundar a compreensão dos complexos mecanismos neuroquímicos subjacentes aos efeitos do exercício físico (EF) agudo na persistência da memória. A pesquisa consiste em três estudos de experimentação animal, conduzidos após a aprovação da Comissão de Ética para Uso de Animais (CEUA) da Universidade Federal do Pampa (UNIPAMPA), conforme detalhado nos Anexos A e B. A tese está estruturada em três partes principais. Na "Parte I", a seção "Introdução" apresenta os conceitos fundamentais que embasam o estudo, seguida pela "Revisão de Literatura", que oferece atualizações sobre as temáticas essenciais relacionadas ao estudo. Nessa primeira parte, também são abordadas a "Justificativa" e os "Objetivos". A "Parte II" inclui os três artigos científicos desenvolvidos durante o período de doutorado, que ampliam nosso entendimento sobre os efeitos e mecanismos do EF agudo na memória. Um artigo já está publicado na revista *Physiology & Behavior*, enquanto os manuscritos 1 e 2 estão em fase de avaliação pelas revistas *Neurochemistry International* (ANEXO C) *Neuroscience* (ANEXO D). Na "Parte III" desta tese, são apresentadas as seções "Discussão" e "Conclusões", onde oferecemos interpretações abrangentes dos resultados obtidos em nossos estudos. Por fim, nas "Perspectivas Futuras", discutimos possíveis direções a serem exploradas com base em nossas descobertas. Ao final do documento, são listadas as referências bibliográficas utilizadas nas seções inicial e final.

PARTE I

1 INTRODUÇÃO

A consolidação da memória é um processo crucial para o armazenamento das informações adquiridas. Esse é um processo prolongado que envolve uma série complexa de eventos moleculares, destinados a transformar a aprendizagem em um traço de memória robusto e resistente ao longo do tempo (MCGAUGH, 2000; ABEL; LATTAL, 2001). Durante esse período, o novo material aprendido passa por uma consolidação gradual, tornando-se suscetível a modificações (ABEL; LATTAL, 2001). Apesar de décadas de estudo sobre os processos de consolidação das memórias de longa duração (MLD), ainda existem lacunas no entendimento dos eventos que induzem a uma memória persistente em comparação com outras (BEKINSCHTEIN et al., 2010; KATCHE; CAMMAROTA; MEDINA, 2013). Portanto, são necessárias investigações adicionais para identificar e compreender os mecanismos neuroquímicos subjacentes às estratégias capazes de fortalecer a consolidação do aprendizado e promover sua persistência ao longo do tempo.

Uma estratégia de modulação da memória que tem ganhado destaque é o exercício físico (EF) agudo, caracterizado por uma única sessão de atividade física, geralmente de intensidade moderada (LOPRINZI; MOORE; LOENNEKE, 2020). Essa estratégia tem sido aplicada em uma janela da consolidação próxima ao aprendizado, e mostra resultados positivos na modulação da memória (BOUCHET et al., 2017; DIEDERICH et al., 2017; VARGAS et al., 2017, 2020). A melhoria na consolidação da memória parece estar relacionada às alterações induzidas pelo EF nas neurotrofinas que estabilizam o traço de memória (LOPRINZI, 2019a). De fato, esta modalidade de EF pode aumentar a expressão do fator neurotrófico derivado do cérebro (BDNF, do inglês *brain-derived neurotrophic factor*) (SOYA et al., 2007; VENEZIA; QUINLAN; ROTH, 2017; VENEZIA et al., 2019), uma proteína crucial para a plasticidade neuronal e processos de consolidação e persistência da memória (BEKINSCHTEIN et al., 2008, 2010; ROSSATO et al., 2009).

Em nosso grupo de pesquisa, dedicamo-nos a explorar os efeitos e mecanismos associados a uma única sessão de EF na consolidação da memória de reconhecimento de objetos (RO) em animais experimentais. A memória de RO, uma forma de memória declarativa episódica, é facilmente comparável a contextos humanos, tornando-a relevante para futuras aplicações desta estratégia (MANNS et al., 2003). Temos observado que uma única sessão de trinta minutos de corrida em

esteira, realizada após a aprendizagem, estende a persistência da memória em pelo menos duas semanas, quando comparada aos animais que não praticaram o EF (VARGAS et al., 2017, 2020; LIMA et al., 2021b). Esse efeito é dependente da ativação dos receptores beta-adrenérgicos (VARGAS et al., 2017) e dopaminérgicos do tipo D1/D5 (VARGAS et al., 2020) no hipocampo, bem como da via da proteína quinase A (PKA) (LIMA et al., 2021b). Considerando a dependência catecolaminérgica no hipocampo para os efeitos do EF agudo, surge a possibilidade de que as áreas cerebrais que enviam projeções contendo esses neurotransmissores ao hipocampo também desempenhem um papel fundamental na ação do EF agudo. Isso nos leva a questionar: *“A área tegmental ventral (ATV) e o locus coeruleus (LC), as principais fontes de dopamina e noradrenalina no hipocampo, são necessárias para os efeitos do EF agudo na memória?”*

Ademais, está amplamente aceito que a síntese proteica no hipocampo é fundamental para a consolidação das MLD, e que a expressão do BDNF desempenha um papel crucial na garantia de sua persistência (BEKINSCHTEIN et al., 2008; ROSSATO et al., 2009; KATCHE et al., 2012). Portanto, existe a hipótese de que o EF agudo, ao estimular a síntese de BDNF e outras neurotrofinas, pode aprimorar a memória através do fenômeno conhecido como marcação e captura sináptica (LOPRINZI; PONCE; FRITH, 2018; LOPRINZI, 2019a). De acordo com esse fenômeno, um estímulo forte (como o EF agudo) pode induzir a expressão e síntese de proteínas relacionadas à plasticidade (PRPs) – como o BDNF, e compartilhar com sinapses paralelas de um aprendizado, contribuindo para sua estabilização como MLD (MONCADA; VIOLA, 2007; REDONDO; MORRIS, 2011). Considerando essa possibilidade, a pergunta que prossegue é: *"A síntese proteica no hipocampo é essencial para os efeitos do EF agudo na consolidação e persistência da memória?".*

Além da modulação da memória durante o período de consolidação inicial, um intervalo de tempo mais tardio, aproximadamente 11 a 12 horas após o aprendizado, tem sido identificado como crucial para os processos subjacentes à persistência das MLD (BEKINSCHTEIN et al., 2007; KATCHE; CAMMAROTA; MEDINA, 2013). Durante essa janela de tempo, ocorre uma série de eventos moleculares e celulares que fortalecem as conexões sinápticas das MLD (KATCHE; CAMMAROTA; MEDINA, 2013). Esses processos envolvem principalmente a ativação dos receptores dopaminérgicos (ROSSATO et al., 2009; KRAMAR et al., 2021), beta-

adrenérgicos (PARFITT et al., 2012) e a expressão do BDNF (BEKINSCHTEIN et al., 2008). Diante do conhecimento de que o EF agudo tem uma forte influência na ativação dessas vias, surge uma terceira pergunta: *"Quais são os efeitos e mecanismos do EF agudo sobre a memória quando praticado na janela de consolidação tardia?".*

Nessa tese, propomos responder às três questões fundamentais apresentadas ao longo deste texto introdutório, cada uma orientando o desenvolvimento de um estudo experimental. Nosso objetivo é aprofundar a investigação dos mecanismos neuroquímicos pelos quais o EF agudo modula a persistência da memória de RO. A compreensão desses mecanismos e a razão pela qual as memórias se tornam mais duradouras quando associadas a essa estratégia podem abrir caminhos para o desenvolvimento de terapias comportamentais ou farmacológicas inovadoras, visando aprimorar a cognição e melhorar a qualidade de vida.

2 REVISÃO DE LITERATURA

2.1 Memórias – definição e importância

Ao conhecer os mecanismos da memória, podemos afirmar que nós *"somos aquilo que recordamos"*, uma vez que só fazemos aquilo que sabemos e que um dia foi aprendido (IZQUIERDO, 2018). Assim, a memória nos torna únicos, seres dos quais não existe outro idêntico, pois as nossas experiências e interpretações acerca delas moldam nosso cérebro singularmente (IZQUIERDO, 2018). Nesse sentido, tanto em seres humanos quanto em outros animais, as memórias provêm de experiências individuais, que requerem a aquisição da informação – fase de aprendizado, consolidação – fase de armazenamento, e evocação – lembrança daquilo que foi armazenado (ABEL; LATTAL, 2001).

As memórias armazenam-se em redes neurais, mais especificamente, nas células nervosas – os neurônios – que se modificam a cada nova informação adquirida e fazem novas conexões a fim de relacionar com aquilo que já foi aprendido e consolidado (CARRILLO-REID, 2022; GUSKJOLEN; CEMBROWSKI, 2023). No cérebro humano, existem aproximadamente 86 bilhões de neurônios (AZEVEDO et al., 2009), cada um transmitindo e recebendo informações por meio de unidades funcionais chamadas sinapses (MAYFORD; SIEGELBAUM; KANDEL, 2012). Quando as informações são armazenadas para uso futuro, ocorrem mudanças tanto bioquímicas quanto físicas nos neurônios ativados durante a aquisição, que podem posteriormente serem reativados durante a recuperação da memória (ORTEGA-DE SAN LUIS; RYAN, 2022). Este fenômeno reflete a plasticidade do cérebro e sua capacidade de aprender e se adaptar ao longo do tempo.

Saber que as memórias provêm de experiências significa que há tantos tipos de memória quanto possibilidades de vivências a serem experienciadas. Isto implica que as memórias podem ser classificadas de diversas formas (IZQUIERDO, 2018). As classificações tradicionais da memória (**Figura 1**) abrangem diferentes aspectos, incluindo (1) sua função, que determina como a memória é usada no contexto diário, sendo a *memória de trabalho*, para gerenciamento imediato da realidade; ou a *propriamente dita*, armazenada para uso posterior; (2) o tempo de duração, que

varia desde as *memórias de curta duração* (MCD), que duram poucas horas, até as MLD, que perduram entre horas, dias, meses ou anos, e; (3) o conteúdo aprendido, que abarca as *memórias declarativas*, que incluem as memórias episódicas detalhadas e os conhecimentos semânticos e as *não declarativas*, relacionadas à memórias motoras e procedurais (IZQUIERDO, 2018; SQUIRE, 2009).

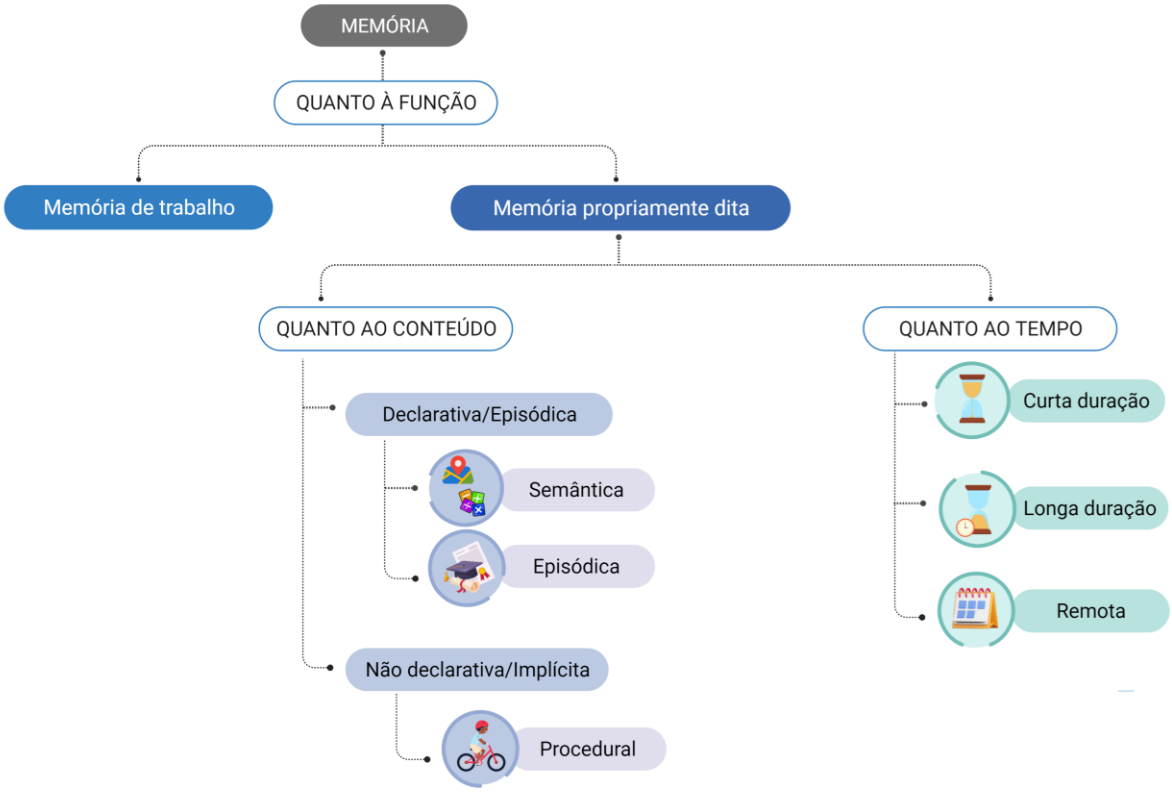

Figura 1 Classificações das memórias. Quanto à função, há dois tipos de memória: a *memória de trabalho*, que é breve e fugaz, mantém a informação por segundos a minutos para o gerenciamento da realidade e envolve apenas atividade elétrica dos neurônios do córtex pré-frontal e hipocampo, sem modificações bioquímicas ou anatômicas; e a *memória propriamente dita*, que é mais complexa e demanda de processos neuroquímicos e do envolvimento de diferentes regiões cerebrais para o armazenamento das informações – abrange as demais classificações de memória. Quanto ao conteúdo, as memórias podem ser: *declarativas* (envolvem principalmente o hipocampo e suas conexões), e conforme o conteúdo registrado, podem ser subdividas em *semânticas* ou *episódicas*, respectivamente – enquanto as *semânticas* estão relacionadas à conhecimentos gerais, como de matemática ou geografia, as *episódicas* são autobiográficas e referem-se a eventos vivenciados, como as lembranças da formatura ou aniversário; ou *não declarativas* (envolvem prioritariamente o núcleo caudado, suas conexões e o cerebelo), que consistem em memórias *procedurais* e referem-se à capacidade ou habilidades motoras e sensoriais, sendo mais fáceis de serem executadas do que descritas, como tocar violão ou andar de bicicleta, por exemplo. Quanto ao tempo que duram, as memórias propriamente ditas podem ser: de *curta duração (MCD)*, quando duram entre 1 e 6 horas; *longa duração (MLD)*, quando duram horas, dias, meses ou anos; ou podem ser *remotas,* quando as MLD duram meses, anos ou décadas. Fonte: elaborado pela autora.

Cada classificação oferece uma janela única para entender a complexidade do funcionamento da memória, revelando a diversidade e a profundidade dos processos cognitivos subjacentes. Além das categorizações tradicionais, é importante ressaltar que as memórias muitas vezes se entrelaçam e se relacionam entre si (IZQUIERDO, 2018). Por exemplo, as memórias episódicas, que capturam eventos específicos e detalhados da nossa vida, frequentemente desempenham um papel fundamental na formação de MLD. Quando recordamos uma experiência pessoal significativa, estamos, de fato, acessando uma memória episódica específica que pode ter sido consolidada como MLD. Essas interconexões entre o conteúdo da memória e seu tempo de duração ilustram como nosso cérebro organiza e utiliza informações do passado para moldar nossa compreensão do presente e nossas ações futuras.

Embora todas as classificações e subtipos de memória sejam relevantes, esta tese enfatizará a memória declarativa episódica de longa duração. A escolha em destacar esse tipo de memória é fundamentada em sua abrangente importância, visto que essas memórias desempenham um papel crítico na capacidade de lembrança consciente de fatos e eventos e na habilidade de interação com o mundo externo (SQUIRE, 2004, 2009). Além disso, ela oferece uma oportunidade valiosa para estudos em modelos de animais experimentais, facilitando a compreensão dos mecanismos subjacentes e das possíveis estratégias de modulação para um armazenamento mais eficaz (SQUIRE, 2004). Essas memórias não apenas definem nossa essência, mas também exercem uma influência decisiva sobre nossas escolhas e comportamentos cotidianos, moldando nossas interações sociais, profissionais, emocionais e nosso relacionamento com o ambiente em que estamos inseridos.

2.2 Memórias declarativas episódicas

As memórias declarativas episódicas são registros de eventos que testemunhamos ou participamos ativamente (MAYFORD; SIEGELBAUM; KANDEL, 2012). Elas se relacionam a fatos e eventos específicos, fornecendo detalhes sobre o contexto espacial e temporal, e permitindo a lembrança de pessoas, lugares e objetos associados (MAYFORD; SIEGELBAUM; KANDEL, 2012). De forma geral, qualquer acontecimento na vida constitui um episódio, que é definido por um objeto ("o que"), espaço ("onde") e tempo ("quando") (ENNACEUR, 2010). A união e recuperação desses três componentes de um episódio juntos é o que torna a memória episódica distinta de outras formas de memória (ENNACEUR, 2010).

Em humanos, exemplos dessas memórias incluem lembranças de eventos familiares, experiências de viagens e ocasiões especiais da vida. Mas, sobretudo, ela está presente nas memórias mais usuais e menos significativas no dia a dia, como lembrar onde deixamos as chaves de casa ou onde estacionamos o carro. Embora essas memórias sejam caracteristicamente humanas, não há dúvida de que os animais têm memória episódica (CRYSTAL, 2010; TEMPLER; HAMPTON, 2013). Os experimentos em animais de laboratório abrangem quase exclusivamente memórias episódicas (reconhecer um evento, lembrar se ele exige fugir ou lutar, se é agradável ou não, reconhecer seu contexto, etc.) (CRYSTAL, 2010). Esses estudos têm ajudado a compreender mais sobre os processos de formação, manutenção e modulação da memória (DICKERSON; EICHENBAUM, 2010).

Um dos exemplos de memória declarativa episódica mais investigado em roedores é a memória de RO, que avalia a capacidade do animal em distinguir um objeto familiar de um novo (MANNS et al., 2003). A tarefa de RO, inicialmente delineada por Ennaceur & Delacour (1988), é inteiramente baseada no comportamento espontâneo dos animais e é comparável aos testes de memória usados em humanos. Baseia-se na tendência natural dos animais em explorar novidades, e dentre as vantagens, não exige treinamento preliminar extenso, não requer exposição a estímulos aversivos, restrição de alimentos ou água e tem sido reproduzido em vários laboratórios, utilizando uma variedade de dispositivos e objetos, tanto em camundongos quanto em ratos (BEVINS; BESHEER, 2006).

Atualmente, existem vários protocolos e adaptações da tarefa de RO (ANTUNES; BIALA, 2012). Em nosso laboratório, padronizamos um protocolo específico para avaliar os processos de consolidação e persistência da memória (NEVES et al., 2020; LIMA et al., 2021b). Esse protocolo envolve algumas etapas: primeiro, os animais são habituados ao aparato, uma caixa de madeira com dimensões de 50 x 50 x 50 cm. Em seguida, há uma sessão de treinamento, na qual os animais são permitidos a explorar livremente dois objetos desconhecidos e distintos. Posteriormente, são realizadas sessões de teste de memória, durante as quais são apresentados um objeto familiar e um novo objeto a cada teste. Os testes são conduzidos 1, 7 e 14 dias após o aprendizado inicial, permitindo-nos investigar tanto a consolidação quanto a persistência da memória ao longo do tempo. A seleção dos objetos é cuidadosamente organizada para evitar qualquer preferência natural dos animais por um objeto específico, um aspecto que foi confirmado em testes piloto (**Figura 2**).

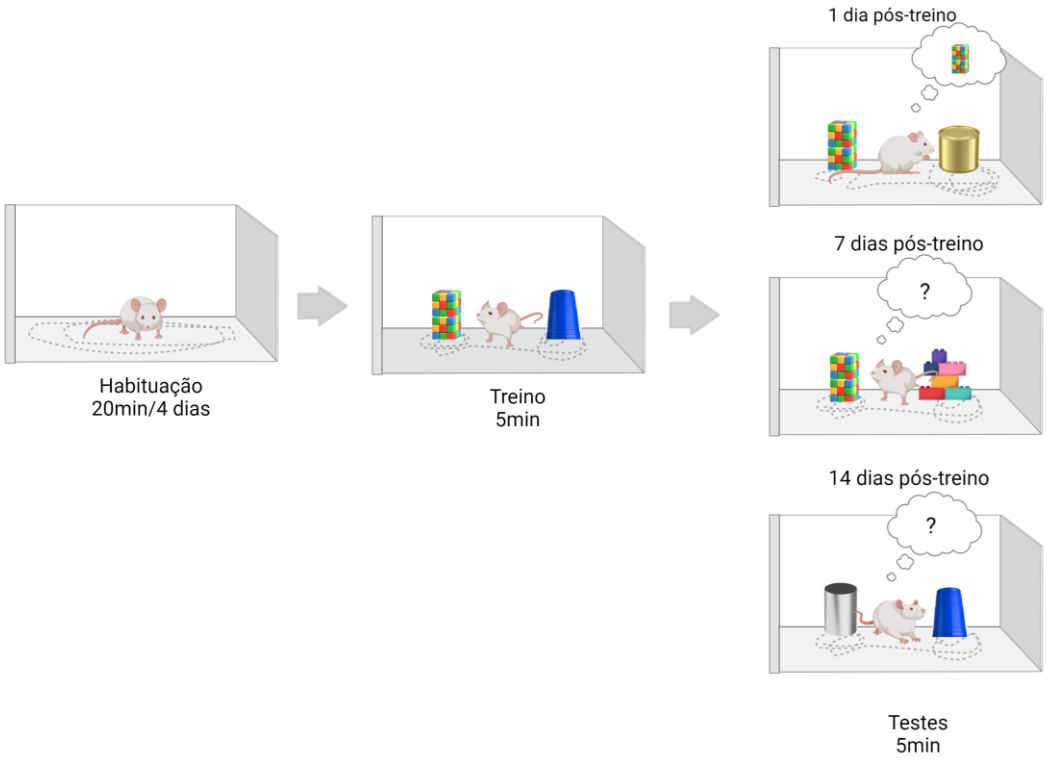

Figura 2 Protocolo da tarefa de reconhecimento de objetos. Inicialmente, os animais são habituados ao aparato (caixa de madeira, 50 x 50 x 50 cm). A habituação envolve a livre exploração do ambiente por 20 minutos diários durante 4 dias consecutivos. Após um período de 24 horas desde o último dia de habituação, os animais passam por uma sessão de treino, com duração de 5 minutos, onde são apresentados a dois objetos distintos e desconhecidos. A avaliação da memória ocorre em

sessões de teste realizadas nos dias 1, 7 e 14 após o treino. Cada sessão de teste, com duração de 5 minutos, permite aos animais explorar um objeto familiar e um novo. A consolidação e persistência da memória são determinadas pela análise da exploração significativa do objeto novo em comparação com o objeto familiar. Nesse protocolo a consolidação, avaliada 1 dia após o treino em animais saudáveis, é observada pela exploração do novo objeto durante um tempo significativamente maior do que do objeto familiar. Nos demais testes, um esquecimento fisiológico é observado, e os animais não distinguem o objeto familiar do novo. Na imagem estão representadas as combinações de objetos utilizadas em cada sessão: A – dois cubos mágicos (um em cima do outro); B – copo de plástico; C – lata circular; D – peça de legos; E – cilindro metálico. Todos os objetos possuem a mesma altura e são fixados no assoalho do aparato, de modo que os animais não consigam movê-lo. Fonte: elaborado pela autora.

Em animais saudáveis, este protocolo de RO promove por si só a consolidação da memória, observada pela exploração significativamente maior do novo objeto comparado ao objeto familiar no teste conduzido 1 dia após o treino. No entanto, é observado um esquecimento fisiológico da memória nos testes conduzidos 7 e 14 dias após. Assim, nosso grupo de pesquisa tem buscado por estratégias comportamentais e farmacológicas que possam modular a consolidação dessa memória e melhorar a sua persistência (VARGAS et al., 2017; NEVES et al., 2020; LIMA et al., 2021b). De fato, essa tarefa tem demonstrado ser uma ferramenta útil para avaliar os processos comportamentais e neurais que medeiam o armazenamento e outros processos relacionados à memória (FURINI et al., 2014, 2020), e os mecanismos de consolidação no hipocampo são tidos como fundamentais para a sua estabilização (IZQUIERDO, 2018).

2.3 Mecanismos de consolidação e persistência das MLD

O hipocampo (**Figura 3**) é uma área do cérebro fundamental para os mecanismos de consolidação e persistência da memória. Esta região recebe projeções do córtex perirrinal, uma área fundamental para o processamento de informações sensoriais como estímulos visuais, olfativos e somatossensoriais, todos relevantes para o RO (CLARKE et al., 2010). Mais especificamente, a região CA1 mostra-se essencial para os processos que medeiam a formação da MLD, incluindo a síntese proteica (ROSSATO et al., 2007; MYSKIW et al., 2008), plasticidade sináptica (CLARKE et al., 2010) e sinalização de uma gama de receptores (FURINI et al., 2010, 2014; MELLO-CARPES et al., 2016), além de mecanismos moleculares específicos (FURINI et al., 2020).

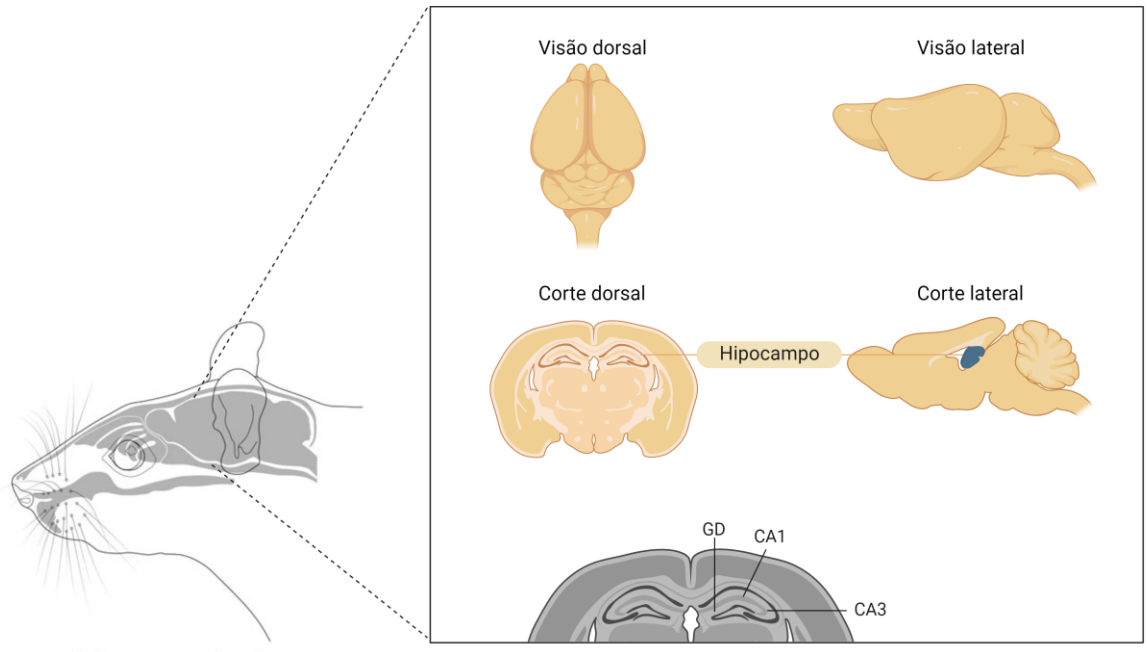

Figura 3 Encéfalo do roedor e localização do hipocampo e suas sub-regiões. Visão dorsal e lateral do encéfalo de um roedor, e seus respectivos cortes destacando a localização do hipocampo na região do lobo temporal. As três principais regiões do hipocampo são destacadas: giro denteado (GD), CA1 e CA3. Fonte: elaborado pela autora.

Estudos sobre memória e neuroplasticidade indicam que a consolidação da memória é tempo-dependente (MCGAUGH, 2000). Após a aquisição e passagem pela memória de trabalho, a informação é armazenada como MCD ou MLD, processos que ocorrem paralelamente e de forma independente (MCGAUGH, 2000).

Enquanto as MCD podem ser evocadas em 1 a 6 horas, as MLD exigem pelo menos 6 horas até sua evocação (IZQUIERDO, 2018). É importante destacar que, embora estes processos ocorram de forma paralela e independente, a informação evocada a partir destas memórias é a mesma, o que as diferencia são os mecanismos que as compõem (IZQUIERDO, 2018). As MCD requerem mecanismos menos complexos do que as MLD – essa complexidade das MLD culmina na síntese proteica, que permite o estabelecimento de modificações neurais para o armazenamento a longo prazo da memória (IZQUIERDO, 2018) (**Figura 4**).

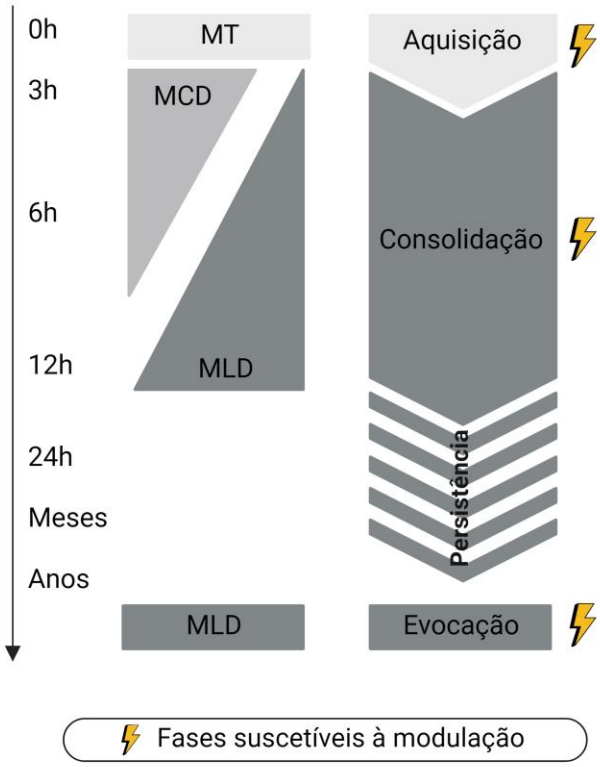

Figura 4 Estabelecimento das memórias de longa duração (MLD). A memória segue três etapas distintas: aquisição, consolidação e evocação, e envolve diferentes tipos de memória em relação ao tempo. Na aquisição, as informações são percebidas e codificadas pelos sentidos para processamento cerebral. A memória de trabalho (MT) retém dados temporários para tarefas mentais imediatas e determina a relevância para a consolidação. Durante a consolidação, as informações estabilizam-se como memórias de curta (MCD) ou longa duração (MLD), envolvendo processos paralelos e independentes. Na evocação, as informações armazenadas são recuperadas conforme necessário. As MCD são evocadas até que as MLD se consolidem, e essas últimas podem persistir por horas, meses ou anos. Todas as fases podem ser moduladas por influências internas ou externas, incluindo estratégias farmacológicas e comportamentais. Fonte: elaborado pela autora.

A consolidação das MLD segue uma sequência de processos bioquímicos baseados nos mecanismos da potenciação de longa duração (LTP, do inglês *long-term potentiation*) (MCGAUGH, 2000; WHITLOCK et al., 2006). A LTP foi identificada

em diversas sinapses no cérebro (BALTACI et al., 2018), no entanto, é no hipocampo que ela se destaca como a forma mais estudada de plasticidade sináptica (LU; CHRISTIAN; LU, 2008). Os eventos bioquímicos desencadeados por um breve estímulo tetânico que iniciam a LTP são conhecidos como indução (BALTACI et al., 2018). Os processos que ocorrem após essa indução e resultam em alterações duradouras na atividade sináptica são denominados expressão (BALTACI et al., 2018). A LTP apresenta uma fase inicial que não requer síntese de proteínas (E-LTP, do inglês *early* LTP) e uma fase tardia (L-LTP, do inglês *late* LTP) que implica ativação de fatores de transcrição, depende da síntese de proteínas e é caracterizada por mudanças estruturais (BALTACI et al., 2018; LU; CHRISTIAN; LU, 2008).

Esse fenômeno eletrofisiológico, que reflete mudanças nas conexões neurais em resposta a estímulos específicos, compartilha muitas semelhanças com os processos relacionados à formação da memória. Por exemplo, assim como a E-LTP, a MCD não induz síntese proteica, da mesma forma em que L-LTP e a MLD apresentam comportamentos similares quanto à indução de síntese de proteínas e ativação de mecanismos de mudança e estabilização sináptica (COSTA-MATTIOLI et al., 2007). Assim, isto leva a hipótese, que hoje é amplamente aceita, de que os mecanismos da LTP são a base da compreensão sobre como as memórias são consolidadas em nível celular e molecular (HERNANDEZ; ABEL, 2008; IZQUIERDO et al., 2008).

De forma geral, a consolidação celular (ou sináptica) – também chamado o processo que ocorre logo após a fase de aquisição – envolve diferentes eventos moleculares que incluem a ativação de cascatas de sinalização em regiões específicas do cérebro, especialmente no hipocampo, para a formação de memórias declarativas (MEDINA et al., 2008; MAYFORD; SIEGELBAUM; KANDEL, 2012). A sequência de eventos moleculares envolvidos na consolidação da MLD começa com a estimulação repetida das células do hipocampo através dos receptores glutamatérgicos ionotrópicos, alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiônico (AMPA) e N-metil-D-aspartato (NMDA), e os metabotrópicos, como o receptor metabotrópico de glutamato (mGlu-R).

Esse processo inicial desencadeia a entrada de íons sódio (Na^{+}) na célula, resultando em despolarização. Consequentemente, o íon magnésio (Mg^{2+}) é expulso da célula, deixando o receptor NMDA livre e funcional para permitir a entrada de íons

cálcio (Ca^{2+}) através dele. Os receptores mGlu-R também são ativados, aumentando a concentração intracelular de Ca^{2+}. Esse aumento de Ca^{2+} intracelular estimula uma série de enzimas proteinoquinases. Essas enzimas regulam a fosforilação de proteínas em sítios específicos, incluindo fatores de transcrição de DNA (ácido desoxirribonucleico), como o CREB (proteína de ligação responsiva ao elemento CRE do AMPc – adenosina 3',5'-monofosfato cíclico), presente no núcleo celular. A ativação do CREB promove a síntese de RNAs (ácido ribonucleico) mensageiros (mRNAs), que por sua vez resultam na síntese de proteínas nos ribossomos celulares. Algumas dessas proteínas são transportadas para os terminais sinápticos das células, alterando sua função (ABEL; LATTAL, 2001; MEDINA et al., 2008).

Esses requisitos moleculares iniciais para a consolidação da MLD são apenas os primeiros passos de uma série de eventos que ocorrem ao longo de um período prolongado, que dura de várias horas a alguns dias (MEDINA et al., 2008). De acordo com o tempo em que esses eventos moleculares ocorrem na janela de consolidação da MLD, estes podem ser denominados “consolidação inicial” ou “consolidação tardia” (KATCHE; CAMMAROTA; MEDINA, 2013). A consolidação inicial é aquela que inicia logo após a aquisição e está relacionada a retenção da memória (IZQUIERDO et al., 2006), enquanto isso, a consolidação tardia é aquela que ocorre cerca de 11 a 12 horas após a aquisição e é essencial para a persistência da memória (ROSSATO et al., 2009; BEKINSCHTEIN et al., 2010; KATCHE et al., 2016). Em ambos os períodos, ocorrem diversos processos moleculares e a memória permanece suscetível à modulação.

Durante as duas fases de consolidação, intervenções farmacológicas ou comportamentais são capazes de modular a persistência da memória. Sabe-se que a força das memórias episódicas varia com o significado emocional dos eventos (MCGAUGH, 2013; MCHAUGH, 2004). Assim, experiências desagradáveis como um acidente automobilístico ou um assalto, e também as agradáveis como aniversários, casamentos e outras ocasiões com envolvimento emocional tendem a formar memórias mais duradouras (MCGAUGH, 2013). O mesmo ocorre em animais experimentais, e isso é facilmente observado em roedores que receberam um estímulo elétrico nas patas em uma tarefa de esquiva inibitória, onde a memória é preservada e apresentada como aversiva mesmo após consecutivas sessões de extinção, processo que visa inibir a expressão da memória aversiva (LIMA et al., 2023).

Dentre os mecanismos moleculares no hipocampo que envolvem o processo de formação e manutenção de MLD persistentes, a síntese proteica hipocampal e ativação de receptores dopaminérgicos e beta-adrenérgicos parecem ser essenciais. Essas vias modulam a retenção do aprendizado tanto na consolidação inicial (FIORITI et al., 2015; VARGAS et al., 2017, 2020) quanto na consolidação tardia (BEKINSCHTEIN et al., 2007, 2010; ROSSATO et al., 2009; PARFITT et al., 2012). Nas próximas seções, serão explorados em detalhes os intricados processos da síntese proteica no hipocampo e a importância do requerimento catecolaminérgico, destacando como esses mecanismos moleculares desempenham um papel decisivo na formação e manutenção de MLD persistentes.

2.3.1 Requisição de síntese proteica

A síntese proteica no hipocampo emerge como um processo crucial na consolidação das MLD, desempenhando um papel crítico ao mediar alterações anatômicas e moleculares nos neurônios, influenciando na conexão sináptica e, por conseguinte, estabilizando a memória (ALBERINI; KANDEL, 2020). Essas evidências são principalmente sustentadas por experimentos farmacológicos realizados em animais experimentais, utilizando uma variedade de paradigmas de memória e aprendizado. Nessas investigações, a administração de fármacos que inibem a síntese proteica ribossomal ou vias de transcrição tem demonstrado prejudicar significativamente a consolidação das MLD quando aplicados em uma janela temporal específica (SCHARF et al., 2002; IZQUIERDO et al., 2008; FURINI et al., 2015).

A anisomicina e a rapamicina são duas substâncias, com mecanismos de ação distintos (**Figura 5**), amplamente empregadas em pesquisas científicas para investigar os mecanismos subjacentes à consolidação das memórias. A anisomicina atua bloqueando a translação do RNA mensageiro (mRNA) para proteínas, interrompendo, assim, a produção de proteínas específicas. Por outro lado, a rapamicina inibe uma proteína denominada mTOR (do inglês, *mammalian target of rapamycin*) e interrompe diversas vias celulares, incluindo aquelas relacionadas à síntese proteica. Em estudos sobre memória, enquanto a anisomicina tem sido empregada para investigar os efeitos da inibição direta da síntese proteica, a rapamicina é frequentemente utilizada para compreender como a inibição das vias de sinalização que medeiam a síntese proteica impacta os processos de consolidação da memória. Muitos estudos usam as duas estratégias farmacológicas a fim de oferecer uma visão mais profunda sobre os eventos moleculares que contribuem para a estabilidade das memórias declarativas (MYSKIW et al., 2014a, 2014b; VARGAS; LIMA; MELLO-CARPES, 2021).

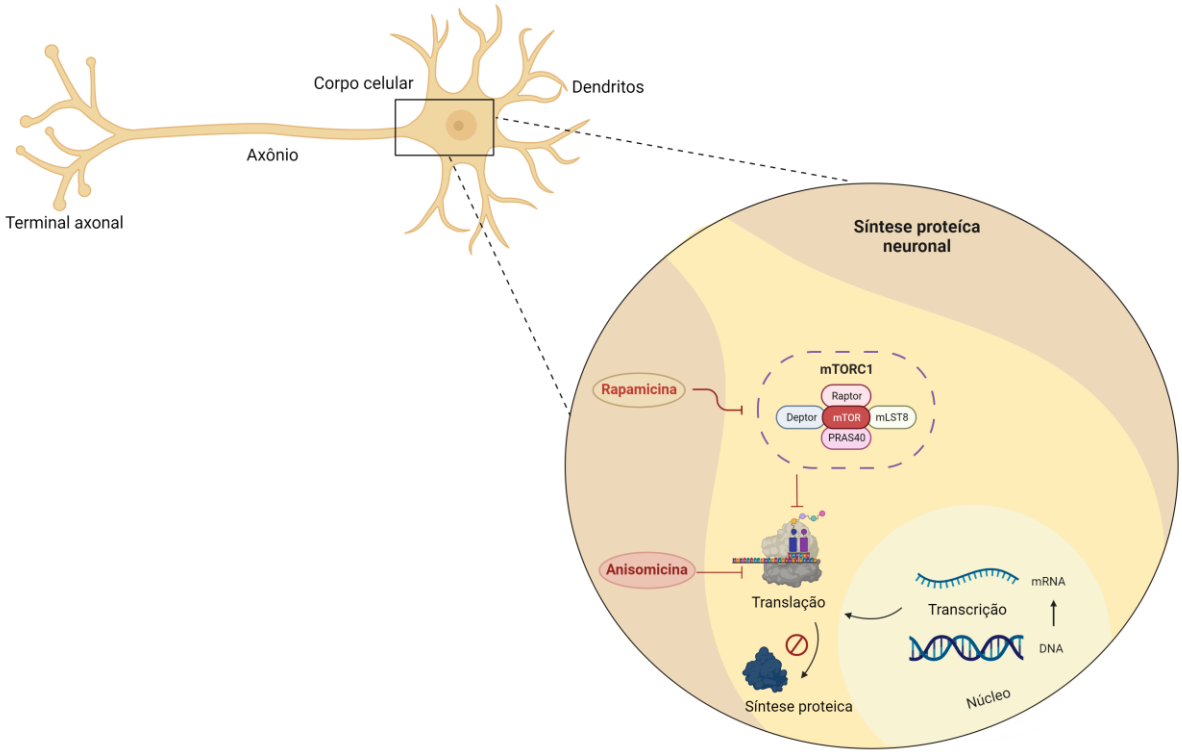

Figura 5 Mecanismos de ação da anisomicina e rapamicina na inibição da síntese proteica neuronal. Os neurônios são compostos pelo corpo celular, dendritos, axônio e terminal axonal, sendo a síntese proteica um produto de reações que ocorrem no corpo celular. O DNA é "transcrito" pelo RNA mensageiro (RNAm) e depois a informação é "traduzida" pelos ribossomos (compostos de RNA ribossômico e moléculas de proteínas) e pelo RNA transportador (RNAt), que transporta os aminoácidos, cuja sequência determinará a proteína a ser formada. A anisomicina é um inibidor potente e reversível da síntese proteica em organismos eucarióticos, atua ligando e inibindo a atividade da peptidil transferase da subunidade ribossômica 60S. A rapamicina é um macrolídeo proveniente de uma bactéria chamada *Streptomyces hygroscopicus*, atua ligando-se ao domínio FKBP12 da mTOR (do inglês, *mammalian target of rapamycin*), mais especificamente no complexo proteico mTORC1, inibindo funções como crescimento celular, proliferação e síntese proteica. Fonte: elaborado pela autora.

Na fase inicial do processo de consolidação, a síntese proteica desempenha um papel crucial na formação da memória, um requisito que tem sido especialmente destacado na região CA1 do hipocampo. A infusão de anisomicina imediatamente após o aprendizado prejudica a consolidação da MLD em ratos testada 24 horas após a aquisição, em diversas tarefas comportamentais, como RO (ROSSATO et al., 2007; FURINI et al., 2015), reconhecimento espacial (OZAWA; YAMADA; ICHITANI, 2017) e esquiva inibitória (MENEZES et al., 2015). De maneira semelhante, a atividade da proteína quinase mTOR no hipocampo dorsal é essencial para a consolidação da memória, incluindo, entre outros paradigmas, a memória de RO (MYSKIW et al., 2008; JOBIM et al., 2012). Estudos que investigaram os efeitos desses inibidores na tarefa de RO indicam que a síntese proteica hipocampal, mediada por ambas as vias, é necessária em uma janela de tempo limitada durante

a consolidação inicial, que se inicia imediatamente após a aquisição e persiste por aproximadamente 3 horas (ROSSATO et al., 2007; MYSKIW et al., 2008).

Por outro lado, durante a fase de consolidação tardia, a síntese proteica hipocampal tem se mostrado fundamental para a persistência, embora não para a consolidação, da memória. Em experimentos nos quais a anisomicina foi infundida na região CA1 do hipocampo 11,5 horas (RAVEN et al., 2021) ou 12 horas (BEKINSCHTEIN et al., 2007, 2008, 2010) após a aquisição nas tarefas de medo condicionado ou esquiva inibitória, a persistência da memória, testada 7 dias após, foi prejudicada, enquanto a consolidação da memória, avaliada 2 dias após o treino, permaneceu intacta. Nessas tarefas, tanto a administração sistêmica de rapamicina em camundongos (MACCALLUM; BLUNDELL, 2020), quanto sua infusão na região CA1 em ratos (BEKINSCHTEIN et al., 2008), realizadas 12 horas após o condicionamento, não afetaram a consolidação ou a persistência da memória, que foram testadas 2 e 7 dias após a aquisição, respectivamente. No entanto, a requisição da síntese proteica e da via mTOR no hipocampo dentro dessa janela de tempo ainda não foi claramente estabelecida na tarefa de RO.

Dentre os produtos resultantes da síntese proteica no hipocampo, o BDNF destaca-se nos processos de consolidação e persistência da memória. O BDNF, uma proteína dimérica de tamanho reduzido, exerce sua função ao se ligar com alta afinidade ao receptor tirosina quinase, conhecido como tropomiosina-relacionada à quinase B (TrkB) (LU; CHRISTIAN; LU, 2008). Tanto o BDNF quanto o TrkB estão amplamente distribuídos em diversas sub-regiões do hipocampo (WEBSTER et al., 2006). O BDNF causa a despolarização dos neurônios tão eficientemente quanto o glutamato (MATSUMOTO et al., 2001), melhorando por si só, ou em cooperação com o glutamato, a transmissão sináptica glutamatérgica (MARTIN; FINSTERWALD, 2011). Além disso, aumenta a fosforilação das subunidades dos receptores de NMDA no hipocampo (LIN et al., 1998) e induz a LTP no hipocampo (YING et al., 2002).

O BDNF é, portanto, uma molécula fundamental envolvida em mudanças plásticas relacionadas à aprendizagem e memória (MIRANDA et al., 2019). A sua expressão durante uma aprendizagem, promove a consolidação da memória, como visto na tarefa de RO, onde o próprio treino na tarefa aumenta os níveis desta proteína no hipocampo dorsal, de forma suficiente para induzir a consolidação da memória, observada 24 horas após (FURINI et al., 2010). Além disso, o BDNF, por

conta própria, promove a persistência da memória, transformando uma MLD não persistente em um traço mais forte e duradouro (BEKINSCHTEIN et al., 2007).

O papel-chave do BDNF na persistência da memória tem sido comprovado em protocolos experimentais conduzidos principalmente na fase de consolidação tardia. O bloqueio da expressão de BDNF na região CA1 do hipocampo 12 horas após a aquisição tem efeitos similares aos da anisomicina e prejudica a persistência da memória avaliada 7, mas não 2 dias, após (BEKINSCHTEIN et al., 2007). Isso demonstra que a atividade endógena do BDNF é necessária durante uma janela de tempo tardia para persistência da MLD. De forma interessante, a infusão de BDNF nesta mesma janela de tempo demonstra ser suficiente para promover a persistência da MLD, mesmo com a inibição paralela da síntese proteica hipocampal por anisomicina (BEKINSCHTEIN et al., 2008). Dentre os sistemas moduladores da expressão de BDNF, o papel dos receptores dopaminérgicos (ROSSATO et al., 2009) e beta-adrenérgicos (FURINI et al., 2010; MELLO-CARPES et al., 2016) é destacado.

2.3.2 Envolvimento do sistema catecolaminérgico

O sistema catecolaminérgico é uma complexa rede de neurotransmissores no cérebro, e é composto principalmente pelos sistemas dopaminérgico e noradrenérgico. Esses sistemas desempenham papéis fundamentais em várias funções cerebrais, incluindo a regulação do humor, atenção, vigilância, recompensa e, notavelmente, a consolidação e persistência da memória (HANSEN, 2017; RANJBAR-SLAMLOO; FAZLALI, 2020). Estudos aprofundados sobre esses sistemas neuroquímicos têm revelado sua importância na modulação da plasticidade sináptica e na influência direta sobre os mecanismos relacionados à memória (LEMON et al., 2009; GHANBARIAN; MOTAMEDI, 2013; HANSEN, 2017),

A ATV e o LC são reconhecidas como as principais fontes de dopamina e noradrenalina no cérebro, apresentando projeções diretas para o hipocampo (**Figura 6**) (LISMAN; GRACE, 2005; SARA, 2009). Enquanto a ATV envia projeções dopaminérgicas, o LC é capaz de liberar tanto dopamina quanto noradrenalina simultaneamente (RANJBAR-SLAMLOO; FAZLALI, 2020). A convergência dessas vias de sinalização dopaminérgica e noradrenérgica no hipocampo, bem como a ativação das ambas as regiões, ATV e LC, sugere que esses sistemas podem exercer efeitos neurofisiológicos paralelos e complementares (TAKEUCHI et al., 2016; MONCADA, 2017).

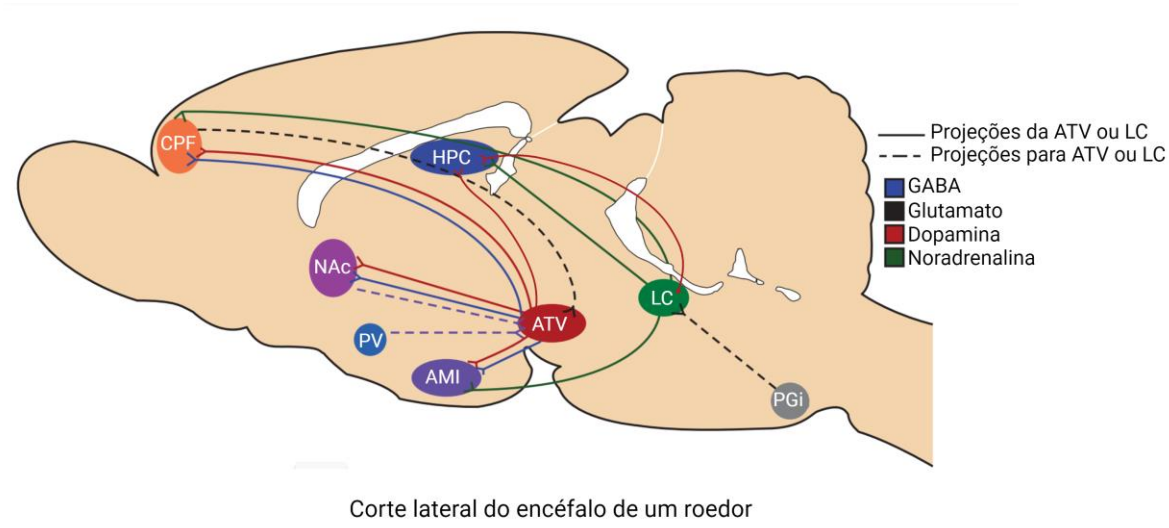

Figura 6 Localização, aferências e eferências da área tegmental ventral (ATV) e locus coeruleus (LC). A ATV e o LC recebem e enviam diferentes tipos de inervações – gabaérgicas (azul),

glutamatérgicas (preto), dopaminérgicas (vermelho) e noradrenérgicas (verde). A ATV envia projeções dopaminérgicas para regiões como hipocampo (HPC), córtex pré-frontal (CPF), núcleo *accumbens* (NAc) e amígdala (AMI) e gabaérgicas para CPF, NAc e AMI. Esta região recebe projeções de regiões como CPF, NAc e palido ventral (PV). O LC envia projeções dopaminérgicas ao HIP e noradrenérgicas, além do HIP, ao CPF e AMI. O núcleo paragigantocelular (PGi) envia projeções glutamatérgicas ao LC. Fonte: Adaptado de MAZEI-ROBISON & NESTLER, 2012.

A ativação da ATV confirma ser essencial para a consolidação e persistência das MLD por mecanismos dependentes do tempo. Esta região é crucial para a consolidação de diversos tipos de memórias declarativas, incluindo a memória aversiva (ROSSATO et al., 2009; MAHMOODI; SHAHIDI; HASANEIN, 2011) e a memória de RO (ROSSATO et al., 2013), uma vez que sua inativação logo após a aquisição prejudica a consolidação da memória avaliada 24 horas após. Corroborando com esses achados, a estimulação elétrica nesta região, dentro de uma janela de tempo restrita de 60 minutos antes ou após a aquisição, melhora a persistência da memória, ativando receptores D1/D5 e promovendo síntese proteica no hipocampo (MONCADA, 2017).

Quando realizada 12 horas após a aquisição, a inibição da ATV prejudica a memória avaliada 14, mas não 2 dias, depois (ROSSATO et al., 2009). Nesse mesmo contexto, a estimulação dessa região a partir da infusão do agonista glutamatérgico NMDA ou do agonista dopaminérgico SKF38393 no mesmo intervalo de tempo tardio promove a persistência da memória, por mecanismo intrinsecamente relacionado com a indução da expressão de BDNF no hipocampo (ROSSATO et al., 2009). Juntos, esses achados ressaltam o papel crucial da ATV na regulação temporal da consolidação e persistência das memórias declarativas, evidenciando conexões complexas entre a ativação da ATV, a expressão de BDNF e a síntese proteica no hipocampo.

De maneira semelhante, há evidências que destacam a importância do LC nos processos de consolidação e persistência da memória. A estimulação elétrica do LC, realizada 60 minutos antes ou após a aquisição do aprendizado, promove a formação de MLD, quando apenas o treinamento por si só não seria suficiente para esse efeito (MONCADA, 2017). Além disso, a inibição do LC imediatamente após e três horas após a aquisição compromete a memória de RO, testada 24 horas mais tarde (MELLO-CARPES; IZQUIERDO, 2013). Embora os efeitos do LC na memória tenham ressaltado a relevância das projeções noradrenérgicas (MELLO-CARPES et al., 2016; MONCADA, 2017), o tônus dopaminérgico no hipocampo apresenta ter

forte influência oriunda do LC (KEMPADOO et al., 2016). Curiosamente, os neurônios do LC contribuem com 73% de todo o tônus dopaminérgico mensurável no hipocampo dorsal, sugerindo que a liberação de dopamina do LC pode ter uma influência mais significativa sobre a atividade desta região do que a ATV (KEMPADOO et al., 2016) (**Figura 7**).

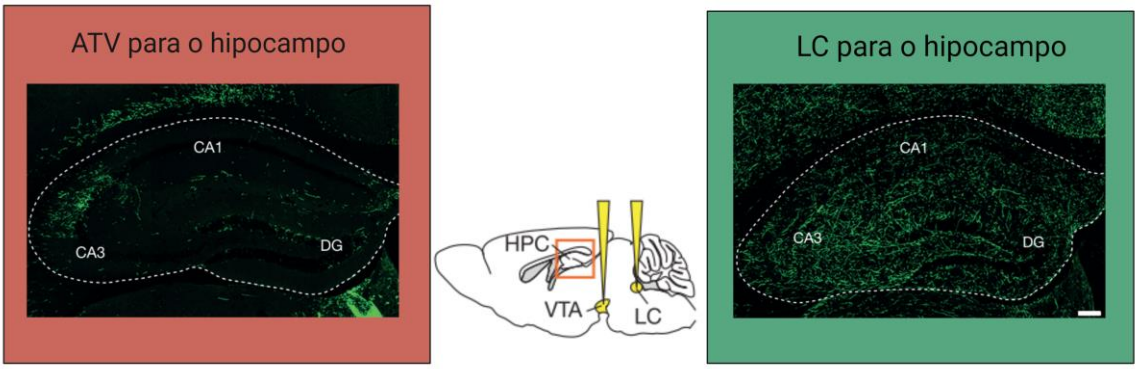

Figura 7 Ativação hipocampal a partir de projeções da área tegmental ventral (ATV) e *locus coerules* (LC). Tanto a ATV quanto o LC se projetam para o hipocampo (HPC) dorsal, mas as projeções de LC são mais densas que as da ATV. Os neurônios de ambas as regiões podem promover a persistência da memória através de mecanismos dependentes de receptores dopaminérgicos D1/D5 no hipocampo e, portanto, presumivelmente através da liberação direta de dopamina de seus axônios. Fonte: Adaptado de TAKEUCHI et al., 2016.

No hipocampo, as projeções da ATV e LC têm efeitos notáveis ao interagir com os receptores dopaminérgicos D1/D5 e beta-adrenérgicos. A inibição dos receptores D1/D5 na região CA1 do hipocampo prejudica o efeito da estimulação da ATV sobre a formação da MLD (MONCADA, 2017). De maneira semelhante, a ativação dos receptores beta-adrenérgicos no hipocampo é crucial para o processo de consolidação após a estimulação do LC (MONCADA, 2017). Além disso, os receptores D1/D5 mostraram-se essenciais no hipocampo para os efeitos das projeções do LC sobre a memória (KEMPADOO et al., 2016). A necessidade desses dois tipos de receptores no hipocampo, tanto os beta-adrenérgicos quanto os dopaminérgicos do tipo D1/D5 foi confirmada como essencial durante a fase inicial de consolidação na tarefa de RO para a formação da MLD (FURINI et al., 2014; MELLO-CARPES et al., 2016). Na fase de consolidação tardia, esses receptores desempenham um papel crucial no processo de persistência da memória (ROSSATO et al., 2009; PARFITT et al., 2012).

Essas descobertas sublinham a complexidade dos mecanismos moleculares por trás da consolidação e persistência das MLD, destacando a interligação

complexa entre os sistemas dopaminérgicos e noradrenérgicos no hipocampo. Compreender essa rede neuroquímica é fundamental para o desenvolvimento de abordagens que possam fortalecer a retenção da informação ao longo do tempo, impactando positivamente a qualidade e a durabilidade das memórias. Além disso, ressaltam a necessidade crucial de investigar não apenas as bases biológicas, mas também estratégias comportamentais que possam aprimorar a persistência da memória.

2.4 EF agudo como estratégia moduladora da memória

O EF agudo refere-se a uma única sessão de exercício, geralmente de intensidade moderada, realizada em um curto período de tempo, e tem sido destacado como uma importante estratégia de modulação da memória (BASSO; SUZUKI, 2017; LOPRINZI, 2019a). Pode envolver atividades como corrida, natação, levantamento de peso ou qualquer outra forma de exercício que demande esforço físico significativo e seja de curta duração. Ainda que seus efeitos positivos sobre a memória tenham sido observados em uma ampla gama de estudos em humanos (LUDYGA et al., 2016; PERINI et al., 2016; LOPRINZI et al., 2019, 2022), os mecanismos cerebrais pelos quais o EF agudo atua ainda são pouco compreendidos (BASSO; SUZUKI, 2017). Nesse contexto, a investigação utilizando modelos de animais experimentais é fundamental para a compreensão das vias e sistemas pelos quais essa estratégia modula a memória.

Embora o EF regular seja amplamente reconhecido pelos benefícios de longo prazo na cognição, no humor e na prevenção de doenças relacionadas à memória, o EF agudo tem sido investigado devido ao seu potencial de modulação imediato no aprendizado (BOUCHET et al., 2017; STEIN et al., 2017; VARGAS et al., 2017, 2020). Esse impacto positivo pode ser, em parte, atribuído ao aumento do estado de alerta mental e à ativação do sistema catecolaminérgico durante a sessão de EF (VARGAS et al., 2017, 2020; MCMORRIS, 2021; MEDRANO et al., 2021). O estado de alerta, ou *arousal*, é substancialmente influenciado pelo EF, levando a uma melhora temporária na vigilância, atenção e processamento cognitivo (MCMORRIS et al., 2016; BASSO; SUZUKI, 2017). Essa ativação catecolaminérgica está associada a melhorias na memória, aprendizado e em outras funções cognitivas, proporcionando uma explicação neuroquímica para os benefícios EF agudo na memória (MCMORRIS et al., 2016).

Em nosso grupo de pesquisa, temos dedicado esforços à investigação dos efeitos e mecanismos envolvidos em diferentes modalidades de EF agudo na memória de ratos. Nossas pesquisas revelam que uma única sessão de 30 minutos de EF de intensidade moderada em esteira, realizada logo após a aquisição de memória de RO, aprimora significativamente a sua persistência, estendendo o

período de retenção por pelo menos 2 semanas além do observado em animais que não realizaram o exercício (VARGAS et al., 2017, 2020). Os mecanismos subjacentes a esses efeitos do EF envolvem a ativação dos receptores beta-adrenérgicos (VARGAS et al., 2017) e dos receptores dopaminérgicos D1/D5 (VARGAS et al., 2020) no hipocampo, juntamente com a ativação crítica da via de sinalização PKA (LIMA et al., 2021b). Além disso, uma única sessão de EF agudo é suficiente para elevar os níveis de dopamina e noradrenalina no hipocampo 30 minutos após a sua prática (VARGAS et al., 2017, 2020).

Em descobertas adicionais de nossas pesquisas, o mesmo protocolo de EF agudo revelou-se eficaz na promoção da formação de MLD em animais com comprometimento cognitivo, causado pela privação materna (SOSA et al., 2019) e pela infusão de beta-amiloide no hipocampo (DARÉ et al., 2020). No último estudo mencionado, além dos efeitos de EF em esteira, observamos efeitos benéficos do EF anaeróbico, realizado por meio de uma única sessão de levantamento de peso em escada. A modalidade anaeróbica melhorou a persistência da memória de RO de animais saudáveis e promoveu a consolidação da memória em animais com déficit de aprendizado induzido pela infusão intra-CA1 de beta-amiloide (DARÉ et al., 2020). Esses achados ressaltam a versatilidade do EF agudo como uma estratégia promissora para melhorar a função cognitiva em diferentes contextos, inclusive em condições de comprometimento cognitivo.

Outros estudos têm avançado na compreensão dos mecanismos cerebrais pelos quais o EF agudo aprimora a memória. Protocolos de sessão única de corrida de baixa a moderada intensidade em esteira por 30 minutos promovem o aumento na expressão de genes relacionados à memória, incluindo o mRNA de BDNF (SOYA et al., 2007; VENEZIA; QUINLAN; ROTH, 2017; VENEZIA et al., 2019). Além disso, o EF agudo tem sido associado ao aumento da matriz metaloproteinase-9, uma enzima crucial na regulação da plasticidade neuronal (NISHIJIMA; KAWAKAMI; KITA, 2015). Diferentes aspectos da neuroplasticidade também têm sido vinculados ao EF agudo em várias modalidades e protocolos. Uma única sessão de EF de resistência, por exemplo, melhora a cognição e aumenta a expressão de proteínas no hipocampo que são essenciais para os processos de ativação da maquinaria pré e pós-sináptica para a consolidação da memória. Dentre elas inclui-se a sinapsina, sinaptofisina e PSD-95 (do inglês, *post synaptic density protein 95*), (FERNANDES et al., 2016), além do BDNF, através da sinalização de receptores de NMDA (LEITE

et al., 2023). Importantemente, os efeitos benéficos do EF agudo não vêm acompanhados de efeitos prejudiciais significativos sobre os parâmetros de estresse oxidativo, ressaltando ainda mais o seu potencial para a saúde cerebral e memória (ACIKGOZ et al., 2006; AKSU et al., 2009).

Além dos mecanismos citados, tem sido discutido um potencial conjunto de mecanismos pelos quais o EF agudo pode influenciar a função da memória episódica. Uma das teorias sugere que o EF agudo estimula áreas cerebrais como a ATV e o LC, que enviam projeções para estruturas do hipocampo, como o giro denteado, CA3 e CA1, aumentando os níveis de neurotransmissores, como noradrenalina e dopamina, desencadeando processos que favorecem a LTP (LOPRINZI; PONCE; FRITH, 2018). Além disso, outra hipótese, se baseia no potencial mecanismo do EF agudo em facilitar a formação da MLD através da marcação e captura sináptica (LOPRINZI; PONCE; FRITH, 2018). Sugere-se que o EF agudo possa produzir PRPs que sejam capturadas por sinapses marcadas por eventos prévios, fortalecendo a memória que ocorre em paralelo (LOPRINZI; PONCE; FRITH, 2018). Nesse contexto, o EF agudo pode ser considerado um fenômeno de metaplasticidade, induzindo alterações fisiológicas e bioquímicas em neurônios e sinapses que modulam a plasticidade desencadeada por eventos que ocorrem em uma janela próxima do tempo. Estudos que utilizam a novidade, uma estratégia que compartilha muitos mecanismos similares ao EF agudo, sustentam essa ideia (MONCADA; VIOLA, 2007; BALLARINI et al., 2009).

Embora tenhamos feito avanços significativos no entendimento dos mecanismos subjacentes ao EF agudo, existem ainda muitas lacunas no conhecimento sobre como exatamente essa estratégia modula a aprendizagem e a memória. Investigar detalhadamente os mecanismos moleculares e neurofisiológicos envolvidos é essencial para desvendar completamente os benefícios do EF agudo para a função cognitiva. Assim, pesquisas adicionais nesta área têm o potencial de fornecer conhecimentos valiosos que permitirão a otimização do uso do EF agudo para aprimorar a memória. Esses avanços podem não apenas impulsionar o desenvolvimento de estratégias mais eficazes, mas também incentivar uma adoção mais ampla dessa abordagem em ambientes educacionais e clínicos.

3 JUSTIFICATIVA

Nesta tese, dedicamo-nos a aprofundar a compreensão dos mecanismos que estão por trás dos efeitos do EF agudo na consolidação e persistência da memória. Embora já se saiba que essa estratégia tem um impacto positivo na persistência da memória em roedores e em seres humanos (PERINI et al., 2016; VARGAS et al., 2017, 2020; LOPRINZI et al., 2022), ainda existem lacunas consideráveis no entendimento dos processos pelos quais ela opera. Nosso objetivo é preencher algumas dessas lacunas e proporcionar uma visão mais abrangente dos mecanismos e efeitos do EF agudo na memória episódica, dada a relevância desse tipo de memória no contexto humano (IZQUIERDO, 2018).

O primeiro estudo busca compreender o papel específico das regiões cerebrais ATV e LC nos efeitos do EF agudo sobre a memória. Ambas as regiões têm se mostrado críticas para a modulação da memória, mediando a liberação dos neurotransmissores noradrenalina e dopamina ao hipocampo e promovendo mecanismos relacionados à plasticidade sináptica (ROSSATO et al., 2009; KEMPADOO et al., 2016; MONCADA, 2017). No entanto, a dependência destas regiões para o efeito do EF agudo sobre a cognição é ainda uma hipótese não avaliada (LOPRINZI; PONCE; FRITH, 2018). A compreensão da dependência dessas regiões fornecerá informações valiosas sobre as vias que regulam os níveis de dopamina e noradrenalina durante a prática do EF, possibilitando o desenvolvimento de terapias e estratégias farmacológicas mais precisas e direcionadas.

No segundo estudo desta tese, nossa investigação se concentra na compreensão da síntese proteica hipocampal, fundamental para a consolidação da memória (ROSSATO et al., 2007; MYSKIW et al., 2008; FURINI et al., 2015), e explora a hipótese de que o EF agudo pode operar por meio de mecanismos de marcação e captura sináptica (LOPRINZI; PONCE; FRITH, 2018). O objetivo é determinar se os efeitos do EF agudo na memória dependem da síntese proteica ribossômica ou da via mTOR e promovem o aumento de BDNF no hipocampo. Além de aprimorar nosso conhecimento sobre a plasticidade neuronal, essa investigação pode indicar se o EF agudo atua por mecanismos que contornam ou não a inibição da síntese proteica no hipocampo realizada após a aquisição da memória. Esses

resultados não apenas ampliarão nossa compreensão do processo neural, mas também podem ter relevância para o tratamento e serem adotados como estratégias de aprendizagem para transtornos que envolvem uma redução na síntese proteica hipocampal como a doença de Alzheimer (HERNÁNDEZ-ORTEGA et al., 2016; MAINA et al., 2018).

Finalmente, a partir do terceiro estudo, busca-se explorar o impacto do EF agudo na fase tardia de consolidação da memória, 11 horas após a aquisição. Essa janela temporal é menos compreendida em comparação com a fase inicial, e poucas estratégias comportamentais tem sido destacadas como potenciais moduladoras da persistência da memória nesta janela de tempo (PARFITT et al., 2012; YANG et al., 2013; TOMAIUOLO et al., 2015a). Entender como o EF agudo influencia a persistência da memória nesse estágio pode ser fundamental para adaptar protocolos de intervenção, personalizando tratamentos para diferentes condições neurológicas ou estágios de aprendizado.

Assim, compreender os efeitos do EF agudo pode ter um impacto transformador na sociedade. Esta é uma estratégia simples de ser aplicada e não acarreta efeitos colaterais significativos, especialmente quando comparada aos tratamentos farmacológicos. É importante ressaltar que os efeitos do EF agudo não devem substituir os benefícios da prática regular de EF, que é conhecida por seus impactos a longo prazo e por mediar uma série de alterações neurais que promovem a neuroplasticidade (DIEDERICH et al., 2017; PIETRELLI et al., 2018). No entanto, esta pode ser uma estratégia útil para modular a memória em momentos pontuais tanto para pessoas saudáveis quanto para aquelas com déficits cognitivos. Além disso, pode ser uma alternativa valiosa para indivíduos que, por razões de saúde ou falta de acompanhamento, estejam impossibilitados de praticar atividade física regularmente.

4 OBJETIVOS

4.1 Objetivo geral

Investigar a requisição das vias noradrenérgicas e dopaminérgicas e da síntese proteica hipocampal no efeito modulatório do EF agudo sobre a persistência da memória de RO.

4.2 Objetivos específicos

i. Investigar a participação da ATV e LC na promoção da persistência da memória de RO induzida pelo EF agudo;
ii. Investigar a participação da ATV e LC após o EF agudo sob os níveis de dopamina e noradrenalina no hipocampo;
iii. Investigar a requisição de síntese proteica hipocampal para a promoção da consolidação e persistência da memória de RO induzida pelo EF agudo;
iv. Investigar o efeito do EF agudo sob a expressão de BDNF no hipocampo;
v. Investigar os efeitos do EF agudo realizado na janela de consolidação tardia da memória, 11 horas após o aprendizado, sob a persistência da memória de RO;
vi. Investigar a dependência de síntese proteica no hipocampo para os efeitos do EF agudo na memória na janela de consolidação tardia da memória, 11 horas após o aprendizado;
vii. Investigar a dependência dos receptores beta-adrenérgicos no hipocampo para os efeitos do EF agudo na memória na janela de consolidação tardia da memória, 11 horas após o aprendizado;
viii. Investigar a dependência dos receptores dopaminérgicos do tipo D1/D5 no hipocampo para os efeitos do EF agudo na memória na janela de consolidação tardia da memória, 11 horas após o aprendizado.

PARTE II

ARTIGO 1

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Acute physical exercise improves recognition memory *via* locus coeruleus activation but not *via* ventral tegmental area activation

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ABSTRACT

Both animals and humans have been studied to explore the impact of acute physical exercise (PE) on memory. In rats, a single session of PE enhances the persistence of novel object recognition (NOR) memory, which depends on dopamine and noradrenaline activity in the hippocampus. However, limited research has examined the involvement of other brain regions in this phenomenon. In this study, we investigated the role of the ventral tegmental area (VTA) and locus coeruleus (LC) in modulating the persistence of NOR memory induced by acute PE. After NOR training, some animals underwent a 30 min treadmill PE session, followed by infusion of either vehicle (VEH) or muscimol (MUS) in either the VTA or LC. Other animals did not undergo PE and only received VEH, MUS, or NMDA within the same time window. We evaluated memory recall 1, 7, and 14 days later. Acute PE promoted memory persistence for up to 14 days afterward, similar to NMDA glutamatergic stimulation of the VTA or LC. Moreover, only the LC region was required for the memory improvement induced by acute PE since blocking this region with MUS impaired NOR encoding. Our findings suggest that acute PE can improve learning within a closed time window, and this effect depends on LC, but not VTA, activity.

1. Introduction

Acute physical exercise (PE) has been demonstrated to enhance memory performance in both humans [1,2] and rodents [3,4] by promoting biochemical activations in the brain. However, the specific neurochemical mechanisms involved in this process are not yet well understood. In rodents, a single PE session applied immediately after learning can promote memory persistence for several days, with the hippocampus being a crucial brain area in supporting this effect [3,5,6]. The close timing window allows for a temporary increase in neurotransmitters and other neuromodulators through PE, potentially strengthening synaptic connections for parallel learning and improving its consolidation [7–9]. Although the hippocampus is critical to memory-related processes, it is crucial to understand the involvement of other brain areas in the PE effects [10].

Studies of our research group have indicated that noradrenaline and dopamine in the hippocampus play essential roles in the memory persistence promoted by acute PE in rodents [3,5,6]. By blocking catecholamine receptors in the CA1 region, we found that the positive effects of acute PE on memory were impaired. Conversely, in the same studies, the drug stimulation of these receptors promoted memory persistence, suggesting that brain regions involved in the secretion of these neurotransmitters may be critical in mediating the memory-enhancing effect.

The ventral tegmental area (VTA) is a brain region that releases dopamine to other areas, such as the hippocampus, while the locus coeruleus (LC) can release both noradrenaline and dopamine [11]. Both regions have been implicated in modulating learning and memory processes [12,13]. The activation of these brain areas controls hippocampal protein synthesis, which mediates the consolidation of long-term memories [14]. Although both regions are involved in arousal, attention, vigilance, reward, plasticity, and memory formation, their projections to the hippocampus differ in density and location, which can contribute to distinct effects on memory processes [13,15,16]. Recent studies have demonstrated that these brain areas can act in parallel or overlap in the modulation of memory [14,17].

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The PE appears to potentiate excitatory inputs for neurons in the VTA and LC [18,19], but a direct relationship between the stimulation of these regions by acute PE and its effects on memory is not yet known. We suggest that the VTA and LC may be involved in the memory-enhancing induced by acute PE. It may stimulate these brain regions, increasing dopamine and noradrenaline release and improving parallel learning by activating downstream signaling pathways involved in neural strengthening [10,18]. Here, we investigated the involvement of these brain areas in modulating the persistence of recognition memory by acute PE to clarify whether the VTA, LC, neither, or both mediate their effects. Our results showed that only the blockade of the LC, but not the VTA, after one single session of PE impaired memory persistence, suggesting its critical role in this process.

2. Material and methods

2.1. Animals

Adult male Wistar rats (3 months old, weighing 300–350 g) were obtained from the University of Santa Maria Central Vivarium (RS, Brazil). They were housed four per cage and maintained in a 12 h light/dark cycle (lights on from 07:00 to 19:00) at a constant temperature of 23 ± 2 °C and humidity of 50 ± 10 %, with food and water provided *ad libitum*. The experiments were initiated after one week of acclimatization in the housing environment and were conducted during the light phase. To minimize stress, all animals were handled by the experimenters before the start of the experiments. All experiments were performed following the protocol approved by the Local Institutional Animal Care and Use Committee (protocol 029/2021).

2.2. Experimental design

We investigated the involvement of the VTA and LC in the modulation of memory persistence induced by acute PE. The experiments were divided into two sets, in which we assessed (i) the role of VTA and (ii) the role of LC (Fig. 1). In both experiments, animals were divided into five groups: vehicle (VEH), NMDA, muscimol (MUS), PE + VEH, and PE + MUS. Animals subjected to PE were previously habituated to a treadmill one week before the memory tasks and exercise session. Since habituation alone does not affect NOR memory, the non-exercised groups were not subjected to habituation [3]. All animals underwent stereotaxic surgery for bilateral cannula implantation in one of the brain regions. Following this, we subjected the animals to the novel object recognition (NOR) protocol. Some animals received VEH, NMDA, or MUS 30 min after NOR training, while others engaged in an acute PE session (30 min at moderate intensity) immediately after training. Subsequently, according to the study, the exercised animals received VEH or MUS to assess the region's requirement. Animals' memory (n = 7–10/group) was tested 1, 7, and 14 days after. However, some animals (n = 3–8/group), with the addition of NAÏVE, did not undergo memory testing and were euthanized 30 min after drug infusion. Their hippocampus was removed for quantification of noradrenaline and dopamine levels.

2.3. Drugs

MUS (M1523, Sigma-Aldrich) and NMDA (M3262, Sigma-Aldrich) were dissolved in saline 0.9 % and stored at −20 °C, protected from light until use. Before infusion, an aliquot was thawed and diluted to a

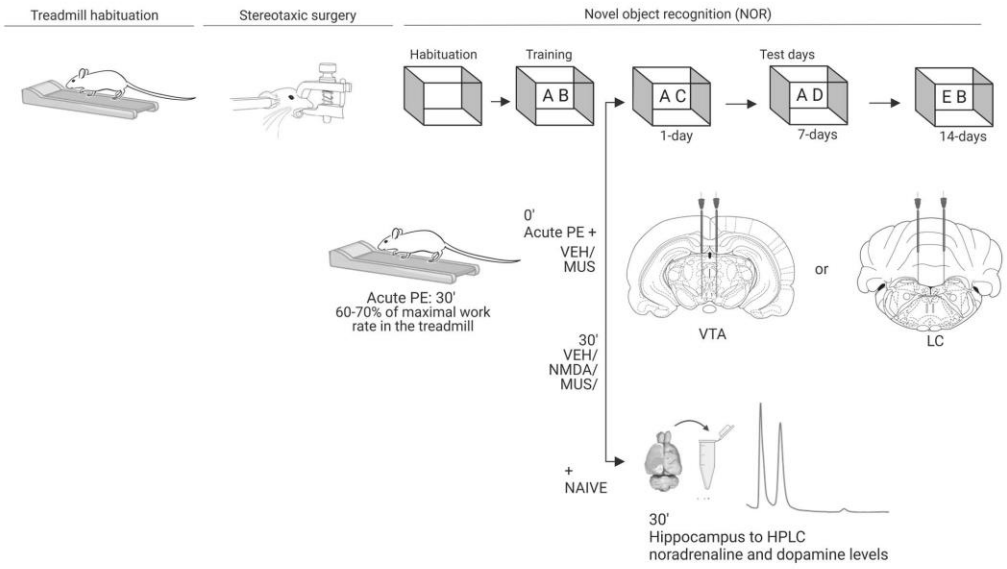

Fig. 1. Experimental design. Animals from acute physical exercise (PE) groups were habituated to the treadmill. All experimental groups were submitted to stereotaxic surgery for bilateral cannula implantation in the ventral tegmental area (VTA) or locus coeruleus (LC). We use the novel object recognition (NOR) task for memory evaluation. Animals were habituated, trained, and had their memory tested in the sessions conducted 1, 7, and 14 days after training (n = 7–10/group). Immediately after NOR training, some animals underwent a 30 min session of PE on a treadmill, with a moderate intensity of 60–70 % of the maximal work rate on the treadmill. Following PE, we infused vehicle (VEH) or muscimol (MUS), a GABAergic agonist for region inhibition, in the VTA or LC. Other animals did not undergo PE and only received VEH, MUS, or NMDA (a glutamatergic agonist for region stimulation) within the same time window. Some animals (n = 3–8/group) were not submitted to the NOR tests, and 30 min after the infusion, we euthanized them and removed their hippocampi for analysis of noradrenaline and dopamine levels using high-performance liquid chromatography (HPLC). In the HPLC, we included a NAÏVE group whose animals did not undergo any protocol except handling by the experimenters. The two-letter combinations included in the draw of the NOR box represent the different objects used per NOR session.

working concentration of 0.1 μg/μL in 0.9 % saline at pH 7.2. The drug concentration was based on previous studies [20,21].

2.4. Surgery and drug infusion

The animals underwent stereotaxic surgery for bilateral guide cannula (9.0 × 0.7 mm) implantation under deep anesthesia (i.p., ketamine 75 mg/kg plus xylazine 10 mg/kg). The cannulas were inserted to reach the VTA (A - 4.8 mm; *L* ± 1.0 mm; V - 8.0 mm) or LC (A - 9.7 mm; *L* ± 1.3 mm; V - 7.1 mm) areas, according to the anatomical atlas of Paxinos and Watson. We considered V - 3.0 mm at surgery, and the brain regions were reached during drug infusion. Dental cement was used to fix the cannulas in place. Animals were allowed to recover from surgery for four days before undergoing further procedures.

The drug infusion occurred 30 min after NOR training and followed PE in some groups. We used a Hamilton syringe connected to fine bore tubing (38 gauge, 15 cm) and a needle (30 gauge) at the end. At the time of drug delivery, the infusion needle was fitted into the guide cannula and inserted 5.0 mm deeper to reach the VTA region or 4.1 mm to LC. Infusions (0.5 μL/side) were performed for 60 s, and the infusion needle was left in place for 60 s to minimize backflow.

2.5. Acute PE

A motorized treadmill designed for rodents (Insight Ltd., Brazil) was utilized in the study. The animals of the acute PE groups were habituated to the apparatus for one week before the subsequent experiments to prevent novelty or stress effects. The habituation period was 10 min per day. The animals were placed on the off-treadmill on the first day. In the following two days, the animals ran at 2 – 5 m/min, and the velocity was increased to 8 m/min in the next three days.

An incremental treadmill test to exhaustion was performed one day after the last habituation session. This test determines the individual maximal work rate and involves starting at a low velocity of 1 m/min, increasing by 5 m/min every 3 min until the rat can no longer continue running.

The animals performed an acute PE session lasting 30 min after NOR training at an intermediate intensity of 60–70 % of the maximal work rate, as previously described [3,5,6].

2.6. NOR memory

The NOR task was carried out in a wooden box (50 × 50 × 50 cm) and is based on the natural tendency of animals to explore novel objects in a familiar context [22].

The animals were habituated to the wooden box for 20 min daily for four days. Next, the NOR training was conducted, and the animals were allowed to explore two different and unknown objects (A and B) for 5 min. The test sessions were conducted 1 (A and C), 7 (A and D), and 14 (E and B) days after training, during which the animals could explore a novel and a familiar object for 5 min. The letters A, B, C, D, and E correspond to a magic cube, a plastic cup, a circular can, Lego pieces, and a metallic cylinder, respectively. As confirmed by previous pilot studies, the animals did not prefer any of the objects.

The experimental room was unoccupied, and the rats' behavior was recorded using a video camera placed above the arena. An experimenter blinded to the experimental conditions measured the exploration time of each object using manual stopwatches. Exploration was defined as the rats touching the object with their front paws or sniffing it with their nose. Sitting on or turning around the objects was not considered exploratory behavior. To prevent olfactory preferences, the objects and apparatus were cleaned with 70 % alcohol after each trial.

2.7. Control behavioral

Behavioral control tests were conducted to ensure that any procedures or environmental changes cause behavior changes that could affect the results of memory tests. The tests were carried out approximately 3 h after each NOR test session.

The open field (OF) apparatus consisted of a wooden box (50 × 50 × 50 cm) with black lines drawn on the floor to divide it into 12 equal quadrants. We evaluated for 5 min the number of crossings and rearing as locomotor and exploratory activity measures.

The elevated plus maze (EPM) apparatus consists of a cross-platform that is 1 m high with 40 cm arms, two of which have high walls (closed arms) and two without walls (open arms). The animals were placed in the maze's center and allowed to explore freely for 5 min. We counted the number of entries and the time spent with open arms, which indicates anxiety-like behavior.

The behavior of the rats was recorded by a video camera positioned over the apparatus in an empty experimentation room. A blind experimenter rated the behavior of the animals, and a manual stopwatch was used when necessary.

2.8. Cannula placement

Cannula placement was confirmed through histological examination postmortem. After the last behavioral test, animals were anesthetized using intraperitoneal injection of ketamine (75 mg/kg) and xylazine (10 mg/kg) and then received a 4 % methylene blue solution (0.5 μL/side) into their VTA or LC. The animals were then perfused with 0.9 % saline solution (200 mL), followed by 10 % formaldehyde (200 mL). The brains were extracted and fixed in 10 % formaldehyde (30 mL) for four hours and then cryopreserved in 30 % sucrose solution (30 mL) for 72 h at 4 °C. The brains were dried and stored at −80 °C until analysis. Coronal brain sections with a thickness of 40 μm were sliced using a Cryostat (LEICA CM3050S) and examined using an optical microscope (Olympus CX21). The area in which the methylene blue solution reached was used to indicate the potential drug diffusion.

2.9. Neurotransmitters levels

We measured the levels of noradrenaline and dopamine in the hippocampus using high-performance liquid chromatography (HPLC). After quickly collecting the entire hippocampus 30 min after acute PE or at an equivalent time for other groups, we placed the samples in Eppendorf tubes and immersed them in liquid nitrogen for preservation. Then, we stored the samples in a freezer at −80 °C until analysis.

We added 1000 μL of a solution containing acetonitrile and 0.5 M HCl (96:4 % v/v) to the sample. The samples were homogenized for 1 min and then centrifuged at 10,000 RPM for 10 min at 4 °C. The supernatant was collected and filtered through a 13 mm hydrophobic polytetrafluoroethylene (PTFE) syringe filter with a pore size of 0.22 μm. The filtered sample was then transferred to an amber vial and kept at 4 °C for a maximum of 2 h until analysis.

To quantify the compounds, we used HPLC equipment coupled with a Young Lin (YL) 9100 diode array detector (HPLC-DAD) and an Inertsil ODS-3 5 μm column (4.6 × 250 mm) equipped with a Security GuardTM guard cartridge (Phenomenex KJ0-42B2). We separated the compounds using mobile phases of water acidified with phosphoric acid to pH 2.55 (A) and methanol (B). We considered a specific time, elution gradient, and flow of the chromatographic method (see Tab. S1 in Supplemental Material).

We injected 20 μL of the sample, and the compounds were identified by comparing their retention time and DAD spectrum with the reference standards from the analytical curve. We constructed the analytical curve with noradrenaline and dopamine (at 198 nm) standards, which comprised 5 points at concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, and 10.0 mg/L for each compound.

2.10. Statistical analysis

We checked the data for normality of distribution using the Shapiro–Wilk test. We used the Mann-Whitney test to compare the maximal work rate on the treadmill between exercised groups. The NOR results were expressed as the percentage of total time spent exploring each object. We analyzed the data using a one-sample Student's *t*-test, assuming a 50 % theoretical mean. We considered the animal's ability to spend over 50 % of the total time exploring the novel object as memory retention. We also performed a comparison between groups, so NOR data were converted to a discrimination index (DI = [(*t* novel − *t* familiar) / (*t* novel + *t* familiar) x 100]), where 't' is the time spent exploring the object. We analyzed this using one-way ANOVA followed by Tukey's post hoc test. A higher DI suggests a greater capacity to discriminate the objects and explore the novel object for more time. The object exploration total time, OF, and EPM results were analyzed using one-way ANOVA followed by Tukey's post hoc test and were used as control parameters. The HPLC results were normalized considering the mean percentage relative to the control. The HPLC data were analyzed in triplicate and compared using Kruskal-Wallis, followed by Dunn's multiple comparison tests. We considered the differences significant when $P < 0.05$ for all analyses.

3. Results

3.1. The role of the VTA in modulating the persistence of NOR memory induced by acute PE

3.1.1. The maximal work rate on the treadmill was similar between exercised groups

We evaluated the maximal work rate in the treadmill of the animals from PE groups and randomly distributed them into the groups. We did not find a difference in this parameter between the groups ($U = 31.50$, $P = 0.3062$; Fig. 2).

3.1.2. Blocking VTA activity impairs memory consolidation and persistence, but acute PE prevents this impairment

The animals underwent NOR task training, followed by acute PE and/or drug manipulations into the VTA. Memory was tested 1, 7, and 14 days later (Fig. 3A). The drug's reach into the VTA was confirmed through histological evaluation (Fig. 3B). During the NOR training session, the animals explored both unknown objects for a similar time ($P > 0.05$; Fig. 3C), and all groups exhibited a similar DI ($F_{(4, 38)} = 0.8357$, $P = 0.1374$; Fig. 3D).

We assessed the rats' ability to recognize a novel object 1 day after NOR training and the interventions. Animals that received only VEH into VTA could remember the familiar object and spent more time exploring the novel object ($P < 0.0001$; Fig. 3E). The same was observed in animals that received NMDA ($P = 0.0001$; Fig. 3E) or practiced acute PE and received VEH ($P = 0.0156$; Fig. 3E) or MUS ($P = 0.0013$; Fig. 3E) into VTA. However, when MUS was infused in VTA, animals did not present memory retention ($P = 0.6021$; Fig. 3E) and presented a lower DI than other groups ($F_{(4,35)} = 1.503$, $P = 0.0004$; $P = 0.0001$ vs. VEH; $P = 0.0044$ vs. NMDA; $P = 0.0257$ vs. PE + VEH; $P = 0.0092$ vs. PE+MUS; Fig. 3F). These results suggest that VTA inhibition impairs memory consolidation, which can be compensated by prior acute PE practice – that seems to act independently of this pathway.

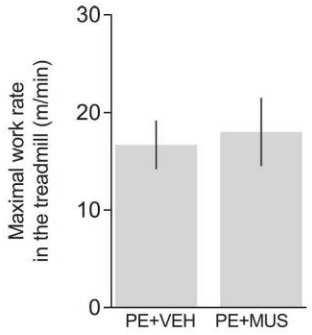

Fig. 2. The maximal work rate in the treadmill in the VTA study was similar between exercised groups. Data are presented as mean ± standard deviation (SD). $P > 0.05$ in the Mann-Whitney test (n = 9–10/group).

We evaluate the memory persistence 7 and 14 days after NOR training. Animals that received VEH were unable to remember the familiar object in the 7-day test, which is an indicator of natural forgetting ($P = 0.2597$; Fig. 2G). However, NMDA into VTA ($P = 0.0019$; Fig. 3G) or PE practice with or without MUS infusion in VTA ($P = 0.0003$, PE + VEH; $P = 0.0017$, PE + MUS; Fig. 3G) promoted the novel object discrimination, as animals spent more time exploring it. As expected, animals that received MUS into VTA without previous PE practice did not discriminate the objects ($P = 0.3936$; Fig. 3G). The DI comparisons ($F_{(4,37)} = 0.9542$, $P < 0.0001$; Fig. 3H) showed that NMDA ($P = 0.0029$ vs. VEH; $P = 0.0172$ vs. MUS; Fig. 3H), PE + VEH ($P = 0.0012$ vs. VEH; $P = 0.0096$ vs. MUS; Fig. 3H), and PE + MUS ($P = 0.0048$ vs. VEH; $P = 0.0302$ vs. MUS; Fig. 3H) groups presented higher novel object DI than the other groups.

We obtained similar results in the 14-day test. Animals that received VEH ($P = 0.8308$; Fig. 3I) or MUS ($P = 0.1679$; Fig. 3I) infusions did not recognize the novel object and spent a similar time exploring both objects. In contrast, the NMDA ($P = 0.0009$; Fig. 3I) and PE groups continued to demonstrate memory persistence ($P = 0.0042$, PE + VEH; $P = 0.0059$, PE + MUS; Fig. 3I). When comparing the groups' DI ($F_{(4,38)} = 0.2151$, $P = 0.0004$; Fig. 3J), NMDA had a higher DI than VEH and MUS ($P = 0.0003$ vs. VEH; $P = 0.0183$ vs. MUS; Fig. 3J), and the PE groups also had a significantly higher DI than VEH ($P = 0.0252$, PE + VEH; $P = 0.0362$, PE + MUS; Fig. 3J).

Together, these results indicate that acute PE promotes the persistence of NOR memory, an effect independent of VTA activation. Its effect is as good as direct drug stimulation into this brain area. The behavioral control parameters evaluated did not differ between VTA groups on test days (Tab. S2, Supplemental Material).

3.1.3. Neither PE nor VTA drug manipulations affected the levels of catecholamines in the hippocampus

We quantified the levels of noradrenaline and dopamine in the hippocampus 30 min after both PE and VTA drug infusion (Fig. 4A). Our analysis revealed no significant differences in the noradrenaline levels ($W_{(6111)} = 5.313$, $P = 0.3789$; Fig. 4B) or dopamine levels ($W_{(6117)} = 6.421$, $P = 0.2674$; Fig. 4C).

3.2. The role of LC in modulating the persistence of NOR memory induced by acute PE

3.2.1. The maximal work rate on the treadmill was similar between the exercised groups

Similar to the first study, the animals were randomly assigned to their respective groups in the LC study. We found no difference in the groups during the maximal work rate in the treadmill test ($U = 13.50$, $P = 0.2284$; Fig. 5).

3.2.2. LC inhibition impairs memory consolidation and persistence, even in the presence of acute PE practice

The NOR protocol was the same as described previously, but drugs were infused into the LC area (Fig. 6A). The effectiveness of drug delivery was verified by histological analysis (Fig. 6B). During NOR training, all animals explored both objects for a similar time ($P > 0.05$; Fig. 6C), and no significant DI differences were found between the

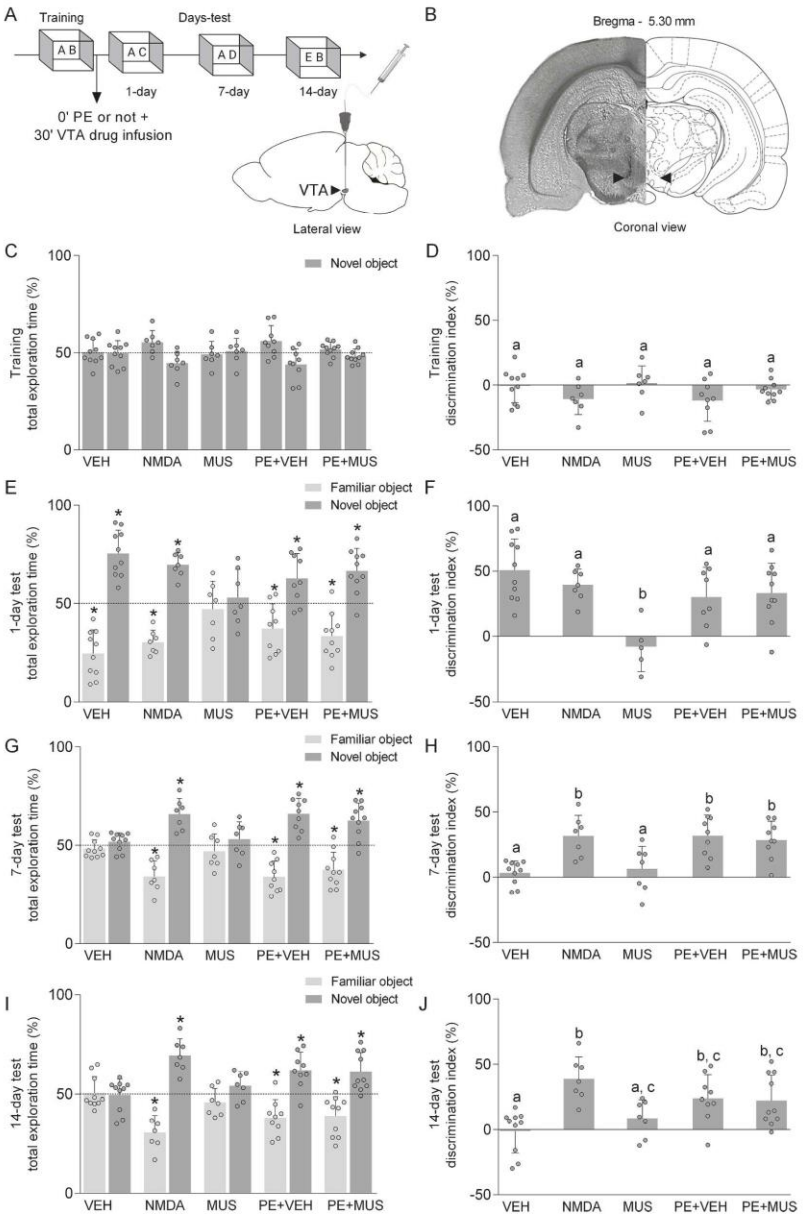

Fig. 3. Inhibiting the VTA impairs memory consolidation and persistence, but acute PE can prevent this effect. A) Experimental design included: an acute physical exercise (PE) session after the novel object recognition (NOR) training; the bilateral infusion into the ventral tegmental area (VTA) of vehicle (VEH), NMDA, or muscimol (MUS) after PE (or in equivalent time for non-exercised groups); and the NOR memory tests performed 1, 7 and 14 days after training. B) Histological image sample of the exact local infusion into VTA. C-J) NOR memory evaluation with total exploration time (C, E, G, and I) and group comparison by a novel object's discrimination index (DI; D, F, H, and J) in the training and testing performed 1, 7 and 14 days after training. Data are presented as mean ± standard deviation (SD). * $P < 0.05$ in One sample *t*-test; different letters indicate $P < 0.05$ in ANOVA followed by Tukey's post hoc ($n = 7$–10/group).

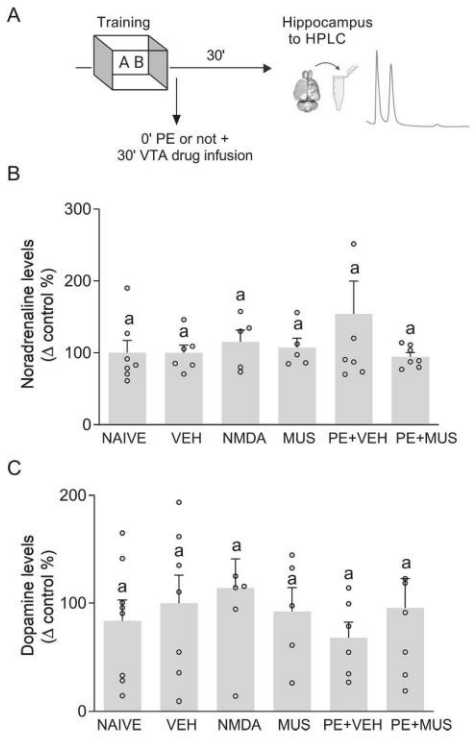

Fig. 4. Neither PE nor VTA drug manipulations affected the levels of catecholamines in the hippocampus. A) Experimental design included: an acute physical exercise (PE) session performed after the novel object recognition (NOR) training; bilateral infusion into the ventral tegmental area (VTA) of vehicle (VEH), NMDA, or muscimol (MUS) after PE or in equivalent time for non-exercised groups; and hippocampus separation 30 min after to HPLC analysis. A NAÏVE group of animals was also included in this experiment as absolute control. B) Noradrenaline levels. C) Dopamine levels. Data are presented as mean percentage relative to control ± standard error of the mean (SEM). Equal letters indicate $P > 0.05$ in the Kruskal-Wallis, followed by Dunn's multiple comparison tests, analyzed in triplicate ($n = 5$–8/group).

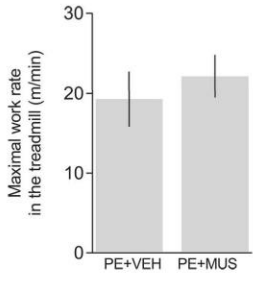

Fig. 5. The indirect maximal work rate in the treadmill in the LC study was similar between exercised groups. Data are presented as mean ± standard deviation (SD). $P > 0.05$ in the Mann-Whitney test ($n = 7$/group).

groups ($F_{(4, 32)} = 0.8322$, $P = 0.1402$; Fig. 6D).

We tested the memory consolidation 1 day after NOR training, and animals that received VEH ($P = 0.0156$; Fig. 6E), NMDA ($P = 0.0018$; Fig. 6E), or practiced PE receiving VEH infusion ($P = 0.0488$; Fig. 6E) discriminated the novel object by exploring it for more than 50 % of the time. However, LC inhibition by MUS prevented memory consolidation regardless of PE practice ($P = 0.3241$, MUS; $P = 0.7031$, PE + MUS; Fig. 6E). In this same context, the VEH ($P = 0.0043$ vs. PE + MUS; Fig. 6F), NMDA ($P = 0.0495$ vs. MUS; $P = 0.0036$ vs. PE + MUS; Fig. 6F), and PE + VEH ($P = 0.0147$ vs. PE + MUS; Fig. 6F) groups presented higher DI than others ($F_{(4, 31)} = 1.311$, $P = 0.0005$; Fig. 6F). Together, these data reveal that LC inhibition impairs consolidation and prevents the PE effect on memory modulation.

Memory persistence was evaluated in the 7 and 14-day tests. The VEH infusion did not promote NOR memory on the 7-day test ($P = 0.1894$; Fig. 6G). However, animals that received NMDA ($P = 0.0161$; Fig. 6G) or practiced PE ($P = 0.0233$; PE+VEH; Fig. 6G) explored the novel object for a longer time than the familiar object. Animals that received MUS without ($P = 0.6257$; Fig. 6G) or with previous PE ($P = 0.5042$; Fig. 6G) did not recognize the new object. Similarly, NMDA ($P = 0.0086$ vs. VEH; $P = 0.0453$ vs. MUS; $P = 0.0287$ vs. PE + MUS; Fig. 6H) and PE + VEH ($P = 0.0059$ vs. VEH; $P = 0.0291$ vs. MUS; $P = 0.0187$ vs. PE + MUS; Fig. 6H) groups showed higher DI than other groups ($F_{(4, 31)} = 0.6318$, $P = 0.0005$; Fig. 6H).

In the 14-day test, the results were similar to those described earlier. Only the NMDA ($P = 0.0003$; Fig. 6H) and PE+VEH ($P = 0.0003$; Fig. 6H) groups showed persistence of memory, discriminating the objects. Meanwhile, animals in the VEH ($P = 0.1010$; Fig. 6H), MUS ($P = 0.4739$, Fig. 6H), and PE + MUS ($P = 0.7097$, Fig. 6H) groups continued not to recognize the new object. Differences between groups were also observed ($F_{(4, 33)} = 0.9497$, $P < 0.0001$; Fig. 6I), and DI was higher for the NMDA ($P = 0.0140$ vs. VEH; $P = 0.0042$ vs. MUS; $P = 0.0039$ vs. PE + MUS; Fig. 6I) and PE + VEH ($P = 0.0039$ vs. VEH; $P = 0.0011$ vs. MUS; $P = 0.0011$ vs. PE + MUS; Fig. 6I) groups.

These findings reveal that the LC is a crucial brain area for both the consolidation and persistence of NOR memory as well as the effect of acute PE on memory. Moreover, no differences were observed in the behavioral control parameters that could have affected the memory tests (Tab. S3, Supplemental Material).

3.2.3. Neither PE nor LC drug manipulations affected the levels of catecholamines in the hippocampus

We measured the levels of noradrenaline and dopamine in the hippocampus 30 min after PE and LC drug infusion (Fig. 7A). There was a difference in noradrenaline levels between groups ($W_{(6102)} = 23.14$, $P = 0.0003$; Fig. 7B). However, this difference occurred in the NAÏVE group compared to VEH ($P = 0.0002$; Fig. 7B) or MUS ($P = 0.0026$; Fig. 7B). No difference was found in the multiple comparisons test to dopamine levels ($W_{(6114)} = 11.23$, $P = 0.0469$; Fig. 7C).

4. Discussion

This work supports evidence that although both the VTA and LC brain regions are critical for the consolidation of NOR memory, only the LC region is essential for the memory persistence induced by an acute session of PE after NOR learning. In our previous studies, we have observed the same effect of acute PE on the NOR task [3,5,6]. However, while the catecholaminergic requirement for this effect was well-known, it was unclear which brain regions beyond the hippocampus were directly involved.

When applied after NOR training, MUS infusion into the VTA decreases this brain area's neural activity and impaired memory consolidation. Consequently, it seems that hippocampal memory consolidation is a process dependent on the VTA functions [23,24] since the inactivation of this area suppresses the magnitude of long-term potentiation (LTP) in the CA1 [23]. LTP is a key cellular mechanism in the brain that

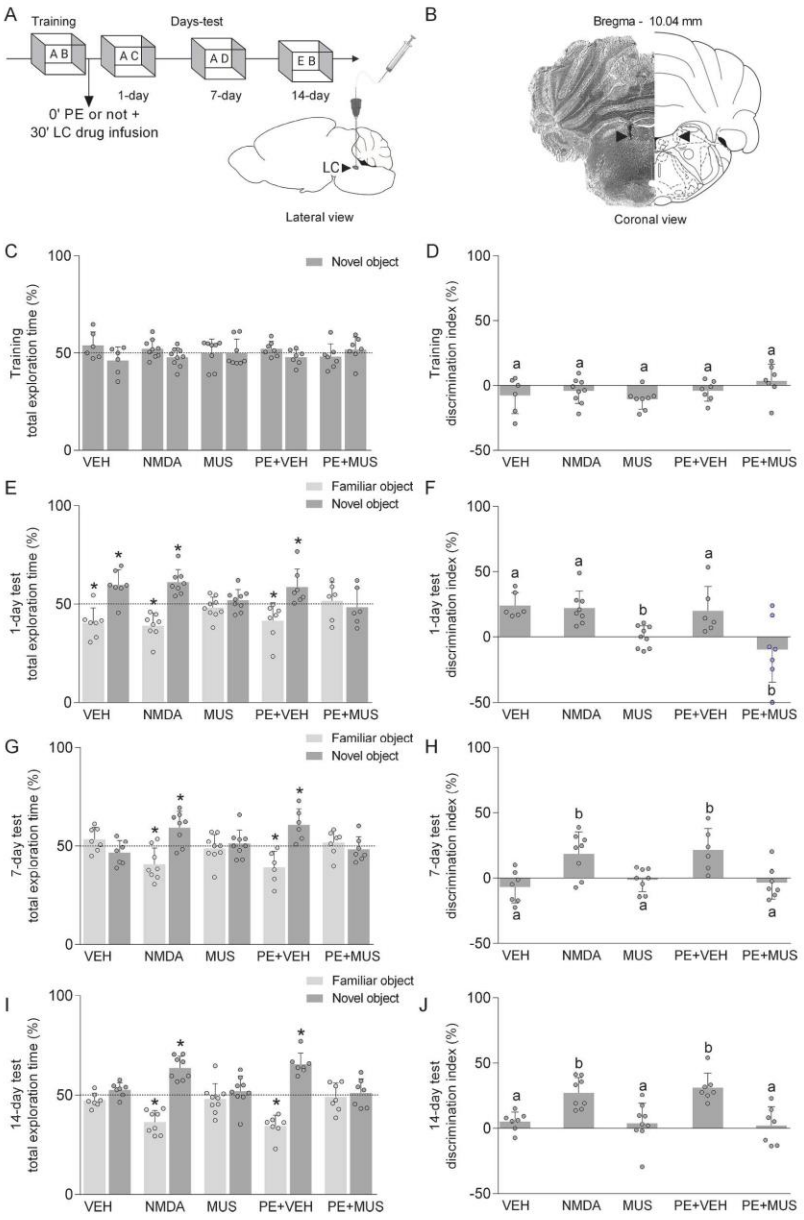

Fig. 6. LC inhibition impairs memory consolidation and persistence even during acute PE practice. A) Experimental design included: an acute physical exercise (PE) session after the novel object recognition (NOR) training; the bilateral infusion into locus coeruleus (LC) of vehicle (VEH), NMDA, or muscimol (MUS) after PE (or in equivalent time for non-exercised groups); and the NOR memory tests performed 1, 7 and 14 days after training. B) Histological image sample of the exact infusion into LC. C-J) Memory evaluation with total exploration time (C, E, G, and I) and group comparison by a novel object's discrimination index (DI; D, F, H, and J). Data are presented as mean ± standard deviation (SD). * $P < 0.05$ in One sample *t*-test; different letters indicate $P < 0.05$ in ANOVA followed by Tukey's post hoc (n = 7–9/group).

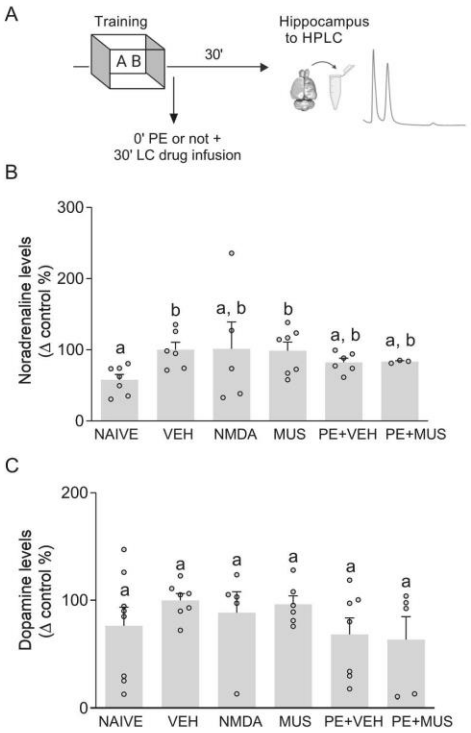

Fig. 7. Neither PE nor LC drug manipulations affected the levels of catecholamines in the hippocampus. A) Experimental design included: an acute physical exercise (PE) session performed after the novel object recognition (NOR) training; bilateral infusion into locus coeruleus (LC) of vehicle (VEH), NMDA, or muscimol (MUS) after PE or in equivalent time for non-exercised groups; and the hippocampus separation 30 min after to HPLC analysis. A NAÏVE group of animals was also included in this experiment as absolute control. B) Noradrenaline levels. C) Dopamine levels. Data are presented as mean percentage relative to control ± standard error of the mean (SEM). Different letters indicate $P < 0.05$ in the Kruskal-Wallis, followed by Dunn's multiple comparison tests, analyzed in triplicate (n = 3–8/group).

underlies learning and memory processes, and its suppression may be related to a decrease in dopamine resulting from VTA inhibition [25].

Interestingly, our findings reveal that a single 30 min bout of PE prevents the memory-impairing effects of MUS into VTA, allowing the animals to persist in their memory over time. Based on these findings, we hypothesize that acute PE provides a strong enough stimulus to recruit other brain pathways, compensating the VTA inhibition and thereby contributing to memory consolidation. These pathways likely involve regions associated with attention, arousal, and emotional processing, all of which are recognized to modulate memory formation [26].

Certain regions potentially involved in the acute PE effect have been highlighted, including those projecting to various memory-related structures within and beyond the hippocampus, such as CA3, CA1, dentate gyrus, basolateral complex of the amygdala, and prefrontal cortex (PFC) [10]. These pathways are influenced by various neurotransmitters, such as catecholamine stimulation in the nucleus tractus solitarius (NTS), the LC, and substantia nigra, serotonin augmentation in the raphe nuclei, and acetylcholine enhancement in the medial septal nucleus [10]. However, further studies are required to confirm these roles in the effects of acute PE.

Building upon the aforementioned and considering the catecholaminergic effects of acute PE and its essential role played by hippocampal D1/D5-dopaminergic and β-adrenergic receptors [3,5], it is crucial to consider the involvement of other brain regions that release these neurotransmitters. Therefore, we investigated the role of the LC in the impact of acute PE and demonstrated that NOR memory is impaired by inhibiting this area, with or without previous acute PE. This highlights the crucial involvement of the LC in memory and the neuromodulator effects of acute PE. Studies have shown the requirement of the LC in various cognitive and behavioral processes [14,17,27], as well as in the consolidation of NOR memory [28]. The dopamine and noradrenaline co-release to the dorsal hippocampal CA1 region from the LC improves the LTP and underlies memory improvement [13,15,17]. This effect seems to be independent since only noradrenaline or dopamine receptor block in the CA1 can impair memory processes [3,5].

The role of the VTA and LC in cognition is strongly evidenced, and the stimulation of these structures is associated with improved memory [12,15,29]. The activity of these brain areas is independent, but when both are stimulated, their effects can be additive, resulting in stronger memory, possibly by recruiting the activity of more neurotransmitters [14]. Both the VTA and LC are important for regulating the synthesis of plasticity-related proteins (PRPs), which, when captured by synaptic tags formed in parallel learning, can strengthen it [14]. This mechanism is known as Synaptic Tagging and Capturing (STC) and has great potential to explain the effects of acute PE on memory [10]. Our findings provide evidence that the effect of acute PE on memory consolidation and persistence depends on the LC but not the VTA. We hypothesize that the co-release of noradrenaline and dopamine is essential for memory processes by STC, and when VTA blockade occurs, it is compensated for by the action of the LC.

We still do not have a clear understanding of whether the effect of acute PE mediated by the LC is due to an increase in noradrenaline, dopamine, or both. Further studies investigating this mechanism would be interesting. However, the studies highlight positive effects on behavioral and biochemical processes for both neurotransmitters within the LC-hippocampus circuit [15,30]. Interestingly, catecholamine neurons of the LC create significantly more dopaminergic connections in the dorsal hippocampus to the VTA, suggesting that the LC dopamine increase may be more crucial for the processes involved in this brain area [15]. This could be an additional explanation for why the effect of acute PE depends on the LC rather than the VTA. Our view is supported by a body of evidence that highlights the dorsal hippocampus as a critical region for memory persistence processes [31,32].

Unfortunately, our biochemical analyses did not provide conclusive answers regarding the effects of acute PE and drug intervention on catecholamine levels in the hippocampus. Neither PE nor manipulations of the VTA or LC affected catecholamine levels in the hippocampus. However, a previous study using the same evaluation method reported an increase in these neurotransmitters 30 min after acute PE [3]. It is worth noting that our study did not detect a decrease in neurotransmitter levels in the VTA or LC when inhibiting them with MUS, nor did we observe an increase in levels when applying NMDA stimulation to the VTA or LC, as projected to these brain regions [17,27,33]. It is important to highlight a limitation of our study, which lies in the methodology employed to directly measure catecholamine levels. Factors such as the timing of hippocampus collection and storing, neurotransmitter degradation during the tissue extraction process, or limitations of the HPLC method may have compromised the accuracy of our measurements. Given that neurotransmission involves rapid events, real-time microdialysis measurement could have been an interesting alternative to consider. Another option is to explore c-Fos expression in the studied brain area as a potential indicator of region-specific neural activity [34].

Additionally, while the importance of the hippocampus in NOR memory and its modulation by acute PE have been well described [3,5,

6], it is also crucial to consider that other brain regions may receive catecholaminergic projections and be influenced by acute PE and modulate memory processes. Besides the hippocampus, LC projects, among various regions, to the PFC, which is implicated in memory function [35]. These projections release noradrenaline and dopamine neurotransmitters to the medial (mPFC) by the LC [36], thus affecting learning and long-term memory. One possibility is that these anatomical connections facilitate functional connectivity, allowing acute PE to positively influence NOR memory by increasing catecholamine release. This is hypothesized since the mPFC is involved in recognition memory [37], and catecholamine neurotransmitters are key modulators of cognitive function in this region [38]. While there is currently limited direct evidence for the effects of acute PE on NOR memory via the mPFC, it is indeed a plausible pathway for mediating the observed effects. Therefore, further research is needed to explore these alternative pathways and other brain areas beyond what we currently know.

Our findings provide insight into the mechanisms underlying the memory-enhancing effects of acute PE. We reveal that activation of the LC is crucial in this process. Most everyday memories are often forgotten, so a session of acute PE close to a new learning experience can strengthen the memory trace and prevent forgetting. This approach could be strategically used in educational or clinical contexts, especially during complex tasks, exams, or tests requiring greater executive function control [39]. In this sense, acute moderate PE is a relevant strategy for healthy individuals and those with cognitive deficits; it stimulates the release of neurotransmitters and other important factors that support memory function [1,40]. Furthermore, acute PE is an easy-to-apply intervention that can provide significant benefits.

5. Conclusions

The LC, but not the VTA, plays a critical role in modulating the persistence of NOR memory induced by acute PE. This downstream signaling may strengthen neural connections and improve the performance of parallel learning applied within a closed time window. These findings provide insight into the underlying brain mechanisms of the beneficial effects of acute PE and support its potential use as a relevant strategy in educational or clinical contexts to develop new strategies for individuals with health or memory deficits.

CRediT authorship contribution statement

Karine Ramires Lima: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ben-Hur Souto das Neves:** Investigation, Writing – review & editing. **Guilherme Salgado Carrazoni:** Investigation, Writing – review & editing. **Ana Carolina de Souza da Rosa:** Investigation, Writing – review & editing. **Murilo Ricardo Sigal Carriço:** Data curation, Investigation, Writing – review & editing. **Rafael Roehrs:** Methodology, Resources, Software, Supervision, Writing – review & editing. **Pâmela Billig Mello-Carpes:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2023.114370.

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MANUSCRITO 1

Submetido para: Neurochemistry International

ACUTE PHYSICAL EXERCISE PREVENTS MEMORY AMNESIA CAUSED BY PROTEIN SYNTHESIS INHIBITION IN RATS HIPPOCAMPUS

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Abstract

The benefits of physical exercise (PE) on memory consolidation have been well-documented in both healthy and memory-impaired animals. However, the underlying mechanisms through which PE exerts these effects are still unclear. In this study, we aimed to investigate the role of hippocampal protein synthesis in memory modulation by acute PE in rats. After novel object recognition (NOR) training, rats were subjected to a 30-minute moderate-intensity acute PE on the treadmill, while control animals did not undergo any procedures. Using anisomycin (ANI) and rapamycin (RAPA), compounds that inhibit protein synthesis through different mechanisms, we manipulated protein synthesis in the CA1 region of the hippocampus to examine its contribution to memory consolidation. Memory was assessed on days 1, 7, and 14 post-training. Our results showed that inhibiting protein synthesis by ANI or RAPA impaired NOR memory consolidation in control animals. However, acute PE prevented this impairment without affecting memory persistence. We also evaluated brain-derived neurotrophic factor (BDNF) levels after acute PE at 0.5h, 2h, and 12h afterwards and found no differences in levels compared to animals that did not engage in acute PE or were only habituated to the treadmill. Therefore, our findings

suggest that acute PE could serve as a non-pharmacological intervention to enhance memory consolidation and prevent memory loss in conditions associated with hippocampal protein synthesis inhibition. This mechanism appears not to depend on BDNF synthesis in the early hours after exercise.

Keywords: BDNF; a bout of exercise; treadmill; neuroplasticity; treadmill.

1 Introduction

The ability of memories to last after the consolidation is known as persistence, a critical aspect of long-term memory (LTM) (Bekinschtein et al., 2010). However, not all learning experiences lead to durable LTM. Recent studies have suggested that acute physical exercise (PE) may be a potential strategy to enhance memory persistence (Bouchet et al., 2017; Fernandes et al., 2016). When applied after a learning that generates only a brief LTM, acute PE can extend memory for several days (Vargas et al., 2017). Although the underlying mechanisms of this effect are not yet fully understood, it is believed that acute PE acts on memory consolidation processes that facilitate a long-lasting LTM by supporting the growth and maintenance of synapses and enhancing neural plasticity (Fernandes et al., 2016; Soya et al., 2007; Venezia et al., 2019). However, it's uncertain whether acute PE depends on hippocampal protein synthesis, an essential mechanism for LTM formation, to improve memory.

While there are known mechanisms of acute PE that explain its modulatory effects on episodic memory, at least to our best knowledge, none specifically address the protein synthesis requirement. Studies have shown that acute PE increases dopamine and noradrenaline levels in the hippocampus, which act on the D1/D5 dopamine and β-adrenergic receptors in the CA1 area to achieve their effects (Vargas et al., 2020, 2017). Additionally, acute PE has been linked to the synthesis of growth factors such as brain-derived neurotrophic factor (BDNF), which plays a critical role in modulating synaptic plasticity and memory consolidation (Aguiar et al., 2011; Huang et al., 2006). The interaction between exercise-induced changes in neurotransmitter levels and growth factor synthesis may provide a mechanism by which acute PE could enhance memory persistence and suggests a possible dependence on protein synthesis in the hippocampus.

The behavioral tagging (BT) hypothesis is a potential mechanism to explain the effects of acute PE on memory persistence (Loprinzi et al., 2018). This hypothesis suggests that the plasticity-related mechanisms activated during memory formation can also be strengthened by other neural events that occur close in time (Ballarini et al., 2009; Moncada and Viola, 2007). The molecular mechanism behind this is explained by the parallel event protein-related plasticity (PRP) synthesis, which is subsequently captured by synapses tagged by memory stimuli (Straube et al.,

2003). This avoids the long-term potentiation (LTP) decay, which is fundamental for forming a stable LTM (Li et al., 2003; Tomaiuolo et al., 2015). This phenomenon usually explains the effect of novelty on memory improvement. Exposure to a novel context shortly after learning in the novel object recognition (NOR) task, which typically generates only a transient LTM, can promote memory persistence for several days (Lima et al., 2021b). Interestingly, novelty and acute PE share very similar neural mechanisms, both acting on dopaminergic mechanisms in the hippocampus and requiring PKA signaling (Lima et al., 2021b, 2021a). Furthermore, both stimulate the hippocampal dopamine release (Menezes et al., 2015; Vargas et al., 2017), a neurotransmitter strictly required to synthesize PRP (Duszkiewicz et al., 2019). Thus, acute PE is a potential plasticity tool to BT, triggering physiological and biochemical changes in neurons and synapses that can modulate the plasticity induced by parallel events, such as memory stimuli.

The hypothesis that acute PE stimulates the synthesis of PRP in the hippocampus raises questions about the mechanisms by which it modulates memory (Loprinzi et al., 2018). Specifically, it is unclear whether protein de novo synthesis in the hippocampus is necessary for the effects of acute PE on memory persistence or if acute PE can enhance learning through other mechanisms. Some studies have proposed that stimulus that increases arousal can consolidate memories even in the presence of protein synthesis inhibitors (Alberini, 2008; Gold, 2008), suggesting that acute PE may enhance memory consolidation through multiple mechanisms beyond PRP synthesis. Therefore, further research is necessary to fully understand the complex processes underlying the effects of acute PE on learning and memory.

One possible approach involves using compounds such as anisomycin (ANI), which inhibits protein synthesis by preventing the translocation of ribosomes, or rapamycin (RAPA), which blocks protein synthesis through the inhibition of the mammalian target of rapamycin (mTOR) signaling (Myskiw et al., 2013). Thus, using both compounds can determine whether the protein synthesis inhibition alone is sufficient to prevent memory consolidation or if the inhibition of the mTOR pathway also plays a crucial role. By manipulating protein synthesis with these compounds, we could investigate the contribution of hippocampal protein synthesis to the modulatory effects of acute PE on NOR memory consolidation and persistence. Additionally, we explored whether the acute PE protocol we employed could enhance BDNF synthesis in the hippocampus during the initial hours following exercise.

2 Material and methods

2.1 Animals

We obtained adult male Wistar rats (3 months old; weighing 300-350 g) from the University of Santa Maria Central Vivarium (RS, Brazil). The rats were housed in four per cage and provided with food and water ad libitum in a room maintained at a constant temperature of 23 ± 2°C and humidity of 50 ± 10%, and with a 12-hour light/dark cycle (lights on from 07:00 to 19:00). The animals were acclimatized to the housing environment for one week, and, to minimize stress, they were handled by the experimenters before the start of the experiments. All experiments were conducted during the light phase. The experimental protocol was initiated after approval by the Local Institutional Animal Care and Use Committee (protocol 029/2021).

2.2 Experimental design

We performed pharmacological-behavioral and biochemical experiments to assess the impact of acute PE on memory (**Figure 1**).

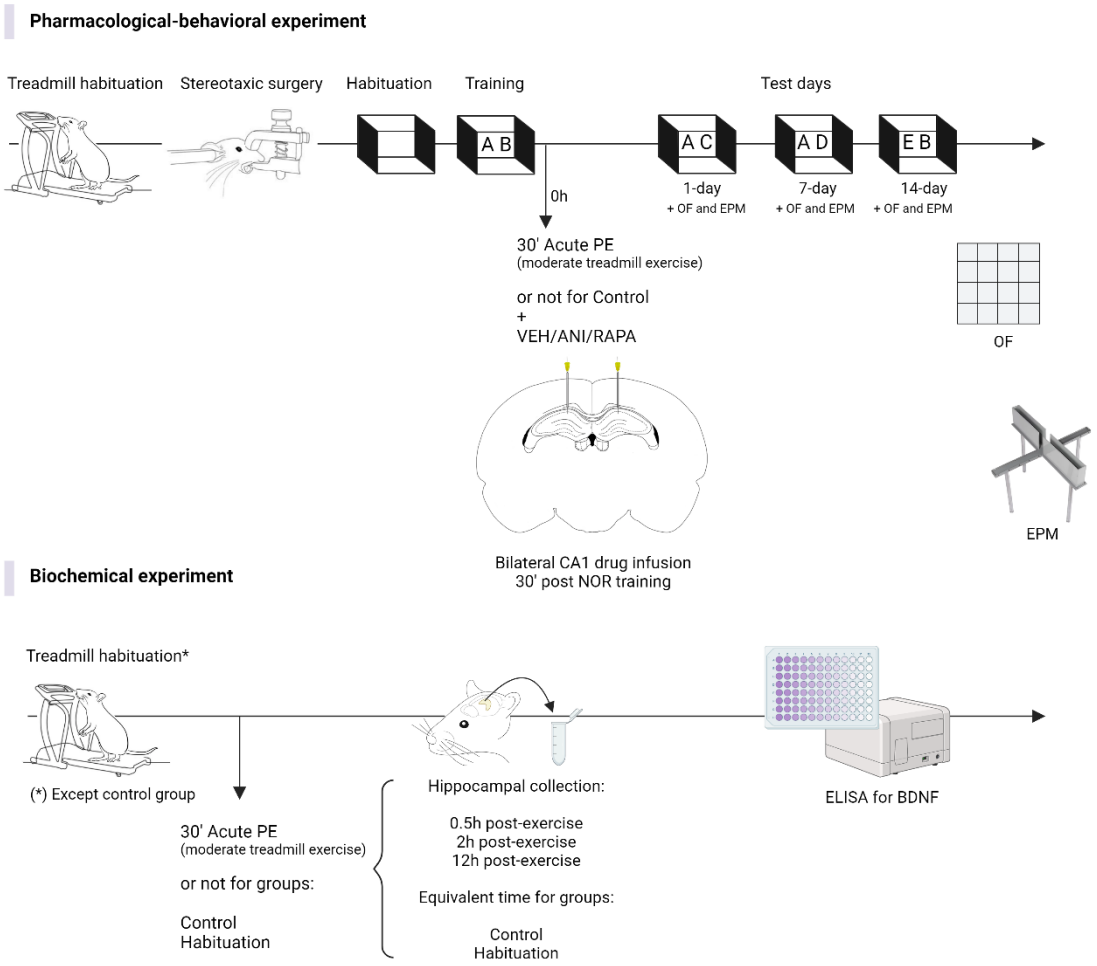

Figure 1 Experimental design. In the pharmacological-behavioral, animals were habituated to the novel object recognition (NOR) task for 4 days and underwent a training session the following day. Memory tests were conducted on days 1, 7, and 14 post-training. To investigate the modulation of learning by Physical Exercise (PE), immediately after NOR training, some animals underwent a 30-minute session of PE on a treadmill, with a moderate intensity set at 60-70% of their indirect maximal oxygen uptake (VO_{2MAX}). Following the PE session, we infused either vehicle (VEH), anisomycin (ANI), or rapamycin (RAPA) into the CA1 region of the hippocampus to inhibit protein synthesis. Other animals did not undergo PE and were named as the control groups. These animals only received VEH, ANI, or RAPA within the same time window, 30 minutes after NOR training. The two-letter combinations used in the NOR box draw represent the combination of objects used in each NOR session. In the biochemical experiments, we assessed the expression of BDNF following an acute PE session, considering distinct time intervals of 0.5h, 2h, or 12h post-exercise. Animals in the control and habituation groups did not undergo acute PE,

and their hippocampi were extracted at corresponding time points to ensure a consistent comparison with the other groups.

First, we investigate whether protein synthesis in the CA1 area of the hippocampus is necessary for the modulatory effect of acute PE on memory. Animals were divided into six groups (n = 7-11): vehicle (VEH), ANI, RAPA, PE + VEH, PE + ANI, and PE + RAPA. Animals subjected to PE were previously habituated to a treadmill one week before the memory tasks and exercise session. Since habituation alone does not affect NOR memory, we did not include a non-exercised group subjected to habituation (Vargas et al., 2017). All animals were implanted with bilateral cannulas in the CA1 area. We conducted memory evaluations using the NOR task, which comprised a habituation phase, a training session with two unfamiliar objects, and test sessions on days 1, 7, and 14 post-training to assess memory consolidation and persistence by measuring the exploration of a novel and a familiar object. After the training session, animals underwent a 30-minute moderate-intensity acute PE on the treadmill, while the control group did not undergo any exercise. Following the PE session or an equivalent time for control animals, some rats received into CA1 either vehicle (VEH), ANI, or RAPA to investigate the protein synthesis requirement. Control behavioral tests were conducted after testing to verify locomotion, exploratory and anxious behavior.

In the biochemical experiments, we explored the impact of acute PE on the temporal modulation of BDNF levels in the hippocampus. Animals were categorized into distinct groups (n = 6): control, habituation, PE 0.5h, PE 2h, and PE 12h. All groups, except the control, underwent treadmill habituation one week before the acute PE. Following a 30-minute session of moderate-intensity acute PE, animals were euthanized at 0.5h, 2h, or 12h intervals, or at equivalent times for the control and habituation to treadmill groups (at the same day of euthanasia of other groups) — groups that did not undergo the acute PE session. BDNF levels were measured by Sandwich ELISA kit.

2.3 Drugs

The ribosomal translation inhibitor, ANI (A9789), and the mTOR-mediated protein synthesis inhibitor, RAPA (R0395), were purchased from Sigma-Aldrich. The

drugs were dissolved in DMSO 2% (diluted in 0.9% saline) and stored at -20ºC, protected from light until use. The drug concentrations were based on previous studies (Myskiw et al., 2015; Vargas et al., 2019), with ANI at 80 µg/µL and RAPA at 5 pg/µL.

2.4 Surgery and drug infusion

Animals were anesthetized with intraperitoneal (i.p.) injections of a mixture of ketamine (75 mg/kg) and xylazine (10 mg/kg). They were submitted to stereotaxic surgery to the 22-gauge bilateral guide cannula implantation into the CA1 area of the hippocampus (anterior, − 4.2 mm; lateral, ± 3.0 mm; ventral, − 2.0 mm). Dental cement was used to fix the cannulas onto the skull. The animals were given four days to recover from surgery before additional procedures were performed.

The drug infusion occurred 30 minutes after NOR training and followed the PE in some groups. We utilized a Hamilton syringe connected to fine bore tubing (38-gauge, 15 cm) and had a needle (30-gauge, 10 mm) at the end. When administering the drug, the infusion needle was placed into the guide cannula and advanced 1.0 mm deeper to reach the target region. Infusions (1 µL/side) were carried out for 60 seconds, and the infusion needle was left in place for an additional 60 seconds to minimize backflow.

2.5 Acute physical exercise (PE)

We used a motorized treadmill built for rodents (Insight Ltd., Brazil). Animals from PE groups were habituated to the apparatus for one week to avoid stress or novelty effects. The habituation consisted of 10-minute daily sessions, starting with off-treadmill placement on day one, then running at 2-5 m/min on the next two days, and increasing to 8 m/min on days four to six.

An indirect maximal volume of oxygen uptake (VO_{2MAX}) test was conducted to determine each animal's exercise intensity. This test involved starting at a low velocity (1 m/min) and increasing by 5 m/min every 3 min until the animal could no longer continue running. The work volume (m/min) was considered an indirect measure of indirect VO_{2MAX}. The test was conducted one day after the last habituation session on the treadmill. An intermediate intensity of 60-70% of indirect

VO_{2MAX} was used for the acute PE session, which lasted 30 minutes and was performed after NOR training (Lima et al., 2021a; Vargas et al., 2020, 2017).

2.6 Novel object recognition (NOR)

The NOR task was based on the natural tendency to explore new objects in a familiar setting (Ennaceur and Delacour, 1988). The task was conducted in a wooden box measuring 50 x 50 x 50 cm designed for experimental purposes and coated with waterproof paint. This coating effectively prevents the penetration of odors, such as urine, as well as the 70% alcohol used for cleaning between trials.

Before training, the animals were habituated to the empty box for 20 minutes daily for four days. During training, the animals were allowed to explore the box containing two unknown objects (A and B) for 5 minutes. Test sessions were conducted on days 1 (A and C), 7 (A and D), and 14 (E and B) after training, each one of them using a familiar and an unknown object. During the test sessions, the animals were given 5 minutes to explore the objects.

The experiments used various objects represented here by different letters, including a magic cube, a plastic cup, a circular can, Lego pieces, and a metallic cylinder, respectively. The animals did not exhibit a preference for any specific object. The animals' behavior in the empty experimentation room was recorded by a video camera placed above the arena, and a blind experimenter measured the exploration time using a manual stopwatch. Exploration was defined as the animals touching or sniffing the object with their front paws and nose, respectively. Sitting on or turning around the objects was not considered exploratory behavior. To avoid olfactory preferences, the objects and apparatus were cleaned with 70% alcohol.

2.7 Behavioral control

Behavioral control tests were conducted approximately 3 hours after each NOR test session to ensure that experimental procedures or environmental conditions did not cause behavioral changes that could affect the memory assessments.

The open field (OF) test was utilized to evaluate animals' locomotor and exploratory activities. The apparatus was a 50 × 50 × 50 cm wooden box with black

lines dividing it into 12 quadrants. The animals were observed for 5 minutes, and the number of crossings and rearing were recorded as measures of their activities.

Anxiety behavior was measured using the elevated plus maze (EPM), consisting of a cross-platform with 40 cm arms, two of which have high walls (closed arms) and two without walls (open arms), and is 1 meter high. The animals were placed in the maze's center and allowed to explore freely for 5 minutes. The time spent and the number of open-arms entries were counted, indicating non-anxious behavior.

The behavior of the rats was recorded using a video camera positioned above the apparatus in an empty experimental room. A blind experimenter rated the animals' behavior, and a manual stopwatch was used as necessary.

2.8 Histology

Cannula placement was achieved through histological examination after the animals were sacrificed. In the end, some animals were anesthetized using an i.p. injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). We infused a 4% methylene blue solution (1 µL/side) into the CA1 area of the hippocampus. Rats were then perfused with 0.9% saline solution (200 mL), followed by 10% formaldehyde (200 mL). The brains were extracted and fixed in 10% formaldehyde (30 mL) for four hours and cryopreserved in 30% sucrose solution (30 mL) for 72 hours at 4°C. The brains were dried and stored at -80°C until analysis. Coronal brain sections with a thickness of 40 µm were sliced using a Cryostat (LEICA CM3050S) and examined using an optical stereo microscope (Olympus). The area where the methylene blue solution reached was used to indicate potential drug diffusion.

2.9 Sandwich ELISA assay

The BDNF Sandwich ELISA kit (CYT306) was purchased from Merck S. A. Samples were promptly diluted upon collection according to the kit specifications, and the resulting supernatants from the preparation were stored at -80ºC until analysis. The assay instructions were meticulously followed, including the preparation of the standard curve in duplicate, mirroring the sample setup. The plate was sealed with a plate sealer and incubated overnight at 2-8ºC on a shaker. Subsequently, the plate

sealer was removed, and the plate was washed at least 4 times with 250uL of diluted Wash Buffer using an automatic plate washer. 100 uL of the diluted biotinylated mouse anti-BDNF monoclonal antibody was added to each well, the plate was covered, and incubated at room temperature for 2-3 hours on a shaker. After incubation, the plate was washed again. Following the wash, 100 uL of the diluted streptavidin-HRP conjugate solution was added to each well. The plate was covered and incubated at room temperature for 1 hour on a shaker, followed by another round of washing. Subsequently, 100 uL of TMB/E Substrate was added to each well, and the plate was incubated at room temperature for 15 minutes. The reaction was halted by adding 100 uL of Stop Solution to each well, causing the blue color to transition to yellow. The plate was immediately read at 450 nm.

2.10 Statistical analysis

We use the GraphPad Prism 8 statistics package for analysis. First, we checked the data for normality of distribution using the Shapiro–Wilk test. We used the Kruskal-Wallis test to compare the indirect VO_{2MAX} between exercised groups. The NOR results were expressed as the percentage of total time spent exploring each object, and we analyzed the data using a one-sample Student's t-test, assuming a 50% theoretical mean. We considered the animal's ability to spend over 50% of the total time exploring the novel object as memory retention. We also performed a comparison between groups, so NOR data were converted to a discrimination index (DI = [(t novel - t familiar)/(t novel + t familiar) x 100]), where ‘t’ is the time spent exploring the objects. We analyzed this using two-way ANOVA followed by Sidak’s post hoc test. A higher DI suggests a greater capacity to discriminate the objects and explore the novel object for more time. The object exploration time, OF, and EPM results were analyzed using two-way ANOVA and were used as control parameters. The BDNF levels were analyzed using one-way ANOVA. Data are presented as mean ± standard deviation (SD). We considered the differences significant when $P < 0.05$ for all analyses.

3 Results

3.1 Exercised groups presented a similar indirect VO_{2MAX}

After habituation to the treadmill, the animals underwent an indirect VO_{2MAX} test. They were randomly distributed among the different acute PE groups, and no significant differences were observed in their indirect VO_{2MAX} values ($K_{(3, 28)}$ = 2.013, P = 0.3655; **Figure 2**). The mean indirect VO_{2MAX} values for the groups were comparable: VEH, 22.50 ± 3.54; ANI, 20.71 ± 3.45; and RAPA 23.18 ± 3.37 m/min.

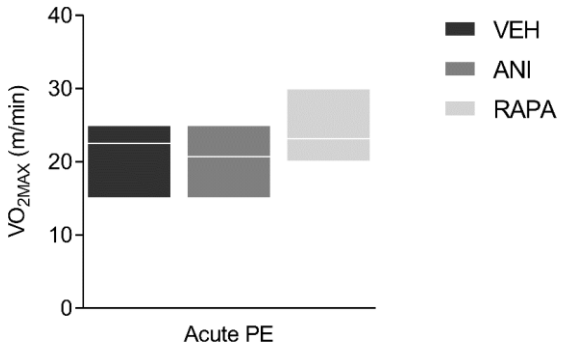

Figure 2 Exercised groups presented a similar indirect VO_{2MAX}. Data are presented as line at mean. P > 0.05 in the Kruskal-Wallis test (n = 7-11/group).

3.2 Inhibition of protein synthesis in the CA1 impairs memory consolidation, and acute PE prevents this effect

In the NOR protocol (**Figure 3A**), the animals were first habituated to the apparatus. After training, the control groups received no intervention, while the acute PE groups practiced a 30-minute treadmill session. To assess the role of protein synthesis, both groups received VEH (to pharmacological control), ANI, or RAPA in the CA1 region of the hippocampus either immediately after acute PE or at an equivalent time point for control animals. The effectiveness of cannula positioning and drug infusion was confirmed by the presence of methylene blue dye upon post-mortem examination (**Figure 3B;** see more in **Figure S1** in the supplementary material).

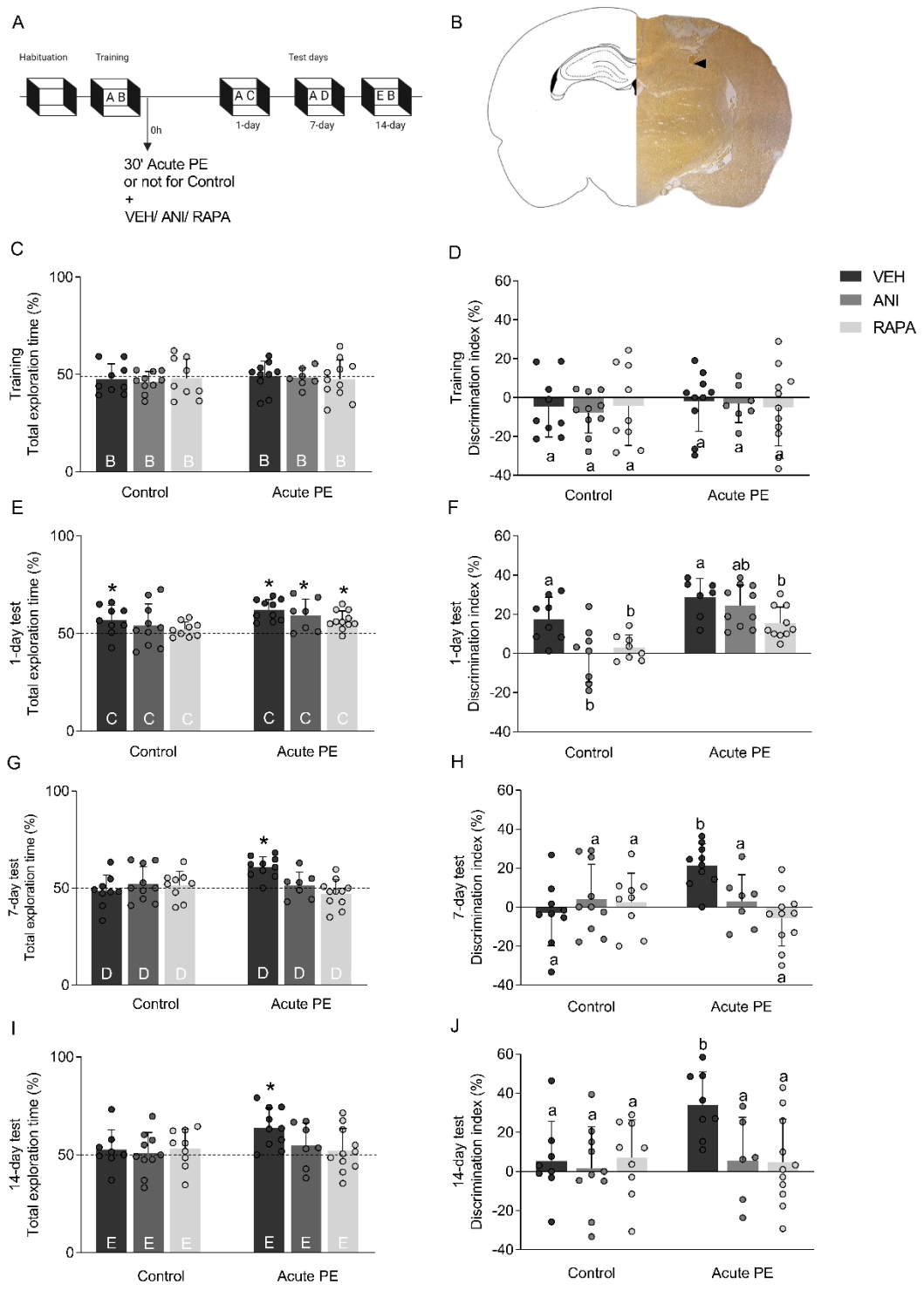

Figure 3 Acute PE prevents the amnesic effect of hippocampal protein synthesis inhibition on memory consolidation. A) Experimental paradigm on novel object recognition (NOR) task. The drugs, vehicle (VEH), anisomycin (ANI), or rapamycin (RAPA) were infused into the CA1 area of the hippocampus after de acute physical exercise (PE) and 30 minutes after novel NOR training in the control group. B) The surgical procedure and drug infusion effectively reached the CA1 region of the

hippocampus. The figure illustrates a coronal section with the CA1 region of the hippocampus highlighted by an arrow. C-J) Memory evaluation. Percentual of total exploration time (C, E, G, and I). Data are presented as mean ± SD. * P < 0.05 in One sample t-test. Groups' comparison by a novel object's discrimination index (DI; D, E, H, and J). P < 0.05 when different letters when comparing intragroup and intergroup in two-way ANOVA followed by Sidak's post hoc (n = 7-11/group).

In training, the animals were exposed to two different and unknown objects. There was no preference between the objects since the animals explored both for about 50% of the time (P > 0.05; **Figure 3C**) and no interaction ($F_{(2,50)}$ = 0.1219; P = 0.8855; **Figure 3D**) or effect in the variables was found ($F_{(1,50)}$ = 0.2488; P = 0.6201 at interventions; $F_{(2,50)}$ = 0.0.0760; P = 0.9269 at drug infused; **Figure 3D**).

To assess memory consolidation, we examined rats' behavior in the NOR 1-day after training, during a 5-min test session in which a familiar object was placed with a novel object. Animals that received VEH spent more than 50% of the total time exploring the novel object (P = 0.0235; **Figure 3E**), indicating memory consolidation. The same was observed for all animals that underwent acute PE (P < 0.0001, PE+VEH; P = 0.0241, PE+ANI; P = 0.0008, PE+RAPA; **Figure 3E**). Interestingly, protein synthesis inhibitors impaired consolidation only when applied in the absence of acute PE (P = 0.2606, ANI; P = 0.1202, RAPA; **Figure 3E**), as indicated by similar exploration times for both objects.

In addition, on the 1-day test, we also observed effects between interventions ($F_{(1,45)}$ = 29.63, P < 0.0001; **Figure 3F**) and infused drugs ($F_{(2,45)}$ = 7.846, P = 0.0012; **Figure 3F**). Acute PE prevented the decrease in DI caused by ANI (P < 0.0001; **Figure 3F**) and RAPA (P = 0.0452; **Figure 3F**). In the control groups, ANI (P = 0.0041; **Figure 3F**) and RAPA (P = 0.0200; **Figure 3F**) presented lower DI than VEH, confirming memory impairment. In the PE groups, only RAPA was able to decrease the DI (P = 0.0349; **Figure 3F**) compared to VEH. These data confirm that acute PE overcomes the effect of inhibition of hippocampal protein synthesis on memory consolidation.

We also assessed the persistence of memory 7 days after the training session. Animals in the control group that received VEH showed physiological forgetfulness, exploring a novel and a familiar object for a similar time (P = 0.5916; **Figure 3G**). As expected, animals receiving only ANI (P = 0.4895; **Figure 3G**) or

RAPA (P = 0.5970; **Figure 3G**) still did not discriminate between objects. Acute PE promoted memory persistence, and the animals explored the novel object for a longer time (P = 0.0001; **Figure 3G**). However, PE did not prevent forgetting in animals that received ANI (P = 0.5892; **Figure 3G**) or RAPA (P = 0.2228; **Figure 3G**).

Confirming our findings, an interaction effect was observed ($F_{(2.50)}$ = 6.423, P = 0.0033; **Figure 3H**); animals that received VEH after acute PE had a higher DI than those that received VEH in the absence of PE (P = 0.0022; **Figure 3H**). As observed, the inhibition of protein synthesis in the hippocampus hindered the impact of acute PE on memory persistence and treatment with ANI (P = 0.0384; **Figure 3H**) or RAPA (P = 0.0004; **Figure 3H**) after acute PE led to a decrease in DI compared to animals that received VEH after exercise.

Similar results were obtained when memory was evaluated in the 14-day test, only animals exposed to acute PE showed LTM persistence. They explored the novel object for a longer time while showing recognition of the familiar one (P = 0.0019; **Figure 3H**). Control animals that received VEH (P = 0.4805; **Figure 3I**), ANI (P = 0.8279; **Figure 3I**), or RAPA (P = 0.3189; **Figure 3I**) explored both objects for a similar time, like those treated with acute PE associated to ANI (P = 0.2980; **Figure 3I**) or RAPA (P = 0.5101; **Figure 3I**). The post hoc test revealed memory persistence in exercised animals; specifically, animals that underwent a PE session and received VEH showed a higher DI compared to the control group that only received VEH (P = 0.0277; **Figure 3J**).

We examined several behavioral factors that could have potentially influenced the results of the NOR task. However, all animals exhibited comparable levels of locomotor and exploratory activity and similar levels of anxiety (**Table S1** in the supplementary material).

3.3 Acute PE did not lead to an increase in BDNF levels in the hippocampus over time

We investigated BDNF levels in the hippocampus at specific time points after acute PE: 0.5, 2, and 12-hours post-exercise (**Figure 4A**). Some animals were habituated to the treadmill one week before, while others were not and served as the

control group. No significant differences were observed among the groups ($F_{(4,25)}$ = 1.255, P = 0.3137; **Figure 4B**).

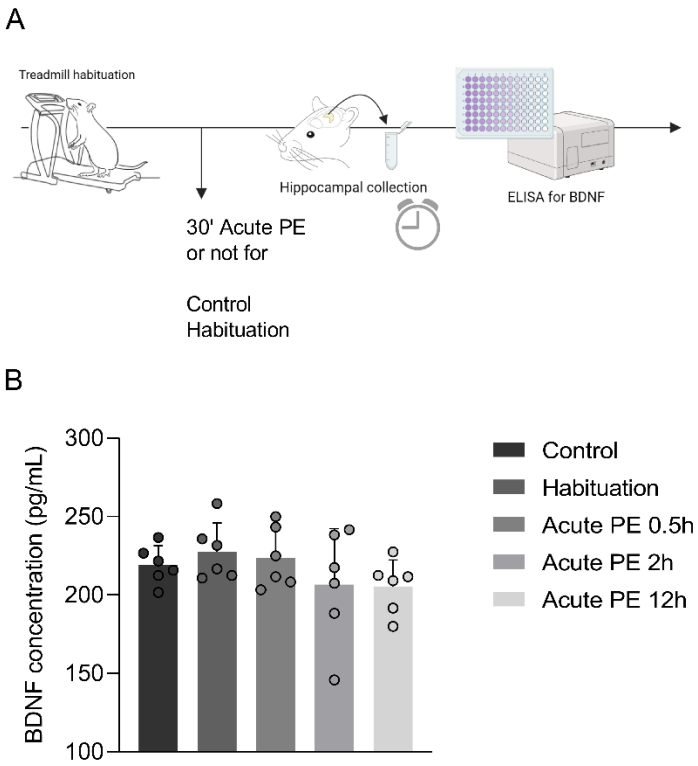

Figure 4 Acute PE did not increase the concentration of BDNF in the hippocampus over the hours. A) Experimental design: Rats were habituated to the treadmill one week before the experiment, except for the control group. Animals in the acute PE groups underwent a 30-minute PE session, while the control and habituation groups did not. Hippocampal collection occurred 0.5, 2, or 12-hours post-exercise, or at equivalent times for other groups. BDNF levels were assessed using an ELISA assay. B) The levels of BDNF in the hippocampus were similar among the groups. Data are presented as mean ± SD. P > 0.05 in one-way ANOVA (n = 6/group).

4 Discussion

Here, we investigated the role of hippocampal protein synthesis in the modulation of NOR memory consolidation and persistence by acute PE. After task training, animals performed a 30-minute running treadmill exercise or did not and were kept as the control group. Following, they received an infusion of VEH, ANI, or RAPA into the CA1 area, and their memory was tested 1, 7, and 14 days later in the NOR task. Our findings revealed that inhibiting protein synthesis impaired the consolidation of NOR memory in animals that did not undergo acute PE. Interestingly, acute PE prevented the amnesia caused by the drugs, preventing the impairment of memory consolidation without affecting persistence. We investigated whether this acute PE protocol is capable of increasing BDNF synthesis in the hippocampus during the initial hours following exercise and did not detect an increase in this protein was not detected at 0.5h, 2h, or 12h post-exercise.

Our NOR task protocol involves a learning session that is sufficient by itself to promote a transient consolidation of memory, which means that the memory is forgotten within a few days (Lima et al., 2021b; Neves et al., 2020). When infused into the CA1 area 30 minutes after training, both ANI and RAPA impaired memory consolidation in the control group, indicating that this memory requires both ribosomal and mTOR-mediated protein synthesis in the hippocampus. Previous findings support that hippocampal protein synthesis is essential for NOR memory consolidation, indicating a critical period within a few hours after learning about this requirement (Furini et al., 2015; Rossato et al., 2007). A body of evidence suggests the requirement for protein synthesis in the consolidation of various hippocampus-dependent memories (Bekinschtein et al., 2007; Menezes et al., 2015; Ozawa et al., 2017), and the dependence of this synthesis for LTM, but not short-term memory (STM), is well-established.

The LTP is a cellular mechanism underlying the formation of LTM that involves strengthening synaptic connections between neurons (Izquierdo et al., 2008). The molecular mechanisms underlying late LTP (L-LTP) and LTM formation both involve the synthesis of new proteins that are required for the maintenance and growth of synapses (Scharf et al., 2002). Inhibiting protein synthesis in the CA1 region of the hippocampus impairs L-LTP (Stanton and Sarvey, 1984) while blocking the synthesis of specific proteins expressions, such as CREB, Arc, Zif268, and BDNF, also impairs

the formation of both L-LTP and LTM (Abraham and Williams, 2008). Moreover, the maintenance of L-LTP is prevented by inhibiting the synthesis of new proteins in neurons by ribosomal (Frey et al., 1988; Mochida et al., 2001) or mTOR inhibition (Tang et al., 2002). These findings provide substantial evidence to support the idea that maintaining L-LTP requires an initial phase of stimulated protein synthesis. This process may enhance the adjacent mechanisms responsible for stabilizing synapses and promoting enduring memories.

Given the critical role of hippocampal protein synthesis in consolidating long-lasting memories, we investigated whether inhibiting protein synthesis after a 30-minute treadmill running session would affect NOR memory in rats. Acute PE alone has been shown to promote the persistence of learning in the NOR task for several days, preventing the physiological forgetting observed after just over 24 hours, as seen in previous studies (Lima et al., 2021a; Vargas et al., 2020, 2017). Our results reinforce these findings, as the animals were able to retain their memory for up to 14 days after training. It is interesting to note that despite the blockade of ribosomal protein synthesis or mTOR, the memory of the exercised animals remained intact one day after the infusion, suggesting successful memory consolidation. However, while acute PE prevented amnesia caused by protein synthesis inhibition, the memory formed was not able to persist over time, as commonly occurs due to the PE modulation of memory consolidation.

Our findings are supported by other research, which has demonstrated intact memories formed despite extensive inhibition of protein synthesis. Specific behavioral or pharmacological manipulations can protect memory formation from the amnestic effects of protein synthesis inhibitors. For example, increasing foot shock (Díaz-Trujillo et al., 2009; González-Franco et al., 2019; González-Salinas et al., 2015) or adding a pretraining (Quevedo et al., 1999) in inhibitory avoidance have been shown to attenuate amnesia. Exposure to an OF shortly before inhibitory avoidance training has also been found to protect memory from impairments by anisomycin or scopolamine (Colettis et al., 2014). Moreover, a variety of drugs such as clonidine, isoproterenol, caffeine, fluoxetine, nicotine, naloxone, and corticosterone have been found to enhance performance in memory tasks even while animals are under the influence of various protein synthesis inhibitors (Routtenberg and Rekart, 2005). However, it is important to note that there are variations in

dosage, route of administration, targeted brain areas, and behavioral protocols from these studies.

Generally, the strategies mentioned as protective against the effects of protein synthesis inhibitors seem to act on arousal, attention, and memory formation. Arousal is a state of heightened physiological and psychological activation characterized by increased alertness closely related to memory enhancement (McGaugh, 2018). Some authors have discussed the possibility of enhancing memory by mimicking the brain's natural response to arousing or stressful situations through the increase of epinephrine and cortisol levels (McGaugh, 2013). Therefore, experiences or drugs that increase arousal could reverse the amnesia produced by protein synthesis inhibitors (Gold, 2008). This effect is thought to occur because the decrease in catecholamine levels caused by protein synthesis inhibitors may lead to a depression of arousal levels, impairing memory formation. However, this hypothesis is not fully supported, as it disregards the importance of hippocampal protein synthesis in memory consolidation.

The most likely hypothesis suggests that strong memories may engage quantitative or qualitative differences in protein synthesis, making them more resistant to amnesic treatments (Alberini, 2008). It seems that the critical level of protein synthesis inhibition necessary to impair long-term memory is at least 90% (Squire and Barondes, 1976). However, it has been argued that strategies that can rescue memory impairments caused by protein synthesis inhibitors may act by increasing the synthesis of the small proportion of proteins that are not inhibited by the blockers (Alberini, 2008; Gold, 2006). It is also possible that these tools that rescue memory impairments caused by protein synthesis inhibitors could act on other cellular mechanisms that promote protein synthesis or modulate mechanisms in other brain areas involved in memory formation.

Considering these ideas, we have explored potential explanations for the protein synthesis independence of acute PE in enhancing memory. One possibility is that acute PE primarily acts on catecholamine mechanisms, heightening arousal and facilitating memory retention (Grinspun et al., 2019; Loprinzi et al., 2018). Another possibility is that the initial modulations induced by acute PE can significantly impact parallel learning, thus reducing the effects of subsequently administered drugs. Additionally, acute PE stimulates protein synthesis and gene expression, which regulate factors such as BDNF, cyclic adenosine monophosphate (cAMP) response

element-binding protein (CREB), and other neuroplasticity-related factors in the hippocampus (Aguiar et al., 2011; Soya et al., 2007; Venezia et al., 2019). Consequently, there is a potential for additional protein synthesis induced by acute PE, which could compensate for or enhance the protein synthesis triggered by the initial novel object recognition (NOR) training, overcoming temporal and anatomical limitations.

Here we demonstrate that acute PE did not elevate the levels of BDNF protein in the first hours following its practice. However, previous research employing similar acute PE protocols has shown an increase in either BDNF protein or its mRNA expression within the hippocampus. For instance, studies have reported that 30 minutes of high-intensity treadmill running (15-17m/min), but not moderate-intensity (12m/min), resulted in an increase in BDNF IV mRNA expression in mice immediately after exercise (Venezia et al., 2019). Similarly, a single 45-minute (followed by 15 minutes of rest) bout of high-intensity exercise (18m/min) led to an increase in total mRNA BDNF expression, while both high- and moderate-intensity (12m/min) exercises increased mRNA BDNF IV expression in mice (Venezia et al., 2017). Furthermore, in Wistar rats, 30-minute of acute moderate PE (15m/min) increased hippocampal BDNF protein levels, whereas acute severe exercise (12m/min plus 3m/min increments up to 24m/min) increased both BDNF protein and mRNA levels in animals 2 hours post-exercise (Huang et al., 2006). Considering these varying times of expression and protein synthesis, we chose different time points to assess BDNF levels, anticipating a possible modulation in the first hours after exercise (0.5h, 2h, and 12h). However, our observations suggest that acute PE may induce an increase in protein levels beyond this time window. Therefore, future studies should explore this modulation after 12 hours, and, in addition, considering others factors such as learning in the NOR task and pharmacological infusion.

In our study it was employed two distinct pharmacological agents, rapamycin and anisomycin, to disrupt mTOR-dependent and ribosomal protein synthesis, respectively. The chosen doses were based on previous studies that evaluated memory encoding dependence on protein synthesis (Power et al., 2006; Rossato et al., 2007; Vargas et al., 2019). However, it is important to note that drugs can have different effects at different doses. For example, anisomycin at low doses (12 or 25 µg/side) did not suppress neuronal activity but prevented long-term potentiation, while higher doses (62–500 µg/side) can reduced the neuronal activity (Remaud et

al., 2014; Sharma et al., 2012; Shires et al., 2012). Therefore, it would be interesting to investigate the acute PE and anisomycin interaction at doses below 25µg. Furthermore, it is important not to overlook additional effects that these drugs may have. For instance, rapamycin is known to induce autophagy, a cellular process involved in the degradation and recycling of cellular components (Li et al., 2014). On the other hand, anisomycin has been shown to activate MAPK cascades, which play crucial roles in cell signaling and regulation (Xiong et al., 2006). These secondary effects could contribute to the overall outcomes observed in our study and should be taken into consideration when interpreting the results.

Our results suggest that acute PE may be a promising non-pharmacological intervention to prevent the harmful effects of protein synthesis inhibition on memory consolidation. It is interesting because it corroborates what we know about acute PE as a potent intervention for memory improvement, significant for both healthy animals and those with mnemonic deficits (Daré et al., 2020; Sosa et al., 2019). Moreover, this strategy can be easily translated to humans and used as a tool, especially in clinical and educational settings. Indeed, the fact that acute PE is not affected by hippocampal protein synthesis inhibition is significant as it implies that exercise could serve as a valuable tool in preventing memory issues, particularly those associated with the inhibition or depletion of protein synthesis in the hippocampus. This is particularly relevant for individuals with memory deficits, such as Alzheimer's disease, since impairments in protein synthesis may be one of the earliest neurochemical alterations contributing to memory deficits in this pathology (Ding et al., 2005).

5 Conclusion

The results of our study demonstrate the importance of hippocampal protein synthesis in the consolidation and persistence of NOR memory, as well as the beneficial effects of acute PE on memory formation. We found that inhibiting protein synthesis into the CA1 area of the hippocampus with ANI or RAPA impaired the consolidation of NOR memory in non-exercised animals. However, acute PE prevented the amnesia caused by the drugs, thereby preventing the impairment of consolidation. Finally, acute PE was able to promote memory persistence, but this effect was not observed when PE was associated to intra-hippocampus ANI or RAPA infusions. These results suggest that acute PE can serve as a non-pharmacological intervention to enhance memory and prevent memory loss in conditions associated with hippocampal protein synthesis inhibition. This could be useful for treating disorders that involve this deficit, such as neurodegenerative diseases.

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Conflict of interest

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

Author contribution

K.R.L. and P.B.M.C. conceived and designed the experiments. K.R.L., B.S.N., G.J.S., and A.C.S.R. performed the behavioral experiments. K.R.L., G.C.G.M., and M.G.G. performed the biochemical analysis. K.R.L. and P.B.M.C. analyzed and interpreted the data, wrote, and reviewed the manuscript. All authors, K.R.L., B.S.N., G.J.S., A.C.S.R., G.C.G.M., M.G.G., and P.B.M.C. approved the final version of the manuscript.

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Supplementary material

Figure S1 The surgical procedure and drug infusion effectively reached the dorsal hippocampus. The figure illustrates a coronal section with the dorsal hippocampus highlighted by an arrow, in comparison with the histological slice in the anatomical atlas.

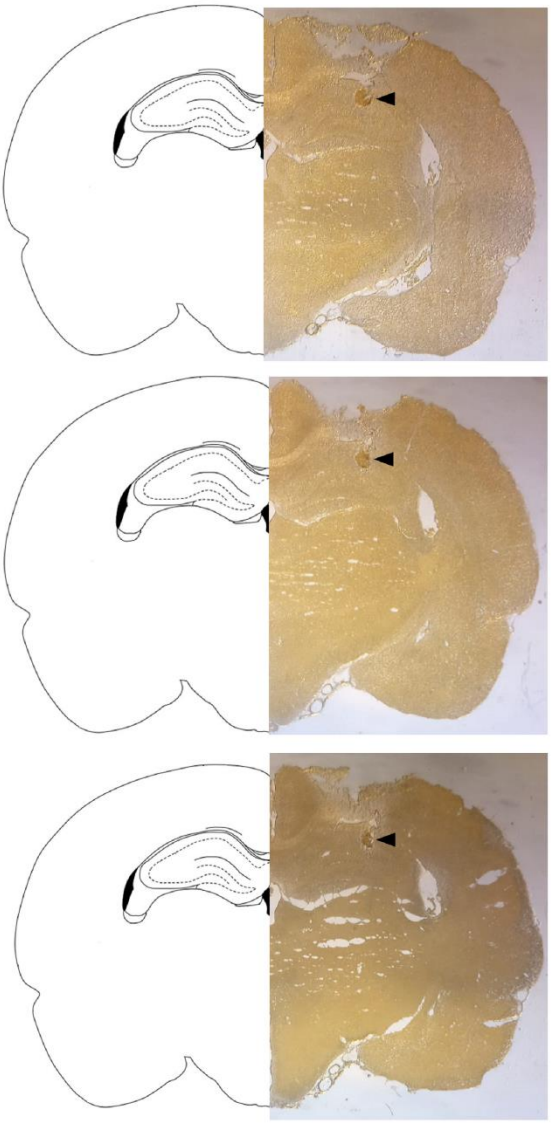

Table S1 No behavioral control differences were found among the experimental groups. Novel object recognition (NOR) total exploration time, locomotor (by crossings number) and exploratory (by rearing number) activities, and anxiety behavior (by entries and time spent in the open arms) were similar between groups. VEH = vehicle; ANI = anisomycin; RAPA = rapamycin. Data are presented as mean ± SD. P > 0.05 in two-way ANOVA.

	Control			Acute PE			Two-way ANOVA		
Number of animals per group	VEH n = 9	ANI n = 10	RAPA n = 9	VEH n = 10	ANI n =7	RAPA n = 11	Interaction	Intervention	Drug
NOR total exploration time (s)									
Training	66.44 ± 24.62	74.70 ± 22.86	89.11 ± 12.65	71.70 ± 31.30	78.14 ± 29.13	70.09 ± 27.98	0.2702	0.6198	0.4301
1 d test	70.89 ± 16.64	67.50 ± 17.81	79.00 ± 12.99	72.40 ± 12.99	79.71 ± 16.09	56.82 ± 13.47	0.0821	0.9518	0.9403
7 d test	67.11 ± 20.85	70.90 ± 27.45	71.56 ± 27.04	65.70 ± 37.62	83.57 ± 21.98	64.64 ± 22.13	0.5306	0.6251	0.2992
14 d test	51.00 ± 20.49	61.20 ± 30.86	56.67 ± 16.18	52.30 ± 25.56	64.86 ± 12.69	56.36 ± 33.71	0.9730	0.8219	0.4184
Crossings (n)									
1 d test	76.11 ± 52.10	57.00 ± 35.72	61.89 ± 37.24	80.00 ± 24.35	79.43 ± 52.67	74.55 ± 29.46	0.7778	0.2197	0.6712
7 d test	94.00 ± 49.19	82.10 ± 29.40	69.89 ± 35.58	69.30 ± 26.52	81.57 ± 22.74	79.18 ± 35.40	0.2990	0.5701	0.7584
14 d test	79.22 ± 27.62	68.30 ± 26.24	73.22 ± 16.72	84.70 ± 33.80	85.14 ± 30.84	86.36 ± 31.77	0.8307	0.1308	0.8616
Rearings (n)									
1 d test	19.89 ± 10.46	17.90 ± 10.39	19.44 ± 9.14	20.30 ± 6.90	21.43 ± 12.70	21.73 ± 8.07	0.8868	0.4250	0.9587
7 d test	22.22 ± 9.11	24.40 ± 9.50	20.22 ± 8.54	22.80 ± 8.24	23.90 ± 6.02	22.09 ± 9.25	0.8752	0.8500	0.6498
14 d test	27.67 ± 6.89	25.80 ± 9.72	26.11 ± 3.69	24.60 ± 8.33	24.43 ± 4.50	26.36 ± 6.65	0.7692	0.4707	0.8762
Entries in open arms (n)									
1 d test	7.22 ± 1.92	7.20 ± 3.01	8.00 ± 2.29	6.60 ± 3.37	8.86 ± 3.85	8.45 ± 2.46	0.7874	0.1256	0.9005
7 d test	9.22 ± 1.09	8.70 ± 4.37	7.22 ± 3.63	7.10 ± 2.28	10.14 ± 1.95	8.73 ± 3.16	0.1209	0.7374	0.3158
14 d test	13.00 ± 4.77	9.20 ± 3.82	10.11 ± 4.51	8.00 ± 2.54	11.00 ± 2.65	9.82 ± 2.40	0.1433	0.0746	0.3831
Time in open arms (s)									
1 d test	182.9 ± 72.68	179.1 ± 57.80	186.3 ± 72.05	147.3 ± 66.12	193.1 ± 56.32	189.6 ± 49.34	0.4590	0.7209	0.4670
7 d test	155.9 ± 32.2	145.0 ± 60.71	133.0 ± 80.36	143.3 ± 52.30	172.9 ± 17.99	146.8 ± 50.17	0.5246	0.5077	0.5727
14 d test	170.3 ± 32.98	168.7 ± 66.72	160.8 ± 60.71	140.6 ± 54.45	182.4 ± 10.15	152.4 ± 40.09	0.4309	0.5438	0.4095

MANUSCRITO 2

Submetido para: Neuroscience

A MODERATE-INTENSITY TREADMILL EXERCISE PERFORMED DURING THE LATE CONSOLIDATION PHASE IMPROVES MEMORY PERSISTENCE BY HIPPOCAMPAL PROTEIN SYNTHESIS AND CATECHOLAMINE MODULATION

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Abstract

Memory persistence is a crucial aspect of long-term memory (LTM) and involves late consolidation processes that modulate memory stability over time. Acute physical exercise (PE) has emerged as a potential strategy to module memory consolidation and enhance memory persistence. While its effects have been extensively explored in the early consolidation phase, its impact on the late phase remains unexplored. In this study, we investigated the effects of a moderate treadmill exercise session on the late consolidation window of novel object recognition (NOR) memory in rats. A 30-minute running session applied 11 hours after NOR memory acquisition significantly increased memory persistence for up to 14 days. Our exploration of the mechanisms underlying this enhancement revealed the involvement of protein synthesis and the requirement of beta-adrenergic and dopaminergic D1/D5 receptors activation in the dorsal hippocampus. These findings provide valuable insights into PE as a potential

memory modulator, contributing to expanding our understanding of memory consolidation dynamics and acute PE effects.

Keywords: long-term memory; novel object recognition; dopamine; noradrenaline.

1 Introduction

The storage of long-term memory (LTM) is a dynamic process, and molecular mechanisms that come into play during the consolidation period can influence memory establishment and stability (MCGAUGH, 2000). This labile state may have evolved to facilitate the integration of memories with new experiences, potentially leading to positive modulation (ABEL; LATTAL, 2001). In this sense, a late-consolidation phase, which occurs approximately 11 to 12 hours after memory acquisition, has been identified as a crucial window for modulating memory persistence (BEKINSCHTEIN et al., 2007, 2008; TOMAIUOLO et al., 2015a; KATCHE et al., 2016). Limited evidence exists on non-pharmacological strategies that modulate memory within this specific time window. Current research highlights the effectiveness of novelty exposure (TOMAIUOLO et al., 2015a) and moderate stress by immobilization (PARFITT et al., 2012) or cold water (YANG et al., 2013) as positive memory modulators during this designated timeframe. Nevertheless, the influence of one session of moderate physical exercise on memory modulation within this critical period remains to be explored.

Over the years, a wealth of accumulating evidence has clarified the potential impact of acute physical exercise (PE), a modality involving one or a few exercise sessions, on memory modulation (LOPRINZI, 2019b; LOPRINZI; MOORE; LOENNEKE, 2020). Studies in both humans and rats have shown that acute PE can improve memory by enhancing retention and cognitive performance (PERINI et al., 2016; BOUCHET et al., 2017). Based on that, exercise can acutely induce a cascade of events in the brain, impacting different organic systems according to the type, mode, duration, and intensity of exercise (CEYLAN et al., 2023). In this sense, different acute PE protocols are effective in modulating rodents' brains, both aerobic on a treadmill (SOYA et al., 2007; AGUIAR et al., 2011; STEIN et al., 2017; VENEZIA et al., 2019) or running wheel (SIETTE; REICHELT; WESTBROOK, 2014; BOUCHET et al., 2017; DIEDERICH et al., 2017) and strength training (FERNANDES et al., 2016; DARÉ et al., 2020).

In our laboratory, we have demonstrated that a single bout of moderate-intensity treadmill exercise for 30 minutes improves memory retention for weeks when applied closely to memory acquisition time (VARGAS et al., 2017). We have highlighted the involvement of dorsal hippocampus dopaminergic and noradrenergic

systems in the effects of this modality of PE, playing critical roles in modulating memory-related processes (VARGAS et al., 2017, 2020; LIMA et al., 2021b). Specifically, the release of neurotransmitters such as dopamine and norepinephrine during the PE practice enhances the signaling mechanisms involved in memory consolidation, relying on D1/D5 dopaminergic and beta-adrenergic receptors in the CA1 area (VARGAS et al., 2017, 2020).

Furthermore, the concept of behavioral tagging (BT), which revolves around the interaction between learning-related events and subsequent experiences, serves as a potential mechanism that helps to explain the exercise-induced modulatory effects on memory (LOPRINZI; PONCE; FRITH, 2018). BT proposes that memory-enhancing effects are maximized when learning is combined with another experience within a specific time window (BALLARINI et al., 2009). This experience needs to be potent to induce the activation of beta-adrenergic and dopaminergic receptors and plasticity-related proteins (PRPs) synthesis, which are captured by synapses activated by parallel learning, thereby strengthening them (BALLARINI et al., 2009; MONCADA et al., 2011), promoting LTM. The idea that acute PE acts through this mechanism gains support as it stimulates the synthesis of brain-derived neurotrophic factor (BDNF) (SOYA et al., 2007; VENEZIA et al., 2019), a potential plasticity-related protein (PRP) within the BT phenomenon (OKUDA et al., 2020).

Exploring the mechanisms of acute PE reveals several compelling reasons why it can potentially enhance LTM persistence during the late time consolidation window. The late consolidation involves catecholaminergic influence modulation since blocking D1/D5 dopamine receptors in the dorsal hippocampus 11 hours after acquisition impairs the modulation of memory persistence in rats (TOMAIUOLO et al., 2015a). In this same sense, intra-CA1 infusion of either dopamine or noradrenaline agonist 12 hours after training enhances the memory trace, inducing a persistent state (ROSSATO et al., 2009; PARFITT et al., 2012). These results emphasize a labile period between 11 and 12 hours post-acquisition when memory becomes susceptible to modifications through the influence of catecholaminergic pathways.

Additionally, the late consolidation phase depends on protein synthesis, where BDNF emerges as a particularly crucial molecule for inducing memory persistence (BEKINSCHTEIN et al., 2008; ROSSATO et al., 2009). Importantly, other key molecules, such as PKA and c-Fos, recognized for their substantial roles in the late

consolidation phase (ROSSATO et al., 2009; KATCHE et al., 2010), may also be influenced by acute PE (SOYA et al., 2007; LIMA et al., 2021b), in conjunction with dopamine, noradrenaline, and BDNF, as previously mentioned. This further underscores the potential of acute PE as an approach to enhancing memory retention in the late consolidation phase.

Understanding how acute PE influences memory processes during this late consolidation phase could offer novel insights into the temporal dynamics of memory consolidation and provide valuable information for designing memory-enhancing strategies. By deciphering the precise mechanisms and time-dependent effects of exercise on memory persistence, we can use exercise as a non-pharmacological tool to optimize memory function and alleviate memory deficits in healthy individuals and those with memory-related impairments. In this study, we aimed to explore the effects of a 30-minute moderate treadmill exercise session performed 11 hours after the initial learning on recognition memory persistence. Additionally, we sought to uncover potential hippocampal mechanisms through which this type of PE might operate within this specific time frame.

2 Material and methods

2.1 Animals

Male Wistar rats (3 months old; weighing 300-350 g) were obtained from the University of Santa Maria Central Vivarium (RS, Brazil). The rats were housed in four per cage at a temperature of 23 ± 2°C and humidity of 50 ± 10%, with food and water *ad libitum*, under a 12-hour light/dark cycle (lights on from 07:00 AM to 07:00 PM). Before the experiments began, the animals were acclimatized to the housing environment for one week and were handled by the experimenters to minimize stress. The experimental procedures followed the guidelines of the Local Institutional Animal Care and Use Committee (protocol 029/2021).

2.2 Experimental design

Animals were divided into ten groups (n = 7 – 11 per group). Animals in the acute PE groups underwent treadmill habituation one week before the memory task. As habituation alone does not impact novel object recognition (NOR) memory (Vargas et al., 2017), we did not include a non-exercised group subjected to treadmill habituation. All animals were surgically implanted with bilateral cannulas in the dorsal hippocampus aiming at the CA1 area. The memory was evaluated in the NOR task, which involved a habituation phase, a training session with two unfamiliar objects, and test sessions on days 1, 7, and 14 after training to assess memory consolidation and persistence. In the tests, we measured the exploration of novel and familiar objects. Certain animals engaged in a moderate-intensity treadmill exercise session 11 hours after NOR task training; others did not and were considered as control. This time window was chosen to respect the 11-12 hours post-acquisition period, when the memory trace is subjected to modulation. After exercise (or in an equivalent period for the control group – 11.5 hours post-acquisition) some animals received vehicle (VEH) in the dorsal hippocampus for control purposes. In contrast, other animals received, in the same brain region, anisomycin (ANI) or rapamycin (RAPA) to investigate protein synthesis requirements or timolol (TIM) or SCH23390 (SCH) to assess beta-adrenergic or D1/D5 dopaminergic receptors requirement, respectively.

After the testing, control behavioral tests were conducted to verify the locomotion, exploratory, and anxiety behavior (**Figure 1**).

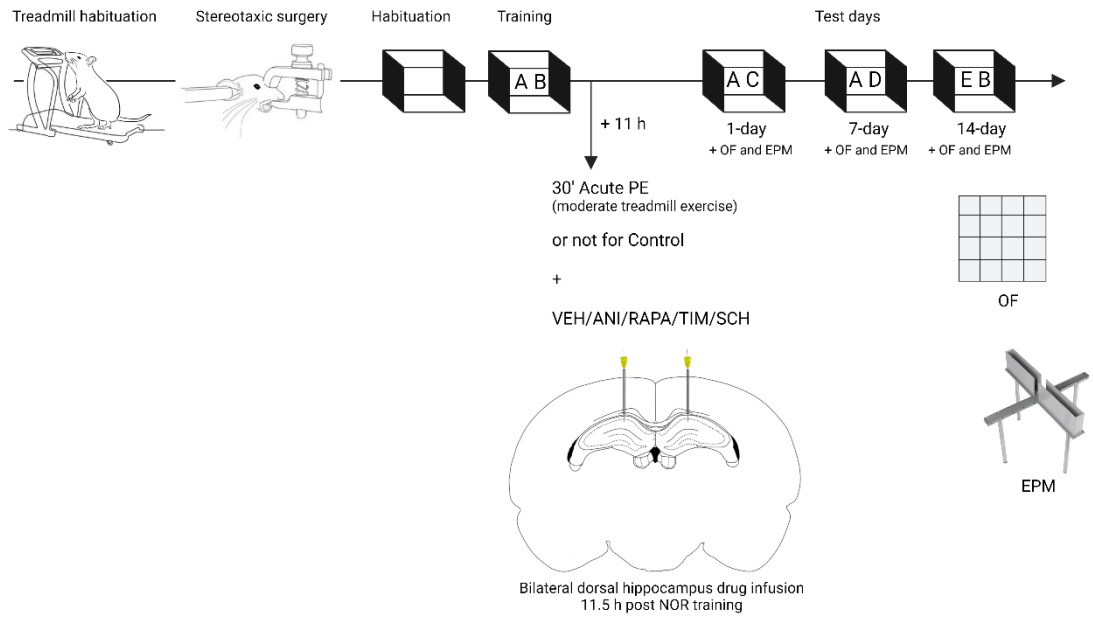

Figure 1 Experimental Design Overview. Animals in the acute PE groups underwent habituation on the treadmill one week before the memory task. All animals were implanted with bilateral cannulas in the dorsal hippocampus, considering CA1 area coordinates. Memory evaluations were performed using the NOR task, which included habituation to the apparatus for 20 minutes for 4 days, training with unfamiliar objects, and tests on days 1, 7, and 14 post-training in 5-minute sessions. Some animals underwent a moderate treadmill exercise session 11 hours after NOR task training, while others did not (control groups). After the acute PE or an equivalent time for the control groups, animals received vehicle (VEH) or specific drug infusions (anisomycin, ANI; rapamycin, RAPA; timolol, TIM; SCH23390, SCH) in the dorsal hippocampus to investigate protein synthesis, beta-adrenergic or D1/D5 dopamine receptors activation requirements. Control behavioral tests were conducted after each memory test to assess locomotion and exploratory behavior (in the open field, OF) and anxiety behavior (in the elevated plus maze, EPM).

2.3 Drugs

The drugs, ANI (A9789, anisomycin), a ribosomal translation inhibitor; RAPA (R0395, rapamycin), an mTOR-mediated protein synthesis inhibitor; TIM (T6394,

timolol), a beta-adrenergic antagonist; and SCH (D054, SCH23390), a D1/D5 dopamine antagonist, were purchased from Sigma-Aldrich. These drugs were dissolved in DMSO, stored at -20ºC, and protected from light until use. Before infusion, an aliquot was thawed and diluted to a working concentration in 0.9% saline. The drug concentrations used were as follows: ANI at 80 µg/µL, RAPA at 5 pg/µL, and TIM and SCH at 1 µg/µL, based on previous studies (Myskiw et al., 2015; Vargas et al., 2017; Vargas et al., 2019; Lima et al., 2021).

2.4 Surgery and drug infusion

The animals were anesthetized using intraperitoneal (i.p.) injections of a ketamine (75 mg/kg) and xylazine (10 mg/kg) mixture. During the stereotaxic surgery, performed by highly experienced trained researchers, their skulls were exposed and carefully leveled to create a flat surface, aligning the lambda and bregma at the same level. Subsequently, 22-gauge bilateral guide cannulas were implanted into the dorsal hippocampus at the following CA1 region coordinates (anterior, −4.2 mm; lateral, ±3.0 mm; ventral, −2.0 mm). The cannulas were securely affixed to the skull using dental cement. Following the surgery, a four-day recovery period was provided for the animals before proceeding with subsequent procedures.

The drug infusion occurred 11.5 hours minutes after NOR training and followed the moderate treadmill exercise session in certain groups. For drug delivery, we utilized a Hamilton syringe connected to fine bore tubing (38-gauge, 15 cm) with a needle (30-gauge, 10 mm) at the end. When administering the drug, the infusion needle was inserted into the guide cannula and advanced 1.0 mm deeper to reach the target region. Infusions (1 µL/side) were carried out for 60 seconds, and the infusion needle was left in place for an additional 60 seconds to minimize backflow.

2.5 Moderate treadmill exercise session

We used a motorized treadmill designed for rodents (Insight Ltd., Brazil). Animals from the acute PE groups were habituated to the treadmill for one week to minimize stress or novelty effects. The habituation process comprised 10-minute daily sessions, starting with off-treadmill placement on day one, followed by running

at 2-5 m/min on the subsequent two days, and gradually increasing to 8 m/min on days four to six.

One day after the last habituation session, an indirect assessment of the oxygen uptake (VO_2) peak was carried out according to previous studies (SCOPEL et al., 2006; MELLO et al., 2008; CECHETTI et al., 2012). The test involved starting at a low velocity of 1 m/min and increasing by 5 m/min every 3 minutes until the animal's exhaustion. Exhaustion was identified as the point at which the animal could no longer continue running, resulting in its inability to keep pace with the treadmill and eventually leaving its body behind. The time to fatigue (in min) and workload (in m/min) were taken as indexes of capacity for exercise, which was taken as indirect VO_2 maximum. A single evaluator assessed this procedure to prevent potential biases or variations in the evaluation process.

For the acute PE, the animals ran for 30 minutes at an intensity of exercise corresponding to moderate exercise, equivalent to 60% of the indirect VO_2 maximum. This intensity exercise classification follows the American College of Sports Medicine guidelines (MLA, 2000) and studies using rats as experimental models (VARGAS et al., 2017, 2020; LIMA et al., 2021b; DE CARVALHO et al., 2022).

2.6 Novel object recognition (NOR)

The NOR task is based on the innate tendency of animals to explore new objects in a familiar environment (ENNACEUR; DELACOUR, 1988). The task was conducted in a wooden box measuring 50 x 50 x 50 cm. Various objects were used in the experiments, represented by the following letters: A - magic cube, B - plastic cup, C - circular can, D - Lego pieces, and E - metallic cylinder. Rats did not exhibit any preference for a specific object in a pilot study.

Before training, the animals underwent a 20-minute daily habituation to the empty box for four days. During the training phase, the animals were allowed to explore the box containing two unfamiliar objects (A and B) for 5 minutes. The training was always performed between 8:00 - 10:30 AM. Test sessions were conducted on days 1 (A and C), 7 (A and D), and 14 (E and B) after training, with each session comprising one familiar and one unknown object. The objects were consistently placed in the same positions during all the test sessions.

To measure the exploration time, a video camera placed above the arena recorded the animals' behavior in the empty experimentation room, and blinded experimenters used a manual stopwatch to quantify the time spent exploring. Exploration was defined as the animals touching or sniffing the object with their front paws and nose, respectively. Sitting on or turning around the objects was not considered exploratory behavior. To avoid olfactory preferences, the objects and the apparatus were cleaned with 70% alcohol.

2.7 Behavioral control

After the NOR memory test sessions, behavioral control tests were conducted to ensure that experimental procedures or environmental conditions did not cause behavioral changes that could affect the memory assessments.

The open field (OF) test was used to assess animals' locomotor and exploratory activities (adapted from Hall and Ballachey, 1932). The apparatus was a 50 × 50 × 50 cm wooden box divided into 12 quadrants by black lines. The animals were observed for 5 min, and the number of crossings and rearings were recorded as measures of their activities.

Anxiety behavior was measured using the elevated plus maze (EPM) task (adapted from Pellow et al., 1985). The apparatus consisted of a cross-platform with 40 cm arms, two of them having high walls (closed arms) and two without walls (open arms), and it was 1 meter high. The animals were observed for 5 min, and the time spent and the number of entries in the open arms were counted as indicators of non-anxious behavior.

The behavior of the rats was recorded using a video camera positioned above the apparatus in an empty experimental room. A blinded experimenter rated the animals' behavior, and a manual stopwatch was used to record the time spent on specific activities.

2.8 Histology

Considering all the protocols and experiments performed, the rats’ euthanasia occurred approximately 25 days after surgery, so some animals lost the cannulas

during this time. However, after all the experimental procedures, animals with intact cannulas (approximately 10%) underwent a verification process to confirm the accurate placement of the cannulas. Animals were anesthetized with an i.p. injection of ketamine (75 mg/kg) and xylazine (10 mg/kg). We infused a 4% methylene blue solution (1 µL/side) into the dorsal hippocampus. After infusion, rats were perfused with 0.9% saline solution (200 mL), followed by 10% formaldehyde (200 mL). The brains were then extracted and fixed in 10% formaldehyde (30 mL) for four hours and cryopreserved in a 30% sucrose solution (30 mL) for 72 hours at 4°C. Subsequently, the brains were dried and stored at -80°C until analysis. To examine the cannula's placement and potential drug diffusion, coronal brain sections with a thickness of 40 µm were sliced using a Cryostat (LEICA CM3050S) and analyzed with an optical microscope (Olympus CX21). The area where the methylene blue solution reached was used to indicate the potential extent of drug diffusion.

2.9 Data preparation and Statistical analysis

We assessed the normality of distribution in the data using the Shapiro–Wilk test. For the comparison of the indirect VO_2 maximum, we employed the Kruskal-Wallis test.

The NOR results are presented as the percentage of total time spent exploring the novel object. During the training session, two objects were novel, and we selected one of them for representation in the graph. To evaluate the results in a dichotomic way (remember/no remember), we analyzed this data using a one-sample Student's t-test, assuming a 50% theoretical mean. An animal's ability to spend over 50% of the total time exploring a novel object was considered memory retention.

To compare data between groups, we converted the NOR results into a discrimination index (DI = [(t novel - t familiar)/(t novel + t familiar) x 100]), where 't' represents the time spent exploring the objects. We analyzed the DI using two-way ANOVA, followed by Sidak’s post hoc test. A higher DI indicates a greater capacity to discriminate the objects and explore the novel object for a longer time.

For object total exploration time, OF, and EPM results, we conducted a two-way ANOVA to groups’ comparison.

All data are presented as mean ± standard deviation (SD), and we considered differences significant when $\alpha < 0.05$.

3 Results

3.1 The indirect VO_2 maximum was similar between groups, and dorsal hippocampus cannula placement was successfully verified

Animals from the acute PE groups underwent treadmill habituation, followed by an indirect VO_2 test to evaluate the highest velocity they could run. Subsequently, the animals were randomly distributed into groups according to the drugs received in the dorsal hippocampus. The animals' average maximum speed run in the test was 21 ± 4 m/min, and no significant differences were observed in the group comparison ($W_{(5,\ 47)}$ = 5.116, p = 0.2756; **Figure 2A**). Additionally, histological assessment confirmed accurate cannula placement and effective drug delivery into the dorsal hippocampus (**Figure 2B**).

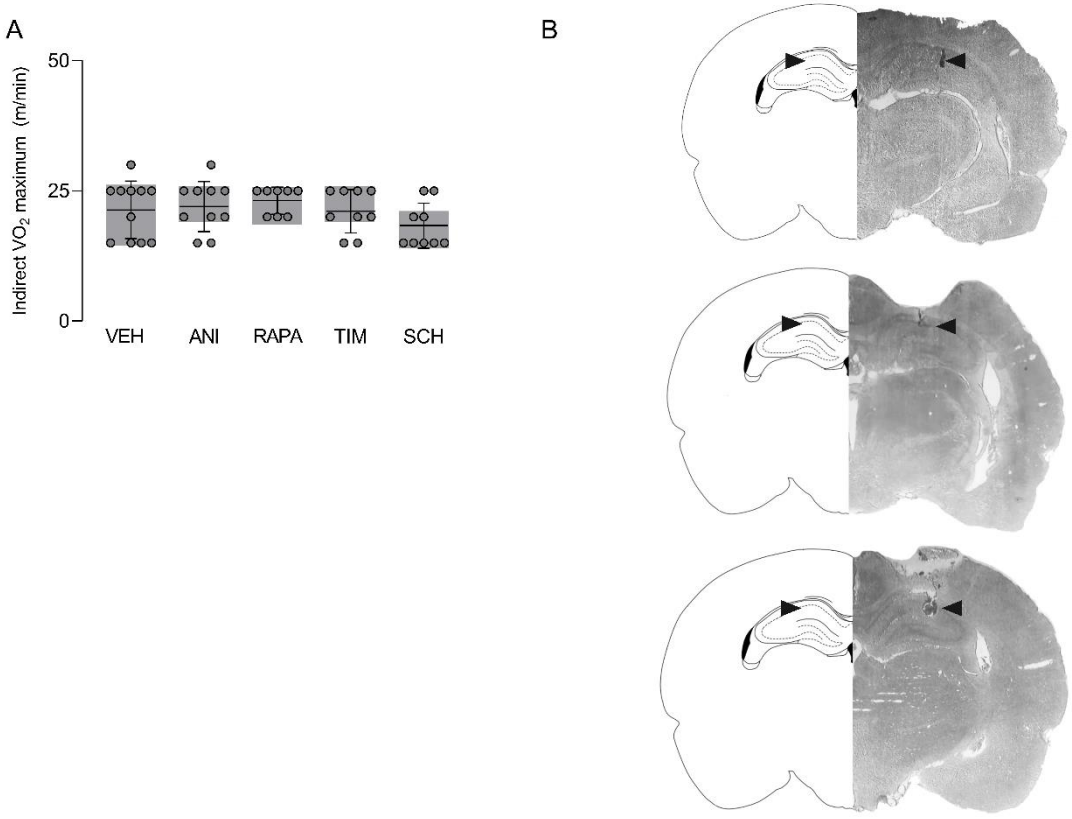

Figure 2 The indirect VO_2 maximum demonstrated consistency across the acute PE groups, and the successful verification of cannula placement was confirmed. A) Animals from the acute PE groups underwent an indirect VO_2 maximum test, and this parameter did not differ between groups. p > 0.05 in the

Kruskal-Wallis test. N = 8 – 11 per group. B) Samples of coronal sections of three rats with the infused dye solution that effectively reached the dorsal hippocampus, as indicated by the arrows.

3.2 Memory formation remains robust despite dorsal hippocampus modulation by drugs or by a moderate treadmill exercise session applied in the late consolidation phase

In the NOR protocol, animals were initially habituated to the apparatus. During the late consolidation period, 11 hours after NOR training, animals underwent a 30-minute moderate treadmill exercise session, while the control group did not perform any PE. Both groups received VEH for pharmacological control. Some animals received ANI or RAPA to evaluate protein synthesis requirements, while others received TIM or SCH to investigate beta-adrenergic and D1/D5 dopamine receptor activation requirements. Initially, we assessed memory consolidation conducted 1-day after the training session (**Figure 3A**).

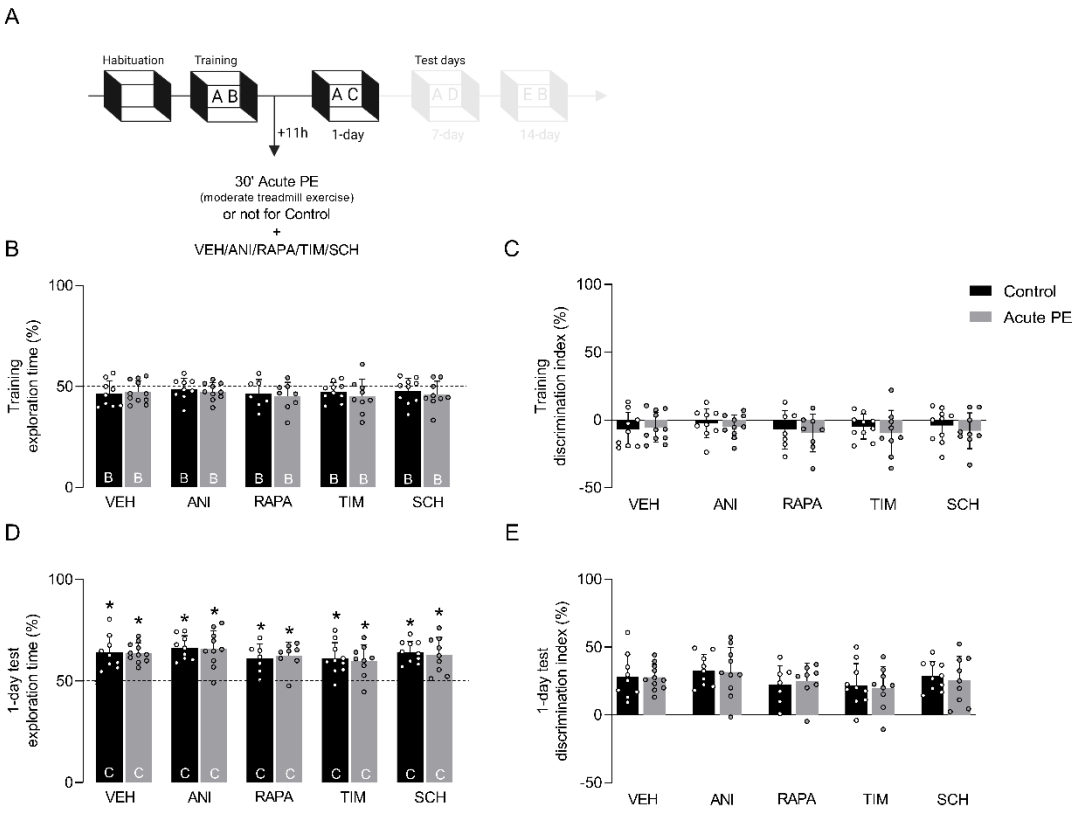

Figure 3 Memory consolidation remains unaffected by protein synthesis blockade or catecholamine receptor inhibition in the dorsal hippocampus during the late consolidation memory window. A) The figure emphasizes the initial protocol involving novel object recognition (NOR) habituation, training with the exploration of two novel objects, and the memory test conducted 1-day after training, which assessed long-term memory consolidation. Memory modulation occurred between 11- and 11.5-hours post-training, with moderate treadmill exercise session and/or drug infusion (vehicle, VEH; anisomycin, ANI; rapamycin, RAPA; timolol, TIM; or SCH23390, SCH) in the dorsal hippocampus. B-E) Memory evaluation presented as the percentage of total exploration time (B and D; * $p < 0.05$ in one sample t-test compared to a theoretical mean of 50%) and groups' comparison using the novel object's discrimination index (DI; C and E; two-way ANOVA followed by Sidak's post hoc; $p > 0.05$). N = 7 – 11 per group.

During the training phase, the animals were exposed to two different and unfamiliar objects, labeled as A and B. There was no preference between the objects, as the animals explored both for approximately equal amounts of time ($p > 0.05$; **Figure 3B**). Additionally, no significant interaction ($F_{(4,82)} = 0.1972$; $p = 0.9392$; **Figure 3C**) or effect on the different drugs infused or PE were observed ($F_{(4,82)} = 0.3691$; $p = 0.83$; $F_{(1,82)} = 0.8184$; $p = 0.3683$; **Figure 3C**).

To assess memory consolidation, we examined rats' behavior 1-day after training, during a 5-minute test session in which a familiar object (A) was placed with a novel object (C). Interestingly, all animals demonstrated memory consolidation, spending more than 50% of the total time exploring the novel object ($p > 0.05$; **Figure 3D**). In the DI analysis, no significant interaction ($F_{(4,82)} = 0.0918$; $p = 0.9848$; **Figure 3E**) or effect on the variables drug-infused or PE was observed ($F_{(4,82)} = 1.655$; $p = 0.1684$; $F_{(1,82)} = 0.0798$; p = 0.0.7783; **Figure 3E**).

3.3 Moderate treadmill exercise session performed in the late consolidation phase improves memory persistence, an effect that depends on protein synthesis and catecholaminergic mechanisms in the dorsal hippocampus

Given the evidence that interventions during the late memory consolidation phase (11-12h after learning) can directly affect memory persistence (BEKINSCHTEIN et al., 2007, 2008), we conducted memory tests on the animals over a specific period. Therefore, after assessing memory consolidation, we evaluated memory persistence by subjecting the animals to retesting at 7 and 14 days after NOR training (**Figure 4A**).

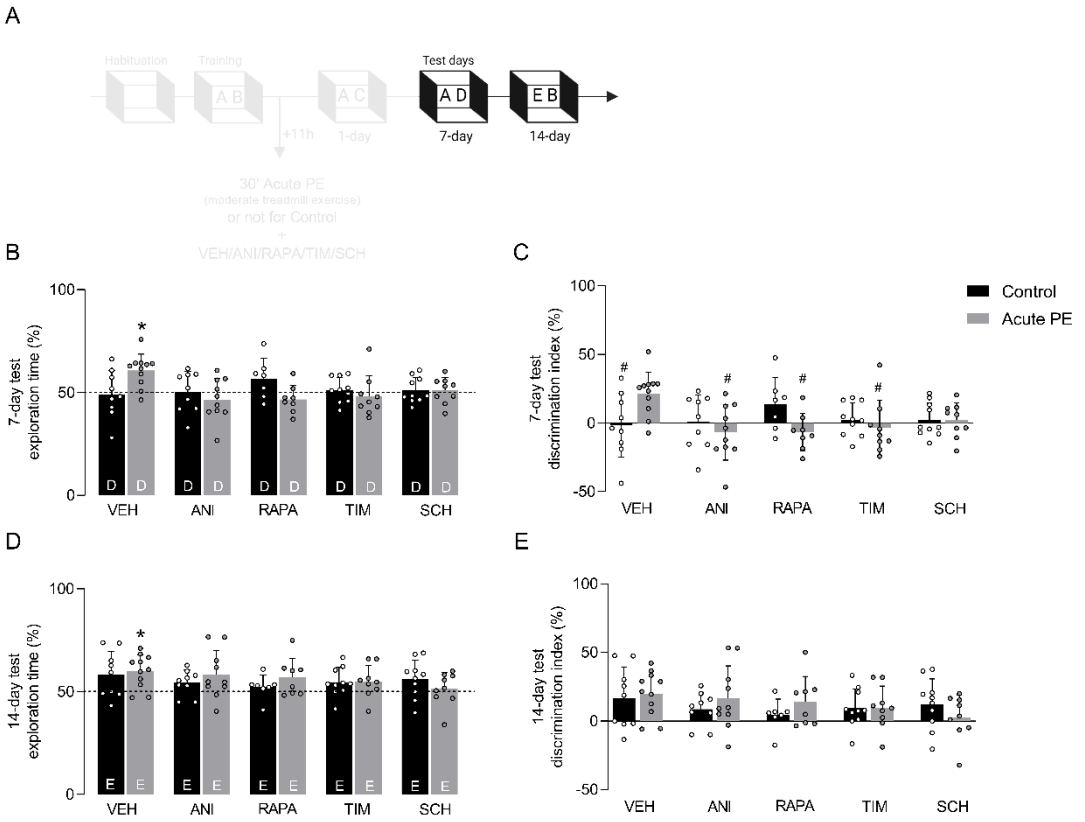

Figure 4 Moderate treadmill exercise in the late consolidation phase improves memory persistence and depends on protein synthesis and catecholaminergic receptors activation in the dorsal hippocampus. A) The figure focuses on memory tests conducted 7 and 14 days following novel object recognition (NOR) training to evaluate memory persistence. The earlier protocol, which involved NOR habituation, training with the exploration of two novel objects, and the memory test conducted one day after training to assess memory consolidation, is implied in the image. This was followed by memory modulation between 11- and 11.5 hours post-training, involving moderate treadmill exercise practice and/or drug infusion (vehicle, VEH; anisomycin, ANI; rapamycin, RAPA; timolol, TIM; or SCH23390, SCH) in the dorsal hippocampus. B-E) Memory evaluation presented as the percentage of total

exploration time (B and D; * $p < 0.05$ in one sample t-test compared to a theoretical mean of 50%) and groups' comparison using the novel object's discrimination index (DI; C and E; $^{\#}$ $p < 0.05$ vs. acute PE + VEH in two-way ANOVA followed by Sidak's post hoc). N = 7 – 11 per group.

In the 7-day post-training test, only animals that engaged in moderate treadmill exercise demonstrated memory persistence ($p = 0.0011$, acute PE + VEH; **Figure 4B**), as they explored the novel object (D) for a longer time, differing from a theoretical mean of 50%. On the other hand, the other groups explored both the novel and familiar objects for a time equivalent to 50% ($p > 0.05$; **Figure 4B**). Our findings were confirmed by observing an interaction effect ($F_{(4,\ 82)} = 3.826$, $p = 0.0067$; **Figure 4C**). Specifically, the animals submitted to moderate treadmill exercise showed a significantly higher DI than the control group when the animals received VEH in the dorsal hippocampus ($p = 0.0195$; **Figure 4C**). Furthermore, the animals that engaged in an exercise session and received ANI ($p = 0.0033$; **Figure 4C**), RAPA ($p = 0.0076$; **Figure 4C**), or TIM ($p = 0.0177$; **Figure 4C**) presented a lower DI than animals that received VEH in the dorsal hippocampus after running on the treadmill.

In the 14-day test, similar results were observed in the one-sample t-test, and animals that practiced moderate treadmill exercise in the late consolidation window explored the novel object (E) for a longer time while presenting recognition of the familiar one (B) ($p = 0.0023$, acute PE + VEH; **Figure 4D**). Conversely, the other groups exhibited no memory persistence, as they explored both objects for approximately 50% of the time ($p > 0.05$; **Figure 4D**). However, in the DI analysis, we did not find an interaction ($F_{(4,82)} = 0.8629$; $p = 0.4899$; **Figure 4E**) or effects for drug-infusion or PE practice ($F_{(4,\ 82)} = 1.168$; $p = 0.3311$; $F_{(1,82)} = 0.3981$, $p = 0.5298$; **Figure 4E**).

3.4 Behavioral control tasks results

In addition to the memory tests, we investigated several behavioral factors that could potentially impact the outcomes of the NOR task and memory evaluation. However, all animals displayed similar levels of total time object exploration,

locomotor, and exploratory activity and exhibited similar performance in anxiety-like behavior assessment (**Table S1** in the Supplementary Material).

4 Discussion

In this study, we examined the impact of a moderate treadmill exercise session on the late consolidation window of NOR memory in rats. A 30-minute session of acute PE performed 11 hours after the memory acquisition improved NOR memory and led to extended novel object exploration time during the 7 and 14-day tests. Our observations revealed that blocking protein synthesis by ribosomal translation or mTOR synthesis inhibition into the dorsal hippocampus hindered the effect of the acute PE on memory persistence evaluated at 7 and 14 days, while it had no impact on memory consolidation, tested 1 day after acquisition. Similarly, assessing the requirement of beta-adrenergic and D1/D5 dopaminergic receptors yielded comparable results.

We assessed memory data through two analyses: the dichotomous approach (learn or not learn) and the novel object DI, allowing group comparison. These methods are widely used in NOR task memory assessment, offering additional evidence for data interpretation (DENNINGER; SMITH; KIRBY, 2018). Here, a moderate treadmill exercise session led to over 50% exploration time dedicated to the novel object in the 7 and 14-day tests, indicating memory persistence from the familiar object. However, only in 7-day test animals present higher DI than others. It may imply a temporal evolution in memory expression, with the acute effect of moderate exercise on object discrimination being more pronounced in the short term (7 days) than in the longer term (14 days). In a recent study applying acute PE immediately after acquisition, the DI for the novel object was higher than in other groups in the 14-day test, hinting at potentially greater memory modulation in the early window than in the late one. However, this remains an initial speculation, and additional tests are necessary for a conclusive assessment.

As part of our control group, a subset of animals received drug infusions into the dorsal hippocampus at the 11.5-hour mark after acquisition, without undergoing a moderate treadmill exercise session. In these non-exercised animals, infusions of VEH, ANI, RAPA, TIM, or SCH did not disrupt memory consolidation when assessed 1 day later. However, 7 and 14 days later evaluations revealed no significant memory persistence. Consistent with these findings, our NOR protocol primarily supports memory consolidation, but not its persistence, resulting in natural memory fading

over time (NEVES et al., 2020; LIMA et al., 2021b). This study then offers evidence that the infused drugs during the late consolidation phase did not alter the consolidation of NOR memory. Similar evidence has been revealed in other memory types (ROSSATO et al., 2009; TOMAIUOLO et al., 2015a; KATCHE et al., 2016). The most plausible explanation is that during this timeframe, the memory has already undergone consolidation, enabling recall within 24 hours. However, the modulation influences its persistence, suggesting the presence of biochemical cascades that occur cyclically over time, extending the retention of information (KATCHE et al., 2010; KATCHE; CAMMAROTA; MEDINA, 2013).

Most of these previous studies have primarily focused on investigating late consolidation using tasks such as inhibitory avoidance (IA) (BEKINSCHTEIN et al., 2007, 2008; ROSSATO et al., 2009; PARFITT et al., 2012). Although this task constitutes a different memory type from recognition memory, they are reliant on hippocampal processes (ROSSATO et al., 2007) and appear to involve similar late-stage mechanisms. Using a protocol in which IA training was robust enough to induce memory persistence, the intra-CA1 infusion of ANI (BEKINSCHTEIN et al., 2007, 2008), SCH (ROSSATO et al., 2009), or propranolol via i.p. (PARFITT et al., 2012) 12 hours after the acquisition did not affect memory retention when assessed 2 days later. However, it did hinder persistence when tested 7 days later. These findings support that the late phase of protein synthesis and catecholamine receptor activity in the rat hippocampus is crucial for maintaining LTM persistence but not for its initial formation. Similarly, the inhibition of endogenous BDNF activity yielded comparable effects, indicating that BDNF is essentially required for memory maintenance (BEKINSCHTEIN et al., 2007, 2008).

A few strategies are proposed to enhance memory persistence when training falls short. In this context, exposing rats to a novel environment 11 hours after weak IA training emerges as a promising approach, enhancing persistence tested thirteen days after (TOMAIUOLO et al., 2015a). The authors suggest that a consolidated but nonpersistent memory exhibits a delayed and transient late time window after learning, during which it becomes feasible to utilize PRPs obtained from a distinct experience to confer persistence upon it (TOMAIUOLO et al., 2015a). In addition to novelty, interventions that induce stress 12 hours after contextual fear conditioning selectively extend the persistence of this LTM (PARFITT et al., 2012; YANG et al.,

2013). Here, for the first time, we demonstrate that a 30-minute moderate treadmill exercise session conducted 11 hours after acquisition leads to the persistence of NOR memory.

The mechanisms underlying acute PE effects share similarities with those previously demonstrated as potent to positively modulate memory during late consolidation. Both acute PE and novelty lead to elevated levels of noradrenaline and dopamine, necessitating CA1 receptor activation to facilitate memory persistence (MENEZES et al., 2015; VARGAS et al., 2017, 2020; LIMA et al., 2021b). Moreover, investigations utilizing compounds like SKF 38393, a D1/D5 receptor agonist administered into the dorsal CA1 region 12 hours post-acquisition, have exhibited augmented memory persistence (ROSSATO et al., 2009). These discoveries offer insightful implications, indicating that strategies intrinsically cultivating these mechanisms, such as novelty, as elucidated in prior studies, or acute PE by moderate intensity running, as demonstrated in our research, hold the potential to yield effects comparable to, if not surpassing, those achieved through external interventions.

Recognizing stress as a potential factor influencing late consolidation (PARFITT et al., 2012; YANG et al., 2013) and considering that treadmill exercise conditions may elevate cortisol or other glucocorticoid levels, exploring post-exercise hormone levels becomes intriguing, and this is a limitation of our study. However, existing research indicates that corticosterone levels rise significantly only with high intensity running at 25 m/min, not at 15 m/min intensity (SOYA et al., 2007). In our study, the animals maintained an average running speed of approximately 12.6 m/min during the acute PE session. Moreover, the animals underwent multiple treadmill habituation sessions to mitigate potential stress during the exercise session, familiarizing them with the apparatus and the running task. Hence, we posit that the memory modulation induced by exercise is not directly linked to stress.

Acute PE in treadmill tends to increase BDNF levels in the hippocampus (SOYA et al., 2007; VENEZIA et al., 2019), a critical protein involved in the later post-acquisition stages, contributing to the induction of LTM storage persistence (BEKINSCHTEIN et al., 2008; ROSSATO et al., 2009). This supports the concept proposed that acute PE triggers PRPs that strengthen the synaptic connections formed during parallel learning, thereby enhancing memory (LOPRINZI; PONCE;

FRITH, 2018). In line with this, we have extensively emphasized the early effects of this exercise type on memory persistence modulation (VARGAS et al., 2017, 2020; LIMA et al., 2021b). In this study, our findings further extend the potential of a moderate treadmill exercise session, supporting the notion that learning, which consolidates a non-persistent memory, establishes a delayed tag well after training that captures PRPs like BDNF induced by acute PE, thereby improving the memory trace. Our findings support this concept since inhibiting protein synthesis in the dorsal hippocampus with ANI or RAPA prevents the impact of exercise on memory. Notably, ribosomal translation and mTOR-mediated protein synthesis intrinsically relate to BDNF regulation on synaptic plasticity (AAKALU et al., 2001; TAKEI et al., 2004; INAMURA; NAWA; TAKEI, 2005).

Still, some studies have highlighted the role of mTOR in the pathways and processes required for noradrenergic or dopaminergic memory consolidation and persistence modulation. For example, the norepinephrine-mediated enhancement of Long-Term Potential (LTP), an electrophysiological phenomenon that many authors consider as the basis for long-term memory consolidation (IZQUIERDO et al., 2008) was reduced by inhibition of mTOR (MAITY et al., 2020). Furthermore, a previous study demonstrated that the activation of cortical dopaminergic receptors D1/D5 mediated by mTOR and dependent on protein synthesis facilitates memory consolidation mechanisms in an auditory memory task (REICHENBACH et al., 2015), and repeated systemic pharmacological stimulation of D1 receptors is sufficient to induce generalized seizures leading to the overactivation of hippocampal mTOR signaling, disrupting hippocampal plasticity, and impairing long-term recognition memories (GANGAROSSA et al., 2014). These researches indicated the importance of mTOR in memory consolidation processes and the relationship between its function and the memory-modulatory role of dopamine or noradrenaline.

While our study provides valuable insights into the impact of moderate treadmill exercise on memory, it is essential to acknowledge certain limitations. One limitation lies in our reliance on indirect assessment of VO_2 rather than direct measurements, which may introduce some variability in estimating oxygen consumption. Additionally, the absence of a direct evaluation of stress levels, such as cortisol measurements, limits our comprehensive understanding of the physiological responses to exercise and potential stress modulation effects on memory. These

limitations highlight areas for improvement and future exploration, emphasizing the need for more comprehensive assessments and diversified approaches to unravel the intricacies of the relationship between exercise, stress, and memory.

In summary, our findings further reinforce the idea that the hippocampus remains dynamically engaged in memory processing even after establishing LTM. Subsequent rounds of consolidation-like events occur well beyond the initial learning phase, with a delayed surge in mRNA and protein synthesis (BEKINSCHTEIN et al., 2007; KATCHE et al., 2010), involving noradrenaline (PARFITT et al., 2012) and dopamine (ROSSATO et al., 2009) in the dorsal hippocampus, being critical for maintaining, rather than forming, persistent memory. Here, we demonstrate the efficacy of a moderate treadmill exercise session during the later memory consolidation phase, approximately 11 hours post-training, in modulating and promoting enduring memory persistence in male rats through similar underlying mechanisms.

Based on these findings, new insights emerge regarding potential applications of acute PE, offering valuable guidance for future studies aimed at applications in humans. Previous research investigating novelty exposition in different time windows as memory modulatory in a naturalistic school scenario shows that when it was faced around 10 h after learning, the novel experience improved the memory persistence tested 7 days later (RAMIREZ BUTAVAND et al., 2020). Our discovery opens exciting possibilities for its application of acute PE in educational settings, where students could engage in a structured exercise routine during specific post-learning intervals to optimize memory consolidation. Additionally, in clinical contexts, such as rehabilitation programs or interventions for individuals with memory-related challenges, incorporating targeted exercise sessions might promote enduring memory persistence. The implications of this research extend beyond the laboratory, offering tangible avenues for harnessing the benefits of exercise for cognitive enhancement.

5 Conclusion

Here, we demonstrated that a 30-minute moderate-intensity treadmill session performed 11 hours after NOR memory acquisition improves NOR memory for 14 days. Furthermore, our investigation into the mechanisms behind this enhancement highlighted the involvement of protein synthesis, beta-adrenergic, and D1/D5 dopamine receptors in the dorsal hippocampus, providing valuable insights into underlying processes contributing to memory modulation.

Declaration of interest

The authors declare no conflict of interest. We certify that the submission is original work and is not under review at any other publication.

Author's contribution

K.R.L. and P.B.M.C. conceived and designed the experiments. K.R.L., A.C.S.R., G.C.M.G., G.J.S., and A.C.P.V.S. performed the experiments. K.R.L. and P.B.M.C. analyzed and interpreted the data, wrote, and reviewed the manuscript. All authors, K.R.L., A.C.S.R., G.C.M.G., G.J.S., A.C.P.V.S., and P.B.M.C. approved the final version of the manuscript.

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Supplementary material

Table S1 No differences were found among the experimental groups on behavioral control tasks. Total object exploration time in novel object recognition (NOR) task (in seconds), locomotor (by crossings number) and exploratory (by rearing number) activities, and anxiety behavior (by entries and time spent in the open arms in seconds) were similar between groups. Data are presented as mean ± SD. $\alpha \geq 0.05$ in two-way ANOVA.

	Control					Acute PE					Two-way ANOVA		
	VEH	ANI	RAPA	TIM	SCH	VEH	ANI	RAPA	TIM	SCH	Interaction	Intervention	Drug
Total number of rats (n)	9	9	7	10	10	11	10	8	9	9			
Total object exploration time (s)													
Training	80.3 ± 14.7	92.7 ± 16.1	85.4 ± 13	98.1 ± 14.1	94.1 ± 20.3	94.3 ± 15.9	90.2 ± 20.1	84.4 ± 18	76.4 ± 18.2	94.8 ± 16.7	0.05	0.56	0.48
1-d test	76.8 ± 23.7	78.3 ± 20.3	77.1 ± 19.2	91.4 ± 32.8	94.7 ± 23.5	92.5 ± 21.8	82.8 ± 20.5	73.9 ± 29.5	64 ± 30.3	76.9 ± 19.3	0.06	0.28	0.69
7-d test	79 ± 24.2	83.6 ± 17.5	84.1 ± 18.6	97.9 ± 26.8	91.2 ± 15.4	88.4 ± 22.2	78.6 ± 21.8	91.1 ± 22.2	83.3 ± 34.8	85.2 ± 18.4	0.49	0.71	0.72
14-d test	59.8 ± 11.8	76 ± 16.1	60.9 ± 14.8	67.7 ± 27.7	71.4 ± 24.9	71.9 ± 18.4	60 ± 17.4	82.6 ± 21.5	65.4 ± 31.9	84.4 ± 21.1	0.08	0.21	0.41
Crossings (n)													
1-d test	65.4 ± 17.1	71.5 ± 10.9	69.1 ± 27.9	65.8 ± 21.6	73.6 ± 19.7	75.1 ± 15.2	62.5 ± 17.2	54.7 ± 15.8	59.8 ± 14.9	59.7 ± 16.3	0.26	0.08	0.65
7-d test	99.9 ± 27	86.9 ± 28.7	108.3 ± 26.8	88.5 ± 28.2	102.4 ± 26	97.4 ± 18.1	86.1 ± 18.4	74.9 ± 22.4	90.5 ± 29.3	93.1 ± 9	0.23	0.09	0.46
14-d test	76.9 ± 27.4	84.2 ± 20.9	87.1 ± 29.4	81.3 ± 27.7	98.5 ± 26.4	92.5 ± 23.3	74.7 ± 31.9	82.5 ± 21.9	71.9 ± 26.3	83.7 ± 13.8	0.38	0.4	0.46
Rearings (n)													
1-d test	22.5 ± 7.6	20.1 ± 5.7	16.6 ± 8.6	19.3 ± 7.2	20.7 ± 7.2	20.1 ± 5	16.9 ± 7.9	15.2 ± 8.7	19.4 ± 9.9	17.5 ± 10.5	0.96	0.23	0.4
7-d test	31 ± 12.9	27.8 ± 7.5	35.3 ± 10.4	31.5 ± 6.1	33.6 ± 7.2	34.2 ± 8.4	32.6 ± 7.3	26.6 ± 7.2	32 ± 10.2	32.9 ± 7.5	0.21	0.92	0.82
14-d test	25.5 ± 12.3	30.8 ± 7.6	30.1 ± 8.4	27.7 ± 9.6	32 ± 8.3	34.2 ± 9.5	28.3 ± 12.5	28.2 ± 10.3	28.5 ± 7.4	31.9 ± 9.6	0.41	0.62	0.82
Entries in open arms (n)													
1-d test	7.7 ± 1.6	6.5 ± 1.5	7.4 ± 3.9	8.1 ± 1.6	7.9 ± 1.9	8 ± 2.1	7.8 ± 3.7	7.9 ± 1.8	7.6 ± 1.8	7.4 ± 2.7	0.8	0.67	0.91
7-d test	9.5 ± 1.3	9.5 ± 2.2	10 ± 3.4	10.2 ± 2.3	10.9 ± 1.4	10.9 ± 2	8.9 ± 3.5	9.9 ± 2.9	9.1 ± 3.5	10.1 ± 1.8	0.58	0.62	0.57
14-d test	10.6 ± 1.7	9.1 ± 4.4	11.1 ± 3.3	9.8 ± 2.5	11.5 ± 3.1	10.5 ± 3.5	9.4 ± 2.4	10.2 ± 1.9	7.8 ± 1.7	9.8 ± 2.4	0.65	0.15	0.13
Time in open arms (s)													
1-d test	153.4 ± 57.2	186 ± 30.6	157.3 ± 57.1	176.8 ± 24	188.7 ± 31	176.9 ± 27.4	173.6 ± 44.9	188.7 ± 38.8	146.2 ± 45.6	171.3 ± 46.4	0.14	0.9	0.56
7-d test	137.8 ± 30.9	154.8 ± 31.3	173.6 ± 27.8	162.8 ± 20.7	165.6 ± 26.4	175.9 ± 26.8	144.6 ± 53	158.5 ± 36.6	151.4 ± 40.4	179.4 ± 20	0.07	0.66	0.25
14-d test	135.9 ± 33.8	139 ± 58	147 ± 21.7	150.8 ± 26.2	163.7 ± 43.5	178.7 ± 21.9	159.8 ± 33.4	158.9 ± 25.8	131.9 ± 44.7	151.9 ± 28.2	0.06	0.23	0.6

PARTE III

DISCUSSÃO

Nesta tese, investigamos a influência das vias noradrenérgicas e dopaminérgicas, bem como a síntese proteica hipocampal, no efeito modulatório do EF agudo sobre a persistência da memória de RO em ratos machos. Nós já sabíamos que uma sessão de 30 minutos de EF em esteira após a aquisição – na janela de consolidação inicial – melhora a persistência da memória de roedores por pelo menos 14 dias a mais em comparação ao grupo controle, fenômeno que depende da ativação de receptores dopaminérgicos e beta-adrenérgicos do hipocampo dorsal (VARGAS et al., 2017, 2020; LIMA et al., 2021b). Através dos três estudos conduzidos nesta tese, contribuímos significativamente para a melhor compreensão dos mecanismos pelos quais o EF agudo impacta a memória. Em resumo, destacamos a importância do LC, uma região fundamental na inervação dopaminérgica e noradrenérgica do hipocampo, para os efeitos do EF agudo na memória, ao passo que tal relevância não foi observada para a ATV. Além disso, de forma intrigante, demonstramos que os efeitos do EF agudo superam a inibição da síntese proteica hipocampal, prevenindo a amnésia induzida por fármacos que inibem o processo de translação. Ainda, evidenciamos que o EF agudo não aumenta os níveis de BDNF nas primeiras horas (0,5h, 2h ou 12h) após a sua prática. Por último, evidenciamos que o EF agudo não apenas modula a consolidação inicial, mas também influencia a janela de consolidação tardia, que ocorre 11 após a aquisição.

Os efeitos do EF agudo foram avaliados na memória de RO, uma forma declarativa de memória dependente de um sistema de estruturas anatomicamente ligadas ao lobo temporal, com destaque para o papel significativo do hipocampo em seu processo de consolidação (FURINI et al., 2020). Esta memória reflete a habilidade de avaliar um item recentemente encontrado como familiar, sendo bem documentada tanto em animais de laboratório quanto em seres humanos (MANNS et al., 2003). É crucial ressaltar que a memória de reconhecimento é composta por diversos componentes associativos, abordando o "o que", "onde" e "quando", proporcionando a capacidade de discernir se um estímulo é familiar, novo e/ou se foi previamente associado a outros estímulos, contextos ou lugares (CHAO et al., 2022). Assim, a modulação dessa forma específica de memória é de relevância, uma vez que a persistência prolongada da memória de RO desempenha um papel crucial

em diversos aspectos da vida cotidiana e do aprendizado. Portanto, o propósito central desta tese foi investigar como o EF agudo pode influenciar a modulação da persistência memória de RO e identificar os mecanismos subjacentes a essa modulação. Para tanto, utilizamos um protocolo que, por si só, é eficaz na consolidação da memória em roedores, embora não estimule sua persistência ao longo do tempo.

No primeiro estudo, exploramos a contribuição da ATV e LC na promoção da persistência da memória de RO induzida pelo EF agudo. Evidenciamos que, embora ambas as regiões sejam fundamentais para a consolidação da memória de RO, apenas o LC desempenha um papel essencial nos efeitos induzidos pelo EF agudo. Nossos achados que revelam que a inibição da ATV ou LC prejudica a consolidação da memória estão de acordo com estudos prévios que indicam a relevância destas regiões para este processo (MELLO-CARPES et al., 2016; MONCADA, 2017). No entanto, quando a inibição destas regiões foi associada ao EF agudo prévio, somente a inibição do LC prejudicou a persistência da memória promovida pelo EF. Sabe-se que tanto a ATV quanto o LC desempenham papéis cruciais na regulação da síntese proteica necessária para o processo de marcação e captura sináptica, um processo crucial na estabilização de memórias de longa duração (MONCADA, 2017). Curiosamente, o LC parece influenciar a consolidação da memória de forma independente da ATV, embora a ativação coordenada de ambas as regiões atue de maneira complementar para esse fim (MONCADA, 2017). Isso pode esclarecer por que a inibição da ATV não compromete a consolidação ou persistência da memória quando a prática do EF agudo a antecede.

É amplamente documentado que a ATV apresenta exclusivamente eferências dopaminérgicas ao hipocampo, enquanto o LC exibe ambas, eferências dopaminérgicas e noradrenérgicas (YAMASAKI; TAKEUCHI, 2017). Essa diferenciação neuroquímica sugere a possibilidade de que a co-liberação destes neurotransmissores seja essencial para os efeitos do EF agudo, dado o envolvimento de receptores D1/D5 (VARGAS et al., 2020; LIMA et al., 2021b) e beta-adrenérgicos (VARGAS et al., 2017) na modulação da memória por esta prática. Assim, especula-se que a ativação do LC, sozinho ou juntamente com outras regiões associadas aos processos de *arousal*, possa superar a inibição específica da ATV. Contudo, permanece em aberto se a contribuição do LC para os efeitos do EF na

persistência da memória depende predominantemente de mecanismos dopaminérgicos, noradrenérgicos, ou de uma interação complexa entre ambos. Para esclarecer esse ponto, futuras pesquisas são imperativas, permitindo a observação direta da ativação dessas vias no hipocampo durante a prática do EF.

Nossas descobertas alinham-se a um estudo anterior que sublinha a importância da novidade na potencialização da retenção da memória episódica, especificamente através de mecanismos mediados pelo LC, mas não pela ATV (TAKEUCHI et al., 2016). Esse estudo revelou a notável sensibilidade do LC ao estímulo da novidade, evidenciando que os neurônios que expressam tirosina-hidroxilase (TH^+), enzima frequentemente encontrada em neurônios dopaminérgicos, estendem projeções mais abrangentes para o hipocampo a partir do LC em relação à ATV (TAKEUCHI et al., 2016). A ativação optogenética desses neurônios no LC reproduziu de maneira fiel o efeito de novidade, e o aprimoramento da memória associado a essa novidade não foi afetado pela inativação da ATV (TAKEUCHI et al., 2016). A novidade consiste na exposição a algo novo, como um ambiente ou um sabor, por exemplo, em uma janela temporal próxima da aquisição (MONCADA; VIOLA, 2007; BALLARINI et al., 2009). Embora o EF agudo e a novidade sejam intervenções distintas para a modulação da memória, ambos compartilham mecanismos semelhantes para a promoção da persistência da memória de RO, no qual inclui-se o requerimento de vias catecolaminérgicas que dependem da ação de dopamina e noradrenalina no hipocampo (MONCADA et al., 2011; LIMA et al., 2021b, 2021a). Essa convergência de resultados ressalta a importância do LC como uma via que independe da ATV para a modulação da memória.

Ao explorar as convergências nos mecanismos neurais associados à novidade e ao EF agudo, surge a indagação sobre a possibilidade de o EF agudo operar por meio da hipótese de marcação e captura sináptica, um mecanismo clássico que fundamenta os efeitos da novidade (MONCADA; VIOLA, 2007; MONCADA et al., 2011). Essa teoria, inicialmente comprovada *in vitro* (FREY; MORRIS, 1997), propõe que o aprendizado desencadeia a formação de uma "etiqueta sináptica", que temporariamente marca uma sinapse após sua ativação (MONCADA; VIOLA, 2007; MONCADA et al., 2011). Quando ocorre um estímulo robusto, como a novidade (ou, conforme nossa hipótese, o EF agudo), em uma janela temporal próxima a essa ativação sináptica, ele tem a capacidade de fornecer

PRPs (MONCADA; VIOLA, 2007; MONCADA et al., 2011). Essas proteínas são capturadas em sinapses específicas que foram previamente marcadas pela atividade sináptica, fortalecendo assim a trajetória do aprendizado de forma paralela (MONCADA; VIOLA, 2007; MONCADA et al., 2011). Neste sentido, sabe-se que a inibição de síntese proteica hipocampal prejudica os efeitos da novidade sobre a consolidação da memória (MONCADA; VIOLA, 2007; MENEZES et al., 2015). Dessa forma, indaga-se se o EF agudo é, de fato, uma ferramenta de plasticidade que requer síntese proteica no hipocampo para manifestar seus efeitos sobre a memória. Esta indagação central foi prontamente abordada pelo segundo estudo desta tese.

No segundo estudo, de maneira notável, demonstramos que o EF agudo, mesmo quando seguido pela inibição da síntese proteica hipocampal, seja de maneira indireta pela via mTOR ou diretamente pela via ribossomal, consegue prevenir a amnésia provocada pelos inibidores (observada quando os fármacos são aplicados na ausência do EF agudo). O efeito dos inibidores na ausência do EF agudo se alinha com estudos anteriores que sustentam a ideia de que a síntese proteica desempenha um papel essencial na consolidação da memória de RO (ROSSATO et al., 2007; FURINI et al., 2015). Isso corrobora com a ideia de que a síntese de novas proteínas e expressão gênica no hipocampo induzem modificações funcionais e estruturais em populações neuronais importantes para a manutenção da memória (KATCHE; CAMMAROTA; MEDINA, 2013). Entretanto, nosso estudo revela que o EF agudo transcende os efeitos da inibição da síntese proteica hipocampal, impedindo o efeito amnésico observado no teste de consolidação.

Em concordância com nossos achados, a literatura discute que a relação entre a síntese proteica e a consolidação da memória não é um fenômeno dicotômico de "tudo ou nada" (ROUTTENBERG; REKART, 2005). Portanto, a codificação e retenção da memória na presença de inibidores de síntese proteica podem ser facilitadas por meio de modificações nos procedimentos de treinamento, como o aumento da intensidade do estímulo elétrico em tarefas de condicionamento aversivo (DÍAZ-TRUJILLO et al., 2009; GONZÁLEZ-SALINAS et al., 2015; GONZÁLEZ-FRANCO et al., 2019), ou a introdução de um pré-treinamento na esquiva inibitória (QUEVEDO et al., 1999). Além disso, substâncias estimulantes do sistema nervoso, como cafeína, nicotina e corticosterona, têm sido documentadas como capazes de superar os efeitos dos inibidores de síntese proteica

(ROUTTENBERG; REKART, 2005). Essa diversidade de abordagens destaca a plasticidade e a adaptabilidade do sistema de consolidação da memória diante de diferentes influências.

Nossa busca por explicações sobre a superação dos efeitos inibitórios da síntese proteica sobre a consolidação da memória pelo EF agudo nos conduziu a considerar diversas hipóteses. Inicialmente, sugere-se que memórias mais fortes podem apresentar diferenças quantitativas ou qualitativas na síntese de proteínas, conferindo-lhes maior resistência a tratamentos amnésicos (ALBERINI, 2008). Assim, estratégias capazes de prevenir prejuízos de memória causados por inibidores da síntese proteica podem atuar aumentando a síntese da pequena proporção de proteínas não inibidas pelos bloqueadores (GOLD, 2006; ALBERINI, 2008). É possível, portanto, que o EF agudo aumente a síntese proteica de forma suficiente a superar o efeito farmacológico dos inibidores. Além disso, ressalta-se que inibidores de síntese proteica, como a anisomicina, não apenas desempenham tal função, mas também reduzem os níveis endógenos de noradrenalina e dopamina (ALBERINI, 2008), neurotransmissores essenciais para os processos de consolidação da memória de RO (FURINI et al., 2010, 2014). Dessa forma, estímulos que aumentam o *arousal*, caracterizado por maior estado de alerta e intimamente relacionado ao aprimoramento da memória, imitam a resposta natural do cérebro a estímulos excitantes, elevando consequentemente os níveis desses neurotransmissores (MCGAUGH, 2018). Nesse contexto, sabendo que o EF agudo promove o aumento desses neurotransmissores no hipocampo (VARGAS et al., 2017, 2020), sugere-se que esse acréscimo possa atuar atenuando os efeitos amnésicos associados à inibição da síntese proteica.

Com base nos resultados comportamentais, consideramos a hipótese de que o EF agudo possa estar influenciando a modulação da memória através do aumento dos níveis de BDNF no hipocampo. Isso porque evidências destacam que o BDNF desempenha um papel crítico na regulação da plasticidade sináptica e na consolidação da memória (HUANG et al., 2006; AGUIAR et al., 2011). Para investigar essa relação, avaliamos os níveis de BDNF no hipocampo de animais submetidos a 30 minutos de EF agudo e de animais não submetidos a esse exercício. No entanto, não observamos um aumento nos níveis dessa proteína em relação aos diferentes tempos de coleta - 0,5h, 2h ou 12h após o exercício - nem

entre os grupos exercitados, nos animais apenas habituados à esteira, ou grupo controle (animais não submetidos a nenhum procedimento). Isso sugere que o EF agudo não eleva os níveis dessa proteína nessas primeiras horas após o exercício. No entanto, acreditamos que esse aumento possa ocorrer em um momento posterior, já que a expressão do mRNA do BDNF no hipocampo foi observada em momentos posteriores ao EF agudo (VENEZIA; QUINLAN; ROTH, 2017; VENEZIA et al., 2019). Além disso, é importante ressaltar que outras proteínas podem ser influenciadas pelo EF agudo, como a proteína de ligação ao elemento responsivo ao AMPc (CREB, do inglês response element-binding protein) e o monofosfato cíclico de adenosina 3',5' (cAMP, do inglês cyclic adenosine monophosphate), conforme demonstrado em estudos anteriores com sessões curtas de EF agudo (4-6 minutos) (AGUIAR et al., 2011). Portanto, é possível que o EF agudo aumente os níveis de BDNF em um tempo tardio após sua prática, e/ou que ele eleve os níveis de outras proteínas relacionadas à plasticidade em momento próximo ou tardio à sua realização.

Nos dois primeiros estudos desta tese, nós investigamos os efeitos e mecanismos neuroquímicos do EF agudo sobre a fase inicial de consolidação da memória, que ocorre nas primeiras horas após a aquisição. No terceiro estudo, nossa investigação se voltou aos efeitos do EF agudo em uma janela temporal distinta, a consolidação tardia, que se desenrola aproximadamente 11 a 12 horas após a aquisição. Nesse estudo, o EF agudo foi aplicado 11 horas após a sessão de treino na tarefa de RO, enquanto a manipulação farmacológica ocorreu na janela de 11,5 horas, sendo aplicada após o EF agudo ou em tempo equivalente para os grupos controle. Nossas evidências destacaram que o EF agudo continua aprimorando a persistência da memória nessa nova janela temporal. Além disso, revelamos que esse efeito é intrinsecamente vinculado à síntese proteica no hipocampo dorsal, bem como à ativação dos receptores D1/D5 dopaminérgicos e beta-adrenérgicos nesta mesma região.

Em nossos achados, a manipulação farmacológica na região CA1 do hipocampo, na ausência do EF agudo, não comprometeu a consolidação da memória de RO avaliada um dia após o treino na tarefa. Esses resultados indicam que a consolidação da memória de RO não depende da síntese proteica hipocampal, nem da ativação dos receptores D1/D5 dopaminérgicos e beta-

adrenérgicos no hipocampo dorsal 11,5 horas após a aquisição. Essa observação sugere que os processos neuroquímicos cruciais para a consolidação devem ocorrer em estágios anteriores, conforme documentado em estudos que investigaram outros tipos de memória (ROSSATO et al., 2009; TOMAIUOLO et al., 2015a; KATCHE et al., 2016). Isto reforça a concepção de que a janela de consolidação tardia está mais relacionada aos processos de persistência da memória, os quais exigem que novas cascata bioquímicas, similares àquelas da consolidação inicial, ocorram ao longo do tempo para estender a retenção da informação (KATCHE et al., 2010; KATCHE; CAMMAROTA; MEDINA, 2013).

O EF agudo revelou-se uma estratégia eficaz na modulação da persistência da memória de RO durante a janela de consolidação tardia. Nossas evidências apontam que a eficácia dessa estratégia está intrinsecamente associada à síntese proteica hipocampal, assim como à ativação dos receptores D1/D5 dopaminérgicos e beta-adrenérgicos nessa região. Estes resultados estão alinhados com estudos prévios que indicaram que o bloqueio dessas vias nesse intervalo temporal específico compromete a persistência, sem influenciar a consolidação, da memória relacionada a um aprendizado robusto (BEKINSCHTEIN et al., 2007, 2008; ROSSATO et al., 2009; PARFITT et al., 2012). Isso fortalece a ideia de que o hipocampo mantém um envolvimento dinâmico no processamento da memória mesmo após a sua consolidação, que depende de expressão proteica (BEKINSCHTEIN et al., 2007; KATCHE et al., 2010) e da ativação de vias catecolaminérgicas (ROSSATO et al., 2009; PARFITT et al., 2012).

Com base nos resultados dos três estudos conduzidos nesta tese, contribuímos significativamente para a compreensão dos efeitos e mecanismos do EF agudo na modulação da memória. Agora, temos uma compreensão mais sólida de que o EF agudo não apenas aprimora a consolidação da memória na fase inicial, mas também na fase tardia, a fim de promover a sua persistência. Aqui, nós demos destaque a alguns dos principais mecanismos do EF agudo, com ênfase na ativação do LC, mas não da ATV; no impedimento da amnésia causada pela infusão de inibidores de síntese proteica no hipocampo; além de destacar a importância da ativação de receptores D1/D5 dopaminérgicos, beta-adrenérgicos e da síntese proteica na região CA1 para os efeitos do EF agudo na janela de consolidação tardia (**Figura 8**).

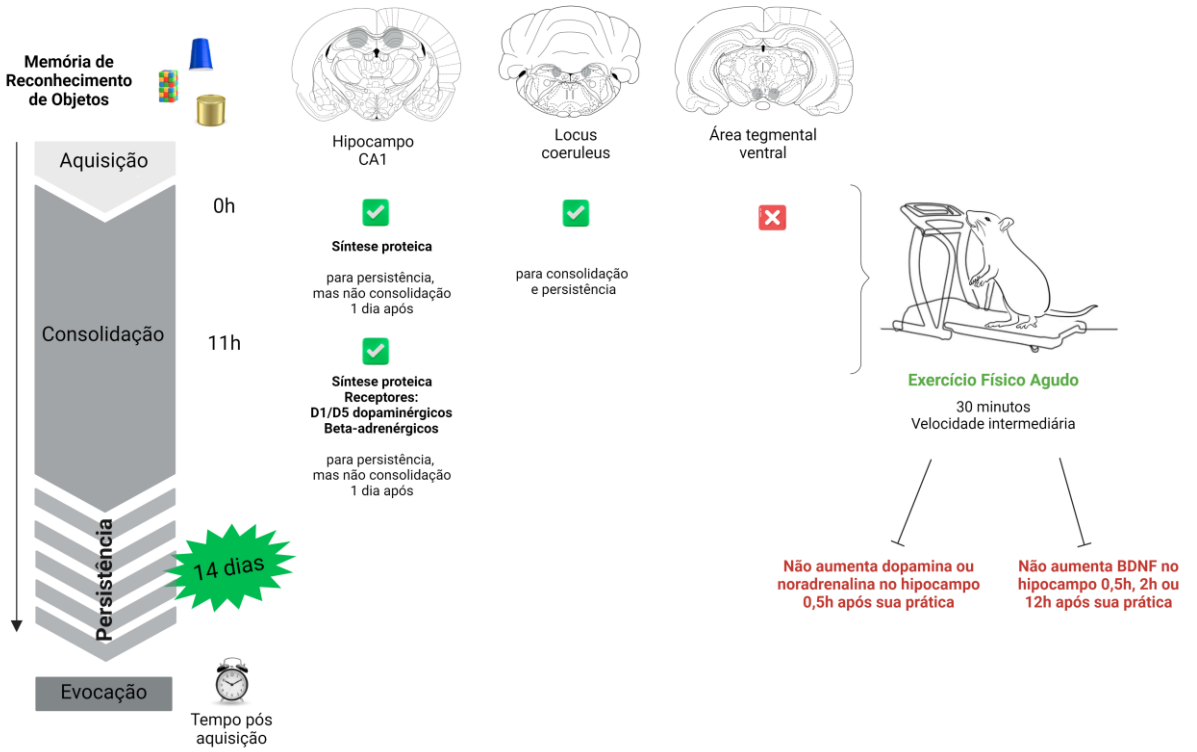

Figura 8 Representação esquemática dos efeitos e mecanismos do exercício físico (EF) agudo na memória evidenciados a partir dos estudos desenvolvidos na tese. Os efeitos do EF agudo foram examinados utilizando o protocolo da tarefa de reconhecimento de objetos (RO). Neste procedimento, os animais passam por uma fase de aquisição, na qual são expostos a dois objetos novos e distintos. A consolidação tem início nas primeiras horas após a aquisição, sendo testada 1 dia depois, enquanto a persistência da memória é avaliada nos dias 7 e 14 seguintes. Os animais submetidos ao EF agudo, seja imediatamente após a aquisição (0h; fase de consolidação inicial) ou 11 horas depois (fase de consolidação tardia), apresentaram uma persistência da memória que se estendeu por até 14 dias. Esse fenômeno mostrou ser dependente da ativação da região CA1 do hipocampo (0h e 11h pós aquisição) e do *locus coeruleus* (LC; 0h pós aquisição), mas não da área tegmental ventral (ATV; 0h pós aquisição). Além disso, evidenciou depender da síntese proteica hipocampal e da ativação de receptores dopaminérgicos D1/D5 e beta-adrenérgicos nessa região específica em ambas as fases, consolidação inicial e tardia. Ademais, o protocolo de EF agudo não aumentou os níveis de dopamina ou noradrenalina no hipocampo coletado 0,5h após a sua prática, bem como não alterou os níveis do fator neurotrófico derivado do cérebro (BDNF, do inglês *brain-derived neurotrophic factor)* nas primeiras horas pós exercício (0,5h, 2h e 12h).

A partir desses achados, novos questionamentos emergem acerca dos efeitos e mecanismos do EF agudo na memória. Conforme evidenciado ao longo desta tese, uma variedade de estudos tem salientado a relevância do EF agudo na regulação da memória, elucidando os principais mecanismos neurobiológicos associados a esse fenômeno. Contudo, é importante notar que nenhum desses estudos explorou a dualidade entre os sexos. Nesse contexto, nosso grupo de pesquisa recentemente divulgou as primeiras evidências acerca da influência do EF agudo sobre a memória de fêmeas (Jaques et al., 2024). Este estudo revelou que o

EF agudo em ratas fêmeas apresenta efeitos semelhantes na memória quando comparado aos observados em ratos machos. Entretanto, é essencial observar que 30 minutos de corrida em esteira aumentaram apenas os níveis de noradrenalina, não sendo observadas alterações nos níveis de dopamina no hipocampo das ratas. Dessa forma, mais investigações são necessárias para elucidar os mecanismos pelos quais o EF agudo atua na modulação da memória em ratas fêmeas. Embora efeitos positivos tenham sido observados em ambos os sexos, é possível que os mecanismos subjacentes apresentem variações.

Ademais, a compreensão sobre como o EF agudo pode ser utilizado para a melhora da memória em seres humanos é crucial. Entretanto, pesquisas que exploram esses efeitos ainda são escassas. Em idosos saudáveis, evidências indicam que uma única sessão de EF não apenas promove maior ativação cerebral, mas também amplifica os processos neurais associados à ativação da memória semântica (WON et al., 2019). Resultados similares foram observados em um estudo envolvendo idosos com comprometimento cognitivo leve, onde o EF não apenas melhorou a memória, mas também aumentou significativamente os níveis endógenos de noradrenalina (SEGAL; COTMAN; CAHILL, 2012). Em uma população mais jovem (média de 20 anos), tanto o EF agudo de resistência (WEINBERG et al., 2014) quanto o EF aeróbico (COLES; TOMPOROWSKI, 2008), quando aplicados na janela de consolidação inicial, demonstraram eficácia ao aprimorar a memória episódica. Em síntese, a busca por entender de maneira abrangente os impactos do EF agudo na cognição humana destaca-se como uma área de pesquisa promissora, onde os benefícios transcendem diferentes faixas etárias e estados de saúde.

Por fim, os achados delineados ao longo desta pesquisa abrem novas perspectivas e desafios significativos no entendimento dos efeitos do EF agudo na modulação da memória. A constatação de que o EF agudo não apenas aprimora a consolidação inicial, mas também pode ter efeitos na fase tardia da consolidação, influenciando a persistência da memória, destaca sua relevância como uma ferramenta dinâmica para otimização dos processos mnemônicos. A identificação de mecanismos específicos, como a ativação do LC, o não envolvimento da ATV, e a independência da síntese proteica hipocampal para a consolidação, oferece noções valiosas que podem orientar futuras investigações. Diante desse cenário, a

continuação das pesquisas nessa área torna-se fundamental, não apenas para aprofundar o entendimento dos mecanismos subjacentes, mas também para explorar aplicações práticas em contextos clínicos e educacionais. A interação complexa entre o EF agudo e os processos mnemônicos merece uma investigação mais aprofundada, visando maximizar seus benefícios e contribuir para estratégias inovadoras no campo da cognição e aprendizagem.

CONCLUSÕES

Com base nos resultados obtidos nos três estudos que fundamentaram esta tese, é possível concluir que:

i. A ativação do LC, mas não da ATV, é crucial para a promoção da persistência da memória de RO induzida pelo EF agudo realizado após a aprendizagem;

ii. A inibição ou estimulação farmacológica na ATV e LC, ou ação do EF agudo realizado logo após a aprendizagem, não alteraram os níveis de dopamina e noradrenalina no hipocampo;

iii. A inibição da síntese proteica ribossomal ou pela via mTOR no hipocampo não impediu o efeito do EF agudo realizado logo após a aprendizagem na consolidação da memória, embora tenha comprometido a persistência;

iv. O EF agudo não aumenta a expressão de BDNF no hipocampo 0,5, 2 ou 12 horas após a sua prática;

v. O EF agudo realizado na janela de consolidação tardia da memória, 11 horas após o aprendizado, melhora a persistência da memória de RO;

vi. Os efeitos do EF agudo na janela de consolidação tardia da memória, 11 horas após o aprendizado, sob a persistência da memória dependem de síntese proteica no hipocampo;

vii. Os efeitos do EF agudo na janela de consolidação tardia da memória, 11 horas após o aprendizado, sob a persistência da memória dependem da ativação de receptores beta-adrenérgicos no hipocampo;

viii. Os efeitos do EF agudo na janela de consolidação tardia da memória, 11 horas após o aprendizado, sob a persistência da memória dependem da ativação de receptores dopaminérgicos do tipo D1/D5 no hipocampo.

Em conjunto, nossos resultados indicam que uma única sessão de EF de 30 minutos, com intensidade moderada, tem efeitos benéficos na melhoria da persistência da memória em duas janelas temporais de consolidação: imediatamente após a aquisição e em uma fase tardia, cerca de 11 horas após a aquisição. Identificamos a importância do LC, mas não da ATV, logo após a aquisição, no

desencadeamento dos processos de persistência da memória mediados pelo EF agudo. Enquanto a síntese proteica no hipocampo é crucial para a persistência induzida pelo EF agudo, esta estratégia parece superar a inibição da síntese proteica após a aquisição, promovendo a consolidação da memória. Além disso, na fase tardia, os efeitos do EF agudo na persistência dependem da ação de catecolaminas nos receptores beta-adrenérgicos e dopaminérgicos do tipo D1/D5 no hipocampo, juntamente com a síntese proteica. Essas descobertas fornecem conhecimentos significativos sobre os mecanismos envolvidos nos efeitos do EF agudo na memória, revelando sua complexidade e seu potencial para otimizar a consolidação e a persistência das memórias.

PERSPECTIVAS FUTURAS

A partir dos resultados desta tese, emergem novos questionamentos que servirão de guia para pesquisas futuras sobre os mecanismos e impactos do EF agudo em diversos contextos. Essas descobertas abrem portas para investigações mais aprofundadas, explorando nuances específicas e aplicações práticas do EF agudo em diferentes cenários:

i. Exploração de Diferentes Protocolos de EF Agudo:

Seria de grande relevância investigar como distintas intensidades, durações e modalidades de EF agudo podem modular de maneira única os processos de consolidação e persistência da memória.

ii. Estudo sobre os Efeitos do Treino e Destreino:

A análise dos efeitos do treino e destreino no contexto do EF agudo oferece uma perspectiva intrigante. Pesquisas futuras podem comparar os impactos do EF agudo na memória, considerando animais sedentários e previamente treinados, proporcionando uma compreensão mais abrangente dessa dinâmica.

iii. Pesquisas com Ratas:

Para aprofundar a compreensão dos efeitos do EF agudo em ratas, futuras investigações devem explorar de maneira mais detalhada os mecanismos moleculares subjacentes ao aumento da persistência da memória considerando o sexo como uma variável.

iv. Estudos com Seres Humanos:

No âmbito humano, é essencial expandir as pesquisas sobre os efeitos do EF físico agudo na cognição. Investigar como o EF agudo influencia a memória em escolares e estudantes universitários é valioso. Além disso, compreender o tempo adequado para a aplicação do EF agudo na memória seria interessante, abrangendo aplicações tanto na janela de consolidação inicial quanto na consolidação tardia.

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ANEXOS

ANEXO A – Certificado de aprovação da Comissão de Ética em Uso de Animais – CEUA da UNIPAMPA

CERTIDÃO

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

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: 20/08/2021

Período de vigência do projeto: 01/12/2023

Pesquisadores(a): Pâmela Billig Mello Carpes

Campus: Uruguaiana

Telefone: (55) 99661-2454

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Finalidade	**( ) Ensino (x) Pesquisa**
Espécie / Linhagem / Raça	Ratos Wistar
N° de animais	350
Peso / Idade	300-350g/90 dias
Sexo	Machos
Origem	Biotério da Universidade Federal de Pelotas/RS

Assinado eletronicamente por **CATIA ALINE VEIVERBERG, PROFESSOR DO MAGISTERIO SUPERIOR**, em 25/08/2021, às 14:24, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

A autenticidade deste documento pode ser conferida no site

ANEXO B – Certificado de aprovação da Comissão de Ética em Uso de Animais – CEUA da UNIPAMPA – Acréscimo de animais e prazo

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

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Finalidade	**( ) Ensino (X) Pesquisa**
Espécie / Linhagem / Raça	Ratos Wistar
N° 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 n° 23100.014088/2021-57 SEI n° 0943385

ANEXO C – Submissão de manuscrito para a revista *Neurochemistry International*

Neurochemistry International

ACUTE PHYSICAL EXERCISE PREVENTS MEMORY AMNESIA CAUSED BY PROTEIN SYNTHESIS INHIBITION IN RATS' HIPPOCAMPUS

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	BDNF; a bout of exercise; treadmill; neuroplasticity; treadmill.
Corresponding Author:	Pamela Mello-Carpes Universidade Federal do Pampa Uruguaiana, RS Brazil
First Author:	Karine Ramires Lima
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	Gabriela Cristiane Mendes Gomes
	Marcelo Gomes de Gomes
	Pamela Billig Mello-Carpes, PhD
Abstract:	The benefits of physical exercise (PE) on memory consolidation have been well-documented in both healthy and memory-impaired animals. However, the underlying mechanisms through which PE exerts these effects are still unclear. In this study, we aimed to investigate the role of hippocampal protein synthesis in memory modulation by acute PE in rats. After novel object recognition (NOR) training, rats were subjected to a 30-minute moderate-intensity acute PE on the treadmill, while control animals did not undergo any procedures. Using anisomycin (ANI) and rapamycin (RAPA), compounds that inhibit protein synthesis through different mechanisms, we manipulated protein synthesis in the CA1 region of the hippocampus to examine its contribution to memory consolidation. Memory was assessed on days 1, 7, and 14 post-training. Our results showed that inhibiting protein synthesis by ANI or RAPA impaired NOR memory consolidation in control animals. However, acute PE prevented this impairment without affecting memory persistence. We also evaluated brain-derived neurotrophic factor (BDNF) levels after acute PE at 0.5h, 2h, and 12h afterward, and found no differences in levels compared to animals that did not engage in acute PE or were only habituated to the treadmill. Therefore, our findings suggest that acute PE could serve as a non-pharmacological intervention to enhance memory consolidation and prevent memory loss in conditions associated with hippocampal protein synthesis inhibition. This mechanism appears not to depend on BDNF synthesis in the early hours after exercise.
Suggested Reviewers:	Cristiane Furini, Professor Hospital Sao Lucas da PUCRS cristianefurini@hotmail.com
	Joao Quevedo UTHealth ms.psychiatry@uth.tmc.edu
	Rudi D'Hooge KU Leuven rudi.dhooge@kuleuven.be
Opposed Reviewers:	

ANEXO D – Submissão de manuscrito para a revista *Neuroscience*

Neuroscience

PERFORMING A PHYSICAL EXERCISE SESSION DURING THE LATE CONSOLIDATION PHASE IMPROVES MEMORY PERSISTENCE BY HIPPOCAMPAL PROTEIN SYNTHESIS AND CATECHOLAMINE MODULATION

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Section/Category:	Behavioral and Cognitive Neuroscience
Keywords:	CA1 area; long-term memory; novel object recognition; dopamine; noradrenaline.
Corresponding Author:	Pâmela Billig Mello-Carpes, Ph.D. Federal University of Pampa - Uruguaiana Campus BRAZIL
First Author:	Karine Ramires Lima
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	Gabriela Cristiane Mendes Gomes
	Gabriela Jaques Sigaran
	Anna Cecilia Perreto
	Pâmela Billig Mello-Carpes, Ph.D.
Abstract:	Memory persistence is a crucial aspect of long-term memory (LTM) and involves late consolidation processes that modulate memory stability over time. Acute physical exercise (PE) has emerged as a potential strategy to module memory consolidation and enhance memory persistence. While its effects have been extensively explored in the early consolidation phase, its impact on the late phase remains unexplored. In this study, we investigated the effects and mechanisms of PE modulation of the late consolidation window of novel object recognition (NOR) memory in rats. A 30-minute treadmill session applied 11 hours after NOR memory acquisition significantly increased memory persistence for up to 14 days. Our exploration of the mechanisms underlying this enhancement revealed the involvement of protein synthesis, and beta-adrenergic and dopaminergic D1/D5 receptors activation in the CA1 region of the hippocampus. These findings provide valuable insights into PE as a potential memory modulator, contributing to expanding our understanding of memory consolidation dynamics and PE effects.
Suggested Reviewers:	Fernando Benetti Federal University of Rio Grande do Sul fernando.benetti@ufrgs.br Expert on neurobiology of learning and memory
	Andresa Germano Chemnitz University of Technology andresa.germano@hsw.tu-chemnitz.de Expert on physical exercise acute effects
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Opposed Reviewers:	