UNIVERSIDADE FEDERAL DE SANTA CATARINA

CENTRO DE CIÊNCIAS BIOLÓGICAS

PROGRAMA DE PÓS-GRADUAÇÃO EM NEUROCIÊNCIAS

Anderson Camargo

GUANOSINA COMO UM AGENTE POTENCIALIZADOR DOS EFEITOS TIPO-ANTIDEPRESSIVO E PRÓ-SINAPTOGÊNICO DESENCADEADOS PELA CETAMINA

Florianópolis

2022

Anderson Camargo

GUANOSINA COMO UM AGENTE POTENCIALIZADOR DOS EFEITOS TIPO-ANTIDEPRESSIVO E PRÓ-SINAPTOGÊNICO DESENCADEADOS PELA CETAMINA

Tese submetida ao Programa de Pós-Graduação em Neurociências da Universidade Federal de Santa Catarina para a obtenção do título de Doutor em Neurociências

Orientadora: Prof. Dra. Ana Lúcia Severo Rodrigues

Florianópolis

2022

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Anderson Camargo

Guanosina como um agente potencializador dos efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela cetamina

O presente trabalho em nível de doutorado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

Prof.ª Josiane Budni, Dr.ª
Universidade do Extremo Sul Catarinense

Prof.ª Carla Inês Tasca, Dr.ª
Universidade Federal de Santa Catarina

Prof.ª Manuella Pinto Kaster, Dr.ª
Universidade Federal de Santa Catarina

Certificamos que esta é a **versão original e final** do trabalho de conclusão que foi julgado adequado para obtenção do título de doutor em Neurociências.

_____

Prof. Aderbal Silva Aguiar Junior, Dr.
Coordenação do Programa de Pós-Graduação

_____

Prof.ª Ana Lúcia Severo Rodrigues, Dr.ª
Orientadora

Florianópolis, 2022.

À minha avó Maria Salete Camargo

(in memorian)

AGRADECIMENTOS

RESUMO

Evidências convincentes têm demonstrado que a cetamina, um antagonista do receptor NMDA, exerce efeito antidepressivo rápido e duradouro, bem como efeito profilático, por promover a formação de espinhos dendríticos via ativação de mTORC1. Considerando que o uso da cetamina apresenta algumas limitações, estratégias direcionas em potencializar suas respostas antidepressivas, mas com menor potencial para efeitos indesejáveis são bem-vindas. Neste sentido, estudos recentes mostraram que a guanosina, uma purina neuroprotetora endógena, apresenta mecanismos de ação sobrepostos à cetamina e, portanto, poderia ser um novo candidato para potencializar suas ações. Diante disso, o objetivo desta tese foi investigar a guanosina como um agente potencializador dos efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela cetamina em camundongos, bem como o papel da via de sinalização mediada por mTORC1 nestas possíveis respostas. A coadministração única com doses sub-efetivas de cetamina (0,1 mg/kg, i.p.) e guanosina (0,01 mg/kg, p.o.) foi capaz de produzir um efeito tipo-antidepressivo após 1 h e 24 h, mas não após 7 dias, em camundongos. Este efeito comportamental foi acompanhado por um aumento rápido (iniciado em 1 h) e transitório (de volta aos níveis do controle em 24 h) no imunoconteúdo de BDNF, p-Akt (Ser^{473}), p-GSK-3β (Ser^9), p-mTORC1 (Ser^{2448}), p-p70S6K (Thr^{389}) no hipocampo, mas não no córtex pré-frontal. Por outro lado, a associação de cetamina e guanosina aumentou o imunoconteúdo PSD-95 e GluA1 no córtex pré-frontal, mas não no hipocampo após 1 h, enquanto um aumento no imunoconteúdo dessas proteínas em ambas as estruturas encefálicas foi observado após 24 h. Contudo, o aumento no imunoconteúdo de PSD-95 e GluA1 promovido pela combinação de cetamina e guanosina não persistiu após 7 dias. Além disso, a administração combinada de cetamina e guanosina aumentou a densidade de espinhos dendríticos na porção ventral do giro denteado da formação hipocampal e no córtex pré-frontal após 24 h. Não obstante, a administração de rapamicina (0,2 nmol/sítio, i.c.v., um inibidor seletivo de mTORC1) anulou completamente os efeitos tipo-antidepressivo e pró-sinaptogênico desencadeadas pela combinação de cetamina e guanosina, indicando que estas respostas são dependentes, pelo menos em parte, da ativação de mTORC1. Adicionalmente, a combinação com doses sub-efetivas de cetamina (0,1 mg/kg, i.p.) e guanosina (0,01 mg/kg, p.o.) foi capaz de reverter o comportamento tipo-depressivo e os déficits no imunoconteúdo de BDNF, p-Akt (Ser^{473}), p-GSK-3β (Ser^9), PSD-95, GluA1 e sinapsina no hipocampo de camundongos submetidos à administração crônica com corticosterona (20 mg/kg, p.o., por 21 dias). É importante destacar que estes efeitos foram abolidos pela administração de rapamicina, reforçando o envolvimento de mTORC1 nos efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela associação de cetamina e guanosina. Além disso, a administração profilática de cetamina (5 mg/kg, i.p.), mas não de guanosina (1 ou 5 mg/kg, p.o.) ou a associação de cetamina (1 mg/kg, i.p.) e guanosina (5 mg/kg, p.o.) preveniu o comportamento tipo-depressivo e reduções no imunoconteúdo de PSD-95, GluA1 e sinapsina no hipocampo de camundongos submetidos à administração crônica com corticosterona (20 mg/kg, p.o., por 21 dias). Estes resultados reforçam a noção que a administração profilática de cetamina é capaz de produzir uma resposta pró-resiliência contra o comportamento tipo-depressivo induzido por estresse. Os resultados deste trabalho indicam que a combinação de cetamina e guanosina é capaz de produzir um efeito tipo-antidepressivo e uma resposta pró-sinaptogênica de maneira tempo-dependente no hipocampo e córtex pré-frontal. Além disso, este estudo sugere que a guanosina em combinação com a cetamina pode constituir uma estratégia segura e promissora para auxiliar pacientes com diagnóstico de depressão no futuro, por permitir a redução da dose terapeuticamente eficaz de cetamina, uma prova de conceito que merece uma investigação mais aprofundada.

Palavras-chave: Cetamina. Depressão. Guanosina. mTORC1. Sinaptogênese.

ABSTRACT

Compelling evidence has demonstrated that ketamine, an NMDA receptor antagonist, elicits fast and long-lasting antidepressant responses, besides prophylactic actions, by stimulating dendritic spines formation via mTORC1 activation. Considering that ketamine's use has some limitations, strategies targeting to potentiate its antidepressant responses but devoid of undesirable effects are welcome. Within this scenario, recent studies have shown that guanosine, an endogenous neuroprotective purine, presents overlapping mechanisms to ketamine e thereby, it could be a novel candidate to potentiate its actions. Given this background, this thesis aimed to investigate guanosine as an augmenting agent of antidepressant-like and pro-synaptogenic effects triggered by ketamine in mice, as well as the role of the mTORC1 signaling pathway in these putative responses. A single combined administration of subthreshold doses of ketamine (0.1 mg/kg, i.p.) and guanosine (0.01 mg/kg, p.o.) was effective to produce a fast (1 h – 24 h), but not long-lasting (7 days) antidepressant-like effect in mice. This behavioral effect was paralleled by a rapid (started in 1 h) and transient (back to baseline in 24 h) increase on BDNF, p-Akt (Ser^{473}), p- GSK-3β (Ser^{9}), p-mTORC1 (Ser^{2448}), p-p70S6K (Thr^{389}) immunocontent in the hippocampus, but not in the prefrontal cortex. Conversely, ketamine plus guanosine increased PSD-95 and GluA1 immunocontent in the prefrontal cortex, but not the hippocampus after 1 h, whereas increased levels of these proteins in both brain structures were observed after 24 h. However, the increase in the immunocontent of PSD-95 and GluA1 elicited by the combination of ketamine and guanosine did not persist after 7 days. Furthermore, the combined administration of ketamine plus guanosine raised the dendritic spines density in the ventral dentate gyrus of the hippocampus and prefrontal cortex after 24 h. Nonetheless, the rapamycin (0.2 nmol/site, i.c.v.) administration completely abrogated the antidepressant-like effect and pro- synaptogenic responses triggered by ketamine plus guanosine, indicating that these responses are dependent, at least in part, on mTORC1 activation. Additionally, a low-dose combination of ketamine (0.1 mg/kg, i.p.) and guanosine (0.01 mg/kg, p.o.) was effective in reversing the depressive-like behavior and deficits on the immunocontent of BDNF, p-Akt (Ser^{473}), p- GSK-3β (Ser^{9}), PSD-95, GluA1, and synapsin in the hippocampus of mice subjected to the chronic administration of corticosterone (20 mg/kg, p.o., for 21 days). It is important to note that these responses were abolished by rapamycin administration, further reinforcing the involvement of mTORC1 in the antidepressant-like and pro-synaptogenic effects elicited by a low-dose combination of ketamine and guanosine. Moreover, a single prophylactic administration of ketamine (5 mg/kg, i.p.), but not guanosine (1 or 5 mg/kg, p.o.) or the association of ketamine (1 mg/kg, i.p.) plus guanosine (5 mg/kg, p.o.), was able to prevent the depressive-like behavior and the reduction in the immunocontent of PSD-95, GluA1, and synapsin in the hippocampus of mice subjected to the chronic administration of corticosterone (20 mg/kg, p.o., for 21 days). These results reinforce the notion that the prophylactic administration of ketamine is effective in producing a pro-resilience response against depressive-like behavior induced by stress. The results of this study indicate that ketamine plus guanosine combination is effective to produce an antidepressant-like effect and a pro-synaptogenic response in a time-dependent manner in the hippocampus and prefrontal cortex. Furthermore, this study suggests that guanosine in combination with ketamine could constitute an effective and safe strategy to assist patients diagnosed with depression in the future by allowing ketamine's therapeutically effective dose to be lowered, a proof-of-concept that deserves further investigation.

Keywords: Ketamine. Depression. Guanosine. mTORC1. Synaptogenesis.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

4E-BP – Proteína ligadora do fator de alongamento 4E

ACTH – Hormônio adrenocorticotrófico

ADP – Adenosina-5'-difosfato

Akt – Proteína cinase B

AMP – Adenosina- 5´-monofosfato

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

ATC – Antidepressivo tricíclico

ATP – Adenosina-5'-trifosfato

BDNF – Fator neurotrófico derivado do encéfalo

CA – *Cornu ammonis*

CaMKII – Complexo cálcio/calmodulina cinase II

CASK – Proteína serina cinase dependente de cálcio

CCVD – Canais de cálcio dependentes de voltagem

CRH – Hormônio liberador de corticotrofina

DNQX – 6,7-dinitroquinoxalina-2,3-diona

DSM – Manual de diagnóstico e estatístico de transtornos mentais

EAAT1 – Transportadores de aminoácidos excitatórios do tipo 1

EAAT2 – Transportadores de aminoácidos excitatórios do tipo 2

FDA – Food and Drug Administration

GABA – Ácido γ-aminobutírico

GDP – Guanosina-5'-difosfato

GluA1 – Subunidade 1 de receptores AMPA

GMP – Guanosina-5'-monofosfato

GSK3β – Glicogênio sintase cinase 3β

GTP – Guanosina-5'-trifosfato

HHA – Hipotálamo-hipófise-adrenal

HSP – Proteína de choque térmico

i.c.v. – Intracerebroventricular

i.p. – Intraperitoneal

iMAO – Inibidor da monoamina oxidase

ISRS –Inibidor seletivo de recaptação de serotonina

LPS – Lipopolissacarídeo

LTP – Potenciação de longa duração

MAO – Monoamina oxidase

mGLU – Receptor metabotrópico de glutamato

mRNA –Ácido ribonucleico mensageiro

mTORC1 – Complexo 1 da proteína alvo mecanístico da rapamicina

NLRP3 – Receptor do tipo NOD com domínio pirina 3

NMDA – N-metil-D-aspartato

p.o. – Per os

p70S6K – Proteína ribossomal S6 cinase de 70 kDa

PI3K – Fosfatidilinositol 3' cinase

PKA – Proteína cinase A

PRAS40 – Proteína substrato de Akt rico em prolina de 40 kDa

PSD-95 – Proteína de densidade sináptica de 95 kDa

Ser – Serina

SNC – Sistema nervoso central

TARP – Proteína transmembrana reguladora de receptores AMPA

TDM – Transtorno depressivo maior

Thr – Treonina

TNF-α – Fator de necrose tumoral-alfa

TrkB – Receptor tropomiosina cinase B

SUMÁRIO

1 INTRODUÇÃO

1.1 TRANSTORNO DEPRESSIVO MAIOR

O transtorno depressivo maior (TDM) ou depressão, é uma condição psiquiátrica grave e altamente prevalente que acarreta perda considerável de produtividade e qualidade de vida, bem como déficits no relacionamento social (OTTE et al., 2016). Destaca-se que este transtorno é a principal causa de incapacitação a nível global e de mortes por suicídio anualmente. Em 2017, a Organização Mundial da Saúde estimou que mais de 300 milhões de pessoas são acometidas pelo TDM globalmente, um índice que equivale a cerca 6% da população adulta mundial, com prevalência de 1 a cada 6 adultos ao longo da vida (WORLD HEALTH ORGANIZATION, 2017). O Brasil, em particular, é o país com o maior índice de indivíduos com depressão na América Latina, com especial destaque para a cidade de São Paulo que apresenta uma prevalência anual de 10,4%, superando os índices mundiais (OTTE et al., 2016). Estes dados refletem um número considerável de indivíduos com uma necessidade potencial de usar os serviços de saúde (MUNHOZ et al., 2016). Contudo, estima-se que após a pandemia causada pelo vírus SARS-CoV-2 (Coronavírus - COVID-19) houve um adicional de 53,2 milhões casos de TDM em todo o mundo (um aumento de cerca de 27,6%), exacerbando drasticamente os anos vividos com incapacidade pelos indivíduos (SANTOMAURO et al., 2021). Estes dados reforçam a urgência crescente em fortalecer os sistemas de saúde mundialmente, tornando-se um desafio socioeconômico no atual cenário.

O TDM é diagnosticado de acordo com critérios presentes no Manual de Diagnóstico e Estatístico dos Distúrbios Mentais. Do ponto de vista sintomatológico, um indivíduo com TDM deve apresentar pelo menos cinco dos seguintes sintomas (Figura 1), por um período mínimo de duas semanas, e quase que diariamente: i) humor deprimido evidente; ii) diminuição no interesse ou prazer em todas ou quase todas as atividades (anedonia); iii) diminuição ou aumento no apetite, associado com alterações no peso sem dieta alimentar aparente; iv) insônia ou hipersônia; v) agitação ou retardo psicomotor; vi) sensação de fadiga; vii) sentimento excessivo de inutilidade ou culpa; viii) diminuição na capacidade de concentração e tomada de decisões; ix) pensamentos recorrentes de morte ou ideação suicida (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Além disso, é necessário que humor deprimido e/ou anedonia estejam presentes, e o que os sintomas apresentados causem prejuízo nas áreas sociais ou ocupacionais. É conveniente enfatizar ainda, que os sintomas apresentados não devem ser

atribuídos ao uso de qualquer substância ou fármaco pelo indivíduo bem como associados a qualquer outra condição médica como por exemplo, episódios de mania, esquizofrenia ou qualquer outro transtorno (ARMOUR; JANA; ELHAI, 2016; OTTE et al., 2016).

Figura 1. Critérios diagnósticos do TDM

O Manual de Diagnóstico e Estatístico dos Distúrbios Mentais que define que um episódio de TDM é caracterizado pela constatação de no mínimo cinco entre os nove sintomas mostrados na figura acima e exige a presença de pelo

menos um dos dois primeiros sintomas mencionados (humor deprimido ou perda de interesse/prazer) presentes com uma duração mínima de duas semanas (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Figura elaborada usando imagens do Mind the Graph e Servier Medical Art. Fonte: autor.

Apesar de sua alta prevalência e gravidade, a fisiopatologia do TDM ainda não está completamente estabelecida. Este fenômeno está associado a alta complexidade clínica e neurobiológica observada no TDM, que inclui diferentes mecanismos, sistemas e regiões encefálicas, o que dificulta a identificação de uma única causa subjacente e manejá-la de maneira adequada (NEMEROFF, 2007; OTTE et al., 2016). Além disso, o TDM também tem sua origem derivada de uma interação entre múltiplas suscetibilidades envolvendo fatores genéticos e ambientais (BERTON; NESTLER, 2006).

Historicamente, uma das primeiras teorias acerca da neurobiologia do TDM foi baseada no sistema monoaminérgico. O papel das monoaminas na fisiopatologia do TDM surgiu após a observação de que a reserpina, um alcaloide anti-hipertensivo que promove a depleção de noradrenalina, causava como efeito adverso sintomas depressivos (CELANO et al., 2011; FREIS, 1954). Em paralelo a essa descoberta, o papel do sistema monoaminérgico na neurobiologia do TDM foi ainda reforçado com a descoberta dos primeiros agentes antidepressivos, a imipramina e a iproniazida (PEREIRA; HIROAKI-SATO, 2018).

Particularmente, em 1957 demonstrou-se pela primeira vez que a imipramina, um fármaco anti-histamínico, apresentou propriedades antidepressivas em pacientes com TDM após tratamento diário por até 6 semanas (DOMINO, 1999; KUHN, 1958). Neste sentido, a imipramina configurou-se como o primeiro antidepressivo descoberto, classificado como antidepressivo tricíclico (ATC), o qual possui como mecanismo de ação a inibição da recaptação de serotonina e noradrenalina (HAMON; BLIER, 2013). No mesmo ano, constatou-se que a iproniazida, um fármaco antituberculínico, foi eficaz em produzir efeito antidepressivo em pacientes diagnosticados com TDM (CRANE, 1957; LÓPEZ-MUÑOZ; ALAMO, 2009). O mecanismo de ação da iproniazida consiste na inibição da enzima monoamina oxidase (MAO), responsável pela degradação das monoaminas serotonina, noradrenalina e dopamina (HAMON; BLIER, 2013).

A descoberta dos mecanismos de ação da iproniazida e da imipramina foi essencial para a formulação da primeira teoria etiológica do TDM, a teoria monoaminérgica (Figura 2). De especial interesse, a teoria baseada no sistema monoaminérgico destaca que pacientes com TDM apresentam uma redução nos níveis dos neurotransmissores monoaminérgicos (basicamente serotonina, noradrenalina e dopamina) na fenda sináptica (DELGADO, 2000).

Neste contexto, postula-se que estratégias capazes de inibir a recaptação destes neurotransmissores ou sua degradação pelas enzimas MAO podem ser capazes de produzir respostas antidepressivas (NESTLER et al., 2002; OTTE et al., 2016).

Figura 2. Hipótese monoaminérgica do TDM
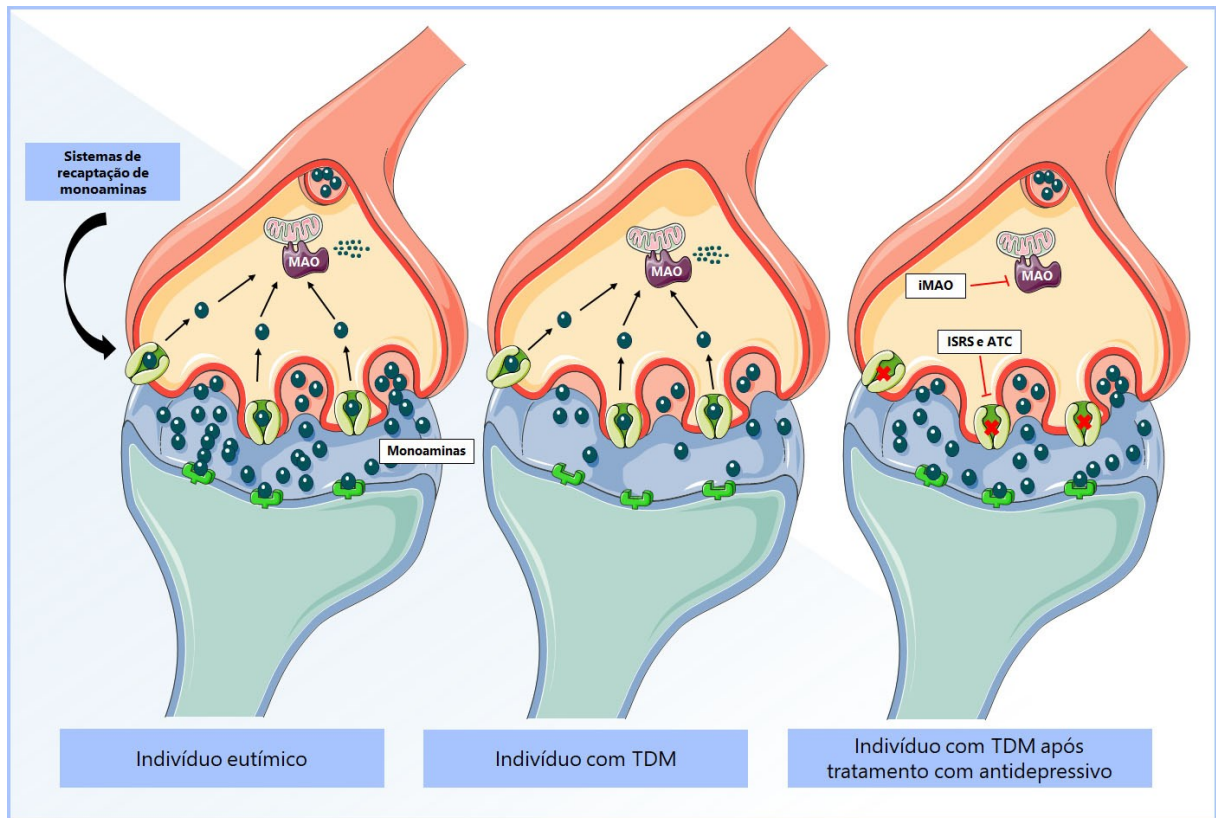
Em indivíduos com humor eutímico, serotonina, noradrenalina ou dopamina (representadas em círculos azuis) contidas em vesículas sinápticas são liberadas na fenda sináptica onde exercem suas funções nos neurônios pós-sinápticos, por meio da ativação dos seus respectivos receptores. Após a interação, estas monoaminas são recaptadas por sistemas de recaptação de volta ao neurônio pré-sináptico, onde são degradadas pela MAO, originando metabólitos que podem ser reciclados para a produção de novos neurotransmissores. Em indivíduos com TDM, ocorre uma redução na biodisponibilidade de monoaminas na fenda sináptica, acarretando numa menor interação e ativação de receptores do neurônio pós-sináptico, o que por sua vez propicia o aparecimento de sintomas depressivos (DELGADO, 2000). Além disso, as enzimas MAOs continuam exercendo a sua função de degradação de monoaminas amplificando ainda mais este déficit. Contudo, após o início do tratamento com antidepressivos, como os ISRS ou os ATCs, ocorre o bloqueio dos sistemas de recaptação de monoaminas, contribuindo para um aumento na biodisponibilidade destes neurotransmissores na fenda sináptica, enquanto os iMAO atuam inibindo a atividade desta enzima e reduzindo a degradação dos neurotransmissores. Postula-se que estes mecanismos em conjunto promovam uma melhora nas condições de humor do paciente. Abreviaturas: ATC: antidepressivos tricíclicos; iMAO: inibidores da enzima monoamina oxidase; ISRS: inibidores seletivos da recaptação de serotonina; MAO monoamina oxidase. Figura elaborada usando imagens do Mind the Graph e Servier Medical Art. Fonte: autor.

Embora a descoberta da imipramina e da iproniazida tenha revolucionado a farmacoterapia do TDM, estes fármacos apresentavam uma série de efeitos adversos, tendo como destaque a hepatotoxicidade (PEREIRA; HIROAKI-SATO, 2018). Dentro deste cenário,

a postulação da teoria monoaminérgica possibilitou a busca por agentes antidepressivos mais seletivos, o que por sua vez poderia diminuir a frequência e a probabilidade de efeitos adversos (LÓPEZ-MUÑOZ; ALAMO, 2009). Neste sentido, em 1984 descobriu-se a fluoxetina, um inibidor seletivo da recaptação de serotonina (ISRS). A fluoxetina teve eficácia comprovada no tratamento do TDM com a vantagem de causar menos efeitos adversos quando comparado aos ATCs e iMAOS (MONTGOMERY, 1989). Dentro deste contexto, a fluoxetina foi considerada a pílula da felicidade, foi o antidepressivo mais vendido de todos os tempos e o segundo medicamento mais vendido no mundo (LÓPEZ-MUÑOZ; ALAMO, 2009). Somado a este fato, considerando a crescente necessidade de fármacos para o manejo do TDM e tendo a fluoxetina como protótipo, outros ISRS foram sintetizados e liberados no mercado posteriormente. Desta forma, a descoberta dos ISRS é considerada um momento de fundamental importância na psicofarmacologia (PEREIRA; HIROAKI-SATO, 2018).

Contudo, apesar da expectativa muito grande sobre os ISRS, estes fármacos não resolveram vários dos problemas anteriores relacionados ao uso dos antidepressivos (LÓPEZ-MUÑOZ; ALAMO, 2009). De fato, os antidepressivos atuais que se baseiam primariamente em aumentar os níveis sinápticos de monoaminas apresentam uma séria de limitações, tais como: baixa taxa de efetividade, sendo que até 50% dos pacientes não atinge a remissão completa dos sintomas, mesmo após serem tratados com vários antidepressivos; alto índice de refratariedade; demora considerável na resposta terapêutica, uma vez que podem levar de semanas a meses para promover uma resposta terapêutica; e uma série de efeitos adversos como dores de cabeça, alterações de peso e sono, e disfunção sexual (PAPAKOSTAS; IONESCU, 2015). Além disso, esses fármacos têm se mostrado ineficazes para o tratamento da ideação suicida em pacientes com TDM grave (PENN; TRACY, 2012). Essas limitações ressaltam a necessidade de desenvolver novas estratégias antidepressivas com efeito mais rápido e com maior eficácia (KASTER et al., 2016).

De fato, a teoria monoaminérgica tem sido considerada excessivamente simplista, uma vez que os antidepressivos supracitados restabelecem os níveis de monoaminas poucas horas após a administração, mas com a resposta terapêutica só ocorrendo apenas após várias semanas de tratamento (OTTE et al., 2016). Desta forma, tendo em vista que os neurotransmissores monoaminérgicos modulam uma série de vias de sinalização, estudos têm buscado identificar novos alvos moleculares subjacentes ao efeito terapêutico tardio dos antidepressivos convencionais (HAMON; BLIER, 2013). Neste sentido, observou-se que a administração crônica com antidepressivos baseados no sistema monoaminérgico foi capaz de diminuir os

níveis de ácido ribonucleico mensageiro (mRNA) que codifica as subunidades do receptor glutamatérgico do subtipo N-metil-D-aspartato (NMDA), atenuando por sua vez a função deste receptor (SKOLNICK, 1999). Além disso, foi reportado que a administração de antagonistas do receptor NMDA produziu efeito tipo-antidepressivo em roedores (TRULLAS; SKOLNICK, 1990). Diante dessas evidências, em 1999 Phil Skolnick propôs os antidepressivos para o novo milênio, os quais se baseariam na modulação do sistema glutamatérgico (SKOLNICK, 1999).

1.2 SISTEMA GLUTAMATÉRGICO E SINAPTOGÊNESE

Um grande conjunto de evidências científicas tem apontado o papel essencial de outros sistemas biológicos além das monoaminas na fisiopatologia do TDM, em particular, o sistema glutamatérgico (MARMIROLI; CAVALETTI, 2012; TOMASETTI et al., 2019). O glutamato é um dos principais neurotransmissores excitatórios do sistema nervoso central (SNC) de mamíferos, e do ponto de vista fisiológico, está envolvido em uma série de funções, com destaque para a proliferação, migração, diferenciação e sobrevivência neuronal, bem como para a formação e maturação de sinapses (SANACORA; TRECCANI; POPOLI, 2012). Neste sentido, é bem estabelecido que o sistema glutamatérgico é essencial para uma série de processos, incluindo a modulação do humor, o aprendizado e a memória (HILLHOUSE; PORTER, 2015).

A síntese do glutamato em neurônios pré-sinápticos ocorre pela desaminação da glutamina, uma etapa mediada pela enzima glutaminase. Além disso, o glutamato também pode ser formado a partir do α-cetoglutarato, em uma reação catalisada pela enzima glutamatodesidrogenase. Consequentemente, o glutamato é armazenado em vesículas sinápticas por meio dos transportadores vesiculares de glutamato e pode ser liberado por exocitose, de maneira dependente de potencial de ação. Por sua vez, o glutamato liberado na fenda sináptica pode interagir com receptores específicos, os receptores metabotrópicos e os ionotrópicos (NICIU; KELMENDI; SANACORA, 2012). Os receptores metabotrópicos de glutamato (nomeados de mGlu1 a mGlu8) incluem os receptores glutamatérgicos acoplados à proteína G estimulatória ou inibitória, enquanto os receptores ionotrópicos incluem os receptores NMDA, alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico (AMPA) e cainato (MARMIROLI; CAVALETTI, 2012). Basicamente, os receptores ionotrópicos permitem a entrada de íons catiônicos (como o cálcio e o sódio) quando ativados pelo glutamato. Os três receptores são tetrâmeros e formados por subunidades específicas, sendo que os receptores

NMDA (GluN1, GluN2A, GluN2B, GluN2C, GluN2D, GluN3A e GluN3B) são heterotetrâmeros, enquanto os receptores AMPA (GluA1, GluA2, GluA3 e GluA4) e cainato (GluK1, GluK2, GluK3, GluK4 e GluK5) podem ser homotetrâmeros ou heterotetrâmeros (NICIU; KELMENDI; SANACORA, 2012).

Subsequentemente à interação com estes receptores, o glutamato é removido da fenda sináptica majoritariamente por transportadores de aminoácidos excitatórios do tipo 1 e 2 (EAAT1 e EAAT2), localizados em astrócitos, e convertido em glutamina por meio da enzima glutamina sintetase (TOMASETTI et al., 2019). Posteriormente, a glutamina pode sofrer efluxo para o meio extracelular seguido de influxo para o interior neuronal via transportadores específicos. Desta forma, a glutamina pode ser convertida novamente em glutamato pela enzima glutaminase no interior dos neurônios (NICIU; KELMENDI; SANACORA, 2012). É importante destacar que a remoção do glutamato da fenda mediado pelos astrócitos compreende um processo essencial para a homeostase sináptica, uma vez que o excesso deste neurotransmissor pode desencadear eventos de sinaptotoxicidade e, ultimamente, atrofia e morte neural (POPOLI et al., 2011). Este fenômeno ocorre devido ao elevado influxo de cálcio mediado principalmente pelos receptores NMDA (MURROUGH; ABDALLAH; MATHEW, 2017). Dentro deste contexto, estudos robustos demonstraram um aumento de glutamato no córtex e plasma de pacientes diagnosticados com TDM, sugerindo que o excesso deste neurotransmissor poderia estar subjacente ao desenvolvimento deste transtorno (HASHIMOTO; SAWA; IYO, 2007; KÜÇÜKIBRAHIMOĞLU et al., 2009). Contudo, é conveniente enfatizar que pacientes com TDM também podem apresentar uma redução ou mesmo inalteração nos níveis de glutamato no cérebro, no líquido cefalorraquidiano e no plasma (MORIGUCHI et al., 2018; SANACORA et al., 2012).

É importante destacar que o glutamato desempenha um papel crucial na neuroplasticidade, a qual é definida como a capacidade do SNC de adaptar-se e moldar-se a nível estrutural e funcional quando sujeito a novas experiências (DUMAN; LI, 2012). A plasticidade sináptica em particular, um dos aspectos da neuroplasticidade, vem sendo cada vez mais estudadas nos últimos anos (DUMAN; DUMAN, 2014), e distúrbios neste processo têm sido observado no encéfalo de indivíduos diagnosticados com TDM (DURIC et al., 2013; HOLMES et al., 2019). O processo de plasticidade sináptica pode envolver a formação de novos espinhos dendríticos (espinogênese), alteração na morfologia e composição proteica dos espinhos dendríticos, eventos que são fundamentais para formação de novas sinapses (sinaptogênese) e fortalecimento das sinapses já existentes (DUMAN; DUMAN, 2014).

Um dos vários fenômenos que contribuem para a plasticidade sináptica é a potenciação de longa duração (LTP), a qual compreende o processo no qual estímulos elétricos de alta frequência desencadeiam mudanças estruturais e funcionais que geram uma resposta subsequente com maior magnitude (BALTACI; MOGULKOC; BALTACI, 2019; DUMAN; AGHAJANIAN, 2012). O fenômeno de LTP ocorre nas sinapses excitatórias maduras encontradas nos espinhos dendríticos, sendo mediado pela liberação de glutamato na fenda sináptica que ativa principalmente os receptores do tipo AMPA e NMDA (BALTACI; MOGULKOC; BALTACI, 2019; KIM; NA, 2016). Em particular, a ativação dos receptores AMPA resulta em um influxo de íons sódio que causam o aumento do potencial de membrana e despolarização do neurônio. Por sua vez, a despolarização acarreta na remoção do magnésio do poro do canal do receptor NMDA, e a ativação deste receptor ocorre em resposta à ligação da glicina (co-agonista) e do glutamato de forma concomitante à despolarização (HARDINGHAM, 2019). O influxo de íons cálcio via receptores NMDA levará à ativação de uma série de proteínas cinases, com destaque para o complexo cálcio/calmodulina cinase II (CaMKII) e a proteína cinase A (PKA) (HERRING; NICOLL, 2016; LISMAN; YASUDA; RAGHAVACHARI, 2012; YATES, 2016).

É conveniente enfatizar ainda que a LTP envolve a inserção de novos receptores AMPA na densidade pós-sináptica, e o tráfego destes receptores pode ser modulado pela fosforilação de suas subunidades (HERRING; NICOLL, 2016; WATT et al., 2004). Particularmente, a fosforilação dos sítios Ser^{831} e Ser^{845} na subunidade GluA1 promove o deslocamento dos receptores AMPA para a membrana plasmática. A fosforilação em Ser^{845} resultante da ação da PKA induz a inserção de receptores AMPA na membrana perissináptica por meio de mecanismos ainda não totalmente esclarecidos (PATRIARCHI; BUONARATI; HELL, 2018). Posteriormente, os receptores AMPA atingem a densidade pós-sináptica por difusão lateral, onde eles são estabilizados por meio da ancoragem da proteína de densidade pós-sináptica de 95kDa (PSD-95), um processo mediado pela proteína transmembrana reguladora de receptores AMPA (TARP) (PAYNE, 2008). Além disso, a CaMKII promove a fosforilação de GluA1 em Ser^{831} aumentando a atividade do canal (PATRIARCHI; BUONARATI; HELL, 2018). Desta forma, a PKA e CAMKII também podem fosforilar e modular a atividade das sinapsinas, proteínas pré-sinápticas responsáveis pela mobilização de vesículas sinápticas (SONG; AUGUSTINE, 2015).

A inserção de novos receptores AMPA na membrana plasmática amplifica o influxo de íons sódio, que por sua vez promove despolarização do neurônio, acarretando na ativação e

abertura dos canais de cálcio dependentes de voltagem (CCVD) (MATEOS-APARICIO; RODRÍGUEZ-MORENO, 2020). De especial interesse, o influxo de cálcio através dos CCVD leva à exocitose de vesículas sinápticas contendo o fator neurotrófico derivado do encéfalo (BDNF), uma neurotrofina com papel crucial no desenvolvimento e sobrevivência neural, bem como responsável pela formação, maturação e fortalecimento das sinapses (YOSHII; CONSTANTINE-PATON, 2010). A ativação dos receptores tropomiosina cinase B (TrkB) pelo BDNF culmina na ativação de vias de sinalização intracelular que regulam a síntese de novas proteínas sinápticas importantes para os processos de espinogênese e sinaptogênese (LEAL; BRAMHAM; DUARTE, 2017). De fato, evidências consistentes demonstraram que o BDNF regula o transporte de mRNA bem como as fases de iniciação e alongamento da síntese de proteínas nos dendritos, contribuindo para a LTP e plasticidade sináptica local (LEAL; COMPRIDO; DUARTE, 2014).

Particularmente, a ativação de TrkB resulta na autofosforilação e dimerização deste receptor, o qual desencadeia a fosforilação e ativação da fosfatidilinositol 3-cinase (PI3K). A fosforilação de PI3K promove o recrutamento da proteína cinase B (Akt) para a membrana plasmática, onde essa proteína será fosforilada (Ser^{473}) e ativada (LEAL; COMPRIDO; DUARTE, 2014; TEJEDA; DÍAZ-GUERRA, 2017). Por sua vez, a Akt fosforila a proteína substrato de Akt rico em prolina de 40 kDa (PRAS40 - Thr^{246}) que tem efeito inibitório sobre o complexo 1 da proteína alvo mecanístico da rapamicina (mTORC1) (HAAR et al., 2007). Assim o complexo se autofosforila (Ser^{2481}) e fosforila a proteína ribossomal S6 cinase de 70 kDa (p70S6K – Thr^{389}), a qual pode fosforilar o mTORC1 (Ser^{2448}), permanecendo em uma alça de fosforilação (MAGNUSON; EKIM; FINGAR, 2011). Subsequentemente, a p70S6K fosforila a subunidade menor do ribossomo (S6 – Ser^{236}), modulando positivamente a síntese de proteínas. Além disso, o mTORC1 pode fosforilar e reprimir a proteína 1 ligadora do fator de alongamento 4E (4E-BP1 – Thr$^{37/46}$), que tem efeito inibitório sobre a síntese de proteínas (HOEFFER; KLANN, 2010). Dentre as proteínas que tem sua síntese regulada pelo mTORC1, destacam-se as proteínas sinápticas GluA1, PSD-95 e sinapsina (DUMAN; VOLETI, 2012). Desta forma, o mTORC1 regula a síntese de proteínas envolvidas na formação e maturação de novos espinhos dendríticos, componentes essenciais para a sinaptogênese (DUMAN et al., 2012). Um esquema ilustrativo resumindo os mecanismos envolvidos no processo de sinaptogênese é mostrado na Figura 3.

Figura 3. Mecanismos envolvidos no processo de sinaptogênese
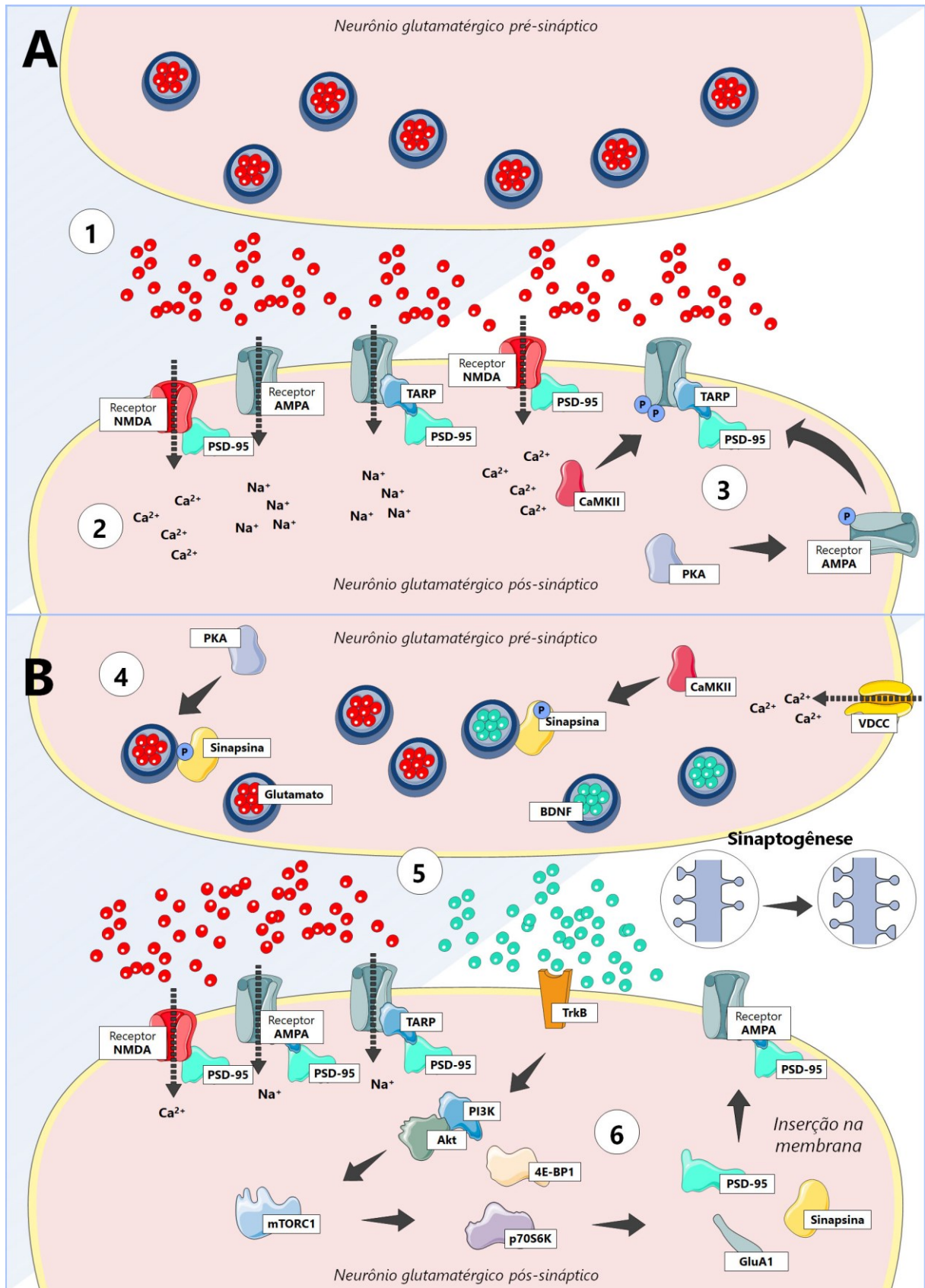
A LTP ocorre nas sinapses excitatórias maduras encontradas nos espinhos dendríticos após uma série de estímulos elétricos de alta frequência neurônio pré-sináptico (A). Estes potenciais de ação levam milissegundos para ativar

os CCVD, liberando cálcio no meio intracelular e favorecendo a exocitose de vesículas contendo glutamato (1). Em questão de segundos, o glutamato ativa os receptores AMPA resultando em um influxo de íons sódio que causam a despolarização do neurônio. Após alguns minutos, o influxo de íons cálcio via receptores NMDA levará à ativação de uma série de proteínas cinases, com destaque para a CaMKII e a PKA (2). A fosforilação em Ser845 resultante por PKA induz a inserção de receptores AMPA na membrana perissináptica. Posteriormente, os receptores AMPA atingem a densidade pós-sináptica por difusão lateral, onde eles são estabilizados por meio da ancoragem da PSD-95, um processo mediado pela TARP. A CaMKII promove a fosforilação de GluA1 em Ser831 aumentando a atividade do canal (3). Além disso, a PKA e CAMKII também podem fosforilar e modular a atividade das sinapsinas, proteínas pré-sinápticas responsáveis pela mobilização de vesículas sinápticas (4). A inserção de novos receptores AMPA na membrana plasmática amplifica o influxo de íons sódio, que por sua vez promove despolarização do neurônio, acarretando na ativação e abertura dos CCVD (B). O influxo de cálcio através dos CCVD leva à exocitose de vesículas sinápticas contendo o BDNF (5). A ativação dos receptores TrkB pelo BDNF culmina na ativação da via de sinalização mediada por PI3K/AKT/mTORC1 e p70S6K/4E-BP1 que regula a síntese de novas proteínas sinápticas (6). Dentre as proteínas que tem sua síntese regulada pelo mTORC1, destacam-se as proteínas sinápticas GluA1, PSD-95 e sinapsina, as quais são importantes para a formação de novos espinhos dendríticos e sinaptogênese. *4E-BP1: proteína 1 ligadora do fator de alongamento 4E; Akt: proteína cinase B; AMPA: alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiônico; BDNF: fator neurotrófico derivado do encéfalo; CaMKII: complexo cálcio/calmodulina cinase II; CCVD: cálcio dependentes de voltagem; GluA1: subunidade 1 de receptores AMPA; LTP: potenciação de longa duração; mTORC1: complexo 1 da proteína alvo mecanístico da rapamicina; NMDA: N-metil-D-aspartato; p70S6K: proteína ribossomal S6 cinase de 70 kDa; PI3K: fosfatidilinositol 3-cinase; PKA: proteína cinase A; PSD-95: proteína de densidade pós-sináptica de 95kDa; TARP: proteína transmembrana reguladora de receptores AMPA; TrkB: tropomiosina cinase B.* Figura elaborada usando imagens do Mind the Graph e Servier Medical Art. Fonte: autor.

Após 1 a 2 horas da indução da LTP, ocorre a formação de novos espinhos dendríticos ou a maturação dos espinhos pré-existentes, e consequentemente a sinaptogênese (DUMAN; AGHAJANIAN, 2012; DUMAN; LI, 2012). Os processos de espinogênese e consequentemente de sinaptogênese configuram-que como eventos complexos caracterizados por mudanças neuroquímicas e morfológicas em elementos pré e/ou pós-sinápticos (CITRI; MALENKA, 2007; HARRIS, 2020). Os espinhos dendríticos são pequenas protuberâncias especializadas que se localizam nos dendritos neuronais e configuram-se como componentes pós-sinápticos da maioria das sinapses excitatórias (CITRI; MALENKA, 2007; QIAO et al., 2016).

Pesquisas substanciais demonstraram que a densidade e a remodelação da morfologia dos espinhos dendríticos desempenham um papel funcional crucial na plasticidade sináptica (CHIDAMBARAM et al., 2019; SEGAL, 2005; YUSTE, 2011). Morfologicamente, o aspecto estrutural de um espinho dendrítico é definido pela presença de uma cabeça, a qual possui componentes pós-sinápticos, conectada às hastes dendríticas por uma estrutura denominada de pescoço (NISHIYAMA, 2019; SEGAL, 2005). Os espinhos dendríticos são enriquecidos de F-actina, uma molécula responsável pela manutenção da estrutura, formato e estabilidade do espinho, além da organização da maquinaria da sinalização pós-sináptica (BASU; LAMPRECHT, 143AD; HOTULAINEN; HOOGENRAAD, 2010). É bem estabelecido que a

morfogênese e o remodelamento dos espinhos dendríticos é dependente da atividade sináptica (LIPPMAN; DUNAEVSKY, 2005).

Em relação à morfogênese dos espinhos dendríticos, tem sido observado que espinhos com forma semelhante a dedos, chamados de 'filopódios', emergem dos dendritos e formam sinapses com axônios adjacentes (BOSCH; HAYASHI, 2012). Inicialmente, o contato entre dois neurônios é dependente de moléculas de adesão, as quais são fundamentais para a formação das sinapses. Dentre essas moléculas, destacam-se as neurexinas e as neuroliginas (THALHAMMER; CINGOLANI, 2014). Particularmente, as neurexinas ligam-se a sítios específicos da proteína serina cinase dependente de cálcio (CASK), uma proteína de ancoragem, promovendo o recrutamento da maquinaria pré-sináptica (GOMEZ; TRAUNMÜLLER; SCHEIFFELE, 2021; SÜDHOF, 2017). Por sua vez, as neuroliginas ligam-se à PSD-95 estimulando o recrutamento de receptores NMDA e AMPA para a densidade pós-sináptica (SÜDHOF, 2018; WU et al., 2019). É importante destacar que estes eventos favorecem a estabilidade do citoesqueleto, um processo de fundamental importância para a maturação da sinapse recém-desenvolvida (BASU; LAMPRECHT, 143AD; CHIDAMBARAM et al., 2019).

Subsequentemente, mudanças na composição proteica do espinho dendríticos em crescimento passam a ser observadas, particularmente proteínas envolvidas com a maturação do mesmo (BOSCH; HAYASHI, 2012). Após o contato sináptico, acredita-se que os espinhos tipo filopódio se transformam morfologicamente em espinhos com sinapses mais funcionais (CHIDAMBARAM et al., 2019). De especial interesse, os filopódios podem sofrer alterações morfológicas formando espinhos do tipo fino (do inglês *thin*) ou achatado (do inglês *stubby*), os quais apresentam pequenas regiões de densidade pós-sinápticas e são considerados estruturas imaturas (BERRY; NEDIVI, 2017; CITRI; MALENKA, 2007). No entanto, o processo final de maturação do espinho dendrítico envolve mudanças na composição proteica da densidade pós-sináptica e no citoesqueleto acarretando na alteração estrutural da arquitetura do espinho, particularmente gerando uma forma mais estável e madura, a qual é denominada de espinho do tipo cogumelo (do inglês *mushrroom*) (BOSCH; HAYASHI, 2012; HELM et al., 2021). A partir de então, o espinho dendrítico do tipo cogumelo pode se integrar com outros espinhos dendríticos de neurônios adjacentes, aprimorando e fortalecendo a transmissão sináptica (CHIDAMBARAM et al., 2019).

1.3 REGIÕES ENCEFÁLICAS ENVOLVIDAS NO TDM

É bem estabelecido que disfunções na homeostase dendrítica, incluindo a redução na densidade, alteração na morfologia e composição proteica dos espinhos dendríticos, contribuem para a conectividade disfuncional nos circuitos sinápticos e atrofia sináptica (DUMAN; DUMAN, 2014; LICZNERSKI; DUMAN, 2013). De especial interesse, tem sido evidenciado que estas disfunções estão majoritariamente presentes no córtex pré-frontal e na formação hipocampal, duas regiões encefálicas envolvidas, dentre outras funções, com a modulação do humor (GODSIL et al., 2013). Portando, este trabalho destacará o córtex pré-frontal e a formação hipocampal.

A região hipocampal, em particular, é constituída pela formação hipocampal e a região parahipocampal, diferindo no número de camadas e características celulares e de conectividade (SCHULTZ; ENGELHARDT, 2014). A formação hipocampal dos mamíferos é dividida nas áreas *Cornu Ammonis* (CA1), CA2, CA3 e uma sub-região denominada de giro denteado (HANNULA; DUFF, 2017; SCHARFMAN, 2016; SENZAI, 2019). Tem sido postulado que a formação hipocampal parece ter duas porções funcionalmente distintas, nomeadamente a porção dorsal e a porção ventral. Evidências sugerem que a porção ventral parece estar associada à modulação do estresse, emoção e afeto, enquanto a zona dorsal desempenha principalmente funções cognitivas (BANNERMAN et al., 2004; FANSELOW; DONG, 2010). De especial interesse, o giro denteado é uma estrutura com três camadas, sendo a primeira delas denominada camada molecular; a segunda camada corresponde a camada granular e a terceira camada é chamada de polimórfica ou hilar. Entre a camada granular e acima do hilo encontra-se a zona subgranular, um dos principais nichos neurogênicos do cérebro adulto de mamíferos (HANNULA; DUFF, 2017; MOSER; MOSER; MCNAUGHTON, 2017; TANTI; BELZUNG, 2013). Além disso, vale ressaltar que essa organização anatômica é semelhante em roedores e primatas (HANNULA; DUFF, 2017; SCHULTZ; ENGELHARDT, 2014).

A camada molecular é composta basicamente por dendritos das células granulares que estão na zona granular. A camada granular, por sua vez, compreende os corpos celulares dos neurônios granulares propriamente ditos, os quais estão orientadas de forma estereotipada. Por último, a camada hilar ou polimórfica é composta pelas fibras musgosas (FANSELOW; DONG, 2010; HANNULA; DUFF, 2017). É conveniente enfatizar ainda que, as fibras musgosas se projetam das camadas hilar e granular para a região CA3, enquanto da região CA3 outras fibras são projetadas para a região CA1, formando as vias colaterais de Schaffer. Adicionalmente, da

região CA1 são enviadas projeções para o córtex entorrinal e para o subículo (SCHULTZ; ENGELHARDT, 2014). Por sua vez, as fibras que deixam o córtex entorrinal em direção ao hipocampo constituem a chamada via perfurante e inervam os dendritos das células granulares na região da camada molecular do giro denteado. Por fim, é importante destacar que esta circuitaria utiliza o glutamato como principal neurotransmissor e é denominada via tri-sináptica (STRANGE et al., 2014).

O córtex pré-frontal, por sua vez, é uma região encefálica crítica para muitas habilidades cognitivas e executivas, como atenção e tomada de decisão, além de configurar-se como um sistema neural essencial para o funcionamento social e emocional (DIXON et al., 2017; TEFFER; SEMENDEFERI, 2012). Embora muitos estudos de neuroimagem buscam estabelecer a exata localização funcional e a divisão do córtex pré-frontal, as suas subdivisões e extensão podem variar. Contudo, postula-se que o córtex pré-frontal compreenda o córtex infralímbico, córtex pré-límbico, córtex orbital medial, córtex orbital ventral, córtex orbital dorsolateral e córtex orbital lateral (CARLÉN, 2017). Do ponto de vista morfo-comparativo, vale destacar que o córtex pré-frontal em primatas é totalmente homotípico, isto é, exibe uma estrutura interna de seis camadas, enquanto o córtex pré-frontal em roedores é heterotípico, ou seja, apresenta ausência da camada granular quatro (CARLÉN, 2017).

Por fim, vale ressaltar que diversos estudos mostraram que distúrbios na conectividade sináptica e atrofia neuronal foram observados no córtex pré-frontal e na formação hipocampal de indivíduos diagnosticados com TDM (BOLDRINI et al., 2013; DURIC et al., 2013; FEYISSA et al., 2009; HOLMES et al., 2019; HUANG et al., 2013; KANG et al., 2012; NUNINGA et al., 2019; RAFALO-ULINSKA et al., 2016). Essas evidências sugerem que estratégias direcionadas ao remodelamento da plasticidade sináptica na formação hipocampal e córtex pré-frontal podem apresentar respostas antidepressivas.

1.4 CETAMINA

Um avanço significativo para o tratamento do TDM foi a descoberta de que uma única administração com uma dose subanestésica de cetamina, um antagonista do receptor NMDA, produz respostas antidepressivas rápidas e duradouras em pacientes com TDM, mesmo naqueles com ideação suicida (BERMAN et al., 2000; ZARATE et al., 2006). A cetamina é um derivado das fenciclidinas (pó de anjo), vendida comercialmente como uma mistura racêmica de dois isômeros (Figura 4), a R-cetamina e a S-cetamina (KURDI; THEERTH; DEVA, 2014).

O isômero S-cetamina apresenta uma afinidade de 3 a 4 vezes maior pelos receptores NMDA do que isômero R-cetamina. É conveniente enfatizar que na clínica esta molécula é frequentemente utilizada como anestésico, devido a sua capacidade de produzir inconsciência e imobilidade (GAO; REJAEI; LIU, 2016).

Figura 4. Estrutura química da cetamina
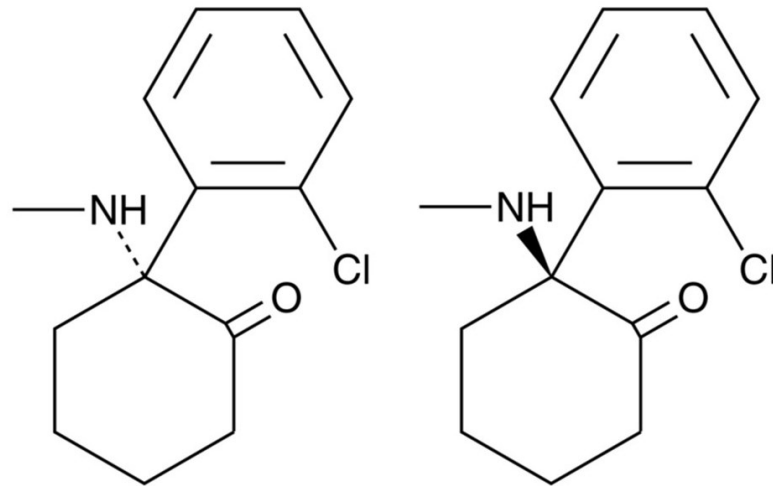
A cetamina é um fármaco derivado das fenciclidinas, sintetizado a partir do brometo de ciclopentilo, o-clorobenzonitrilo e metilamina. Comercialmente, este fármaco é utilizado como uma mistura racêmica de dois isômeros opticamente ativos, a R-cetamina (esquerda) e a S-cetamina (direita). Fonte: PubChem CID: 3821.

Particularmente, Berman et al. (2000) demonstraram que uma única administração endovenosa e subanestésica de cetamina produziu um rápido efeito antidepressivo em pacientes com TDM, uma resposta que foi observada dentro de 4 horas enquanto um efeito progressivo foi também evidenciado por até 3 dias. Notavelmente, este efeito foi reforçado e expandido por Zarate et al. (2006), os quais demonstraram que uma única administração subanestésica de cetamina promoveu um efeito antidepressivo rápido (dentro de 2 horas) e sustentado (por até 7 dias) em pacientes refratários ao tratamento convencional. Essas descobertas despertaram um interesse significativo da comunidade científica em estudar os mecanismos subjacentes às ações antidepressivas da cetamina. Evidências robustas têm demonstrado que a cetamina exerce seu efeito antidepressivo por promover a formação de espinhos dendríticos e sinaptogênese no hipocampo (FRAGA et al., 2020, 2021) e no córtex pré-frontal (LI et al., 2010, 2011) de roedores, regiões encefálicas envolvidas na modulação do humor (LIU et al., 2017).

Do ponto de vista mecanístico (Figura 5), o efeito antidepressivo da cetamina parece ser desencadeado por meio do antagonismo dos receptores NMDA em interneurônios

GABAérgicos, impedindo a ação inibitória deste sistema sobre o tônus glutamatérgico, em função da diminuição do influxo de íons cloreto pelos receptores $GABA_A$ (ABDALLAH et al., 2016). Os neurônios glutamatérgicos promovem a liberação de glutamato na fenda sináptica, o qual ativa preferencialmente os receptores AMPA, visto que os receptores NMDA ainda se encontram bloqueados pela cetamina. Uma vez ativados, os receptores AMPA promovem um influxo transitório de íons sódio, aumentando o potencial de membrana com consequente ativação e abertura dos CCVD (DUMAN et al., 2012). A entrada de cálcio através dos CCVD, promove a exocitose de vesículas sinápticas contendo o BDNF, que então pode ativar seus receptores TrkB (LEPACK et al., 2014; CASAROTTO et al., 2021).

Figura 5. Vias de sinalização implicadas nos efeitos antidepressivo e pró-sinaptogênico desencadeados pela cetamina
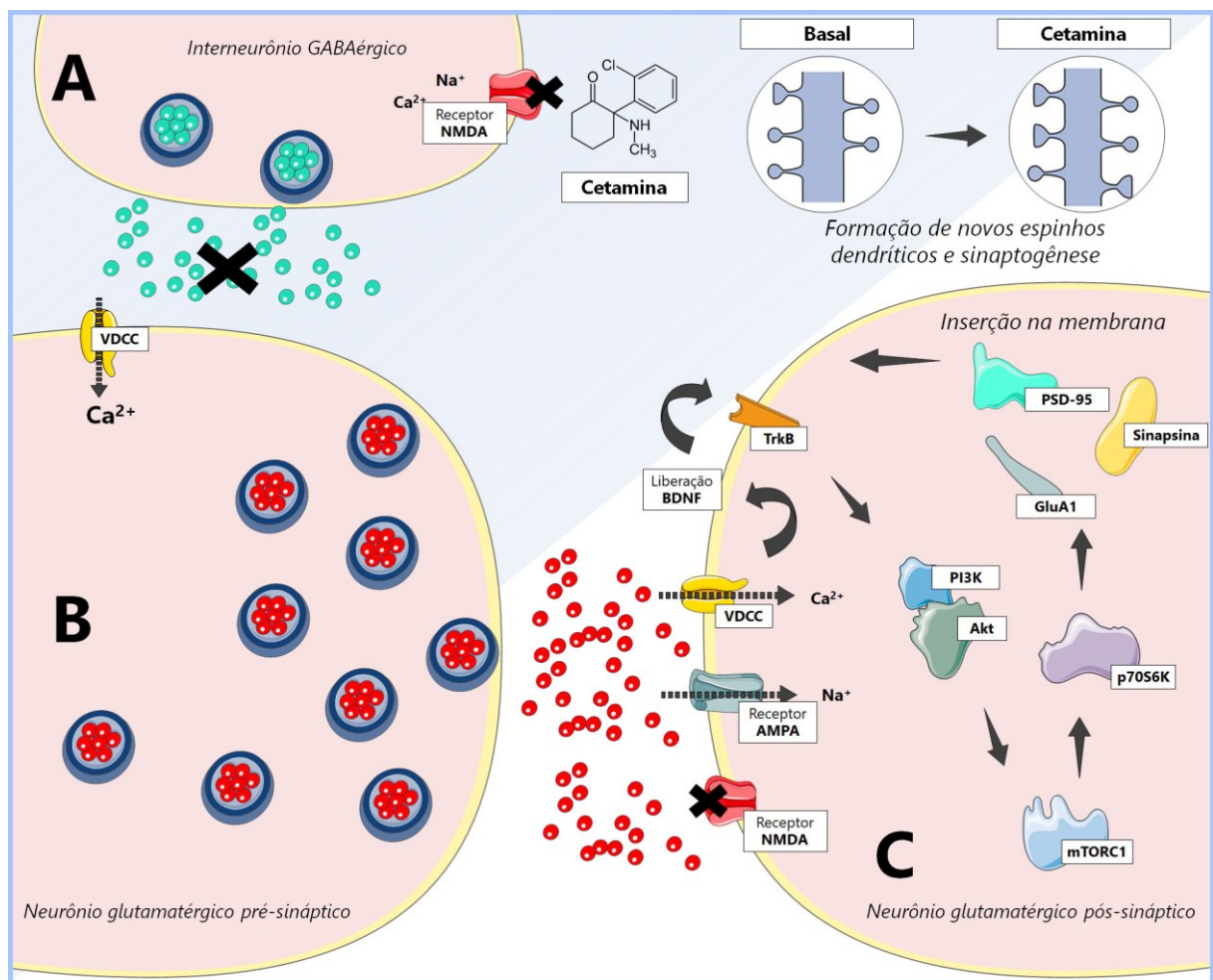
A cetamina, ao promover antagonismo dos receptores NMDA em interneurônios GABAérgicos (A), impede a ação inibitória deste sistema sobre o tônus glutamatérgico, resultando na liberação de glutamato na fenda sináptica (B). O glutamato ativa preferencialmente os receptores AMPA, que promovem um influxo transitório de cargas positivas aumentando o potencial de membrana com consequente ativação e abertura dos CCVD. A entrada de cálcio promove a exocitose de BDNF, que ativa os receptores TrkB. A ativação destes receptores culmina na

estimulação da via de sinalização PI3K/Akt/mTORC1/p70S6K que promove a síntese de proteínas sinápticas como a PSD-95, GluA1 e a sinapsina (C), as quais são requeridas para espinogênese e sinaptogênese. *Akt: proteína cinase B; AMPA: alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiônico; BDNF: fator neurotrófico derivado do encéfalo; CCVD: cálcio dependentes de voltagem; GluA1: subunidade 1 de receptores AMPA; mTORC1: complexo 1 da proteína alvo mecanístico da rapamicina; NMDA: N-metil-D-aspartato; p70S6K: proteína ribossomal S6 cinase de 70 kDa; PI3K: fosfatidilinositol 3-cinase; PSD-95: proteína de densidade pós-sináptica de 95kDa; TrkB: tropomiosina cinase B.* Figura elaborada usando imagens do Mind the Graph e Servier Medical Art. Fonte: autor.

Os receptores TrkB provocam a estimulação da via de sinalização intracelular mediada por PI3K/Akt que culmina na inibição da proteína glicogênio sintase cinase-3β (GSK-3β) e ativação do mTORC1 (LI et al., 2010; LIU et al., 2013). Conforme mencionado anteriormente, o mTORC1 regula a etapa inicial da síntese de proteínas requeridas para a formação de novos espinhos dendríticos e sinaptogênese. Particularmente, a ativação de mTORC1 controla a síntese das proteínas sinápticas PSD-95, subunidades de receptores AMPA GluA1 e sinapsina, via fosforilação e ativação da p70S6K (ABDALLAH; AVERILL; KRYSTAL, 2015). De fato, a liberação de BDNF, a ativação da via de sinalização mediada por mTORC1 e o aumento sinaptogênese desempenham um papel crucial para respostas antidepressivas rápidas e sustentadas (DUMAN et al., 2012). Portanto, as estratégias direcionadas ao aumento da sinaptogênese via mTORC1 podem produzir respostas antidepressivas de ação rápida e duradoura.

Vale destacar que quase 20 anos após a descoberta do rápido efeito antidepressivo da cetamina, em março de 2019, o FDA (*Food and Drug Administration*) dos EUA aprovou o uso de spray nasal de (S)-cetamina (Spravato ™) para o tratamento de pacientes com TDM refratário (WEI; CHANG; HASHIMOTO, 2020). No mesmo ano, o spray nasal de (S)-cetamina foi aprovado para uso em pacientes com TDM resistente ao tratamento na Europa. No Brasil, a Agência Nacional de Vigilância Sanitária (Anvisa) aprovou o uso do Spravato ™ para pacientes com depressão refratária no final do ano de 2020. No entanto, a principal limitação referente ao uso da cetamina está associada aos efeitos colaterais indesejáveis que ela pode produzir, notadamente efeitos psicotomiméticos e dissociativos, além de seu potencial de abuso (GAO; REJAEI; LIU, 2016). Portanto, a investigação de novos antidepressivos que apresentam ações semelhantes às da cetamina, mas que são desprovidos de efeitos adversos, são um dos principais focos de desenvolvimento de drogas no campo de pesquisa da neuropsicofarmacologia (ABDALLAH; AVERILL; KRYSTAL, 2015).

Apesar dos resultados encorajadores com outros moduladores do canal do receptor NMDA em estudos pré-clínicos, essas drogas não foram capazes de exercer todo o espectro de ações da cetamina em ensaios clínicos (NEWPORT et al., 2015; POCHWAT; NOWAK;

SZEWCZYK, 2019). Diante desse cenário, estratégias que possam aumentar as respostas antidepressivas da cetamina com menor potencial para efeitos indesejáveis são bem-vindas. As estratégias de aumento (*augmentation strategies*) que consistem na utilização da medicação antidepressiva combinada com agentes não antidepressivos podem ser uma ferramenta interessante para o tratamento do TDM quando o medicamento de primeira escolha tem limitações (HAN et al., 2014; PAPAKOSTAS et al., 2005; PARK, 2020; ZHOU et al., 2019). A terapia de aumento apresenta vantagens em relação à monoterapia, uma vez que pode induzir a uma remissão mais significativa dos sintomas depressivos (MALHI et al., 2020; MORET, 2005; ZHOU et al., 2015). Esta estratégia também permite a redução da dose do antidepressivo, o que poderia reduzir seus efeitos adversos (BAROWSKY; SCHWARTZ, 2006; MORET, 2005).

1.5 ESTRESSE E RESILIÊNCIA

O estresse configura-se como uma reação comum frente a uma adversidade, e a exposição do indivíduo a um agente estressor promove a ativação do eixo hipotálamo-hipófise-adrenal (HHA), o qual estimula o organismo a iniciar respostas adaptativas e de enfrentamento para manter a homeostase (KELLER et al., 2017; MCEWEN, 2007; RIBONI; BELZUNG, 2017). Neste contexto, em situações de estresse o núcleo paraventricular do hipotálamo é estimulado a secretar o hormônio liberador de corticotrofina (CRH) que irá, por sua vez, estimular a liberação de hormônio adrenocorticotrófico (ACTH) pela hipófise (também conhecida como pituitária) anterior ou adeno-hipófise. O ACTH liberado na corrente sanguínea estimulará a secreção de cortisol (ou corticosterona em roedores) pelo córtex das glândulas adrenais (INCOLLINGO RODRIGUEZ et al., 2015). Um esquema mostrando o funcionamento do eixo HHA está ilustrado na Figura 6.

Figura 6. O eixo HHA
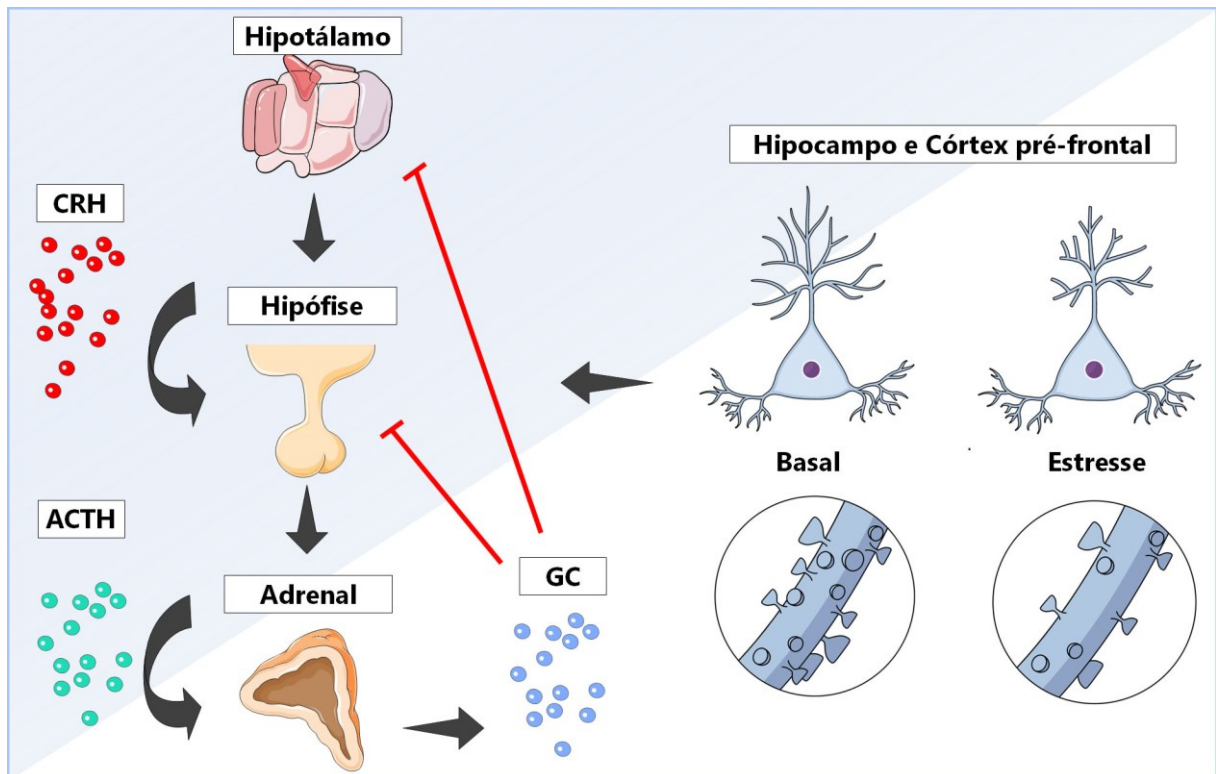
Em situações de estresse o núcleo paraventricular do hipotálamo é estimulado a secretar o CRH que irá, por sua vez, estimular a liberação de ACTH pela hipófise (também conhecida como pituitária) anterior ou adeno-hipófise. O ACTH liberado na corrente sanguínea estimulará a secreção de GC como cortisol em humanos ou corticosterona em roedores pelas glândulas adrenais. O cortisol é um hormônio esteroide que irá atuar em diversos tecidos corporais desencadeando diversas funções, principalmente relacionadas ao comportamento de luta ou fuga. O cortisol ou a corticosterona (roedores) também exercem uma influência inibitória sobre o eixo HHA através de um sistema de retroalimentação negativa, atuando a nível do núcleo paraventricular e da hipófise. A regulação do eixo HHA também parece ser dependente do hipocampo e do córtex pré-frontal, estrutura encefálicas que expressam grande densidade de receptores glicocorticoides. Contudo, o estresse crônico pode levar ao aumento dos níveis plasmáticos de glicocorticoides, que podem causar danos aos neurônios do hipocampo e córtex pré-frontal, incluindo déficits sinápticos, atrofia e morte neuronal. Em conjunto, estes eventos reduzem o mecanismo de retroalimentação negativa e amplificam a disfunção do eixo HHA, eventos que parecem estar subjacentes ao desenvolvimento do TDM. *ACTH: hormônio adrenocorticotrófico; CRH: hormônio liberador de corticotrofina; GC: glicocorticoides*. Figura elaborada usando imagens do Mind the Graph e Servier Medical Art. Fonte: autor.

O cortisol é um hormônio esteroide sintetizado a partir da molécula de colesterol que irá atuar em diversos tecidos corporais desencadeando diversas funções, principalmente relacionadas ao comportamento de luta ou fuga. É importante destacar que estas ações são mediadas por meio da interação com receptores específicos, os receptores de mineralocorticoides e os receptores de glicocorticoides (GROENEWEG et al., 2012; JURUENA, 2014). Os receptores de mineralocorticoides que recebem esta denominação por terem alta afinidade pela aldosterona, possuem uma alta afinidade pelo cortisol, portanto, são ativados mesmo quando os níveis deste glicocorticoide estão baixos (JOËLS; KARST; SARABDJITSINGH, 2018). Contudo, os receptores de glicocorticoides que apresentam uma

alta afinidade por glicocorticoides sintéticos, apresentam uma afinidade mais baixa pelo cortisol, sendo ativados apenas quando as concentrações deste hormônio estão elevadas (KADMIEL; CIDLOWSKI, 2013). Os receptores de glicocorticoide localizam-se no citoplasma em seu estado de repouso, e nestas condições eles permanecem acoplados a um complexo multimérico de proteínas chaperonas incluindo as proteínas de choque térmico (HSP56 e HSP90) e imunofilinas. Após a interação com os glicocorticoides, esses receptores são ativados e se dissociam do complexo de proteínas chaperonas e imunofilinas, resultando na sua translocação para o núcleo, onde controlam a expressão gênica (KADMIEL; CIDLOWSKI, 2013). Além disso, as vias de sinalização medidas pelo receptor de glicocorticoide culmina na ativação de mecanismos de resposta rápida (não-genômicos) ou de resposta lenta (através de alterações na transcrição gênica), os quais contribuem coletivamente para uma adaptação comportamental bem-sucedida (JOËLS; PASRICHA; KARST, 2013).

É importante destacar ainda que os glicocorticoides também exercem uma influência inibitória sobre o eixo HHA, particularmente atuando a nível do núcleo paraventricular e da hipófise, através de um sistema de retroalimentação negativa (GJERSTAD; LIGHTMAN; SPIGA, 2018). Além destas alças de controle inibitório, a regulação do eixo HHA também parece ser dependente do hipocampo e do córtex pré-frontal, estruturas encefálicas que expressam grande densidade de receptores glicocorticoides (JACOBSON; SAPOLSKY, 1991; MCKLVEEN et al., 2013; MIZOGUCHI et al., 2003). Contudo, o estresse crônico pode levar ao aumento dos níveis plasmáticos de glicocorticoides, que podem causar danos aos neurônios do hipocampo e córtex pré-frontal e reduzir o mecanismo de retroalimentação negativa do eixo HHA (JOËLS et al., 2009; LEE; OGLE; SAPOLSKY, 2002; MIZOGUCHI et al., 2003). De fato, evidências têm demonstrado de forma robusta que modelos de estresse crônico são capazes de promover disfunções no eixo HHA bem como déficits sinápticos, atrofia e morte neuronal na formação hipocampal e no córtex pré-frontal de roedores (ALFAREZ et al., 2009; DALMAGRO et al., 2020; FRAGA et al., 2021; LI et al., 2011; PAZINI et al., 2016), eventos que parecem estar subjacentes ao desenvolvimento de comportamento tipo-depressivo.

Reforçando essa premissa, evidências convincentes demonstraram que o estresse e disfunção do eixo HHA configuram-se como fatores de riscos para o desenvolvimento do TDM (JURUENA, 2014; OTTE et al., 2016; PARIANTE; LIGHTMAN, 2008). De forma consistente e robusta, demonstrou-se previamente que cerca de 50% dos pacientes acometidos pelo TDM apresentam níveis aumentados de cortisol (DETTENBORN et al., 2012; GOODYER et al., 2000; HARRIS et al., 2000; KNORR et al., 2010; STETLER; MILLER, 2011), bem como

disfunções no eixo HHA (FRODL; O'KEANE, 2013; GOLD, 2015; MARIC; ADZIC, 2013). Além disso, indivíduos com TDM apresentam uma redução na função dos receptores de glicocorticoide (ANACKER et al., 2011), bem como déficits sinápticos e atrofia neuronal no córtex pré-frontal e no hipocampo (BOLDRINI et al., 2013; DURIC et al., 2013; FEYISSA et al., 2009; HOLMES et al., 2019; KANG et al., 2012; RAFALO-ULINSKA et al., 2016). Desta forma, postula-se o estresse crônico pode causar más adaptações moleculares e celulares que contribuem para o desenvolvimento do TDM (KLOET; JOËLS; HOLSBOER, 2005; MCEWEN, 2007).

Embora seja bem estabelecido que o estresse comumente precipite o TDM, nem todos os indivíduos expostos a agentes estressores desenvolvem este transtorno, isto é, alguns indivíduos são suscetíveis enquanto outros são resilientes (FAYE et al., 2018; RUSSO et al., 2012). A resiliência é definida como o processo de adaptação frente a situações adversas e estressoras, visando o manter a funcionalidade homeostática física e mental (HAN; NESTLER, 2017). Apesar de alguns estudos apontarem fatores que promovem a suscetibilidade a doenças psiquiátricas induzidas pelo estresse, poucos estudos investigaram o que torna os indivíduos resilientes (FEDER; NESTLER; CHARNEY, 2009). Até recentemente, os estudos sobre a resiliência ao estresse foram baseados na premissa de que esta é uma propriedade passiva, relacionada a ausência dos fatores de risco que tornam os indivíduos suscetíveis (RUSSO et al., 2012). No entanto, modelos animais sugerem que a resiliência ao estresse pode ser também mediada por processos ativos, por mecanismos distintos e paralelos aos da suscetibilidade (BRACHMAN et al., 2016; CAMARGO et al., 2021; KRISHNAN et al., 2007; LAGAMMA et al., 2018; PARISE et al., 2021).

É importante enfatizar ainda que embora uma extensa pesquisa tenha focado em tratamentos farmacológicos que possam neutralizar as patologias relacionadas ao estresse, poucos estudos investigaram se os tratamentos farmacológicos poderiam aumentar a resiliência frente a agentes estressores (GURURAJAN et al., 2019). Com base neste cenário, aumentar a resiliência ao estresse poderia proteger contra o desenvolvimento de transtornos psiquiátricos, como o TDM (CATHOMAS et al., 2019), mas estratégias terapêuticas para esta finalidade ainda são poucos estudadas. Além disso, a identificação das vias de sinalização e alvos moleculares subjacentes à resiliência ao estresse poderia sustentar o desenvolvimento de novas abordagens preventivas, especialmente para populações vulneráveis em risco de desenvolver TDM.

Embora a descoberta da cetamina como antidepressivo rápido tenha marcado a mudança na história do desenvolvimento de agentes antidepressivos, estudos inovadores mostraram sua capacidade de produzir efeito profilático e promotor de resiliência frente ao estresse em roedores (BRACHMAN et al., 2016; MASTRODONATO et al., 2018; MCGOWAN et al., 2018) e humanos (MA et al., 2019). O efeito pro-resiliência da cetamina foi observado em protocolos experimentais nos quais ela foi administrada 7 dias antes do início da exposição crônica ao estresse, incluindo os modelos de estresse de derrota social, paradigma de desamparo aprendido, estresse crônico e imprevisível e estresse crônico de restrição (BRACHMAN et al., 2016; CHEN et al., 2020; KRZYSTYNIAK et al., 2019; MASTRODONATO et al., 2018; PARISE et al., 2021). Além disso, a administração profilática de cetamina foi capaz de aumentar a resiliência contra o comportamento do tipo-depressivo induzido por estressores inflamatórios, particularmente o lipopolissacarídeo (LPS) e fator de necrose tumoral-alfa (TNF-α) (CAMARGO et al., 2021; MASTRODONATO et al., 2020). Também reportou-se que a cetamina pode atenuar uma resposta ao medo em um paradigma de medo condicionado ao contexto quando administrada uma semana antes, mas não quando administrada imediatamente antes ou depois de um episódio indutor de estresse (MCGOWAN et al., 2017). Estes achados reforçam a noção de que a cetamina apresenta uma janela de eficácia terapêutica muito além da sua meia-vida de algumas horas e é capaz de desencadear uma cascata de eventos que se sustenta por vários dias (BRACHMAN et al., 2016). Mais importante, estes resultados também sugerem que a cetamina pode emergir como uma nova estratégia potencial para reduzir a incidência e manejar os pacientes sob condições de alto estresse em risco de desenvolver TDM.

Pesquisas subsequentes reportaram que o mecanismo de ação profilático da cetamina parece ser associado ao aumento da formação de espinhos dendríticos e aumento da eficácia sináptica na região CA3 da formação hipocampal (KRZYSTYNIAK et al., 2019; MASTRODONATO et al., 2018) e no córtex pré-frontal (DOLZANI et al., 2018; KRZYSTYNIAK et al., 2019). É importante destacar, que o efeito profilático da cetamina, ao contrário do efeito antidepressivo, parece não envolver o aumento de processo de neurogênese (LAGAMMA et al., 2018). Além disso, o fenótipo pro-resiliente induzido pela administração profilática frente aos estressores inflamatórios LPS e TNF-α foi associado a supressão da via de sinalização mediada pelo inflamassoma NLRP3 (receptor do tipo NOD com domínio pirina 3) no hipocampo de camundongos (CAMARGO et al., 2021). É interessante observar que um estudo recente forneceu evidências de que os mecanismos subjacentes ao efeito profilático e

promotor de resiliência desencadeados pela cetamina é semelhante aos observados em sua resposta antidepressiva rápida, particularmente a ativação da via da sinalização mediada por Akt/mTORC1 com consequentemente aumento da eficácia sináptica (PARISE et al., 2021). Portanto, esses achados sugerem que a via de sinalização mediada por mTORC1 pode ser um alvo subjacente ao aumento da resiliência contra transtornos relacionados ao estresse. De especial interesse, demonstrou-se que o efeito profilático e promotor de resiliência da cetamina parece ser associado a modulação no sistema purinérgico, sendo que o aumento das formas mono e difosfatadas de guanosina poderiam estar subjacentes a este efeito (MCGOWAN et al., 2018). Essa evidência sugere que o sistema purinérgico poderia ser um componente importante para respostas promotoras de resiliência.

1.6 SISTEMA PURINÉRGICO

O sistema purinérgico constitui-se como um complexo sistema celular que é crucial para diversas funções fisiológicas. De especial interesse, as purinas que compõem o sistema purinérgico podem desempenhar uma série de funções, tais como a estruturação do ácido desoxirribonucleico (DNA) e RNA, como componentes do metabolismo energético e segundos mensageiros nos mecanismos de transdução de sinal, bem como neurotransmissores e neuromoduladores (BURNSTOCK, 2020). Desta forma, as purinas apresentam um papel fundamental em diversos processos fisiológicos, incluindo respostas imunes, inflamação, dor, mecanismos de sinalização intracelular e extracelular, proliferação e morte celular (BURNSTOCK, 2016; HEINE; SYGNECKA; FRANKE, 2016; MIRAS-PORTUGAL et al., 2016; OLIVEIRA; ILLES; ULRICH, 2016; RIBEIRO et al., 2016; RODRIGUES; MARQUES; CUNHA, 2019).

Em 1972, postulou-se pela primeira vez o conceito de sinalização purinérgica, no qual sugeriu-se que o ATP poderia agir como neurotransmissor (BURNSTOCK, 1972). Inicialmente, esta ideia gerou uma série de críticas já que o papel energético do ATP estava bem estabelecido. Consistentemente, estudos pioneiros demonstraram a presença de receptores transmembrana para o sistema purinérgico, os quais foram categorizados em dois grandes grupos de acordo com seus ligantes. Os purinoceptores do tipo 1 (P1) possuem a adenosina como ligante enquanto, os receptores purinérgicos do tipo 2 (P2) interagem com o ATP ou ADP (BURNSTOCK, 2007). Particularmente, os receptores P1 configuram-se como receptores metabotrópicos e são divididos em quatro subtipos: A_1, $A_{2}A$, $A_{2}B$ e A_3. Em particular, os

purinoceptores A_1 são acoplados à proteína G inibitória levando a uma diminuição da atividade da adenilato ciclase e consequente diminuição nos níveis de AMP cíclico intracelular. Contrariamente, os purinoceptores A_2A são acoplados à proteína G estimulatória, levando ao aumento dos níveis de AMP cíclico. Os receptores P2 são subdivididos em P_2X (receptores ionotrópicos) e P_2Y (receptores metabotrópicos) (BURNSTOCK, 2020). É importante destacar que os receptores A_1 e A_2A são amplamente distribuídos no SNC sendo encontrados nos terminais pré-sinápticos e pós-sinápticos em diferentes regiões do cérebro e são os principais responsáveis pelos efeitos centrais da adenosina, enquanto os receptores A_2B e A_3 parecem ter uma menor expressão no SNC (BURNSTOCK, 2008).

Vale destacar ainda que embora um grande conjunto de evidências tenha demonstrado o importante papel fisiológico das purinas à base de adenina, os derivados da guanina têm recebido menos atenção nesta perspectiva. Além disso, apesar do importante papel dos derivados da guanina como componentes das cascatas de sinalização intracelular, evidências robustas têm evidenciado que os derivados da guanina podem também atuar como moléculas de sinalização extracelular (SCHMIDT; LARA; SOUZA, 2007). Dentre os derivados da guanina, destaca-se o nucleosídeo guanosina (Figura 7). A principal fonte de guanosina é sua produção por astrócitos, os quais liberam nucleotídeos da guanina no espaço extracelular que são rapidamente catabolizados pelas ecto-5'-nucleotidases, produzindo este nucleosídeo (CICCARELLI et al., 2001; SCHMIDT; LARA; SOUZA, 2007).

Figura 7. Estrutura química da guanosina
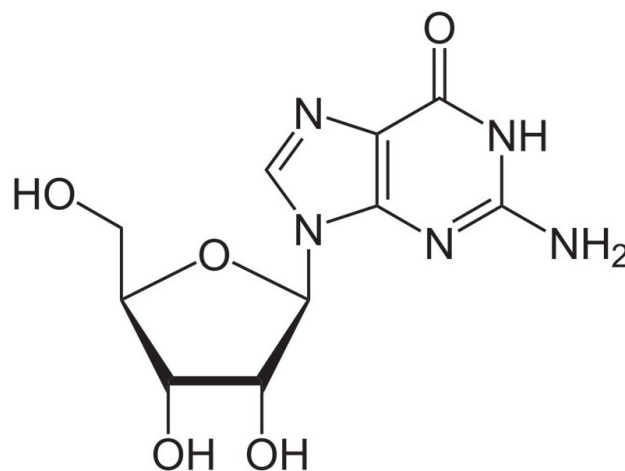
A guanosina é um nucleosídeo da guanina que ocorre naturalmente no sistema nervoso central, cuja estrutura que compreende uma guanina ligada a um anel de ribose. $C_{10}H_{13}N_5O_5$; peso molecular: 283.244 g/mol. Fonte: PubChem CID: 6802.

A guanosina é liberada no espaço extracelular em condições fisiológicas e especialmente durante eventos patológicos, sendo capaz de desencadear uma ampla gama de funções em várias regiões encefálicas, achados que postulam que a mesma poderia ser um neuroprotetor endógeno (CICCARELLI et al., 1999; LANZNASTER et al., 2016). Consistentemente, o efeito neuroprotetor da guanosina foi demonstrado por diversos estudos *in vitro* e *in vivo*, particularmente em modelos de isquemia (DAL-CIM et al., 2011, 2013, 2016; HANSEL et al., 2014; RAMOS et al., 2016; THOMAZ et al., 2016), septicemia (PETRONILHO et al., 2012), doença de Alzheimer (LANZNASTER et al., 2017), doença de Parkinson (MARQUES; MASSARI; TASCA, 2019; MASSARI et al., 2020, 2017), epilepsia (OLIVEIRA et al., 2004; TORRES et al., 2010), e excitotoxicidade glutamatérgica (MOLZ et al., 2011). Também demonstrou-se que a guanosina é capaz de promover efeitos antinociceptivo e neurotróficos (SCHMIDT et al., 2009, 2010). De especial interesse, postula-se que a guanosina exerça seus efeitos neuroprotetores por estimular vias de sinalização que culminam na síntese e liberação de fatores neurotróficos, bem como respostas antioxidantes e anti-inflamatórias (BETTIO; GIL-MOHAPEL; RODRIGUES, 2016; TASCA et al., 2018). Apesar do efeito neuroprotetor promovido pela guanosina ser bem estabelecido, o seu exato sítio de ação ainda não está bem elucidado (LANZNASTER et al., 2016).

Estudos buscaram estabelecer se os efeitos neuroprotetores da guanosina seriam de fato atribuídas a este nucleosídeo e não a algum efetor. Neste sentido, evidenciou-se que os níveis plasmáticos de guanosina aumentam de maneira dose e tempo-dependentes após sua administração intraperitoneal (GIULIANI et al., 2012). Particularmente, averiguou-se que a concentração de guanosina encontra-se duplicada após 90 minutos da administração (JIANG et al., 2008). É bem estabelecido que a guanosina é amplamente distribuída após administração sistêmica, sendo capaz de entrar no SNC em 7,5 min. Além disso, suas concentrações continuam a aumentar no SNC, atingindo um pico 30 min após administração intraperitoneal (GIULIANI et al., 2012; JIANG et al., 2008).

É importante destacar que um padrão semelhante também foi observado após administração da guanosina por via oral (SCHMIDT et al., 2010; VINADÉ et al., 2005). De fato, a guanosina pode ser captada por transportadores de nucleosídeos, que são amplamente encontrados em células intestinais, microvasos e na barreira hematoencefálica (JONES; HAMMOND, 1995; KALARIA; HARIK, 1988; NAGASAWA et al., 2007; PATIL; UNADKAT, 1997; PENG et al., 2005). É conveniente destacar ainda que a guanosina pode ser convertida em guanina através da atividade da enzima purina nucleosídeo fosforilase

(SCHMIDT; LARA; SOUZA, 2007). Neste sentido, estudos prévios mostraram que as concentrações máximas dos metabólitos da guanosina, particularmente guanina, xantina e ácido úrico, foram detectados no plasma após 15-30 min da sua administração, indicando que esse nucleosídeo pode ser rapidamente metabolizado após administração exógena (GIULIANI et al., 2012; JIANG et al., 2008).

De especial interesse, demonstrou-se que os níveis de guanosina estão reduzidos no soro de pacientes com TDM (ALI-SISTO et al., 2016; MOCKING et al., 2021), o que sugere o envolvimento deste nucleosídeo na neurobiologia deste transtorno. Particularmente, nosso grupo de pesquisa foi o primeiro a demonstrar que a guanosina é capaz de exercer uma resposta tipo-antidepressiva em camundongos após administração única (BETTIO et al., 2012) ou repetida (BETTIO et al., 2016), um efeito reforçado por outros grupos de pesquisa (ALMEIDA et al., 2020, 2021; MARQUES et al., 2019; PIERMARTIRI et al., 2020). Vale destacar que a guanosina apresenta mecanismos de ação similares à cetamina e, portanto, poderia ser um novo candidato para produzir respostas tipo-antidepressivas rápidas ou potencializar as ações da cetamina (CAMARGO; RODRIGUES, 2019).

Do ponto de vista mecanístico (Figura 8), o efeito tipo-antidepressivo provocado pela guanosina no teste de suspensão pela cauda parece envolver a modulação dos receptores NMDA, visto que a administração de NMDA (um agonista do receptor NMDA) ou D-serina (um co-agonista do receptor NMDA) aboliram completamente este efeito (BETTIO et al., 2012). Além disso, a administração combinada de guanosina e MK-801 (um antagonista do receptor NMDA) produziram um efeito tipo-antidepressivo no teste de suspensão pela cauda em camundongos (BETTIO et al., 2012). O efeito tipo-antidepressivo deste nucleosídeo foi completamente abolido pela administração de DNQX (um antagonista do receptor AMPA) ou verapamil (um bloqueador dos CCVD) (ROSA et al., 2021). A liberação de BDNF e a ativação do receptor TrkB também parecem ser necessários para o efeito tipo-antidepressivo da guanosina, uma vez que a administração do anticorpo anti-BDNF ou do K-252a (um antagonista do receptor TrkB) aboliu esta resposta no teste de suspensão pela cauda (ROSA et al., 2021). Além disso, esta resposta comportamental foi acompanhada por um aumento significativo nos níveis de BDNF no hipocampo e no córtex pré-frontal de camundongos após 1 h do tratamento com guanosina (ROSA et al., 2021).

Figura 8. Mecanismos envolvidos no efeito tipo-antidepressivo da guanosina
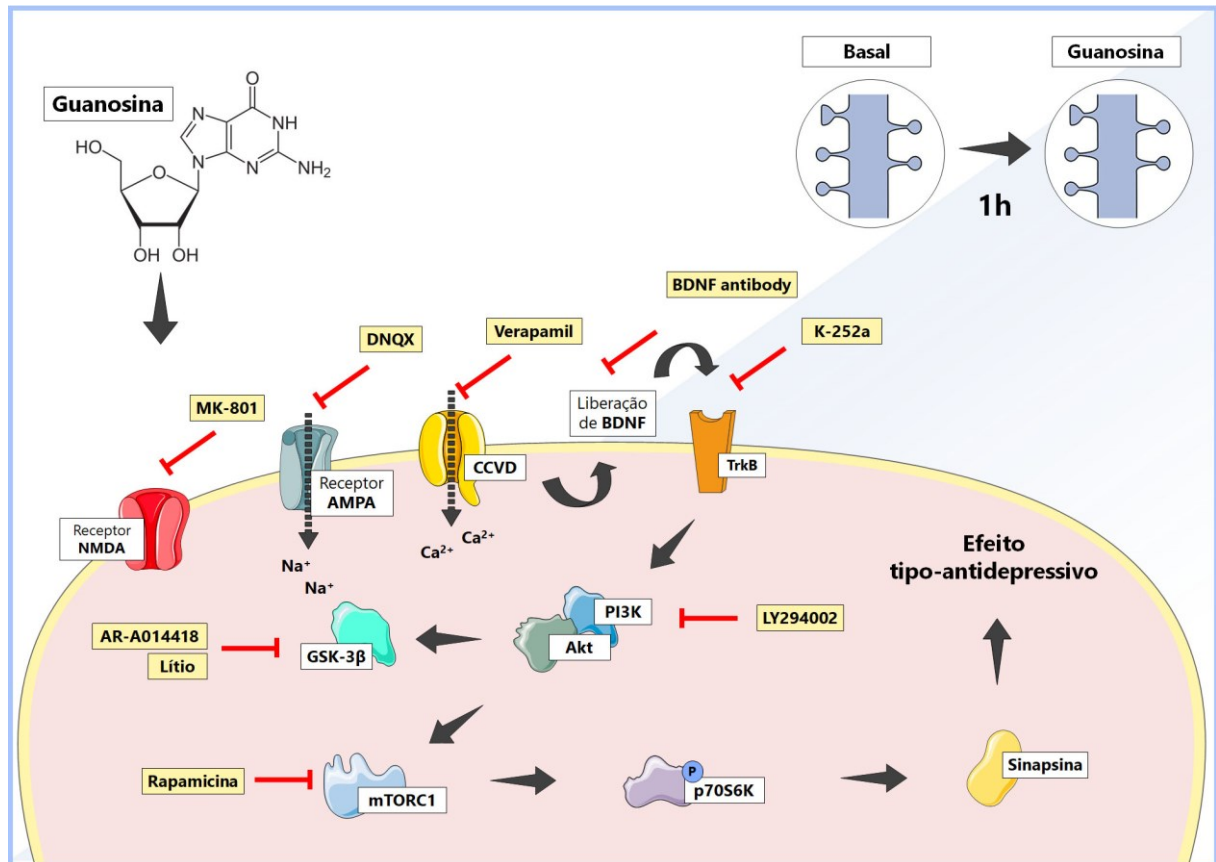
O efeito tipo-antidepressivo da guanosina no teste de suspensão pela cauda parece envolver a modulação dos receptores NMDA e AMPA e a ativação dos CCVD, bem como a liberação de BDNF e a ativação do receptor TrkB. O efeito tipo-antidepressivo desencadeado pela guanosina está associado à ativação da via de sinalização PI3K/Akt/GSK-3β/mTORC1 com consequentemente aumento na fosforilação de p70S6K e no imunoconteúdo de sinapsina no hipocampo de camundongos. No entanto, uma única administração de guanosina não aumentou a densidade e a maturação de espinhos dendríticos na porção ventral do giro denteado a formação hipocampal de camundongos após 1 h do tratamento. *Akt: proteína cinase B; AMPA: alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico; BDNF: fator neurotrófico derivado do encéfalo; CCVD: cálcio dependentes de voltagem; DNQX: 6,7-dinitroquinoxaline-2,3-dione; GSK-3β: glicogênio sintase cinase-3β; GluA1: subunidade 1 de receptores AMPA; mTORC1: complexo 1 da proteína alvo mecanístico da rapamicina; NMDA: N-metil-D-aspartato; p70S6K: proteína ribossomal S6 cinase de 70 kDa; PI3K: fosfatidilinositol 3-cinase; PSD-95: proteína de densidade pós-sináptica de 95kDa; TrkB: tropomiosina cinase B. Figura elaborada usando imagens do Mind the Graph e Servier Medical Art. Fonte: autor.*

O efeito tipo-antidepressivo da guanosina também está associado à estimulação de via de sinalização PI3K/Akt/GSK-3β/mTORC1. Em particular, a administração de LY294002 (um inibidor de PI3K) ou rapamicina (um inibidor seletivo de mTORC1) anulou completamente as respostas tipo-antidepressivas da guanosina no teste de suspensão pela cauda (BETTIO et al., 2012). A coadministração com doses sub-efetivas de guanosina e cloreto de lítio (um inibidor não seletivo de GSK-3β) ou AR-A014418 (um inibidor seletivo de GSK-3β) produziu um efeito tipo-antidepressivo no teste de suspensão pela cauda (ROSA et al., 2019). A administração de guanosina também foi capaz de aumentar significativamente a fosforilação de p70S6K e o

imunoconteúdo de sinapsina no hipocampo de camundongos após 1 h do tratamento (ROSA et al., 2021). Reforçando a noção de que a cetamina e a guanosina compartilham respostas comportamentais e alvos moleculares comuns, uma única administração de guanosina foi capaz de reverter o comportamento tipo-depressivo induzido pela bulbectomia olfatória em camundongos submetidos ao teste de borrifagem com sacarose e ao teste de reconhecimento de objetivos (ALMEIDA et al., 2020). Entretanto, este efeito não foi observado quando os camundongos foram pré-tratados com a rapamicina, indicando que o efeito tipo-antidepressivo da guanosina é dependente da estimulação de mTORC1 (ALMEIDA et al., 2020). Contudo, é importante destacar que uma única administração de guanosina, ao contrário da cetamina, não aumentou a densidade e a maturação de espinhos dendríticos na porção ventral do giro denteado da formação hipocampal de camundongos 1h após sua administração (ROSA et al., 2021).

Recentemente, nosso grupo de pesquisa demostrou que uma administração aguda de guanosina, de forma semelhante à cetamina, apresentou um efeito tipo-antidepressivo no teste da alimentação suprimida pela novidade (CAMARGO et al., 2019; ROSA et al., 2021), um paradigma comportamental sensível a uma única administração de agentes antidepressivos com rápido efeito, mas que não responde a uma única administração de antidepressivos convencionais (NEIS et al., 2020; PAZINI et al., 2020). Além disso, a capacidade da guanosina em aumentar o efeito tipo-antidepressivo de doses sub-efetivas de cetamina no teste da alimentação suprimida pela novidade também foi demonstrada por nosso grupo de pesquisa (CAMARGO et al., 2019). Este efeito comportamental observado no teste da alimentação suprimida pela novidade após a combinação de cetamina e guanosina não foi detectado quando a rapamicina foi previamente administrada aos camundongos, indicando que o efeito tipo-antidepressivo é dependente da ativação de mTORC1 (CAMARGO et al., 2019). Reforçando o papel da via de sinalização mediada por mTORC1 neste efeito, a coadministração com doses sub-efetivas de cetamina e guanosina aumentou significativamente a fosforilação de mTORC1 no hipocampo de camundongos (CAMARGO et al., 2019).

Apesar das evidências mostrando que a guanosina é efetiva em produzir efeito tipo-antidepressivo (BETTIO et al., 2012; CAMARGO et al., 2019; ROSA et al., 2021), ainda não foi determinado se a guanosina é eficaz em potencializar o efeito tipo-antidepressivo e pró-sinaptogênico rápido e sustentado desencadeado pela cetamina. Mais importante, ainda precisa ser elucidado o papel da via de sinalização mediada por mTORC1 e a formação de espinhos dendríticos nos possíveis efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela coadministração de doses sub-efetivas de cetamina e guanosina. Além disso, ainda não há

evidências que mostrem que a administração única de doses sub-efetivas de cetamina e guanosina poderia exercer efeitos tipo-antidepressivo e pró-sinaptogênico em um modelo animal que mimetiza alterações comportamentais e moleculares observadas em paciente com TDM. Por fim, ainda precisa ser determinado se a guanosina é capaz de produzir um efeito pró-resiliência bem como potencializar o fenótipo pró-resiliente promovido pela cetamina contra o comportamento tipo-depressivo e disfunções sinápticas induzidas por estresse crônico.

2 JUSTIFICATIVA

Considerando que vários antagonistas do receptor NMDA falharam em exercer todo o espectro de ações da cetamina em ensaios clínicos (NEWPORT et al., 2015; POCHWAT; NOWAK; SZEWCZYK, 2019), estratégias direcionas em potencializar as respostas antidepressivas desta molécula, com menor potencial para efeitos indesejáveis são bem-vindas. Notavelmente, há um crescente conjunto de evidências mostrando que os moduladores glutamatérgicos endógenos podem produzir efeito tipo-antidepressivo rápido e até mesmo aumentar as ações antidepressivas da cetamina por estimular a sinalização mediada por mTORC1 (CAMARGO; RODRIGUES, 2019). Neste sentido, destaca-se que guanosina, uma purina neuroprotetora endógena a base de guanina. Estudos recentes demonstraram que a guanosina apresenta mecanismos de ação sobrepostos à cetamina e, portanto, poderia ser um novo candidato para produzir respostas tipo-antidepressivas rápidas ou potencializar as ações da cetamina (CAMARGO; RODRIGUES, 2019). Em vista das limitações do uso da cetamina, a estratégia de combinação da cetamina com a guanosina, em doses sub-efetivas, pode constituir uma estratégia promissora para auxiliar pacientes com diagnóstico de TDM no futuro, particularmente por permitir a redução da dose terapeuticamente eficaz da cetamina e possivelmente os efeitos adversos. Estes dados nos incentivam a investigar a guanosina como um agente potencializador dos efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela cetamina em camundongos, bem como o papel da via de sinalização mediada por mTORC1 nesta possível resposta.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Investigar a guanosina como um agente potencializador dos efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela cetamina em camundongos, bem como o papel da via de sinalização mediada por mTORC1 nesta possível resposta.

3.2 OBJETIVOS ESPECÍFICOS

a) Avaliar o efeito tipo-antidepressivo da administração de cetamina, guanosina e a combinação de doses sub-efetivas de cetamina e guanosina;

b) Investigar se a possível resposta tipo-antidepressiva desencadeada pela coadministração de doses sub-efetivas de cetamina e guanosina está associada com a via de sinalização pró-sinaptogênica mediada por mTORC1;

c) Analisar se a guanosina é capaz de potencializar o efeito tipo-antidepressivo após 1 h, 24 h e 7 dias da administração de cetamina, e investigar se esta resposta relacionada a estimulação da via pró-sinaptogênica mediada por mTORC1;

d) Avaliar a densidade de espinhos dendríticos na porção ventral do giro denteado da formação hipocampal e no córtex pré-frontal de animais tratados com cetamina e/ou guanosina após 1 h, 24 h e 7 dias;

e) Analisar se a possível capacidade da guanosina em potencializar o efeito tipo-antidepressivo e pró-sinaptogênico desencadeado pela cetamina é dependente da ativação de mTORC1;

f) Investigar o efeito tipo-antidepressivo e pró-sinaptogênico da combinação de doses sub-efetivas de cetamina e guanosina em animais submetidos à administração crônica de corticosterona e o papel da via de sinalização mediada por mTORC1;

g) Verificar se o efeito tipo-antidepressivo e pró-sinaptogênico desencadeado pela associação de doses sub-efetivas de cetamina e guanosina em animais submetidos à administração crônica de corticosterona é dependente da ativação da via de sinalização mediada por mTORC1;

h) Avaliar se a administração profilática de cetamina, guanosina e a associação de cetamina e guanosina é capaz de prevenir o comportamento tipo-depressivo induzido pela administração crônica de corticosterona, e se este efeito está associado a via de sinalização mediada por mTORC1.

4 MATERIAIS, MÉTODOS E RESULTADOS

Esta Tese de Doutorado apresenta os principais resultados obtidos durante o período de doutoramento. Os resultados estão divididos em 4 capítulos contendo os manuscritos completos publicados nos respectivos periódicos.

CAPÍTULO 1

"Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: Possible role of pro-synaptogenic signaling pathway"

*Manuscrito completo referente aos objetivos **a** e **b** publicado no periódico Journal of Affective Disorders, v. 271, p. 100-108, 2020.*

CAPÍTULO 2

"Guanosine boosts the fast, but not sustained, antidepressant-like and pro-synaptogenic effects of ketamine by stimulating mTORC1-driven signaling pathway"

*Manuscrito completo referente aos objetivos **c, d** e publicado no periódico European Neuropsychopharmacology, v. 57, p. 15-29, 2022.*

CAPÍTULO 3

"A low-dose combination of ketamine and guanosine counteracts corticosterone-induced depressive-like behavior and hippocampal synaptic impairments via mTORC1 signaling"

*Manuscrito completo referente aos objetivos **f** e **g** publicado no periódico Progress in Neuro-Psychopharmacology and Biological Psychiatry, v. 111, p. 110371, 2021.*

CAPÍTULO 4

"Ketamine, but not guanosine, as a prophylactic agent against corticosterone-induced depressive-like behavior: Possible role of long-lasting pro-synaptogenic signaling pathway"

*Manuscrito completo referente ao objetivo **h** publicado no Experimental Neurology, v. 334, p. 113459, 2020.*

4.1 CAPÍTULO 1

Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: Possible role of pro-synaptogenic signaling pathway

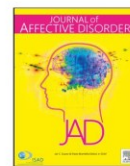*Journal of Affective Disorders, v. 271, p. 100-108, 2020.*

Anderson Camargo, Ana Paula Dalmagro, Ana Lúcia B. Zeni, Ana Lúcia S. Rodrigues

Fator de impacto: 4.839 (JCR 2020)

Research paper

Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: Possible role of pro-synaptogenic signaling pathway

Anderson Camargo[a], Ana Paula Dalmagro[b], Ana Lúcia B. Zeni[b], Ana Lúcia S. Rodrigues[a,*]

[a] Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis, 88040-900, SC, Brazil
[b] Department of Natural Sciences, Center of Exact and Natural Sciences, Universidade Regional de Blumenau, Blumenau, CEP 89030-903, Santa Catarina, Brazil

ABSTRACT

Background Augmentation therapies may be effective strategies to potentiate the ketamine's actions with lower potential for knock-on effects. Thus, this study investigated the ability of combined administration of guanosine plus ketamine to elicit an antidepressant-like effect associated with mTOR pathway modulation. The ability of this combined administration to exert an antidepressant-like effect in a model of depression was also evaluated.

Methods Mice were administered with subthreshold doses of ketamine (0.1 mg/kg, i.p.) and guanosine (0.01 mg/kg, p.o.) and submitted to the tail suspension test, and immunoblotting analyses (p-mTOR, p-p70S6K, PSD-95, GluA1, and synapsin) in the hippocampus and prefrontal cortex. The antidepressant-like effect of ketamine plus guanosine in mice subjected to administration of corticosterone (20 mg/kg, p.o., 21 days) was also evaluated.

Results Ketamine plus guanosine treatment elicited an antidepressant-like effect, which was associated with increased mTOR (Ser^{2448}) and p70S6K (Thr^{389}) phosphorylation in the hippocampus, but not in the prefrontal cortex. Furthermore, increased PSD-95 and GluA1 immunocontent were observed in the prefrontal cortex, but not in the hippocampus of ketamine plus guanosine-treated mice. Reinforcing the notion that guanosine may potentiate the ketamine's behavioral response, a single administration of subthreshold doses of ketamine plus guanosine counteracted the corticosterone-induced depressive-like behavior.

Conclusions Our results indicate that guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine, an effect likely associated with the stimulation of synaptogenic pathway in the hippocampus and prefrontal cortex, although with a different profile. The augmentation effect of ketamine by guanosine could have therapeutic relevance for patients with treatment-resistant depression.

1. Introduction

Depression is a burdensome neuropsychiatric disorder whose lifetime prevalence is nearly 20% (Otte et al., 2016). Among the multiples causes that underlie the pathophysiology of depression, the mood-related circuitry disruption in brain structures such as the hippocampus and prefrontal cortex has received special attention (Duman et al., 2016). Neuroplasticity alterations in the hippocampus and prefrontal cortex have been associated with low remission rates and impaired clinical response to conventional antidepressant drugs (MacQueen et al., 2008; Savitz and Drevets, 2009). These drugs are modestly effective since they require weeks to months to provide complete remission of symptoms, besides being effective to only about one-third of patients (Crisafulli et al., 2011; Papakostas and Ionescu, 2015). Given this scenario, these limitations underscore the need for the development of novel, more efficacious and fast-acting antidepressant agents, not primarily based on the monoaminergic system.

Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, has emerged as a fast-acting antidepressant that elicits rapid and long-lasting effects (Berman et al., 2000) even in treatment-refractory individuals with suicidal ideation (Zarate et al., 2006). These findings have spurred several research groups to conduct preclinical studies aimed at elucidating the ketamine's mechanisms of action. The activation of mammalian target of rapamycin (mTOR) that controls the translation of synaptic proteins such as postsynaptic density protein-95 kDa (PSD-95), alpha-amino-3-hydroxy-methyl-5-4-isoxazole propionic acid (AMPA) receptor subunits 1 (GluA1) and, synapsin has been reported to underpin the fast-acting antidepressant effect elicited by ketamine (Li et al., 2010). These synaptic proteins promote

---

* Corresponding author.
  E-mail address: ana.l.rodrigues@ufsc.br (A.L.S. Rodrigues).

spinogenesis and synaptogenesis in the prefrontal cortex and hippo-campus, which in turn could be associated with antidepressant responses (Li et al., 2011, 2010; Liu et al., 2016; Zhou et al., 2014). However, the use of ketamine may cause psychotomimetic and neurotoxic effects, limiting its widespread clinical use (Gao et al., 2016). Therefore, strategies to augment the ketamine's antidepressant effects with lower potential for knock-on effects are needed.

Augmentation strategy is generally used to provide greater improvement than monotherapy and to allow antidepressant dose reduction, thereby attenuating its adverse/side effects (Barowsky and Schwartz, 2006; Han et al., 2014; Moret, 2005). Guanosine, a guanine-based nucleoside, has been shown to present overlapping mechanisms of action to ketamine and could be an augmenting agent (Bettio et al., 2012; Camargo and Rodrigues, 2019; Camargo et al., 2019). Accordingly, it promotes antidepressant-like effect with a behavioral profile similar to ketamine, particularly by stimulating phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt)/mTOR signaling (Bettio et al., 2012; Camargo et al., 2019). Moreover, a single administration of guanosine affords augmentation of ketamine's effects in the novelty-suppressed feeding test (Camargo et al., 2019). Therefore, this study sought to investigate: a) the effect of administration of subthreshold doses of ketamine and guanosine in the mouse tail suspension test; b) whether the administration of subthreshold doses of ketamine and guanosine is effective to modulate mTOR signaling pathway increasing synaptic proteins in the hippocampus and prefrontal cortex; c) the ability of a single administration of subthreshold doses of ketamine and guanosine to counteract the depressive-like behavior induced by sub-chronic corticosterone (CORT) administration in mice.

2. Material and methods

2.1. Animals

The behavioral experiments were conducted using male *Swiss* mice (30–40 g, 45–60 days of age), maintained under controlled temperature (21 ± 1 °C) and humidity (50 ± 20%) with a 12:12 h light/dark cycle (lights on at 7:00 a.m.). Animals were maintained with free access to food and water in groups of 8–10 in a $41 \times 34 \times 16$ cm cage and the behavioral tests were carried out between 9.00 a.m. and 04.00 p.m. The experiments were carried out according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were performed after approval of the protocol by the Institutional Ethics Committee. All efforts were done to minimize animal suffering and to reduce their number to the minimum necessary to demonstrate consistent effects in the experiments.

2.2. Drugs and treatments

The first set of experiments was designed to obtain the dose-response curve of ketamine and guanosine in the tail suspension test (TST). To develop the first protocol, mice were randomly assigned to seven experimental groups (n = 8/group): control (vehicle), ketamine (Ket − 0.1, 1 and 5 mg/kg), and guanosine (Guo − 0.01, 0.05 and 1 mg/kg). Ketamine and guanosine, obtained from Sigma Chemical Co., St. Louis, U.S.A, were dissolved in distilled water and saline (0.9%), respectively. Guanosine was administered orally (p.o.) while ketamine was administered intraperitoneally (i.p.), both in a volume of 10 ml/kg. Mice were submitted to the TST 60 min after guanosine treatment and 30 min after ketamine administration. Ten min after the TST, mice were submitted to the open field test (OFT) (Fig. 1A). In the second set of experiments, in order to investigate the potential augmentation effect of ketamine by guanosine in the TST, mice (n = 8/group) were administered with subthreshold doses of ketamine (Ket − 0.1 mg/kg, i.p.) and/or guanosine (Guo − 0.01 mg/kg, p.o.) and submitted to the TST and OFT 10 min apart (Fig. 1B). Mice were immediately euthanized by decapitation and the hippocampus and prefrontal cortex were collected

for western blotting analysis immediately after the OFT. The third set of experiments was performed to test whether guanosine is effective to potentiate the effect of ketamine in an animal model of depression induced by CORT (Fig. 1C). To conduct the protocol, mice were divided into the following groups: vehicle + vehicle; vehicle + ketamine (Ket − 0.1 mg/kg); vehicle + guanosine (Guo − 0.01 mg/kg); vehicle + ketamine (Ket − 0.1 mg/kg)/guanosine (Guo − 0.01 mg/kg); CORT + vehicle; CORT + ketamine (Ket − 0.1 mg/kg); CORT + guanosine (Guo − 0.01 mg/kg); and CORT + ketamine (Ket − 0.1 mg/kg)/guanosine (Guo − 0.01 mg/kg). CORT (Sigma Chemical Co., St. Louis, U.S.A) was dissolved in distilled water with 2% Tween 80 and 0.2% DMSO and administered orally at 20 mg/kg once a day for 21 days. Ketamine and/or guanosine treatments were administered in a single dose immediately after the last CORT administration. On the 22nd day, 24 h after the treatments, animals were submitted to the TST and OFT (after 10 min). All doses and time points of administration were chosen based on previous studies (Bettio et al., 2012; Ludka et al., 2013; Pazini et al., 2016), and the drugs were freshly prepared before administration. A diagram of all experimental schedules (treatment regimen, behavioral and biochemical evaluation) is provided in Fig. 1.

2.3. Tail suspension test (TST)

The total immobility time of mice suspended by the tail was measured as previously proposed (Steru et al., 1985). Briefly, visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period by an experienced observer blind to the experimental groups. Mice were considered immobile only when they hung passively and completely motionless.

2.4. Open-field test (OFT)

Ten min after the TST the locomotor activity was assessed in the OFT in a wooden box measuring 40×60 cm $\times 50$ cm height with the floor divided into 12 equal squares (Pazini et al., 2016). At the start of each trial, mice were placed in the left corner of the field and allowed to freely explore the arena. The number of crossings (squares crossed with all paws) was registered for 6 min. The arena floor was cleaned with 10% ethanol between tests.

2.5. Western blotting

Hippocampus and prefrontal cortex were quickly dissected and snap-frozen with liquid nitrogen before storage at −80 °C until use. Briefly, samples were mechanically homogenized in 400 μl of 50 mM TRIS pH 7.0, 1 mM EDTA, 100 mM NaF, 0.1 mM PMSF, 2 mM Na_3VO_4, 1 % Triton X-100, 10 % glycerol, Sigma Protease Inhibitor Cocktail (P2714). Lysates were centrifuged (10,000 g for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in 100 mM TRIS pH 6.8, 4 mM EDTA, 8 % SDS, and boiled for 5 min. Thereafter, sample dilution (40 % glycerol, 100 mM TRIS, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β-mercaptoethanol (final concentration 8 %) were added to the samples. Protein content was quantified using bovine serum albumin (BSA) as a standard (Peterson, 1977). The samples containing 60 μg protein/track were separated by SDS-PAGE (miniVE Vertical Electrophoresis System TM, GE Healthcare Life Sciences, Piscataway, NJ, USA) using 7–10 % gel and the proteins were transferred to nitrocellulose membranes using a semi-dry blotting apparatus (1.2 mA/cm2; 1.5 h). Subsequently, the membranes were blocked with 5% BSA in TBS (10 mM Tris, 150 mM NaCl, pH 7.5). The immunocontent of total and phosphorylated forms of mTOR (Ser2448) and p70S6K (Thr389), as well as PSD-95, GluA1, synapsin, and β-actin (loading control) immunocontent were detected using specific antibodies (obtained from Cell Signaling Technology −1:1000 dilution) incubated overnight diluted in TBS-T (10 mM Tris,
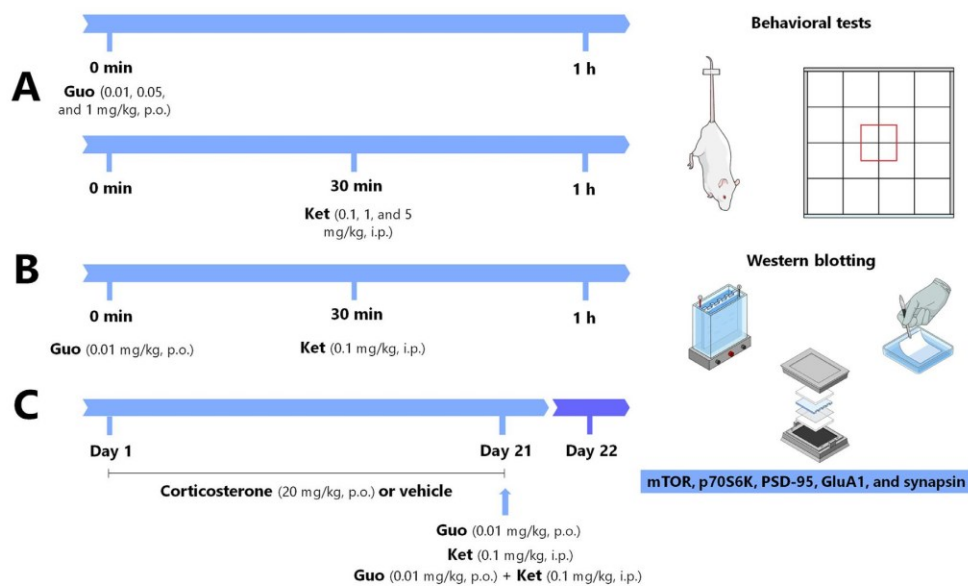
A. Camargo, et al.

Fig. 1. Schematic representation of the treatment protocol and behavioral and biochemical evaluation. (A) In order to obtain the dose-response curve of ketamine and guanosine, mice received ketamine (Ket – 0.1, 1 and 5 mg/kg, i.p.) or guanosine (Guo – 0.01, 0.05 and 1 mg/kg, p.o.), and subsequently, they were submitted to the TST and OFT. (B) To investigate the augmentation effect of ketamine by guanosine, mice received subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) and, subsequently they were subjected to the TST and OFT. After the behavioral tests, mice were immediately euthanized by decapitation and the hippocampus and prefrontal cortex were collected for western blotting. (C) To investigate the effect of augmentation effect of ketamine by guanosine in an animal model of depression, mice were treated or not with corticosterone orally (20 mg/kg, p.o.), once a day, for 21 consecutive days to induce depressive-like behavior. Ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) solutions were administered in a single dose immediately following the last corticosterone administration. On the 22nd day, 24 h after the treatments, animals were submitted to TST and OFT. Figure designed using images from Mind the Graph.
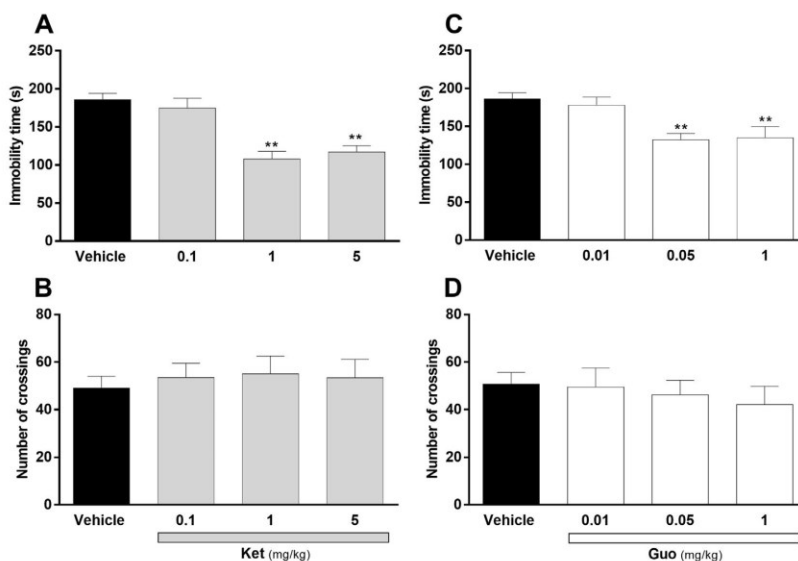
Fig. 2. Effect of a single administration of ketamine (Ket – 0.01, 1 and 5 mg/kg, i.p.) or guanosine (Guo – 0.01, 0.05 and 1 mg/kg, p.o.) in mice submitted to the TST and OFT. Ketamine or guanosine was administered 30 min or 60 min prior to the test, respectively. Values are expressed as means ± S.E.M (n = 8). **$P < 0.01$ as compared with the vehicle-treated control (one-way ANOVA followed by Newman-Keuls *post-hoc* test).

150 mM NaCl, 0.1 % Tween-10, pH 7.5) containing 2.5 % BSA. After, the membranes were incubated with anti-rabbit antibody horseradish peroxidase-conjugated secondary antibody (Cell Signaling, 1:2500) for 60 min, and the immunoreactive bands were developed using a chemiluminescence kit (Amersham ECL Select, Piscataway, USA). All

blocking and incubation steps were followed by three washes (5 min) of the membranes with TBS-T (Leal et al., 2002). The optical density (OD) of the bands was quantified using Image Lab Software® 4.1 (Bio-Rad Laboratories). The phosphorylation levels of mTOR and p70S6K were determined as a ratio of OD of the phosphorylated band over OD of the

total band. The immunocontent of PSD-95, GluA1, and synapsin was determined as a ratio of OD of PSD-95, GluA1, and synapsin band over the OD of the β-actin band. Results are expressed as compared to control group (100%).

2.6. Statistical analysis

The results are expressed as means ± S.E.M. Differences among experimental groups were determined by one-way or two-way analysis of variance (ANOVA) followed by Newman-Keuls *post hoc* test, when appropriate. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Effect of ketamine or guanosine alone or in combination in the TST

In the first set of experiments, to obtain the effective and sub-threshold doses of ketamine and guanosine in the TST, a dose-response curve was performed. The acute administration of ketamine (Ket – 1 and 5 mg/kg; $F_{(3, 28)} = 15.65$, $P < 0.01$) or guanosine (Guo – 0.05 and 1 mg/kg; $F_{(3, 28)} = 6.59$, $P < 0.01$) significantly decreased the immobility time in the TST (Fig. 2A and C), whereas ketamine (0.1 mg/kg) or guanosine (0.01 mg/kg) caused no effect. In addition, the number of crossings in the OFT (Fig. 2B and D) was not altered in mice treated with ketamine or guanosine ($F_{(3, 28)} = 0.15$, $P = 0.93$, and $F_{(3, 28)} = 0.32$, $P = 0.80$, respectively).

In a next step, subthreshold doses of ketamine and guanosine were coadministered to test the hypothesis that guanosine is effective to augment the effect of ketamine in the TST (Fig. 3A). Statistical analysis showed that subthreshold doses of ketamine (0.1 mg/kg) and guanosine (0.01 mg/kg) significantly reduced the immobility time in the TST as compared to the control group ($F_{(3, 28)} = 7.77$, $P < 0.01$ – Fig. 3A). This effect in the TST was not associated with any alteration in the locomotor activity ($F_{(3, 28)} = 0.08$, $P = 0.96$; Fig. 3B).

3.2. mTOR-mediated signaling pathway is associated with the augmentation effect of ketamine by guanosine

Considering previous evidence on the role of the mTOR signaling pathway for the antidepressant-like effect of ketamine (Li et al., 2010), we investigated the ability of the combined administration of ketamine and guanosine to activate mTOR signaling pathway leading to increased levels of synaptic proteins in the hippocampus and prefrontal cortex.

The results illustrated Fig. 4 show the hippocampal (4A and 4C) and prefrontocortical (4B and 4D) immunocontent of phospho-mTOR (Ser^{2448}) and phospho-p70S6K (Thr^{389}) in mice treated with subthreshold doses of guanosine and/or ketamine. Statistical analysis indicated that the combined administration of ketamine and guanosine increased hippocampal phospho-mTOR (Ser^{2448}) as compared to the control group ($F_{(3, 24)} = 10.01$, $P < 0.01$). In addition, no significant effect was observed on hippocampal total mTOR immunocontent ($F_{(3, 24)} = 0.44$, $P = 0.72$). Furthermore, statistical analysis showed that a single coadministration with subthreshold doses of ketamine and guanosine increased hippocampal phospho-p70S6K (Thr^{389}) as compared to the control group ($F_{(3, 24)} = 25.24$, $P < 0.01$). No significant effect was observed on hippocampal total p70S6K immunocontent ($F_{(3, 24)} = 1.22$, $P = 0.32$). One-way ANOVA revealed no significant differences for treatment on prefrontocortical phospho-mTOR ($F_{(3, 24)} = 1.70$, $P = 0.19$) and on prefrontocortical phospho-p70S6K ($F_{(3, 24)} = 0.46$, $P = 0.70$). In addition, the immunocontent of prefrontocortical total mTOR ($F_{(3, 24)} = 0.32$, $P = 0.80$) and prefrontocortical total p70S6K treatment ($F_{(3, 24)} = 0.44$, $P = 0.72$) was not affected by any treatment.

The results depicted in Fig. 5 show the hippocampal (5A and 5C) and prefrontocortical (5B and 5D) immunocontent of synaptic proteins
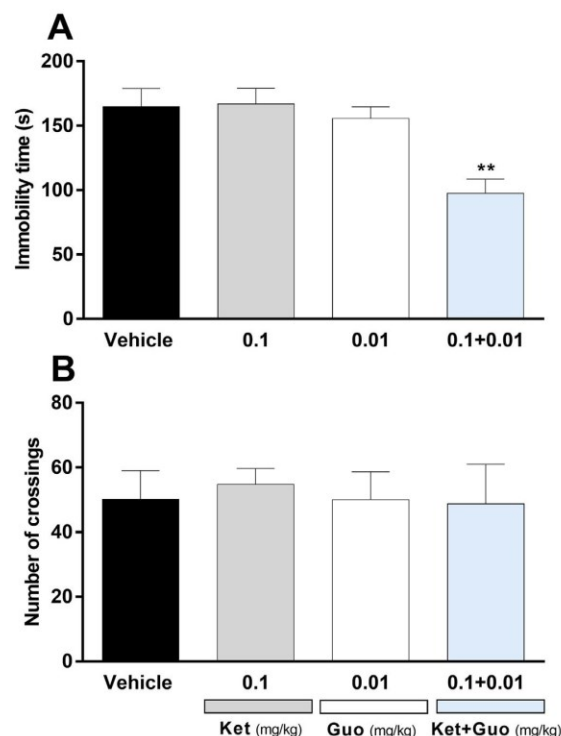
Fig. 3. Effect of a single administration with a subthreshold dose of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) in mice submitted to the TST and OFT. Ketamine and/or guanosine were administered 30 min or 60 min prior to the test, respectively. Values are expressed as means ± S.E.M ($n = 8$). $**P < 0.01$ as compared with the vehicle-treated control (one-way ANOVA followed by Newman-Keuls *post-hoc* test).

PSD-95, GluA1, and synapsin in mice treated with subthreshold doses of guanosine and/or ketamine. One-way ANOVA revealed no significant differences for treatment on hippocampal PSD-95 immunocontent ($F_{(3, 24)} = 1.30$, $P = 0.29$), on hippocampal GluA1 immunocontent ($F_{(3, 24)} = 0.18$, $P = 0.90$), as well as on hippocampal synapsin immunocontent ($F_{(3, 24)} = 0.31$, $P = 0.81$). However, statistical analysis indicated that a single coadministration with subthreshold doses of ketamine plus guanosine significantly increased the prefrontocortical immunocontent of PSD-95 ($F_{(3, 24)} = 8.35$, $P < 0.01$) and, GluA1 ($F_{(3, 24)} = 4.54$, $P < 0.01$) as compared to the control group. One-way ANOVA revealed no significant differences for treatment ($F_{(3, 24)} = 1.14$, $P = 0.35$) on prefrontocortical synapsin immunocontent.

3.3. Effect of a single dose of ketamine and/or guanosine on the CORT-induced depressive-like behavior

To investigate the possible augmentation effect of ketamine by guanosine in a model that mimics depressive state caused by stress, CORT-treated mice received a single administration of subthreshold doses of ketamine plus guanosine. Two-way ANOVA revealed significant differences for treatment ($F_{(3, 56)} = 31.73$, $P < 0.01$) and CORT administration ($F_{(1, 56)} = 121.80$, $P < 0.01$), but not for treatment × CORT administration interaction ($F_{(3, 56)} = 2.31$, $P = 0.08$). *Post-hoc* analysis showed that CORT administration significantly increased the immobility time in mice submitted to the TST ($P < 0.01$ – Fig. 6A), while this behavioral alteration was significantly counteracted by coadministration of subthreshold doses of ketamine

Fig. 4. Effect of a single administration with subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) on phospho-mTOR and phospho-p70S6K immunocontent in the hippocampus (A and C) and prefrontal cortex (B and D) of mice submitted to the TST and OFT. Values are expressed as means ± S.E.M (n = 7). **$P < 0.01$ as compared with the vehicle-treated control (one-way ANOVA followed by Newman-Keuls post-hoc test).

and guanosine. Ketamine or guanosine alone did not present any behavioral effect. A single coadministration with ketamine and/or guanosine did not significantly alter the number of crossings in the OFT (treatment (F $_{(3, 56)}$ = 0.20, P = 0.89), CORT administration (F $_{(1, 56)}$ = 0.01, P = 0.89), treatment × CORT administration interaction (F $_{(3, 56)}$ = 0.15, P = 0.92), Fig. 6B).

4. Discussion

We demonstrated that guanosine potentiates the antidepressant-like effect of ketamine, a response associated with the pro-synaptogenic signaling pathway, although with a different profile in the hippocampus and prefrontal cortex of mice. Noteworthy, the effectiveness of this

Fig. 5. Effect of a single administration with subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) on PSD-95, GluA1 and synapsin immunocontent in the hippocampus (A and C) and prefrontal cortex (B and D) of mice submitted to the TST and OFT. Values are expressed as means ± S.E.M (n = 7). **$P < 0.01$ as compared with the vehicle-treated control (one-way ANOVA followed by Newman-Keuls *post-hoc* test).

Fig. 6. Effect of a single administration with ketamine (Ket – 1 mg/kg, i.p.), guanosine (Guo – 0.05 mg/kg, p.o.), and th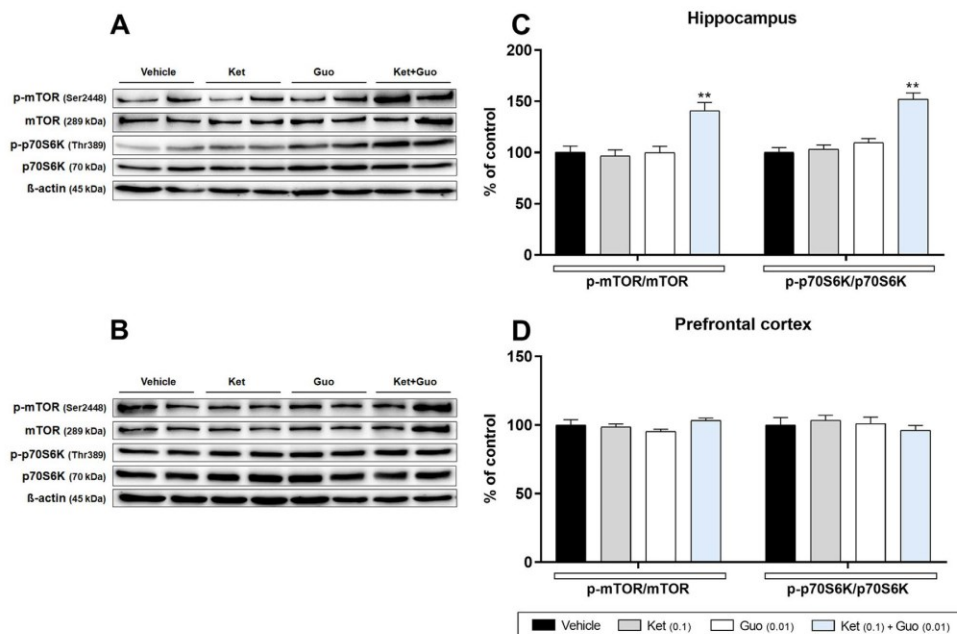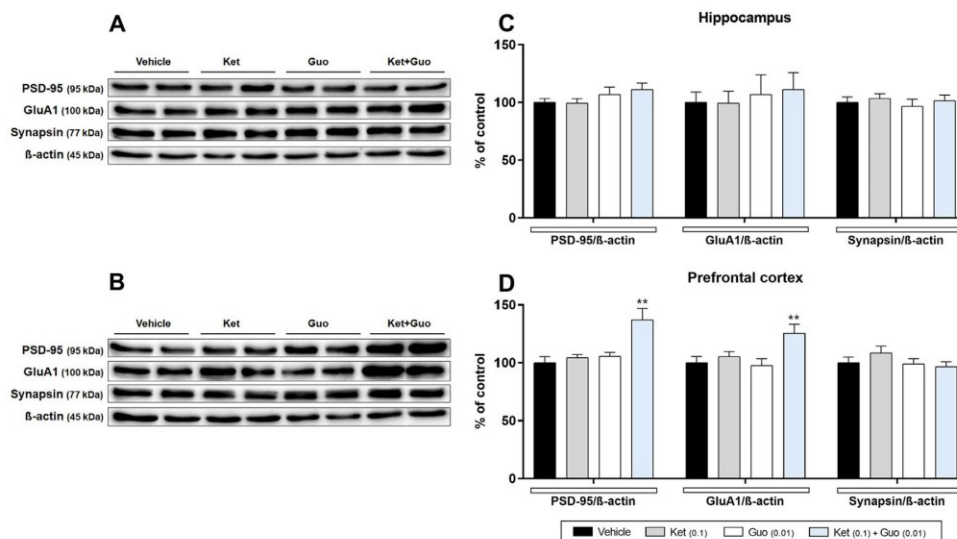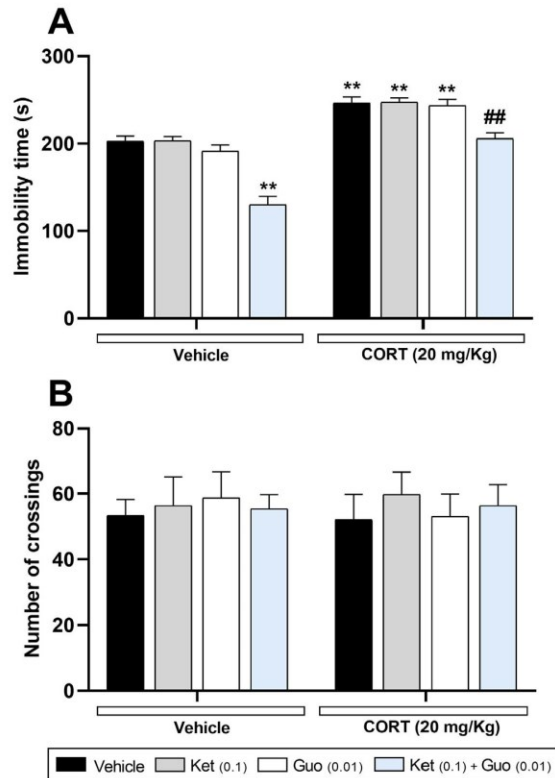e coadministration with a sub-threshold dose of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) in mice treated or not with corticosterone (CORT – 20 mg/kg, p.o.) and submitted to TST and OFT. Values are expressed as means ± S.E.M ($n = 8$). **$P < 0.01$ as compared with the vehicle-treated group; ##$P < 0.01$ as compared with the CORT-treated group (two-way ANOVA followed by Newman-Keuls *post-hoc* test).

putative augmentation strategy is also observed in an animal model of depression induced by repeated CORT administration.

Guanosine is an endogenous nucleoside that exerts a core role in the central nervous system, especially in brain regions involved with mood regulation (Bettio et al., 2016; Lanznaster et al., 2016). It is proposed that guanosine could play a role in the pathophysiology of depression, particularly due to a compelling finding demonstrating that plasma levels of this nucleoside were reduced in patients with depression (Ali-Sisto et al., 2016). Reinforcing this notion, our research group has provided evidence that guanosine produces an antidepressant-like effect in behavioral tests predictive of antidepressant activity in mice, the TST (0.05, 0.1 and 0.5 mg/kg, p.o.) and forced swim test (0.5, 1 and 5 mg/kg, p.o.) in experiments conducted in male and female mice homogeneously distributed into the groups (Bettio et al., 2012). We confirm and extend these results here by showing that acute administration of guanosine (0.05 and 1 mg/kg, p.o.) exerts an antidepressant-like effect in the TST in male mice, without affecting the locomotor performance of mice. Moreover, in agreement with literature data (Ludka et al., 2013), we showed that ketamine was effective to reduce the immobility time in the TST at 1 and 5 mg/kg. In the present study, we also confirm that the acute coadministration with subthreshold doses of ketamine (0.1 mg/kg, i.p.) and guanosine (0.01 mg/kg, p.o.) are effective to produce an antidepressant-like effect in the TST, in

agreement with a previous result (Bettio et al., 2012). Reinforcing the notion that the effect of ketamine may be augmented by guanosine coadministration, we recently reported a similar augmentation response in the novelty-suppressed feeding test in mice (Camargo et al., 2019).

Considering the relevance of strategies to increase the rapid action of ketamine, we decided to obtain deeper insight into the mechanisms associated with the effects of the combined administration of ketamine plus guanosine in circuitries/brain regions that underlie fast-acting antidepressant responses, the hippocampus, and prefrontal cortex. Particularly, we investigated the role of the mTOR-mediated signaling pathway and its synaptic downstream proteins. Although the mTOR hypothesis to explain the rapid effect of ketamine has been questioned (Popp et al., 2016; Abdallah et al., 2018), a great body of evidence has shown that the mTOR-dependent synapse formation is a substantial target for the effect of fast-acting antidepressant agents (Li et al., 2010; 2011; Réus et al., 2015). Importantly, it would be interesting to investigate how ketamine plus guanosine treatment affects the hippocampus and prefrontal cortex, specifically whether distinct or even inverse patterns on mTOR signaling occur in these brain regions after the coadministration of these compounds. mTOR is a ubiquitously expressed serine/threonine kinase that controls a core of cell processes, particularly the translation via the assembly of multi-protein signaling complexes (Hoeffer and Klann, 2010). Remarkably, mTOR signaling is involved in the behavioral and synaptic plasticity effects of ketamine (Li et al., 2010), scopolamine (Voleti et al., 2013), and creatine (Pazini et al., 2016). Our results showed that ketamine plus guanosine treatment increased mTOR phosphorylation on Ser^{2448}, indicative of its activation, following their administration (1 h after guanosine and 30 min after ketamine) in the hippocampus, but not in the prefrontal cortex, of mice. This result is consistent with prior evidence that reported increased mTOR phosphorylation in the hippocampus of mice following 1 h of coadministration with subthreshold doses of ketamine and guanosine (Camargo et al., 2019).

Active mTORC1 promotes protein synthesis through the phosphorylation of key effectors, as the 70 kDa ribosomal protein S6 kinase (p70S6K) (Ersahin et al., 2015; Soliman et al., 2010). p70S6K phosphorylation at Thr^{389} residue by mTORC1 promotes translation initiation and elongation since this kinase stimulates components of the translational machinery such as ribosomal protein S6 (rpS6) and eukaryotic elongation factor 2 kinase (eEF2K) (Ma and Blenis, 2009). Therefore, we investigated whether ketamine plus guanosine treatment affects p70S6K phosphorylation in the hippocampus and prefrontal cortex. Herein, we observed that the combined administration of ketamine plus guanosine increased p70S6K phosphorylation at Thr^{389} in the hippocampus, but not in the prefrontal cortex. Importantly, activation of p70S6K has become a suitable target for readout of mTORC1 activation, reinforcing the present results (Soliman et al., 2010). mTOR-mediated signaling controls the translation of synaptic proteins such as PSD-95, AMPA receptor subunits 1 (GluA1) and synapsin, which are important to promote new dendritic spines formation and synaptogenesis (Duman et al., 2016; Li et al., 2010). Notably, deficits on PSD95 (Feyissa et al., 2009), GluA1 (Rafalo-Ulinska et al., 2016) and synapsin (Kang et al., 2012) immunocontent were showed in the prefrontal cortex of postmortem tissue of depressed subjects, reinforcing the notion that these proteins are core targets implicated in depression. We observed herein an increase on the immunocontent of PSD-95 and GluA1 in the prefrontal cortex, but not in the hippocampus, after a single administration of ketamine plus guanosine. The absence of effect in the hippocampus agrees with a previous study in which no alteration in PSD-95, GluA1, and synapsin was observed in mice previously subjected to the novelty-suppressed feeding test (Camargo et al., 2019). The distinct regional pattern of synaptogenic signaling pathway activation was firstly observed in the present study after the augmentative ketamine plus guanosine treatment. Indeed, emotion-related circuitries/brain regions that underlie the neurobiology of depression and
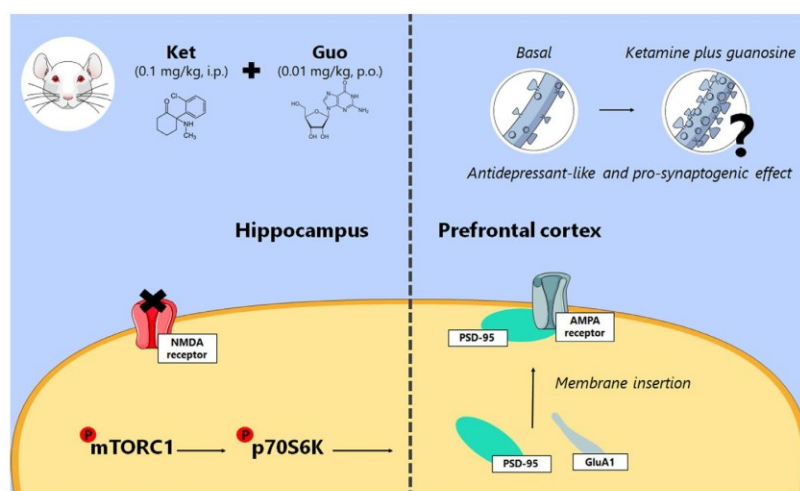
Fig. 7. Putative signaling pathways implicated in the augmentation effect evoked by guanosine over ketamine's antidepressant-like and pro-synaptogenic effects. The mammalian target of rapamycin complex 1 (mTORC1) is a ubiquitously expressed serine/threonine kinase that controls protein synthesis through the phosphorylation of 70 kDa ribosomal protein S6 kinase (p70S6K). Among the proteins regulated by mTORC1 stands out AMPA receptor subunits 1 (GluA1) and postsynaptic density-9 (PSD-95). These synaptic proteins are subsequently inserted into the cell membrane contributing to new dendritic spines formation and synaptogenesis (Hoeffer and Klann, 2010). We provided evidence that a single administration with subthreshold doses of ketamine (0.1 mg/kg, i.p.) and guanosine (0.01 mg/kg, p.o.) increased mTOR (Ser2448) and p70S6K (Thr389) phosphorylation in the hippocampus, but not in the prefrontal cortex (1 h after guanosine and 30 min after ketamine). Conversely, it was detected an increase in the immunocontent of PSD-95 and GluA1 in the prefrontal cortex, but not in the hippocampus, after a single administration of ketamine plus guanosine. These results raise the supposition that ketamine plus guanosine treatment affects the hippocampus and prefrontal cortex in a distinct pattern on synaptogenic signaling, specifically, this mechanism seems to occur in a faster manner in the prefrontal cortex, than the hippocampus. Figure designed using images from Servier Medical Art and Mind the Graph.

fast-acting antidepressant responses have intrinsic features (Liu et al. 2017). We provide evidence that ketamine plus guanosine treatment affects the hippocampus and prefrontal cortex in a distinct pattern on synaptogenic signaling. Therefore, considering data presented herein one may suppose that the neurochemical effects dependent on mTOR activation could occur faster in the prefrontal cortex since increased immunocontent of synaptic proteins targets of mTOR were observed in this region after the combined treatment with guanosine plus ketamine, whereas in the hippocampus an event upstream to synaptic protein translation (mTOR/p70S6K phosphorylation) was detected. These findings provide novel insights into the region specificity of the mechanism underlying the augmentation response of ketamine by guanosine.

To shed light on the possible rapid augmentation effect of ketamine by guanosine, the ability of the combined administration of these drugs to counteract the depressive-like behavior induced by CORT was tested. Notably, the stress induced by repeated administration of CORT is a useful model to mimic physiological and behavioral alterations that occur in human depression (Hare et al., 2017). Accordingly, this animal model is responsive only to chronic, but not acute, administration of conventional antidepressants, which mirrors the clinical situation (Pazini et al., 2016; Zeni et al., 2019). However, this model is sensitive to a single administration of fast-acting antidepressants (Koike et al., 2013; Neis et al., 2018; Pazini et al., 2016). We demonstrated herein that administration of CORT for 21 days was effective to induce depressive-like behavior by increasing the immobility in the TST, a result consistent with prior reports (Pazini et al., 2016; Zhao et al., 2008). Of note, a single treatment with subthreshold doses of ketamine and guanosine rescued the depressive-like phenotype of mice exposed to CORT, reinforcing the notion that the combined administration of these drugs is effective to elicit a fast antidepressant-like response.

The exact mechanism by which guanosine acts to potentiate the antidepressant-like and pro-synaptogenic effects of subthreshold doses of ketamine remains unclear, although some hypotheses may be raised. The antidepressant-like effect of guanosine has been proposed to be associated with a modulation of NMDA receptor, similar to ketamine, since its antidepressant-like effect in the TST was abolished by NMDA (NMDA receptor agonist) and D-serine (glycine-site NMDA receptor agonist) (Bettio et al., 2012). Of note, the PI3K inhibitors (LY294002 or wortmannin) and mTORC1 inhibitor (rapamycin) abolished the antidepressant-like effect of guanosine, suggesting the PI3k/mTOR pathway is required for its behavioral effect (Bettio et al., 2012), similarly to ketamine. Thus, we may suppose that guanosine could share similar molecular targets to ketamine, which in turn could trigger convergent mechanisms, including the mTOR pathway that could be responsible for the antidepressant-like effect elicited by the combined administration of subthreshold doses of guanosine and ketamine. However, the initial targets by which guanosine acts remain to be determined, even in the glutamatergic system, and a guanosine receptor have not been characterized until now (Lanznaster et al., 2016). In addition, an interaction between ketamine and guanosine/guanosinergic system may account for the augmentation effect observed when both drugs were administered at subthreshold doses. Accordingly, a single administration of ketamine raises guanosine precursors (GTP and GDP) after 2 weeks of the treatment, suggesting that ketamine could modulate the guanine-based purinergic system (McGowan et al., 2018). Based on these findings, one may also suppose that ketamine and guanosine could interact with each other, a hypothesis that should be addressed in a future study.

5. Limitations

The present study has some limitations. First, this study does not ascertain the causal relationship between mTOR signaling, synaptic plasticity, and behavioral outcomes. Second, considering that convergent evidence supports the evidence regarding the stimulation on the synaptogenic pathway independent on the stimulation of the mTOR signaling pathway, we cannot rule out that the increase in synaptic proteins occurred regardless mTOR-mediated signaling, i.e, by other protein translation mechanisms. Further experiments using mTORC1 inhibitor (rapamycin) and general protein synthesis inhibitors (such as anisomycin, sparsomycin, and others) could be carried out in futures studies to ascertain this issue. Third, although our study has provided evidence supporting the role of the pro-synaptogenic signaling pathway for the antidepressant-like effect of combined administration of ketamine and guanosine, the formation and function of new synapses were not addressed.

6. Conclusion

We provided evidence regarding the potentiation effect triggered by guanosine onto ketamine's antidepressant-like response, and this behavioral effect was associated with the stimulation of pro-synaptogenic signaling pathway in the prefrontal cortex and hippocampus, although with a distinct profile (Fig. 7). The augmentation effect of ketamine by guanosine could have therapeutic relevance as a potential adjunctive treatment for the management of treatment-resistant depression. Therefore, this study opens perspectives for the design of proof-of-concept trials for the combined use of ketamine and guanosine for depression.

Funding

No external funding was used for this study.

Authors' contribution

Ana Lúcia S. Rodrigues and Anderson Camargo designed the study and wrote the protocol. Anderson Camargo, Ana P. Dalmagro, and Ana Lúcia B. Zeni administered the drugs, performed the behavioral tests and western blotting analysis. All authors contributed to undertook the statistical analysis and wrote the first draft of the manuscript, as well as approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2020.03.186.

References

Abdallah, C., Averill, L.A., Gueorguieva, R., Goktas, S., Purohit, P., Ranganathan, M., D'Souza, D.C., Formica, R., Southwick, S.M., Duman, R.S., Sanacora, G., Krystal, J., 2018. Rapamycin, an immunosuppressant and mTORC1 inhibitor, triples the antidepressant response rate of ketamine at 2 weeks following treatment: A double-blind, placebo-controlled, cross-over, randomized clinical trial. Preprint at 10.1101/500959.

Ali-Sisto, T., Tolmunen, T., Toffol, E., Viinamäki, H., Mäntyselkä, P., Valkonen-Korhonen, M., Honkalampi, K., Ruusunen, A., Velagapudi, V., Lehto, S.M., 2016. Purine metabolism is dysregulated in patients with major depressive disorder. Psychoneuroendocrinology 70, 25–32.

Barowsky, J., Schwartz, T.L., 2006. An evidence-based approach to augmentation and combination strategies for treatment resistant depression. Psychiatry 3, 42–61.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry 47, 351–354.

Bettio, L.E.B., Cunha, M.P., Budni, J., Pazini, F.L., Oliveira, Á., Colla, A.R., Rodrigues, A.L.S., 2012. Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. Behav. Brain Res. 234, 137–148.

Bettio, L.E.B., Gil-Mohapel, J., Rodrigues, A.L.S., 2016. Guanosine and its role in neuropathologies. Purinergic Signal 12, 411–426.

Camargo, A., Rodrigues, A.L.S., 2019. Novel targets for fast antidepressant responses: Possible role of endogenous neuromodulators. Chronic Stress 3, 1–13.

Camargo, A., Pazini, F.L., Rosa, J.M., Wolin, I.A.V., Moretti, M., Rosa, P.B., Neis, V.B., Rodrigues, A.L.S., 2019. Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway. J. Psychiatr. Res. 115, 103–112.

Crisafulli, C., Fabbri, C., Porcelli, S., Drago, A., Spina, E., De Ronchi, D., Serretti, A., 2011. Pharmacogenetics of antidepressants. Front. Pharmacol. 1–21 FEB.

Duman, R.S., Aghajanian, G.K., Sanacora, G., Krystal, J.H., 2016. Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. Nat. Med. 22, 238–249.

Ersahin, T., Tuncbag, N., Cetin-Atalay, R., 2015. PI3K/AKT/mTOR Interactive Pathway. Mol. Biosyst. 1, 1–9.

Feyissa, A.M., Chandran, A., Stockmeier, C.A., Karolewicz, B., 2009. Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 70–75.

Gao, M., Rejaei, D., Liu, H., 2016. Ketamine use in current clinical practice. Acta Pharmacol. Sin. 37, 865–872.

Han, C., Wang, S., Seo, H., Chul, B., Jin, H., Kim, W., Kwak, K., Pae, C., 2014. Aripiprazole augmentation, antidepressant combination or switching therapy in patients with major depressive disorder who are partial- or non-responsive to current antidepressants : a multi-center, naturalistic study. J. Psychiatr. Res. 49, 75–82.

Hare, B.D., Ghosal, S., Duman, R.S., 2017. Rapid acting antidepressants in chronic stress models: molecular and cellular mechanisms. Chronic Stress 1, 1–12.

Hoeffer, C.A., Klann, E., 2010. mTOR signaling: At the crossroads of plasticity, memory and disease. Trends Neurosci. 33, 67–75.

Kang, H.J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C.A., Licznerski, P., Lepack, A., Majik, M.S., Jeong, L.S., Banasr, M., Son, H., Duman, R.S., 2012. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat. Med. 18, 1413–1417.

Koike, H., Iijima, M., Chaki, S., 2013. Effects of ketamine and LY341495 on the depressive-like behavior of repeated corticosterone-injected rats. Pharmacol. Biochem. Behav. 107, 20–23.

Lanznaster, D., Dal-Cim, T., Piermartiri, T.C.B., Tasca, C.I., 2016. Guanosine: A neuromodulator with therapeutic potential in brain disorders. Aging Dis. 7, 657–679.

Leal, R.B., Cordova, F.M., Herd, L., Bobrovskaya, L., Dunkley, P.R., 2002. Lead-stimulated p38MAPK-dependent Hsp27 phosphorylation. Toxicol. Appl. Pharmacol. 178, 44–51.

Li, N., Lee, B., Liu, R.-J., Banasr, M., Dwyer, J.M., Iwata, M., Li, X.-Y., Aghajanian, G., Duman, R.S., 2010. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA Antagonists. Science 329, 959–965.

Li, N., Liu, R.J., Dwyer, J.M., Banasr, M., Lee, B., Son, H., Li, X.Y., Aghajanian, G., Duman, R.S., 2011. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol. Psychiatry 69, 754–761.

Liu, W.X., Wang, J., Xie, Z.M., Xu, N., Zhang, G.F., Jia, M., Zhou, Z.Q., Hashimoto, K., Yang, J.J., 2016. Regulation of glutamate transporter 1 via BDNF-TrkB signaling plays a role in the anti-apoptotic and antidepressant effects of ketamine in chronic unpredictable stress model of depression. Psychopharmacology 233, 405–415.

Liu, W., Ge, T., Leng, Y., Pan, Z., Fan, J., Yang, W., Cui, R., 2017. The role of neural plasticity in depression: from hippocampus to prefrontal cortex. Neural Plast. 2017, 6871089.

Ludka, F.K., Zomkowski, A.D.E., Cunha, M.P., Dal-Cim, T., Zeni, A.L.B., Rodrigues, A.L.S., Tasca, C.I., 2013. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the l-arginine-nitric oxide-cyclic guanosine monophosphate pathway and increases BDNF levels. Eur. Neuropsychopharmacol. 23, 400–412.

Ma, X.M., Blenis, J., 2009. Molecular mechanisms of mTOR-mediated translational control. Nat. Rev. 10, 307–318.

MacQueen, G.M., Yucel, K., Taylor, V.H., Macdonald, K., Joffe, R., 2008. Posterior hippocampal volumes are associated with remission rates in patients with major depressive disorder. Biol. Psychiatry 64, 880–883.

McGowan, J.C., Hill, C., Mastrodonato, A., LaGamma, C.T., Kitayev, A., Brachman, R.A., Narain, N.R., Kiebish, M.A., Denny, C.A., 2018. Prophylactic ketamine alters nucleotide and neurotransmitter metabolism in brain and plasma following stress. Neuropsychopharmacology 43, 1813–1821.

Moret, C., 2005. Combination / augmentation strategies for improving the treatment of depression. Neuropsychiatr. Dis. Treat. 1, 301–309.

Neis, V.B., Bettio, L.B., Moretti, M., Rosa, P.B., Olescowicz, G., Fraga, D.B., Goncalves, F.M., Freitas, A.E., Heinrich, I.A., Lopes, M.W., Leal, R.B., Rodrigues, A.L.S., 2018. Single administration of agmatine reverses the depressive-like behavior induced by corticosterone in mice: Comparison with ketamine and fluoxetine. Pharmacol. Biochem. Behav. 173, 44–50.

Otte, C., Gold, S.M., Penninx, B.W., Pariante, C.M., Etkin, A., Fava, M., Mohr, D.C., Schatzberg, A.F., 2016. Major depressive disorder. Nat. Rev. Dis. Primers 2, 16065.

Papakostas, G.I., Ionescu, D.F., 2015. Towards new mechanisms: An update on therapeutics for treatment-resistant major depressive disorder. Mol. Psychiatry 20, 1142–1150.

Pazini, F.L., Cunha, M.P., Rosa, J.M., Colla, A.R.S., Lieberknecht, V., Oliveira, Á., Rodrigues, A.L.S., 2016. Creatine, similar to ketamine, counteracts depressive-like behavior induced by corticosterone via PI3K/Akt/mTOR pathway. Mol. Neurobiol. 53, 6818–6834.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83, 346–356.

Popp, S., Behl, B., Joshi, J.J., Lanz, T.A., Spedding, M., Schenker, E., Jay, T.M., Svenningsson, P., Caudal, D., Cunningham, J.I., Deaver, D., Bespalo, A., 2016. In search of the mechanisms of ketamine's antidepressant effects: How robust is the evidence behind the mTor activation hypothesis. F1000Res634, 1–13.

Rafalo-Ulinska, A., Piotrowska, J., Kryczyk, A., Sowa-kucma, M., Misztak, P., Rajkowska, G., Stockmeier, C.A., Datka, W., Nowak, G., Szewczyk, B., 2016. Zinc transporters

protein level in postmortem brain of depressed subjects and suicide victims. J. Psychiatr. Res. 83, 220–229.

Réus, G.Z., Quevedo, J., Rodrigues, A.L.S., 2015. mTOR signaling in the neuropathophysiology of depression: current evidence. J. Recept. Ligand Channel Res. 8, 65–74.

Savitz, J., Drevets, W.C., 2009. Bipolar and major depressive disorder: Neuroimaging the developmental-degenerative divide. Neurosci. Biobehav. Rev. 33, 699–771.

Soliman, G.A., Acosta-Jaquez, H.A., Dunlop, E.A., Ekim, B., Maj, N.E., Tee, A.R., Fingar, D.C., 2010. mTOR Ser-2481 autophosphorylation monitors mTORC-specific catalytic activity and clarifies rapamycin mechanism of action. J. Biol. Chem. 285, 7866–7879.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: A new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Voleti, B., Navarria, A., Liu, R., Banasr, M., Li, N., Terwilliger, R., Sanacora, G., Eid, T., Aghajanian, G., Duman, R.S., 2013. Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. Biol. Psychiatry 74, 742–749.

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H., 2006. a randomized trial of an n-methyl-d-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry 63, 856–864.

Zeni, A.L.B., Camargo, A., Dalmagro, A.P., 2019. Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities. Pharmacol. Biochem. Behav. 179, 63–72.

Zhao, Y., Ma, R., Shen, J., Su, H., Xing, D., Du, L., 2008. A mouse model of depression induced by repeated corticosterone injections. Eur. J. Pharmacol. 581, 113–120.

Zhou, W., Wang, N., Yang, C., Li, X.M., Zhou, Z.Q., Yang, J.J., 2014. Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus and prefrontal cortex. Eur. Psychiatry 29, 419–423.

4.2 CAPÍTULO 2

Guanosine boosts the fast, but not sustained, antidepressant-like and pro-synaptogenic effects of ketamine by stimulating mTORC1-driven signaling pathway

European Neuropsychopharmacology, v. 57, p. 15-29, 2022.

Anderson Camargo, Ana Paula Dalmagro, Eslen Delanogare, Daiane B. Fraga, Ingrid A. V. Wolin, Ana Lúcia B. Zeni, Patricia S. Brocardo, Ana Lúcia S. Rodrigues

Fator de impacto: 4.600 (JCR 2020)

Guanosine boosts the fast, but not sustained, antidepressant-like and pro-synaptogenic effects of ketamine by stimulating mTORC1-driven signaling pathway

Anderson Camargo[a], Ana Paula Dalmagro[d], Eslen Delanogare[a], Daiane B. Fraga[a], Ingrid A.V. Wolin[b], Ana Lúcia B. Zeni[d], Patricia S. Brocardo[a,c], Ana Lúcia S. Rodrigues[a,b,*]

[a] Neuroscience Graduate Program, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis, 88040-900 SC, Brazil
[b] Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis, 88040-900 SC, Brazil
[c] Department of Morphological Sciences, Center of Biological Sciences, Federal University of Santa Catarina, Florianopolis, 88040-900 SC, Brazil
[d] Laboratory of Evaluation of Bioactive Substances, Department of Natural Sciences, Universidade Regional de Blumenau, 89030-903 Blumenau, SC, Brazil

Abstract
The mTORC1-dependent dendritic spines formation represents a key mechanism for fast and long-lasting antidepressant responses, but it remains to be determined whether this mechanism may account for the ability of guanosine in potentiating ketamine's actions. Here, we investigated the ability of ketamine plus guanosine to elicit fast and sustained antidepressant-like and pro-synaptogenic effects in mice and the role of mTORC1 signaling in these responses. The combined administration of subthreshold doses of ketamine (0.1 mg/kg, i.p.) and guanosine (0.01 mg/kg, p.o.) caused a fast (1 h – 24 h), but not long-lasting (7 days) reduction in

*Corresponding author at: Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis 88040-900, SC, Brazil.
E-mail address: ana.l.rodrigues@ufsc.br (A.L.S. Rodrigues).

the immobility time in the tail suspension test. This behavioral effect was paralleled by a rapid (started in 1 h) and transient (back to baseline in 24 h) increase on BDNF, p-Akt (Ser[473]), p-GSK-3β (Ser[9]), p-mTORC1 (Ser[2448]), p-p70S6K (Thr[389]) immunocontent in the hippocampus, but not in the prefrontal cortex. Conversely, ketamine plus guanosine increased PSD-95 and GluA1 immunocontent in the prefrontal cortex, but not the hippocampus after 1 h, whereas increased levels of these proteins in both brain structures were observed after 24 h, but these effects did not persist after 7 days. The combined administration of ketamine plus guanosine raised the dendritic spines density in the ventral hippocampal DG and prefrontal cortex after 24 h Rapamycin (0.2 nmol/site, i.c.v.) abrogated the antidepressant-like effect and pro-synaptogenic responses triggered by ketamine plus guanosine. These results indicate that guanosine may boost the antidepressant-like effect of ketamine for up to 24 h by a mTORC1-dependent mechanism.

1. Introduction

Major depressive disorder (MDD), a widespread psychiatric condition associated with an increased risk of suicide, has considerable social and economic consequences (World Health Organization, 2017). Despite the high prevalence and severity of MDD, its harmful effects are compounded by the lack of fast and efficacious antidepressant therapies (Otte et al., 2016). The current monoaminergic-targeted antidepressants can take weeks to months to promote a therapeutic response, and a significant percentage of patients do not achieve symptom remission even after being treated with multiple antidepressants (Papakostas and Ionescu, 2015). In addition, these drugs are ineffective for the treatment of suicide ideation in patients with severe MDD (Penn and Tracy, 2012). These drawbacks underscore the necessity for developing new antidepressant strategies with a faster onset effect and greater efficacy.

A significant advancement for the treatment of MDD was the discovery that a single administration of a subanesthetic dose of ketamine, an NMDA (N-methyl-D-aspartate) receptor antagonist, produces fast and long-lasting antidepressant responses in patients with MDD (Berman et al., 2000; Zarate et al., 2006), even in those with suicidal ideation (DiazGranados et al., 2010). These findings have triggered a significant interest of the scientific community to study the mechanisms underlying the antidepressant-like actions of ketamine. Accumulating evidence suggests that ketamine exerts its antidepressant effect by promoting dendritic spine formation and synaptogenesis in the hippocampus (Fraga et al., 2020, 2021) and prefrontal cortex (Li et al., 2010, 2011), key regions involved in mood modulation. More importantly, compelling reports showed that ketamine's antidepressant and synaptic effects are activity-dependent and result from a burst of glutamate, brain-derived neurotrophic factor (BDNF) release, and activation of the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway (Lepack et al., 2014; Li et al., 2010). Particularly, mTORC1 activation promotes synaptic protein synthesis, such as postsynaptic density protein-95 kDa (PSD-95), alpha-amino-3-hydroxy-methyl-5-4-isoxazole propionic acid (AMPA) receptor subunits 1 (GluA1), and synapsin via phosphorylation and activation of 70 kDa ribosomal protein S6 kinase (p70S6K) (Abdallah et al., 2016; Huang and Fingar, 2014). Of note, BDNF release and the activation of mTORC1 signaling have been proposed as crucial mechanisms for fast and long-lasting antidepressant and pro-synaptogenic responses (Abdallah et al., 2016). Therefore, strategies targeting synaptogenesis via mTORC1 could afford rapid and sustained antidepressant responses.

The main limitation regarding ketamine use is that it may produce undesirable side effects, notably psychotomimetic and dissociative symptoms, besides its abuse potential (Gao et al., 2016). Therefore, novel antidepressants that present ketamine-like actions, but are devoid of adverse effects are one of the major focuses of current drug development. Despite the encouraging results with other NMDA receptor channel blockers in preclinical studies, these drugs have failed to exert the full spectrum of ketamine's actions in clinical trials (Newport et al., 2015). Given this scenario, strategies to increase the antidepressant responses of ketamine with a lower potential for undesirable effects are welcome. Augmentation strategy with ongoing antidepressant medication combined with non-antidepressant agents may be a valuable tool for managing MDD when the first-choice drug has drawbacks (Han et al., 2014; Papakostas et al., 2005; Park, 2020; Zhou et al., 2019). Importantly, augmentation therapy acts within the fast-response window and is preferred over monotherapy because it may significantly improve depressive symptoms (Malhi et al., 2020; Moret, 2005). Interestingly, this strategy allows the curtail of the antidepressant dose, which may reduce its knock-on effects (Barowsky and Schwartz, 2006; Moret, 2005).

Guanosine, an endogenous purine, has become a promising candidate to exert a fast antidepressant response or augment the ketamine's actions (Camargo and Rodrigues, 2019). Particularly, the antidepressant-like effect of guanosine involves the modulation of NMDA and AMPA receptors and the stimulation of BDNF/mTORC1 signaling (Bettio et al., 2012; Rosa et al., 2021). A single administration of guanosine, similar to ketamine, was able to reverse the depressive-like behavior induced by olfactory bulbectomy in mice via the mTORC1 pathway (Almeida et al., 2020). Interestingly, the ability of guanosine in boosting the rapid antidepressant-like and pro-synaptogenic effects of subthreshold doses of ketamine has been demonstrated by our research group (Camargo et al., 2019, 2020). Noteworthy, we showed in a recent study that mTORC1 underlies the antidepressant-like and pro-synaptogenic responses dis-

played by a low-dose combination of ketamine and guanosine in a mouse model of stress (Camargo et al., 2021). However, whether guanosine is effective in potentiating the long-lasting antidepressant-like actions of ketamine remains to be determined. More importantly, the exact role of the mTORC1 signaling pathway and dendritic spine formation in the antidepressant-like effect elicited by ketamine plus guanosine remains to be thoroughly investigated.

Within this context, this study investigated the ability of guanosine to potentiate the rapid and long-lasting antidepressant-like effect of subthreshold doses of ketamine and whether these responses could be associated with an increase in protein synthesis and dendritic spines formation by stimulating the mTORC1 signaling pathway.

2. Material and methods

2.1. Animals

Male *Swiss* mice (30–40 g, 45–60 days of age, substrain Swiss-UFSC) were housed in groups of 8 in a cage (41 × 34 × 16 cm), maintained under controlled temperature (21 ± 1 °C) and humidity (50 ± 20%) with a 12:12 h light/dark cycle (lights on at 7:00 a.m.), and with free access to food and water. The experiments were carried out according to the National Institute of Health Guide for the Care and Use of Laboratory Animals and were performed after approval of the protocol by the Institutional Ethics Committee (N° 8420,231,120). Mice were provided by the Central Animal Care Facility of Universidade Federal de Santa Catarina, Florianópolis, Brazil.

2.2. Drugs and treatments

The present study was divided into two experimental approaches. The first set of experiments was designed to investigate the time course of the antidepressant-like and pro-synaptogenic effects elicited by subthreshold doses of ketamine plus guanosine. Mice were randomly assigned to four experimental groups (n = 7/group): a) control group that received vehicle (i.p.) plus vehicle (p.o.); b) ketamine group that received vehicle (p.o.) plus ketamine (0.1 mg/kg, i.p.); c) guanosine group that received guanosine (0.01 mg/kg, p.o.) plus vehicle (i.p.); d) ketamine plus guanosine group that received guanosine (0.01 mg/kg, p.o.) plus vehicle ketamine (0.1 mg/kg, i.p.). Ketamine and guanosine (obtained from Sigma Chemical Co., St. Louis, U.S.A) were dissolved in saline (0.9%) and distilled water, respectively. Mice were administered orally (p.o.) with guanosine (0.01 mg/kg) and 30 min later intraperitoneally (i.p.) with ketamine (0.1 mg/kg). Then, independent groups of mice were tested 30 min, 24 h, or 7 days after the administration of ketamine in the tail suspension test (TST) and subsequently, after 10 min, in the open-field test (OFT). All doses and time points of administration were chosen based on previous studies (Camargo et al., 2020; Ludka et al., 2013).

The second set of experiments was performed to test whether the mTORC1-driven signaling pathway underlies the ability of guanosine in augmenting ketamine's effects. To address this hypothesis, mice were randomly assigned to four experimental groups (n = 7/group): a) control group that received vehicle (i.c.v.), vehicle (i.p.) plus vehicle (p.o.); b) ketamine plus guanosine group that received vehicle (i.c.v.), ketamine (0.1 mg/kg, i.p.) plus guanosine (0.01 mg/kg, p.o.); c) rapamycin group that received rapamycin (0.2 nmol/site, i.c.v.) plus vehicle (i.p) plus vehicle (p.o.); d) rapamycin (0.2 nmol/site) plus ketamine (0.1 mg/kg) plus guanosine

(0.01 mg/kg) group. Rapamycin (a selective mTORC1 inhibitor) was administered 15 and 45 min before guanosine and ketamine administration, respectively. Mice were subjected to TST 1 h or 24 h later, followed by the OFT after 10 min. Rapamycin was dissolved in sterile saline with dimethyl sulfoxide (DMSO) at a final concentration of 1% and administered by intracerebroventricular route (i.c.v.) in a volume of 3 μl per mouse (0.2 nmol/site). The i.c.v. injections were performed by employing a freehand method under isoflurane anesthesia according to the procedure described previously (Camargo et al., 2019, 2021; ; Pazini et al., 2016, 2020). Briefly, a 0.4-mm external diameter hypodermic needle attached to a cannula linked to a 25-μl Hamilton syringe was inserted perpendicularly through the skull (no more than 2 mm into the brain of each mouse). Rapamycin was administered into the left lateral ventricle, at the following coordinates from bregma taken from the atlas of Franklin and Paxinos (1997): anterioposterior (AP) = −0.1 mm; mediolateral (ML) = 1 mm; and dorsoventral (DV) = −3 mm. The injection was given over 30 s, and the needle remained in place for another 30 s to avoid the reflux of the substances injected. The injection site was 1 mm to the left from the midpoint on a line drawn through to the ears' anterior base and performed by an experienced investigator. After dissection of the animal's brain, the success of the injection was examined, macroscopically, discarding results from mice presenting misplacement of the injection site or any sign of cerebral hemorrhage (<5%).

In both sets of experiments, one cohort of mice (n = 7/group) was immediately euthanized by decapitation, and hippocampus and prefrontal cortex were collected for ELISA and Western Blotting analysis, and a second cohort of animals (n = 6/group) was deeply anesthetized and subjected to transcardial perfusion with 0.9% NaCl for morphological analysis of the hippocampus and prefrontal cortex.

2.3. Tail suspension test (TST)

The total immobility time of mice suspended by the tail was measured as previously proposed (Steru et al., 1985). Visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period by an experienced observer blind to the experimental groups. Mice were considered immobile only when they hung passively and completely motionless.

2.4. Open-field test (OFT)

Mice were individually subjected to the OFT as previously described (Fraga et al., 2018). The apparatus consisted of a wooden box (40 × 60 × 50 cm high) with the floor divided into 12 equal squares. The number of squares crossed was registered for 6 min and considered as a parameter of locomotor activity.

2.5. Brain-derived neurotrophic factor (BDNF) measurement

A BDNF ELISA kit (Promega® Inc., USA) was used to measure mature BDNF protein levels in hippocampal and prefrontal cortex homogenates, according to the manufacturer's instructions.

2.6. Western blotting

Hippocampus and prefrontal cortex were quickly dissected and snap-frozen with liquid nitrogen before storage at −80 °C until use.

Briefly, samples were mechanically homogenized in 400 μl of 50 mM TRIS pH 7.0, 1 mM EDTA, 100 mM NaF, 0.1 mM PMSF, 2 mM Na$_3$VO$_4$, 1% Triton X-100, 10% glycerol, Sigma Protease Inhibitor Cocktail (P2714). Lysates were centrifuged (10,000 g for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in 100 mM TRIS pH 6.8, 4 mM EDTA, 8% SDS, and boiled for 5 min. In the next step, samples were diluted in 40% glycerol, 100 mM TRIS, bromophenol blue (pH 6.8) in the ratio 25:100 (v/v), and β-mercaptoethanol at a final concentration of 8% was added to each sample, as previously described (Pazini et al., 2020; Rosa et al., 2019). Protein content was quantified using bovine serum albumin (BSA) as a standard (Peterson, 1977). The samples containing 60 μg protein/track were electrophoresed in SDS-PAGE (miniVE Vertical Electrophoresis System TM, GE Healthcare Life Sciences, Piscataway, NJ, USA) using 7-10% gel, and the separated proteins were transferred to nitrocellulose membranes using a semi-dry blotting apparatus (1.2 mA/cm2; 1.5 h). Subsequently, the membranes were blocked with 5% BSA in TBS (10 mM Tris, 150 mM NaCl, pH 7.5). By using specific antibodies (Cell Signaling, 1:1000) diluted in TBS-T (10 mM Tris, 150 mM NaCl, 0.1% Tween-10, pH 7.5) containing 2.5% BSA and incubated overnight (Leal et al., 2020), the immunocontent of total and phosphorylated forms of protein kinase B (Akt – Ser^{473}, #9271) glycogen synthase kinase 3β (GSK-3β – Ser^9, #9336), mTORC1 (Ser^{2448}, #2971) and 70 kDa ribosomal protein S6 kinase (p70S6K – Thr^{389}, #9205), as well as the total immunocontent of Akt (#9272), GSK-3β (#9315), mTORC1 (#2972), p70S6K (#9202), PSD-95 (#2507), GluA1 (#13,185), synapsin (#2312), and β-actin (loading control, #4970) were detected. After the membranes were incubated with anti-rabbit antibody horseradish peroxidase-conjugated secondary antibody (Cell Signaling, 1:2500) for 60 min, the immunoreactive bands were developed using a chemiluminescence kit (Amersham ECL Select, Piscataway, USA). All blocking and incubation steps were followed by three washes (5 min) of the membranes with TBS-T. The optical density (OD) of the bands was quantified using Image Lab Software® 4.1 (Bio-Rad Laboratories). The phosphorylation levels of Akt, GSK-3β, mTORC1, and p70S6K were determined as a ratio of OD of the phosphorylated band over OD of the total band. The immunocontent of PSD-95, GluA1, and synapsin was determined as a ratio of the specific protein band over the OD of the β-actin band. Results are expressed as compared to the control group (100%). The horizontally uncropped images of Western Blotting are available in the Suppl. Material.

2.7. Golgi staining and dendrite spine analysis

Under analgesia with lidocaine (7 mg/kg, i.p.) and deep anesthesia with thiopental (100 mg/kg, i.p.), mice were transcardially perfused with 0.9% NaCl solution. Brains were immediately removed, placed in vials containing 20 mL modified Golgi-Cox solution, and stored at room temperature in the dark for 14 days (Fraga et al., 2020, 2021). Following this period, brains were protected in 30% sucrose and cut into 200 μm coronal sections (Vibratome Series 1000; Vibratome, St Louis, USA), collected in 6% sucrose, and blotted dry onto cleaned, 2% gelatin-coated microscope slides. The sections obtained were alkalinized in ammonium hydroxide and fixed in Kodak rapid fixer (Kannangara et al., 2014). The specimens were then dehydrated through a graded series of increasing concentrations of ethanol, cleared in xylene, mounted, coverslipped, and imaged on an IX83 inverted microscope under bright field illumination (Olympus, Tokyo, Japan). Golgi-impregnated cells were selected if they fulfilled the following criteria: had consistent impregnation throughout the extent of the cell body and dendrites, were able to be distinguished from neighboring impregnated cells, and had intact dendritic trees. For dendritic spine density analysis, secondary and tertiary dendritic branches of neurons in the dentate gyrus (DG) of the ventral hippocampus and the prefrontal cortex (within the layers I, II/III, and V) were ac-

quired using a 100 $\times$ magnification oil objective. For this study, 8 segments were analyzed per animal. Starting from the origin of the branch (as a reference point) and continuing away from the cell soma, spines were counted manually along with the selected dendrite segment using ImageJ software (National Institute of Health, Bethesda Maryland, USA). Total dendritic spines in each segment were counted and normalized by the total length of the dendritic segment (Fraga et al., 2020; Freitas et al., 2020; Oliveira et al., 2021). The images had brightness and contrast linearly altered, but spine counting was performed without any image manipulation.

2.8. Statistical analysis

The D'Agostino-Pearson test was used to assess data normality. The differences among experimental groups were determined by one-way or two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test, when appropriate. The description of statistical data is listed in Suppl. Table 1 and Suppl. Table 2. Data are presented as mean $\pm$ standard error of mean (SEM). A value of $P<0.05$ was considered significant.

3. Results

3.1. Subthreshold doses of ketamine plus guanosine elicit antidepressant-like and pro-synaptogenic effects in a time-dependent manner

In the first set of experiments, we investigated the antidepressant-like and pro-synaptogenic effects elicited by a single administration of subthreshold doses of ketamine plus guanosine 1 h after the treatment (Fig. 1A). Ketamine administered in combination with guanosine significantly decreased the immobility time in mice subjected to the TST ($P<0.01$; Fig. 1B), without altering the number of crossings in the OFT (Fig. 1C), a result consistent with an antidepressant-like effect. Regarding the neurochemical findings, ketamine plus guanosine raised the BDNF immunocontent in the hippocampus ($P<0.01$; Fig. 1D) and prefrontal cortex ($P<0.01$; Fig. 1E). Moreover, the combined administration of these drugs increased the phosphorylation of Akt (Ser^{473}), GSK-3β (Ser^9), mTORC1 (Ser^{2448}), and p70S6K (Thr^{389}) in the hippocampus of mice ($P<0.01$; Fig. 1F and G), when compared to the vehicle-treated mice. No significant alterations were detected on the immunocontent of PSD-95, GluA1, and synapsin in the hippocampus of ketamine plus guanosine-treated mice. Conversely, the combined administration of these drugs increased PSD-95 and GluA1 immunocontent in the prefrontal cortex ($P<0.01$; Fig. 1H and I) but did not alter the phosphorylation levels of Akt (Ser^{473}), GSK-3β (Ser^9), mTORC1 (Ser^{2448}), and p70S6K (Thr^{389}) as well as total immunocontent of synapsin in the prefrontal cortex.

In the second set of experiments, we examined the antidepressant-like and pro-synaptogenic effects elicited by ketamine plus guanosine 24 h after the treatment (Fig. 2A). Single coadministration of these drugs significantly reduced the immobility time in the TST ($P<0.01$; Fig. 2B). This result indicates that the combined treatment with these drugs
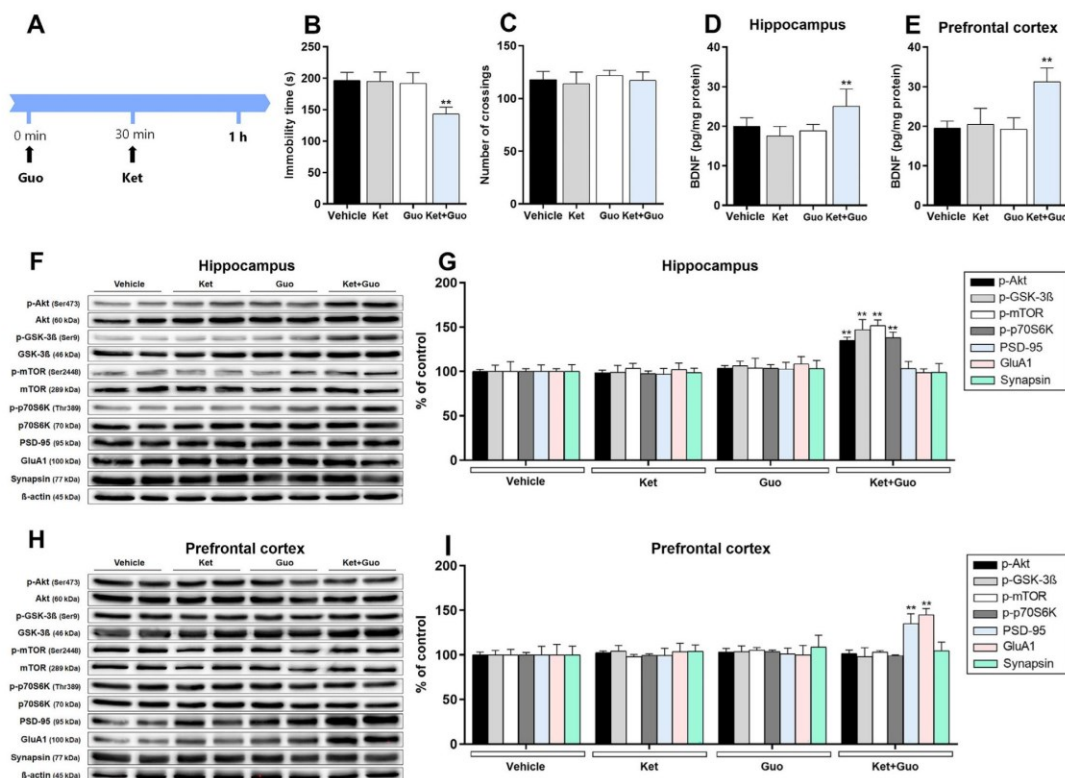
Fig. 1 Effect of a single administration of subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) plus guanosine (Guo – 0.01 mg/kg, p.o.) in the depression-related behavioral tests and mTORC1 signaling pathway following 1 h Experimental timeline (**A**). Panels represent respectively the immobility time in the TST (**B**) and the number of crossings in the OFT (**C**). BDNF immunocontent in the hippocampus (**D**) and prefrontal cortex (**E**). Representative bands of phospho-Akt (Ser^{473}), Akt, phospho-GSK-3β (Ser^9), GSK-3β, phospho-mTOR (Ser^{2448}), mTOR, phospho-p70S6K (Thr^{389}), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the hippocampus (**F**) and prefrontal cortex (**H**) of mice. Densitometric quantification of these proteins in the hippocampus (**G**) and prefrontal cortex (**I**). Statistical analysis was performed by Tukey's *post hoc* test (one-way ANOVA). Values are expressed as means $\pm$ S.E.M ($n = 7$). **$P<0.01$ as compared with the vehicle-treated group.

caused an antidepressant-like effect since no significant alterations in the number of crossings in the OFT were observed (Fig. 2C). No significant changes were observed in the BDNF immunocontent in the hippocampus (Fig. 2D) and prefrontal cortex (Fig. 2E). Conversely, ketamine plus guanosine treatment increased PSD-95 and GluA1 immunocontent in the hippocampus ($P<0.01$; Fig. 2F and G) and prefrontal cortex ($P<0.01$; Fig. 2H and I).

In the next step, we investigated the antidepressant-like and pro-synaptogenic effects elicited by ketamine plus guanosine 7 days after the treatment (Fig. 3A). The immobility time in the TST (Fig. 3B) and the number of crossings in the OFT were not affected (Fig. 3C) by ketamine and/or guanosine administration. No significant alterations were detected on the BDNF immunocontent in the hippocampus (Fig. 3D) and prefrontal cortex (Fig. 3E). Likewise, phosphorylation levels of Akt (Ser^{473}), GSK-3β (Ser^9), mTORC1 (Ser^{2448}), and p70S6K (Thr^{389}) as well as total immunocontent of PSD-95, GluA1, and synapsin in the hippocampus

(Fig. 3F and G) and prefrontal cortex were not significantly altered by any treatment (Fig. 3H and I).

The ability of a single administration of subthreshold doses of ketamine (0.1 mg/kg, i.p.) plus guanosine (0.01 mg/kg, p.o.) to increase the dendritic spines density following 1 h (Fig. 4A), 24 h (Fig. 4F), or 7 days (Fig. 4K) was next investigated. Statistical analysis indicated no significant alterations on the total dendritic spine density in the ventral DG of the hippocampus (Fig. 4B and C) and the prefrontal cortex (Fig. 4D and E) 1 h after ketamine and/or guanosine administration. However, a single administration of ketamine plus guanosine increased the dendritic spine density in the ventral DG of the hippocampus ($P<0.01$; Fig. 4G and H) and the prefrontal cortex ($P<0.01$; Fig. 4I and J) following 24 h when compared to the vehicle-treated group. However, no significant effects on the total dendritic spine density in the ventral hippocampal DG (Fig. 4L and M) and the prefrontal cortex (Fig. 4N and O) after ketamine plus guanosine were observed 7 days after the treatment.
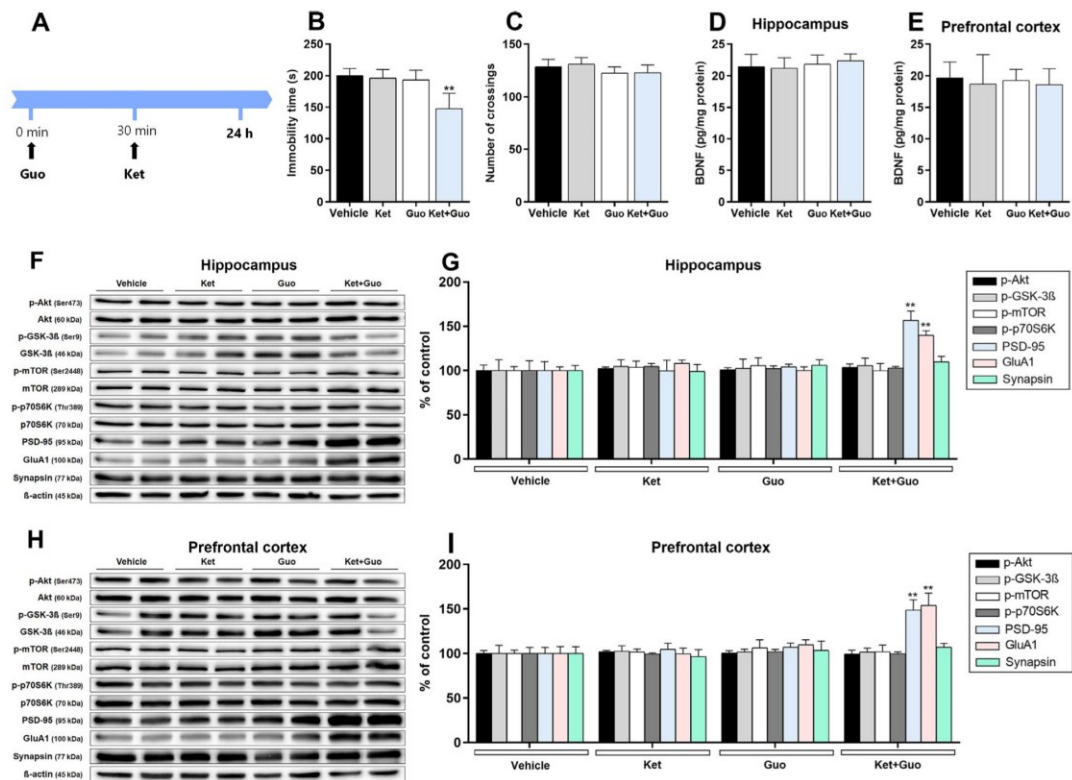
A. Camargo, A.P. Dalmagro, E. Delanogare et al.

Fig. 2 Effect of a single administration of subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) plus guanosine (Guo – 0.01 mg/kg, p.o.) in the depression-related behavioral tests and mTORC1 signaling pathway following 24 h Experimental timeline (**A**). Panels represent respectively the immobility time in the TST (**B**) and the number of crossings in the OFT (**C**). BDNF immunocontent in the hippocampus (**D**) and prefrontal cortex (**E**). Representative bands of phospho-Akt (Ser^{473}), Akt, phospho-GSK-3β (Ser^9), GSK-3β, phospho-mTOR (Ser^{2448}), mTOR, phospho-p70S6K (Thr^{389}), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the hippocampus (**F**) and prefrontal cortex (**H**) of mice. Densitometric quantification of these proteins in the hippocampus (**G**) and prefrontal cortex (**I**). Statistical analysis was performed by Tukey's *post hoc* test (one-way ANOVA). Values are expressed as means $\pm$ S.E.M ($n = 7$). **$P<0.01$ as compared with the vehicle-treated group.

3.2. mTORC1-driven signaling is required for the antidepressant-like and pro-synaptogenic effects displayed by ketamine plus guanosine

To ascertain the hypothesis that mTORC1 participates in the antidepressant-like and pro-synaptogenic effects triggered by ketamine by guanosine following 1 h (Fig. 5A and 7A) and 24 h (Fig. 6A and 7F), mice received rapamycin (a selective mTORC1 inhibitor, 0.2 nmol/site, i.c.v.) prior the treatment of ketamine plus guanosine.

Rapamycin was effective to prevent the decreased immobility time in the TST observed 1 h after the administration of ketamine plus guanosine ($P<0.01$; Fig. 5B). None of the treatments caused any effect on the number of crossings in the OFT (Fig. 5C). Ketamine plus guanosine administration raised the phosphorylation levels of mTOR (Ser^{2448}) and p70S6K (Thr^{389}) in the hippocampus ($P<0.01$; Fig. 5D and E) and the total immunocontent of PSD-95 and GluA1 in the prefrontal cortex ($P<0.01$; Fig. 5F and G). All these neu-

rochemical alterations induced by the combined administration of ketamine and guanosine were prevented by rapamycin infusion ($P<0.01$). No alterations were detected on PSD-95, GluA1, and synapsin immunocontent in the hippocampus after ketamine plus guanosine and/or rapamycin. Likewise, no changes were observed on the phosphorylation levels of mTOR (Ser^{2448}) and p70S6K (Thr^{389}) as well as total immunocontent of synapsin in the prefrontal cortex after ketamine plus guanosine treatment and/or rapamycin.

Rapamycin administration was also effective to prevent the reduced immobility time observed 24 h after the coadministration of subthreshold doses of ketamine and guanosine ($P<0.01$; Fig. 6B). No alterations were observed in the locomotor activity (Fig. 6C). Regarding the neurochemical data, ketamine plus guanosine increased the immunocontent of PSD-95 and GluA1 in the hippocampus ($P<0.01$; Fig. 6D and E) and prefrontal cortex ($P<0.01$; Fig. 6F and G), but this response was blocked by rapamycin pretreatment ($P<0.01$). None of the treatments caused any changes
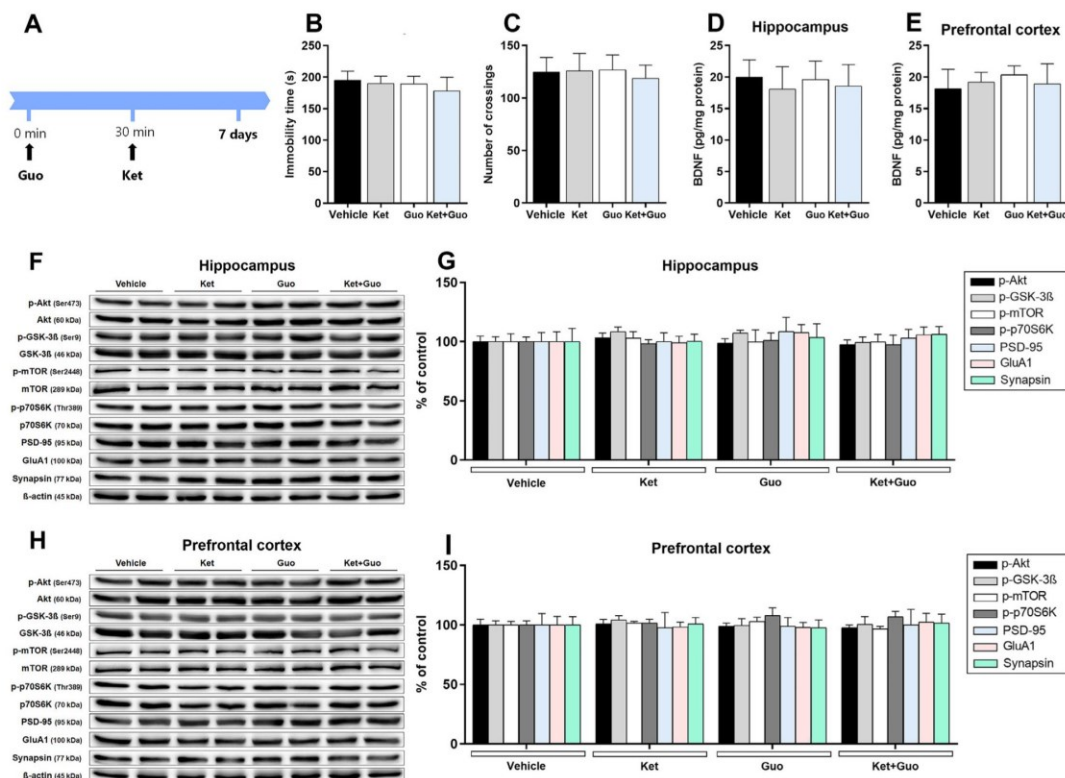
Fig. 3 Effect of a single administration of subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) plus guanosine (Guo – 0.01 mg/kg, p.o.) in the depression-related behavioral tests and mTORC1 signaling pathway following 7 days. Experimental timeline (**A**). Panels represent respectively the immobility time in the TST (**B**) and the number of crossings in the OFT (**C**). BDNF immunocontent in the hippocampus (**D**) and prefrontal cortex (**E**). Representative bands of phospho-Akt (Ser^{473}), Akt, phospho-GSK-3β (Ser^9), GSK-3β, phospho-mTOR (Ser^{2448}), mTOR, phospho-p70S6K (Thr^{389}), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the hippocampus (**F**) and prefrontal cortex (**H**) of mice. Densitometric quantification of these proteins in the hippocampus (**G**) and prefrontal cortex (**I**). Statistical analysis was performed by Tukey's *post hoc* test (one-way ANOVA). Values are expressed as means $\pm$ S.E.M ($n = 7$).

in the phosphorylation levels of mTOR (Ser^{2448}) and p70S6K (Thr^{389}), as well as the immunocontent of synapsin in the hippocampus and prefrontal cortex.

Concerning the morphological analysis carried out 1 h after treatments, no significant effects were observed in total dendritic spine density in the ventral DG of the hippocampus (Fig. 7B and C) and the prefrontal cortex (Fig. 7D and E). However, after 24 h, the pretreatment with rapamycin was able to abolish the increase in dendritic spine density in the ventral hippocampal DG ($P<0.01$; Fig. 7G and H) and the prefrontal cortex (Fig. 7I and J; $P<0.01$), indicating the involvement of mTORC1 signaling.

4. Discussion

In this study, we reported that a single combined administration of subthreshold doses of ketamine and guanosine, which had no effect alone, effectively produced a fast, but not prolonged reduction of immobility time in the TST in mice. Importantly, this behavioral effect was accompa-

nied by rapid and transient activation of the mTORC1-driven signaling pathway with a consequent increase in synaptic protein synthesis and dendritic spines density in a time-dependent manner in the hippocampus and prefrontal cortex.

The discovery that the NMDA receptor antagonist ketamine is effective in producing fast and sustained antidepressant actions was a great breakthrough in the development of MDD pharmacotherapy. Compelling studies have demonstrated that ketamine exerts its antidepressant effect by promoting dendritic spines formation and synaptogenesis, effects associated with the activation of the mTORC1 signaling pathway (Fraga et al., 2020, 2021; Li et al., 2010, 2011). Supporting these findings, substantial evidence also showed disturbances on mTORC1 and synapse connectivity in the prefrontal cortex and hippocampus of MDD subjects (Duric et al., 2013; Holmes et al., 2019; Jernigan et al., 2011; Kang et al., 2012). Therefore, the activation of mTORC1 signaling with subsequent increased synaptic plasticity in the hippocampus and prefrontal cortex could lead to fast-onset and sustained antidepressant-
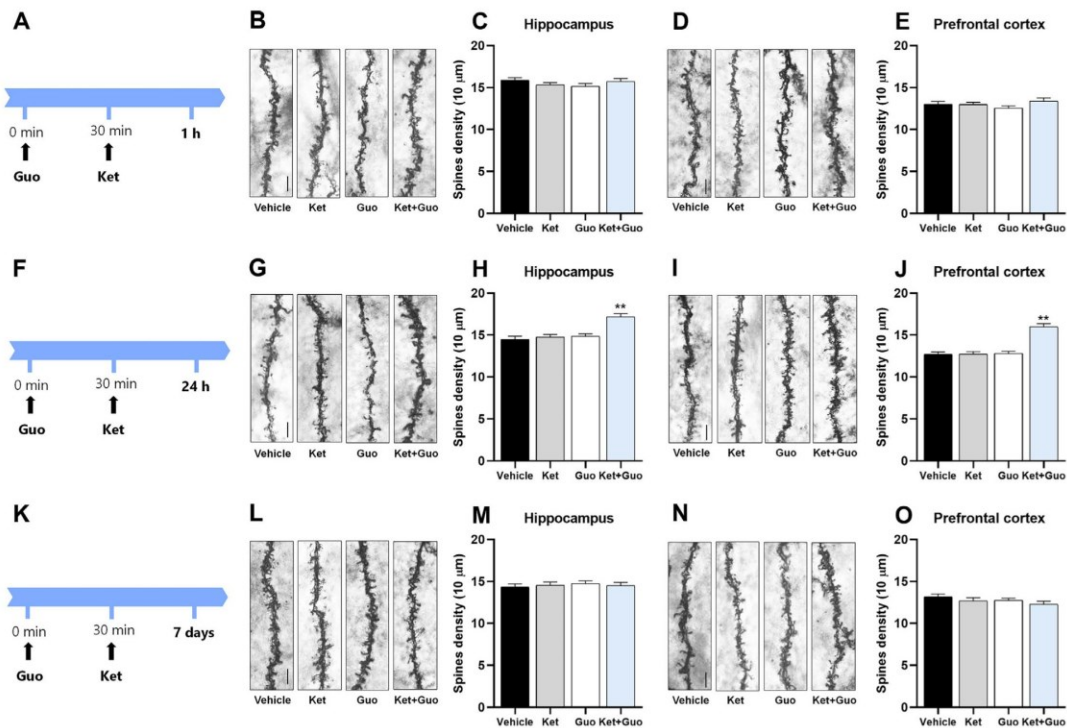
Fig. 4 Effect of a single administration of subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) plus guanosine (Guo – 0.01 mg/kg, p.o.) on dendritic spines density following 1 h, 24 h, or 7 days. Experimental timeline (**A**). Representative images of different dendritic segments in the ventral DG of the hippocampus following 1 h (**B**). Total spine density in the ventral DG of the hippocampus following 1 h (**C**). Representative images of different dendritic segments in the prefrontal cortex following 1 h (**D**). Total spine density in the prefrontal cortex following 1 h (**E**). Experimental timeline (**F**). Representative images of different dendritic segments in the ventral DG of the hippocampus following 24 h (**G**). Total spine density in the ventral DG of the hippocampus following 24 h (**H**). Representative images of different dendritic segments in the prefrontal cortex following 24 h (**I**). Total spine density in the prefrontal cortex following 24 h (**J**). Experimental timeline (**K**). Representative images of different dendritic segments in the ventral DG of the hippocampus following 7 days (**L**). Total spine density in the ventral DG of the hippocampus following 7 days (**M**). Representative images of different dendritic segments in the prefrontal cortex following 7 days (**N**). Total spine density in the prefrontal cortex following 7 days (**O**). Statistical analysis was performed by Tukey's *post hoc* test (one-way ANOVA). Values are expressed as means $\pm$ S.E.M ($n = 6$). Scale bar: 2 μm.

like responses. Aside from selective modulators of NMDA receptors, there is a growing body of studies showing that endogenous glutamatergic neuromodulators may produce fast antidepressant-like effects and augment ketamine's actions by activating the mTORC1 pathway (Almeida et al., 2020; Camargo et al., 2020; ; Freitas et al., 2020; Neis et al., 2016; Pazini et al., 2016; Rosa et al., 2021). In this regard, substantial studies have prospectively shown that guanosine presents overlapping mechanisms of action to ketamine and could be a novel candidate to produce fast antidepressant responses or even potentiate ketamine's actions (Camargo and Rodrigues, 2019). Although guanosine has been reported to be an augmenting agent over the fast antidepressant-like effect of ketamine (Camargo et al., 2020, 2021), whether guanosine effectively potentiates the long-lasting antidepressant-like and pro-synaptogenic actions of ketamine by stimulating the mTORC1 pathway

remains to be fully investigated. Moreover, if mTORC1-mediated synaptic protein synthesis and dendritic spine formation underlie the rapid actions induced by subthreshold doses of ketamine plus guanosine also remains to be resolved.

Herein, we reinforced the notion that a single administration of a subthreshold dose of guanosine is capable of augmenting the antidepressant-like effect of a subthreshold dose of ketamine in the TST, a sensitive and reliable behavioral test widely used to detect antidepressant activity in mice (Cryan et al., 2005). Importantly, we provided novel evidence that this response occurred in a time-dependent manner, starting 1 h after the treatment and lasting up for 24 h, but not for 7 days. Of note, ketamine plus guanosine treatment did not alter the number of crossings in the OFT, indicating that the anti-immobility effect was not due to a psychostimulant response. Further rein-
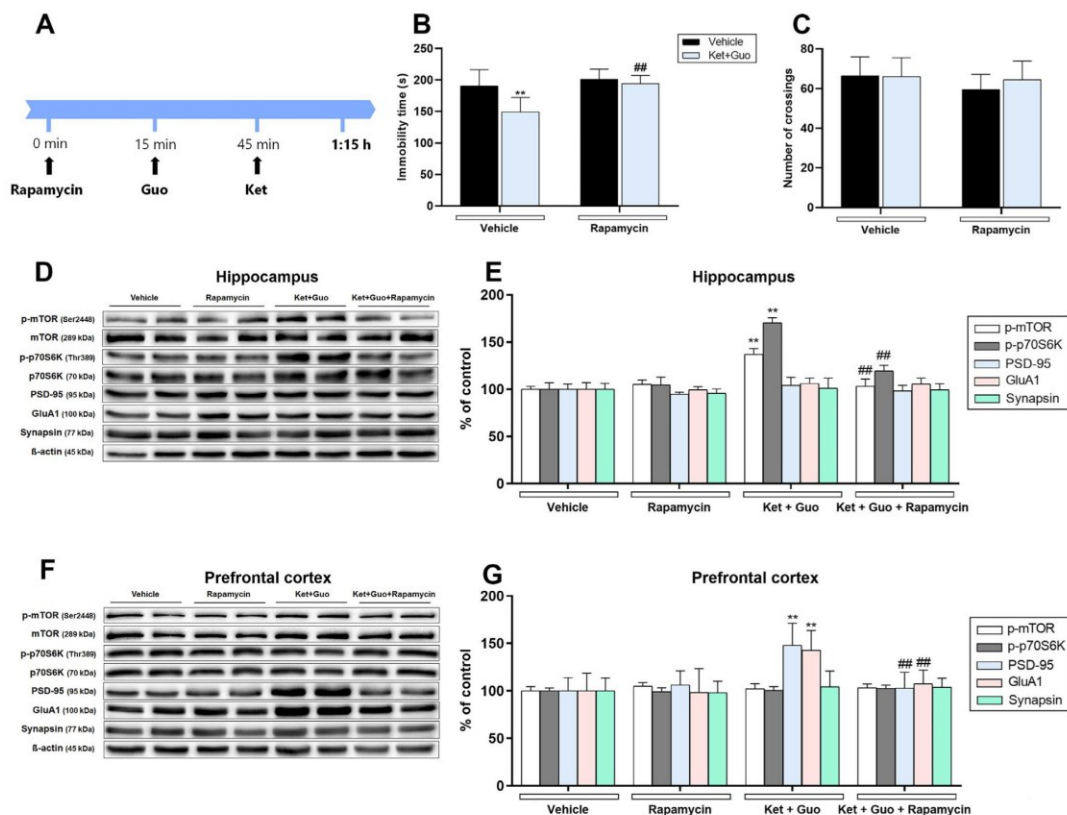
Fig. 5 Effect of rapamycin (a selective mTORC1 inhibitor, 0.2 nmol/site, i.c.v.) in the behavioral and molecular effects triggered by subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) plus guanosine (Guo – 0.01 mg/kg, p.o.) following 1 h Experimental timeline (**A**). Panels represent the immobility time in the TST (**B**) and the number of crossings in the OFT (**C**). Representative bands of phospho-mTOR (Ser^{2448}), mTOR, phospho-p70S6K (Thr^{389}), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the hippocampus (**D**) and prefrontal cortex (**F**) of mice. Densitometric quantification of these proteins in the hippocampus (**E**) and prefrontal cortex (**G**). Statistical analysis was performed by Tukey's *post hoc* test (two-way ANOVA). Values are expressed as means $\pm$ S.E.M ($n = 7$). **$P < 0.01$ as compared with the vehicle-treated group; ##$P < 0.01$ as compared with the ketamine plus guanosine-treated group.

forcing the possible antidepressant-like effect elicited by a single coadministration of subthreshold doses of ketamine plus guanosine, our group previously showed that the combination of these drugs (same dose used here) was effective in the novelty-suppressed feeding test (Camargo et al., 2019) and TST (Camargo et al., 2020) 1 h after their administration. Guanosine has also been previously reported to potentiate the antidepressant-like effect of a subthreshold dose of ketamine 24 h after the chronic administration of corticosterone in mice (Camargo et al., 2021). Notably, our results are in line with a prior study showing that the combination of sub-effective doses of ketamine and lithium, which had no effect alone, significantly decreased the immobility time of rats in the forced swim test, starting in 30 min and lasting up for 7 days (Liu et al., 2013). Similarly, the coadministration of subthreshold doses of ketamine and agmatine induced a fast (1 h and 24 h) and sustained (7 days) antidepressant-like effect in mice (Freitas et al., 2020). However, differently from these studies that reported a sus-

tained effect after 7 days (Freitas et al., 2020; Liu et al., 2013), or even with a study that investigated the effect of ketamine *per se* (Autry et al., 2011), we report here that the combined treatment of ketamine plus guanosine was only effective to produce a fast (1 h – 24 h), but not sustained (7 days) effect in the TST.

In the next step, we decided to elucidate whether the mTORC1-driven signaling pathway is associated with the antidepressant-like effect elicited by ketamine plus guanosine. Our experiments unveil that a single subthreshold dose of ketamine, which had no effect by itself, given in combination with a subthreshold dose of guanosine increased BDNF levels and mTORC1 signaling, molecular targets involved in fast and sustained antidepressant and pro-synaptogenic responses (Duman et al., 2012), in a time-dependent manner in the hippocampus and prefrontal cortex. Ketamine plus guanosine raised BDNF levels both in the hippocampus and prefrontal cortex 1 h after administration, but this effect was not observed in 24 h or 7 days. Moreover,
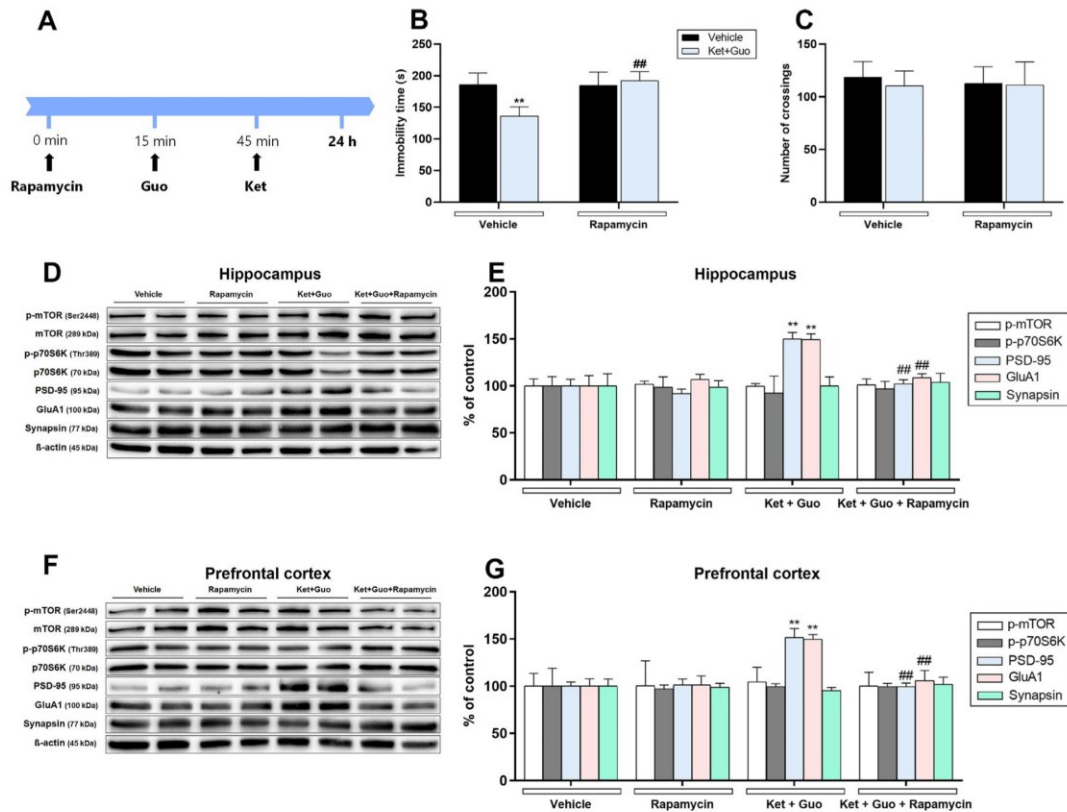
Fig. 6 Effect of rapamycin (a selective mTORC1 inhibitor, 0.2 nmol/site, i.c.v.) in the behavioral and molecular effects triggered by subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) plus guanosine (Guo – 0.01 mg/kg, p.o.) following 24 h Experimental timeline (**A**). Panels represent the immobility time in the TST (**B**) and the number of crossings in the OFT (**C**). Representative bands of phospho-mTOR (Ser^{2448}), mTOR, phospho-p70S6K (Thr^{389}), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the hippocampus (**D**) and prefrontal cortex (**F**) of mice. Densitometric quantification of these proteins in the hippocampus (**E**) and prefrontal cortex (**G**). Statistical analysis was performed by Tukey's *post hoc* test (two-way ANOVA). Values are expressed as means ± S.E.M ($n = 7$). **$P<0.01$ as compared with the vehicle-treated group; ##$P<0.01$ as compared with the ketamine plus guanosine-treated group.

ketamine plus guanosine rapidly increased (1 h after administration) Akt, GSK-3β, mTORC1, and p70S6K phosphorylation in the hippocampus, but not in the prefrontal cortex, and this effect was not sustained (back to baseline in 24 h). Our results also revealed a time-dependent upregulation of PSD-95 and GluA1 immunocontent in the hippocampus and prefrontal cortex after the combined administration of ketamine plus guanosine. Particularly, the immunocontent of PSD-95 and GluA1 was upregulated in the prefrontal cortex 1 h after ketamine plus guanosine coadministration, an effect not observed in the hippocampus. However, an increase in the immunocontent of PSD-95 and GluA1 was detected 24 h after ketamine plus guanosine treatment both in the hippocampus and prefrontal cortex of mice, while no effects were observed after 7 days. These results concur with previous findings that showed the ability of ketamine plus guanosine in stimulating the mTORC1-driven signaling pathway in a time-dependent manner with a distinct regional pattern (Camargo et al., 2020). In addition, we pro-

vide novel evidence regarding the distinct regional and time course (24 h and 7 days) patterns in upstream and downstream targets of the mTORC1-driven signaling pathway. These results also suggest that the activation of mTORC1-driven pro-synaptogenic signaling in the hippocampus and prefrontal cortex is time-dependent.

This study also demonstrated for the first time that the combination of sub-effective doses of ketamine plus guanosine, but not either alone, increased the dendritic spines formation in the hippocampus and prefrontal cortex with a distinct time course. Our results indicated that ketamine plus guanosine increased the dendritic spine density in the ventral DG of the hippocampus and the prefrontal cortex 24 h after their administration, but failed to cause any significant alteration after 1 h or 7 days. In agreement, the combination of low doses of ketamine plus lithium increased dendritic spine density, spine head diameter, and excitatory postsynaptic currents in the prefrontal cortex of rats after 24 h, and these effects lasted up to 7 days after
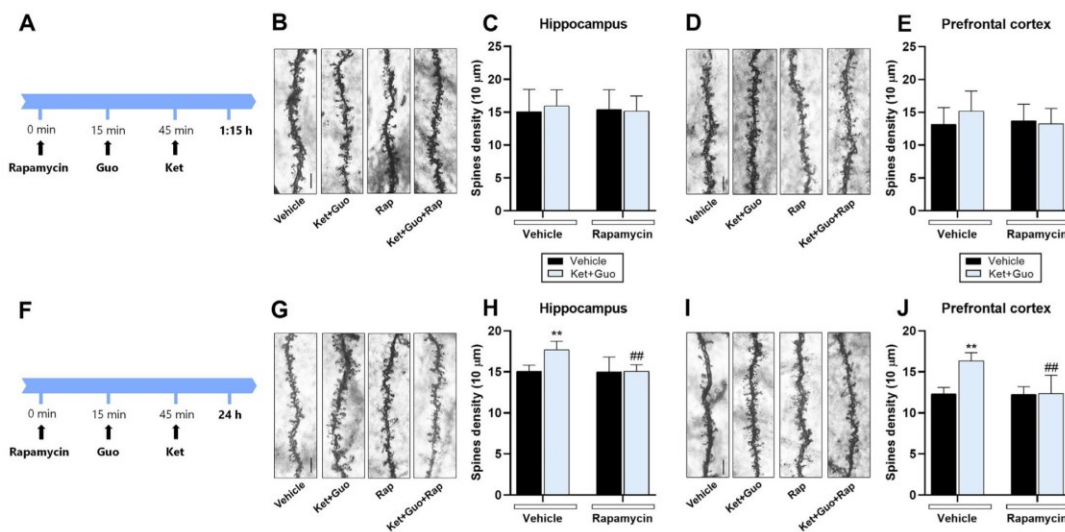
Fig. 7 Effect of rapamycin (a selective mTORC1 inhibitor, 0.2 nmol/site, i.c.v.) on dendritic spines formation elicited by subthreshold doses of ketamine (Ket – 0.1 mg/kg, i.p.) plus guanosine (Guo – 0.01 mg/kg, p.o.) following 1 h or 24 h Experimental timeline (**A**). Representative images of different dendritic segments in the ventral DG of the hippocampus following 1 h (**B**). Total spine density in the ventral DG of the hippocampus following 1 h (**C**). Representative images of different dendritic segments in the prefrontal cortex following 1 h (**D**). Total spine density in the prefrontal cortex following 1 h (**E**). Experimental timeline (**F**). Representative images of different dendritic segments in the ventral DG of the hippocampus following 24 h (**G**). Total spine density in the ventral DG of the hippocampus following 24 h (**H**). Representative images of different dendritic segments in the prefrontal cortex following 24 h (**I**). Total spine density in the prefrontal cortex following 24 h (**J**). Statistical analysis was performed by Tukey's *post hoc* test (one-way ANOVA). Values are expressed as means $\pm$ S.E.M ($n = 6$). Scale bar: 2 μm.

the treatment, which could explain the fast and sustained antidepressant-like response of this treatment (Liu et al., 2013). Additionally, the coadministration of subthreshold doses of ketamine and agmatine raised the synaptic protein synthesis and dendritic spines in the prefrontal cortex of mice after 1 h Importantly, this effect was sustained for 24 h and 7 days and was accompanied by an antidepressant-like effect elicited by the combined administration of these drugs (Freitas et al., 2020). Notably, ketamine by itself (active dose) was effective in exerting an antidepressant-like effect and increasing the dendritic spines density in the ventral DG of the hippocampus (Fraga et al., 2020) and the prefrontal cortex (Li et al., 2010). Considering that the increase in synaptic protein synthesis and dendritic spines formation is implicated in fast antidepressant response (Duman and Duman, 2014), one may suppose that these morphological alterations could be associated with the ability of ketamine plus guanosine to induce a rapid antidepressant-like effect. However, future studies are necessary to characterize the volume of spines, spine morphology and architecture as well as dendrite arborization, which are critical components in regulating synaptic formation (Duman and Duman, 2014). Noteworthy, the ability of ketamine plus guanosine treatment in modulating dendritic spines density and shape in animal models that induce behavioral, molecular, and morphological alterations similar to the ones observed in MDD remains to be investigated.

To obtain deeper insights regarding the role of mTORC1 in guanosine's ability to boost antidepressant-like and pro-synaptogenic effects triggered by ketamine, mice were pretreated with the mTORC1 inhibitor rapamycin. Our results demonstrated that the ability of ketamine plus guanosine to reduce the immobility time in the TST requires mTORC1 activation, since rapamycin completely prevented this behavioral effect. In agreement with our results, the pretreatment with rapamycin was reported to effectively abolish the effect elicited by the combined administration of ketamine plus guanosine in the novelty-suppressed feeding test in mice (Camargo et al., 2019). Accordingly, the administration of rapamycin completely prevented the ability of guanosine to potentiate the antidepressant-like effect of subthreshold doses of ketamine in mice subjected to the chronic administration of corticosterone, reinforcing the notion that mTORC1 signaling may be crucial for this response (Camargo et al., 2021). Furthermore, the rapamycin administration has been shown to abrogate the antidepressant-like response elicited by either ketamine or guanosine, suggesting that the mTORC1 pathway is required for the antidepressant response of these drugs (Almeida et al., 2020; Bettio et al., 2012; Li et al., 2010). Here, we also demonstrated that the ketamine plus guanosine-induced increase on phosphorylation of mTORC1 and p70S6K and total immunocontent of synaptic proteins (PSD-95 and GluA1) were prevented by rapamycin administration. These results indicate that the pro-synaptogenic effect triggered by ketamine plus guanosine in the hippocampus and prefrontal cortex was dependent on mTORC1 signaling. These findings resemble a previous study reporting that
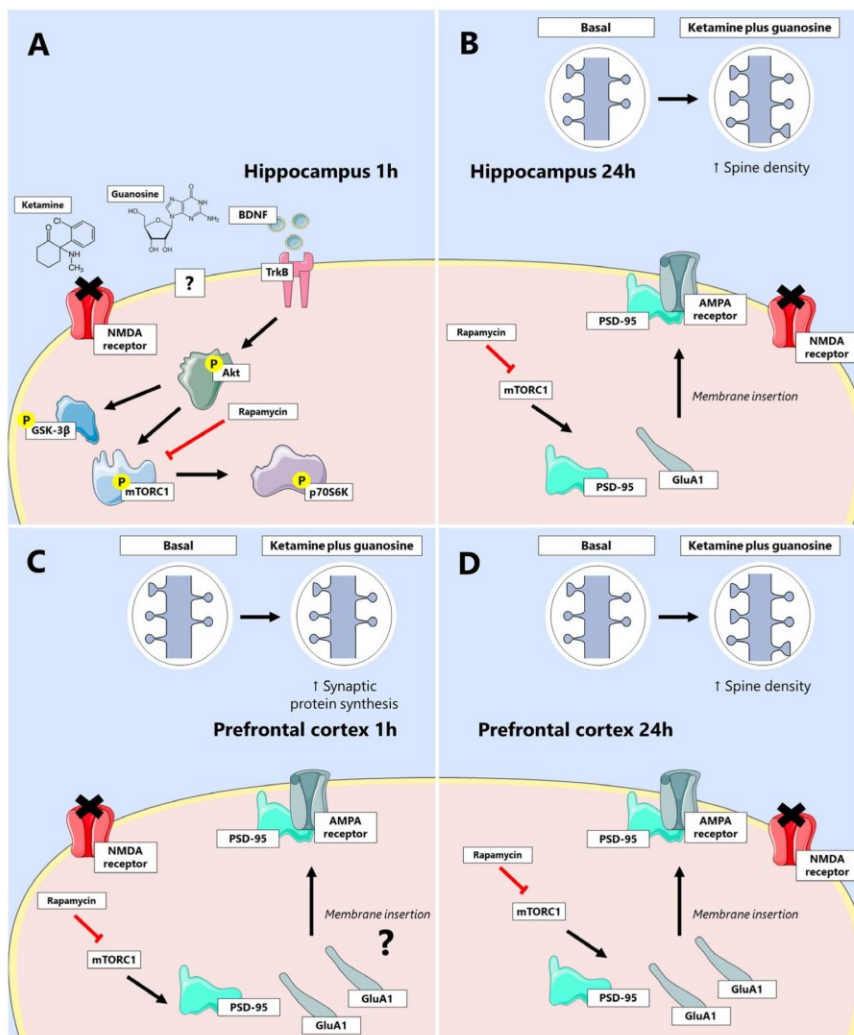
Fig. 8 Supposed intracellular signaling pathway implicated in the antidepressant-like and pro-synaptogenic effects in the hippocampus (A and B) and prefrontal cortex (C and D) elicited by combined administration of subthreshold doses of ketamine and guanosine following 1 h and 24 h Ketamine antagonizes the NMDA receptors in GABAergic interneurons, attenuating the inhibitory action of this system on the glutamatergic tonus. This event results in glutamate release that activates AMPA receptors. The activation of these receptors promotes a transient sodium influx that depolarizes the cell and activates the voltage-dependent calcium channels, causing exocytosis of synaptic vesicles containing BDNF. The activation of TrkB by BDNF culminates in Akt activation (Ser^{473}), which can phosphorylate and inactivate GSK-3β at Ser^9. Akt also activates mTORC1 at Ser^{2448} that, in turn, phosphorylates and activates p70S6K at Thr^{389}. These events stimulate synaptic proteins synthesis including AMPA receptor subunits 1 (GluA1) and postsynaptic density-95 (PSD-95) that contribute to dendritic spines formation. In the present study, we provide evidence that a single coadministration of subthreshold doses of ketamine (0.1 mg/kg, i.p.) plus guanosine (0.01 mg/kg, p.o.) following 1 h increased the phosphorylation of Akt (Ser^{473}), GSK-3β (Ser^9), mTORC1 (Ser^{2448}), and p70S6K (Thr^{389}) in the hippocampus, but not in the prefrontal cortex. Ketamine plus guanosine increased PSD-95 and GluA1 immunocontent in the prefrontal cortex, but not in the hippocampus, 1 h after their coadministration. Interestingly, ketamine plus guanosine raised the PSD-95 and GluA1 immunocontent both in the hippocampus and prefrontal cortex 24 h after the treatment. Moreover, ketamine plus guanosine increased the dendritic spine density in the ventral DG of the hippocampus and the prefrontal cortex only following 24 h Importantly, rapamycin pretreatment (mTORC1 inhibitor) completely abolished the antidepressant-like and pro-synaptogenic responses elicited by ketamine plus guanosine. These results suggest that guanosine potentiates the antidepressant-like and pro-synaptogenic effects of subthreshold doses of ketamine by activating mTORC1 signaling in the hippocampus and prefrontal cortex in a time-dependent manner. Figure designed using images from Servier Medical Art and Mind the Graph.

the efficacy of ketamine plus guanosine low-dose combination in reversing corticosterone-induced reduction in synaptic proteins in the hippocampus was dependent on mTORC1 (Camargo et al., 2021). Of note, the administration of rapamycin by itself did not affect mTORC1 and p70S6K phosphorylation and synaptic proteins immunocontent, which agree with previous studies that reported its inability to alter mTORC1 and its downstream targets in naïve and stress-exposed rodents (Li et al., 2010, 2011; Pazini et al., 2020). More importantly, our results also unveil that the mTORC1-driven signaling pathway is required for the ability of ketamine plus guanosine to rapidly induce an increase in dendritic spines density in the ventral DG of the hippocampus and the prefrontal cortex following 24 h, since the pretreatment with rapamycin completely abolished these responses. Therefore, our findings agree with the assumption that stimulating mTORC1-dependent dendritic spines formation and protein synthesis may lead to fast-onset antidepressant-like responses (Duman and Duman, 2014; Li et al., 2010).

Taken together, the results reinforce and extend the notion that guanosine may boost the antidepressant-like and pro-synaptogenic effects of ketamine by activating mTORC1 signaling (Fig. 8). The ability of ketamine plus guanosine treatment to elicit a pro-synaptogenic effect occurred in a region-specific and time-dependent manner. Given the behavioral, neurochemical, and morphological outcomes observed when subthreshold doses of ketamine and guanosine were coadministered, one may suppose that even a low quantity of ketamine or guanosine may reach the brain to exert beneficial effects. However, we cannot rule out that ketamine was converted into several metabolites such as norketamine and hydroxynorketamine that could be responsible for the effects observed in the present study, since the antidepressant-like properties of these metabolites have been reported (Zanos et al., 2016, 2019). Therefore, additional studies are necessary to address these topics. Of note, the association of subthreshold doses of ketamine and guanosine is effective to produce a fast, but not sustained, antidepressant-like response, in contrast to the long-lasting effect obtained when a higher dose of ketamine is administered (Autry et al., 2011; Liu et al., 2013). Despite this drawback, the administration of guanosine in combination with a very low dose of ketamine has the advantage of potentially being able to reduce the side effects of ketamine. Therefore, this study suggests that guanosine in combination with ketamine could constitute an effective and safe strategy to assist patients diagnosed with MDD, an issue that warrants future investigation.

Conflict of Interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Anderson Camargo: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Writing – original draft. **Ana Paula Dalmagro:** Formal analysis, Investigation, Methodology. **Eslen Delanogare:** Investigation, Methodology. **Daiane B. Fraga:** Investigation, Methodology. **Ingrid A.V. Wolin:** Investigation,

Methodology. **Ana Lúcia B. Zeni:** Investigation, Methodology. **Patricia S. Brocardo:** Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft. **Ana Lúcia S. Rodrigues:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft.

Role of funding source

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2021.12.010.

References

Abdallah, C.G., Adams, T.G., Kelmendi, B., Esterlis, I., Sanacora, G., Krystal, J.H., 2016. Ketamine's mechanism of action: a path to rapid-acting antidepressants. Depress. Anxiety 33, 689-697.

Almeida, R.F., Pocharski, C.B., Rodrigues, A.L.S., Elisabetsky, E., Souza, D.O., 2020. Guanosine fast onset antidepressant-like effects in the olfactory bulbectomy mice model. Sci. Rep. 10, 8429.

Autry, A.E., Adachi, M., Nosyreva, E., Na, E.S., Los, M.F., Cheng, P.F., Kavalali, E.T., Monteggia, L.M., 2011. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 475, 91-96.

Barowsky, J., Schwartz, T.L., 2006. An evidence-based approach to augmentation and combination strategies for treatment resistant depression. Psychiatry 3, 42-61.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry 47, 351-354.

Bettio, L.E.B., Cunha, M.P., Budni, J., Pazini, F.L., Oliveira, Á., Colla, A.R., Rodrigues, A.L.S., 2012. Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. Behav. Brain Res. 234, 137-148.

Camargo, A., Pazini, F.L., Rosa, J.M., Wolin, I.A.V., Moretti, M., Rosa, P.B., Neis, V.B., Rodrigues, A.L.S., 2019. Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway. J. Psychiatr. Res. 115, 103-112.

Camargo, A., Rodrigues, A.L.S., 2019. Novel targets for fast antidepressant responses: possible role of endogenous neuromodulators. Chronic Stress 3, 247054701985808.

Camargo, A., Dalmagro, A.P., Zeni, A.L.B., Rodrigues, A.L.S., 2020. Guanosine potentiates the antidepressant-like effect of sub-threshold doses of ketamine: possible role of pro-synaptogenic signaling pathway. J. Affect. Disord. 271, 100-108.

Camargo, A., Dalmagro, A.P., Wolin, I.A.V., Siteneski, A., Zeni, A.L.B., Rodrigues, A.L.S., 2021. A low-dose combination of ketamine and guanosine counteracts corticosterone-induced depressive-like behavior and hippocampal synaptic impairments via mTORC1 signaling. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 111, 110371.

Cryan, J.F., Mombereau, C., Vassout, A., 2005. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neurosci. Biobehav. Rev. 29, 571-625.

DiazGranados, N., Ibrahim, L.A., Brutsche, N.E., Ameli, R., Henter, I.D., Luckenbaugh, D.A., Machado-Vieira, R., Zarate, Jr, C.A., 2010. Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. J. Clin. Psychiatry 71, 1605-1611.

Duman, C.H., Duman, R.S., 2014. Spine synapse remodeling in the pathophysiology and treatment of depression. Neurosci. Lett. 601, 20-29.

Duman, R.S., Li, N., Liu, R.J., Duric, V., Aghajanian, G., 2012. Signaling pathways underlying the rapid antidepressant actions of ketamine. Neuropharmacology 62, 35-41.

Duric, V., Banasr, M., Stockmeier, C.A., Simen, A.A., Newton, S.S., Overholser, J.C., Jurjus, G.J., Dieter, L., Duman, R.S., 2013. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. Int. J. Neuropsychopharmacol. 16, 69-82.

Fraga, D.B., Camargo, A., Olescowicz, G., Azevedo Padilha, D., Mina, F., Budni, J., Brocardo, P.S., Rodrigues, A.L.S., 2021. A single administration of ascorbic acid rapidly reverses depressive-like behavior and hippocampal synaptic dysfunction induced by corticosterone in mice. Chem. Biol. Interact. 342, 109476.

Fraga, D.B., Costa, A.P., Olescowicz, G., Camargo, A., Pazini, F.L., E. Freitas, A., Moretti, M., S. Brocardo, P., .S, Rodrigues, A.L., 2020. Ascorbic acid presents rapid behavioral and hippocampal synaptic plasticity effects. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 96, 109757.

Fraga, D.B., Olescowicz, G., Moretti, M., Siteneski, A., Tavares, M.K., Azevedo, D., Colla, A.R.S., Rodrigues, A.L.S., 2018. Anxiolytic effects of ascorbic acid and ketamine in mice. J. Psychiatr. Res. 100, 16-23.

Freitas, A., Heinrich, I., Moura, T., Fraga, D., Costa, A., Azevedo, D., Brocardo, P., Kaster, M., Leal, R., Rodrigues, A.L., 2020. Agmatine potentiates antidepressant and synaptic actions of ketamine: effects on dendritic arbors and spines architecture and Akt/S6 kinase signaling. Exp. Neurol. 333, 113398.

Gao, M., Rejaei, D., Liu, H., 2016. Ketamine use in current clinical practice. Acta Pharmacol. Sin. 37, 865-872.

Han, C., Wang, S., Seo, H., Chul, B., Jin, H., Kim, W., Kwak, K., Pae, C., 2014. Aripiprazole augmentation, antidepressant combination or switching therapy in patients with major depressive disorder who are partial- or non-responsive to current antidepressants : a multi-center, naturalistic study. J. Psychiatr. Res. 49, 75-82.

Holmes, S.E., Scheinost, D., Finnema, S.J., Naganawa, M., Davis, M.T., DellaGioia, N., Nabulsi, N., Matuskey, D., Angarita, G.A., Pietrzak, R.H., Duman, R.S., Sanacora, G., Krystal, J.H., Carson, R.E., Esterlis, I., 2019. Lower synaptic density is associated with depression severity and network alterations. Nat. Commun. 10, 1-10.

Huang, K., Fingar, D.C., 2014. Growing knowledge of the mTOR signaling network. Semin. Cell Dev. Biol. 36, 79-90.

Jernigan, C.S., Goswami, D.B., Austin, M.C., Iyo, A.H., Chandran, A., Stockmeier, C.A., Karolewicz, B., 2011. The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 1774-1779.

Kang, H.J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C.A., Licznerski, P., Lepack, A., Majik, M.S., Jeong, L.S., Banasr, M., Son, H., Duman, R.S., 2012. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat. Med. 18, 1413-1417.

Kannangara, T.S., Bostrom, C.A., Ratzlaff, A., Thompson, L., Cater, R.M., Gil-Mohapel, J., Christie, B.R., 2014. Deletion of the NMDA receptor GluN2A subunit significantly decreases dendritic growth in maturing dentate granule neurons. PLoS ONE 9, 1-10.

Leal, R.B., Lopes, M.W., Formolo, D.A., de Carvalho, C.R., Hoeller, A.A., Latini, A., Sousa, D.S., Wolf, P., Prediger, R.D., Bortolotto, Z.A., Linhares, M.N., Lin, K., Walz, R., 2020. Amygdala levels of the GluA1 subunit of glutamate receptors and its phosphorylation state at serine 845 in the anterior hippocampus are biomarkers of ictal fear but not anxiety. Mol. Psychiatry 25, 655-665.

Lepack, A.E., Fuchikami, M., Dwyer, J.M., Banasr, M., Duman, R.S., 2014. BDNF release is required for the behavioral actions of ketamine. Int. J. Neuropsychopharmacol. 18, 1-6.

Li, N., Lee, B., Liu, R.-.J., Banasr, M., Dwyer, J.M., Iwata, M., Li, X.-.Y., Aghajanian, G., Duman, R.S., 2010. mTOR-Dependent Synapse Formation Underlies the Rapid Antidepressant Effects of NMDA Antagonists. Science 329, 959-965.

Li, N., Liu, R.J., Dwyer, J.M., Banasr, M., Lee, B., Son, H., Li, X.Y., Aghajanian, G., Duman, R.S., 2011. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol. Psychiatry 69, 754-761.

Liu, R.J., Fuchikami, M., Dwyer, J.M., Lepack, A.E., Duman, R.S., Aghajanian, G.K., 2013. GSK-3 inhibition potentiates the synaptogenic and antidepressant-like effects of subthreshold doses of ketamine. Neuropsychopharmacology 38, 2268-2277.

Ludka, F.K., Zomkowski, A.DE., Cunha, M.P., Dal-Cim, T., Zeni, A.L.B., Rodrigues, A.L.S., Tasca, C.I., 2013. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the L-arginine-nitric oxide-cyclic guanosine monophosphate pathway and increases BDNF levels. Eur. Neuropsychopharmacol. 23, 400-412.

Malhi, G.S., Morris, G., Bell, E., Hamilton, A., 2020. A New Paradigm for Achieving a Rapid Antidepressant Response. Drugs 1-10.

Moret, C., 2005. Combination /augmentation strategies for improving the treatment of depression. Neuropsychiatr. Dis. Treat. 1, 301-309.

Neis, V.B., Moretti, M., Bettio, L.E.B., Ribeiro, C.M., Rosa, P.B., Gonçalves, F.M., Lopes, M.W., Leal, R.B., Rodrigues, A.L.S., 2016. Agmatine produces antidepressant-like effects by activating AMPA receptors and mTOR signaling. Eur. Neuropsychopharmacol. 26, 959-971.

Newport, D.J., Carpenter, L.L., McDonald, W.M., Potash, J.B., Tohen, M., Nemeroff, C.B., 2015. Ketamine and other NMDA antagonists: early clinical trials and possible mechanisms in depression. Am. J. Psychiatry 172, 950-966.

Oliveira, M.M., Lourenco, M.V., Longo, F., Kasica, N.P., Yang, W., Ureta, G., Ferreira, D.D.P., Mendonça, P.H.J., Bernales, S., Ma, T., De Felice, F.G., Klann, E., Ferreira, S.T., 2021. Correction of eIF2-dependent defects in brain protein synthesis, synaptic plasticity, and memory in mouse models of Alzheimer's disease. Sci. Signal. 14, 1-12.

Otte, C., Gold, S., Penninx, B., Pariante, C., Etkin, A., Fava, M., Mohr, D., Schatzberg, A., 2016. Major depressive disorder. Nat. Rev. Dis. Prim. 2, 1-20.

Papakostas, G.I., Ionescu, D.F., 2015. Towards new mechanisms: an update on therapeutics for treatment-resistant major depressive disorder. Mol. Psychiatry 20, 1142–1150.

Papakostas, G.I., Petersen, T.J., Green, C., Iosifescu, D.V., Yeung, A.S., Nierenberg, A.A., Fava, M., Posternak, M.A., 2005. A description of next-step switching versus augmentation practices for outpatients with treatment-resistant major depressive disorder enrolled in an academic specialty clinic. Ann. Clin. Psychiatry 17, 161–165.

Park, Y.M., 2020. The hypothesis on the prediction of treatment response with buspirone augmentation along with serotonergic antidepressant in patients with major depressive disorder using loudness dependence of auditory evoked potentials: two cases and review of the liter. Psychiatry Investig 17, 222–224.

Pazini, F.L., Cunha, M.P., Rosa, J.M., Colla, A.R.S., Lieberknecht, V., Oliveira, Á., Rodrigues, A.L.S., 2016. Creatine, Similar to Ketamine, Counteracts Depressive-Like Behavior Induced by Corticosterone via PI3K/Akt/mTOR Pathway. Mol. Neurobiol. 53, 6818–6834.

Pazini, F.L., Rosa, J.M., Camargo, A., Fraga, D.B., Moretti, M., Siteneski, A., Rodrigues, A.L.S., 2020. mTORC1-dependent signaling pathway underlies the rapid effect of creatine and ketamine in the novelty-suppressed feeding test. Chem. Biol. Interact. 332, 109281.

Penn, E., Tracy, D.K., 2012. The drugs don't work? antidepressants and the current and future pharmacological management of depression. Ther. Adv. Psychopharmacol. 2, 179–188.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83, 346–356.

Rosa, J.M., Pazini, F.L., Olescowicz, G., Camargo, A., Moretti, M., Gil-Mohapel, J., Rodrigues, A.L.S., 2019. Prophylactic effect of physical exercise on Aβ_{1-40}-induced depressive-like behavior: role of BDNF, mTOR signaling, cell proliferation and survival in the hippocampus. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 94, 109646.

Rosa, P.B., Bettio, L.E.B., Neis, V.B., Moretti, M., Kaufmann, F.N., Tavares, M.K., Werle, I., Dalsenter, Y., Platt, N., Rosado, A.F., Fraga, D.B., Heinrich, I.A., Freitas, A.E., Leal, R.B., Rodrigues, A.L.S., 2021. Antidepressant-like effect of guanosine involves activation of AMPA receptor and BDNF/TrkB signaling. Purinergic Signal. 1–17.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

, 2017. Depression and Other Common Mental disorders: Global Health Estimates. World Health Organization, pp. 1–24.

Zanos, P., Moaddel, R., Morris, P.J., Georgiou, P., Fischell, J., Elmer, G.I., Alkondon, M., Yuan, P., Pribut, H.J., Singh, N.S., Dossou, K.S.S., Fang, Y., Huang, X.P., Mayo, C.L., Wainer, I.W., Albuquerque, E.X., Thompson, S.M., Thomas, C.J., Zarate, C.A., Gould, T.D., 2016. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 533, 481–486.

Zanos, P., Highland, J.N., Stewart, B.W., Georgiou, P., Jenne, C.E., Lovett, J., Morris, P.J., Thomas, C.J., Moaddel, R., Zarate, C.A., Gould, T.D., 2019. (2R,6R)-hydroxynorketamine exerts mGlu2 receptor-dependent antidepressant actions. Proc. Natl. Acad. Sci. U. S. A. 116, 6441–6450.

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H., 2006. A Randomized Trial of an N-methyl-D-aspartate Antagonist in Treatment-Resistant Major Depression. Arch. Gen. Psychiatry 63, 856–864.

Zhou, J., Wang, W., Yang, J., Zhu, X., Feng, L., Xiao, L., Wang, G., 2019. Scopolamine augmentation of a newly initiated escitalopram treatment for major depressive disorder: study protocol for a randomized controlled trial. Trials 20, 1–7.

4.3 CAPÍTULO 3

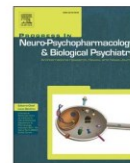**A low-dose combination of ketamine and guanosine counteracts corticosterone-induced depressive-like behavior and hippocampal synaptic impairments via mTORC1 signaling**

Anderson Camargo, Ana Paula Dalmagro, Ingrid A. V. Wolin, Aline Siteneski, Ana Lúcia B. Zeni, Ana Lúcia S. Rodrigues

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

A low-dose combination of ketamine and guanosine counteracts corticosterone-induced depressive-like behavior and hippocampal synaptic impairments via mTORC1 signaling

Anderson Camargo [a,b], Ana Paula Dalmagro [c], Ingrid A.V. Wolin [b], Aline Siteneski [a,b], Ana Lúcia B. Zeni [c], Ana Lúcia S. Rodrigues [a,b,*]

[a] Neuroscience Postgraduate Program, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis, 88040-900, SC, Brazil
[b] Department of Biochemistry, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis, 88040-900, SC, Brazil
[c] Laboratory of Evaluation of Bioactive Substances, Department of Natural Sciences, Universidade Regional de Blumenau, 89030-903, Blumenau, SC, Brazil

ARTICLE INFO

ABSTRACT

Ketamine exhibits rapid and sustained antidepressant responses, but its repeated use may cause adverse effects. Augmentation strategies have been postulated to be useful for the management/reduction of ketamine's dose and its adverse effects. Based on the studies that have suggested that ketamine and guanosine may share overlapping mechanisms of action, the present study investigated the antidepressant-like effect of subthreshold doses of ketamine and guanosine in mice subjected to repeated administration of corticosterone (CORT) and the role of mTORC1 signaling for this effect. The ability of the treatment with ketamine (0.1 mg/kg, i.p.) plus guanosine (0.01 mg/kg, p.o.) to counteract the depressive-like behavior induced by CORT (20 mg/kg, p.o., for 21 days) in mice, was paralleled with the prevention of the CORT-induced reduction on BDNF levels, Akt (Ser^{473}) and GSK-3β (Ser^9) phosphorylation, and PSD-95, GluA1, and synapsin immunocontent in the hippocampus. No changes on mTORC1 and p70S6K immunocontent were found in the hippocampus and prefrontal cortex of any experimental group. No alterations on BDNF, Akt/GSK-3β, mTORC1/p70S6K, and synaptic proteins were observed in the prefrontal cortex of mice. The antidepressant-like and pro-synaptogenic effects elicited by ketamine plus guanosine were abolished by the pretreatment with rapamycin (0.2 nmol/site, i.c.v., a selective mTORC1 inhibitor). Our results showed that the combined administration of ketamine and guanosine at low doses counteracted CORT-induced depressive-like behavior and synaptogenic disturbances by activating mTORC1 signaling. This study supports the notion that the combined administration of guanosine and ketamine may be a useful therapeutic strategy for the management of MDD.

1. Introduction

Major depressive disorder (MDD), a highly prevalent psychiatric condition, represents a serious public health concern. This disorder affects more than 300 million individuals and is the leading cause of disability worldwide (World Health Organization, 2017). The hypothalamic-pituitary-adrenal (HPA) axis dysfunction is a key component underpinning the onset of depressive symptoms (Mizoguchi et al., 2003; Myers et al., 2014), and compelling evidence have prospectively shown that increased cortisol levels are a risk factor for MDD (Goodyer et al., 2000; Harris et al., 2000). Glucocorticoid levels are

tightly regulated by a negative feedback mechanism, but in depressive patients this might be impaired due to an HPA axis dysfunction (Watson and Mackin, 2006). In this respect, prolonged exposure to glucocorticoids may damage the hippocampus and prefrontal cortex, brain regions involved in the HPA axis autoregulation and mood control, contributing to the depressive symptoms (Lee et al., 2002; McKlveen et al., 2013). Importantly, synaptic loss and deficits in functional connectivity were widely reported in the hippocampus and prefrontal cortex of MDD patients (Duric et al., 2013; Feyissa et al., 2009; Holmes et al., 2019; Kang et al., 2012; Murrough et al., 2016) and rodents subjected to stress models (Fraga et al., 2021; Li et al., 2011; Wang et al., 2013).
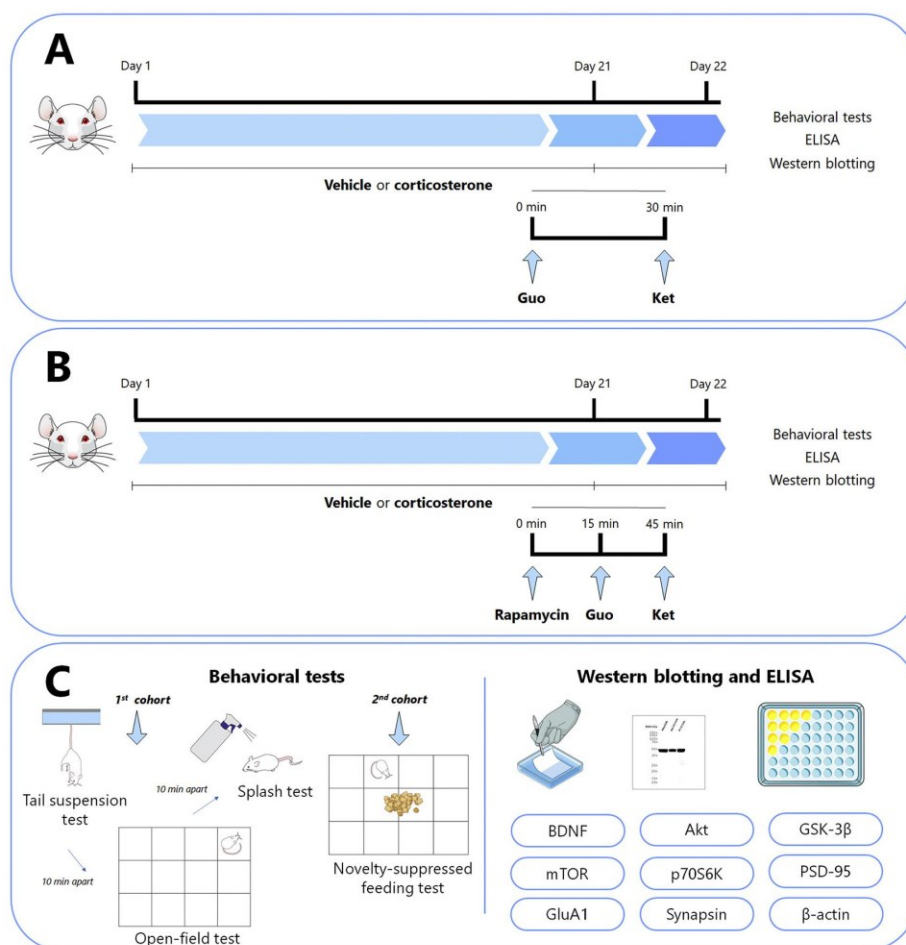
Fig. 1. Schematic representation of the treatment regimen. In the first set of experiments, mice received vehicle or CORT (20 mg/kg, p.o.) once a day, for 21 days. On the 21st day, after the last CORT administration, mice received a single administration of guanosine (0.01 mg/kg) and following 30 min they were treated with ketamine (0.1 mg/kg). On the 22nd day, 24 h after the treatments, mice were subjected to the behavioral, ELISA and western blotting analyses (A). In the second set of experiments, mice underwent chronic administration of vehicle or CORT (20 mg/kg, p.o.), and rapamycin (0.2 nmol/site, i.c.v.) was given 15 and 45 min before guanosine and ketamine administration, respectively. On the 22nd day, 24 h after ketamine plus guanosine treatment, the behavioral tests, ELISA and western blotting were performed (B). The behavioral tests included the tail suspension test, open-field test, splash test, and novelty-suppressed feeding test. One cohort of animals was subjected to tail suspension test, open-field test, and splash test (10 min apart). The second cohort of animals was subjected to the novelty-suppressed feeding test. The BDNF immunocontent was measured by ELISA analysis. The immunocontent of Akt, GSK-3β, mTOR, p70S6K, PSD-95, GluA1, and synapsin was evaluated by western blotting analysis (C).

Despite all the efforts in establishing the neurobiological basis of this medical condition and developing effective therapeutic approaches for its management, the pharmacological treatment for MDD is still a challenge (Otte et al., 2016). The conventional treatment for MDD has been aimed at modulating the monoaminergic system, however, these antidepressants are effective only in some patients (about 50% fail to achieve remission) and act slowly (Papakostas and Ionescu, 2015). Moreover, about 30% of the patients do not achieve remission of the depressive symptoms even after treatment with multiple drugs and are considered treatment-resistant (Kaster et al., 2016). These limitations underscore the need to develop new antidepressants that provide a faster antidepressant effect and greater efficacy. The most promising approach for the treatment of MDD emerged in 2000 when Berman et al. (2000) demonstrated in a clinical study that ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, produced a rapid and sustained antidepressant effect. Importantly, these rapid actions displayed by ketamine were also observed even in treatment-refractory patients with suicidal ideation (Price et al., 2009; Zarate et al., 2006). These findings became the focus of several research groups that aimed at elucidating the mechanisms of action of ketamine.

Accumulating evidence suggests that ketamine exerts its antidepressant effect through the release of brain-derived neurotrophic factor (BDNF) with subsequent activation of the protein kinase B (Akt)/mechanistic target of rapamycin protein complex 1 (mTORC1) (Lepack et al., 2014; Li et al., 2010, 2011). In turn, mTORC1 activation culminates in the translation of synaptic proteins important to dendritic spines formation and synaptogenesis, such as postsynaptic density protein-95 kDa (PSD-95), alpha-amino-3-hydroxy-methyl-5-4-isoxazole propionic acid (AMPA) receptor subunits 1 (GluA1), and synapsin (Abdallah et al., 2015). Despite the major breakthrough that ketamine represented for

MDD pharmacotherapy, its repeated use has been reported to cause imaginative and/or dissociative states, making it a drug with abuse potential (Gao et al., 2016). Considering these limitations, the investigation of compounds that may produce ketamine-like effects or that augment its actions without causing side effects is welcome.

Augmentation strategies that comprise the association of the antidepressant drug with non-antidepressant agents could be an approach to overcome ketamine's drawbacks, since it may produce more considerable improvement than monotherapy and allow for a reduction in the dose used, which may contribute to lowering the adverse effects (Barowsky and Schwartz, 2006; Han et al., 2014; Moret, 2005; Papakostas et al., 2005). Our research group has provided evidence that guanosine, a guanine-based nucleoside, could be a candidate to induce a fast-acting antidepressant response or even augment ketamine's effects (Camargo and Rodrigues, 2019). Particularly, the antidepressant-like effect of guanosine involves the modulation of NMDA and AMPA receptors and stimulation of BDNF/mTORC1 signaling (Bettio et al., 2012; Rosa et al., 2021;). A single administration of guanosine, similar to ketamine, was able to reverse the depressive-like behavior induced by olfactory bulbectomy in mice via mTORC1 pathway (Almeida et al., 2020). The ability of guanosine to potentiate the antidepressant-like and pro-synaptogenic effects of subthreshold doses of ketamine in naïve mice were recently demonstrated (Camargo et al., 2019, 2020a). Of note, guanosine was effective in potentiating the antidepressant-like effect of a subthreshold dose of ketamine in the mice subjected to repeated administration of CORT, an animal model that mimics human MDD (Camargo et al., 2020b). Despite this evidence, the role of mTORC1 signaling in the antidepressant-like and pro-synaptogenic effects triggered by subthreshold doses of ketamine plus guanosine remains to be investigated.

Given this scenario, the present study investigated the antidepressant-like and pro-synaptogenic effects of single administration with low doses of ketamine and/or guanosine in corticosterone-treated mice and the role of mTORC1-driven signaling pathway in this response.

2. Material and methods

2.1. Animals

The experiments were conducted using male *Swiss* mice (45–60 days of age, 30–40 g), maintained under controlled temperature (21 ± 1 °C) and humidity (50 ± 20%) with a 12:12 h light/dark cycle (lights on at 7:00 a.m.). Animals were housed in groups of 8 in a cage (41 × 34 × 16 cm) with free access to food and water, except when they were 24-h food-deprived before the novelty-suppressed feeding test. The experiments were performed after approval of the protocol by the Institutional Ethics Committee and according to the National Institute of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs and treatments

Mice received vehicle or corticosterone (CORT – 20 mg/kg, dissolved in distilled water with 2% Tween 80 and 0.2% DMSO) once a day for 21 days. Ketamine (0.1 mg/kg) and/or guanosine (0.01 mg/kg) were dissolved in saline (0.9%) and distilled water, respectively, and administered in a single dose immediately after the last CORT administration. On the 22nd day, mice were subjected to the behavioral tests 24 h after the treatments (Fig. 1A). Subsequently, mice were immediately euthanized by decapitation, and the hippocampus and prefrontal cortex were collected for neurochemical analyses. Vehicle, CORT, and guanosine were administered orally (p.o.) by gavage, while ketamine was administered intraperitoneally (i.p.). All drugs (obtained from Sigma Chemical Co., St. Louis, USA) were freshly prepared and administered in a volume of 10 ml/kg body weight. All doses and time points of administration were chosen based on previous studies (Camargo et al., 2019; Ludka

et al., 2013; Pazini et al., 2016).

In the second set of experiments, to investigate the role of mTORC1 pathway in the effects elicited by ketamine plus guanosine coadministration, rapamycin (a selective mTORC1 inhibitor) was administered 15 and 45 min before guanosine and ketamine administration, respectively. The animals were subjected to behavioral tests 24 h later (Fig. 1B). Rapamycin was dissolved in sterile saline with dimethyl sulfoxide (DMSO) at a final concentration of 1% and administered by intracerebroventricular route (i.c.v.) in a volume of 3 μl per mouse (0.2 nmol/site). Appropriate vehicle-treated groups were also assessed simultaneously. The i.c.v. injections were performed by employing a freehand method under isoflurane anesthesia according to the procedure described previously (Camargo et al., 2019). Briefly, a 0.4-mm external diameter hypodermic needle attached to a cannula linked to a 25-μl Hamilton syringe was inserted perpendicularly through the skull (no more than 2 mm into the brain of each mouse). Rapamycin was administered into the left lateral ventricle. The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. The injection site was 1 mm to the left from the midpoint on a line drawn through to the anterior base of the ears. Then, i.c.v. injections were performed by an experienced investigator, and after dissection of the brain of the animal, the success of the injection was examined, macroscopically, discarding results from mice presenting misplacement of the injection site or any sign of cerebral hemorrhage (<5%).

Two cohorts of animals were used in this study. One cohort of animals was subjected to tail suspension test, open-field test, and splash test (10 min apart). The second cohort of animals was subjected to the novelty-suppressed feeding test. All manipulations and behavioral tests were carried out between 11:00 and 16:00 h.

2.3. Tail suspension test

The total immobility time of mice suspended by the tail was measured as previously proposed (Steru et al., 1985). Visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was manually recorded during a 6-min period using a stopwatch by an experienced and blind observer. Mice were considered immobile only when they hung passively and completely motionless.

2.4. Open-field test

Mice were individually subjected to the open-field test as previously described (Dalmagro et al., 2019). The apparatus consisted of a wooden box (40 × 60 × 50 cm high) with the floor divided into 12 equal squares. The number of squares crossed, a parameter indicative of locomotor activity, was manually registered for 6 min by an experienced and blind observer.

2.5. Splash test

The splash test consists of squirting a 10% sucrose solution (w/v) on the dorsal coat of mice placed in clear boxes (9 × 7 × 11 cm). Due to its viscosity, the sucrose solution dirties the mice which then initiates a grooming behavior (Rosa et al., 2014). After applying the sucrose solution, the latency time to the first grooming and the total time spent grooming were manually recorded for 5 min using a stopwatch by an experienced and blind observer, as indices of self-care and motivational behavior (Willner, 2005).

2.6. Novelty-suppressed feeding test

The novelty-suppressed feeding test performed as previously proposed (Bodnoff et al., 1988; Pazini et al., 2020) measures the latency of mice in approaching and eating food in a novel environment following
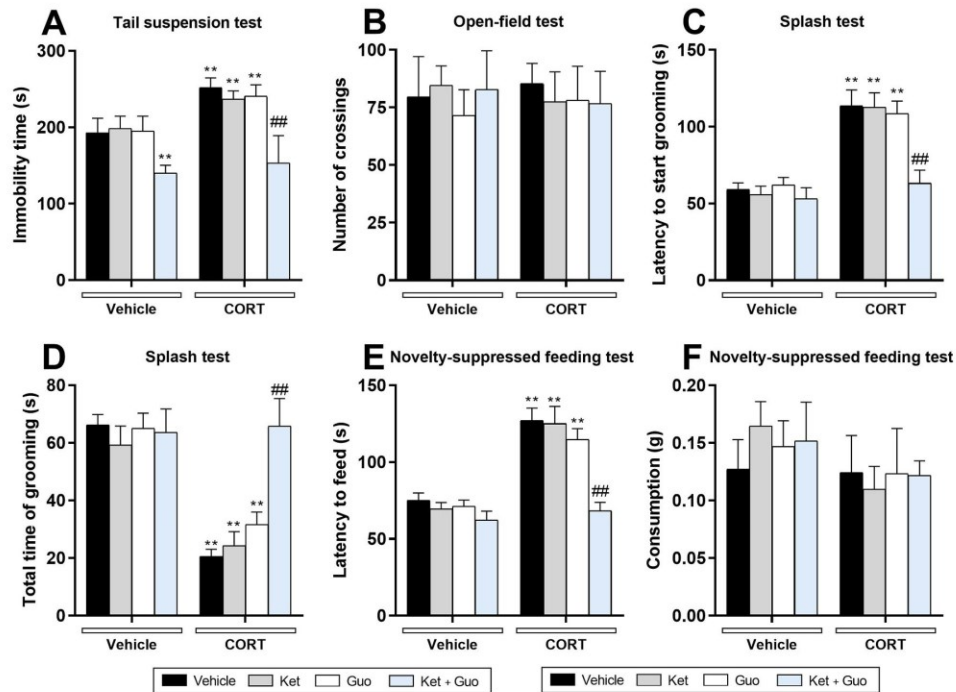
Fig. 2. Effect of a single administration with a subthreshold dose of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) in mice treated with vehicle or corticosterone (CORT – 20 mg/kg, p.o.) and subjected to the behavioral tests. Panels represent the immobility time in the tail suspension test (A), number of crossings in the open-field test (B), grooming latency (C) and total time of grooming (D) in the splash test, and latency to feed (E) and food consumption (F) in the novelty-suppressed feeding test. Values are expressed as means ± S.E.M (n = 7). **$P < 0.01$ as compared with the vehicle-treated control group; ##$P < 0.01$ as compared with the CORT-treated control group (two-way ANOVA followed by Newman-Keuls *post-hoc* test).

an extended period (up to 24 h) of food deprivation. The latency to begin eating reflects how the animal copes with a behavioral conflict, and because the ability to solve conflicts is inversely related to anxiety/MDD, this test has been used to investigate behaviors related to these disorders (Dulawa and Hen, 2005). Mice were weighed and all food was removed from their cages, although water continued to be provided with free access. 24 h after the removal of the food, mice were placed in an illuminated wooden box (40 × 60 cm and 50 cm height). A small piece of mouse chow was positioned in the center of the box and each mouse was placed in the corner of the testing arena, and the time until the first feeding episode was manually recorded within 10 min using a stopwatch by an experienced and blind observer. Subsequently, the tested animal was placed alone in a cage with a weighed piece of chow for 5 min and, at the end of this period, the amount of food consumed was determined by weighing the piece of chow (Fraga et al., 2020; Iijima et al., 2012). After, mice returned to the cage with free access to food and water.

2.7. BDNF measurement

A BDNF ELISA kit (Promega® Inc., USA) was used to measure mature BDNF protein levels in hippocampal and prefrontocortical homogenates, according to the manufacturer's instructions. Briefly, hippocampal or prefrontocortical tissues were homogenized in lysis buffer, centrifuged at 14,000*g* for 30 min at 4 °C, and the supernatants were collected and stored at −80 °C until assay. All samples and standards were applied in duplicate into 96-well plates pre-coated with rabbit anti-human BDNF antibodies, which were incubated overnight on a shaker at 4 °C. After washing four times, biotinylated mouse anti-BDNF antibodies were added, and the plates were incubated for 3 h at room temperature. Next,

the plates were incubated with streptavidin–HRP conjugate solution at room temperature for 1 h and subsequently with TMB/E substrate for 15 min. The sample absorbances were read using a microplate reader at 450 nm.

2.8. Western blotting

The hippocampus and prefrontal cortex were quickly dissected and snap-frozen with liquid nitrogen before storage at −80 °C until use. The samples were mechanically homogenized in 400 μl of 50 mM TRIS pH 7.0, 1 mM EDTA, 100 mM NaF, 0.1 mM PMSF, 2 mM Na_3VO_4, 1%Triton X-100, 10% glycerol, Sigma Protease Inhibitor Cocktail (P2714). Lysates were centrifuged (10,000 *g* for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in 100 mM TRIS pH 6.8, 4 mM EDTA, 8% SDS, and boiled for 5 min. Thereafter, sample dilution (40% glycerol, 100 mM TRIS, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β-mercaptoethanol (final concentration 8%) were added to the samples (Camargo et al., 2020c). Protein content was quantified using bovine serum albumin (BSA) as a standard (Peterson, 1977). The samples containing 60 μg protein/track were separated by SDS-PAGE (miniVE Vertical Electrophoresis System TM, GE Healthcare Life Sciences, Piscataway, NJ, USA) using 7–10% gel and the proteins were transferred to nitrocellulose membranes using a semi-dry blotting apparatus (1.2 mA/cm2; 1.5 h). To verify the transfer efficiency process, membranes were stained with Ponceau and were subsequently blocked with 5% BSA in TBS (10 mM Tris, 150 mM NaCl, pH 7.5). The immunocontent of total and phosphorylated forms of Akt (Ser[473]), glycogen synthase kinase-3β (GSK-3β – Ser[9]), mTORC1 (Ser[2448]), and 70 kDa ribosomal protein S6 kinase (p70S6K – Thr[389]), as well as PSD-95, GluA1,

synapsin, and β-actin (loading control) immunocontent were detected using specific antibodies (Cell Signaling, 1:1000) diluted in TBS-T (10 mM Tris, 150 mM NaCl, 0.1% Tween-10, pH 7.5) containing 2.5% BSA and incubated overnight. Subsequently, the membranes were incubated with anti-rabbit antibody horseradish peroxidase-conjugated secondary antibody (Cell Signaling, 1:2500) for 60 min, and the immunoreactive bands were developed using a chemiluminescence kit (Amersham ECL Select, Piscataway, USA). All blocking and incubation steps were followed by three washes (5 min) of the membranes with TBS-T. The optical density (OD) of the bands was quantified using Image Lab Software® 4.1 (Bio-Rad Laboratories). The phosphorylation levels of Akt, GSK-3β, mTORC1, and p70S6K were determined as a ratio of OD of the phosphorylated band over OD of the total band. The immunocontent of PSD-95, GluA1, and synapsin was determined as a ratio of the specific protein band over the OD of the β-actin band. Results are expressed as compared to the control group 100%.

2.9. Statistical analysis

The D'Agostino-Pearson test was used to assess data normality. The differences among experimental groups were determined by two-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test, when appropriate. Data are presented as mean ± standard error of mean (SEM). A value of $P < 0.05$ was considered significant.

3. Results

3.1. Subthreshold doses of ketamine and guanosine when given in combination elicit antidepressant-like effect

The first set of experiments was designed to validate and reinforce herein the antidepressant-like effect of ketamine plus guanosine in mice subjected to the chronic administration of CORT.

Two-way ANOVA revealed significant differences for treatment [$F_{(3, 48)} = 50.92$, $P < 0.01$], CORT [$F_{(1, 48)} = 59.03$, $P < 0.01$], and treatment × CORT interaction [$F_{(3, 48)} = 3.67$, $P < 0.05$]) in the tail suspension test (Fig. 2A). *Post-hoc* analysis indicated that ketamine plus guanosine significantly decreased the immobility time in mice subjected to the tail suspension test when compared to vehicle-treated control ($P < 0.01$). Moreover, CORT administration significantly increased the immobility time when compared to vehicle-treated control ($P < 0.01$). However, this alteration was only counteracted by a single coadministration of guanosine plus ketamine ($P < 0.01$). No alteration was observed in the number of crossings in the open-field test (Fig. 2B) in any experimental group (treatment [$F_{(3, 48)} = 0.11$, $P = 0.94$], CORT [$F_{(1, 48)} = 0.02$, $P = 0.98$], treatment × CORT interaction [$F_{(3, 48)} = 0.14$, $P = 0.93$]).

Concerning the splash test (Fig. 2C and D), two-way ANOVA revealed significant differences for grooming latency (treatment [$F_{(3, 48)} = 6.49$, $P < 0.01$], CORT [$F_{(1, 48)} = 61.85$, $P < 0.01$], treatment × CORT interaction [$F_{(3, 48)} = 4.13$, $P < 0.05$]), and total time of grooming (treatment [$F_{(3, 48)} = 6.01$, $P < 0.01$], CORT [$F_{(1, 48)} = 42.82$, $P < 0.01$], treatment × CORT interaction [$F_{(3, 48)} = 5.94$, $P < 0.01$]). *Post-hoc* analysis showed that CORT administration significantly increased the grooming latency and reduced the total time of grooming (Fig. 2C and D, respectively) when compared to vehicle-treated control ($P < 0.01$), but these alterations were significantly abolished by ketamine plus guanosine treatment ($P < 0.01$).

Additionally, two-way ANOVA revealed significant differences for treatment [$F_{(3, 48)} = 11.61$, $P < 0.01$], CORT [$F_{(1, 48)} = 67.94$, $P < 0.01$] and treatment × CORT interaction [$F_{(3, 48)} = 5.68$, $P < 0.01$] in latency to feed in the novelty-suppressed feeding test (Fig. 2E). *Post-hoc* analysis indicated that CORT administration significantly increased the latency to feed when compared to vehicle-treated control ($P < 0.01$), but this effect was not observed in ketamine plus guanosine-treated mice ($P < 0.01$). No alteration was observed in the food consumption (Fig. 2F) in the novelty-suppressed feeding test (treatment [$F_{(3, 48)} = 0.07$, $P =$
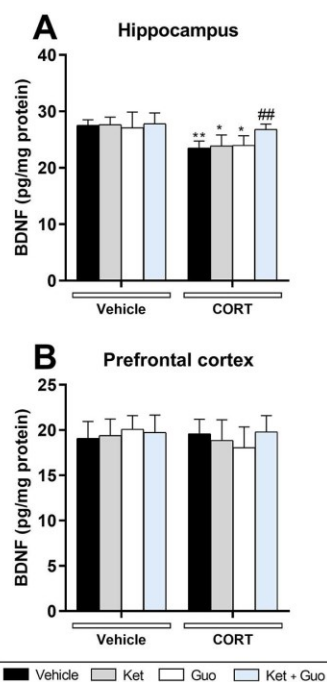
Fig. 3. Effect of a single administration with a subthreshold dose of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) on BDNF levels in the hippocampus (A) and prefrontal cortex (B) of mice treated with vehicle or corticosterone (CORT – 20 mg/kg, p.o.). Values are expressed as means ± S.E.M ($n = 7$). *$P < 0.05$ and **$P < 0.01$ as compared with the vehicle-treated control group; ##$P < 0.01$ as compared with the CORT-treated control group (two-way ANOVA followed by Newman-Keuls *post-hoc* test).

0.97], CORT [$F_{(1, 48)} = 2.10$, $P = 0.15$], treatment × CORT interaction [$F_{(3, 48)} = 0.31$, $P = 0.82$]).

3.2. The influence of BDNF/Akt/GSK-3β/mTORC1 signaling pathway in the augmentation effect triggered by ketamine plus guanosine

We next sought to investigate whether the BDNF and mTORC1-driven signaling pathway may be associated with the antidepressant-like effect elicited by ketamine plus guanosine.

Two-way ANOVA revealed significant main effects for BDNF levels (Fig. 3A and B) in the hippocampus (treatment [$F_{(3, 48)} = 3.55$, $P < 0.05$], CORT [$F_{(1, 48)} = 43.15$, $P < 0.01$], and treatment × CORT interaction [$F_{(3, 48)} = 2.85$, $P < 0.05$]), but not in the prefrontal cortex (treatment [$F_{(3, 48)} = 0.38$, $P = 0.76$], CORT [$F_{(1, 48)} = 0.95$, $P = 0.33$], treatment × CORT interaction [$F_{(3, 48)} = 1.18$, $P = 0.32$]). *Post-hoc* analysis showed that CORT administration significantly reduced BDNF levels ($P < 0.01$) in the hippocampus (Fig. 3A), but not in the prefrontal cortex (Fig. 3B), when compared to vehicle-treated control. However, this alteration was only abolished by single coadministration of ketamine plus guanosine ($P < 0.01$).

Additionally, two-way ANOVA revealed significant differences for phospho-Akt (treatment [$F_{(3, 48)} = 7.70$, $P < 0.01$], CORT [$F_{(1, 48)} = 35.20$, $P < 0.01$], treatment × CORT interaction [$F_{(3, 48)} = 4.94$, $P < 0.01$]) and phospho-GSK-3β (treatment [$F_{(3, 48)} = 17.48$, $P < 0.01$], CORT [$F_{(1, 48)} = 47.12$, $P < 0.01$], treatment × CORT interaction [$F_{(3, 48)} = 4.29$, $P < 0.01$]) in the hippocampus. *Post-hoc* analysis showed that CORT administration significantly reduced Akt (Ser[473]) and GSK-3β (Ser[9]) phosphorylation ($P < 0.01$) in the hippocampus (Fig. 4A and B)
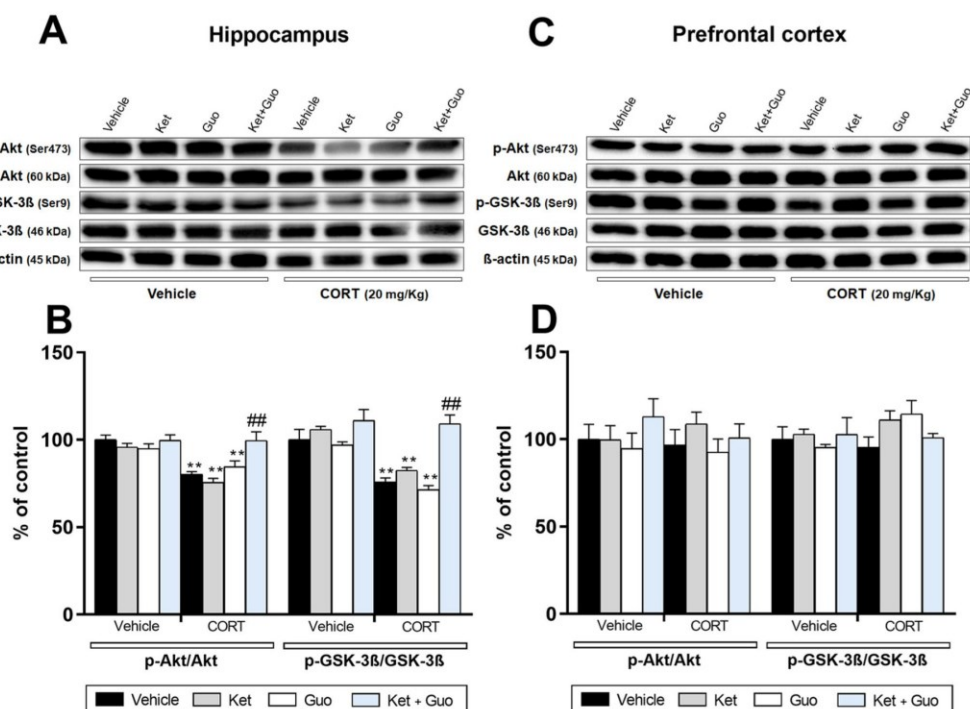
Fig. 4. Effect of a single administration with a subthreshold dose of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) on phospho-Akt (Ser^{473}) and phospho-GSK-3β (Ser^9) immunocontent in the hippocampus (A and B) and prefrontal cortex (C and D) of mice treated with vehicle or corticosterone (CORT – 20 mg/kg, p.o.). Panels A and C show respectively the representative bands of phospho-Akt (Ser^{473}), Akt, phospho-GSK-3β (Ser^9), GSK-3β, and β-actin in the hippocampus and prefrontal cortex. Panels B and D show the quantification of these proteins in the hippocampus and prefrontal cortex, respectively. Values are expressed as means ± S.E.M (n = 7). **P < 0.01 as compared with the vehicle-treated control group; ##P < 0.01 as compared with the CORT-treated control group (two-way ANOVA followed by Newman-Keuls *post-hoc* test).

when compared to vehicle-treated control, but this alteration was completely rescued by single coadministration of ketamine plus guanosine (P < 0.01). No changes were observed on the immunocontent of phospho-Akt (treatment [$F_{(3, 48)} = 1.07$, P = 0.39], CORT [$F_{(1, 48)} = 0.12$, P = 0.72], treatment × CORT interaction [$F_{(3, 48)} = 0.52$, P = 0.65]) and phospho-GSK-3β (treatment [$F_{(3, 48)} = 0.91$, P = 0.44], CORT [$F_{(1, 48)} = 1.49$, P = 0.22], treatment × CORT interaction [$F_{(3, 48)} = 0.62$, P = 0.19]) in the prefrontal cortex (Fig. 4C and D).

No changes were observed on the phosphorylation levels and total immunocontent of phospho-mTOR (treatment [$F_{(3, 48)} = 0.97$, P = 0.41], CORT [$F_{(1, 48)} = 1.70$, P = 0.19], treatment × CORT interaction [$F_{(3, 48)} = 0.88$, P = 0.45]) and phospho-p70S6K (treatment [$F_{(3, 48)} = 0.46$, P = 0.70], CORT [$F_{(1, 48)} = 0.01$, P = 0.94], treatment × CORT interaction [$F_{(3, 48)} = 0.39$, P = 0.75]) in the hippocampus (Fig. 5A and B). Also, no significant effects were observed on the phosphorylation levels and total immunocontent of phospho-mTOR (treatment [$F_{(3, 48)} = 2.44$, P = 0.07]; CORT [$F_{(1, 48)} = 0.06$, P = 0.79]; treatment × CORT interaction [$F_{(3, 48)} = 1.34$, P = 0.27]) and phospho-p70S6K (treatment [$F_{(3, 48)} = 0.30$, P = 0.83], CORT [$F_{(1, 48)} = 0.012$, P = 0.91], treatment × CORT interaction [$F_{(3, 48)} = 0.067$, P = 0.97]) in the prefrontal cortex (Fig. 5C and D).

Additionally, two-way ANOVA revealed significant differences for PSD-95 (treatment [$F_{(3, 48)} = 5.53$, P < 0.01], CORT [$F_{(1, 48)} = 108.20$, P < 0.01], and treatment × CORT interaction [$F_{(3, 48)} = 8.36$, P < 0.01]), GluA1 (treatment [$F_{(3, 48)} = 12.72$, P < 0.01], CORT [$F_{(1, 48)} = 107.60$, P < 0.01], and treatment × CORT interaction [$F_{(3, 48)} = 19.10$, P < 0.01]), as well as synapsin (treatment [$F_{(3, 48)} = 5.12$, P < 0.01], CORT [$F_{(1, 48)} = 66.90$, P < 0.01], and treatment × CORT interaction [$F_{(3, 48)} = 2.91$, P

< 0.05]) in the hippocampus (Fig. 6A and B). *Post-hoc* analysis demonstrated that CORT administration significantly decreased the PSD-95, GluA1, and synapsin immunocontent in the hippocampus (Fig. 6A and B) when compared to vehicle-treated control (P < 0.01), but these alterations were counteracted by single coadministration of ketamine plus guanosine (P < 0.01). No alterations were observed on the immunocontent of PSD-95 (treatment [$F_{(3, 48)} = 0.16$, P = 0.92], CORT [$F_{(1, 48)} = 0.23$, P = 0.62], treatment × CORT interaction [$F_{(3, 48)} = 1.11$, P = 0.35]), GluA1 (treatment [$F_{(3, 48)} = 2.13$, P = 0.10], CORT [$F_{(1, 48)} = 2.93$, P = 0.09], treatment × CORT interaction [$F_{(3, 48)} = 1.59$, P = 0.20]) as well as synapsin (treatment [$F_{(3, 48)} = 0.52$, P = 0.66], CORT [$F_{(1, 48)} = 0.30$, P = 0.58], treatment × CORT interaction [$F_{(3, 48)} = 1.32$, P = 0.27]) in the prefrontal cortex (Fig. 6C and D),

3.3. The role of mTORC1 in the antidepressant-like and pro-synaptogenic effects triggered by ketamine plus guanosine

To give insight into the role of mTORC1 signaling in the antidepressant-like and pro-synaptogenic effects elicited by ketamine plus guanosine low-dose combination, mice were pretreated with rapamycin, a selective mTORC1 inhibitor.

Two-way ANOVA revealed a significant main effect for treatment (treatment [$F_{(3, 48)} = 21.50$, P < 0.01]) in the tail suspension test (Fig. 7A), with only ketamine plus guanosine-treated mice presenting a reduction in the immobility time (P < 0.01) regardless of the treatment with vehicle or CORT. A significant main effect of CORT administration was also detected $F_{(1, 48)} = 99.38$, P < 0.01]), with CORT-treated mice spending more time immobile in the tail suspension test as compared
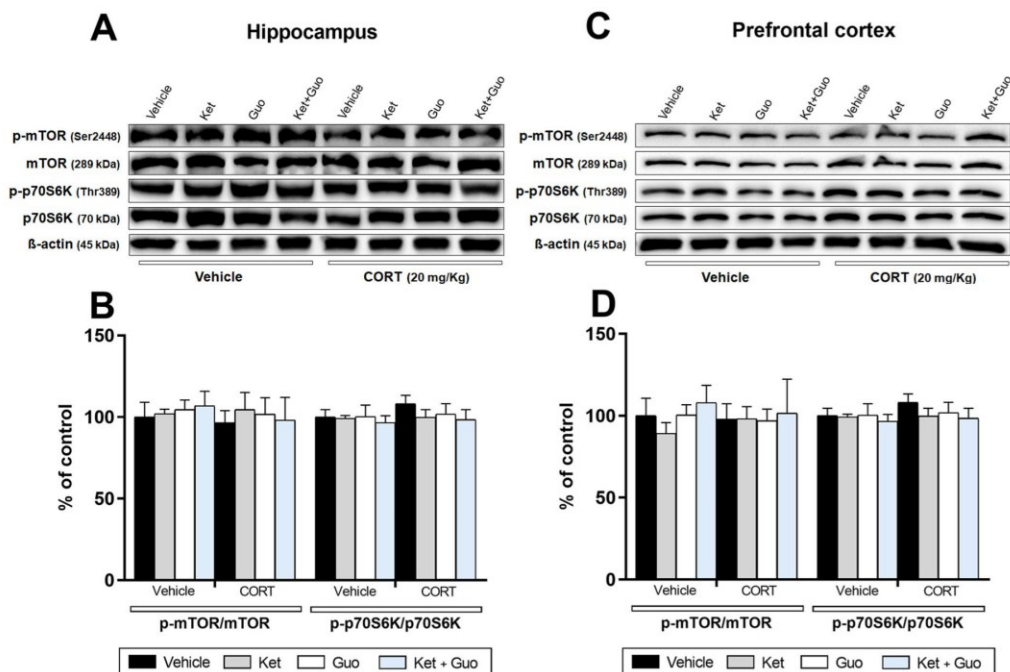
Fig. 5. Effect of the administration with a subthreshold dose of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) on phospho-mTORC1 and phospho-p70S6K immunocontent in the hippocampus (A and B) or prefrontal cortex (C and D) of mice treated with vehicle or corticosterone (CORT – 20 mg/kg, p.o.). Panels A and C illustrate the representative western blots, while panels B and D show the densitometric quantitation of blots. Values are expressed as means ± S.E.M (n = 7). Two-way ANOVA followed by Newman-Keuls *post-hoc* test.

Fig. 6. Effect of a single administration with a subthreshold dose of ketamine (Ket – 0.1 mg/kg, i.p.) and/or guanosine (Guo – 0.01 mg/kg, p.o.) on PSD-95, GluA1, and synapsin immunocontent in the hippocampus (A and B) and prefrontal cortex (C and D) of mice treated with vehicle or corticosterone (CORT – 20 mg/kg, p.o.). Panels A and C show the representative bands of PSD-95, GluA1, synapsin, and β-actin in the hippocampus and prefrontal cortex, respectively. Panels B and D show the quantification of these proteins in the hippocampus and prefrontal cortex, respectively. Values are expressed as means ± S.E.M (n = 7). **P < 0.01 as compared with the vehicle-treated control group; ##P < 0.01 as compared with the CORT-treated control group (two-way ANOVA followed by Newman-Keuls *post-hoc* test).

Fig. 7. Effect of treatment with ketamine (0.1 mg/kg, i.p.) plus guanosine (0.01 mg/kg, p.o.) and/or rapamycin (0.2 nmol/site, i.c.v.) on depression-related behaviors in mice subjected to the vehicle or corticosterone (CORT – 20 mg/kg, p.o.) administration. Panels represent the immobility time in the tail suspension test (A), number of crossings in the open-field test (B), grooming latency (C) and total time of grooming (D) in the splash test, and latency to feed (E) and food consumption (F) in the novelty-suppressed feeding test. Values are expressed as means ± S.E.M (n = 7). @@P < 0.01 as compared CORT-treated mice with their vehicle-treated counterparts (i.e., significant main effect of CORT administration); &&P < 0.01 as compared with vehicle-treated mice (i.e., significant main effect of treatment). **P < 0.01 as compared with the vehicle-treated group; ##P < 0.01 as compared with the CORT-treated group. $$P < 0.01 as compared with the ketamine plus guanosine-treated group (two-way ANOVA followed by Newman-Keuls *post-hoc* test when appropriate).

with vehicle-treated mice ($P < 0.01$). No significant treatment × CORT interaction ([$F_{(3, 48)} = 0.14$, $P = 0.93$]) was observed in the tail suspension test. No effect was observed in the number of crossings (treatment [$F_{(3, 48)} = 0.0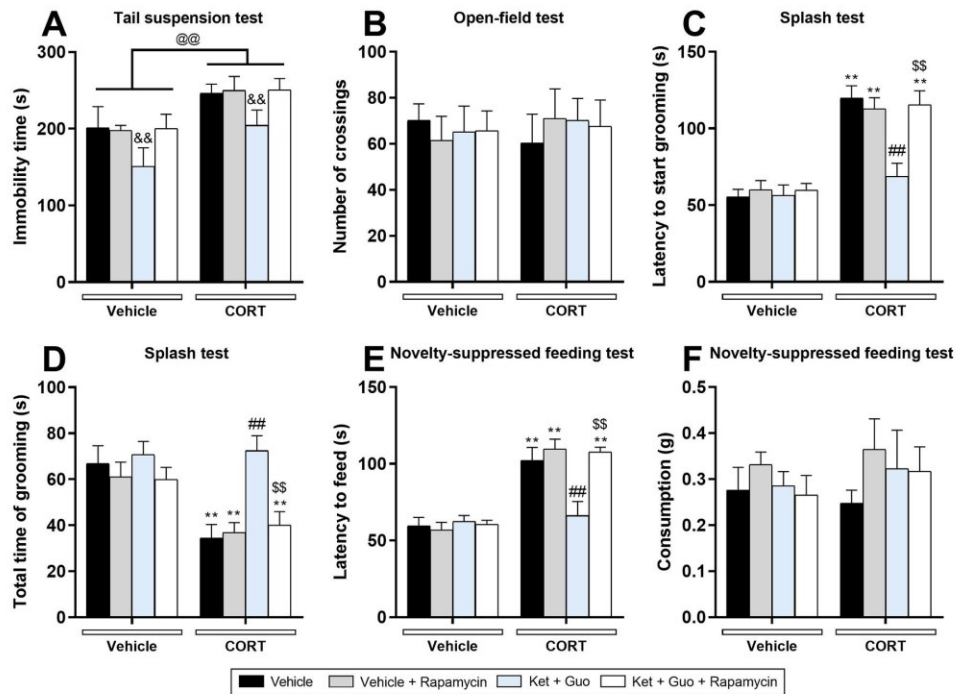1$, $P = 0.99$], CORT [$F_{(1, 48)} = 0.04$, $P = 0.82$], treatment × CORT interaction [$F_{(3, 48)} = 0.30$, $P = 0.82$]) in the open-field test (Fig. 7B).

Additionally, two-way ANOVA revealed significant differences for grooming latency (treatment [$F_{(3, 48)} = 6.06$, $P < 0.01$], CORT [$F_{(1, 48)} = 86.27$, $P < 0.01$], treatment × CORT interaction [$F_{(3, 48)} = 5.36$, $P < 0.05$]) and total time of grooming (treatment [$F_{(3, 48)} = 6.39$, $P < 0.01$], CORT [$F_{(1, 48)} = 18.78$, $P < 0.01$], treatment × CORT interaction [$F_{(3, 48)} = 2.86$, $P < 0.05$]) in mice subjected to the splash test (Fig. 7C and D). *Post-hoc* analysis showed that CORT administration significantly increased the latency to start grooming ($P < 0.01$) and reduced the time spent grooming ($P < 0.01$) in mice subjected to the splash test (Fig. 7C and D, respectively). A single administration of ketamine plus guanosine was effective in counteracting CORT-induced alterations in the splash test ($P < 0.01$), but these responses were completely abolished by the pretreated with rapamycin ($P < 0.01$).

Concerning the novelty-suppressed feeding test (Fig. 7E), two-way ANOVA revealed significant differences for latency to feed (treatment [$F_{(3, 48)} = 4.83$, $P < 0.01$], CORT [$F_{(1, 48)} = 74.41$, $P < 0.01$], treatment × CORT interaction [$F_{(3, 48)} = 6.91$, $P < 0.01$]). *Post-hoc* analysis indicated that CORT administration significantly increased the latency to feed when compared with vehicle-treated mice ($P < 0.01$). Of note, CORT-induced increase in the latency to feed was counteracted by a single administration with subthreshold doses of ketamine plus

guanosine ($P < 0.01$), but these responses were abrogated by the pretreatment with rapamycin ($P < 0.01$). No alterations were observed in the food consumption (treatment [$F_{(3, 48)} = 0.97$, $P = 0.41$], CORT [$F_{(1, 48)} = 0.40$, $P = 0.52$], treatment × CORT interaction [$F_{(3, 48)} = 0.23$, $P = 0.86$]) in the novelty-suppressed feeding test in any experimental group (Fig. 7F).

No alterations were observed on the phosphorylation levels and total immunocontent of phospho-mTOR (treatment [$F_{(3, 48)} = 0.31$, $P = 0.81$], CORT [$F_{(1, 48)} = 1.12$, $P = 0.29$], treatment × CORT interaction [$F_{(3, 48)} = 0.58$, $P = 0.62$]) and phospho-p70S6K (treatment [$F_{(3, 48)} = 0.78$, $P < 0.05$], CORT [$F_{(1, 48)} = 1.78$, $P = 0.18$], treatment × CORT interaction [$F_{(3, 48)} = 0.43$, $P = 0.72$]) in the hippocampus (Fig. 8A and B) of any experimental group. No changes were observed on the phosphorylation levels and total immunocontent of phospho-mTOR (treatment [$F_{(3, 48)} = 0.12$, $P = 0.94$]; CORT [$F_{(1, 48)} = 0.63$, $P = 0.42$]; treatment × CORT interaction [$F_{(3, 48)} = 0.17$, $P = 0.91$]) and phospho-p70S6K (treatment [$F_{(3, 48)} = 1.88$, $P = 0.14$], CORT [$F_{(1, 48)} = 2.07$, $P = 0.15$], treatment × CORT interaction [$F_{(3, 48)} = 0.10$, $P = 0.95$]) in the prefrontal cortex (Fig. 8C and D) of any experimental group.

Two-way ANOVA revealed significant differences for PSD-95 (treatment [$F_{(3, 48)} = 4.66$, $P < 0.01$], CORT [$F_{(1, 48)} = 16.89$, $P < 0.01$], and treatment × CORT interaction [$F_{(3, 48)} = 3.61$, $P < 0.05$]), GluA1 (treatment [$F_{(3, 48)} = 3.10$, $P < 0.05$], CORT [$F_{(1, 48)} = 16.90$, $P < 0.01$], and treatment × CORT interaction [$F_{(3, 48)} = 3.61$, $P < 0.05$]), as well as synapsin (treatment [$F_{(3, 48)} = 6.40$, $P < 0.01$], CORT [$F_{(1, 48)} = 3.98$, $P < 0.05$], and treatment × CORT interaction [$F_{(3, 48)} = 5.12$, $P < 0.01$]) in the hippocampus (Fig. 9A and B). *Post-hoc* analysis indicated
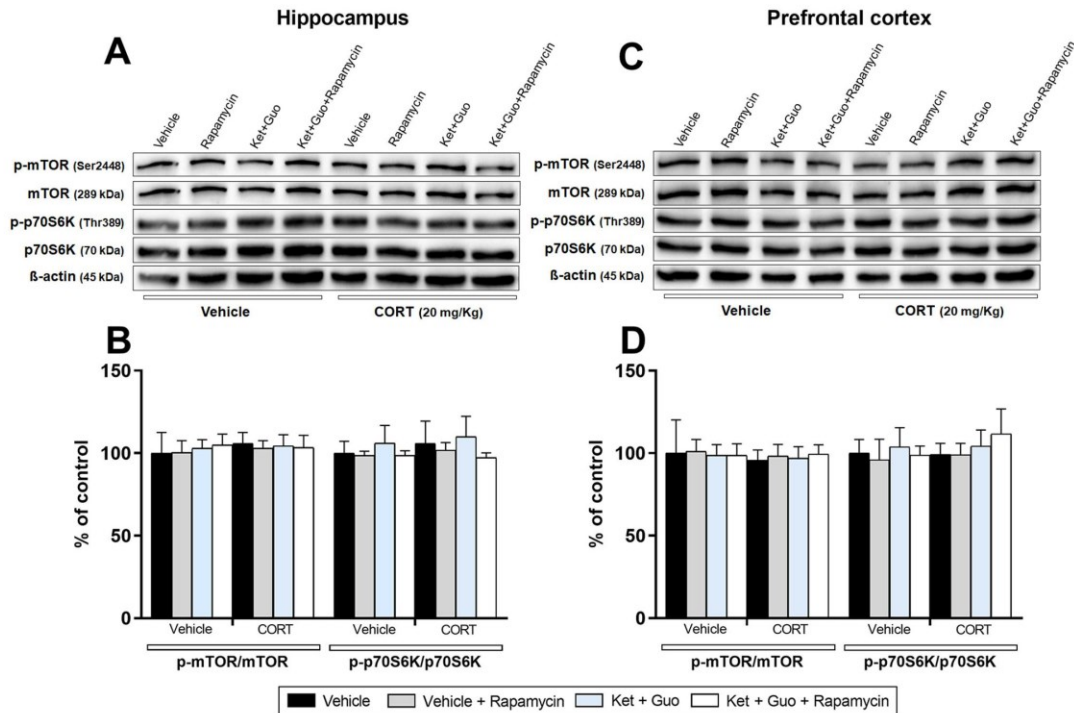
Fig. 8. Effect of treatment with ketamine (0.1 mg/kg, i.p.) plus guanosine (0.01 mg/kg, p.o.) and/or rapamycin (0.2 nmol/site, i.c.v.) on phospho-mTORC1 and phospho-p70S6K immunocontent in the hippocampus (panels A and B) and prefrontal cortex (panels C and D) of mice that underwent the vehicle or corticosterone (CORT – 20 mg/kg, p.o.) administration. Panels A and C illustrate the representative western blots, while panels B and D show the densitometric quantitation of blots. Values are expressed as means ± S.E.M (n = 7). Two-way ANOVA followed by Newman-Keuls *post-hoc* test.

that CORT administration significantly reduced the immunocontent of PSD-95 (P < 0.01), GluA1 (P < 0.01), and synapsin (P < 0.01) in the hippocampus, but these alterations were not observed in the ketamine plus guanosine-treated mice (P < 0.01). Notably, the ability of ketamine plus guanosine in counteracting the reductions in PSD-95 and GluA1 immunocontent induced by CORT was completely abolished by rapamycin (P < 0.01). However, rapamycin administration was not able to abolish the upregulation of synapsin immunocontent induced by ketamine plus guanosine in the hippocampus of CORT-treated mice. Additionally, no alterations were observed on the immunocontent of PSD-95 (treatment $[F_{(3, 48)} = 0.01, P = 0.99]$, CORT $[F_{(1, 48)} = 1.56, P = 0.21]$, treatment × CORT interaction $[F_{(3, 48)} = 0.11, P = 0.95]$), GluA1 (treatment $[F_{(3, 48)} = 0.40, P = 0.98]$, CORT $[F_{(1, 48)} = 0.53, P = 0.43]$, treatment × CORT interaction $[F_{(3, 48)} = 0.11, P = 0.94]$) as well as synapsin (treatment $[F_{(3, 48)} = 0.05, P = 0.98]$, CORT $[F_{(1, 48)} = 0.66, P = 0.41]$, treatment × CORT interaction $[F_{(3, 48)} = 0.31, P = 0.80]$) in the prefrontal cortex after CORT, ketamine plus guanosine, and/or rapamycin administrations (Fig. 9C and D).

4. Discussion

The present study reinforces and extends the notion that a subthreshold dose of ketamine, which had no effect by itself, when given in combination with a subthreshold dose of guanosine produces an antidepressant-like effect in mice chronically administered with CORT, a rodent model that resembles MDD (Sterner and Kalynchuk, 2010). Additionally, we showed for the first time a link between the upregulation of proteins upstream and downstream to mTORC1 in the antidepressant-like response elicited by ketamine plus guanosine in this

model. Noteworthy, our results unveiled that the ability of ketamine plus guanosine in abolishing the CORT-induced depressive-like behavior and synaptogenic impairments in the hippocampus is dependent on mTORC1 signaling.

In this study, CORT administration for 21 days was effective to induce a depressive-like behavior as evidenced by increased immobility time in the tail suspension test and latency to feed in the novelty-suppressed feeding test, as well as reduced self-care in the splash test. In line with our results, previous studies reported that chronic exposure to CORT is capable of causing a depressive-like phenotype (David et al., 2009; Pazini et al., 2016; Zhao et al., 2009). This behavioral alteration induced by CORT has been reported to be abolished by chronic, but not acute administration of typical monoaminergic antidepressants (Ago et al., 2013; Ampuero et al., 2010; Morais et al., 2014; Zeni et al., 2019), but it is reversed by a single administration of ketamine (Koike et al., 2013; Neis et al., 2018; Pazini et al., 2016). Therefore, this experimental approach allows the screening of putative rapid-acting antidepressant agents. In this study, CORT-induced depressive-like behavior was successfully abolished by a single administration of sub-effective doses of ketamine plus guanosine as efficiently as the effect elicited by a higher dose of ketamine (Koike et al., 2013; Neis et al., 2018). Additionally, we provide evidence that the ketamine plus guanosine-induced antidepressant-like effect in CORT-exposed mice is dependent on the activation mTORC1 signaling, since rapamycin (a selective mTORC1 inhibitor) consistently abolished the behavioral responses. Of note, an acute i.c.v. administration of rapamycin per se had no effect on depression-related behaviors. This result agrees with previous studies demonstrating that rapamycin was unable to affect depression-related phenotypes after a single central administration (Li et al., 2010, 2011;
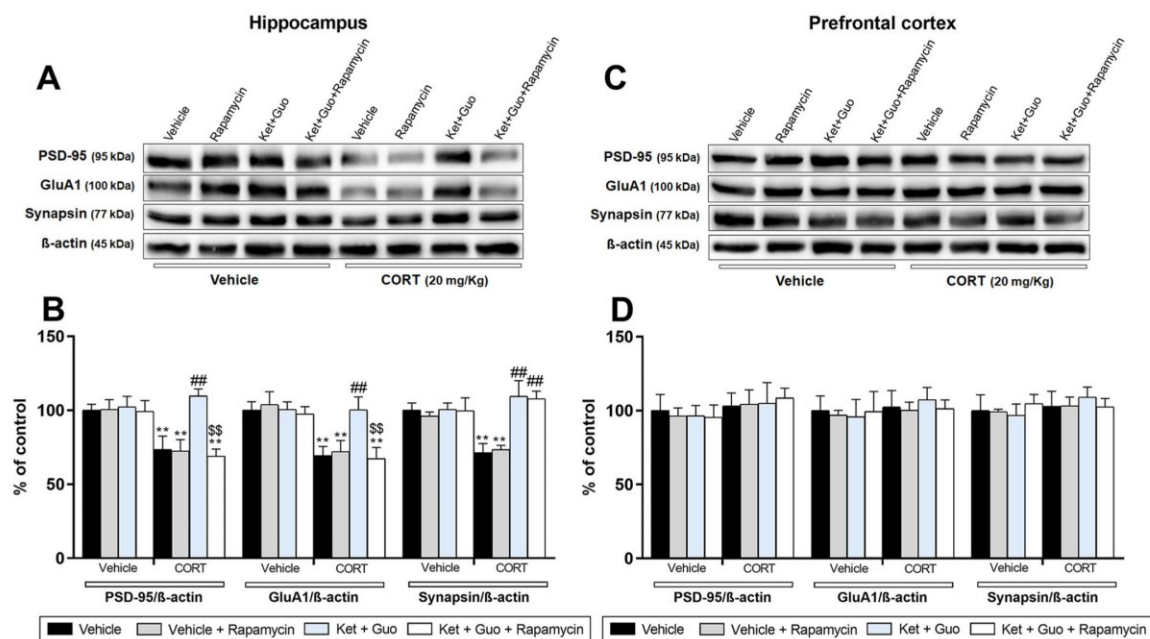
Fig. 9. Effect of treatment with ketamine (0.1 mg/kg, i.p.) plus guanosine (0.01 mg/kg, p.o.) and/or rapamycin (0.2 nmol/site, i.c.v.) on PSD-95, GluA1, and synapsin immunocontent in the hippocampus (A and B) and prefrontal cortex (C and D) of mice treated with vehicle or corticosterone (CORT – 20 mg/kg, p.o.). Panels A and C show the representative bands of PSD-95, GluA1, synapsin, and β-actin in the hippocampus and prefrontal cortex, respectively. Panels B and D show the quantification of these proteins in the hippocampus and prefrontal cortex, respectively. Values are expressed as means ± S.E.M (n = 7). **$P < 0.01$ as compared with the vehicle-treated group; ##$P < 0.01$ as compared with the CORT-treated group; \$\$$P < 0.01$ as compared with the ketamine plus guanosine-treated group (two-way ANOVA followed by Newman-Keuls post-hoc test).

Neis et al., 2020; Pazini et al., 2016), but it was effective in modulating depression-related behaviors after repeated systemic administration (Hadamitzky et al., 2018; Halloran et al., 2012). Supporting our results, mTORC1 activation has been recently reported to be required for the acute behavioral effects of ketamine plus guanosine treatment in the novelty-suppressed feeding test (Camargo et al., 2019) and guanosine's antidepressant response in the olfactory bulbectomy model (Almeida et al., 2020) in mice. Therefore, these findings demonstrated that a low-dose combination of ketamine and guanosine is effective to counteract corticosterone-induced depressive-like behavior via activating mTORC1 signaling.

To reinforce the role of mTORC1-driven signaling pathway in the antidepressant-like effect elicited by a low-dose combination of ketamine and guanosine, proteins upstream and downstream to mTORC1 were next investigated. Although questions have been raised about the role of the mTORC1 in the antidepressant effect of ketamine in clinical and preclinical studies (Abdallah et al., 2020; Popp et al., 2016), numerous reports have shown that the mTORC1 signaling is a crucial target for fast antidepressant responses (Li et al., 2010; Neis et al., 2020; Réus et al., 2015; Voleti et al., 2013). Classically, it has been postulated that ketamine blocks NMDA receptors located on inhibitory GABAergic interneurons, resulting in a disinhibition of hippocampal pyramidal cells and consequent release of glutamate in the synaptic cleft (Abdallah et al., 2015). Glutamate by activating AMPA receptors causes sodium influx and depolarization, leading to the activation of voltage-dependent calcium channels and BDNF release (Lepack et al., 2014). In the present study, CORT administration reduced BDNF levels in the hippocampus, but not in the prefrontal cortex, and this alteration concurs partially with previous studies (Freitas et al., 2016; Pazini et al., 2016; Weng et al., 2016). Since the hippocampus presents a high density of glucocorticoid receptors, this feature could make it particularly more

vulnerable to the effects of chronic CORT administration than the prefrontal cortex (Lee et al., 2002). Of note, this assumption may explain the BDNF reduction (and its downstream targets evidenced throughout this paper) only in the hippocampus. However, only the combined administration of subthreshold doses of ketamine and guanosine rescued the reduction on hippocampal BDNF levels induced by CORT, suggesting that BDNF could be associated with antidepressant-like effect triggered by ketamine plus guanosine combination. Consistent with these findings, previous studies demonstrated that BDNF upregulation was associated with the antidepressant-like effects elicited by guanosine (Rosa et al., 2021) and ketamine (Lepack et al., 2014). Regarding the modulation of BDNF by guanosine, we recently showed that hippocampal BDNF levels were increased 1, 6 and 24 h after a single administration of guanosine. However, in the prefrontal cortex, BDNF level was increased only 1 h after guanosine treatment, suggesting that guanosine may affect the hippocampus and the prefrontal cortex with a different profile (Rosa et al., 2021) .

The activation of tropomyosin receptor kinase B (TrkB) by BDNF culminates in Akt phosphorylation, which plays a role in cell metabolism, growth, and survival (Tejeda and Díaz-Guerra, 2017). In line with a prior report, the present study showed that CORT exposure reduced Akt (Ser473) phosphorylation in the hippocampus (Dong et al., 2019), but this alteration was not observed in ketamine plus guanosine-treated mice. Reinforcing these results, we also found that ketamine plus guanosine treatment counteracted the CORT-induced reduction on phosphorylation (Ser9) of glycogen synthase kinase-3β (GSK-3β), a downstream target of Akt. Importantly, Akt activation and GSK-3β inhibition represent a key mechanism for the rapid-acting actions of ketamine, an effect that was observed at 30 min and 60 min after its administration, respectively (Beurel et al., 2011; Li et al., 2010). The antidepressant-like and synaptogenic effects of a sub-effective dose of
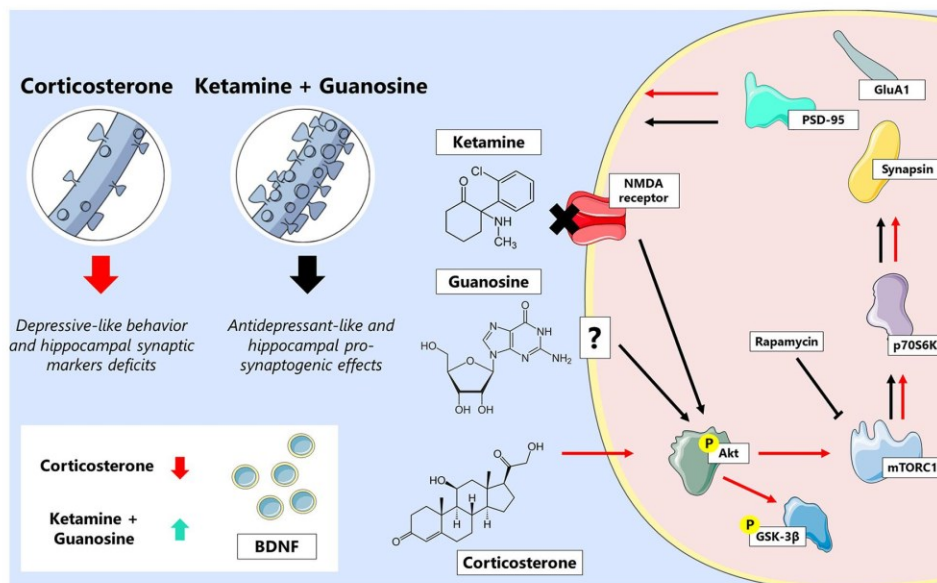
Fig. 10. This schematic representation postulates how the combination of ketamine plus guanosine at low doses could activate the mTORC1-driven signaling pathway to produce antidepressant-like and pro-synaptogenic effects. We demonstrated that corticosterone (20 mg/kg, p.o., for 21 days) administration reduced the BDNF levels, Akt/GSK-3β phosphorylation, and PSD-95, GluA1, and synapsin immunocontent (synaptic proteins downstream to mTORC1/p70S6K) in the hippocampus. A single combined administration of subthreshold doses of ketamine and guanosine was able to counteract all these corticosterone-induced alterations. Moreover, the pretreatment with rapamycin, a mTORC1 inhibitor, completely abolished the antidepressant-like and pro-synaptogenic effect elicited by ketamine plus guanosine. These findings suggest that the modulation of mTORC1-driven signaling pathway contribute to the antidepressant-like effect triggered by ketamine plus guanosine. Figure designed using images from Servier Medical Art and Mind the Graph.

ketamine were potentiated by a sub-effective dose of lithium, a GSK-3β inhibitor (Liu et al., 2013). Previous studies also demonstrated that the antidepressant-like effect of guanosine is dependent on PI3K/Akt (Bettio et al., 2012) and GSK-3β signaling pathways (Rosa et al., 2019), reinforcing the results of this study. Therefore, these results suggest that Akt/GSK-3β signaling could contribute to the antidepressant-like effect elicited by the combined administration of ketamine and guanosine.

Akt can activate mTORC1 by phosphorylating and inactivating proline-rich Akt substrate of 40 kDa (PRAS40) at Thr^{246} (Haar et al., 2007). Subsequently, mTORC1 phosphorylates 70 kDa ribosomal protein S6 kinase (p70S6K) at Thr^{389}, which also phosphorylates and activates mTORC1 at Ser^{2448} via a feedback loop (Magnuson et al., 2011). mTORC1 plays an essential role in the regulation of protein synthesis and is consistently associated with key neuronal functions as axonal sprouting and dendritic spine growth (Laplante and Sabatini, 2012). Notably, deficits on mTORC1/p70S6K immunocontent were observed in the prefrontal cortex of subjects diagnosed with MDD (Jernigan et al., 2011) and in the hippocampus and prefrontal cortex of rodents that underwent the chronic unpredictable stress and CORT administration protocols (Dong et al., 2019; Pazini et al., 2016; Zhu et al., 2018). Here, no alterations on mTOR1/p70S6K phosphorylation in the hippocampus and prefrontal cortex were observed after the administration of CORT (21 days, 20 mg/kg) or rapamycin, in agreement with previous results (Camargo et al., 2020c; Li et al., 2010, 2011). Likewise, no alterations on hippocampal or prefrontocortical mTORC1 and p70S6K phosphorylation were observed 24 h after a single administration of ketamine plus guanosine. This result is in contrast with a rapid increase on mTORC1/p70S6K phosphorylation detected 1 h after the combined administration of subthreshold doses of ketamine and guanosine in the hippocampus, but not in the prefrontal cortex (Camargo et al., 2020a). We may

hypothesize that the absence of significant effects on mTORC1/p70S6K phosphorylation observed in the present study may be due to the long time period (24 h) elapsed between the administration of ketamine plus guanosine and the western blotting analysis. Indeed, the activation of mTORC1 and p70S6K phosphorylation is a dynamic process that rapidly triggers downstream activation of molecular targets such as protein translation (Hoeffer and Klann, 2010). In line with this notion, ketamine has been found to induce a rapid (started in 30 min) and transient (back to baseline in 2 h) increase on mTORC1/p70S6K phosphorylation after its administration in the prefrontal cortex of rats (Li et al., 2010). Conversely, a previous study demonstrated that 24 h after ketamine administration no effect was observed on mTORC1/p70S6K phosphorylation (Li et al., 2011), suggesting that ketamine stimulates the mTORC1 signaling transiently, in a time-dependent manner, but future studies investigating additional time points are necessary to address this issue.

mTORC1/S6K signaling regulates protein translation, such as PSD-95, GluA1, and synapsin, which are required for the formation, maturation, and function of new synapses (Duman and Voleti, 2012). Remarkably, rapid-acting antidepressant agents, such as GLYX-13 (Liu et al., 2017), scopolamine (Voleti et al., 2013), and AZD6765 (Neis et al., 2020) have convergent effects on the pro-synaptogenesis signaling pathway. The rapid upregulation on PSD-95, GluA1, and synapsin is consistent with the possibility that increased synapse formation and connectivity underlie the fast-acting responses (Li et al., 2010; Voleti et al., 2013). Importantly, robust reductions in these synaptic proteins were reported in the hippocampus and prefrontal cortex of depressed subjects (Duric et al., 2013; Feyissa et al., 2009; Kang et al., 2012; Rafalo-Ulinska et al., 2016) and rodents subjected to chronic stress and CORT administration (Camargo et al., 2020c; Li et al., 2011; Pazini et al.,

2016). Here, CORT caused a downregulation on PSD-95, GluA1, and synapsin immunocontent in the hippocampus of mice, but not in the prefrontal cortex, corroborating prior studies (Camargo et al., 2020c; Pazini et al., 2016). Nonetheless, this study revealed for the first time that a single administration of low doses of ketamine plus guanosine, which had no effect alone, when given in combination was effective in reversing the alterations on PSD-95, GluA1, and synapsin immunocontent induced by CORT in the hippocampus. Furthermore, we also demonstrated that the ability of ketamine plus guanosine to reverse CORT-induced deficits on PSD-95 and GluA1 in the hippocampus was abolished by the administration of rapamycin, indicating the involvement of mTORC1 in this response. Therefore, we may speculate that the modulation of synaptic proteins via mTORC1 signaling could be associated with the antidepressant-like response triggered by ketamine plus guanosine. The administration of rapamycin by itself had no effect on synaptic proteins immunocontent, which is in line with previous evidence that reported its inability to affect mTORC1 downstream targets after an acute central administration in naïve (Li et al., 2010) and stress-exposed rodents (Li et al., 2011). It is intriguing to note that the ability of ketamine plus guanosine to counteract CORT-induced reduction in synapsin immunocontent was not rescued by rapamycin administration. This result suggests the participation of additional mechanisms on the modulation of this protein, and further investigation is necessary to ascertain this issue.

Altogether, our study reinforces and extends the notion that a single administration of ketamine plus guanosine at low doses robustly reverses CORT-induced depressive-like behavior. Noteworthy, this study provides novel evidence that the combined administration of ketamine and guanosine at low doses counteracted CORT-induced reductions on the immunocontent of BDNF, Akt/GSK-3β, and synaptic proteins (PSD-95, GluA1, and synapsin) in the hippocampus, a response that could contribute to the antidepressant-like effect elicited by these drugs (Fig. 10). Although no alterations on mTORC1/p70S6K phosphorylation were observed after ketamine plus guanosine and/or CORT, by using rapamycin we provide convincing evidence of the essential role of mTORC1 activation in the antidepressant-like and pro-synaptogenic effects elicited by ketamine plus guanosine. Despite the time point chosen in this study (24 h) to evaluate the phosphorylation of mTORC1/p70S6K failed to detect any changes in these parameters, our experiments were optimized to study the modulation of synaptic proteins (PSD-95, GluA1, and synapsin) that are crucial for antidepressant responses (Duman and Voleti, 2012). In this regard, we demonstrated that ketamine plus guanosine treatment upregulated synaptic proteins, but further studies are necessary to ascertain the ability of this pharmacological strategy to enhance the number and function of dendritic spines. Overall, this strategy could have therapeutic relevance for the treatment of MDD.

Disclosure conflict of interest

The authors declare that they have no conflict of interest.

Role of funding source

Acknowledgments

Author statement

All authors have agreed to the submission and approved the final version of the manuscrip, which is not currently being considered for publication by any other print or electronic journal.

Ethical statement

The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals and the experiments were performed after approval of the protocol by the Committee on Ethics of Animal Experimentation of the Federal University of Santa Catarina. The authors declare that no financial support or compensation has been received from any individual or corporate entity over the past three years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

References

Abdallah, C.G., Sanacora, G., Duman, R.S., Krystal, J.H., 2015. Ketamine and rapid-acting antidepressants: a window into a new neurobiology for mood disorder therapeutics. Annu. Rev. Med. 66, 509–523.

Abdallah, C.G., Averill, L.A., Gueorguieva, R., Goktas, S., Purohit, P., Ranganathan, M., Sherif, M., Ahn, K.H., D'Souza, D.C., Formica, R., Southwick, S.M., Duman, R.S., Sanacora, G., Krystal, J.H., 2020. Modulation of the antidepressant effects of ketamine by the mTORC1 inhibitor rapamycin. Neuropsychopharmacology 45, 990–997.

Ago, Y., Yano, K., Araki, R., Hiramatsu, N., Kita, Y., Kawasaki, T., Onoe, H., Chaki, S., Nakazato, A., Hashimoto, H., Baba, A., Takuma, K., Matsuda, T., 2013. Metabotropic glutamate 2/3 receptor antagonists improve behavioral and prefrontal dopaminergic alterations in the chronic corticosterone-induced depression model in mice. Neuropharmacology 65, 29–38.

Almeida, R.F., Pocharski, C.B., Rodrigues, A.L.S., Elisabetsky, E., Souza, D.O., 2020. Guanosine fast onset antidepressant-like effects in the olfactory bulbectomy mice model. Sci. Rep. 10, 8429.

Ampuero, E., Rubio, F.J., Falcon, R., Sandoval, M., Diaz-Veliz, G., Gonzalez, R.E., Earle, N., Dagnino-Subiabre, A., Aboitiz, F., Orrego, F., Wyneken, U., 2010. Chronic fluoxetine treatment induces structural plasticity and selective changes in glutamate receptor subunits in the rat cerebral cortex. Neuroscience 169, 98–108.

Barowsky, J., Schwartz, T.L., 2006. An evidence-based approach to augmentation and combination strategies for treatment resistant depression. Psychiatry 3, 42–61.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry 47, 351–354.

Bettio, L.E.B., Cunha, M.P., Budni, J., Pazini, F.L., Oliveira, Á., Colla, A.R., Rodrigues, A. L.S., 2012. Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. Behav. Brain Res. 234, 137–148.

Beurel, E., Song, L., Jope, R.S., 2011. Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. Mol. Psychiatry 16, 1068–1070.

Bodnoff, S.R., Suranyi-Cadotte, B., Aitken, D.H., Quirion, R., Meaney, M.J., 1988. The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology 95, 298–302.

Camargo, A., Rodrigues, A.L.S., 2019. Novel targets for fast antidepressant responses: possible role of endogenous neuromodulators. Chron. Stress 3, 247054701985808.

Camargo, A., Pazini, F.L., Rosa, J.M., Wolin, I.A.V., Moretti, M., Rosa, P.B., Neis, V.B., Rodrigues, A.L.S., 2019. Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway. J. Psychiatr. Res. 115, 103–112.

Camargo, A., Dalmagro, A.P., Zeni, A.L.B., Rodrigues, A.L.S., 2020a. Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: possible role of pro-synaptogenic signaling pathway. J. Affect. Disord. 271, 100–108.

Camargo, A., Dalmagro, A.P., Rosa J, M., Zeni AL, B., Kaster M, P., Tasca, C.I., S. Rodrigues, A.L., 2020b. Subthreshold doses of guanosine plus ketamine elicit antidepressant-like effect in a mouse model of depression induced by corticosterone: role of GR/NF-κB/IDO-1 signaling. Neurochem. Int. 139, 104797.

Camargo, A., Dalmagro, A.P., de Souza, M.M., Zeni, A.L.B., Rodrigues, A.L.S., 2020c. Ketamine, but not guanosine, as a prophylactic agent against corticosterone-induced depressive-like behavior: possible role of long-lasting pro-synaptogenic signaling pathway. Exp. Neurol. 334, 113459.

Dalmagro, A.P., Camargo, A., Rodrigues, A.L.S., Zeni, A.L.B., 2019. Involvement of PI3K/Akt/GSK-3β signaling pathway in the antidepressant-like and neuroprotective effects of Morus nigra and its major phenolic, syringic acid. Chem. Biol. Interact. 314, 108843.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.P., Artymyshyn, R.P., Gardier, A.M., Gerald, C., Antonijevic, I.A., Leonardo, E.D., Hen, R., 2009. Neurogenesis-dependent and

-independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62, 479–493.

Dong, S.Q., Wang, S.S., Zhu, J.X., Mu, R.H., Li, C.F., Geng, D., Liu, Q., Yi, L.T., 2019. Oleanolic acid decreases SGK1 in the hippocampus in corticosterone-induced mice. Steroids 149, 108419.

Dulawa, S.C., Hen, R., 2005. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. Neurosci. Biobehav. Rev. 29, 771–783.

Duman, R.S., Voleti, B., 2012. Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. Trends Neurosci. 35, 47–56.

Duric, V., Banasr, M., Stockmeier, C.A., Simen, A.A., Newton, S.S., Overholser, J.C., Jurjus, G.J., Dieter, L., Duman, R.S., 2013. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. Int. J. Neuropsychopharmacol. 16, 69–82.

Feyissa, A.M., Chandran, A., Stockmeier, C.A., Karolewicz, B., 2009. Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33, 70–75.

Fraga, D.B., Costa, A.P., Olescowicz, G., Camargo, A., Pazini, F.L., Freitas A., E., Moretti, M., Brocardo, P.S., Rodrigues, A.L.S., 2020. Ascorbic acid presents rapid behavioral and hippocampal synaptic plasticity effects. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 96, 109757.

Fraga, D.B., Camargo, A., Olescowicz, G., Padilha, D.A., Mina, F., Budni, J., Brocardo, P. S., Rodrigues, A.L.S., 2021. A single administration of ascorbic acid rapidly reverses depressive-like behavior and hippocampal synaptic dysfunction induced by corticosterone in mice. Chem. Biol. Interact. 342, 109476.

Freitas, A.E., Egea, J., Buendia, I., Gómez-Rangel, V., Parada, E., Navarro, E., Casas, A.I., Wojnicz, A., Ortiz, J.A., Cuadrado, A., Ruiz-Nuño, A., Rodrigues, A.L.S., Lopez, M.G., 2016. Agmatine, by improving neuroplasticity markers and inducing Nrf2, prevents corticosterone-induced depressive-like behavior in mice. Mol. Neurobiol. 53, 3030–3045.

Gao, M., Rejaei, D., Liu, H., 2016. Ketamine use in current clinical practice. Acta Pharmacol. Sin. 37, 865–872.

Goodyer, I.M., Herbert, J., Tamplin, A., Altham, P.M.E., 2000. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. Br. J. Psychiatry 179, 499–504.

Haar, E. Vander, Lee, S. Il, Bandhakavi, S., Griffin, T.J., Kim, D.H., 2007. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat. Cell Biol. 9, 316–323.

Hadamitzky, M., Herring, A., Kirchhof, J., Bendix, I., Haight, M.J., Keyvani, K., Lückemann, L., Unteroberdörster, M., Schedlowski, M., 2018. Repeated systemic treatment with rapamycin affects behavior and amygdala protein expression in rats. Int. J. Neuropsychopharmacol. 21, 595–602.

Halloran, J., Hussong, S.A., Burbank, R., Podlutskaya, N., Fischer, K.E., Sloane, L.B., Austad, S.N., Strong, R., Richardson, A., Hart, M.J., Galvan, V., 2012. Chronic inhibition of mammalian target of rapamycin by rapamycin modulates cognitive and non-cognitive components of behavior throughout lifespan in mice. Neuroscience 223, 102–113.

Han, C., Wang, S., Seo, H., Chul, B., Jin, H., Kim, W., Kwak, K., Pae, C., 2014. Aripiprazole augmentation, antidepressant combination or switching therapy in patients with major depressive disorder who are partial- or non-responsive to current antidepressants: a multi-center, naturalistic study. J. Psychiatr. Res. 49, 75–82.

Harris, T.O., Borsanyi, S., Messari, S., Stanford, K., Cleary, S.E., Shiers, H.M., Brown, G. W., Herbert, J., 2000. Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. Br. J. Psychiatry 177, 505–510.

Hoeffer, C.A., Klann, E., 2010. mTOR signaling: at the crossroads of plasticity, memory and disease. Trends Neurosci. 33, 67–75.

Holmes, S.E., Scheinost, D., Finnema, S.J., Naganawa, M., Davis, M.T., DellaGioia, N., Nabulsi, N., Matuskey, D., Angarita, G.A., Pietrzak, R.H., Duman, R.S., Sanacora, G., Krystal, J.H., Carson, R.E., Esterlis, I., 2019. Lower synaptic density is associated with depression severity and network alterations. Nat. Commun. 10, 1–10.

Iijima, M., Fukumoto, K., Chaki, S., 2012. Acute and sustained effects of a metabotropic glutamate 5 receptor antagonist in the novelty-suppressed feeding test. Behav. Brain Res. 235, 287–2892.

Jernigan, C.S., Goswami, D.B., Austin, M.C., Iyo, A.H., Chandran, A., Stockmeier, C.A., Karolewicz, B., 2011. The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 35, 1774–1779.

Kang, H.J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C.A., Licznerski, P., Lepack, A., Majik, M.S., Jeong, L.S., Banasr, M., Son, H., Duman, R.S., 2012. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. Nat. Med. 18, 1413–1417.

Kaster, M.P., Moretti, M., Cunha, M.P., Rodrigues, A.L.S., 2016. Novel approaches for the management of depressive disorders. Eur. J. Pharmacol. 771, 236–240. https://doi.org/10.1016/j.ejphar.2015.12.029.

Koike, H., Iijima, M., Chaki, S., 2013. Effects of ketamine and LY341495 on the depressive-like behavior of repeated corticosterone-injected rats. Pharmacol. Biochem. Behav. 107, 20–23.

Laplante, M., Sabatini, D.M., 2012. Review mTOR signaling in growth control and disease. Cell 149, 274–293.

Lee, A.L., Ogle, W.O., Sapolsky, R.M., 2002. Stress and depression: possible links to neuron death in the hippocampus. Bipolar Disord. 4, 117–128.

Lepack, A.E., Fuchikami, M., Dwyer, J.M., Banasr, M., Duman, R.S., 2014. BDNF release is required for the behavioral actions of ketamine. Int. J. Neuropsychopharmacol. 18, 1–6.

Li, N., Lee, B., Liu, R.-J., Banasr, M., Dwyer, J.M., Iwata, M., Li, X.-Y., Aghajanian, G., Duman, R.S., 2010. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329, 959–965.

Li, N., Liu, R.J., Dwyer, J.M., Banasr, M., Lee, B., Son, H., Li, X.Y., Aghajanian, G., Duman, R.S., 2011. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol. Psychiatry 69, 754–761.

Liu, R., Duman, C., Kato, T., Hare, B., Lopresto, D., Bang, E., Burgdorf, J., Moskal, J., Taylor, J., Aghajanian, G., Duman, R.S., 2017. GLYX-13 produces rapid antidepressant responses with key synaptic and behavioral effects distinct from ketamine. Neuropsychopharmacology 42, 1231–1242.

Liu, R.J., Fuchikami, M., Dwyer, J.M., Lepack, A.E., Duman, R.S., Aghajanian, G.K., 2013. GSK-3 inhibition potentiates the synaptogenic and antidepressant-like effects of subthreshold doses of ketamine. Neuropsychopharmacology 38, 2268–2277.

Ludka, F.K., Zomkowski, A.D.E., Cunha, M.P., Dal-Cim, T., Zeni, A.L.B., Rodrigues, A.L. S., Tasca, C.I., 2013. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the l-arginine-nitric oxide-cyclic guanosine monophosphate pathway and increases BDNF levels. Eur. Neuropsychopharmacol. 23, 400–412.

Magnuson, B., Ekim, B., Fingar, D.C., 2011. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochem. J. 441, 1–21.

McKlveen, J.M., Myers, B., Flak, J.N., Bundzikova, J., Solomon, M.B., Seroogy, K.B., Herman, J.P., 2013. Role of prefrontal cortex glucocorticoid receptors in stress and emotion. Biol. Psychiatry 74, 672–679.

Mizoguchi, K., Ishige, A., Aburada, M., Tabira, T., 2003. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. Neuroscience 119, 887–897.

Morais, M., Santos, P.A.R., Mateus-Pinheiro, A., Patrício, P., Pinto, L., Sousa, N., Pedroso, P., Almeida, S., Filipe, A., Bessa, J.M., 2014. The effects of chronic stress on hippocampal adult neurogenesis and dendritic plasticity are reversed by selective MAO-A inhibition. J. Psychopharmacol. 28, 1178–1183.

Moret, C., 2005. Combination/augmentation strategies for improving the treatment of depression. Neuropsychiatr. Dis. Treat. 1, 301–309.

Murrough, J.W., Abdallah, C.G., Anticevic, A., Collins, K.A., Geha, P., Averill, L.A., Schwartz, J., DeWilde, K.E., Averill, C., Jia-Wei Yang, G., Wong, E., Tang, C.Y., Krystal, J.H., Iosifescu, D.V., Charney, D.S., 2016. Reduced global functional connectivity of the medial prefrontal cortex in major depressive disorder. Hum. Brain Mapp. 37, 3214–3223.

Myers, B., McKlveen, J.M., Herman, J.P., 2014. Glucocorticoid actions on synapses, circuits, and behavior: implications for the energetics of stress. Front. Neuroendocrinol. 35, 180–196.

Neis, V.B., Bettio, L.B., Moretti, M., Rosa, P.B., Olescowicz, G., Fraga, D.B., Goncalves, F. M., Freitas, A.E., Heinrich, I.A., Lopes, M.W., Leal, R.B., Rodrigues, A.L.S., 2018. Single administration of agmatine reverses the depressive-like behavior induced by corticosterone in mice: comparison with ketamine and fluoxetine. Pharmacol. Biochem. Behav. 173, 44–50.

Neis, V.B., Moretti, M., Rosa, P.B., Dalsenter, Y. De O., Werle, I., Platt, N., Kaufmann, F. N., Rosado, A.F., Besen, M.H., Rodrigues, A.L.S., 2020. The involvement of PI3K/Akt/mTOR/GSK3β signaling pathways in the antidepressant-like effect of AZD6765. Pharmacol. Biochem. Behav. 198, 173020.

Otte, C., Gold, S.M., Penninx, B.W., Pariante, C.M., Etkin, A., Fava, M., Mohr, D.C., Schatzberg, A.F., 2016. Major depressive disorder. Nat. Rev. Dis. Primers 2, 1–21.

Papakostas, G.I., Ionescu, D.F., 2015. Towards new mechanisms: an update on therapeutics for treatment-resistant major depressive disorder. Mol. Psychiatry 20, 1142–1150.

Papakostas, G.I., Petersen, T.J., Green, C., Iosifescu, D.V., Yeung, A.S., Nierenberg, A.A., Fava, M., Posternak, M.A., 2005. A description of next-step switching versus augmentation practices for outpatients with treatment-resistant major depressive disorder enrolled in an academic specialty clinic. Ann. Clin. Psychiatry 17, 161–165.

Pazini, F.L., Cunha, M.P., Rosa, J.M., Colla, A.R.S., Lieberknecht, V., Oliveira, Á., Rodrigues, A.L.S., 2016. Creatine, similar to ketamine, counteracts depressive-like behavior induced by corticosterone via PI3K/Akt/mTOR pathway. Mol. Neurobiol. 53, 6818–6834.

Pazini, F.L., Rosa, J.M., Camargo, A., Fraga, D.B., Moretti, M., Siteneski, A., Rodrigues, A. L.S., 2020. mTORC1-dependent signaling pathway underlies the rapid effect of creatine and ketamine in the novelty-suppressed feeding test. Chem. Biol. Interact. 332, 109281.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83, 346–356.

Popp, S., Behl, B., Joshi, J.J., Lanz, T.A., Spedding, M., Schenker, E., Jay, T.M., Svenningsson, P., Caudal, D., Cunningham, J.I., Deaver, D., Bespalov, A., 2016. In search of the mechanisms of ketamine's antidepressant effects: how robust is the evidence behind the mTOR activation hypothesis. F1000Res. 5, 1–9.

Price, R.B., Nock, M.K., Charney, D.S., Mathew, S.J., 2009. Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. Biol. Psychiatry 66, 522–526.

Rafalo-Ulinska, A., Piotrowska, J., Kryczyk, A., Sowa-kucma, M., Misztak, P., Rajkowska, G., Stockmeier, C.A., Datka, W., Nowak, G., Szewczyk, B., 2016. Zinc transporters protein level in postmortem brain of depressed subjects and suicide victims. J. Psychiatr. Res. 83, 220–229.

Rosa, P.B., Ribeiro, C.M., Bettio, L.E.B., Colla, A., Lieberknecht, V., Moretti, M., Rodrigues, A.L.S., 2014. Folic acid prevents depressive-like behavior induced by chronic corticosterone treatment in mice. Pharmacol. Biochem. Behav. 127, 1–6.

Réus, G.Z., Quevedo, J., Rodrigues, A.L.S., 2015. mTOR signaling in the neuropathophysiology of depression: current evidence. J. Receptor. Ligand Channel 8, 65–74.

Rosa, P.B., Bettio, L.E.B., Neis, V.B., Moretti, M., Kaufmann, F.N., Tavares, M., Werle, I., Dalsenter, Y., Platt, N., Rosado, A., Fraga, D.B., Heinrich, I., Freitas, A.E., Leal, R.B., Rodrigues, A.L.S., 2021. Antidepressant-like effect of guanosine involves activation of AMPA receptor and BDNF/TrkB signaling. Purinergic Signal. 17, 285–301.

Rosa, P.B., Bettio, L.E.B., Neis, V.B., Moretti, M., Werle, I., Leal, R.B., Rodrigues, A.L.S., 2019. The antidepressant-like effect of guanosine is dependent on GSK-3β inhibition and activation of MAPK/ERK and Nrf2/heme oxygenase-1 signaling pathways. Purinergic Signal 15, 491–504.

Sterner, E.Y., Kalynchuk, L.E., 2010. Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: relevance to depression. Prog. Neuro Psychopharmacol. Biol. Psychiatry 34, 777–790.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Tejeda, G.S., Díaz-Guerra, M., 2017. Integral characterization of defective BDNF/TrkB signalling in neurological and psychiatric disorders leads the way to new therapies. Int. J. Mol. Sci. 18, 1–24.

Voleti, B., Navarria, A., Liu, R., Banasr, M., Li, N., Terwilliger, R., Sanacora, G., Eid, T., Aghajanian, G., Duman, R.S., 2013. Scopolamine rapidly increases mammalian target of rapamycin complex 1 signaling, synaptogenesis, and antidepressant behavioral responses. Biol. Psychiatry 74, 742–749.

Wang, G., Cheng, Y., Gong, M., Liang, B., Zhang, M., Chen, Y., Zhang, C., Yuan, X., Xu, J., 2013. Systematic correlation between spine plasticity and the anxiety/depression-like phenotype induced by corticosterone in mice. Neuroreport 24, 682–687.

Watson, S., Mackin, P., 2006. HPA axis function in mood disorders. Psychiatry 5, 166–170.

Weng, L., Guo, X., Li, Y., Yang, X., Han, Y., 2016. Apigenin reverses depression-like behavior induced by chronic corticosterone treatment in mice. Eur. J. Pharmacol. 774, 50–54.

Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90–110.

World Health Organization, 2017. Depression and Other Common Mental Disorders: Global Health Estimates. World Health Organization, pp. 1–24.

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry 63, 856–864.

Zeni, A.L.B., Camargo, A., Dalmagro, A.P., 2019. Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities. Pharmacol. Biochem. Behav. 179, 63–72.

Zhao, Y., Xie, W., Dai, J., Wang, Z., Huang, Y., 2009. The varying effects of short-term and long-term corticosterone injections on depression-like behavior in mice. Brain Res. 1261, 82–90.

Zhu, J.-X., Shan, J.-L., Hu, W.-Q., Zeng, J.-X., Shu, J.-C., 2018. Gallic acid activates hippocampal BDNF-Akt-mTOR signaling in chronic mild stress. Metab. Brain Dis. 34, 93–101.

4.4 CAPÍTULO 4

Ketamine, but not guanosine, as a prophylactic agent against corticosterone-induced depressive-like behavior: Possible role of long-lasting pro-synaptogenic signaling pathway

Anderson Camargo, Ana Paula Dalmagro, Márcia M. de Souza, Ana Lúcia B. Zeni, Ana Lúcia S. Rodrigues
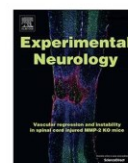
Research paper

Ketamine, but not guanosine, as a prophylactic agent against corticosterone-induced depressive-like behavior: Possible role of long-lasting pro-synaptogenic signaling pathway

Anderson Camargo[a], Ana Paula Dalmagro[b], Márcia M. de Souza[b], Ana Lúcia B. Zeni[c], Ana Lúcia S. Rodrigues[a],*

[a] Neuroscience Postgraduate Program, Center of Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis 88040-900, SC, Brazil
[b] Pharmaceutical Sciences Postgraduate Program, Center of Health Sciences, Universidade do Vale do Itajaí, CEP 88302-202 Itajaí, Santa Catarina, Brazil
[c] Department of Natural Sciences, Center of Exact and Natural Sciences, Universidade Regional de Blumenau, CEP 89030-903 Blumenau, Santa Catarina, Brazil

ARTICLE INFO

ABSTRACT

Ketamine has been reported to exert a prophylactic effect against stress-induced depressive-like behavior by modulating the guanosine-based purinergic system. However, the molecular pathways underlying its prophylactic effect and whether guanosine also elicits a similar effect remain to be determined. Here, we investigated the prophylactic effect of ketamine and guanosine against corticosterone (CORT – 20 mg/kg, p.o.)-induced depressive-like behavior in mice. Furthermore, we characterized if the prophylactic response may be associated with mTORC1-driven signaling in the hippocampus and prefrontal cortex. A single administration of ketamine (5 mg/kg, i.p.), but not guanosine (1 or 5 mg/kg, p.o.), given 1 week before the pharmacological stress prevented CORT-induced depressive-like behavior in the tail suspension test (TST) and splash test (SPT). Fluoxetine treatment for 3 weeks did not prevent CORT-induced behavioral effects. A single administration of subthreshold doses of ketamine (1 mg/kg, i.p.) plus guanosine (5 mg/kg, p.o.) partially prevented the CORT-induced depressive-like behavior in the SPT. Additionally, CORT reduced Akt (Ser^{473}) and GSK-3β (Ser^9) phosphorylation and PSD-95, GluA1, and synapsin immunocontent in the hippocampus, but not in the prefrontal cortex. No alterations on mTORC1/p70S6K immunocontent were found in both regions in any experimental group. CORT-induced reductions on PSD-95, GluA1, and synapsin immunocontent were prevented only by ketamine treatment. Collectively, these findings suggest that ketamine, but not guanosine, exerts a prophylactic effect against depressive-like behavior, an effect associated with the stimulation of long-lasting pro-synaptogenic signaling in the hippocampus.

1. Introduction

Major depressive disorder (MDD), a prevalent and pervasive psychiatric condition affecting more than 300 million individuals, is the most cause of disability worldwide (World Health Organization, 2017). Importantly, stress exposure is the main risk factor for the onset of depressive symptoms (Otte et al., 2016) and several lines of evidence have shown that increased levels of cortisol are associated with depressive episodes (Goodyer et al., 2000; Harris et al., 2000). For instance, compelling evidence has revealed associations among subjective well-being, MDD, anxiety, and alexithymic traits during the COVID-19 pandemic, a high-stress condition that may increase suicide risk (De Berardis et al., 2017; Pappa et al., 2020; Tang et al., 2020). Of note, depressive-like behavior has been reported in rodents subjected to chronic unpredictable stress or repeated administration of corticosterone (Li et al., 2011; Moretti et al., 2012; Pazini et al., 2016; Zeni et al., 2019). However, current monoamine-based antidepressant drugs have reduced efficacy and large time-lag for exerting a therapeutic response, and these are significant limitations for patients with severe MDD and at risk of suicide (Kaster et al., 2016; Papakostas and Ionescu,

A. Camargo, et al.

2015). Therefore, novel antidepressants with new mechanisms of action are a major focus of current drug development. Reinforcing this assumption, agomelatine that targets the melatonergic system has been reported to be effective as an antidepressant drug, which could be a novel intriguing antidepressant option (Pompili et al., 2013).

However, the most promising advance for the treatment of MDD emerged with the discovery of ketamine, an N-Methyl-D-aspartate (NMDA) receptor antagonist that produces fast and sustained antidepressant responses (Berman et al., 2000; Zarate et al., 2006). Ketamine can relieve depressive symptoms within hours after a single dose and its effects can last up to 2 weeks, even in treatment-resistant patients with suicidal ideation (DiazGranados et al., 2010; Price et al., 2009; Zarate et al., 2006). Compelling reports have shown that the antidepressant effect of ketamine is dependent on the mechanistic target of rapamycin protein complex 1 (mTORC1) signaling pathway and synaptic protein synthesis (Li et al., 2011; Li et al., 2010; Sarkar and Kabbaj, 2016). mTORC1 stimulation controls synaptic protein translation such as postsynaptic density protein-95 kDa (PSD-95), alpha-amino-3-hydroxy-methyl-5-4-isoxazole propionic acid (AMPA) receptor subunits 1 (GluA1), and synapsin, which are essential for spinogenesis and synaptogenesis (Abdallah et al., 2016). Notably, esketamine was approved for adults with treatment-resistant MDD (Wei et al., 2020). A study by Brachman et al. (2016) has provided groundbreaking evidence that ketamine is also effective as a prophylactic agent against stress-induced depressive-like behavior, opening novel perspectives for the design of strategies to prevent MDD. Of special interest, McGowan et al. (2018) showed that the prophylactic effect of ketamine against stress was associated with increased levels of guanosine precursors, guanosine's diphosphate and triphosphate, both in the hippocampus and prefrontal cortex, suggesting an interaction between ketamine and the purinergic system.

We previously reported that guanosine, an endogenous guanine-based purine, could be a novel agent to exert fast antidepressant-like responses or even potentiate the actions of ketamine (Almeida et al., 2020; Camargo et al., 2020a; Camargo and Rodrigues, 2019). This nucleoside is a neuromodulator with remarkable neurotrophic and neuroprotective effects (Bettio et al., 2016; Di Liberto et al., 2016; Tasca et al., 2018). Of note, guanosine administered systemically or centrally produces an antidepressant-like effect in the tail suspension test (TST) and forced swimming test (FST) through the modulation of NMDA receptors and the mTORC1 pathway (Bettio et al., 2012). Moreover, a single administration of guanosine exerted a fast onset antidepressant-like effect in the olfactory bulbectomy mice model (Almeida et al., 2020). The ability of guanosine to augment the antidepressant-like and pro-synaptogenic effects of subthreshold doses of ketamine in the novelty-suppressed feeding test (NSF) and TST was also demonstrated (Camargo et al., 2019; Camargo et al., 2020a). Noteworthy, a single combined administration with subthreshold doses of ketamine and guanosine was effective in counteracting corticosterone (CORT)-induced depressive-like behavior 24 h after treatment (Camargo et al., 2020b). However, it remains to be determined if guanosine could be effective as a prophylactic agent or even potentiate the prophylactic actions of ketamine against stress.

Considering that ketamine-induced mTORC1-driven pro-synaptogenic effect has a long-lasting window (Li et al., 2011; Moda-Sava et al., 2019), we hypothesized that this response could be associated with its prophylactic effect against CORT-induced depressive-like behavior. Moreover, taking into account that ketamine may modulate the purinergic system (McGowan et al., 2018) and guanosine shares some common molecular targets to ketamine (Camargo and Rodrigues, 2019), we also hypothesized that this nucleoside could elicit a prophylactic effect or would be able to potentiate the ketamine's effects. Therefore, the present study had three major purposes. First, we aimed to validate the prophylactic effect of ketamine against the depressive-like behavior elicited by the repeated administration of corticosterone (a protocol that mimics chronic stress) and characterize if this response

may be associated with mTORC1-mediated signaling. Second, we investigated the possible prophylactic effect of guanosine against this same protocol of corticosterone administration and whether the modulation of mTORC1 signaling could be associated with this response. Third, we evaluated the ability of the combined administration of ketamine and guanosine to exert a prophylactic response and the role of mTORC1 signaling.

2. Material and methods

2.1. Animals

Male *Swiss* mice (55–60 days of age) were housed in groups of 8 in a cage (41 × 34 × 16 cm), under controlled temperature (21 ± 1 °C) and humidity (50 ± 20%) with a 12:12 h light/dark cycle (lights on at 7:00 a.m.), and with free access to food and water. The animals were used according to the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the experiments were performed after approval of the protocol by the Institutional Ethics Committee.

2.2. Drugs and treatments

Mice received a single administration of ketamine (1 or 5 mg/kg, i.p., dissolved in 0.9% saline) or guanosine (1 or 5 mg/kg, p.o., dissolved in distilled water) 1 week prior to the administration of vehicle or CORT. Both drugs were administered in a volume of 10 ml/kg body weight. In another set of experiments, mice were daily administered with fluoxetine (10 mg/kg, p.o., dissolved in distilled water) for 3 weeks before the onset of the administration of vehicle or CORT. In the last set of experiments, mice received a single administration of ketamine (1 mg/kg, i.p.) plus guanosine (5 mg/kg, p.o.) 1 week before the administration of vehicle or CORT. Vehicle or CORT (20 mg/kg, dissolved in distilled water with 2% Tween 80 and 0.2% DMSO) was administered by gavage (p.o.) once a day (between 9:00 a.m. and 10 a.m.) for 21 days. On the 22nd day, 24 h after the last CORT administration, animals were subjected to the depression-related behavioral tests (10 min apart). Subsequently, mice were immediately euthanized by decapitation, and the hippocampus and prefrontal cortex were collected for western blotting analysis. The drugs (Sigma Chemical Co., St. Louis, USA) were freshly prepared before administration, and all doses and time points of administration were chosen based on previous studies (Bettio et al., 2012; Brachman et al., 2016; Camargo et al., 2020a; Pazini et al., 2016). A diagram of the treatment regimen, behavioral and neurochemical analyses is provided in Fig. 1.

2.3. Tail suspension test (TST)

The total immobility time of mice suspended by the tail was measured as previously proposed (Steru et al., 1985). Visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded for 6 min by an experienced observer blind to the experimental groups. Mice were considered immobile only when they hung passively and completely motionless.

2.4. Open-field test (OFT)

Mice were individually subjected to the OFT as previously described (Rodrigues et al., 1996). The apparatus consisted of a wooden box measuring 40 × 60 × 50 cm high. The floor of the arena was divided into 12 equal squares. The number of squares crossed for 6 min was considered a parameter of locomotor activity.

2.5. Splash test (SPT)

The SPT consists of squirting a 10% sucrose solution (*w/v*) on the
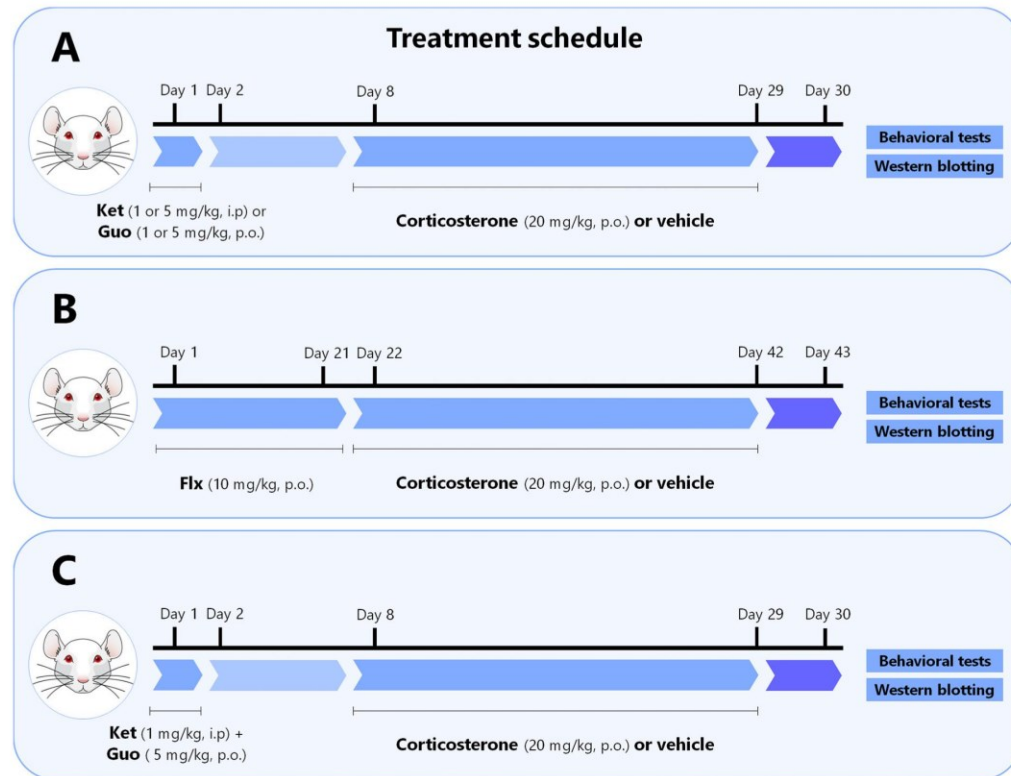
A. Camargo, et al.

Fig. 1. Experimental design and schedule of the treatment regimen, behavioral and neurochemical analyses. a Mice were treated with a single administration of ketamine (1 or 5 mg/kg, i.p.) or guanosine (1 or 5 mg/kg, p.o.) 1 week prior to the administration with vehicle or corticosterone. b Mice were daily administered with fluoxetine (10 mg/kg, p.o.) for 3 weeks before starting the chronic administration with vehicle or corticosterone. c Mice were treated with a single administration of a subthreshold dose of ketamine (1 mg/kg, i.p.) plus a high dose of guanosine (5 mg/kg, p.o.) 1 week before the administration with vehicle or corticosterone. a, b, c Vehicle or corticosterone (20 mg/kg) was administered orally (p.o.), once a day, for 21 consecutive days. On the 22nd day, 24 h after the last corticosterone administration, animals were subjected to the depression-related behavioral tests, namely tail suspension test, open-field test, and splash test (10 min apart). Subsequently, mice were immediately euthanized by decapitation and the hippocampus and prefrontal cortex were collected for western blotting analysis. Figure designed using images from Mind the Graph.

dorsal coat of mice placed in clear boxes (12 × 20 × 30 cm). Due to its viscosity, the sucrose solution dirties the mice which then initiate a grooming behavior. After applying the sucrose solution, the latency time to the first grooming and the total time spent grooming were recorded for 5 min, as an index of self-care and motivational behavior (Rosa et al., 2014; Willner, 2005).

2.6. Western blotting

Western blotting was conducted as previously described (Leal et al., 2020). The hippocampus and prefrontal cortex were mechanically homogenized in 400 μl of 50 mM TRIS pH 7.0, 1 mM EDTA, 100 mM NaF, 0.1 mM PMSF, 2 mM Na_3VO_4, 1%Triton X-100, 10% glycerol, and protease inhibitor cocktail. Lysates were centrifuged (10,000 g for 10 min, at 4 °C) and the supernatants were diluted 1/1 (v/v) in 100 mM TRIS pH 6.8, 4 mM EDTA, 8% SDS, and boiled for 5 min. Thereafter, sample dilution (40% glycerol, 100 mM TRIS, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β-mercaptoethanol (final concentration 8%) were added to the samples. Protein content was quantified using bovine serum albumin (BSA) as a standard (Peterson, 1977). The samples containing 60 μg protein/track were separated by SDS-PAGE using 7–10% gel and the proteins were transferred to nitrocellulose membranes using a semi-dry blotting apparatus (1.2 mA/

cm2; 1.5 h). To verify the transfer efficiency process, membranes were stained with Ponceau and subsequently, the membranes were blocked with 5% BSA in TBS (10 mM Tris, 150 mM NaCl, pH 7.5). The immunocontent of total and phosphorylated forms of protein kinase B (Akt – Ser[473], #9271), glycogen synthase kinase 3β (GSK-3β – Ser[9], #9336), mTORC1 (Ser[2448], #2971) and 70 kDa ribosomal protein S6 kinase (p70S6K – Thr[389,] #9205), as well as the total immunocontent of Akt (#9272), GSK-3β (#9315), mTORC1 (#2972), p70S6K (#9202), PSD-95 (#2507), GluA1 (#13185), synapsin (#2312), and β-actin (loading control, #4970) were detected using specific antibodies (Cell Signaling, 1:1000) diluted in TBS-T (10 mM Tris, 150 mM NaCl, 0.1% Tween-10, pH 7.5) containing 2.5% BSA and incubated overnight. Next, the membranes were incubated with a horseradish peroxidase-conjugated secondary antibody (Cell Signaling, 1:2500) for 60 min, and the immunoreactive bands were developed using a chemiluminescence kit (Amersham ECL Select, Piscataway, USA). The optical density (OD) of the bands was quantified using Image Lab Software® 4.1 (Bio-Rad Laboratories). The phosphorylation levels of Akt, GSK-3β, mTOR, and p70S6K were determined as a ratio of OD of the phosphorylated band over OD of the total band. The immunocontent of PSD-95, GluA1, and synapsin was determined as a ratio of the specific protein band over the OD of the β-actin band. Results are expressed as compared to the control group 100%.

A. Camargo, et al.
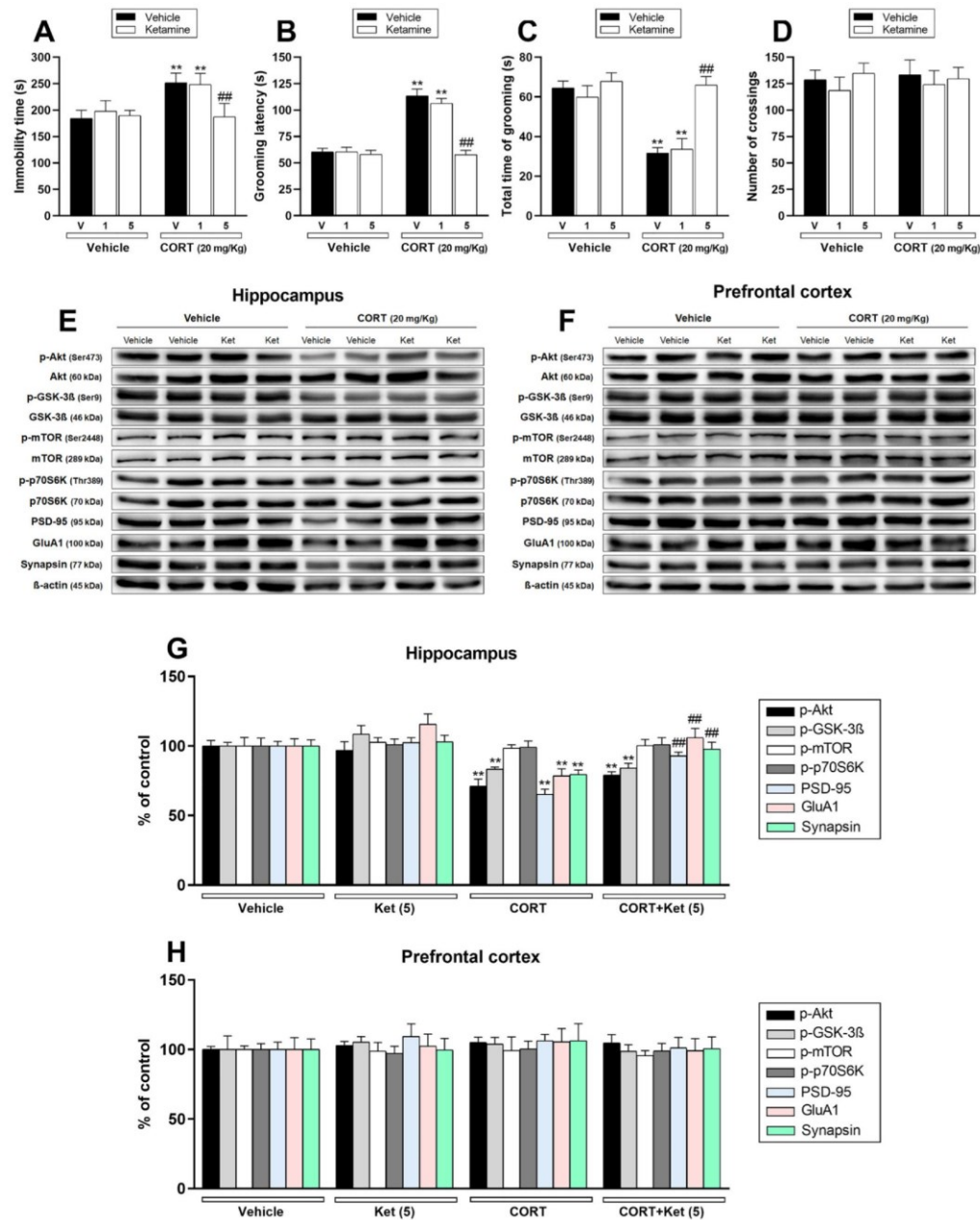
Fig. 2. Effect of a single administration with ketamine (Ket – 1 or 5 mg/kg, i.p.) 1 week prior to the administration with vehicle or corticosterone (CORT – 20 mg/kg, p.o.) on depression-related behaviors and synaptic markers in the hippocampus and prefrontal cortex of mice. a Ketamine (5 mg/kg, i.p.) administration prevented CORT-induced increase in the immobility time in the TST (n = 8). b, c Ketamine (5 mg/kg, i.p.) administration prevented the increase in the grooming latency and reduction in the total time of grooming induced by CORT in the SPT (n = 8). d All groups of mice had comparable number of crossings in the OFT (n = 8). e, f Representative bands of phospho-Akt (Ser[473]), Akt, phospho-GSK-3β (Ser[9]), GSK-3β, phospho-mTOR (Ser[2448]), mTOR, phospho-p70S6K (Thr[389]), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the (e) hippocampus and (f) prefrontal cortex of mice. g, h Quantification of these proteins in the (g) hippocampus and (h) prefrontal cortex. Values are expressed as means ± S.E.M (n = 7). ** $p < .01$ as compared with the vehicle-treated group; ## $p < .01$ as compared with the CORT-treated group (two-way ANOVA followed by Newman-Keuls *post hoc* test).

2.7. Statistical analysis

The D'Agostino-Pearson test was used to assess data normality. The differences among experimental groups were determined by two-way analysis of variance (ANOVA) followed by Newman-Keuls *post hoc* test, when appropriate. Data are presented as mean ± standard error of mean (SEM). A value of $p < .05$ was considered significant.

3. Results

3.1. Ketamine prevents CORT-induced depressive-like behavior and hippocampal synaptogenic markers deficits

The first set of experiments was designed to validate herein the prophylactic effect of ketamine previously shown in C57BL/6NTac mice (Brachman et al., 2016). Chronic CORT administration significantly increased the immobility time (a behavioral despair behavior) in the TST ($p < .01$; Fig. 2a) and the latency to first grooming (indicative of impaired self-care behavior) in the SPT ($p < .01$; Fig. 2b). Additionally, CORT administration reduced the time spent grooming (an anhedonic marker) in the SPT ($p < .01$; Fig. 2c). No effect was observed in the number of crossings in the OFT (Fig. 2d). These results indicate that the CORT protocol induced a depressive-like phenotype. In contrast, a single administration of ketamine at dose 5 mg/kg, i.p., but not 1 mg/kg, i.p., significantly prevented the increase in immobility time and grooming latency ($p < .01$), and the reduced total time of grooming ($p < .01$) induced by CORT. These results suggest that ketamine was effective in producing a prophylactic effect against the depressive-like behavior elicited by CORT administration.

We next sought to investigate whether the mTORC1-mediated signaling pathway may be associated with the prophylactic effect of ketamine. Fig. 2 shows the influence of CORT administration and/or ketamine treatment on the phosphorylated forms of protein kinase B (Akt – Ser^{473}), glycogen synthase kinase 3β (GSK-3β – Ser^9), mTOR (Ser^{2448}), and 70 kDa ribosomal protein S6 kinase (p70S6K – Thr^{389}), as well as the immunocontent of PSD-95, GluA1, synapsin in the hippocampus (Fig. 2e, g) and prefrontal cortex (Fig. 2f, h) of mice. CORT administration significantly reduced Akt and GSK-3β phosphorylation and PSD-95, GluA1, and synapsin immunocontent in the hippocampus of mice ($p < .01$). No significant effects of any treatment were observed on mTOR and p70S6K phosphorylation in the hippocampus. However, a single administration of ketamine (5 mg/kg, i.p.) 1 week prior to the CORT administration significantly prevented the reduction on PSD-95, GluA1, and synapsin immunocontent induced by this hormone in the hippocampus ($p < .01$). Conversely, ketamine treatment was not effective in preventing CORT-induced decrease on hippocampal Akt and GSK-3β phosphorylation. Furthermore, no significant alterations on the phosphorylation levels of Akt, GSK-3β, mTOR, p70S6K, as well as on immunocontent of PSD-95, GluA1, and synapsin were observed in the prefrontal cortex after CORT and/or ketamine administration.

3.2. Fluoxetine is ineffective to prevent the depressive-like behavior and hippocampal synaptogenic markers deficits induced by CORT

The second set of experiments was designed to validate whether fluoxetine fails to exert a prophylactic effect, as previously reported in C57BL/6NTac mice (Brachman et al., 2016). Chronic CORT administration raised the immobility time ($p < .01$; Fig. 3a) and the latency to first grooming ($p < .01$; Fig. 3b) in the TST and SPT, respectively. Furthermore, CORT reduced the time spent grooming ($p < .01$; Fig. 3c) in mice subjected to the SPT. No effect was observed in the number of crossings in the OFT (Fig. 3d). Importantly, repeated fluoxetine administration for 3 weeks before the CORT protocol was ineffective to prevent the depressive-like behavior.

The influence of fluoxetine on mTORC1-mediated signaling was also investigated. Fig. 3 shows the influence of CORT and/or fluoxetine administration on the phosphorylated forms of Akt (Ser^{473}), GSK-3β (Ser^9), mTOR (Ser^{2448}), and p70S6K (Thr^{389}), as well as the immunocontent of PSD-95, GluA1, synapsin in the hippocampus (Fig. 3e, g) and prefrontal cortex (Fig. 3f, h). CORT significantly reduced Akt and GSK-3β phosphorylation as well as PSD-95, GluA1, and synapsin immunocontent in the hippocampus of mice ($p < .01$), but no effect was observed on mTOR and p70S6K phosphorylation in this brain structure. Fluoxetine treatment did not protect against CORT-induced reduction in any hippocampal protein. Additionally, no alterations were observed on mTORC1-related targets in the prefrontal cortex after CORT and/or fluoxetine administration.

3.3. Guanosine is unable to prevent CORT-induced depressive-like behavior and hippocampal synaptogenic markers deficits

We next investigated the possible prophylactic effect of guanosine, a novel target postulated for exhibiting fast antidepressant-like responses (Camargo and Rodrigues, 2019). Chronic CORT administration enhanced the immobility time in the TST ($p < .01$; Fig. 4a) and latency to start the first grooming in the SPT ($p < .01$; Fig. 4b), as well as reduced the time spent grooming in the SPT ($p < .01$; Fig. 4c). No effect was observed in the OFT (Fig. 4d). As opposed to ketamine's results, guanosine was unable to prevent any behavioral alteration induced by the CORT.

The influence of guanosine on mTORC1-mediated signaling was subsequently investigated. Fig. 4 shows the influence of CORT administration and/or guanosine treatment on the phosphorylation levels of Akt (Ser^{473}), GSK-3β (Ser^9), mTOR (Ser^{2448}), and p70S6K (Thr^{389}), as well as the immunocontent of PSD-95, GluA1, synapsin in the hippocampus (Fig. 4e, g) and prefrontal cortex (Fig. 4f, h). CORT protocol significantly decreased Akt and GSK-3β phosphorylation and PSD-95, GluA1, and synapsin immunocontent in the hippocampus of mice ($p < .01$), although no alteration in any protein was observed in the prefrontal cortex. A single administration of guanosine given before the pharmacological stress failed to prevent the reduction induced by CORT on Akt, GSK-3β, PSD-95, GluA1, and synapsin immunocontent. No alterations on mTORC1-related targets were detected in the prefrontal cortex after CORT and/or guanosine administration.

3.4. Ketamine plus guanosine partially prevented CORT-induced reduction on self-care behavior, without altering hippocampal synaptogenic markers deficits induced by CORT

Considering that guanosine potentiates the antidepressant-like of subthreshold doses of ketamine in CORT-administered mice (Camargo et al., 2020b), we next investigated whether this augmentation response could be effective in producing a prophylactic effect. Chronic CORT administration significantly increased immobility time in the TST ($p < .01$; Fig. 5a) and grooming latency in the SPT ($p < .01$; Fig. 5b), and reduced time spent grooming in the SPT ($p < .01$; Fig. 5c). Furthermore, no alteration was observed in the OFT in any group (Fig. 5d). A single coadministration of ketamine plus guanosine partially counteracted the CORT-induced increase in the grooming latency ($p < .05$). However, this combined administration did not prevent the other behavioral alterations induced by the CORT.

The influence of ketamine plus guanosine treatment on mTORC1-mediated signaling was next evaluated. Fig. 5 shows the influence of CORT administration and/or ketamine plus guanosine treatment on the phosphorylation of Akt (Ser^{473}), GSK-3β (Ser^9), mTOR (Ser^{2448}), and p70S6K (Thr^{389}), as well as the immunocontent of PSD-95, GluA1, synapsin in the hippocampus (Fig. 5e, g) and prefrontal cortex (Fig. 5f, h). CORT administration significantly decreased Akt and GSK-3β phosphorylation and PSD-95, GluA1, and synapsin immunocontent in the hippocampus of mice ($p < .01$), while no alterations on these proteins were observed in the prefrontal cortex. A single administration of ketamine plus guanosine failed to prevent the reduction on Akt, GSK-3β,

A. Camargo, et al.
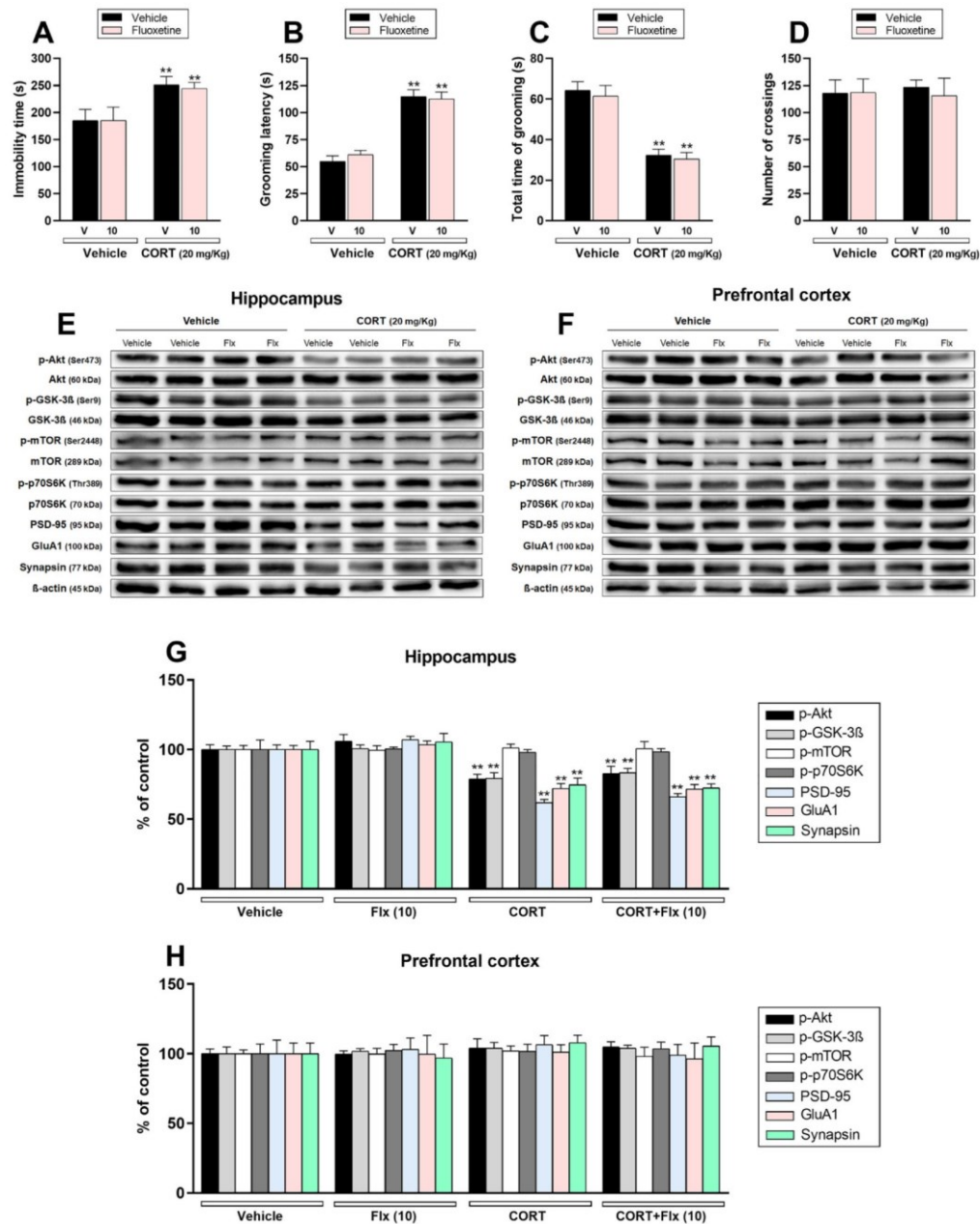
Fig. 3. Effect of repeated treatment with fluoxetine (Flx – 10 mg/kg, p.o.) before the chronic administration with vehicle or corticosterone (CORT – 20 mg/kg, p.o.) on depression-related behaviors and synaptic markers in the hippocampus and prefrontal cortex of mice. a Fluoxetine (10 mg/kg, p.o) administration did not prevent CORT-induced increase in the immobility time in the TST (n = 8). b, c Fluoxetine (10 mg/kg, p.o) administration did not prevent the increase in the grooming latency and reduction in the time spent grooming induced by CORT in the SPT (n = 8). d All groups of mice had comparable number of crossings in the OFT (n = 8). e, f Representative bands of phospho-Akt (Ser^{473}), Akt, phospho-GSK-3β (Ser^9), GSK-3β, phospho-mTOR (Ser^{2448}), mTOR, phospho-p70S6K (Thr^{389}), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the (e) hippocampus and (f) prefrontal cortex of mice. g, h Quantification of these proteins in the (g) hippocampus and (h) prefrontal cortex. Values are expressed as means ± S.E.M (n = 7). ** $p < .01$ as compared with the vehicle-treated group (two-way ANOVA followed by Newman-Keuls *post hoc* test).

Fig. 4. Effect of a single administration with guanosine (Guo – 1 or 5 mg/kg, i.p.) 1 week prior to the administration with vehicle or corticosterone (CORT – 20 mg/kg, p.o.) on depression-related behaviors and synaptic markers in the hippocampus and prefrontal cortex of mice. a Guanosine (1 or 5 mg/kg, p.o) administration did not prevent CORT-induced increase in the immobility time in the TST (n = 8). b, c Guanosine (1 or 5 mg/kg, p.o) administration did not prevent the increase in the grooming latency and reduction in the total time of grooming induced by CORT in the SPT (n = 8). d All groups of mice had comparable number of crossings in the OFT (n = 8). e, f Representative bands of phospho-Akt (Ser[473]), Akt, phospho-GSK-3β (Ser[9]), GSK-3β, phospho-mTOR (Ser[2448]), mTOR, phospho-p70S6K (Thr[389]), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the (e) hippocampus and (f) prefrontal cortex of mice. g, h Quantification of these proteins in the (g) hippocampus and (h) prefrontal cortex. Values are expressed as means ± S.E.M (n = 7). ** $p < .01$ as compared with the vehicle-treated group (two-way ANOVA followed by Newman-Keuls *post hoc* test).

A. Camargo, et al.

Fig. 5. Effect of single administration with a subthreshold dose of ketamine (Ket – 1 mg/kg, i.p.) plus an ineffective dose of guanosine (Guo – 5 mg/kg, p.o.) 1 week before the chronic administration with vehicle or corticosterone (CORT – 20 mg/kg, p.o.) on depression-related behaviors and synaptic markers in the hippocampus and prefrontal cortex of mice. a Ketamine 1 mg/kg, i.p.) plus guanosine (5 mg/kg, p.o) administration did not prevent the increase in the immobility time in the TST induced by CORT (n = 8). b Ketamine 1 mg/kg, i.p.) plus guanosine (5 mg/kg, p.o) administration attenuated CORT-induced increase in the latency to first grooming in the SPT (n = 8). c Ketamine 1 mg/kg, i.p.) plus guanosine (5 mg/kg, p.o) administration did not prevent the reduction in the time spent grooming induced by CORT in the SPT (n = 8). d All groups of mice had comparable number of crossings in the OFT (n = 8). e, f Representative bands of phospho-Akt (Ser473), Akt, phospho-GSK-3β (Ser9), GSK-3β, phospho-mTOR (Ser2448), mTOR, phospho-p70S6K (Thr389), p70S6K, PSD-95, GluA1, synapsin, and β-actin in the (e) hippocampus and (f) prefrontal cortex of mice. g, h Quantification of these proteins in the (g) hippocampus and (h) prefrontal cortex. Values are expressed as means ± S.E.M (n = 7). ** $p < .01$ as compared with the vehicle-treated group; # $p < .05$ as compared with the CORT-treated group (two-way ANOVA followed by Newman-Keuls *post hoc* test).

PSD-95, GluA1, and synapsin induced by CORT.

4. Discussion

This study reinforces and extends the notion that a single administration with a subanesthetic dose of ketamine given before the pharmacological stress protects mice against CORT-induced depressive-like behavior. Additionally, we provide evidence that ketamine's ability in preventing the CORT-induced impairments on synaptogenic markers occurs selectively in the hippocampus of mice, and these findings could be associated with its prophylactic response. We also confirm the previous findings that the repeated fluoxetine administration fails to exert a prophylactic response (Brachman et al., 2016; Chen et al., 2020), and we demonstrated that this drug is also unable to prevent CORT-induced reduction on hippocampal synaptogenic markers. In addition, we have shown that guanosine is not capable of preventing CORT-induced depressive-like behavior and hippocampal synaptic markers impairment, although the combined administration of subthreshold doses of ketamine plus guanosine attenuated the impaired self-care behavior induced by CORT.

The evidence that ketamine may have a prophylactic effect against stress besides producing rapid-onset and long-lasting antidepressant effects has emerged as a possible novel strategy to manage those patients at risk to develop severe MDD (Brachman et al., 2016). However, it remains to be fully resolved whether this prophylactic effect elicited by ketamine translate from mice to humans. In the present study, we extend the investigations regarding the prophylactic effect of ketamine in mice subjected to repeated administration of CORT. This experimental protocol has been postulated to be useful for the study of MDD since it induces behavioral and neurochemical alterations similar to those occurring in patients with this disorder (Sterner and Kalynchuk, 2010; Zhao et al., 2009). Here, we found that CORT administration for 21 days robustly induced a depressive-like behavior, as evidenced by the behavioral alterations in the TST and SPT, in agreement with previous studies (David et al., 2009; Moda-Sava et al., 2019; Rosa et al., 2014; Zhao et al., 2008). Importantly, we showed that a single administration of ketamine (5 mg/kg, i.p.) 1 week before starting the pharmacological stress was effective in preventing the depressive-like behavior, although a lower dose (1 mg/kg, i.p.) that exerts an antidepressant-like response in this model when administered following CORT administration (Neis et al., 2018; Pazini et al., 2016), failed to produce the same effect. The ketamine's prophylactic effect against the behavioral effects elicited by CORT is in agreement with a previous study, which indicated that a single administration of ketamine prevented CORT-induced increase in the grooming latency in the SPT (90 mg/kg, i.p.) and the latency to feed in the NSF test (10 and 90 mg/kg, i.p) in C57BL/6NTac mice (Brachman et al., 2016). Furthermore, the prophylactic efficacy of a low dose of ketamine (3 mg/kg, i.p.) against depressive-like behavior induced by chronic unpredictable stress (14 days) in C57BL/6 J mice subjected to the sucrose preference test was also reported (Krzystyniak et al., 2019). Here, we provide novel evidence that ketamine was effective in preventing the increase in the immobility time in the TST and reduction in the total time of grooming in the SPT induced by CORT administration in *Swiss* mice, reinforcing the notion that ketamine may be a useful prophylactic strategy in at-risk populations. The present study also provides additional evidence that fluoxetine (10 mg/kg, p.o.) administration for 3 weeks before stress did not protect against CORT-induced depressive-like behavior in *Swiss* mice subjected to the TST and SPT. These results are in agreement with previous studies reporting that repeated fluoxetine (18 mg/kg, p.o.) treatment was ineffective to elicit a prophylactic effect against CORT in the SPT and NSF test in C57BL/6NTac mice (Brachman et al., 2016; Chen et al., 2020).

Due to recent reports showing that ketamine may affect the purinergic system and that guanosine shares some common molecular targets with ketamine (Almeida et al., 2020; Camargo et al., 2019;

McGowan et al., 2018), we next investigated whether guanosine would be able to afford prophylactic response against the CORT-induced depressive-like behavior. Guanosine serum levels were shown to be reduced in patients with MDD, reinforcing the notion that this nucleoside may play a role in the pathophysiology of this disorder (Ali-Sisto et al., 2016). However, as opposed to the actions displayed by ketamine, a single administration of guanosine (1 or 5 mg/kg, p.o.) given 1 week before the pharmacological stress was unable to prevent CORT-induced depressive-like behavior. Thus, one may speculate that this nucleoside could be only effective to produce fast (Almeida et al., 2020; Camargo and Rodrigues, 2019), but not long-term responses against stress. Our research group demonstrated that guanosine potentiates the antidepressant-like effect of a subthreshold dose of ketamine in the NSF test and TST in the vehicle- and CORT-administered mice (Camargo et al., 2019; Camargo et al., 2020a; Camargo et al., 2020b). Thus, we next investigated the ability of a single administration with an ineffective dose of guanosine (5 mg/kg, p.o.) in combination with a subthreshold dose of ketamine (1 mg/kg, p.o.) to elicit a prophylactic response. We found that ketamine plus guanosine treatment partially prevented the reduced self-care behavior evoked by CORT, although it did not prevent the CORT-induced behavioral despair and anhedonic-like behavior. However, additional studies are necessary to ascertain the long-lasting beneficial effects of this combined treatment in self-care-related responses. Furthermore, this combined strategy was not capable of exerting the full spectrum of ketamine's prophylactic actions, highlighting the necessity to understand the mechanisms of action associated with its long-lasting effect.

Despite the numerous studies investigating the mechanisms of ketamine's antidepressant effects, few studies have focused on the mechanisms underlying the prophylactic effect of this drug. The available mechanistic studies provide evidence that prophylactic ketamine treatment may act by modulating the neural activity (Dolzani et al., 2018; Mastrodonato et al., 2018) and promoting synaptic plasticity in the hippocampus and prefrontal cortex (Krzystyniak et al., 2019) as well as modulating the immune system (Mastrodonato et al., 2020), effects likely associated with its prophylactic response. Furthermore, the antidepressant-like effect, but not the prophylactic effect of ketamine was associated with its ability to increase pro-neurogenic markers (LaGamma et al., 2018). Regarding this issue, the ketamine's prophylactic effect in socially defeated 129S6/SvEvTac mice was associated with increased ΔFosB expression, a transcriptional regulator of synaptic plasticity, in the prefrontal cortex and hippocampus, whereas ΔFosB transcriptional silencing in the hippocampal CA3 area inhibited the ketamine's behavioral response (Mastrodonato et al., 2018). Additionally, ketamine's prophylactic effect against chronic unpredictable stress in C57BL/6 J mice was associated with an increase in dendritic spines density in the prefrontal cortex and hippocampal CA1 and CA3 areas (Krzystyniak et al., 2019), but the signaling pathways implicated in these effects remain to be determined.

To provide some insight into the molecular targets underpinning the prophylactic effect of ketamine, particularly whether these targets are similar or divergent from its antidepressant response, we investigate if mTORC1-driven long-lasting synaptogenic signaling could be associated with ketamine's prophylactic effect against CORT. Here, we found that CORT robustly reduced the phosphorylation of Akt (Ser^{473}), and its downstream target GSK-3β (Ser^9) in the hippocampus, but not in the prefrontal cortex, while no alterations were detected on mTOR (Ser^{2448})/p70S6K (Thr^{389}) phosphorylation in both structures. Additionally, CORT downregulated PSD-95, GluA1, and synapsin immunocontent in the hippocampus, but not in the prefrontal cortex, suggesting a synaptogenic deficit. These results are partially in line with prior reports showing the distinct effects of CORT administration and chronic stress on mTORC1-related targets in the hippocampus and prefrontal cortex (Freitas et al., 2016; Li et al., 2011; Pazini et al., 2016; Zhu et al., 2018). Particularly, it has been shown that CORT protocol reduced mTOR phosphorylation and synaptic proteins content in the

hippocampus of mice without affecting p70S6K (Thr[389]) (Freitas et al., 2016; Pazini et al., 2016), whereas chronic stress decreased Akt, mTOR, and p70S6K phosphorylation (Zhu et al., 2018) and synaptic proteins levels (Li et al., 2011) in the prefrontal cortex of mice and rats, respectively. The exact reasons for these discrepancies are unclear but considering that the hippocampus expresses a high density of glucocorticoid receptors, this aspect could make this brain region more vulnerable to the deleterious effects of CORT administration rather than the prefrontal cortex (Lee et al., 2002). In addition, these neurochemical divergences may also be attributable to mouse strains differences or testing conditions (Jacobson and Cryan, 2007; Lathe, 2004).

Our results unveil that CORT-induced reduction on PSD-95, GluA1, and synapsin immunocontent was completely prevented by a single administration of ketamine. These results suggest that ketamine is effective in preventing CORT-induced synaptic impairments in the hippocampus and this effect could be associated with its behavioral prophylactic effect. Reinforcing this assumption, repeated fluoxetine treatment or a single administration of guanosine, which had no behavioral prophylactic effect, was not capable of preventing CORT-induced synaptic markers impairment in the hippocampus, indicating that this effect was selectively exerted by ketamine. Therefore, one may speculate that ketamine-induced long-lasting pro-synaptogenic effect in the hippocampus could make this brain region less prone to CORT-induced synaptic impairment and could enhance the resilience of mice against the detrimental effects evoked by this hormone. However, no preventive effect was observed concerning the reduced Akt and GSK-3β phosphorylation in CORT-treated mice. Considering that ketamine affects rapidly and transiently the phosphorylation of Akt and GSK-3β (Beurel et al., 2011; Li et al., 2010; Liu et al., 2013), we cannot rule out that these targets may also be associated with its prophylactic effect in earlier time points. It is worth noting that the combined administration of subthreshold doses of ketamine and guanosine, which partially prevented the reduced self-care behavior, did not prevent CORT-induced detrimental effects on hippocampal PSD-95, GluA1, and synapsin immunocontent, possibly because of the subtle behavioral response, not enough to impact the pro-synaptogenic signaling. However, further investigations are necessary to understand the possible preventive effect triggered by ketamine plus guanosine treatment and the mechanisms associated with this response.

5. Conclusions

Taken together, our results reinforce the notion that a single administration of ketamine robustly exerts a prophylactic effect against CORT administration, and provides evidence that this behavioral response is associated with the stimulation of long-lasting pro-synaptogenic signaling pathway, specifically in the hippocampus. Moreover, we showed that a single administration with subthreshold doses of ketamine plus guanosine partially prevented CORT-induced reduction on self-care behavior possibly conferring a subtle stress resilience that deserves further investigation. The prophylactic effect of ketamine could have therapeutic relevance as a potential novel strategy to manage those patients at risk to develop severe MDD and suicide, *i.e.*, individuals under high-stress conditions. Particularly in the COVID-19 pandemic, medical health workers or people in confinement with psychiatric disorders are good examples of a predictably at-risk patient population (De Berardis et al., 2017; Pappa et al., 2020; Tang et al., 2020).

Nonetheless, this research has important caveats that need to be considered. Taking into account that the causal relationship between mTORC1-driven signaling and behavioral/pro-synaptogenic outcomes elicited by ketamine's prophylactic treatment was not addressed herein, additional experiments using rapamycin (mTORC1 inhibitor) could be undertaken to ascertain this issue. Furthermore, considering that the stimulation of the pro-synaptogenic pathway may occur independently of mTORC1 stimulation, further experiments using general inhibitors of

protein synthesis (such as anisomycin) would give some insight into this issue. Finally, although a single administration of ketamine upregulated the pro-synaptogenic markers (PSD-95, GluA1, and synapsin) in the hippocampus of CORT-treated mice after 4 weeks, the morphological evaluation of spinogenesis and synaptogenesis was not addressed in the present study. Despite these limitations, the data reported herein suggest that targeting the long-lasting pro-synaptogenic signaling pathway can induce a resilience-enhancing effect, protecting against stress-related disorders.

Declaration of Competing Interest

All the authors declare that have no biomedical financial interests or conflict of interest in this study.

References

Abdallah, C.G., Adams, T.G., Kelmendi, B., Esterlis, I., Sanacora, G., Krystal, J.H., 2016. Ketamine's mechanism of action: a path to rapid-acting antidepressants. Depress. Anxiety 33, 689–697. https://doi.org/10.1002/da.22501.

Ali-Sisto, T., Tolmunen, T., Toffol, E., Viinamäki, H., Mäntyselkä, P., Valkonen-Korhonen, M., Honkalampi, K., Ruusunen, A., Velagapudi, V., Lehto, S.M., 2016. Purine metabolism is dysregulated in patients with major depressive disorder. Psychoneuroendocrinology 70, 25–32. https://doi.org/10.1016/j.psyneuen.2016.04.017.

Almeida, R.F., Pocharski, C.B., Rodrigues, A.L.S., Elisabetsky, E., Souza, D.O., 2020. Guanosine fast onset antidepressant-like effects in the olfactory bulbectomy mice model. Sci. Rep. 10, 8429. https://doi.org/10.1038/s41598-020-65300-w.

Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatry 47, 351–354. https://doi.org/10.1016/S0006-3223(99)00230-9.

Bettio, L.E.B., Cunha, M.P., Budni, J., Pazini, F.L., Oliveira, Á., Colla, A.R., Rodrigues, A.L.S., 2012. Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. Behav. Brain Res. 234, 137–148. https://doi.org/10.1016/j.bbr.2012.06.021.

Bettio, L.E.B., Gil-Mohapel, J., Rodrigues, A.L.S., 2016. Guanosine and its role in neuropathologies. Purinergic Signal 12, 411–426. https://doi.org/10.1007/s11302-016-9509-4.

Beurel, E., Song, L., Jope, R.S., 2011. Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. Mol. Psychiatry 16, 1068–1070. https://doi.org/10.1038/mp.2011.47.

Brachman, R.A., McGowan, J.C., Perusini, J.N., Lim, S.C., Pham, T.H., Faye, C., Gardier, A.M., Mendez-David, I., David, D.J., Hen, R., Denny, C.A., 2016. Ketamine as a prophylactic against stress-induced depressive-like behavior. Biol. Psychiatry 79, 776–786. https://doi.org/10.1016/j.biopsych.2015.04.022.

Camargo, A., Rodrigues, A.L.S., 2019. Novel targets for fast antidepressant responses: possible role of endogenous neuromodulators. Chronic Stress 3, 247054701985808. https://doi.org/10.1177/2470547019858083.

Camargo, A., Pazini, F.L., Rosa, J.M., Wolin, I.A.V., Moretti, M., Rosa, P.B., Neis, V.B., Rodrigues, A.L.S., 2019. Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway. J. Psychiatr. Res. 115, 103–112. https://doi.org/10.1016/j.jpsychires.2019.05.017.

Camargo, A., Dalmagro, A.P., Zeni, A.L.B., Rodrigues, A.L.S., 2020a. Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: possible role of pro-synaptogenic signaling pathway. J. Affect. Disord. 271, 100–108. https://doi.org/10.1016/j.jad.2020.03.186.

Camargo, A., Dalmagro, A.P., Rosa, J.M., Zeni, A.L.B., Kaster, M.P., Tasca, C.I., Rodrigues, A.L.S., 2020b. Subthreshold doses of guanosine plus ketamine elicit antidepressant-like effect in a mouse model of depression induced by corticosterone: role of GR/NF-κB/IDO-1 signaling. Neurochem. Int. 139, 104797. https://doi.org/10.1016/j.neuint.2020.104797.

Chen, B.K., Mendez-David, I., Luna, V.M., Faye, C., Gardier, A.M., David, D.J., Denny, C.A., 2020. Prophylactic efficacy of 5-HT4R agonists against stress. Neuropsychopharmacology 45, 542–552. https://doi.org/10.1038/s41386-019-

0540-3.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.P., Artymyshyn, R.P., Gardier, A.M., Gerald, C., Antonijevic, I.A., Leonardo, E.D., Hen, R., 2009. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62, 479–493. https://doi.org/10.1016/j.neuron.2009.04.017.

De Berardis, D., Fornaro, M., Orsolini, L., Valchera, A., Carano, A., Vellante, F., Perna, G., Serafini, G., Gonda, X., Pompili, M., Martinotti, G., Di Giannantonio, M., 2017. Alexithymia and suicide risk in psychiatric disorders: a mini-review. Front. Psychiatry 14, 1–6. https://doi.org/10.3389/fpsyt.2017.00148.

Di Liberto, V., Mudò, G., Garozzo, R., Frinchi, M., Fernandez-Dueñas, V., Di Iorio, P., Ciccarelli, R., Caciagli, F., Condorelli, D.F., Ciruela, F., Belluardo, N., 2016. The guanine-based purinergic system: the tale of an orphan neuromodulation. Front. Pharmacol. 7, 1–15. https://doi.org/10.3389/fphar.2016.00158.

DiazGranados, N., Ibrahim, L., Brutsche, N., Ameli, R., Henter, L., Luckenbaugh, D., Machado-Vieira, R., Zarate, C.A., 2010. Rapid resolution of suicidal ideation after a single infusion of an NMDA antagonist in patients with treatment-resistant major depressive disorder. J. Clin. Psychiatry 71, 1605–1611. https://doi.org/10.4088/JCP.09m05327blu.

Dolzani, S.D., Baratta, M.V., Moss, J.M., Leslie, N.L., Tilden, S.G., Sørensen, A.T., Watkins, L.R., Lin, Y., Maier, S.F., 2018. Inhibition of a descending prefrontal circuit prevents ketamine-induced stress resilience in females. eNeuro 5, 1–18. https://doi.org/10.1523/ENEURO.0025-18.2018.

Freitas, A.E., Egea, J., Buendia, I., Gómez-Rangel, V., Parada, E., Navarro, E., Casas, A.I., Wojnicz, A., Ortiz, J.A., Cuadrado, A., Ruiz-Nuño, A., Rodrigues, A.L.S., Lopez, M.G., 2016. Agmatine, by improving neuroplasticity markers and inducing Nrf2, prevents corticosterone-induced depressive-like behavior in mice. Mol. Neurobiol. 53, 3030–3045. https://doi.org/10.1007/s12035-015-9182-6.

Goodyer, I.M., Herbert, J., Tamplin, A., Altham, P.M.E., 2000. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. Br. J. Psychiatry 177, 499–504. https://doi.org/10.1192/bjp.177.6.499.

Harris, T.O., Borsanyi, S., Messari, S., Stanford, K., Cleary, S.E., Shiers, H.M., Brown, G.W., Herbert, J., 2000. Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. Br. J. Psychiatry 177, 505–510. https://doi.org/10.1192/bjp.177.6.505.

Jacobson, L.H., Cryan, J.F., 2007. Feeling strained? Influence of genetic background on depression-related behavior in mice: a review. Behav. Genet. 37, 171–213. https://doi.org/10.1007/s10519-006-9106-3.

Kaster, M.P., Moretti, M., Cunha, M.P., Rodrigues, A.L.S., 2016. Novel approaches for the management of depressive disorders. Eur. J. Pharmacol. 771, 236–240. https://doi.org/10.1016/j.ejphar.2015.12.029.

Krzystyniak, A., Baczynska, E., Magnowska, M., Antoniuk, S., Roszkowska, M., Zareba-Koziol, M., Das, N., Basu, S., Pikula, M., Wlodarczyk, J., 2019. Prophylactic ketamine treatment promotes resilience to chronic stress and accelerates recovery: correlation with changes in synaptic plasticity in the CA3 subregion of the hippocampus. Int. J. Mol. Sci. 20, 1–15. https://doi.org/10.3390/ijms20071726.

LaGamma, C.T., Tang, W.W., Morgan, A.A., McGowan, J.C., Brachman, R.A., Denny, C.A., 2018. Antidepressant but not prophylactic ketamine administration alters calretinin and calbindin expression in the ventral hippocampus. Front. Mol. Neurosci. 11, 1–14. https://doi.org/10.3389/fnmol.2018.00404.

Lathe, R., 2004. The individuality of mice. Genes Brain Behav. 3, 317–327. https://doi.org/10.1111/j.1601-183X.2004.00083.x.

Leal, R.B., Lopes, M.W., Formolo, D.A., de Carvalho, C.R., Hoeller, A.A., Latini, A., Sousa, D.S., Wolf, P., Prediger, R.D., Bortolotto, Z.A., Linhares, M.N., Lin, K., Walz, R., 2020. Amygdala levels of the GluA1 subunit of glutamate receptors and its phosphorylation state at serine 845 in the anterior hippocampus are biomarkers of ictal fear but not anxiety. Mol. Psychiatry 25, 655–665. https://doi.org/10.1038/s41380-018-0084-7.

Lee, A.L., Ogle, W.O., Sapolsky, R.M., 2002. Stress and depression: possible links to neuron death in the hippocampus. Bipolar Disord. 4, 117–128. https://doi.org/10.1034/j.1399-5618.2002.01144.x.

Li, N., Lee, B., Liu, R.-J., Banasr, M., Dwyer, J.M., Iwata, M., Li, X.-Y., Aghajanian, G., Duman, R.S., 2010. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. Science 329, 959–965. https://doi.org/10.1126/science.1190287.

Li, N., Liu, R.J., Dwyer, J.M., Banasr, M., Lee, B., Son, H., Li, X.Y., Aghajanian, G., Duman, R.S., 2011. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. Biol. Psychiatry 69, 754–761. https://doi.org/10.1016/j.biopsych.2010.12.015.

Liu, R.J., Fuchikami, M., Dwyer, J.M., Lepack, A.E., Duman, R.S., Aghajanian, G.K., 2013. GSK-3 inhibition potentiates the synaptogenic and antidepressant-like effects of subthreshold doses of ketamine. Neuropsychopharmacology 38, 2268–2277. https://doi.org/10.1038/npp.2013.128.

Mastrodonato, A., Martinez, R., Pavlova, I.P., LaGamma, C.T., Brachman, R.A., Robison, A.J., Denny, C.A., 2018. Ventral CA3 activation mediates prophylactic ketamine efficacy against stress-induced depressive-like behavior. Biol. Psychiatry 84, 846–856. https://doi.org/10.1016/j.biopsych.2018.02.011.

Mastrodonato, A., Cohensedgh, O., LaGamma, C.T., McGowan, J.C., Hunsberger, H.C., Denny, C.A., 2020. Prophylactic (R,S)-ketamine selectively protects against inflammatory stressors. Behav. Brain Res. 378, 112238. https://doi.org/10.1016/j.bbr.2019.112238.

McGowan, J.C., Hill, C., Mastrodonato, A., Lagamma, C.T., Kitayev, A., Brachman, R.A., Narain, N.R., Kiebish, M.A., Denny, C.A., 2018. Prophylactic ketamine alters nucleotide and neurotransmitter metabolism in brain and plasma following stress. Neuropsychopharmacology 43, 1813–1821. https://doi.org/10.1038/s41386-018-0043-7.

Moda-Sava, R.N., Murdock, M.H., Parekh, P.K., Fetcho, R.N., Huang, B.S., Huynh, T.N., Witztum, J., Shaver, D.C., Rosenthal, D.L., Alway, E.J., Lopez, K., Meng, Y., Nellissen,

L., Grosenick, L., Milner, T.A., Deisseroth, K., Bito, H., Kasai, H., Liston, C., 2019. Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. Science 364, 1–11. https://doi.org/10.1126/science.aat8078.

Moretti, M., Colla, A., De Oliveira Balen, G., Dos Santos, D.B., Budni, J., De Freitas, A.E., Farina, M., Rodrigues, A.L.S., 2012. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. J. Psychiatr. Res. 46, 331–340. https://doi.org/10.1016/j.jpsychires.2011.11.009.

Neis, V.B., Bettio, L.B., Moretti, M., Rosa, P.B., Olescowicz, G., Fraga, D.B., Goncalves, F.M., Freitas, A.E., Heinrich, I.A., Lopes, M.W., Leal, R.B., Rodrigues, A.L.S., 2018. Single administration of agmatine reverses the depressive-like behavior induced by corticosterone in mice: comparison with ketamine and fluoxetine. Pharmacol. Biochem. Behav. 173, 44–50. https://doi.org/10.1016/j.pbb.2018.08.005.

Otte, C., Gold, S., Penninx, B., Pariante, C., Etkin, A., Fava, M., Mohr, D., Schatzberg, A., 2016. Major depressive disorder. Nat. Rev. Dis. Primers 2, 1–20. https://doi.org/10.1038/nrdp.2016.65.

Papakostas, G.I., Ionescu, D.F., 2015. Towards new mechanisms: an update on therapeutics for treatment-resistant major depressive disorder. Mol. Psychiatry 20, 1142–1150. https://doi.org/10.1038/mp.2015.92.

Pappa, S., Ntella, V., Giannakas, T., Giannakoulis, V.G., Papoutsi, E., Katsaounou, P., 2020. Prevalence of depression, anxiety, and insomnia among healthcare workers during the COVID-19 pandemic: a systematic review and meta-analysis. Brain Behav. Immun. 88, 901–907. https://doi.org/10.1016/j.bbi.2020.05.026.

Pazini, F.L., Cunha, M.P., Rosa, J.M., Colla, A.R.S., Lieberknecht, V., Oliveira, Á., Rodrigues, A.L.S., 2016. Creatine, similar to ketamine, counteracts depressive-like behavior induced by corticosterone via PI3K/Akt/mTOR pathway. Mol. Neurobiol. 53, 6818–6834. https://doi.org/10.1007/s12035-015-9580-9.

Peterson, G.L., 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83, 346–356. https://doi.org/10.1016/0003-2697(77)90043-4.

Pompili, M., Serafini, G., Innamorati, M., Venturini, P., Fusar-Poli, P., Sher, L., Amore, M., Girardi, P., 2013. Agomelatine, a novel intriguing antidepressant option enhancing neuroplasticity: a critical review. World J. Biol. Psychiatry 14, 412–431. https://doi.org/10.3109/15622975.2013.765593.

Price, R.B., Nock, M.K., Charney, D.S., Mathew, S.J., 2009. Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression. Biol. Psychiatry 66, 522–526. https://doi.org/10.1016/j.biopsych.2009.04.029.

Rodrigues, A.L.S., Rocha, J.B.T., Mello, C.F., Souza, D.O., 1996. Effect of perinatal lead exposure on rat behaviour in open-field and two-way avoidance tasks. Pharmacol. Toxicol. 79, 150–156. https://doi.org/10.1111/j.1600-0773.1996.tb00259.x.

Rosa, P.B., Ribeiro, C.M., Bettio, L.E.B., Colla, A., Lieberknecht, V., Moretti, M., Rodrigues, A.L.S., 2014. Folic acid prevents depressive-like behavior induced by chronic corticosterone treatment in mice. Pharmacol. Biochem. Behav. 127, 1–6. https://doi.org/10.1016/j.pbb.2014.10.003.

Sarkar, A., Kabbaj, M., 2016. Sex differences in effects of ketamine on behavior, spine density, and synaptic proteins in socially isolated rats. Biol. Psychiatry 80, 448–456. https://doi.org/10.1016/j.biopsych.2015.12.025.

Sterner, E.Y., Kalynchuk, L.E., 2010. Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: relevance to depression. Prog. Neuro Psychopharmacol. Biol. Psychiatry 34, 777–790. https://doi.org/10.1016/j.pnpbp.2010.03.005.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370. https://doi.org/10.1007/BF00428203.

Tang, W., Hu, T., Yang, L., Xu, J., 2020. The role of alexithymia in the mental health problems of home-quarantined university students during the COVID-19 pandemic in China. Personal. Individ. Differ. 165, 110131. https://doi.org/10.1016/j.paid.2020.110131.

Tasca, C.I., Lanznaster, D., Oliveira, K.A., Fernández-Dueñas, V., Ciruela, F., 2018. Neuromodulatory effects of guanine-based purines in health and disease. Front. Cell. Neurosci. 12, 1–14. https://doi.org/10.3389/fncel.2018.00376.

Wei, Y., Chang, L., Hashimoto, K., 2020. A historical review of antidepressant effects of ketamine and its enantiomers. Pharmacol. Biochem. Behav. 190, 172870. https://doi.org/10.1016/j.pbb.2020.172870.

Willner, P., 2005. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 52, 90–110. https://doi.org/10.1159/000087097.

World Health Organization, 2017. Depression and Other Common Mental Disorders: Global Health Estimates. World Health Organization, pp. 1–24 (doi:CC BY-NC-SA 3.0 IGO).

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatry 63, 856–864. https://doi.org/10.1001/archpsyc.63.8.856.

Zeni, A.L.B., Camargo, A., Dalmagro, A.P., 2019. Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities. Pharmacol. Biochem. Behav. 179, 63–72. https://doi.org/10.1016/j.pbb.2019.02.004.

Zhao, Y., Ma, R., Shen, J., Su, H., Xing, D., Du, L., 2008. A mouse model of depression induced by repeated corticosterone injections. Eur. J. Pharmacol. 581, 113–120. https://doi.org/10.1016/j.ejphar.2007.12.005.

Zhao, Y., Xie, W., Dai, J., Wang, Z., Huang, Y., 2009. The varying effects of short-term and long-term corticosterone injections on depression-like behavior in mice. Brain Res. 1261, 82–90. https://doi.org/10.1016/j.brainres.2008.12.083.

Zhu, J.-X., Shan, J.-L., Hu, W.-Q., Zeng, J.-X., Shu, J.-C., 2018. Gallic acid activates hippocampal BDNF-Akt-mTOR signaling in chronic mild stress. Metab. Brain Dis. 34, 93–101. https://doi.org/10.1007/s11011-018-0328-x.

5 DISCUSSÃO GERAL

O presente estudo demonstra que a coadministração única com doses sub-efetivas de cetamina e guanosina, que não produzem efeito quando administradas isoladamente, é capaz de provocar um efeito tipo-antidepressivo rápido, mas não sustentado, em camundongos. É importante ressaltar que este efeito comportamental foi acompanhado por uma ativação rápida e transitória da via de sinalização mediada por mTORC1 com consequente aumento na síntese de proteínas sinápticas e na densidade de espinhos dendríticos de maneira tempo-dependente no hipocampo e córtex pré-frontal. Além disso, a combinação de doses sub-efetivas de cetamina e guanosina é eficaz em reverter rapidamente o comportamento tipo-depressivo e disfunções na síntese de proteínas sinápticas no hipocampo induzidos pela administração crônica com corticosterona, um efeito dependente da ativação de mTORC1. Por fim, demonstramos que uma única administração profilática de cetamina, mas não de guanosina ou associação de doses sub-efetivas de cetamina e guanosina, é capaz de prevenir o comportamento tipo-depressivo e disfunções na síntese de proteínas sinápticas no hipocampo de camundongos submetidos à administração crônica de corticosterona.

A descoberta de que a cetamina, um antagonista do receptor NMDA, é eficaz em produzir ações antidepressivas rápidas e sustentadas configurou-se como um avanço substancial no desenvolvimento da farmacoterapia do TDM. Tem sido demonstrado que a cetamina exerce respostas antidepressivas por promover o aumento na formação de espinhos dendríticos e sinaptogênese, efeitos associados com a ativação da via de sinalização mediada por mTORC1 (FRAGA et al., 2020, 2021; LI et al., 2010, 2011). Neste sentido, demonstrou-se que diversos moduladores do receptor NMDA, tais como o AZD-6765 (NEIS et al., 2020), GLYX-13 (LIU et al., 2016), Ro 25-6981 (LI et al., 2010) e o NVP-AAM077 (GORDILLO-SALAS et al., 2018) são eficazes em exercer uma resposta tipo-antidepressiva rápida por um mecanismo de ação também dependente da sinalização intracelular mediada por mTORC1. Suportando esses achados, evidências também mostraram que distúrbios nesta via de sinalização e na conectividade sináptica foram observados no córtex pré-frontal e hipocampo de indivíduos diagnosticados com TDM (DURIC et al., 2013; HOLMES et al., 2019; JERNIGAN et al., 2011; KANG et al., 2012). Esses achados reforçam a noção de que a ativação da via de sinalização mediada por mTORC1 com consequente aumento da plasticidade sináptica no hipocampo e córtex pré-frontal podem estar subjacentes a respostas antidepressivas de início rápido e de longa duração. Contudo, além dos moduladores seletivos do receptor

NMDA, há um crescente conjunto de evidências mostrando que os moduladores glutamatérgicos endógenos podem produzir efeito tipo-antidepressivo rápido e até mesmo aumentar as ações antidepressivas da cetamina por estimular a sinalização mediada por mTORC1 (ALMEIDA et al., 2020; CAMARGO et al., 2019; CUNHA et al., 2016; FREITAS et al., 2020; NEIS et al., 2016; PAZINI et al., 2016; ROSA et al., 2021).

Estudos recentes demonstraram que a guanosina apresenta mecanismos de ação sobrepostos à cetamina e, portanto, poderia ser um novo candidato para produzir respostas tipo-antidepressivas rápidas ou potencializar as ações da cetamina (CAMARGO; RODRIGUES, 2019). Em particular, o efeito tipo-antidepressivo da guanosina parece envolver a modulação dos receptores NMDA e AMPA e a ativação dos CCVD, de forma semelhante à cetamina (BETTIO et al., 2012; ROSA et al., 2021). Vale ressaltar que a liberação de BDNF e a ativação do receptor TrkB também parecem ser necessários para o efeito tipo-antidepressivo da guanosina no teste de suspensão pela cauda (ROSA et al., 2021). Além disso, a administração aguda de guanosina aumenta os níveis de BDNF no hipocampo e no córtex pré-frontal de camundongos (ROSA et al., 2021). Reforçando a noção de que a cetamina e a guanosina compartilham respostas comportamentais e alvos moleculares comuns, o efeito tipo-antidepressivo da guanosina também está associado a ativação da via de sinalização PI3K/Akt/GSK-3β/mTORC1 e aumento da fosforilação de p70S6K e do imunoconteúdo de sinapsina no hipocampo de camundongos (BETTIO et al., 2012; ROSA et al., 2021). Destaca-se ainda que uma única administração de guanosina foi capaz de reverter o comportamento tipo-depressivo induzido pela bulbectomia olfatória em camundongos, um efeito dependente da ativação de mTORC1 (ALMEIDA et al., 2020). A administração aguda de guanosina, de forma semelhante à cetamina, apresentou um efeito tipo-antidepressivo no teste da alimentação suprimida pela novidade (ROSA et al., 2021), um paradigma comportamental sensível a uma única administração de agentes antidepressivos com efeito rápido (PAZINI et al., 2020). Além disso, também reportou-se que guanosina é capaz de aumentar o efeito tipo-antidepressivo de doses sub-efetivas de cetamina neste teste, por um mecanismo dependente de mTORC1 (CAMARGO et al., 2019). Considerando este conjunto de evidências, esta estratégia de administração combinada de cetamina e guanosina merece uma investigação mais detalhada em relação aos efeitos comportamentais e mecanismos subjacentes.

No primeiro capítulo desta tese, analisou-se o possível efeito tipo-antidepressivo desencadeado pela administração de doses sub-efetivas de cetamina e guanosina no teste de suspensão pela cauda. Além disso, avaliou-se se esta estratégia seria eficaz em modular a síntese

de proteínas sinápticas via sinalização de mTORC1 no hipocampo e córtex pré-frontal de camundongos. Desta forma, um primeiro conjunto de experimentos foi realizado para obtenção das doses ativas e sub-efetivas de cetamina e guanosina no teste de suspensão pela cauda. Os resultados mostraram que a administração aguda de guanosina (0,05 e 1 mg/kg, p.o.) exerce um efeito tipo-antidepressivo no teste de suspensão pela cauda em camundongos, sem afetar o desempenho locomotor no teste do campo aberto, conforme demonstrado anteriormente (BETTIO et al., 2012; ROSA et al., 2021). Além disso, de acordo com dados da literatura (LUDKA et al., 2013), mostramos que a cetamina foi eficaz em reduzir o tempo de imobilidade no teste de suspensão pela cauda nas doses de 1 e 5 mg/kg. Além disso, a coadministração aguda com doses sub-efetivas de cetamina (0,1 mg/kg, i.p.) e guanosina (0,01 mg/kg, p.o.) foi eficaz em produzir um efeito tipo-antidepressivo no teste de suspensão pela cauda, em concordância com um resultado anterior (BETTIO et al., 2012). Em seguida, decidimos avaliar os mecanismos associados ao efeito tipo-antidepressivo produzido pela administração combinada de cetamina e guanosina em regiões encefálicas que estão subjacentes a respostas antidepressivas de ação rápida, em particular o hipocampo e o córtex pré-frontal. Particularmente, investigamos se o efeito tipo-antidepressivo estaria associado a estimulação da via de sinalização mediada por mTORC1 e a síntese de proteínas sinápticas *downstream*. De especial interesse, avaliamos como o tratamento combinado com cetamina e guanosina afetaria o hipocampo e o córtex pré-frontal, especificamente se padrões distintos ou mesmo inversos na sinalização de mTORC1 poderiam ocorrer nessas regiões encefálicas após a coadministração desses compostos.

Embora a hipótese de que a ativação da via de sinalização intracelular mediada pela mTORC1 para explicar o efeito rápido da cetamina tenha sido questionada (ABDALLAH et al., 2020; POPP et al., 2016), um grande conjunto de evidências demonstrou que a formação de sinapses dependente de mTORC1 é um alvo substancial para o efeito de agentes antidepressivos de ação rápida (ABDALLAH; AVERILL; KRYSTAL, 2015; DUMAN et al., 2012). Neste estudo, fornecemos evidências de que uma administração única com doses sub-efetivas de cetamina e guanosina aumentou a fosforilação de mTORC1 (Ser^{2448}) e p70S6K (Thr^{389}) no hipocampo, mas não no pré-frontal córtex (1 h após a administração de guanosina e 30 min após a cetamina). Por outro lado, foi detectado um aumento no imunoconteúdo de PSD-95 e GluA1 no córtex pré-frontal, mas não no hipocampo, após a associação de cetamina e guanosina. É importante destacar que este padrão regional distinto de ativação da via de sinalização pró-sinaptogênica mediada por mTORC1 após o tratamento combinado de cetamina

e guanosina foi observado pela primeira vez no presente estudo. De fato, o hipocampo e o córtex pré-frontal têm características intrínsecas, que inclui diferenças na organização anatômica, tipos celulares, e atividade neural (LIU et al., 2017), aspectos que podem contribuir para respostas diferenciais. Portanto, considerando os dados aqui apresentados, pode-se supor que os efeitos neuroquímicos dependentes da ativação de mTORC1 poderiam ocorrer mais rapidamente no córtex pré-frontal, uma vez que um aumento no imunoconteúdo de proteínas sinápticas alvos de mTORC1 foi observado nesta região após o tratamento combinado com cetamina e guanosina, enquanto no hipocampo, um evento *upstream* à síntese de proteínas sinápticas (fosforilação mTORC1/p70S6K) foi detectado.

No segundo capítulo desta tese, foi determinado se a guanosina é eficaz em potencializar o efeito tipo-antidepressivo e pró-sinaptogênico rápido e sustentado desencadeados pela cetamina, bem como o papel da via de sinalização mediada por mTORC1 e da formação de espinhos dendríticos nesta resposta. Os resultados deste capítulo reforçaram a noção de que uma única administração com uma dose sub-efetiva de guanosina é capaz de potencializar o efeito tipo-antidepressivo de uma dose sub-efetiva de cetamina no teste de suspensão pela cauda. Contudo, demonstramos que essa resposta ocorreu de maneira tempo-dependente, isto é, iniciando 1 hora após o tratamento e mantendo-se por até 24 h, mas não por 7 dias. Um estudo anterior mostrou que a combinação de doses sub-efetivas de cetamina e lítio diminuiu significativamente o tempo de imobilidade em ratos submetidos ao teste do nado forçado, um efeito que foi observado em apenas 30 min e perdurou por 7 dias (LIU et al., 2013). Além disso, a coadministração única com doses sub-efetivas de cetamina e agmatina, outro modulador glutamatérgico endógeno, produziu um efeito tipo-antidepressivo rápido (1 h e 24 h) e sustentado (7 dias) em camundongos submetidos ao teste de suspensão pela cauda (FREITAS et al., 2020). Entretanto, diferentemente desses estudos que relataram um efeito sustentado após 7 dias com a associação de cetamina e agmatina ou lítio (FREITAS et al., 2020; LIU et al., 2013), ou mesmo um estudo que investigou o efeito sustentado da cetamina *per se* (AUTRY et al., 2011), o tratamento combinado com doses sub-efetivas de cetamina e guanosina foi apenas eficaz em produzir uma resposta tipo-antidepressiva rápida (1 h – 24 h), mas não sustentada (7 dias). Embora a combinação com doses sub-efetivas de cetamina e guanosina tenha sido apenas efetiva em produzir uma resposta tipo-antidepressiva 1 h e 24 h após o tratamento, é importante destacar que os antidepressivos convencionais, mediante uma única administração, não são capazes de produzir tal resposta (FRAGA et al., 2021; NEIS et al., 2018; PAZINI et al., 2016).

Subsequentemente, decidimos ampliar o conhecimento acerca do papel da via de sinalização mediada por mTORC1 no efeito tipo-antidepressivo provocado pela coadministração de cetamina e guanosina. Nossos experimentos revelaram que uma única administração com uma dose sub-efetiva de cetamina, que não teve efeito por si só, quando administrada em combinação com uma dose sub-efetiva de guanosina aumentou os níveis de BDNF e a sinalização de mTORC1, alvos moleculares envolvidos em respostas tipo-antidepressiva e pró-sinaptogênica (DUMAN et al., 2012; ABDALLAH et al., 2016), de forma tempo-dependente no hipocampo e córtex pré-frontal. Particularmente, a administração de cetamina e guanosina aumentou os níveis de BDNF no hipocampo e no córtex pré-frontal 1 h após a administração, mas este efeito não foi observado em 24 h ou 7 dias. Além disso, a administração conjunta de cetamina e guanosina aumentou rapidamente (1 h após a administração) a fosforilação de Akt (Ser^{473}), GSK-3β (Ser^9), mTORC1 (Ser^{2448}) e p70S6K (Thr^{389}) no hipocampo, mas não no córtex pré-frontal, e este efeito não se manteve por 24 h. Nossos resultados também revelaram um aumento tempo-dependente no imunoconteúdo PSD-95 e GluA1 no hipocampo e córtex pré-frontal após a administração combinada de cetamina e guanosina. Em particular, houve um aumento no imunoconteúdo de PSD-95 e GluA1 no córtex pré-frontal 1 h após a coadministração de cetamina e guanosina, um efeito não observado no hipocampo. No entanto, um aumento significativo no imunoconteúdo de PSD-95 e GluA1 foi detectado 24 h após o tratamento com cetamina e guanosina no hipocampo e no córtex pré-frontal de camundongos, mas este efeito não foi verificado após 7 dias. Além disso, estudos anteriores demonstraram que administração de guanosina e cetamina isoladamente, em doses ativas, foi capaz de aumentar os níveis de BDNF (LEPACK et al., 2014; ROSA et al., 2021). Desta forma, neste capítulo, fornecemos novas evidências sobre padrões temporais (24 h e 7 dias) e regionais (hipocampo e córtex pré-frontal) em alvos *upstream* (BDNF, Akt e GSK-3β) e *downstream* (PSD-95 e GluA1) da via de sinalização mediada por mTORC1 após a administração de cetamina e guanosina.

Em seguida, investigamos se a formação de espinhos dendríticos no córtex pré-frontal e no giro denteado da formação hipocampal, um mecanismo essencial para respostas antidepressivas de efeito rápido e duradouro (DUMAN; DUMAN, 2014), poderia estar associado com o efeito tipo-antidepressivo exercido pela coadministração de cetamina e guanosina. De especial interesse, analisamos se a formação de espinhos poderia acontecer na porção ventral do giro denteado da formação hipocampal, considerando que estudos prévios demonstraram que a região ventral (região anterior em primatas) parece estar mais associada à

modulação de estresse, emoção e afeto, enquanto a zona dorsal desempenha majoritariamente funções cognitivas (BANNERMAN et al., 2004; FANSELOW; DONG, 2010). Os resultados mostraram que a combinação com doses sub-efetivas de cetamina e guanosina aumentou a formação de espinhos dendríticos na porção ventral do giro denteado da formação hipocampal e no córtex pré-frontal de maneira tempo-dependente. Em particular, nossos resultados indicaram que coadministração de cetamina e guanosina aumentou a densidade de espinhos dendríticos na porção ventral do giro denteado da formação hipocampal e no córtex pré-frontal 24 h após sua administração, mas não causou qualquer alteração significativa em 1 h ou 7 dias. Um estudo prévio mostrou que a combinação de doses sub-efetivas de cetamina e lítio aumentou a densidade de espinhos dendríticos no córtex pré-frontal de ratos após 24 horas, e esses efeitos se mantiveram por até 7 dias após o tratamento (LIU et al., 2013). Desta forma, estes resultados poderiam explicar a resposta tipo-antidepressiva rápida e sustentada desencadeada pela administração desses compostos (LIU et al., 2013). Além disso, a coadministração de doses sub-efetivas de cetamina e agmatina aumentou a síntese de proteínas sinápticas e o número de espinhos dendríticos no córtex pré-frontal de camundongos após 1 h. Contudo, esse efeito se sustentou por 24 horas e 7 dias, o que também foi relacionado ao efeito do tipo-antidepressivo provocado pela administração combinada dessas moléculas (FREITAS et al., 2020). Estudos prévios mostraram que uma única administração de cetamina *per se* (dose ativa) exerceu um efeito tipo-antidepressivo e aumentou a densidade dos espinhos dendríticos na porção ventral do giro denteado da formação hipocampal (FRAGA et al., 2020) e no córtex pré-frontal (LI et al., 2010) de roedores. Considerando que o aumento na síntese de proteínas sinápticas e formação de espinhos dendríticos estão subjacentes aos efeitos antidepressivos de início rápido (DUMAN; DUMAN, 2014), pode-se supor que estes mecanismos poderiam estar associados à capacidade da combinação de cetamina e guanosina em exercer um rápido efeito tipo-antidepressivo.

Com o intuito de reforçar o papel da via de sinalização mediada por mTORC1 nos efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela coadministração de cetamina e guanosina, os camundongos foram pré-tratados com a rapamicina, um inibidor seletivo de mTORC1. Nossos resultados demonstraram que o efeito tipo-antidepressivo provocado pela combinação de doses sub-efetivas de cetamina e guanosina após 1 h e 24 h em camundongos submetidos ao teste de suspensão pela cauda requer a ativação de mTORC1, uma vez que o pré-tratamento com a rapamicina aboliu completamente este efeito. Em consonância com nossos resultados, um estudo anterior do nosso grupo de pesquisa mostrou que o pré-

tratamento com rapamicina preveniu o efeito tipo-antidepressivo provocado pela administração combinada de cetamina e guanosina no teste da alimentação suprimida pela novidade em camundongos após 1 h (CAMARGO et al., 2019). Além disso, foi reportado anteriormente que a administração de rapamicina anulou completamente o efeito tipo-antidepressivo exercido pela administração isolada das doses ativas de cetamina ou guanosina (ALMEIDA et al., 2020; BETTIO et al., 2012; LI et al., 2010). Também demonstramos que o aumento na fosforilação de mTORC1 e p70S6K e no imunoconteúdo das proteínas sinápticas PSD-95 e GluA1 promovidos pela combinação de cetamina e guanosina, foram completamente prevenidos pela administração de rapamicina. Estes resultados indicam que o efeito pró-sinaptogênico desencadeado pela associação de cetamina e guanosina no hipocampo e córtex pré-frontal é dependente, pelo menos em parte, da ativação de mTORC1. Além disso, esses achados reforçam a noção de que a ativação da via sinalização mediada por mTORC1 parece ocorrer de forma mais rápida no córtex pré-frontal do que no hipocampo, ou seja, iniciando antes de 1 h, conforme sugerido no primeiro capítulo desta tese. Essa suposição é baseada no fato de que, embora não tenha sido verificado nenhum aumento na fosforilação de mTORC1/p70S6K no córtex pré-frontal neste período, o aumento no imunoconteúdo de proteínas sinápticas promovido pela associação de cetamina e guanosina foi abolido pelo pré-tratamento com rapamicina. Nossos resultados também revelam pela primeira vez que a sinalização mediada por mTORC1 está subjacente à capacidade da combinação de cetamina e guanosina em aumentar a densidade de espinhos dendríticos na porção ventral do giro denteado da formação hipocampal e no córtex pré-frontal após 24 horas, uma vez o pré-tratamento com rapamicina aboliu totalmente essa resposta. Portanto, nossos achados concordam com a premissa de que estimular a via de sinalização mediada por mTORC1 com consequente aumento na síntese de proteínas sinápticas e formação de espinhos dendríticos pode levar a respostas tipo-antidepressivas de início rápido (DUMAN; DUMAN, 2014; DUMAN; VOLETI, 2012).

No terceiro capítulo desta tese, avaliamos os efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela administração com doses sub-efetivas de cetamina e guanosina no modelo animal de estresse farmacológico induzido pela administração crônica de corticosterona. Este modelo tem recebido atenção nos últimos anos devido sua capacidade de mimetizar aspectos comportamentais, neuroquímicos e morfológicos observados em indivíduos com TDM (STERNER; KALYNCHUK, 2010). De especial interesse, a administração crônica de corticosterona é capaz de provocar comportamento do tipo-depressivo no teste de suspensão pela cauda, teste do nado forçado e no teste de borrifagem de sacarose (DAVID et al., 2009;

NEIS et al., 2018; PAZINI et al., 2016; ZENI; CAMARGO; DALMAGRO, 2019). Além disso, este modelo promove alterações neuroquímicas e morfológicas que incluem diminuição de BDNF, disfunções na via de sinalização mediada por mTORC1 e na síntese de proteínas sinápticas, bem como déficits na densidade e arquitetura de espinhos dendríticos no hipocampo e no córtex pré-frontal de camundongos (FRAGA et al., 2021; FREITAS et al., 2016; PAZINI et al., 2016). Vale ressaltar que as alterações comportamentais e moleculares induzidas pela corticosterona são abolidas apenas pela administração crônica, mas não aguda, de antidepressivos monoaminérgicos convencionais (AGO et al., 2013; AMPUERO et al., 2010; MORAIS et al., 2014; ZENI; CAMARGO; DALMAGRO, 2019), mas são revertidas por uma única administração de cetamina (KOIKE; IIJIMA; CHAKI, 2013; NEIS et al., 2018; PAZINI et al., 2016). Portanto, este modelo animal configura-se como uma abordagem experimental útil para a investigação de novos agentes antidepressivos com efeito rápido. Os resultados deste capítulo revelaram que a administração com uma dose sub-efetiva de cetamina, que não teve efeito por si só, quando administrada em combinação com uma dose sub-efetiva de guanosina produziu um efeito tipo-antidepressivo em camundongos administrados cronicamente com corticosterona. Particularmente, demonstramos que a administração por 21 dias de corticosterona (20 mg/kg, p.o.) foi capaz de produzir um comportamento do tipo-depressivo em camundongos submetidos ao teste de suspensão pela cauda, teste da borrifagem com sacarose e teste da alimentação suprimida pela novidade. Contudo, o fenótipo tipo-depressivo induzido pela corticosterona não foi observado após 24 h do tratamento combinado com doses sub-efetivas de cetamina e guanosina. Além disso, fornecemos evidências de que o efeito tipo-antidepressivo induzido pela combinação de cetamina e guanosina em camundongos expostos a corticosterona é dependente da ativação de mTORC1, uma vez que a administração de rapamicina aboliu este efeito comportamental.

Para reforçar o papel da via de sinalização mediada por mTORC1 no efeito do tipo-antidepressivo provocado pela associação de cetamina e guanosina, proteínas *upstream* e *downstream* de mTORC1 foram investigadas. Os resultados revelaram que a administração crônica com corticosterona causou uma redução significativa nos níveis de BDNF, na fosforilação de Akt (Ser^{473}) e GSK-3β (Ser^9) e no imunoconteúdo de PSD-95, GluA1 e sinapsina no hipocampo, mas não no córtex pré-frontal de camundongos, e esses resultados concordam parcialmente com estudos anteriores (DONG et al., 2019; FRAGA et al., 2021; FREITAS et al., 2016; PAZINI et al., 2016; WENG et al., 2016). Entretanto, estas alterações moleculares foram completamente revertidas após 24 h de uma única administração da

cetamina e guanosina. Além disso, também demonstramos que a capacidade da combinação de cetamina e guanosina em reverter os déficits no imunoconteúdo de PSD-95 e GluA1 induzidos por corticosterona no hipocampo foi abolida pela administração de rapamicina, indicando o envolvimento de mTORC1 nesta resposta. Além disso, é intrigante notar que a capacidade da associação cetamina e guanosina em atenuar a redução no imunoconteúdo de sinapsina induzida pela corticosterona não foi abolida pela administração de rapamicina, sugerindo a participação de mecanismos adicionais na modulação dessa proteína. Contudo, pode-se especular que a modulação da síntese de proteínas sinápticas via sinalização de mTORC1 pode estar associada à resposta tipo-antidepressiva promovida pela coadministração de doses sub-efetivas de cetamina e guanosina em um modelo animal induzido estresse. Desta forma, estes resultados reforçam a noção de que a administração combinada de guanosina e cetamina pode ser uma estratégia terapêutica útil para o manejo do TDM.

Por fim, é importante destacar que uma única administração prévia de cetamina foi capaz de proteger contra o comportamento tipo-depressivo induzido por estresse, sugerindo que esta molécula além de possuir propriedades antidepressivas rápidas também apresenta ação profilática e promotora de resiliência (BRACHMAN et al., 2016; MASTRODONATO et al., 2018; MCGOWAN et al., 2018). Desta forma, no quarto capítulo desta tese investigamos se a administração profilática de guanosina, de forma semelhante à cetamina, poderia produzir um efeito pró-resiliência. Além disso, avaliamos se a guanosina poderia potencializar a capacidade da cetamina em exercer uma resposta profilática. Os resultados mostraram que a cetamina (5 mg/kg, i.p.), quando administrada 1 semana antes do início da administração crônica de corticosterona (20 mg/kg, p.o., por 21 dias), foi eficaz na prevenção do comportamento tipo-depressivo induzido por este protocolo de estresse farmacológico. No entanto, a administração repetida de fluoxetina (10 mg/kg, p.o., por 21 dias, antidepressivo convencional), uma única administração de guanosina (1 ou 5 mg/kg, p.o.) ou a associação de doses sub-efetivas de cetamina (1 mg/kg, i.p.) e guanosina (5 mg/kg, p.o.) não foram capazes de prevenir o comportamento tipo-depressivo induzido pela corticosterona. Estes dados reforçam a premissa de que a combinação de cetamina e guanosina é capaz de produzir respostas rápidas, mas não sustentadas.

De especial interesse, é importante enfatizar que os alvos moleculares subjacentes à resposta profilática da cetamina estão pouco caracterizados e ainda não são totalmente compreendidos. Somado a este fato, ainda existem poucos estudos investigando se o efeito profilático da cetamina poderia também estar associado à sua cascata de sinalização clássica,

por meio da estimulação da mTORC1 com consequente aumento na síntese de proteínas e sinaptogênese (KRZYSTYNIAK et al., 2019; PARISE et al., 2021). Desta forma, é importante caracterizar se o mecanismo clássico da cetamina poderia estar subjacente ao seu efeito profilático e promotor de resiliência. Nesta tese, verificou-se que a administração crônica com corticosterona reduziu significativamente a fosforilação de Akt (Ser^{473}) e GSK-3β (Ser^9), bem como o imunoconteúdo de PSD-95, GluA1 e sinapsina no hipocampo, mas não no córtex pré-frontal de camundongos, reforçando resultados prévios (DONG et al., 2019; FRAGA et al., 2021; FREITAS et al., 2016; PAZINI et al., 2016). No entanto, foi observado que a redução no imunoconteúdo hipocampal de PSD-95, GluA1 e sinapsina induzida pela corticosterona foi seletivamente prevenida pela administração profilática de cetamina, uma vez que a fluoxetina, a guanosina ou a associação de cetamina e guanosina, não apresentaram o mesmo efeito.

Desta forma, este estudo sugere que o efeito pró-sinaptogênico sustentado no hipocampo exercido pela administração profilática de cetamina poderia tornar esta região encefálica menos propensa aos déficits sinápticos induzidos pela corticosterona e poderia aumentar a resiliência de camundongos contra os efeitos prejudiciais do estresse. Além disso, nossos dados sugerem que estratégias direcionadas a estimulação da via de sinalização pró-sinaptogênica de longa duração poderia aumentar a resiliência e proteger contra transtornos relacionados ao estresse. Somado a isso, os resultados deste estudo também reforçam a noção de que intervenções sinaptoprotetoras, que são capazes de prevenir o comprometimento sináptico induzido por estresse, podem configurar-se como estratégias preventivas contra o TDM (HUZIAN et al., 2021). É importante destacar que nossos dados reforçam a premissa que a administração profilática de cetamina pode emergir como uma nova estratégia potencial para reduzir a incidência e manejar os pacientes sob condições de alto estresse em risco de desenvolver TDM.

6 CONCLUSÕES

A coadministração única com doses sub-efetivas de cetamina e guanosina, que não produzem efeito quando administradas isoladamente, é capaz de provocar um efeito tipo-antidepressivo após 1 h e 24 h, mas não após 7 dias, em camundongos;

O efeito tipo-antidepressivo desencadeado pela combinação de cetamina e guanosina foi acompanhado por uma estimulação da via de sinalização mediada por mTORC1 com consequente aumento na síntese de proteínas sinápticas e na densidade de espinhos dendríticos de maneira tempo-dependente na formação hipocampal e córtex pré-frontal;

A capacidade do tratamento combinado com doses sub-efetivas de cetamina e guanosina em provocar um efeito tipo-antidepressivo e pró-sinaptogênico é dependente da ativação da sinalização de mTORC1;

A combinação com doses sub-efetivas de cetamina e guanosina é eficaz em reverter o comportamento tipo-depressivo e os déficits sinápticos hipocampais induzidos pela administração crônica com corticosterona;

O efeito tipo-antidepressivo e pró-sinaptogênico promovido pela combinação de cetamina e guanosina em camundongos expostos a corticosterona é dependente da ativação de mTORC1;

A administração profilática de cetamina, mas não de guanosina ou a associação de cetamina e guanosina, foi capaz de prevenir o comportamento tipo-depressivo e déficits sinápticos hipocampais induzidos pela administração crônica de corticosterona;

O presente estudo ampliou o conhecimento acerca dos mecanismos envolvidos nos efeitos tipo-antidepressivo e pró-sinaptogênico desencadeados pela coadministração com doses sub-efetivas de cetamina e guanosina;

Este estudo sugere que a guanosina em combinação com a cetamina pode constituir uma estratégia promissora para auxiliar pacientes com diagnóstico de TDM no futuro, por permitir a redução da dose terapeuticamente eficaz de cetamina, uma prova de conceito que merece uma investigação mais aprofundada.

7 PERSPECTIVAS

Investigar se o efeito tipo-antidepressivo e pró-sinaptogênico desencadeados pela coadministração única com doses sub-efetivas de cetamina e guanosina pode se sustentar por 48 h ou 72 h;

Analisar a arquitetura dos espinhos dendríticos na formação hipocampal e córtex pré-frontal após o tratamento combinado com doses sub-efetivas de cetamina e guanosina, a fim de caracterizar a maturação dos espinhos dendríticos;

Investigar se a associação com doses sub-efetivas de cetamina e guanosina é capaz de alterar parâmetros de eletrofisiologia após 1 e 24 h de uma única administração, com intuito de investigar a funcionalidade dos espinhos dendríticos;

Verificar os níveis de fosforilação nos sítios Ser^{831} e Ser^{845} da subunidade GluA1 do receptor AMPA no hipocampo e córtex pré-frontal após a coadministração única com doses sub-efetivas de cetamina e guanosina;

Avaliar os efeitos da associação de cetamina e guanosina sobre a sinaptogênese (avaliação morfológica do número e formato dos espinhos dendríticos) no hipocampo e córtex pré-frontal de animais submetidos ao modelo de estresse farmacológico induzido por corticosterona;

Estudar o envolvimento de outras regiões encefálicas, como giro denteado dorsal, habênula lateral, amígdala e núcleo accumbens, no efeito tipo-antidepressivo promovido pela associação de cetamina e guanosina;

Averiguar se a formação e o remodelamento de espinhos dendríticos no hipocampo e córtex pré-frontal pode estar subjacente ao efeito pró-resiliência promovido pela cetamina.

8 PUBLICAÇÕES

PUBLICAÇÕES RELACIONADAS À TESE:

1. **CAMARGO**, **A**. et al. Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: Possible role of pro-synaptogenic signaling pathway. **Journal of Affective Disorders**, v. 271, p. 100–108, 2020.

2. **CAMARGO**, **A**. et al. Ketamine, but not guanosine, as a prophylactic agent against corticosterone-induced depressive-like behavior: Possible role of long-lasting pro-synaptogenic signaling pathway. **Experimental Neurology**, v. 334, p. 113459, 2020.

3. **CAMARGO**, **A**. et al. A low-dose combination of ketamine and guanosine counteracts corticosterone-induced depressive-like behavior and hippocampal synaptic impairments via mTORC1 signaling. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 111, p. 110371, 2021.

4. **CAMARGO**, **A**. et al. Guanosine boosts the fast, but not sustained, antidepressant-like and pro-synaptogenic effects of ketamine by stimulating mTORC1-driven signaling pathway. **European Neuropsychopharmacology**, v. 57, p. 15–29, 2022.

PUBLICAÇÕES COMO AUTOR NÃO RELACIONADAS À TESE:

1. **CAMARGO**, **A**. et al. Low doses of ketamine and guanosine abrogate corticosterone-induced anxiety-related behavior, but not disturbances in the hippocampal NLRP3 inflammasome pathway. **Psychopharmacology** 2021 238:9, v. 238, n. 9, p. 2555–2568, 2021.

2. **CAMARGO**, **A**. et al. The resilient phenotype elicited by ketamine against inflammatory stressors-induced depressive-like behavior is associated with NLRP3-driven signaling pathway. **Journal of Psychiatric Research**, v. 144, p. 118–128, 2021.

3. **CAMARGO**, **A**. et al. Cholecalciferol abolishes depressive-like behavior and hippocampal glucocorticoid receptor impairment induced by chronic corticosterone administration in mice. **Pharmacology Biochemistry and Behavior**, v. 196, p. 172971, 2020.

4. **CAMARGO**, **A**. et al. Subthreshold doses of guanosine plus ketamine elicit antidepressant-like effect in a mouse model of depression induced by corticosterone: Role of GR/NF-κB/IDO-1 signaling. **Neurochemistry International**, v. 139, p. 104797, 2020.

5. **CAMARGO**, **A**. et al. Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway. **Journal of Psychiatric Research**, v. 115, 2019.

6. **CAMARGO**, **A**.; RODRIGUES, A. L. S. Novel Targets for Fast Antidepressant Responses: Possible Role of Endogenous Neuromodulators. **Chronic Stress**, v. 3, p. 247054701985808, 2019.

PUBLICAÇÕES COMO CO-AUTOR:

1. FRAGA, D. B. et al. A single administration of ascorbic acid rapidly reverses depressive-like behavior and hippocampal synaptic dysfunction induced by corticosterone in mice. **Chemico-Biological Interactions**, v. 342, p. 109476, 2021.

2. FRAGA, D. B. et al. Ketamine, but not fluoxetine, rapidly rescues corticosterone-induced impairments on glucocorticoid receptor and dendritic branching in the hippocampus of mice. **Metabolic Brain Disease**, v. 36, n. 8, p. 2223–2233, 1 dez. 2021.

3. GRUHN, K. et al. Physical exercise stimulates hippocampal mTORC1 and FNDC5/irisin signaling pathway in mice: Possible implication for its antidepressant effect. **Behavioural Brain Research**, v. 400, p. 113040, 2021.

4. COLLA, A. R. S. et al. Ursolic acid abrogates depressive-like behavior and hippocampal pro-apoptotic imbalance induced by chronic unpredictable stress. **Metabolic Brain Disease**, v. 36, n. 3, p. 437–446, 2021.

5. VALVERDE, A. P.; CAMARGO, A.; RODRIGUES, A. L. S. Agmatine as a novel candidate for rapid-onset antidepressant response. **World Journal of Psychiatry**, v. 11, n. 11, p. 981–996, 2021.

6. ROSA, J. M. et al. Physical exercise prevents amyloid $\beta1-40$-induced disturbances in NLRP3 inflammasome pathway in the hippocampus of mice. **Metabolic Brain Disease**, v. 36, n. 2, p. 351–359, 2021.

7. VEBER, B. et al. Red cabbage (Brassica oleracea L.) extract reverses lipid oxidative stress in rats. **Anais da Academia Brasileira de Ciências**, v. 92, n. 1, p. 20180596, 2020.

8. DALMAGRO, A. P. et al. Plumieride exerts anxiolytic-like effect mediated by GABAergic and monoaminergic systems. **Natural Product Research**, v. 35, n. 22, p. 4849-4852, 2021.

9. DALMAGRO, A. P. et al. Morus nigra leaves extract revokes the depressive-like behavior, oxidative stress, and hippocampal damage induced by corticosterone: A pivotal role of the phenolic syringic acid. **Behavioural Pharmacology**, p. 397–406, 2020.

10. FRAGA, D. B. et al. Ascorbic acid presents rapid behavioral and hippocampal synaptic plasticity effects. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 96, p. 109757, 2020.

11. MANOSSO, L. M. et al. Vitamin E for the management of major depressive disorder: possible role of the anti-inflammatory and antioxidant systems. **Nutritional Neuroscience**, v. 23, p. 1–15, 2020.

12. PAZINI, F. L. et al. mTORC1-dependent signaling pathway underlies the rapid effect of creatine and ketamine in the novelty-suppressed feeding test. **Chemico-Biological Interactions**, v. 332, p. 109281, 2020.

13. ROSA, J. M. et al. Prophylactic effect of physical exercise on Aβ1-40-induced depressive-like behavior and gut dysfunction in mice. **Behavioural Brain Research**, v. 393, p. 112791, 2020.

14. SITENESKI, A. et al. Antidepressant-like and pro-neurogenic effects of physical exercise: the putative role of FNDC5/irisin pathway. **Journal of Neural Transmission**, v. 127, n. 3, p. 355–370, 2020.

15. ROSA, J. M. et al. Prophylactic effect of physical exercise on Aβ-1–40-induced depressive-like behavior: Role of BDNF, mTOR signaling, cell proliferation and survival in the hippocampus. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 94, p. 109646, 2019.

16. ZENI, A. L. B. et al. Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities. **Pharmacology Biochemistry and Behavior**, v. 179, p. 63–72, 2019.

17. DALMAGRO, A. P. et al. Involvement of PI3K/Akt/GSK-3β signaling pathway in the antidepressant-like and neuroprotective effects of Morus nigra and its major phenolic, syringic acid. **Chemico-Biological Interactions**, v. 314, p. 108843, 2019.

REFERÊNCIAS

ABDALLAH, C. G. et al. Ketamine's mechanism of action: A path to rapid-acting antidepressants. **Depression and Anxiety**, v. 33, n. 8, p. 689–697, 2016.

ABDALLAH, C. G. et al. Modulation of the antidepressant effects of ketamine by the mTORC1 inhibitor rapamycin. **Neuropsychopharmacology**, v. 45, n. 6, p. 990–997, 2020.

ABDALLAH, C. G.; AVERILL, L. A.; KRYSTAL, J. H. Ketamine as a promising prototype for a new generation of rapid-acting antidepressants. **Annals of the New York Academy of Sciences**, v. 1344, n. 1, p. 66–77, 2015.

AGO, Y. et al. Metabotropic glutamate 2/3 receptor antagonists improve behavioral and prefrontal dopaminergic alterations in the chronic corticosterone-induced depression model in mice. **Neuropharmacology**, v. 65, p. 29–38, 2013.

ALFAREZ, D. N. et al. Corticosterone reduces dendritic complexity in developing hippocampal CA1 neurons. **Hippocampus**, v. 19, n. 9, p. 828–836, 2009.

ALI-SISTO, T. et al. Purine metabolism is dysregulated in patients with major depressive disorder. **Psychoneuroendocrinology**, v. 70, p. 25–32, 2016.

ALMEIDA, R. F. et al. Guanosine fast onset antidepressant-like effects in the olfactory bulbectomy mice model. **Scientific Reports**, v. 10, n. 1, p. 8429, 2020.

ALMEIDA, R. F. et al. Antidepressant-Like Effects of Chronic Guanosine in the Olfactory Bulbectomy Mouse Model. **Frontiers in Psychiatry**, v. 1268, p. 1–14, 2021.

AMERICAN PSYCHIATRIC ASSOCIATION. **Diagnostic and Statistical Manual of Mental Disorders**. 5th. ed. Washington, DC: American Psychiatric Association, 2013.

AMPUERO, E. et al. Chronic fluoxetine treatment induces structural plasticity and selective changes in glutamate receptor subunits in the rat cerebral cortex. **Neuroscience**, v. 169, n. 1, p. 98–108, 2010.

ANACKER, C. et al. The glucocorticoid receptor: Pivot of depression and of antidepressant treatment? **Psychoneuroendocrinology**, v. 36, n. 3, p. 415–425, 2011.

ARMOUR, C.; JANA, M.; ELHAI, J. D. A systematic literature review of PTSD's latent structure in the Diagnostic and Statistical Manual of Mental Disorders: DSM-IV to DSM-5. **Clinical Psychology Review**, v. 44, p. 60–74, 2016.

AUTRY, A. E. et al. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. **Nature**, v. 475, n. 7354, p. 91–96, 2011.

BALTACI, S. B.; MOGULKOC, R.; BALTACI, A. K. Molecular mechanisms of early and late LTP. **Neurochemical research**, v. 44, n. 2, p. 281–296, 2019.

BANNERMAN, D. M. et al. Regional dissociations within the hippocampus - Memory and anxiety. **Neuroscience and Biobehavioral Reviews**, v. 28, n. 3, p. 273–283, 2004.

BAROWSKY, J.; SCHWARTZ, T. L. An evidence-based approach to augmentation and combination strategies for treatment resistant depression. **Psychiatry**, v. 3, n. 7, p. 42–61, 2006.

BASU, S.; LAMPRECHT, R. The Role of Actin Cytoskeleton in Dendritic Spines in the Maintenance of Long-Term Memory. **Frontiers in Molecular Neuroscience**, v. 11, p. 1–8, 2018.

BERMAN, R. M. et al. Antidepressant effects of ketamine in depressed patients. **Biological Psychiatry**, v. 47, n. 4, p. 351–354, 2000.

BERRY, K. P.; NEDIVI, E. Spine dynamics: Are they all the same? **Neuron**, v. 96, n. 1, p. 43–55, 2017.

BERTON, O.; NESTLER, E. J. New approaches to antidepressant drug discovery: Beyond monoamines. **Nature Reviews Neuroscience**, v. 7, n. 2, p. 137–151, 2006.

BETTIO, L. et al. The antidepressant-like effect of chronic guanosine treatment is associated with increased hippocampal neuronal differentiation. **European Journal of Neuroscience**, v. 43, n. 8, p. 1006–15, 2016.

BETTIO, L. E. B. et al. Guanosine produces an antidepressant-like effect through the modulation of NMDA receptors, nitric oxide-cGMP and PI3K/mTOR pathways. **Behavioural Brain Research**, v. 234, n. 2, p. 137–148, 2012.

BETTIO, L. E. B.; GIL-MOHAPEL, J.; RODRIGUES, A. L. S. Guanosine and its role in neuropathologies. **Purinergic Signalling**, v. 12, n. 3, p. 411–426, 2016.

BOLDRINI, M. et al. Hippocampal granule neuron number and dentate gyrus volume in antidepressant-treated and untreated major depression. **Neuropsychopharmacology**, v. 38, n. 6, p. 1068–1077, 2013.

BOSCH, M.; HAYASHI, Y. Structural plasticity of dendritic spines. **Current opinion in neurobiology**, v. 22, n. 3, p. 383–388, 2012.

BRACHMAN, R. A. et al. Ketamine as a prophylactic against stress-induced depressive-like behavior. **Biological Psychiatry**, v. 79, n. 9, p. 776–786, 2016.

BURNSTOCK, G. Purinergic nerves. **Pharmacological Reviews**, v. 24, n. 3, p. 509–581, 1972.

BURNSTOCK, G. Purine and pyrimidine receptors. **Cellular and Molecular Life Sciences**, v. 64, p. 1471–1483, 2007.

BURNSTOCK, G. Purinergic signalling and disorders of the central nervous system. **Nature Reviews Drug Discovery**, v. 7, p. 575–590, 2008.

BURNSTOCK, G. An introduction to the roles of purinergic signalling in neurodegeneration, neuroprotection and neuroregeneration. **Neuropharmacology**, v. 104, p. 4–17, 2016.

BURNSTOCK, G. Introduction to Purinergic Signaling. **Methods in Molecular Biology**, v. 2041, p. 1–15, 2020.

CAMARGO, A. et al. Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway. **Journal of Psychiatric Research**, v. 115, 2019.

CAMARGO, A. et al. The resilient phenotype elicited by ketamine against inflammatory stressors-induced depressive-like behavior is associated with NLRP3-driven signaling pathway. **Journal of Psychiatric Research**, v. 144, p. 118–128, 2021.

CAMARGO, A.; RODRIGUES, A. L. S. Novel Targets for Fast Antidepressant Responses: Possible Role of Endogenous Neuromodulators. **Chronic Stress**, v. 3, p. 247054701985808, 2019.

CARLÉN, M. What constitutes the prefrontal cortex? **Science**, v. 358, n. 6362, p. 478–482, 2017.

CASAROTTO, P. C. et al. Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. **Cell**, v. 184, p. 1299–1312, 2021.

CATHOMAS, F. et al. Neurobiology of Resilience: Interface Between Mind and Body. **Biological Psychiatry**, v. 86, n. 6, p. 410–420, 2019.

CELANO, C. M. et al. Depressogenic effects of medications: a review. **Dialogues in Clinical Neuroscience**, v. 13, n. 1, p. 109–125, 2011.

CHEN, B. K. et al. Sex-specific neurobiological actions of prophylactic (R,S)-ketamine, (2R,6R)-hydroxynorketamine, and (2S,6S)-hydroxynorketamine. **Neuropsychopharmacology**, v. 45, n. 9, p. 1545–1556, 2020.

CHIDAMBARAM, S. B. et al. Dendritic spines: Revisiting the physiological role. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 92, p. 161–193, 8 jun. 2019.

CICCARELLI, R. et al. Rat cultured astrocytes release guanine-based purines in basal conditions and after hypoxia/hypoglycemia. **Glia**, v. 25, n. 1, p. 93–98, 1999.

CICCARELLI, R. et al. Involvement of astrocytes in purine-mediated reparative processes in the brain. **International Journal of Developmental Neuroscience**, v. 19, n. 4, p. 395–414, 2001.

CITRI, A.; MALENKA, R. C. Synaptic plasticity: Multiple forms, functions, and mechanisms. **Neuropsychopharmacology**, v. 33, n. 1, p. 18–41, 2007.

CRANE, G. Iproniazid (marsilid) phosphate, a therapeutic agent for mental disorders and debilitating diseases. **Report of the American Psychiatric Association**, v. 8, p. 142–152, 1957.

CUNHA, M. P. et al. Involvement of PI3K/Akt Signaling Pathway and Its Downstream Intracellular Targets in the Antidepressant-Like Effect of Creatine. **Molecular Neurobiology**, v. 53, n. 5, p. 2954–2968, 2016.

DAL-CIM, T. et al. Guanosine is neuroprotective against oxygen/glucose deprivation in hippocampal slices via large conductance Ca2+-activated K+channels, phosphatidilinositol-3 kinase/protein kinase B pathway activation and glutamate uptake. **Neuroscience**, v. 183, p. 212–220, 2011.

DAL-CIM, T. et al. Guanosine controls inflammatory pathways to afford neuroprotection of hippocampal slices under oxygen and glucose deprivation conditions. **Journal of Neurochemistry**, v. 126, n. 4, p. 437–450, 2013.

DAL-CIM, T. et al. Neuroprotection promoted by guanosine depends on glutamine synthetase and glutamate transporters activity in hippocampal slices subjected to oxygen/glucose deprivation. **Neurotoxicity Research**, v. 29, n. 4, p. 460–468, 2016.

DALMAGRO, A. P. et al. Morus nigra leaves extract revokes the depressive-like behavior, oxidative stress, and hippocampal damage induced by corticosterone: A pivotal role of the phenolic syringic acid. **Behavioural Pharmacology**, p. 397–406, 2020.

DAVID, D. J. et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. **Neuron**, v. 62, n. 4, p. 479–493, 2009.

DELGADO, P. L. Depression: the case for a monoamine deficiency. **Journal of Clinical Psychiatry**, v. 61, n. 6, p. 7–11, 2000.

DETTENBORN, L. et al. Introducing a novel method to assess cumulative steroid concentrations: increased hair cortisol concentrations over 6 months in medicated patients with depression. **Stress**, v. 15, n. 3, p. 348–353, maio 2012.

DIAZGRANADOS, N. et al. Rapid resolution of suicidal ideation after a single infusion of an NMDA antagonist in patients with treatment-resistant major depressive disorder. **Journal of Clinical Psychiatry**, v. 71, n. 12, p. 1605–1611, 2010.

DIXON, M. L. et al. Emotion and the prefrontal cortex: An integrative review. **Psychological bulletin**, v. 143, n. 10, p. 1033–1081, 2017.

DOLZANI, S. D. et al. Inhibition of a descending prefrontal circuit prevents ketamine-induced stress resilience in females. **eNeuro**, v. 5, n. 1, p. 1–18, 2018.

DOMINO, E. F. History of modern psychopharmacology: A personal view with an emphasis on antidepressants. **Psychosomatic Medicine**, v. 61, n. 5, p. 591–598, 1999.

DONG, S. Q. et al. Oleanolic acid decreases SGK1 in the hippocampus in corticosterone-induced mice. **Steroids**, v. 149, p. 108419, 2019.

DUMAN, C. H.; DUMAN, R. S. Spine synapse remodeling in the pathophysiology and treatment of depression. **Neuroscience Letters**, v. 601, p. 20–29, 2014.

DUMAN, R. S. et al. Signaling pathways underlying the rapid antidepressant actions of ketamine. **Neuropharmacology**, v. 62, n. 1, p. 35–41, 2012.

DUMAN, R. S.; AGHAJANIAN, G. K. Synaptic dysfunction in depression: Potential therapeutic targets. **Science**, v. 338, p. 68–72, 2012.

DUMAN, R. S.; LI, N. A neurotrophic hypothesis of depression: Role of synaptogenesis in the actions of NMDA receptor antagonists. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 367, p. 2475–2484, 2012.

DUMAN, R. S.; VOLETI, B. Signaling pathways underlying the pathophysiology and treatment of depression: Novel mechanisms for rapid-acting agents. **Trends in Neurosciences**, v. 35, n. 1, p. 47–56, 2012.

DURIC, V. et al. Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. **International Journal of Neuropsychopharmacology**, v. 16, n. 1, p. 69–82, 2013.

FANSELOW, M. S.; DONG, H. W. Are the Dorsal and Ventral Hippocampus Functionally Distinct Structures? **Neuron**, v. 65, n. 1, p. 7–19, 2010.

FAYE, C. et al. Neurobiological mechanisms of stress resilience and implications for the aged population. **Current Neuropharmacology**, v. 16, n. 3, p. 234, 2018.

FEDER, A.; NESTLER, E. J.; CHARNEY, D. S. Psychobiology and molecular genetics of resilience. **Nature Reviews Neuroscience**, v. 10, n. 6, p. 446–457, 2009.

FEYISSA, A. M. et al. Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. **Progress in Neuropsychopharmacology & Biological Psychiatry**, v. 33, n. 1, p. 70–75, 2009.

FRAGA, D. B. et al. Ascorbic acid presents rapid behavioral and hippocampal synaptic plasticity effects. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 96, p. 109757, 2020.

FRAGA, D. B. et al. A single administration of ascorbic acid rapidly reverses depressive-like behavior and hippocampal synaptic dysfunction induced by corticosterone in mice. **Chemico-Biological Interactions**, v. 342, p. 109476, 2021.

FREIS, E. D. Mental depression in hypertensive patients treated for long periods with large doses of reserpine. **The New England Journal of Medicine**, v. 252, n. 25, p. 1006–1008, 1954.

FREITAS, A. et al. Agmatine potentiates antidepressant and synaptic actions of ketamine: Effects on dendritic arbors and spines architecture and Akt/S6 kinase signaling. **Experimental Neurology**, v. 333, p. 113398, 2020.

FREITAS, A. E. et al. Agmatine, by improving neuroplasticity markers and inducing Nrf2, prevents corticosterone-induced depressive-like behavior in mice. **Molecular Neurobiology**, v. 53, n. 5, p. 3030–3045, 2016.

FRODL, T.; O'KEANE, V. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. **Neurobiology of Disease**, v. 52, p. 24–37, 2013.

GAO, M.; REJAEI, D.; LIU, H. Ketamine use in current clinical practice. **Acta Pharmacologica Sinica**, v. 37, n. 7, p. 865–872, 2016.

GIULIANI, P. et al. Tissue distribution and metabolism of guanosine in rats following intraperitoneal injection. **Journal of Biological Regulators and Homeostatic Agents**, v. 26, n. 1, p. 51–65, 2012.

GJERSTAD, J. K.; LIGHTMAN, S. L.; SPIGA, F. Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. **Stress**, v. 21, n. 5, p. 403–416, 2018.

GODSIL, B. P. et al. The hippocampal–prefrontal pathway: The weak link in psychiatric disorders? **European Neuropsychopharmacology**, v. 23, n. 10, p. 1165–1181, 1 out. 2013.

GOLD, P. W. The organization of the stress system and its dysregulation in depressive illness. **Molecular psychiatry**, v. 20, n. 1, p. 32–47, 2015.

GOMEZ, A. M.; TRAUNMÜLLER, L.; SCHEIFFELE, P. Neurexins: molecular codes for shaping neuronal synapses. **Nature Reviews Neuroscience**, v. 22, n. 3, p. 137–151, 2021.

GOODYER, I. M. et al. Recent life events, cortisol, dehydroepiandrosterone and the onset of major depression in high-risk adolescents. **British Journal of Psychiatry**, v. 177, p. 499–504, 2000.

GORDILLO-SALAS, M. et al. Signaling pathways responsible for the rapid antidepressant-like effects of a GluN2A-preferring NMDA receptor antagonist. **Translational Psychiatry**, v. 8, n. 1, p. 1–9, 2018.

GROENEWEG, F. L. et al. Mineralocorticoid and glucocorticoid receptors at the neuronal membrane, regulators of nongenomic corticosteroid signalling. **Molecular and Cellular Endocrinology**, v. 350, n. 2, p. 299–309, 2012.

GURURAJAN, A. et al. Resilience to chronic stress is associated with specific neurobiological, neuroendocrine and immune responses. **Brain, Behavior, and Immunity**, v. 80, p. 583–594, 1 ago. 2019.

HAAR, E. VANDER et al. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. **Nature Cell Biology**, v. 9, n. 3, p. 316–323, 2007.

HAMON, M.; BLIER, P. Monoamine neurocircuitry in depression and strategies for new treatments. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 45, p. 54–63, 2013.

HAN, C. et al. Aripiprazole augmentation, antidepressant combination or switching therapy in patients with major depressive disorder who are partial- or non-responsive to current antidepressants: A multi-center , naturalistic study. **Journal of Psychiatric Research**, v. 49, p. 75–82, 2014.

HAN, M. H.; NESTLER, E. J. Neural Substrates of Depression and Resilience. **Neurotherapeutics**, v. 14, n. 3, p. 677–686, 2017.

HANNULA, D. E.; DUFF, M. C. **The Hippocampus from Cells to Systems,** Editora Springer, 1ª ed. P. 1-589, 2017.

HANSEL, G. et al. The potential therapeutic effect of guanosine after cortical focal ischemia in rats. **PLoS ONE**, v. 9, n. 2, p. 1–10, 2014.

HARDINGHAM, G. E. NMDA receptor C-terminal signaling in development, plasticity, and disease. **F1000Research**, v. 8, 2019.

HARRIS, K. M. Structural LTP: from synaptogenesis to regulated synapse enlargement and clustering. **Current Opinion in Neurobiology**, v. 63, p. 189–197, 2020.

HARRIS, T. O. et al. Morning cortisol as a risk factor for subsequent major depressive disorder in adult women. **British Journal of Psychiatry**, v. 177, p. 505–510, 2000.

HASHIMOTO, K.; SAWA, A.; IYO, M. Increased levels of glutamate in brains from patients with mood disorders. **Biological Psychiatry**, v. 62, n. 11, p. 1310–1316, 2007.

HEINE, C.; SYGNECKA, K.; FRANKE, H. Purines in neurite growth and astroglia activation. **Neuropharmacology**, v. 104, p. 255–271, 2016.

HELM, M. S. et al. A large-scale nanoscopy and biochemistry analysis of postsynaptic dendritic spines. **Nature Neuroscience**, v. 24, n. 8, p. 1151–1162, 2021.

HERRING, B. E.; NICOLL, R. A. Long-term potentiation: from camkii to ampa receptor trafficking. **Annual Review of Physiology**, v. 78, p. 351–365, 2016.

HILLHOUSE, T. M.; PORTER, J. H. A brief history of the development of antidepressant drugs: From monoamines to glutamate. **Experimental and Clinical Psychopharmacology**, v. 23, n. 1, p. 1–21, 2015.

HOEFFER, C. A.; KLANN, E. mTOR signaling: At the crossroads of plasticity, memory and disease. **Trends in Neurosciences**, v. 33, n. 2, p. 67–75, 2010.

HOLMES, S. E. et al. Lower synaptic density is associated with depression severity and network alterations. **Nature Communications**, v. 10, n. 1, p. 1–10, 2019.

HOTULAINEN, P.; HOOGENRAAD, C. C. Actin in dendritic spines: connecting dynamics to function. **The Journal of Cell Biology**, v. 189, n. 4, p. 619–629, 2010.

HUANG, Y. et al. Structural changes in hippocampal subfields in major depressive disorder: a high-field magnetic resonance imaging study. **Biological psychiatry**, v. 74, n. 1, p. 62–68, 2013.

HUZIAN, O. et al. Stress resilience is associated with hippocampal synaptoprotection in the female rat learned helplessness paradigm. **Neuroscience**, v. 459, p. 85–103, 2021.

INCOLLINGO RODRIGUEZ, A. C. et al. Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: A systematic review. **Psychoneuroendocrinology**, v. 62, p. 301–318, 2015.

JACOBSON, L.; SAPOLSKY, R. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. **Endocrine Reviews**, v. 12, n. 2, p. 118–134, 1991.

JERNIGAN, C. S. et al. The mTOR signaling pathway in the prefrontal cortex is compromised in major depressive disorder. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 35, n. 7, p. 1774–1779, 2011.

JIANG, S. et al. Metabolism and distribution of guanosine given intraperitoneally: Implications for spinal cord injury. **Nucleosides, Nucleotides and Nucleic Acids**, v. 7770, n. 6, p. 673–680, 2008.

JOËLS, M. et al. Corticosteroid effects on cellular physiology of limbic cells. **Brain Research**, v. 1293, p. 91–100, 2009.

JOËLS, M.; KARST, H.; SARABDJITSINGH, R. A. The stressed brain of humans and rodents. **Acta Physiologica**, v. 223, n. 2, p. 1–10, 2018.

JOËLS, M.; PASRICHA, N.; KARST, H. The interplay between rapid and slow corticosteroid actions in brain. **European Journal of Pharmacology**, v. 719, n. 1–3, p. 44–52, 2013.

JONES, K.; HAMMOND, J. Characterization of nucleoside transport activity in rabbit cortical synaptosomes. **Canadian Journal of Physiology and Pharmacology**, v. 73, n. 12, p. 1733–1741, 1995.

JURUENA, M. F. Early-life stress and HPA axis trigger recurrent adulthood depression. **Epilepsy and Behavior**, v. 38, p. 148–159, 2014.

KADMIEL, M.; CIDLOWSKI, J. A. Glucocorticoid receptor signaling in health and disease. **Trends in Pharmacological Sciences**, v. 34, n. 9, p. 518–530, 2013.

KALARIA, R. N.; HARIK, S. I. Adenosine receptors and the nucleoside transporter in human brain vasculature. **Journal of Cerebral Blood Flow and Metabolism**, v. 8, p. 32–39, 1988.

KANG, H. J. et al. Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. **Nature Medicine**, v. 18, n. 9, p. 1413–1417, 2012.

KASTER, M. P. et al. Novel approaches for the management of depressive disorders. **European Journal of Pharmacology**, v. 771, p. 236–240, 2016.

KELLER, J. et al. HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. **Molecular Psychiatry**, v. 22, n. 4, p. 527–536, 2017.

KIM, Y. K.; NA, K. S. Role of glutamate receptors and glial cells in the pathophysiology of treatment-resistant depression. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 70, p. 117–126, 2016.

KLOET, E.; JOËLS, M.; HOLSBOER, F. Stress and the brain: from adaptation to disease. **Nature Reviews Neuroscience**, v. 6, n. 6, p. 463–475, 2005.

KNORR, U. et al. Salivary cortisol in depressed patients versus control persons: A systematic review and meta-analysis. **Psychoneuroendocrinology**, v. 35, n. 9, p. 1275–1286, 2010.

KOIKE, H.; IIJIMA, M.; CHAKI, S. Effects of ketamine and LY341495 on the depressive-like behavior of repeated corticosterone-injected rats. **Pharmacology Biochemistry and Behavior**, v. 107, p. 20–23, 2013.

KRISHNAN, V. et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. **Cell**, v. 131, n. 2, p. 391–404, 2007.

KRZYSTYNIAK, A. et al. Prophylactic ketamine treatment promotes resilience to chronic stress and accelerates recovery: Correlation with changes in synaptic plasticity in the CA3 subregion of the hippocampus. **International Journal of Molecular Sciences**, v. 20, n. 7, p. 1–15, 2019.

KÜÇÜKIBRAHIMOĞLU, E. et al. The change in plasma GABA, glutamine and glutamate levels in fluoxetine- or S-citalopram-treated female patients with major depression. **European Journal of Clinical Pharmacology**, v. 65, n. 6, p. 571–577, 2009.

KUHN, R. The treatment of depressive states with G 22355 (imipramine hydrochloride). **The American Journal of Psychiatry**, v. 115, n. 5, p. 459–464, 1958.

KURDI, M. S.; THEERTH, K. A.; DEVA, R. S. Ketamine: Current applications in anesthesia, pain, and critical care. **Anesthesia: Essays and Researches**, v. 8, n. 3, p. 283–290, 2014.

LAGAMMA, C. T. et al. Antidepressant but not prophylactic ketamine administration alters calretinin and calbindin expression in the ventral hippocampus. **Frontiers in Molecular Neuroscience**, v. 11, 2018.

LANZNASTER, D. et al. Guanosine: A neuromodulator with therapeutic potential in brain disorders. **Aging and disease**, v. 7, n. 5, p. 657–679, 2016.

LANZNASTER, D. et al. Guanosine prevents anhedonic-like behavior and impairment in hippocampal glutamate transport following amyloid-β1–40 administration in mice. **Molecular Neurobiology**, v. 54, n. 7, p. 5482–5496, 2017.

LEAL, G.; BRAMHAM, C. R.; DUARTE, C. B. BDNF and Hippocampal Synaptic Plasticity. **Vitamins and Hormones**, v. 104, p. 153–195, 2017.

LEAL, G.; COMPRIDO, D.; DUARTE, C. B. BDNF-induced local protein synthesis and synaptic plasticity. **Neuropharmacology**, v. 76, p. 639–656, 2014.

LEE, A. L.; OGLE, W. O.; SAPOLSKY, R. M. Stress and depression: Possible links to neuron death in the hippocampus. **Bipolar Disorders**, v. 4, n. 2, p. 117–128, 2002.

LEPACK, A. E. et al. BDNF release is required for the behavioral actions of ketamine. **International Journal of Neuropsychopharmacology**, v. 18, n. 1, p. 1–6, 2014.

LI, N. et al. mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. **Science**, v. 329, n. 5994, p. 959–964, 2010.

LI, N. et al. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. **Biological Psychiatry**, v. 69, n. 8, p. 754–761, 2011.

LICZNERSKI, P.; DUMAN, R. S. Remodeling of axo-spinous synapses in the pathophysiology and treatment of depression. **Neuroscience**, v. 251, p. 33–50, 2013.

LIPPMAN, J.; DUNAEVSKY, A. Dendritic spine morphogenesis and plasticity. **Journal of Neurobiology**, v. 64, n. 1, p. 47–57, 2005.

LISMAN, J.; YASUDA, R.; RAGHAVACHARI, S. Mechanisms of CaMKII action in long-term potentiation. **Nature Reviews Neuroscience**, v. 13, n. 3, p. 169–182, 2012.

LIU, R. et al. GLYX-13 produces rapid antidepressant responses with key synaptic and behavioral effects distinct from ketamine. **Neuropsychopharmacology**, v. 42, n. 6, p. 1231–1242, 2016.

LIU, R. J. et al. GSK-3 inhibition potentiates the synaptogenic and antidepressant-like effects of subthreshold doses of ketamine. **Neuropsychopharmacology**, v. 38, n. 11, p. 2268–2277, 2013.

LIU, W. et al. The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. **Neural Plasticity**, v. 2017, p. 1–12, 2017.

LÓPEZ-MUÑOZ, F.; ALAMO, C. Monoaminergic Neurotransmission : The History of the Discovery of Antidepressants from 1950s Until Today. **Current Pharmaceutical Design**, v. 15, p. 1563–1586, 2009.

LUDKA, F. K. et al. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the l-arginine-nitric oxide-cyclic guanosine monophosphate pathway and increases BDNF levels. **European Neuropsychopharmacology**, v. 23, n. 5, p. 400–412, 2013.

MA, J. H. et al. Prophylactic use of ketamine reduces postpartum depression in Chinese women undergoing cesarean sectio. **Psychiatry Research**, v. 279, p. 252–258, 2019.

MAGNUSON, B.; EKIM, B.; FINGAR, D. C. Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. **Biochemical Journal**, v. 441, n. 1, p. 1–21, 2011.

MALHI, G. S. et al. A New Paradigm for achieving a rapid antidepressant response. **Drugs**, p. 1–10, 2020.

MARIC, N. P.; ADZIC, M. Pharmacological modulation of HPA axis in depression New avenues for potential therapeutic benefits. **Psychiatria Danubina**, v. 25, n. 3, p. 299–305, 2013.

MARMIROLI, P.; CAVALETTI, G. The glutamatergic neurotransmission in the central nervous system. **Current Medicinal Chemistry**, v. 19, n. 9, p. 1269–1276, 2012.

MARQUES, N. F. et al. Guanosine prevents depressive-like behaviors in rats following bilateral dorsolateral striatum lesion induced by 6-hydroxydopamine. **Behavioural Brain Research**, v. 372, 2019.

MARQUES, N. F.; MASSARI, C. M.; TASCA, C. I. Guanosine protects striatal slices against 6-OHDA-induced oxidative damage, mitochondrial dysfunction, and ATP depletion. **Neurotoxicity Research**, v. 35, n. 2, p. 475–483, 2019.

MASSARI, C. et al. Involvement of adenosine A 1 and A 2A receptors on guanosine-mediated anti-tremor effects in reserpinized mice. **Purinergic Signalling**, v. 16, n. 3, p. 379–387, 2020.

MASSARI, C. M. et al. Antiparkinsonian efficacy of guanosine in rodent models of movement disorder. **Frontiers in Pharmacology**, v. 8, p. 4–11, 2017.

MASTRODONATO, A. et al. Ventral CA3 activation mediates prophylactic ketamine efficacy against stress-induced depressive-like behavior. **Biological Psychiatry**, v. 84, n. 11, p. 846–856, 2018.

MASTRODONATO, A. et al. Prophylactic (R,S)-ketamine selectively protects against inflammatory stressors. **Behavioural Brain Research**, v. 378, p. 112238, 2020.

MATEOS-APARICIO, P.; RODRÍGUEZ-MORENO, A. Calcium Dynamics and Synaptic Plasticity. **Advances in Experimental Medicine and Biology**, v. 1131, p. 965–984, 2020.

MCEWEN, B. Physiology and neurobiology of stress and adaptation: central role of the brain. **Physiological Reviews**, v. 87, n. 3, p. 873–904, 2007.

MCGOWAN, J. C. et al. Prophylactic ketamine attenuates learned fear. **Neuropsychopharmacology**, v. 42, n. 8, p. 1577–1589, 2017.

MCGOWAN, J. C. et al. Prophylactic ketamine alters nucleotide and neurotransmitter metabolism in brain and plasma following stress. **Neuropsychopharmacology**, v. 43, n. 9, p. 1813–1821, 2018.

MCKLVEEN, J. M. et al. Role of prefrontal cortex glucocorticoid receptors in stress and emotion. **Biological Psychiatry**, v. 74, n. 9, p. 672–679, 2013.

MIRAS-PORTUGAL, M. T. et al. Nucleotides in neuroregeneration and neuroprotection. **Neuropharmacology**, v. 104, p. 243–254, 2016.

MIZOGUCHI, K. et al. Chronic stress attenuates glucocorticoid negative feedback: Involvement of the prefrontal cortex and hippocampus. **Neuroscience**, v. 119, n. 3, p. 887–897, 2003.

MOCKING, R. J. T. et al. Metabolic features of recurrent major depressive disorder in remission, and the risk of future recurrence. **Translational Psychiatry**, v. 11, n. 1, p. 1–13, 2021.

MOLZ, S. et al. Neuroprotective effect of guanosine against glutamate-induced cell death in rat hippocampal slices is mediated by the phosphatidylinositol-3 kinase/Akt/glycogen synthase kinase 3β pathway activation and inducible nitric oxide synthase inhibition. **Journal of Neuroscience Research**, v. 89, n. 9, p. 1400–1408, 2011.

MONTGOMERY, A. M. The efficacy of fluoxetine as an antidepressant in the short and long term. **International Clinical Psychopharmacology**, v. 4, n. 1, p. 113–119, 1989.

MORAIS, M. et al. The effects of chronic stress on hippocampal adult neurogenesis and dendritic plasticity are reversed by selective MAO-A inhibition. **Journal of Psychopharmacology**, v. 28, n. 12, p. 1178–1183, 2014.

MORET, C. Combination/augmentation strategies for improving the treatment of depression. **Neuropsychiatric Disease and Treatment**, v. 1, n. 4, p. 301–309, 2005.

MORIGUCHI, S. et al. Glutamatergic neurometabolite levels in major depressive disorder: a systematic review and meta-analysis of proton magnetic resonance spectroscopy studies. **Molecular Psychiatry**, v. 24 p. 952–964, 2018.

MOSER, E. I.; MOSER, M. B.; MCNAUGHTON, B. L. Spatial representation in the hippocampal formation: a history. **Nature Neuroscience**, v. 20, n. 11, p. 1448–1464, 2017.

MUNHOZ, T. N. et al. A nationwide population-based study of depression in Brazil. **Journal of Affective Disorders**, v. 192, p. 226–233, 2016.

NAGASAWA, K. et al. Characterization of guanine and guanosine transport in primary cultured rat cortical astrocytes and neurons. **Glia**, v. 55, n. 14, p. 1397–1404, 2007.

NEIS, V. et al. Single administration of agmatine reverses the depressive-like behavior induced by corticosterone in mice: Comparison with ketamine and fluoxetine. **Pharmacology Biochemistry and Behavior**, v. 173, p. 44–50, 2018.

NEIS, V. B. et al. Acute agmatine administration, similar to ketamine, reverses depressive-like behavior induced by chronic unpredictable stress in mice. **Pharmacology Biochemistry and Behavior**, v. 150–151, p. 108–114, 2016.

NEIS, V. B. et al. The involvement of PI3K/Akt/mTOR/GSK3β signaling pathways in the antidepressant-like effect of AZD6765. **Pharmacology Biochemistry and Behavior**, v. 198, p. 173020, 2020.

NEMEROFF, C. B. The burden of severe depression: A review of diagnostic challenges and treatment alternatives. **Journal of Psychiatric Research**, v. 41, n. 3–4, p. 189–206, 2007.

NESTLER, E. J. et al. Neurobiology of depression. **Neuron**, v. 34, n. 1, p. 13–25, 2002.

NEWPORT, D. J. et al. Ketamine and other NMDA antagonists: Early clinical trials and possible mechanisms in depression. **American Journal of Psychiatry**, v. 172, n. 10, p. 950–966, 2015.

NICIU, M. J.; KELMENDI, B.; SANACORA, G. Overview of glutamatergic neurotransmission in the nervous system. **Pharmacology, Biochemistry and Behavior**, v. 100, n. 4, p. 656–664, 2012.

NISHIYAMA, J. Plasticity of dendritic spines: Molecular function and dysfunction in neurodevelopmental disorders. **Psychiatry and Clinical Neurosciences**, v. 73, n. 9, p. 541–550, 2019.

NUNINGA, J. O. et al. Volume increase in the dentate gyrus after electroconvulsive therapy in depressed patients as measured with 7T. **Molecular Psychiatry**, v. 25, n. 7, p. 1559–1568, 2019.

OLIVEIRA, Á.; ILLES, P.; ULRICH, H. Purinergic receptors in embryonic and adult neurogenesis. **Neuropharmacology**, v. 104, p. 272–281, 2016.

OLIVEIRA, D. L. DE et al. Quinolinic acid promotes seizures and decreases glutamate uptake in young rats: Reversal by orally administered guanosine. **Brain Research**, v. 1018, n. 1, p. 48–54, 2004.

OTTE, C. et al. Major depressive disorder. **Nature Reviews Disease Primers**, v. 2, p. 1–20, 2016.

PAPAKOSTAS, G. I. et al. A description of next-step switching versus augmentation practices for outpatients with treatment-resistant major depressive disorder enrolled in an academic specialty clinic. **Annals of Clinical Psychiatry**, v. 17, n. 3, p. 161–165, 2005.

PAPAKOSTAS, G. I.; IONESCU, D. F. Towards new mechanisms: An update on therapeutics for treatment-resistant major depressive disorder. **Molecular Psychiatry**, v. 20, n. 10, p. 1142–1150, 2015.

PARIANTE, C. M.; LIGHTMAN, S. L. The HPA axis in major depression: classical theories and new developments. **Trends in Neurosciences**, v. 31, n. 9, p. 464–468, 2008.

PARISE, E. M. et al. The resilient phenotype induced by prophylactic ketamine exposure during adolescence is mediated by the ventral tegmental area–nucleus accumbens pathway. **Biological Psychiatry**, v. 90, n. 7, p. 482–493, 2021.

PARK, Y. M. The hypothesis on the prediction of treatment response with buspirone augmentation along with serotonergic antidepressant in patients with major depressive disorder using loudness dependence of auditory evoked potentials: Two cases and review of the liter. **Psychiatry Investigation**, v. 17, n. 3, p. 222–224, 2020.

PATIL, S.; UNADKAT, J. Sodium-dependent nucleoside transport in the human intestinal membrane. **American Journal of Physiology**, v. 272, p. 1314–1320, 1997.

PATRIARCHI, T.; BUONARATI, O. R.; HELL, J. W. Postsynaptic localization and regulation of AMPA receptors and Cav1.2 by β2 adrenergic receptor/PKA and Ca2+/CaMKII signaling. **The EMBO Journal**, v. 37, n. 20, p. 99771, 2018.

PAYNE, H. L. The role of transmembrane AMPA receptor regulatory proteins (TARPs) in neurotransmission and receptor trafficking. **Molecular Membrane Biology**, v. 25, n. 4, p. 353–362, 2008.

PAZINI, F. L. et al. Creatine, similar to ketamine, counteracts depressive-like behavior induced by corticosterone via PI3K/Akt/mTOR pathway. **Molecular Neurobiology**, v. 53, n. 10, p. 6818–6834, 2016.

PAZINI, F. L. et al. mTORC1-dependent signaling pathway underlies the rapid effect of creatine and ketamine in the novelty-suppressed feeding test. **Chemico-Biological Interactions**, v. 332, p. 109281, 2020.

PENG, L. et al. Nucleoside Transporter Expression and Function in Cultured Mouse Astrocytes. **Glia**, v. 35, n. 5, p. 25–35, 2005.

PENN, E.; TRACY, D. K. The drugs don't work? antidepressants and the current and future pharmacological management of depression. **Therapeutic Advances in Psychopharmacology**, v. 2, n. 5, p. 179–188, 2012.

PEREIRA, V. S.; HIROAKI-SATO, A. A brief history of antidepressant drug development: from tricyclics to beyond ketamine. **Acta Neuropsychiatric**a, v. 30, n. 6, p. 307–322, 2018.

PETRONILHO, F. et al. Protective effects of guanosine against sepsis-induced damage in rat brain and cognitive impairment. **Brain, Behavior, and Immunity**, v. 26, n. 6, p. 904–910, 2012.

PIERMARTIRI, T. et al. Guanosine Promotes Proliferation in Neural Stem Cells from Hippocampus and Neurogenesis in Adult Mice. **Molecular Neurobiology**, v. 57, n. 9, p. 3814–3826, 2020.

POCHWAT, B.; NOWAK, G.; SZEWCZYK, B. An update on NMDA antagonists in depression. **Expert Review of Neurotherapeutics**, v. 19, n. 11, p. 1055–1067, 2019.

POPOLI, M. et al. The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. **Nature Reviews Neuroscience**, v. 13, n. 1, p. 22–37, 2011.

POPP, S. et al. In search of the mechanisms of ketamine's antidepressant effects: How robust is the evidence behind the mTOR activation hypothesis. **F1000Research**, v. 5, p. 1–9, 2016.

QIAO, H. et al. Dendritic spines in depression: What we learned from animal models. **Neural plasticity**, v. 2016, p. 1–19, 2016.

RAFALO-ULINSKA, A. et al. Zinc transporters protein level in postmortem brain of depressed subjects and suicide victims. **Journal of Psychiatric Research**, v. 83, p. 220–229, 2016.

RAMOS, D. B. et al. Intranasal guanosine administration presents a wide therapeutic time window to reduce brain damage induced by permanent ischemia in rats. **Purinergic Signalling**, v. 12, n. 1, p. 149–159, 2016.

RIBEIRO, F. F. et al. Purine nucleosides in neuroregeneration and neuroprotection. **Neuropharmacology**, v. 104, p. 226–242, 2016.

RIBONI, F. V.; BELZUNG, C. Stress and psychiatric disorders: from categorical to dimensional approaches. **Current Opinion in Behavioral Sciences**, v. 14, p. 72–77, 2017.

RODRIGUES, R. J.; MARQUES, J. M.; CUNHA, R. A. Purinergic signalling and brain development. **Seminars in Cell & Developmental Biology**, v. 95, p. 34–41, 2019.

ROSA, P. B. et al. The antidepressant-like effect of guanosine is dependent on GSK-3β inhibition and activation of MAPK/ERK and Nrf2/heme oxygenase-1 signaling pathways. **Purinergic Signalling**, v. 15, n. 4, p. 491–504, 2019.

ROSA, P. B. et al. Antidepressant-like effect of guanosine involves activation of AMPA receptor and BDNF/TrkB signaling. **Purinergic Signalling**, p. 1–17, 2021.

RUSSO, S. J. et al. Neurobiology of resilience. **Nature Neuroscience**, v. 15, n. 11, p. 1475–1484, 2012.

SANACORA, G.; TRECCANI, G.; POPOLI, M. Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. **Neuropharmacology**, v. 62, n. 1, p. 63–77, 2012.

SANTOMAURO, D. F. et al. Global prevalence and burden of depressive and anxiety disorders in 204 countries and territories in 2020 due to the COVID-19 pandemic. **Lancet**, v. 398, p. 1700–1712, 2021.

SCHARFMAN, H. E. The enigmatic mossy cell of the dentate gyrus. **Nature Reviews Neuroscience**, v. 17, n. 9, p. 562–575, 2016.

SCHMIDT, A. P. et al. Mechanisms involved in the antinociception induced by systemic administration of guanosine in mice. **British Journal of Pharmacology**, v. 159, n. 6, p. 1247–1263, 2010.

SCHMIDT, A. P.; LARA, D. R.; SOUZA, D. O. Proposal of a guanine-based purinergic system in the mammalian central nervous system. **Pharmacology and Therapeutics**, v. 116, n. 3, p. 401–416, 2007.

SCHULTZ, C.; ENGELHARDT, M. Anatomy of the hippocampal formation. **Frontiers of neurology and neuroscience**, v. 34, p. 6–17, 23 2014.

SEGAL, M. Dendritic spines and long-term plasticity. **Nature Reviews Neuroscience**, v. 6, n. 4, p. 277–284, 2005.

SENZAI, Y. Function of local circuits in the hippocampal dentate gyrus-CA3 system. **Neuroscience Research**, v. 140, p. 43–52, 2019.

SKOLNICK, P. Antidepressants for the new millennium. **European Journal of Pharmacology**, v. 375, p. 31–40, 1999.

SONG, S. H.; AUGUSTINE, G. J. Synapsin isoforms and synaptic vesicle trafficking. **Molecules and Cells**, v. 38, n. 11, p. 936, 2015.

STERNER, E. Y.; KALYNCHUK, L. E. Behavioral and neurobiological consequences of prolonged glucocorticoid exposure in rats: Relevance to depression. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 34, n. 5, p. 777-790, 2010.

STETLER, C.; MILLER, G. E. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. **Psychosomatic Medicine**, v. 73, n. 2, p. 114–126, 2011.

STRANGE, B. A. et al. Functional organization of the hippocampal longitudinal axis. **Nature Reviews Neuroscience**, v. 15, n. 10, p. 655–669, 2014.

SÜDHOF, T. C. Synaptic neurexin complexes: A molecular code for the logic of neural circuits. **Cell**, v. 171, n. 4, p. 745–769, 2017.

SÜDHOF, T. C. Towards an understanding of synapse formation. **Neuron**, v. 100, n. 2, p. 276–293, 2018.

TANTI, A.; BELZUNG, C. Neurogenesis along the septo-temporal axis of the hippocampus: are depression and the action of antidepressants region-specific? **Neuroscience**, v. 252, p. 234–252, 12 nov. 2013.

TASCA, C. I. et al. Neuromodulatory effects of guanine-based purines in health and disease. **Frontiers in Cellular Neuroscience**, v. 12, p. 1-14, 2018.

TEFFER, K.; SEMENDEFERI, K. Human prefrontal cortex: evolution, development, and pathology. **Progress in Brain Research**, v. 195, p. 191–218, 2012.

TEJEDA, G. S.; DÍAZ-GUERRA, M. Integral characterization of defective BDNF/TrkB signalling in neurological and psychiatric disorders leads the way to new therapies. **International Journal of Molecular Sciences**, v. 18, n. 2, p. 1–24, 2017.

THALHAMMER, A.; CINGOLANI, L. A. Cell adhesion and homeostatic synaptic plasticity. **Neuropharmacology**, v. 78, n. C, p. 23–30, 2014.

THOMAZ, D. T. et al. Guanosine prevents nitroxidative stress and recovers mitochondrial membrane potential disruption in hippocampal slices subjected to oxygen/glucose deprivation. **Purinergic Signalling**, v. 12, n. 4, p. 707–718, 2016.

TOMASETTI, C. et al. Novel pathways in the treatment of major depression: focus on the glutamatergic system. **Current Pharmaceutical Design**, v. 25, n. 4, p. 381–387, 2019.

TORRES, F. V. et al. Electrophysiological effects of guanosine and MK-801 in a quinolinic acid-induced seizure model. **Experimental Neurology**, v. 221, n. 2, p. 296–306, 2010.

TRULLAS, R.; SKOLNICK, P. Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. **European Journal of Pharmacology**, v. 185, n. 1, p. 1–10, 1990.

VINADÉ, E. R. et al. Effects of chronic administered guanosine on behavioral parameters and brain glutamate uptake in rats. **Journal of Neuroscience Research**, v. 79, n. 1–2, p. 248–253, 2005.

WATT, A. J. et al. A proportional but slower NMDA potentiation follows AMPA potentiation in LTP. **Nature Neuroscience**, v. 7, n. 5, p. 518–524, 2004.

WEI, Y.; CHANG, L.; HASHIMOTO, K. A historical review of antidepressant effects of ketamine and its enantiomers. **Pharmacology Biochemistry and Behavior**, v. 190, p. 172870, 2020.

WENG, L. et al. Apigenin reverses depression-like behavior induced by chronic corticosterone treatment in mice. **European Journal of Pharmacology**, v. 774, p. 50–54, 2016.

WORLD HEALTH ORGANIZATION. Depression and other common mental disorders: Global health estimates. **World Health Organization**, p. 1–24, 2017.

WU, X. et al. Neuroligin-1 signaling controls LTP and NMDA receptors by distinct molecular pathways. **Neuron**, v. 102, n. 3, p. 621- 635, 2019.

YATES, D. A synaptic bridge. **Nature Reviews Neuroscience**, v. 17, n. 3, p. 135–135, 2016.

YOSHII, A.; CONSTANTINE-PATON, M. Post-synaptic BDNF-TrkB signaling in synapse maturation, plasticity and disease. **Developmental Neurobiology**, v. 70, n. 5, p. 304, 2010.

YUSTE, R. Dendritic spines and distributed circuits. **Neuron**, v. 71, n. 5, p. 772–781, 2011.

ZARATE, C. A. et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. **Archives of General Psychiatry**, v. 63, n. 8, p. 856–864, 2006.

ZENI, A. L. B.; CAMARGO, A.; DALMAGRO, A. P. Lutein prevents corticosterone-induced depressive-like behavior in mice with the involvement of antioxidant and neuroprotective activities. **Pharmacology Biochemistry and Behavior**, v. 179, p. 63–72, 2019.

ZHOU, J. et al. Scopolamine augmentation of a newly initiated escitalopram treatment for major depressive disorder: Study protocol for a randomized controlled trial. **Trials**, v. 20, n. 1, p. 1–7, 2019.

ZHOU, X. et al. Atypical Antipsychotic Augmentation for Treatment- Resistant Depression: A systematic review and network meta-analysis. **International Journal of Neuropsychopharmacology**, p. 1–10, 2015.