UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:
BIOQUÍMICA TOXICOLÓGICA

Paula Michelotti

PARÂMETROS COMPORTAMENTAIS E FISIOLÓGICOS EM PEIXES-ZEBRA ADULTOS EXPOSTOS A CETAMINA

Santa Maria, RS
2020

Paula Michelotti

PARÂMETROS COMPORTAMENTAIS E FISIOLÓGICOS EM PEIXES-ZEBRA ADULTOS EXPOSTOS A CETAMINA

Dissertação apresentada ao curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM), como requisito parcial para obtenção do título de **Mestre em Bioquímica Toxicológica.**

Orientadora: Profª. Drª. Maria Ester Pereira

Santa Maria, RS
2020

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

Michelotti, Paula
PARÂMETROS COMPORTAMENTAIS E FISIOLÓGICOS EM PEIXES ZEBRA ADULTOS EXPOSTOS A CETAMINA / Paula Michelotti.- 2020.
78 p.; 30 cm

Orientadora: Maria Ester Pereira
Coorientador: Denis Broock Rosemberg
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, RS, 2020

1. Danio rerio 2. Agressividade 3. Comportamento estereotipado 4. Consolidação da memória 5. Antagonista glutamatérgico I. Pereira, Maria Ester II. Broock Rosemberg, Denis III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, PAULA MICHELOTTI, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Dissertação) foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de qualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

Paula Michelotti

PARÂMETROS COMPORTAMENTAIS E FISIOLÓGICOS EM PEIXES-ZEBRA ADULTOS EXPOSTOS A CETAMINA

Dissertação apresentada ao curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM), como requisito parcial para obtenção do título de **Mestre em Bioquímica Toxicológica.**

Aprovado em 29 de julho de 2020:

Maria Ester Pereira

Maria Ester Pereira, Drª. (UFSM) - Videoconferência
(Presidente/Orientadora)

Denis Broock Rosemberg, Dr. (UFSM) - Videoconferência
(Co-orientador)

Anne M Siebel

Anna Maria Siebel, Drª. (UNOCHAPECÓ) - Videoconferência

Sara Marchesan de Oliveira

Sara Marchesan de Oliveira, Drª. (UFSM) - Videoconferência

Santa Maria, RS
2020

DEDICATÓRIA

Dedico este trabalho aos meus pais, Lourdes e Ivanir, que sempre me apoiaram e não mediram esforços para que alcançasse meus objetivos.

AGRADECIMENTOS

Agradeço a Deus pela vida, por me proporcionar tudo isso, por me guiar, iluminar e me dar tranquilidade para seguir em frente com os meus objetivos e não desanimar com as dificuldades.

Agradeço a minha família pelo apoio incondicional. Aos meus pais, Lourdes e Ivanir, que sempre torceram por mim, a vocês eu devo tudo e todo meu amor. A minha tia Marli, que sempre está presente na minha vida me apoiando.

Toda minha gratidão a minha orientadora profª Drª. Maria Ester Pereira por me acolher e por aceitar me orientar com muita paciência e disposição para contribuir com o meu aprendizado. Também agradeço ao meu co-orientador prof. Dr. Denis Broock Rosemberg por todos ensinamentos.

Agradeço aos colegas do Laboratório de Neuropsicobiologia Experimental, especialmente pelas minhas amigas e colegas Talise, Francini, Flávia e Thiele pelos momentos vividos dentro e fora da universidade, sejam eles bons ou ruins, por toda a ajuda na realização deste trabalho.

Agradeço as minhas amigas Joseane e Viviane por sempre estarem presentes na minha vida e que, longe ou perto, sempre me ajudam.

Agradeço a UFSM e a CAPES pelo apoio financeiro.

“Talvez não tenha conseguido fazer o melhor, mas lutei para que o melhor fosse feito. Não sou o que deveria ser, mas Graças a Deus, não sou o que era antes”.

(Marthin Luther King)

RESUMO

PARÂMETROS COMPORTAMENTAIS E FISIOLÓGICOS EM PEIXES-ZEBRA ADULTOS EXPOSTOS A CETAMINA

AUTOR: Paula Michelotti
ORIENTADOR: Profª. Drª. Maria Ester Pereira

A cetamina é um anestésico amplamente usado na clínica humana e veterinária e também como uma droga de abuso. Possui propriedade anestésica dissociativa que em doses subanestésicas induz analgesia. Embora a cetamina apresente propriedades ansiolíticas e antidepressivas, ela pode induzir efeitos pró-psicóticos e alucinógenos, bem como comportamentos estereotipados após a administração de doses subanestésicas. O uso de modelos animais alternativos, como o peixe-zebra, tem sido importante para estudos toxicológicos e neuropsiquiátricos. O peixe-zebra (*Danio rerio*) é um pequeno teleósteo amplamente utilizado na pesquisa de neurociência comportamental, pois apresenta conservação na fisiologia, neurotransmissores caracterizados e genoma completamente sequenciado e similar aos humanos (70%). Considerando que muitas doenças neuropsiquiátricas apresentam alterações em parâmetros comportamentais e bioquímicos, o estudo sobre os efeitos promovidos por doses subanestésicas de cetamina em peixe-zebra é relevante. Assim, investigou-se os efeitos da exposição à cetamina por 20 min (0, 2, 20 ou 40 mg/L) aguda e repetidamente (7 dias) na agressividade, locomoção, comportamento estereotipado, consolidação da memória e na ansiedade. Além disso, analisamos os níveis de cortisol em corpo inteiro e a atividade da acetilcolinesterase em encéfalo de peixes-zebra adultos. Resultados da exposição aguda mostram um aumento nos fenótipos de agressividade induzido pelo espelho na concentração de 2 mg/L e redução nas concentrações de 20 e 40 mg/L; observamos também um aumento do comportamento estereotipado de nado circular em peixes expostos a concentração de 40 mg/L, e alterações nos parâmetros motores devido a hiperlocomoção nas concentrações de 20 e 40 mg/L. A consolidação da memória foi prejudicada pela exposição à concentração de 40 mg/L. Nenhuma das concentrações alterou a atividade da acetilcolinesterase. Os resultados da exposição repetida mostram que os parâmetros comportamentais como locomoção, agressividade e ansiedade, juntamente com dosagem de cortisol e atividade da acetilcolinesterase não foram alterados pela exposição a cetamina. No entanto, os animais expostos a concentração de 40 mg/L apresentaram comportamento estereotipado. Em suma, verificamos que as alterações comportamentais induzidas pela cetamina dependem da tarefa comportamental e da dose testada. Tomando em conjunto, podemos sugerir que este modelo animal pode ser útil para investigar os efeitos de concentrações subanestésicas de cetamina sobre parâmetros comportamentais; porém, concentrações intermediárias em relação as que foram usadas nesse estudo, outras tarefas comportamentais, assim como outros intervalos de exposição precisam ser estudados.

Palavras-chave: *Danio rerio*. Agressividade. Comportamento estereotipado. Consolidação da memória. Antagonista glutamatérgico.

ABSTRACT

BEHAVIORAL AND PHYSIOLOGICAL PARAMETERS IN ADULT ZEBRAFISH EXPOSED TO KETAMINE

AUTHOR: Paula Michelotti
ADVISOR: Profª. Drª. Maria Ester Pereira

Ketamine is an anesthetic widely used in human and veterinary clinic and also as a drug of abuse. It has dissociative anesthetic property that in subanesthetic doses induces analgesia. Although ketamine displays anxiolytic and antidepressant properties, it may induce pro-psychosis and hallucinogen effects, as well as stereotypic behaviors following acute administration at sub-anesthetic doses. Zebrafish (*Danio rerio*) is a small teleost widely used in behavioral neuroscience research, because it presents conservation in physiology, characterized neurotransmitters and genome completely sequenced and similar to humans (70%). Considering that many neuropsychiatric diseases are related to changes in behavioral and biochemical parameters, the study of the effects promoted by subanesthetic doses of ketamine in zebrafish is relevant. Thus, we investigated the effects of ketamine exposure for 20 min (0, 2, 20 or 40 mg/L) acutely and repeatedly (7 days) on aggression, locomotion, stereotyped behavior, memory consolidation, and anxiety. In addition, we analyze whole-body cortisol levels and brain acetylcholinesterase activity in adult zebrafish. Results of acute exposure show an increase in the phenotypes of aggression induced by the mirror at a concentration of 2 mg/L and a reduction in the concentration of 20 and 40 mg/L; we also observed an increase in stereotyped behavior of circular swimming in fish exposed to concentration of 40 mg/L, and locomotor alterations due to hyperlocomotion in concentrations of 20 and 40 mg/L. Memory consolidation is impaired at concentration of 40 mg/L. None of the doses altered the activity of acetylcholinesterase. The results of repeated exposure show that behavioral parameters such as locomotion, aggression and anxiety, along with whole-body cortisol levels and acetylcholinesterase activity were not altered by exposure to Ketamine. However, animals exposed to a concentration of 40 mg/L showed stereotyped behavior. In short, we found that the behavioral changes induced by ketamine depend on the behavioral task and the tested dose. Taken together, we can suggest that this animal model may be useful to investigate the effects of subanesthetic ketamine concentrations on behavioral parameters; however, intermediate concentration in relation to that used in this study, other behavioral tasks, as well as other exposure intervals need to be studied.

Keywords: *Danio rerio*. Aggressiveness. Stereotyped behavior. Memory consolidation. Glutamatergic antagonistic.

LISTA DE FIGURAS

LISTA DE ABREVITURAS E SIGLAS

AChE	Acetilcolinesterase
ChAT	Colina acetiltransferase
DTNB	Ácido 5,5'-ditiobis-2-nitrobenzoico
GABA	Ácido gama-aminobutírico
hpf	Horas pós-fertilização
HPI	Hipotálamo-pituitária-interrenal
i.p.	Intraperitoneal
MK-801	Dizocilpina
NMDA	N-metil-D-aspartato
pH	Potencial hidrogeniônico
SNC	Sistema nervoso central
TFK	Tampão fosfato de potássio
vAChT	Transportador vesicular de acetilcolina
WT	Linhagem selvagem, do inglês 'wild-type'

SUMÁRIO

1 INTRODUÇÃO

1.1 CETAMINA

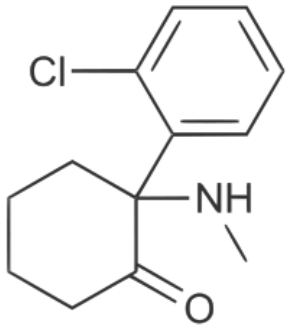
A cetamina é um anestésico amplamente usado em humanos, na veterinária e também como droga de abuso (LIAO et al., 2018; RIEHL et al., 2011). Foi sintetizada no ano de 1962 por Calvin Lee Stevens no laboratório de química orgânica da Wayne State University, nos Estados Unidos, em colaboração com a companhia farmacêutica Parke-Davis. A cetamina foi desenvolvida como uma droga alternativa ao uso da fenciclidina, um anestésico dissociativo desenvolvido em 1956. Este apresentou diversos efeitos adversos como delírios e efeitos psicóticos por mais de 12 horas, o que tornou seu uso indesejável (DOMINO; LUBY, 2012; GREIFENSTEIN et al., 1958; JOHNSTONE; EVANS; BAIGEL, 1959). A primeira dose de cetamina a ser administrada em humanos foi no ano de 1964, onde evidenciaram que esta é uma droga aparentemente segura e eficaz para uso na anestesia clínica (DOMINO; CHODOFF; CORSSEN, 1965; LI; VLISIDES, 2016).

1.1.1 Química e farmacocinética da cetamina

A cetamina, ((RS)-2-(2-clorofenil)-2-(metilamino)ciclohexan-1-ona) (Figura 1) possui a fórmula molecular $C_{13}H_{16}NClO$ (WHITE; WAY; TREVOR, 1982). Ela é comercializada com nome Ketalar ou Cetamin (DOMINO, 2010; WHITE; WAY; TREVOR, 1982). Possui dois estereoisômeros ópticos S (+) e R (-), sendo que o isômero S (+) possui uma potência anestésica de 3 a 4 vezes maior que o isômero R (-) e apresenta menos efeitos adversos. A forma comercial é uma mistura racêmica em solução ácida (pH de 3,5- 5,5 e pKa de 7,5), totalmente solúvel em água, e peso molecular de 237 Da (KHARASCH; LABROO, 1992; WHITE et al., 1985).

Em humanos, a cetamina possui um tempo de meia-vida de 180 minutos. Sua metabolização consiste em reações de N-desmetilação pelas enzimas do citocromo P450 no fígado, sendo elas citocromo P450 3A4 (CYP3A4), citocromo P450 2B6 (CYP2B6) e citocromo P450 2C9 (CYP2C9). As enzimas transformam a cetamina em norcetamina (80%), o qual é um metabólito ativo que possui atividade biológica de 1/3 a 1/5 da cetamina e, posteriormente, em 6-hidroxi-norcetamina (15%) que é excretado na bile e urina após a glicuronoconjugação. A cetamina também apresenta outros metabólitos cujos efeitos são pouco estudados e elucidados (CHEN et al., 2018; SINNER; GRAF, 2008).

Figura 1- Representação da fórmula estrutural da cetamina



Fonte: Modificado de White et al (1982).

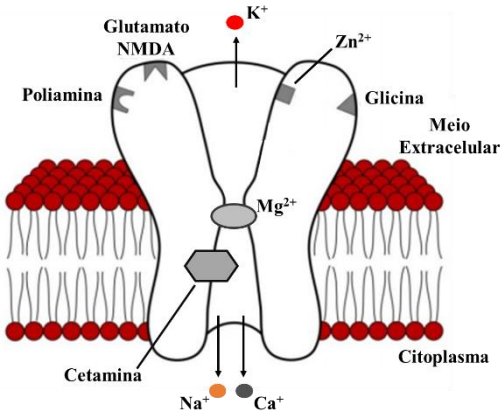
1.1.2 Mecanismos de ação e uso na clínica

A neurofarmacologia da cetamina é muito complexa pois este fármaco atua em diversos tipos de receptores. No sistema glutamatérgico, atua como um antagonista não competitivo de receptor ionotrópico N-metil-D-asparato (NMDA) (RIEHL et al., 2011) (Figura 2). O glutamato é o aminoácido mais abundante no sistema nervoso central (SNC), sendo o principal neurotransmissor excitatório em vertebrados (FEATHERSTONE, 2010). A cetamina age também em receptores opióides, monoaminérgicos, colinérgicos e do ácido gama-aminobutírico (GABA) (HUSTVEIT; MAURSET; OYE, 1995; RIEHL et al., 2011). Basicamente, a cetamina apresenta efeito agonista nos receptores opióides e $GABA_A$ (IRIFUNE et al., 2000; SMITH; WESTFALL; ADAMS, 1982), e apresenta efeito antagonista em receptores muscarínicos (DURIEUX, 1995) e nicotínicos (O'DELL et al., 1991) inibindo a transmissão colinérgica no SNC. Além disso, aumenta a transmissão neural de neurotransmissores monoaminérgicos (serotonina, noradrenalina e dopamina) (CRISP et al., 1991).

A cetamina possui propriedade anestésica dissociativa por induzir perda sensorial marcante, amnésia e paralisia de movimentos sem perda real da consciência, ocorrendo intensa sensação de dissociação do meio (MORGAN et al., 2004). Apresenta também ação analgésica quando utilizada em doses subanestésicas (ENGIN; TREIT; DICKSON, 2009). Sabe-se que em humanos a dose anestésica de cetamina é de 1,5 – 2,0 mg/kg por via endovenosa (WHITE; WAY; TREVOR, 1982); porém, em doses subanestésicas esta droga apresenta diversos efeitos, tais como antidepressivo (ZARATE et al., 2006), alucinógeno e sedativo (DICKERSON et al., 2010; KRYSTAL et al., 2005), dissociativo e pró-psicótico (KRYSTAL et al., 1994; LI et al.,

2011). Estudos demonstram que quando a cetamina é administrada em doses subanestésicas em roedores, estes apresentam um aumento na locomoção (WILSON et al., 2005), comportamentos estereotipados e alterações na interação social (CANEVER et al., 2010), bem como efeitos ansiolíticos e antidepressivos (ENGIN; TREIT; DICKSON, 2009; KRYSTAL et al., 1994).

Figura 2 - Estrutura e principais locais de ligação do receptor NMDA



Fonte: Modificado de Tomek et al (2013).

1.1.3 A cetamina e modelos animais

Conhecendo os diversos efeitos comportamentais e fisiológicos que a cetamina desempenha, seu uso vai além da anestesia; é empregado em diversos modelos experimentais de dor aguda e crônica (NOWACKA; BORCZYK, 2019), depressão (LI et al., 2011), ansiedade (PAPP et al., 2016), esquizofrenia (OLNEY; FARBER, 1995) e memória (NEWCOMER et al., 1999).

Estudo em ratos mostra que a administração repetida de cetamina reduz o efeito hiperalgésico induzido por opióides (LAULIN et al., 2002). Em humanos, estudos usam a cetamina nas diferentes síndromes de dor crônica, como neuropáticas (EIDE et al., 1995), síndrome da dor regional (KIEFER et al., 2008), dor de câncer (MERCADANTE et al., 2000) e fibromialgia (GRAVEN-NIELSEN et al., 2000); e, quando adicionada ao tratamento com opióides na dor aguda evita a depressão respiratória (LUGINBÜHL et al., 2003; NESHER et al., 2009).

A cetamina também é usada em tratamentos de depressão maior, onde a ação antidepressiva rápida (em poucas horas) e sustentada (até 7 dias) acontece após uma única

administração intravenosa da droga (0,5 mg/kg) em pacientes resistentes ao tratamento (ZARATE et al., 2006). Redução do pensamento suicida por 24 horas (WILKINSON et al., 2018) ou 4 h também foi relatado após a infusão endovenosa de cetamina (0,5 mg/kg) (DIAZGRANADOS et al., 2010). O efeito ansiolítico da cetamina administrada agudamente por via intraperitoneal (i.p.) (10mg/kg) também é encontrado em camundongos (FRAGA et al., 2018).

Na esquizofrenia podemos evidenciar o surgimento de alterações comportamentais, como agressividade e ansiedade (HOPTMAN, 2015). A cetamina é usada em modelo experimental para induzir sintomas característicos de esquizofrenia, pois induz sintomas positivos, negativos e déficit cognitivo em roedores (COYLE et al., 2012; JAVITT, 2012). Um estudo recente mostra que a administração aguda de cetamina (15 mg/kg, i.p.) em camundongos diminui a atividade locomotora, mas não tem efeito significativo no comportamento do tipo agressivo. Já em tratamento crônico (7 dias), a mesma dose de cetamina não teve efeito na locomoção, mas diminuiu a ansiedade e agressividade dos animais (SHIN et al., 2019).

A cetamina também pode ser usada em testes de memória (NEWCOMER et al., 1999). Em ratos, os efeitos da cetamina na memória são controversos; um estudo mostra que doses subanestésicas de 50 e 100 mg/kg de cetamina (i.p.) não alteram a memória (CHROBAK; HINMAN; SABOLEK, 2008), enquanto outro estudo verificou comprometimento da consolidação da memória em doses de 20 ou 100 mg/kg (i.p.) (BOULTADAKIS; PITSIKAS, 2011). Em camundongos, também usando doses subanestésicas (5, 10, 20 e 50 mg/kg, i.p.), foi encontrada a interrupção da reconsolidação de memória (WANG et al., 2006). Em humanos também foi relatada diminuição da memória de trabalho (ADLER et al., 1998) e déficit na formação da memória (UMBRICHT et al., 2000) quando a cetamina é administrada por via intravenosa.

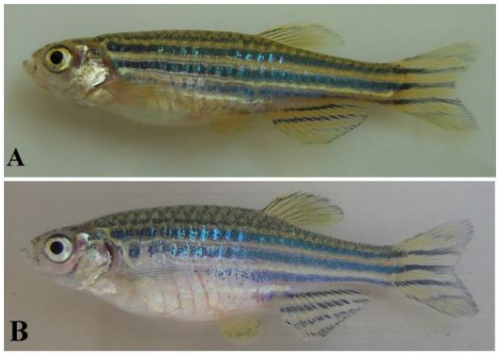
1.2 PEIXE-ZEBRA COMO ORGANISMO MODELO

O uso de modelos alternativos não mamíferos tem sido muito importante em estudos toxicológicos, farmacológicos e neurocomportamentais (BRAGA et al., 2013; HOWE et al., 2013; PIATO et al., 2011). O peixe-zebra (*Danio rerio*) (Figura 3) é uma espécie tropical de água doce conhecida popularmente como “paulistinha”, a qual pertence à família *Cyprinidae* e nativa da Ásia (WHITLOCK; WESTERFIELD, 2000). Dentre as vantagens apresentadas podemos citar o pequeno tamanho (adultos podem medir de 3 a 5 cm), o baixo custo e o pequeno espaço requerido para manutenção. O peixe-zebra apresenta uma grande prole (50 a 200 ovos

por dia para cada fêmea em condições otimizadas de reprodução), ovos translúcidos e rápido desenvolvimento até a fase adulta (aproximadamente 2–3 meses) (DAHM; GEISLER, 2006; LELE; KRONE, 1996).

O peixe-zebra possui características fisiológicas conservadas, genoma completamente sequenciado com similaridade em comparação aos genes de mamíferos (aproximadamente 70%) (HOWE et al., 2013). Além disso, apresenta os principais sistemas de neurotransmissores presentes em mamíferos (RICO et al., 2011), possibilitando a validação de novos modelos experimentais relacionados a doenças humanas (FONTANA et al., 2018; HOWE et al., 2013; LIESCHKE; CURRIE, 2007; PANULA et al., 2006). Uma característica importante deste modelo é a possibilidade da administração de fármacos/compostos hidrossolúveis, uma vez que podem ser diretamente dissolvidos na água do tanque e absorvidas pelos peixes, principalmente pelas brânquias (FONTANA et al., 2018). O peixe-zebra apresenta um conjunto comportamental extenso e bem caracterizado, e com isso, é crescente o uso como um organismo modelo em diferentes áreas do conhecimento, como toxicologia e neuropsiquiatria (BLASER; CHADWICK; MCGINNIS, 2010; EDWARDS; MICHEL, 2002; EGAN et al., 2009; FONTANA et al., 2016; GERLAI, 2003; KALUEFF et al., 2013). Estas características fortalecem o uso do peixe para avaliação dos efeitos de fármacos com ação sobre o SNC, além de sua influência em respostas defensivas e fenótipos relacionados aos diferentes comportamentos (GERLAI, 2010, 2011; MAXIMINO et al., 2010a; WAY et al., 2015).

Figura 3 - Peixe-zebra adulto (*Danio rerio*) tipo selvagem macho (A) e fêmea (B)



Fonte: Avdesh et al (2012).

1.2.1 Parâmetros locomotores

Dentre os comportamentos analisados em peixe-zebra, a atividade locomotora é um parâmetro investigado com recorrência, sendo analisados a velocidade máxima, distância percorrida e ângulo absoluto de giro. Um estudo usando MK-801 (Dizocilpina), mostra um aumento na distância percorrida e velocidade média, caracterizando hiperlocomoção em peixe-zebra (SEIBT et al., 2010). Já o comportamento de rotação (*circular swimming*) é definido pela natação repetida em direção circular (KALUEFF et al., 2013). É um comportamento estereotipado, pois se caracteriza por um padrão de comportamentos rígidos e repetitivos, e que pode ser induzido por drogas alucinógenas (KYZAR et al., 2012).

1.2.2 Comportamento do tipo ansiedade

A ansiedade é um comportamento apresentado à avaliação de risco de uma potencial ameaça, seja ela por uma exposição ao ambiente novo ou um estímulo aversivo no ambiente; embora seja uma reação adaptativa natural em humanos e animais, pode se tornar patológico (BLANCHARD; BLANCHARD, 1988; MAXIMINO et al., 2010b; STEIMER, 2002). O teste do tanque novo é empregado de forma consolidada para avaliar o comportamento inato de nado e fuga em ambientes novos (CACHAT et al., 2010; EGAN et al., 2009; KYSIL et al., 2017). Quando os peixes são expostos à ambientes novos, apresentam um conflito comportamental entre ir para a superfície do aparato em busca de alimento/parceiros ou ir para o fundo em busca de proteção, por isso é um dos testes utilizados indicados para estudo de ansiedade em peixe-zebra (MAXIMINO et al., 2010b). Quando o peixe permanece mais tempo no topo do aparato do teste representa um efeito ansiolítico; drogas antagonistas de receptor NMDA induzem este efeito (SEIBT et al., 2010).

1.2.3 Comportamento do tipo agressividade

A agressividade é um comportamento que ocorre tanto em humanos quanto animais, a qual pode culminar em danos a outros organismos em decorrência de comportamentos impulsivos, violência e luta (GODAR et al., 2016; RAMBO et al., 2017; THEODORIDI; TSALAFOUTA; PAVLIDIS, 2017). O comportamento agressivo pode estar associado a aspectos genéticos, neurofisiológicos e hormonais, que podem sofrer modificações ao longo do tempo devido a experiências traumáticas (COMAI et al., 2012; WAY et al., 2015). A

agressividade pode estar relacionada a vários distúrbios psiquiátricos, mostrando uma alta prevalência em pacientes psicóticos com comportamento antissocial (DAS et al., 2018; SPIDEL et al., 2010). Em peixes-zebra, o comportamento agressivo é caracterizado por natação rápida em direção ao oponente, nadadeiras eretas, movimentos corporais ondulatórios e mordidas (JONES; NORTON, 2015). Peixes-zebra expostos ao MK-801 apresentam uma redução na agressividade (ZIMMERMANN et al., 2016).

1.2.4 Memória

O peixe-zebra é uma espécie capaz de discriminar estímulos aversivos condicionais e incondicionais de diferentes naturezas, tais como alternância espacial, discriminação visual e condicionamento olfativo, dentre outros tipos de memórias (BLANK et al., 2009). A região do cérebro responsável por essa tarefa no peixe-zebra é o telencéfalo, que é uma região análoga ao hipocampo e amígdala em cérebro de mamíferos (RICO et al., 2011). A ativação do receptor NMDA está relacionada com a aprendizagem e memória (NG et al., 2012) e o uso de antagonistas desse receptor, tal como a cetamina, poderia culminar na modulação destas respostas comportamentais (BLANK et al., 2009; NG et al., 2012; SISON; GERLAI, 2011). De fato, estudos demonstram que a exposição ao MK-801 prejudica a aquisição (NG et al., 2012) e consolidação da memória em peixe-zebra (BERTONCELLO et al., 2019; BLANK et al., 2009; FRANSCESCON et al., 2020).

1.3 PARÂMETROS BIOQUÍMICOS

1.3.1 Cortisol

Sabemos que comportamento agressivo e ansiogênico são geralmente associados a distúrbios na regulação das emoções e alterações das funções autonômicas e neuroendócrinas (NEUMANN; VEENEMA; BEIDERBECK, 2010). Um dos parâmetros neuroendócrinos que podemos dosar é o cortisol. Este hormônio corticoesteróide é liberado pelo eixo hipotálamo-pituitária-interrenal (HPI) no peixe-zebra que é homólogo ao eixo hipotálamo-pituitária-adrenal (HPA) em seres humanos, sendo que sua liberação é alterada quando exposto a fatores estressores (OLIVEIRA et al., 2013, 2014). Em um estudo onde peixe-zebra é exposto a cetamina nas concentrações de 20 e 40 mg/L verifica-se diminuição nos níveis de cortisol concomitante ao seu efeito ansiolítico (RIEHL et al., 2011). Em outro estudo verificou-se que

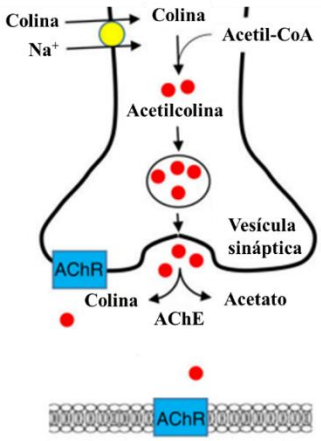
peixe-zebra exposto a cetamina na concentração de 20 mg/L por 2 semanas apresenta aumento nos níveis de cortisol e quando associada a fluoxetina apresenta efeito ansiolítico (PITTMAN; HYLTON, 2015).

1.3.2 Sistema colinérgico: Acetilcolinesterase

A acetilcolina é um neurotransmissor sintetizado no citosol das células neuronais colinérgicas pré-sinápticas do SNC e periférico. É sintetizada a partir da colina e acetil-CoA, proveniente da mitocôndria em reação catalisada pela enzima colina acetiltransferase (ChAT), e posteriormente é armazenada em vesículas. A acetilcolina é liberada do neurônio na fenda sináptica através de exocitose pelo Transportador Vesicular de acetilcolina (vAChT). Exerce seus efeitos via receptores muscarínicos e/ou nicotínicos e é degradada pela enzima acetilcolinesterase (AChE) em colina e acetato (SHIN; DIXON, 2015; ZHANG; ZHOU; YUAN, 2016). Alterações na atividade da AChE permite avaliar a funcionalidade do sistema colinérgico dentro de um determinado contexto (ZUGNO et al., 2013), por alterar os níveis do neurotransmissor na fenda. De fato, alterações na sinalização colinérgica podem contribuir para manifestações neuropsiquiátricas, como alucinações, delírios e comportamento agressivo psicótico (BURT, 2000).

A cetamina pode atuar como antagonista dos receptores nicotínicos e muscarínicos (DURIEUX, 1995; O'DELL et al., 1991). Por exemplo, um estudo com larvas de peixes medaka (*Oryzias latipes*) mostrou que a exposição à cetamina por 14 dias aumentou a atividade da AChE em aproximadamente 14% (LIAO et al., 2018). Em camundongos, a cetamina administrada agudamente por via i.p. aumentou a atividade da AChE em córtex, estriado e hipocampo (CHATTERJEE et al., 2012) e em ratos, a cetamina aumentou a atividade da enzima AChE nas regiões do córtex pré-frontal, hipocampo e estriado (ZUGNO et al., 2015).

Figura 4 - Representação esquemática da fenda sináptica e ação da acetilcolinesterase



Fonte: Modificado de Dulawa et al (2019).

2 JUSTIFICATIVA

O estudo dos mecanismos neuroquímicos e dos fenótipos comportamentais associados à exposição a fármacos é importante para a descoberta de potenciais estratégias terapêuticas. Devido à dificuldade de interpretar diferentes respostas comportamentais é importante a busca de novos modelos animais para melhor compreender os fatores neurobiológicos envolvidos neste comportamento. Portanto, este estudo servirá como suporte para uma melhor compreensão das respostas do comportamento agressivo e ansiolítico, bem como níveis de cortisol, memória e atividade da enzima acetilcolinesterase associada à exposição a diferentes concentrações de cetamina. Ademais, sabendo-se que muitas doenças neuropsiquiátricas estão associadas a alterações em parâmetros comportamentais e bioquímicos, o estudo relacionando os efeitos promovidos por concentrações subanestésicas em peixe-zebra é relevante.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar os efeitos da exposição aguda e repetida a concentrações subanestésicas de cetamina sobre parâmetros comportamentais e bioquímicos em peixe-zebra.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar as possíveis alterações causadas pela cetamina sobre a locomoção, agressividade, ansiedade e memória;
- Avaliar as possíveis alterações causadas pela cetamina no sistema colinérgico através da medida da atividade da enzima acetilcolinesterase de encéfalo;
- Avaliar as possíveis alterações causadas pela cetamina nos níveis de cortisol em corpo inteiro.

4 DESENVOLVIMENTO

As metodologias e os resultados desta dissertação estão apresentados na seguinte forma:

- Artigo publicado em 2018 na revista *Neuroscience Letters* apresentado no formato do Word, respeitando as normas da revista. Título: “Ketamine modulates aggressive behavior in adult zebrafish”.
- Manuscrito em preparação, a ser submetido, e apresentado no formato do Word. Título: “Ketamine effects on memory consolidation and stereotyped behavior in adult zebrafish”.
- Atividade da AChE de encéfalo de peixe-zebra adulto.

4.1 ARTIGO

Ketamine modulates aggressive behavior in adult zebrafish

Paula Michelotti[1], Vanessa A. Quadros[1,2], Maria E. Pereira[2,*] and Denis B. Rosemberg[1,2,3,*]

[1] *Laboratory of Experimental Neuropsychobiology, Department of Biochemistr and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil.*

[2]*Graduate Program in Biological Sciences: Toxicological Biochemistry, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil.*

[3] *The International Zebrafish Neuroscience Research Consortium (ZNRC), 309 Palmer Court, Slidell, LA 70458, USA.*

[*]Correspondence to:

Denis B. Rosemberg

Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil. E-mail: dbrosemberg@gmail.com

Maria E. Pereira

Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil. E-mail: pereirame@yahoo.com.br

Abstract

Ketamine is a non-competitive glutamatergic antagonist that induces analgesia and anesthesia. Although ketamine displays anxiolytic and antidepressant properties, it may induce pro-psychosis and hallucinogen effects, as well as stereotypic behaviors following acute administration in sub-anesthetic doses. Since heightened aggression is maladaptive and usually comorbid with various neuropsychiatric disorders, we aimed to investigate whether ketamine modulates aggressive behavior in adult zebrafish. Fish were acutely exposed to 2, 20, and 40 mg/L ketamine for 20 min and their locomotion, exploratory activity, and aggression towards mirror were further assessed. Ketamine (2 mg/L) increased aggression-related phenotypes, while 20 and 40 mg/L reduced aggression and elicited stereotypic behaviors by causing hyperlocomotion, altering motor patterns, and increasing circling behavior at the higher concentration tested. Collectively, our data expand the utility of zebrafish to investigate the influence of sub-anesthetic concentrations of ketamine on aggression behavior domain in translational neuropsychiatric research field.

Keywords: Aggressive behavior; psychotomimetic drugs; stereotypic behaviors; ketamine; zebrafish.

1. Introduction

Ketamine is a non-competitive antagonist of N-methyl-D-aspartate (NMDA) glutamate receptor widely used in clinical and veterinary medicine for analgesia and anesthesia [1,2]. Ketamine acts as a dissociative anesthetic and modulates opioid, monoaminergic, cholinergic, and GABAergic systems at higher doses [3,4]. Mounting evidence describes the anxiolytic, antidepressant, and hallucinogen properties of ketamine [5,6]. Sub-anesthetic doses of ketamine increase locomotion, stereotypic behavior, as well as promote social interaction deficits [4,7]. Although aggression is a universal behavior with a fundamental adaptive role in organisms [8,9], heightened aggression is maladaptive and usually comorbid with various neuropsychiatric disorders, showing a high prevalence in psychotic patients with anti-social behavior [10,11]. Since the mechanisms underlying the neurochemical effects of ketamine are complex, more studies regarding the behavioral and physiological responses in different experimental models are needed.

Zebrafish (*Danio rerio*) is a suitable model organism in translational neurobehavioral research mainly due to its genetic and physiological conservation when compared to mammals [12–14]. This species presents an extensive behavioral repertoire, which has been characterized previously [15]. In their natural environment, zebrafish are highly social and form larger shoals. Nonetheless, isolated zebrafish facing another conspecific display agonistic behavior, increasing aggression in order to establish social dominance and hierarchy [16,17]. Aggressive zebrafish show a fast swimming towards the opponent associated with erect fins, ondulatory body movements, and bites [18]. These behavioral phenotypes can be measured using the inclined mirror task, a simple protocol that predicts aggression when fish attack their own reflected image [19,20]. Although zebrafish acutely exposed to ketamine display anxiolytic-like behaviors and

aberrant circular swimming [4,21], the influence of ketamine on aggression behavior domain remains unknown. Thus, considering that sub-anesthetic doses may antagonize NMDA receptors inducing psychotic-like symptoms in animal models [4,22], this study investigates whether acute ketamine exposure modulates aggression, a phenotype that may comorbid with various psychiatric disorders, in adult zebrafish.

2. Materials e Methods

2.1. Animals and husbandry

Adult short fin *wild type* (*WT*) zebrafish (*Danio rerio*) were obtained from a local commercial supplier (Hobby Aquários, RS, Brazil). Animals were 4-6 months-old and a 50:50 (male:female) proportion was used for the experiments. Fish were kept in 40-L housing tanks with a maximum density of 4 fish per liter for at least two weeks before the experiments under standard water conditions (27 ± 1ºC, pH 7.0 ± 0.15; dissolved oxygen at 6.0 ± 0.1 mg/L; total ammonia at < 0.01 mg/L). Animals were housed under a 14h/10h light/dark photoperiod (lights on at 7:00 am) and fed thrice daily with Alcon BASIC™ flake fish food (Alcon, Brazil). All protocols were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria (protocol number 6894010616).

2.2. Ketamine exposure and behavioral analyses

All experiments were recorded between 09:00 am and 4:00 pm using n = 12 animals per group. Zebrafish were exposed to ketamine (2, 20, and 40 mg/L) for 20 min as described elsewhere [4]. Importantly, the concentrations selected here are lower than 100 mg/L, which induces anesthesia in zebrafish [23]. Control group were kept for 20 min in drug-free water, while ketamine concentrations were obtained by serial dilution of a stock 10% ketamine choridrate solution (Syntec, São Paulo, Brazil). After the exposure

period, fish were individually placed in the test tank (25 cm length × 15 cm height × 10 cm width) filled with 2.5 L of non-chlorinated water (water column depth of 10 cm). For ketamine exposure and behavioral experiments, water conditions were similar to those of the housing tanks. An inclined mirror (22.5º) was placed in one wall of the tank, which was virtually divided in two areas related to their proximity to mirror (close and far) [19]. All other tank walls were covered with opaque partitions to minimize the influence of environmental cues in fish behavior. Behaviors were recorded for 6 min using automated video-tracking software (ANY-maze^{TM}, Stoelting CO, USA) at 30 frames/s. During the trial, the respective behaviors were assessed: distance traveled, absolute turn angle, and maximum speed (locomotor parameters); number of rotations (circling behavior); transitions and time spent in the close area (exploratory behavior); number and duration of aggressive episodes, and latency to attack the mirror (aggression-related behaviors). Aggression towards mirror was coded by two-trained observers (inter-rater reliability > 0.85) and was defined as the number of events in which fish attack the opponent image displaying fin erection, undulating body movements, fast swimming, and biting towards the mirror in the close area [15,19,20].

2.3. Statistics

Normality and homogeneity of variances were analyzed using Kolmogorov–Smirnov and Bartlett's tests, respectively. Since all data were normally distributed and attend the assumption of homoscedasticity, results were expressed as a mean ± standard error of the mean (S.E.M.) and differences between control and ketamine groups were determined using one-way ANOVA followed by Student-Newman-Keuls multiple comparison test. The significance level was set at $p \leq 0.05$

3. Results

Fig. 1 shows the aggression-related behaviors in zebrafish following acute ketamine exposure. We observed that 2 mg/L ketamine increased the number and duration of aggressive episodes, while 20 and 40 mg/L ketamine reduced aggression ($F_{(3,44)}$ = 43.95, $p < 0.0001$). Moreover, 40 mg/L ketamine increased the latency to attack the mirror ($F_{(3,44)}$ = 4.399, $p = 0.0086$).

The effects of ketamine on locomotion, number of rotations, and exploration are depicted in **Fig. 2**. Fish exposed to 20 and 40 mg/L had increased swimming activity when compared to control and 2 mg/L ketamine groups ($F_{(3,44)} = 6.015$, $p = 0.0016$), while the absolute turn angle decreased following 20 and 40 mg/L ketamine ($F_{(3,44)} = 5.681$, $p = 0.0022$) and the maximum speed did not change ($F_{(3,44)} = 1.428$, $p = 0.2473$). Furthermore, 40 mg/L ketamine increased circular behavior when compared to control ($F_{(3,44)} = 2.9$, $p = 0.0455$). Regarding the exploratory activity, 20 and 40 mg/L ketamine reduced the time spent in the mirror area ($F_{(3,44)} = 7.749$, $p = 0.0003$) and no differences were detected in the number of transitions ($F_{(3,44)} = 2.509$, $p = 0.0711$). The behavioral phenotypes observed following ketamine exposure were depicted as representative track and occupancy plots **(Fig. 3A)** and visual diagrams of behavior were constructed **(Fig. 3B)**.

4. Discussion

Here, we assessed whether sub-anesthetic concentrations of ketamine acutely modulate aggression in adult zebrafish. Our novel findings showed that 2 mg/L ketamine increased aggression towards mirror, whereas 20 and 40 mg/L reduced aggressive behavior. Moreover, the higher ketamine concentrations tested increased locomotion and circling behavior, which could impair the overall swimming pattern and exploratory

activity of fish. Thus, ketamine elicits a dual effect on aggression, depending on the concentration tested.

Sub-anesthetic doses of ketamine alter different behaviors in vertebrates. For example, in humans, ketamine elicits hallucinogen, sedative, dissociative, pre-psychotic, and antidepressant effects [24]. In rodents, anxiolytic- and antidepressant-like properties have been described [1]. Mounting evidence also shows that antagonists of NMDA receptors may exert a biphasic role on aggression, in which lower and higher doses increase and suppress aggression, respectively [25,26]. Here, we described a similar response in zebrafish, showing a dual role of ketamine on aggression towards mirror. Changes in glutamatergic and dopaminergic systems have been associated to ketamine-induced transient psychotic symptoms in humans [27], suggesting a complex interaction among different neurotransmitters in aggression. The relevance of applying cross-species and cross-domain modeling to drug abuse-related phenotypes has been recognized as a key strategy in translational psychopharmacology research. These approaches allow the identification of evolutionarily conserved phenotypes, which represent important features to understand how basic neural mechanisms influence behavioral domains [28,29]. Although fish models do not fully recapitulate all aspects of complex psychiatric disorders, zebrafish show pharmacological and behavioral responses following exposure to sub-anesthetic concentrations of ketamine that parallel with rodents and humans [4,21]. Thus, the assessment of clinically relevant neurobehavioral domains (e.g. locomotor, affective, and social) may improve predictive, face, and construct validity, thereby fostering the use of zebrafish models in psychiatric research field.

The effects of ketamine in zebrafish depend on the exposure protocol and developmental stage. For example, zebrafish exposed to high ketamine concentrations (0.2 to 0.8 mg/mL) for 20 min during early embryogenesis show cephalic disorders and

organ abnormalities [30]. Moreover, ketamine exposure during the 50% epiboly and 1-4 somites stages modulates *shh a* and *nog3* gene expression levels [31], increases thigmotaxis, and decreases avoidance behavior without affecting socialization at 144 h post-fertilization (hpf) [32]. These set of data suggest that early-life exposure to ketamine is highly toxic and elicits prolonged behavioral changes in larvae. Because NMDA receptors subunit genes are expressed after 24 hpf [33], these effects seem to occur by a complex regulation of developmental-related genes independently from NMDA receptor action [30].

Similar to rodents, adult zebrafish acutely exposed to ketamine display anxiolytic-like behaviors [7,34], abnormal locomotor activity, and increased circular movements [34,35]. Although a plausible explanation may be due to hallucinogenic-like effects of ketamine, other hallucinogen drugs, such as lysergic acid diethylamide (LSD) and cocaine did not influence rotation. Since MK-801 elicits circling behavior [36], this phenotype may be linked to the antagonism of NMDA receptors rather than hallucinogenic action in general. Representative traces of ketamine-exposed fish showed increased locomotor activity at the higher concentrations tested. Probably, changes in locomotion and motor patterns observed in 20 mg/L and 40 mg/L groups may reflect stereotypic behaviors, culminating in an impaired exploratory activity in the test tank. Although previous data showed that ketamine elicits abnormal circling behavior without locomotor changes in zebrafish subjected to the novel tank test [4], high sub-anesthetic concentrations of ketamine increase the number of crossings in the light-dark test [7]. Based on the experimental differences observed in different behavioral tasks, we suggest the influence of context on the locomotor effects of ketamine in adult zebrafish. Because ketamine triggers prolonged behavioral effects in larval zebrafish [32] and reduces anxiety-like behavior following chronic ethanol exposure withdrawal [37], further studies

aiming to verify the duration of abnormal behaviors in adult specimens, as well as to clarify the neurochemical mechanisms underlying the behavioral effects of ketamine on different zebrafish models, are needed.

5. Conclusion

In summary, we report an inverted U-shaped effect of ketamine on zebrafish aggression. While lower sub-anesthetic concentrations increase aggression towards mirror, higher ketamine concentrations elicit stereotypic behaviors and reduce aggression. Furthermore, our data expand the influence of ketamine on different behavioral domains and reinforce the growing utility of zebrafish to assess the behavioral effects of psychotomimetic drugs in translational neuropsychiatric research.

Acknowledgments

We recognize the financial support and fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). V.A.Q. was recipient of fellowship from CAPES. D.B.R. and M.E.P. received the CNPq research productivity grant (307595/2015-3 and 311082/2014-9, respectively). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

P.M. and V.A.Q performed the experiments and analyzed the data. D.B.R. and M.E.P. contributed to experimental design and analyzed the data; P.M., D.B.R., and M.E.P. wrote the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that no competing interests exist.

References

[1] E. Engin, D. Treit, C.T. Dickson, Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models, Neuroscience. 161 (2009) 359–369. https://doi.org/10.1016/j.neuroscience.2009.03.038.
[2] T.C. Brown, W.H. Cole, G.H. Murray, Ketamine: A new anaesthetic agent, Aust. N. Z. J. Surg. 39 (1970) 305–310.
[3] O. Hustveit, A. Maurset, I. Oye, Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors, Pharmacol. Toxicol. 77 (1995) 355–359.
[4] R. Riehl, E. Kyzar, A. Allain, J. Green, M. Hook, L. Monnig, K. Rhymes, A. Roth, M. Pham, R. Razavi, J. Dileo, S. Gaikwad, P. Hart, A.V. Kalueff, Behavioral and physiological effects of acute ketamine exposure in adult zebrafish, Neurotoxicol. Teratol. 33 (2011) 658–667. https://doi.org/10.1016/j.ntt.2011.05.011.
[5] J.H. Krystal, E.B. Perry, R. Gueorguieva, A. Belger, S.H. Madonick, A. Abi-Dargham, T.B. Cooper, L. Macdougall, W. Abi-Saab, D.C. D'Souza, Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function, Arch. Gen. Psychiatry. 62 (2005) 985–994. https://doi.org/10.1001/archpsyc.62.9.985.
[6] C.A. Zarate, J.B. Singh, P.J. Carlson, N.E. Brutsche, R. Ameli, D.A. Luckenbaugh, D.S. Charney, H.K. Manji, A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression, Arch. Gen. Psychiatry. 63 (2006) 856–864. https://doi.org/10.1001/archpsyc.63.8.856.
[7] E.G. De Campos, A.T. Bruni, B.S. De Martinis, Ketamine induces anxiolytic effects in adult zebrafish: A multivariate statistics approach, Behav. Brain Res. 292 (2015) 537–546. https://doi.org/10.1016/j.bbr.2015.07.017.
[8] C.L. Rambo, R. Mocelin, M. Marcon, D. Villanova, G. Koakoski, M.S. de Abreu, T.A. Oliveira, L.J.G. Barcellos, A.L. Piato, C.D. Bonan, Gender differences in aggression and cortisol levels in zebrafish subjected to unpredictable chronic stress, Physiol. Behav. 171 (2017) 50–54. https://doi.org/10.1016/j.physbeh.2016.12.032.
[9] A. Theodoridi, A. Tsalafouta, M. Pavlidis, Acute Exposure to Fluoxetine Alters Aggressive Behavior of Zebrafish and Expression of Genes Involved in Serotonergic System Regulation, Front. Neurosci. 11 (2017) 223. https://doi.org/10.3389/fnins.2017.00223.
[10] A. Spidel, T. Lecomte, C. Greaves, K. Sahlstrom, J.C. Yuille, Early psychosis and aggression: predictors and prevalence of violent behaviour amongst individuals with early onset psychosis, Int. J. Law Psychiatry. 33 (2010) 171–176. https://doi.org/10.1016/j.ijlp.2010.03.007.
[11] S. Das, S. Sengupta, K. Pathak, D. Sah, S. Mehta, P.R. Avinash, A. Baruah, S.K. Deuri, A. Sarmah, V. Gogoi, K.N. Kalita, J. Hazarika, Aggression as an independent

entity even in psychosis - The role of cortisol, Psychiatry Res. 259 (2018) 405–411. https://doi.org/10.1016/j.psychres.2017.11.002.
[12] B.D. Fontana, N.J. Mezzomo, A.V. Kalueff, D.B. Rosemberg, The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review, Exp. Neurol. 299 (2018) 157–171. https://doi.org/10.1016/j.expneurol.2017.10.004.
[13] G.J. Lieschke, P.D. Currie, Animal models of human disease: zebrafish swim into view, Nat. Rev. Genet. 8 (2007) 353–367. https://doi.org/10.1038/nrg2091.
[14] P. Panula, V. Sallinen, M. Sundvik, J. Kolehmainen, V. Torkko, A. Tiittula, M. Moshnyakov, P. Podlasz, Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases, Zebrafish. 3 (2006) 235–247. https://doi.org/10.1089/zeb.2006.3.235.
[15] A.V. Kalueff, M. Gebhardt, A.M. Stewart, J.M. Cachat, M. Brimmer, J.S. Chawla, C. Craddock, E.J. Kyzar, A. Roth, S. Landsman, S. Gaikwad, K. Robinson, E. Baatrup, K. Tierney, A. Shamchuk, W. Norton, N. Miller, T. Nicolson, O. Braubach, C.P. Gilman, J. Pittman, D.B. Rosemberg, R. Gerlai, D. Echevarria, E. Lamb, S.C.F. Neuhauss, W. Weng, L. Bally-Cuif, H. Schneider, Zebrafish Neuroscience Research Consortium, Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond, Zebrafish. 10 (2013) 70–86. https://doi.org/10.1089/zeb.2012.0861.
[16] R.F. Oliveira, J.F. Silva, J.M. Simões, Fighting zebrafish: characterization of aggressive behavior and winner-loser effects, Zebrafish. 8 (2011) 73–81. https://doi.org/10.1089/zeb.2011.0690.
[17] M.C. Teles, R.F. Oliveira, Quantifying Aggressive Behavior in Zebrafish, Methods Mol. Biol. Clifton NJ. 1451 (2016) 293–305. https://doi.org/10.1007/978-1-4939-3771-4_20.
[18] L.J. Jones, W.H.J. Norton, Using zebrafish to uncover the genetic and neural basis of aggression, a frequent comorbid symptom of psychiatric disorders, Behav. Brain Res. 276 (2015) 171–180. https://doi.org/10.1016/j.bbr.2014.05.055.
[19] B.D. Fontana, D.L. Meinerz, L.V.C. Rosa, N.J. Mezzomo, A. Silveira, G.S. Giuliani, V.A. Quadros, G.L.B. Filho, R.E. Blaser, D.B. Rosemberg, Modulatory action of taurine on ethanol-induced aggressive behavior in zebrafish, Pharmacol. Biochem. Behav. 141 (2016) 18–27. https://doi.org/10.1016/j.pbb.2015.11.011.
[20] R. Gerlai, M. Lahav, S. Guo, A. Rosenthal, Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects, Pharmacol. Biochem. Behav. 67 (2000) 773–782.
[21] S.M. Zakhary, D. Ayubcha, F. Ansari, K. Kamran, M. Karim, J.R. Leheste, J.M. Horowitz, G. Torres, A behavioral and molecular analysis of ketamine in zebrafish, Synap. N. Y. N. 65 (2011) 160–167. https://doi.org/10.1002/syn.20830.
[22] M. Yadav, M. Parle, D.K. Jindal, S. Dhingra, Protective effects of stigmasterol against ketamine-induced psychotic symptoms: Possible behavioral, biochemical and histopathological changes in mice, Pharmacol. Rep. PR. 70 (2018) 591–599. https://doi.org/10.1016/j.pharep.2018.01.001.
[23] T. Martins, E. Diniz, L.M. Félix, L. Antunes, Evaluation of anaesthetic protocols for laboratory adult zebrafish (Danio rerio), PloS One. 13 (2018) e0197846. https://doi.org/10.1371/journal.pone.0197846.
[24] J.H. Krystal, L.P. Karper, J.P. Seibyl, G.K. Freeman, R. Delaney, J.D. Bremner, G.R. Heninger, M.B. Bowers, D.S. Charney, Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses, Arch. Gen. Psychiatry. 51 (1994) 199–214.

[25] A. Takahashi, K.A. Miczek, Neurogenetics of aggressive behavior: studies in rodents, Curr. Top. Behav. Neurosci. 17 (2014) 3–44. https://doi.org/10.1007/7854_2013_263.
[26] M.M. Torregrossa, A Role for Prefrontal Cortical NMDA Receptors in Murine Alcohol-Heightened Aggression, Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. (2018). https://doi.org/10.1038/npp.2017.286.
[27] S. Aalto, J. Ihalainen, J. Hirvonen, J. Kajander, H. Scheinin, H. Tanila, K. Någren, H. Vilkman, L.L. Gustafsson, E. Syvälahti, J. Hietala, Cortical glutamate-dopamine interaction and ketamine-induced psychotic symptoms in man, Psychopharmacology (Berl.). 182 (2005) 375–383. https://doi.org/10.1007/s00213-005-0092-6.
[28] A. Stewart, K. Wong, J. Cachat, S. Gaikwad, E. Kyzar, N. Wu, P. Hart, V. Piet, E. Utterback, M. Elegante, D. Tien, A.V. Kalueff, Zebrafish models to study drug abuse-related phenotypes, Rev. Neurosci. 22 (2011) 95–105. https://doi.org/10.1515/RNS.2011.011.
[29] N. Neelkantan, A. Mikhaylova, A.M. Stewart, R. Arnold, V. Gjeloshi, D. Kondaveeti, M.K. Poudel, A.V. Kalueff, Perspectives on zebrafish models of hallucinogenic drugs and related psychotropic compounds, ACS Chem. Neurosci. 4 (2013) 1137–1150. https://doi.org/10.1021/cn400090q.
[30] L.M. Félix, L.M. Antunes, A.M. Coimbra, Ketamine NMDA receptor-independent toxicity during zebrafish (Danio rerio) embryonic development, Neurotoxicol. Teratol. 41 (2014) 27–34. https://doi.org/10.1016/j.ntt.2013.11.005.
[31] L.M. Félix, C. Serafim, A.M. Valentim, L.M. Antunes, S. Campos, M. Matos, A.M. Coimbra, Embryonic Stage-Dependent Teratogenicity of Ketamine in Zebrafish (Danio rerio), Chem. Res. Toxicol. 29 (2016) 1298–1309. https://doi.org/10.1021/acs.chemrestox.6b00122.
[32] L.M. Félix, L.M. Antunes, A.M. Coimbra, A.M. Valentim, Behavioral alterations of zebrafish larvae after early embryonic exposure to ketamine, Psychopharmacology (Berl.). 234 (2017) 549–558. https://doi.org/10.1007/s00213-016-4491-7.
[33] J.A. Cox, S. Kucenas, M.M. Voigt, Molecular characterization and embryonic expression of the family of N-methyl-D-aspartate receptor subunit genes in the zebrafish, Dev. Dyn. Off. Publ. Am. Assoc. Anat. 234 (2005) 756–766. https://doi.org/10.1002/dvdy.20532.
[34] E.J. Kyzar, A.V. Kalueff, Exploring Hallucinogen Pharmacology and Psychedelic Medicine with Zebrafish Models, Zebrafish. 13 (2016) 379–390. https://doi.org/10.1089/zeb.2016.1251.
[35] E.J. Kyzar, C. Collins, S. Gaikwad, J. Green, A. Roth, L. Monnig, M. El-Ounsi, A. Davis, A. Freeman, N. Capezio, A.M. Stewart, A.V. Kalueff, Effects of hallucinogenic agents mescaline and phencyclidine on zebrafish behavior and physiology, Prog. Neuropsychopharmacol. Biol. Psychiatry. 37 (2012) 194–202. https://doi.org/10.1016/j.pnpbp.2012.01.003.
[36] H.A. Swain, C. Sigstad, F.M. Scalzo, Effects of dizocilpine (MK-801) on circling behavior, swimming activity, and place preference in zebrafish (Danio rerio), Neurotoxicol. Teratol. 26 (2004) 725–729. https://doi.org/10.1016/j.ntt.2004.06.009.
[37] J. Pittman, A. Hylton, Behavioral, endocrine, and neuronal alterations in zebrafish (Danio rerio) following sub-chronic coadministration of fluoxetine and ketamine, Pharmacol. Biochem. Behav. 139 Pt B (2015) 158–162. https://doi.org/10.1016/j.pbb.2015.08.014.

Figure captions

Fig. 1. Aggression-related behaviors of ketamine-exposed zebrafish. Data were expressed as means ± S.E.M. and analyzed by one-way ANOVA followed by Student-Newman-Keuls multiple comparison test. Different letters indicate statistical differences among groups (n = 12 per group, $p \leq 0.05$).

Fig. 2. Effects of ketamine on locomotion, number of rotations, and exploratory activity. Data were expressed as means ± S.E.M. and analyzed by one-way ANOVA followed by Student-Newman-Keuls multiple comparison test when necessary. Different letters indicate statistical differences among groups. The absence of letters above bars reflects similar results among the groups tested (n = 12 per group, $p \leq 0.05$).

Fig. 3. Main behaviors of ketamine-exposed zebrafish in the inclined mirror test. **(A)** Representative track and occupancy plots showing the swimming traces and time spent in close and far areas. **(B)** Visual diagrams of zebrafish behavior. The number of arrows indicates the frequency of each behavior, while the arrow width depicts their duration. The clock size reflects the latency to attack the virtual conspecific image.

Figure 1

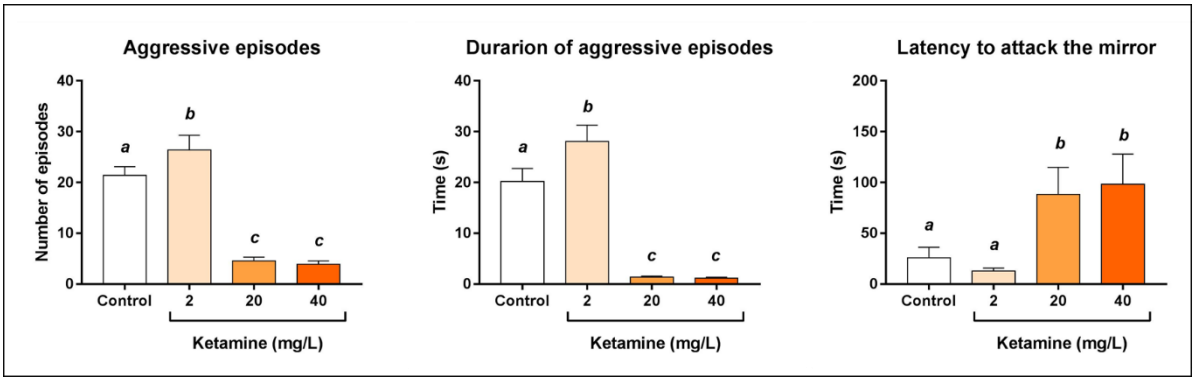


Figure 2

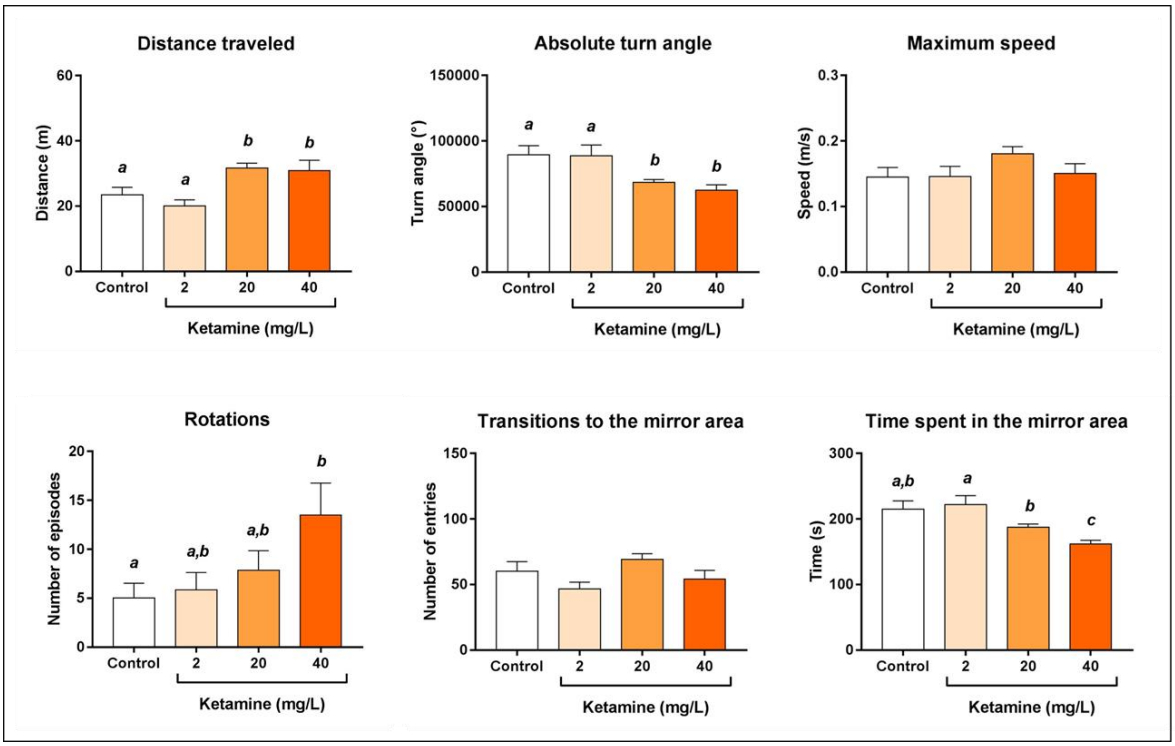
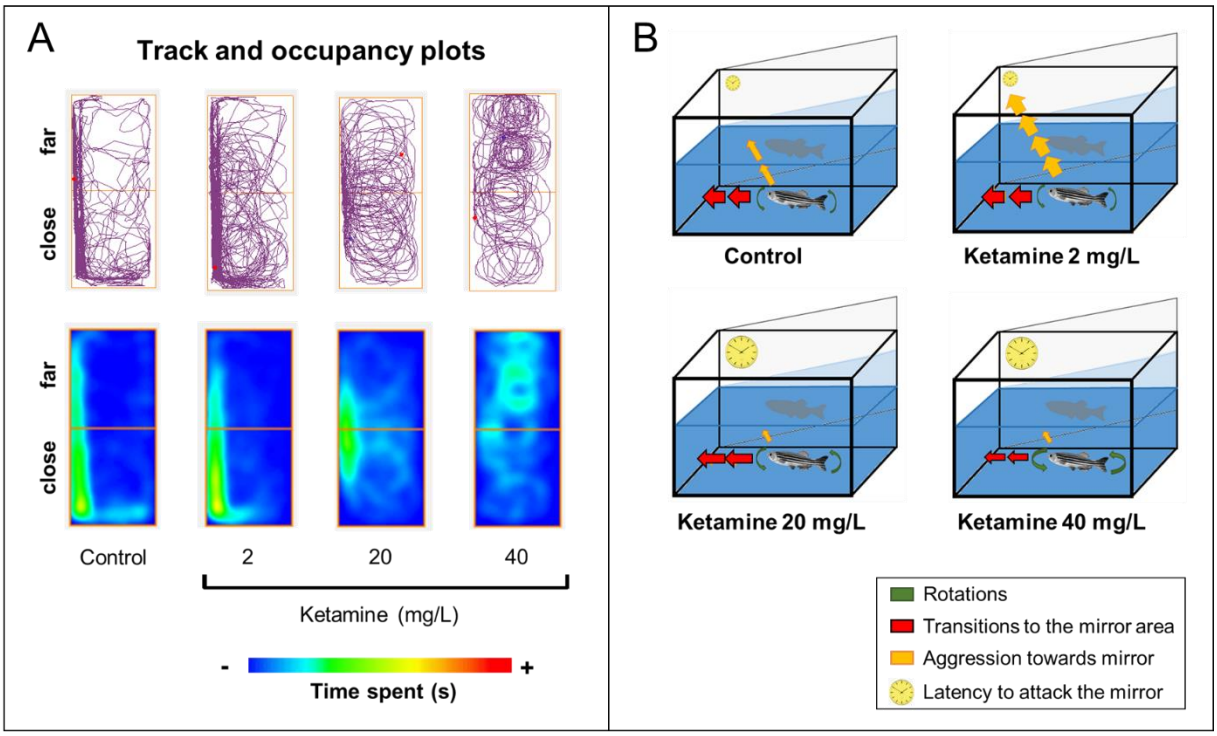


Figure 3


A
Track and occupancy plots
far
close
far
close
Control
2
20
40
Ketamine (mg/L)
-
+
Time spent (s)
B
Control
Ketamine 2 mg/L
Ketamine 20 mg/L
Ketamine 40 mg/L
Rotations
Transitions to the mirror area
Aggression towards mirror
Latency to attack the mirror

4.2 MANUSCRITO

Ketamine effects on memory consolidation and stereotyped behavior in adult zebrafish

Paula Michelotti[1,2], Francini Franscescon[1,2], Talise E. Müller[1,2], Denis B. Rosemberg[1,2*] and Maria E. Pereira[2*]

[1] *Laboratory of Experimental Neuropsychobiology, Department of Biochemistry and Molecular Biology, Center of Natural and Exact Sciences, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105–900, Brazil.*

[2] *Graduate Program in Biological Sciences: Toxicological Biochemistry, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105–900, Brazil.*

[*]Correspondence to:

Maria E. Pereira

Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil. E-mail: pereirame@yahoo.com.br

Denis B. Rosemberg

Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil. E-mail: dbrosemberg@gmail.com

Abstract

Ketamine is a drug employed as a dissociative anesthetic in human and veterinary clinic, as well as abuse drug, and acts on several neurotransmitter systems. The use of alternative animal models, such as zebrafish, is necessary to study the actions of drugs in neuropsychiatric disorders. This study evaluated the effects of different sub-anesthetic ketamine concentrations on memory, locomotion, vertical exploration, aggressive-like behavior and whole-body cortisol levels in adult zebrafish. In acute protocol, the fish were submitted to the task of avoiding inhibition (training and testing with 24-hour intervals) and their locomotion was analyzed after the test session. Immediately after the training session, the fish were exposed to ketamine (0, 2, 20 and 40 mg/L) for 20 minutes. In repeated exposure, animals were exposed to the same concentrations of ketamine during 20 min for 7 days. 24 h after the last exposure, the novel tank task was performed, followed by the mirror-induced aggression test and whole-body cortisol levels. The results show that only the acute higher ketamine concentration (40 mg/L) induced a slight memory deficit without altering locomotion. In repeated exposure, results show that locomotion, vertical exploration, aggressive-like behavior and whole-body cortisol levels were not changed by ketamine. However, zebrafish exposed to ketamine 40 mg/L showed an increase in the number of rotations, characteristic of stereotyped behavior. These results show that amnesia and stereotyped effects of ketamine are not related any locomotor changes. Our data expand the usefulness of zebrafish to investigate the influence of sub-anesthetic ketamine concentrations on memory and neuropsychiatric behavior models.

Keywords: psychotomimetic drugs; ketamine; memory, aggression and anxiety-like behavior; stereotyped behavior; zebrafish.

1. Introduction

Ketamine is a dissociative anesthetic used in humans, veterinary and as drug of abuse (LIAO et al., 2018; RIEHL et al., 2011). This drug has a complex neuropharmacology and acts on several types of receptors in the brain. In the glutamatergic system, ketamine acts as non-competitive antagonist of N-methyl-D-aspartate (NMDA) receptor (RIEHL et al., 2011). In other neurotransmission systems, ketamine acts as an agonist of opioid and GABAa receptors (IRIFUNE et al., 2000; SMITH; WESTFALL; ADAMS, 1982), causes inhibitory effects on nicotinic (O'DELL et al., 1991) and muscarinic receptors (DURIEUX, 1995). Moreover, ketamine increases the neural transmission of monoaminergic neurotransmitters (CRISP et al., 1991). Studies with humans demonstrate that sub-anesthetic doses of ketamine cause several effects, such as antidepressant (ZARATE et al., 2006), hallucinogenic and sedative (DICKERSON et al., 2010; KRYSTAL et al., 2005) dissociative and pro-psychotic (KRYSTAL et al., 1994; LI et al., 2011). In rodents, sub-anesthetic doses of ketamine show an increase in locomotion (WILSON et al., 2005), stereotyped behaviors and impairs social interaction (CANEVER et al., 2010), as well as anxiolytic and antidepressant effects (ENGIN; TREIT; DICKSON, 2009).

Literature shows that psychiatric disorders have a direct relationship with changes in glutamatergic system (ZAKHARY et al., 2011). Studies with rodents show that ionotropic glutamate receptors are involved with the appearance of behaviors that are present, for example, in schizophrenia (ENGIN; TREIT; DICKSON, 2009; MOGHADDAM, 2003). Patients with psychiatric disorders, as in schizophrenia, exhibit different types of behavior. Aggressive behavior is being very studied in recent years, where its appearance occurs due to several factors (LECLERC et al., 2018). In the course of the disease, the risk of relapse and the incidence of suicide attempts increases and

worsens cognitive deficits (GARAY et al., 2015; PALLANTI; CANTISANI; GRASSI, 2013). Ketamine is used in models to induce schizophrenia-like symptoms (FROHLICH; VAN HORN, 2014; JONES; WATSON; FONE, 2011). However, knowing that in this psychiatric disorder we observe the appearance of several behaviors, studying in an alternative animal organism is interesting.

In this sense, the use of alternative animal models is necessary to study the actions of drugs in neuropsychiatric disorders. The zebrafish (*Danio rerio*) is a suitable model organism to assess the biological effects of substances that modulate brain function in vertebrates (FONTANA et al., 2018). This species is highly sensitive to pharmacological manipulations, favoring investigations related to translational neuroscience research (HOWE et al., 2013; KALUEFF et al., 2013)**.** Thereby, regarding that ketamine triggers several behavioral and physiological changes in animal models and it is similar to what occur in neuropsychiatric disorders, the objective of this study is to verify the effects of acute sub-anesthetic doses of ketamine on the consolidation memory, and to analyze the possible effects of repeated ketamine exposure on locomotion, aggression-like behaviors and vertical exploration, as well as cortisol levels on adult zebrafish.

2. Materials and Methods

2.1. Animals and husbandry

Wild-type (WT) zebrafish (*Danio rerio*) ranging from 4 to 6 months of age and 50:50 male:female ratio were used. The animals are reproduced in southeastern Brazil and transported to a local commercial supplier (Hobby Aquarios, RS, Brazil), where the animals were obtained for research. Since the animals are obtained from an external supplier, they are expected to be genetically heterogeneous as a way of better representing the nature of fish populations, thus diminishing the effects of genetic heredity (LIMA et

al., 2016; PARRA; ADRIAN; GERLAI, 2009; SPEEDIE; GERLAI, 2008). Animals were kept in 40-L housing tanks with a maximum density of 4 fish per liter under standard conditions with non-chlorinated water under constant filtration and aeration. The conditions of the tank were: water temperature 27 ± 1 °C, pH 7.0 ± 0.15, dissolved oxygen at 6.0 ± 0.1 mg/L, total ammonia at < 0.01 mg/L, and conductivity of 1500 – 1600 $\mu S.cm^{-1}$. The animals were acclimated for two weeks before the experiments under a 14/10h light/dark photoperiod (lights on at 7:00 AM and off at 9:00 PM) and fed daily with Alcon BASIC^{TM} flake fish food (Alcon, Brazil). All protocols were approved by the Institutional Animal Care and Use Committee of the Federal University of Santa Maria (protocol number 6894010616/2016).

2.2 Ketamine exposure protocol

In this study, we used 2 different protocols. In acute exposure protocol (Fig. 1A) the total number of animals used was 88. Here we investigated the effect of sub-anesthetic doses of ketamine on memory. Zebrafish were submitted to a unique exposition at concentrations of 2, 20 and 40 mg/L of ketamine or drug-free water (control group) in non-chlorinated water for 20 minutes (RIEHL et al., 2011) after the training session in the inhibitory avoidance task. As our goal here is to analyze memory consolidation, exposure to ketamine occurs only once after the training session.

In the repeated exposure protocol (Fig. 1B) the total number of animals used was 64. Here we investigated the effect of sub-anesthetic doses of ketamine on locomotion, anxiety- and aggressive-like behavior and whole-body cortisol levels. The animals were exposed for 7 days at concentrations of 2, 20 and 40 mg/L of ketamine or drug-free water for 20 min for day, according to adapted protocol (ZUGNO et al., 2015). Important to note that the anesthetic concentration in zebrafish starts from 100 mg/L (MARTINS et

al., 2018). Ketamine concentrations were obtained by dilution of a stock 10% ketamine chlorhydrate solution (Syntec, São Paulo, Brazil).

2.3 Behavioral analysis

Behavioral tests were performed 24 h after the last exposure to ketamine. The procedures during the behavioral analysis were performed on a stable surface and with as few environmental distractions as possible. All experiments were recorded between 09:00 AM and 4:00 PM. Data were extracted from automated video-tracking software at a 30 frames/s rate (ANY-maze™, Stoelting CO, USA).

2.3.1 Inhibitory avoidance task

The inhibitory avoidance task was performed based on the protocol described elsewhere (BERTONCELLO et al., 2019). The apparatus consisted of a glass tank (30 cm length x 10 cm width x 10 cm height) filled with 1.3 L of non-chlorinated water. The tank was divided into two equal compartments (black and white) separated by a manually operated guillotine-type partition (10 x 10 cm). The black compartment contained three pairs of metal bars (1 cm diameter) with spacing of 5 cm and connected to a 12 V stimulator. In this test, the animals received a pulsed shock of 100 Hz for 5 seconds. The animals were individually acclimated for three days in accommodation tanks with water circulation and visualization of conspecifics as previously reported (BERTONCELLO et al., 2019). On the fourth day, the animals were submitted to the training session, in which fish were placed individually in the white compartment of the apparatus. After 1 minute of acclimatization, the guillotine-type door was lifted and the fish allowed to enter the black compartment. When the animal crossed into the dark compartment, the door was lowered and a mild electric shock (125 mA, 3 ± 0.2 V) triggered. Then, the animals were

removed from the test tank, exposed to ketamine for 20 minutes, and placed in their accommodation tanks. The test session was performed after 24 h, similarly to the training session, although no electric shock applied. To investigate the acute effects of ketamine on memory consolidation, the animals were exposed to ketamine for 20 minutes immediately after the training session. After the inhibitory avoidance task, animals were individually transferred to the novel tank task to analyze locomotion. Because it is verified that the effect on memory did not interfere with changes in locomotion.

2.3.2 Novel tank task

Animals were placed individually in a tank (25 cm length × 15 cm height × 6 cm width) with 2.5 L of non-chlorinated water (27 ± 1 °C) and their behavior was recorded for 6 min. Locomotor parameters was analyzed individually in the animals placed in the tank and the following endpoints are used: distance traveled, maximum speed, absolute turn angle and number of rotations. To analyze the vertical exploration, the tank is divided into two horizontal sections (top and bottom) using the following endpoints: number of entries and time spent in the top and bottom area (EGAN et al., 2009).

2.3.2 Mirror-induced aggression (MIA) test

Animals were placed individually in a tank (25 cm length ×15 cm height ×6 cm width) with 2.5 L of non-chlorinated water (27 ± 1 °C) and their behavior was recorded for 6 min. MIA test was performed by placing a mirror tilted at 22.5° in one wall of the tank. All other walls of the tank were covered with an opaque material to minimize the influence of external factors in the experiments. The apparatus was virtually divided into two areas in relation to the approach of the mirror (close and far). The following endpoints were performed: number and duration of aggressive episodes, transitions to the close area

the mirror and time spent in this area. Aggression towards mirror was coded by two-trained observers (interrater reliability > 0.85) and was defined as the number of events in which fish attack the opponent image displaying fin erection, undulating body movements, fast swimming, and biting towards the mirror in the close area (GERLAI et al., 2000).

2.4 Whole-body cortisol extraction and measurement

After behavioral analyzes, samples were prepared to determine whole-body cortisol levels adapting protocol previously described (SINK; LOCHMANN; FECTEAU, 2008). The animals were weighed and then frozen in liquid nitrogen and stored at -20°C. The sample is macerated and homogenized in 1 mL of phosphate-saline buffer (PBS) pH 7.4. Soon after, cortisol is extracted using 2.5 mL of ethyl ether, where the lipid portion (supernatant) is removed and allowed to evaporate overnight. The lipid portion that is retained in the tube is resuspended in 100 μL of PBS. Cortisol levels were measured in duplicate using the enzyme immunoassay kit (EIAgen ™ Cortisol test, BioChem ImmunoSystems), where the reading was made by spectrophotometer at 450 nm and data expressed in ng of cortisol/g of tissue.

2.5 Statistics

Normality and homogeneity of the results were analyzed using Kolmogorov–Smirnov and Bartlett's tests, respectively. Non-parametric data of inhibitory avoidance were presented as median ± interquartile range and comparison of latencies between training and test were analyzed by the Wilcoxon matched-pairs signed rank test. Data with normal and homogeneous distribution were presented as mean ± standard error of the mean and analyzed by one-way ANOVA followed by the Student-Newman-Keuls

multiple comparisons test, when necessary. The level of significance was accepted at $p \leq 0.05$.

3. Results

3.1 Effects of acute exposure to sub-anesthetic doses of ketamine on zebrafish memory

The effects of acute ketamine exposure on zebrafish memory consolidation is shown in Figure 2. Wilcoxon matched-pairs signed rank test indicated that the control, 2 and 20 mg/L ketamine groups (W= 131.00, $p = 0.0064$; W= 118.00, $p = 0.0080$ and W= 134.00, $p = 0.0105$, respectively) showed significant memory retention 24 h after the training session. In contrast, 40 mg/L ketamine induced a subtle memory deficit on zebrafish. The effects of ketamine on locomotion 24 h after training session are presented in Fig. 3. One-way ANOVA showed that all ketamine concentrations did not change the distance traveled (Fig. 3A), maximum speed (Fig. 3B), and absolute turn angle (Fig. 3C).

3.2 Effects of repeated exposure to sub-anesthetic doses of ketamine on locomotor parameters, aggressive-like behavior, vertical exploration and cortisol levels in zebrafish

The effects of ketamine on locomotion and number of rotations are depicted in Fig. 4. One-way ANOVA shows that ketamine did not change the distance traveled (Fig.4A), maximum speed (Fig. 4B) and absolute turn angle (Fig. 4C). Regarding rotations number, one-way ANOVA shows significant effect of ketamine ($F_{(3,47)} = 9.751$, $p < 0,0001$) due to the higher number of rotations presented by the 40 mg/L ketamine group (Fig. 4D).

Aggression-related behavior is shown in Figure 5. One-way ANOVA shows that ketamine exposure did not alter the number of aggressive episodes (Fig. 5A), duration of

aggressive episodes (Fig. 5B), transitions to the mirror area (Fig. 5C) and time spend in the mirror area (Fig. 5D). The vertical exploration of the animals is shown in Fig. 6. One-way ANOVA shows that ketamine did not change the number of transitions (Fig. 6A) and the time spent in the top area (Fig. 6B); also did not change the number of transitions (Fig. 6C) and the time spent in the bottom area (Fig. 6D). Figure 7 shows that exposure to different concentrations of ketamine did not alter the whole-body cortisol levels (one-way ANOVA).

4. Discussion

In the current study, we evaluated the effects of sub-anesthetic doses of ketamine on memory consolidation, as well as the effects of repeated exposure on locomotion, aggression-like behavior, vertical exploration and whole-body cotisol levels on adult zebrafish. For first time, the findings show that acute ketamine exposure at 40 mg/L after training session impaired memory consolidation in zebrafish without changing locomotor parameters. Moreover, our results show that repeated exposure to ketamine did not interfer in the locomotion, anxiety- and aggressive-like behavior and whole-body cortisol levels. However, animals exposed to 40 mg/L presented an acentuated increase in the number of rotations.

Ketamine is a dissociative anesthetic (LIAO et al., 2018) that in sub-anesthesic dose causes several effects on CNS (DICKERSON et al., 2010; KRYSTAL et al., 1994, 2005; LI et al., 2011; ZARATE et al., 2006). Here, acute exposure to ketamine at higher sub-anesthetic concentration tested (40 mg/L) caused a mild amnesic effect. Considering that the ketamine is a non-competitive NMDA receptor antagonist, this finding agree with the study that show that a single post-training exposure to MK-801 impairs memory consolidation in adult zebrafish (BLANK et al., 2009; SEIBT et al., 2011). In rodents,

ketamine effects on memory is controversial, despite a study showing that sub-anesthetic doses of 50 and 100 mg/kg of ketamine injected intraperitoneally (i.p.) do not alter memory in rats (CHROBAK; HINMAN; SABOLEK, 2008). Moreover, another study verified memory consolidation impairment in rats with doses of 20 or 100 mg/kg (i.p) (BOULTADAKIS; PITSIKAS, 2011). Still using sub-anesthetic doses (5, 10, 20, and 50 mg/kg, i.p.) in mice, memory reconsolidation disruption was also found (WANG et al., 2006). Finally, decreased working memory (ADLER et al., 1998) and a deficit in memory formation (UMBRICHT et al., 2000) when ketamine is administered intravenously in humans has also been reported. Thereby, our results are in agreement with data in the literature, confirming the involvement of the glutamatergic pathway and NMDA receptor antagonism in the appearance of cognitive deficit in zebrafish, which is similar to what occurs in schizophrenic patients (GARAY et al., 2015).

Our results show that repeated exposure (7 days) to ketamine did not alter parameters related to locomotion, such as maximum speed, distance traveled and absolute turn angle. However, the higher concentration tested (40 mg/L) induced an increase of rotations numbers by zebrafish. The analysis of the number of rotations (circular swimming) is used to characterize the presence of stereotyped behavior, which is a repetitive and abnormal behavior of the animal, and which is induced by hallucinogenic drugs (KALUEFF et al., 2013; KYZAR et al., 2012). In the previous studies we showed that acute exposure to ketamine in the same concentrations used in this work induced hyperlocomotion and rotations (MICHELOTTI et al., 2018), to the contrary of other study that observed absence the effect on locomotion (RIEHL et al., 2011). Studies in rats show that when ketamine (25 mg/kg, i.p.) is administered for 14 days there is an increase in locomotion (ZUGNO et al., 2013). Ketamine (15 mg/kg) acutely administered in mice shows a decrease in locomotion, while in exposure for 7 days shows no change (SHIN et

al., 2019). However, exposure for 7 days to ketamine (100 mg/kg, i.p.) shows increase in the locomotion (CHATTERJEE et al., 2011). These studies reveal controverses results about ketamine on locomotor parameters.

Our results show that repeated exposure to ketamine did not affect the aggressive-like behavior. Previouly we demonstrated that zebrafish exposed to acute ketamine at a concentration of 2 mg/L increased aggressive behavior while concentrations of 20 and 40 mg/L decreased (MICHELOTTI et al., 2018). In addition, exposure to MK-801 5 μM (1,1 mg/L) for 15 min is known to reduce aggression in zebrafish (ZIMMERMANN et al., 2016). These date show that ketamine has different effects on aggressive behavior which depend of the experimental model used and the dose administered.

Vertical exploration of animals, staying longer on top or bottom of the tank, is an adequate test for assessing anxiety-like behavior (MAXIMINO et al., 2010). Our results show that the transitions and time spent to the top and botton area were not altered by ketamine exposure, i.e. ketamine did not present effect on anxiolytic-like behavior. Literature data show that zebrafish acutely exposed to ketamine present anxiolytic behavior at concentrations of 40 and 60 mg/L (DE CAMPOS; BRUNI; DE MARTINIS, 2015) and 20 and 40 mg/L (RIEHL et al., 2011). In mice also was verified anxiolity effect by ketamine (FRAGA et al., 2018). Human studies also show reduction in anxiety with ketamine administration (SALVADORE et al., 2009; ZARATE et al., 2006).

Regarding our results, repeated exposure to ketamine did not change the whole-body cortisol levels. Zebrafish studies show that whole-body cortisol levels increased at a dose of 20 mg/L with exposure for 2 weeks (PITTMAN; HYLTON, 2015) and decreased in doses of 20 and 40 mg/L in acute exposure (RIEHL et al., 2011). Our investigation show that the effect of ketamine on cortisol levels differs of that presented in the literature, as well as on locomotion, aggressive-like behavior and vertical

exploration. We emphasize that these controversial effects can be attributed to several factors such as the use of different doses, protocols and routes of administration, including the difference between animal models.

5. Conclusion

In summary, ketamine in acute exposure at sub-anesthetic concentration of 40 mg/L causes a slight deficit in memory consolidation without altering locomotor activity, suggesting amnesic effects in zebrafish. In addition, locomotion, anxiety- and aggressive-like behavior, and the whole-body cortisol levels were not changed by repeated exposure. The animals presented the steriotyped behavior only et high concentration tested. The number of rotations increased at a dose of 40 mg/L, showing stereotyped behavior. Overall, our findings expand the knowledge regarding the influence of ketamine in different behavioral domains and reinforce the increasing utility of zebrafish to evaluate the behavioral effects of psychotomimetic drugs in translational neuropsychiatric research.

Acknowledgments

We acknowledge the financial support and fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROEX 23038.004173/2019-93). P.M. (88887.210053/2018-00), F.F. (88882.182160/2018-01), and T.E.M. (88882.182130/2018-01) are recipients of fellowships from CAPES. D.B.R. and M.E.P. received the CNPq research productivity grant (305051/2018-0 and 311082/2014-9, respectively). Funding agencies had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Conflict of interest

The authors declare that no competing interests exist.

References

ADLER, C. M. et al. Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. **Biological Psychiatry**, v. 43, n. 11, p. 811–816, 1 jun. 1998.

BERTONCELLO, K. T. et al. Taurine prevents memory consolidation deficits in a novel alcohol-induced blackout model in zebrafish. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 93, p. 39–45, 14 mar. 2019.

BLANK, M. et al. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. **Neurobiology of Learning and Memory**, v. 92, n. 4, p. 529–534, nov. 2009.

BOULTADAKIS, A.; PITSIKAS, N. Anesthetic ketamine impairs rats' recall of previous information: the nitric oxide synthase inhibitor N-nitro-L-arginine methylester antagonizes this ketamine-induced recognition memory deficit. **Anesthesiology**, v. 114, n. 6, p. 1345–1353, jun. 2011.

CANEVER, L. et al. A rodent model of schizophrenia reveals increase in creatine kinase activity with associated behavior changes. **Oxidative Medicine and Cellular Longevity**, v. 3, n. 6, p. 421–427, dez. 2010.

CHATTERJEE, M. et al. Effect of "chronic" versus "acute" ketamine administration and its "withdrawal" effect on behavioural alterations in mice: implications for experimental psychosis. **Behavioural Brain Research**, v. 216, n. 1, p. 247–254, 1 jan. 2011.

CHROBAK, J. J.; HINMAN, J. R.; SABOLEK, H. R. Revealing past memories: proactive interference and ketamine-induced memory deficits. **The Journal of Neuroscience: The Official Journal of the Society for Neuroscience**, v. 28, n. 17, p. 4512–4520, 23 abr. 2008.

CRISP, T. et al. The local monoaminergic dependency of spinal ketamine. **European Journal of Pharmacology**, v. 194, n. 2–3, p. 167–172, 5 mar. 1991.

DE CAMPOS, E. G.; BRUNI, A. T.; DE MARTINIS, B. S. Ketamine induces anxiolytic effects in adult zebrafish: A multivariate statistics approach. **Behavioural Brain Research**, v. 292, p. 537–546, 1 out. 2015.

DICKERSON, D. et al. Ethanol-like effects of thiopental and ketamine in healthy humans. **Journal of Psychopharmacology (Oxford, England)**, v. 24, n. 2, p. 203–211, fev. 2010.

DURIEUX, M. E. Inhibition by ketamine of muscarinic acetylcholine receptor function. **Anesthesia and Analgesia**, v. 81, n. 1, p. 57–62, jul. 1995.

EGAN, R. J. et al. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. **Behavioural Brain Research**, v. 205, n. 1, p. 38–44, 14 dez. 2009.

ENGIN, E.; TREIT, D.; DICKSON, C. T. Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. **Neuroscience**, v. 161, n. 2, p. 359–369, 30 jun. 2009.

FONTANA, B. D. et al. The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review. **Experimental Neurology**, v. 299, n. Pt A, p. 157–171, jan. 2018.

FRAGA, D. B. et al. Anxiolytic effects of ascorbic acid and ketamine in mice. **Journal of Psychiatric Research**, v. 100, p. 16–23, 2018.

FROHLICH, J.; VAN HORN, J. D. Reviewing the ketamine model for schizophrenia. **Journal of Psychopharmacology (Oxford, England)**, v. 28, n. 4, p. 287–302, abr. 2014.

GARAY, R. P. et al. Investigational drugs for anxiety in patients with schizophrenia. **Expert Opinion on Investigational Drugs**, v. 24, n. 4, p. 507–517, abr. 2015.

GERLAI, R. et al. Drinks like a fish: zebra fish (Danio rerio) as a behavior genetic model to study alcohol effects. **Pharmacology, Biochemistry, and Behavior**, v. 67, n. 4, p. 773–782, dez. 2000.

HOWE, K. et al. The zebrafish reference genome sequence and its relationship to the human genome. **Nature**, v. 496, n. 7446, p. 498–503, 25 abr. 2013.

IRIFUNE, M. et al. Evidence for GABA(A) receptor agonistic properties of ketamine: convulsive and anesthetic behavioral models in mice. **Anesthesia and Analgesia**, v. 91, n. 1, p. 230–236, jul. 2000.

JONES, C. A.; WATSON, D. J. G.; FONE, K. C. F. Animal models of schizophrenia. **British Journal of Pharmacology**, v. 164, n. 4, p. 1162–1194, out. 2011.

KALUEFF, A. V. et al. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. **Zebrafish**, v. 10, n. 1, p. 70–86, mar. 2013.

KRYSTAL, J. H. et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. **Archives of General Psychiatry**, v. 51, n. 3, p. 199–214, mar. 1994.

KRYSTAL, J. H. et al. Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. **Archives of General Psychiatry**, v. 62, n. 9, p. 985–994, set. 2005.

KYZAR, E. J. et al. Effects of hallucinogenic agents mescaline and phencyclidine on zebrafish behavior and physiology. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 37, n. 1, p. 194–202, 27 abr. 2012.

LECLERC, M. P. et al. Some neuroanatomical insights to impulsive aggression in schizophrenia. **Schizophrenia Research**, v. 201, p. 27–34, 2018.

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, 15 abr. 2011.

LIAO, P.-H. et al. Illicit drug ketamine induces adverse effects from behavioral alterations and oxidative stress to p53-regulated apoptosis in medaka fish under environmentally relevant exposures. **Environmental Pollution (Barking, Essex: 1987)**, v. 237, p. 1062–1071, jun. 2018.

LIMA, M. G. et al. Time-dependent sensitization of stress responses in zebrafish: A putative model for post-traumatic stress disorder. **Behavioural Processes**, v. 128, p. 70–82, jul. 2016.

MARTINS, T. et al. Evaluation of anaesthetic protocols for laboratory adult zebrafish (Danio rerio). **PloS One**, v. 13, n. 5, p. e0197846, 2018.

MAXIMINO, C. et al. Measuring anxiety in zebrafish: a critical review. **Behavioural Brain Research**, v. 214, n. 2, p. 157–171, 25 dez. 2010.

MICHELOTTI, P. et al. Ketamine modulates aggressive behavior in adult zebrafish. **Neuroscience Letters**, v. 684, p. 164–168, 25 2018.

MOGHADDAM, B. Bringing order to the glutamate chaos in schizophrenia. **Neuron**, v. 40, n. 5, p. 881–884, 4 dez. 2003.

O'DELL, T. J. et al. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. **Proceedings of the National Academy of Sciences of the United States of America**, v. 88, n. 24, p. 11285–11289, 15 dez. 1991.

PALLANTI, S.; CANTISANI, A.; GRASSI, G. Anxiety as a core aspect of schizophrenia. **Current Psychiatry Reports**, v. 15, n. 5, p. 354, maio 2013.

PARRA, K. V.; ADRIAN, J. C.; GERLAI, R. The synthetic substance hypoxanthine 3-N-oxide elicits alarm reactions in zebrafish (Danio rerio). **Behavioural Brain Research**, v. 205, n. 2, p. 336–341, 28 dez. 2009.

PITTMAN, J.; HYLTON, A. Behavioral, endocrine, and neuronal alterations in zebrafish (Danio rerio) following sub-chronic coadministration of fluoxetine and ketamine. **Pharmacology, Biochemistry, and Behavior**, v. 139 Pt B, p. 158–162, dez. 2015.

RIEHL, R. et al. Behavioral and physiological effects of acute ketamine exposure in adult zebrafish. **Neurotoxicology and Teratology**, v. 33, n. 6, p. 658–667, dez. 2011.

SALVADORE, G. et al. Increased anterior cingulate cortical activity in response to fearful faces: a neurophysiological biomarker that predicts rapid antidepressant response to ketamine. **Biological Psychiatry**, v. 65, n. 4, p. 289–295, 15 fev. 2009.

SEIBT, K. J. et al. Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (Danio rerio). **Behavioural Brain Research**, v. 224, n. 1, p. 135–139, 10 out. 2011.

SHIN, S. Y. et al. Chronic administration of ketamine ameliorates the anxiety- and aggressive-like behavior in adolescent mice induced by neonatal maternal separation. **The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology**, v. 23, n. 1, p. 81–87, jan. 2019.

SINK, T. D.; LOCHMANN, R. T.; FECTEAU, K. A. Validation, use, and disadvantages of enzyme-linked immunosorbent assay kits for detection of cortisol in channel catfish, largemouth bass, red pacu, and golden shiners. **Fish Physiology and Biochemistry**, v. 34, n. 1, p. 95–101, mar. 2008.

SMITH, D. J.; WESTFALL, D. P.; ADAMS, J. D. Assessment of the potential agonistic and antagonistic properties of ketamine at opiate receptors in the guinea-pig ileum. **Neuropharmacology**, v. 21, n. 7, p. 605–611, jul. 1982.

SPEEDIE, N.; GERLAI, R. Alarm substance induced behavioral responses in zebrafish (Danio rerio). **Behavioural Brain Research**, v. 188, n. 1, p. 168–177, 17 mar. 2008.

UMBRICHT, D. et al. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. **Archives of General Psychiatry**, v. 57, n. 12, p. 1139–1147, dez. 2000.

WANG, J. H. et al. Ketamine affects memory consolidation: differential effects in T-maze and passive avoidance paradigms in mice. **Neuroscience**, v. 140, n. 3, p. 993–1002, 7 jul. 2006.

WILSON, C. et al. Naloxone increases ketamine-induced hyperactivity in the open field in female rats. **Pharmacology, Biochemistry, and Behavior**, v. 81, n. 3, p. 530–534, jul. 2005.

ZAKHARY, S. M. et al. A behavioral and molecular analysis of ketamine in zebrafish. **Synapse (New York, N.Y.)**, v. 65, n. 2, p. 160–167, fev. 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, ago. 2006.

ZIMMERMANN, F. F. et al. Oxytocin reversed MK-801-induced social interaction and aggression deficits in zebrafish. **Behavioural Brain Research**, v. 311, p. 368–374, 15 2016.

ZUGNO, A. I. et al. Rivastigmine reverses cognitive deficit and acetylcholinesterase activity induced by ketamine in an animal model of schizophrenia. **Metabolic Brain Disease**, v. 28, n. 3, p. 501–508, set. 2013.

ZUGNO, A. I. et al. Omega-3 fatty acids prevent the ketamine-induced increase in acetylcholinesterase activity in an animal model of schizophrenia. **Life Sciences**, v. 121, p. 65–69, 15 jan. 2015.

Figure captions

Fig. 1. Schematic representation of the experimental design. (A) We evaluated the acute effects of sub-anesthetic ketamine concentrations on zebrafish the inhibitory avoidance task, in which ketamine exposure occurred immediately after the training session. After 24 h, the test session was performed and locomotion was evaluated in the novel tank test. (B) We evaluated the effects of repeated exposure of sub-anesthetic ketamine concentrations on zebrafish. After 24 h of the last exposure, novel tank task and mirror-induced aggression test were performed. Immediately after the behavioral tests, the sample was prepared for analysis of the whole-body cortisol levels.

Fig. 2. Inhibitory avoidance task of zebrafish acutely exposed to ketamine. Data are presented as median ± interquartile range and analyzed by Wilcoxon matched-pairs signed rank test. Asterisks above bars express significant differences between sessions (n = 19 - 22 animals per group; * $p < 0.05$ and ** $p < 0.01$).

Fig. 3. Locomotor parameters of zebrafish 24 h after acute ketamine exposure. Data are presented as mean ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA (n = 12 animals per group).

Fig. 4. Locomotor parameters of zebrafish repeatedly exposed to ketamine. Date are presented as mean ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA and followed by Student-Newman-Keuls multiple comparison test when necessary. Asterisks above bars express significant difference between groups (n = 10 – 15 animals per group, ***$p < 0.001$, ****$p < 0.0001$).

Fig. 5. Aggression-related behaviors of zebrafish repeatedly exposed to ketamine. Date are presented as mean ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA (n = 10 – 15 animals per group).

Fig. 6. Vertical exploration of zebrafish repeatedly exposed to ketamine. Date are presented as mean ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA (n = 11 – 16 animals per group).

Fig. 7. Whole-body cortisol levels (ng/g body weight for each individual fish) of zebrafish repeatedly exposed to ketamine. Data are presented as mean ± standard error of the mean (S.E.M.) and analyzed by one-way ANOVA (n = 8 - 9 animals per group).

Figure 1

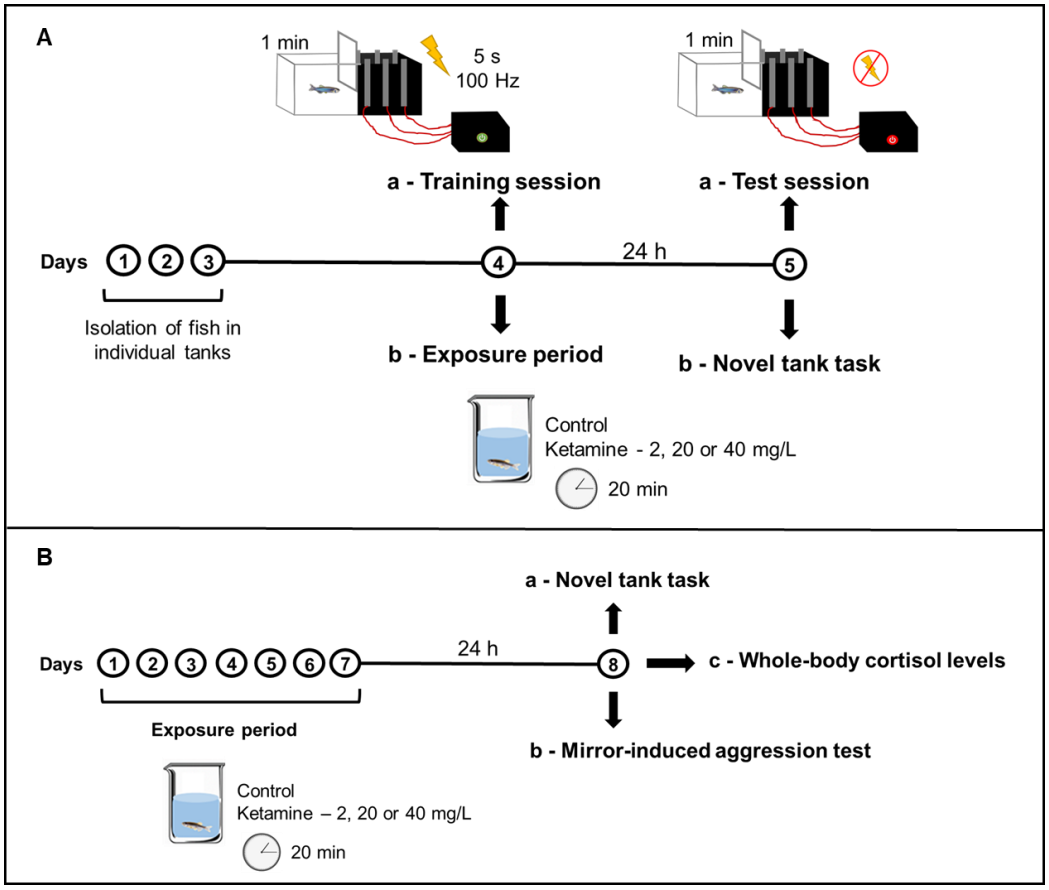


Figure 2

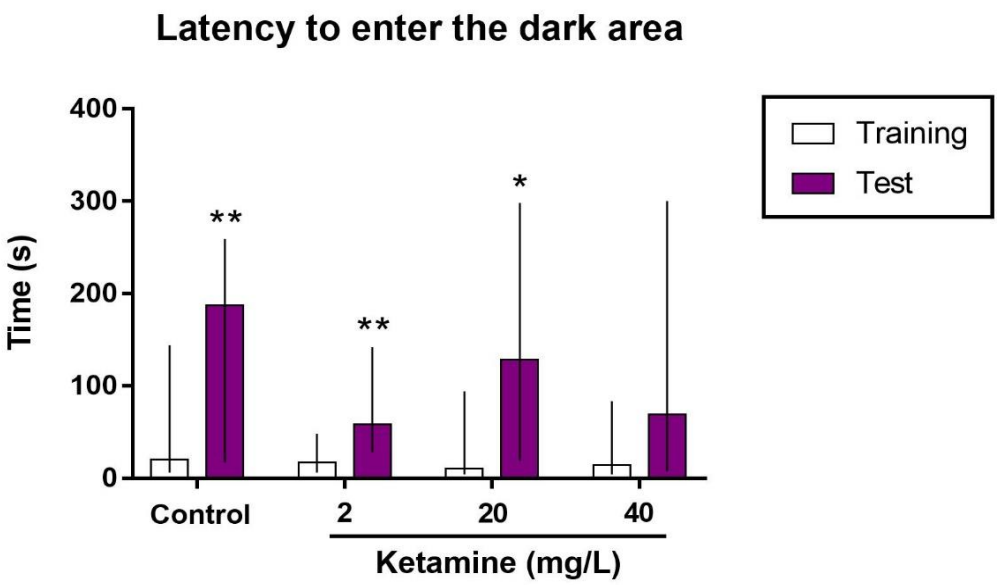


Figure 3

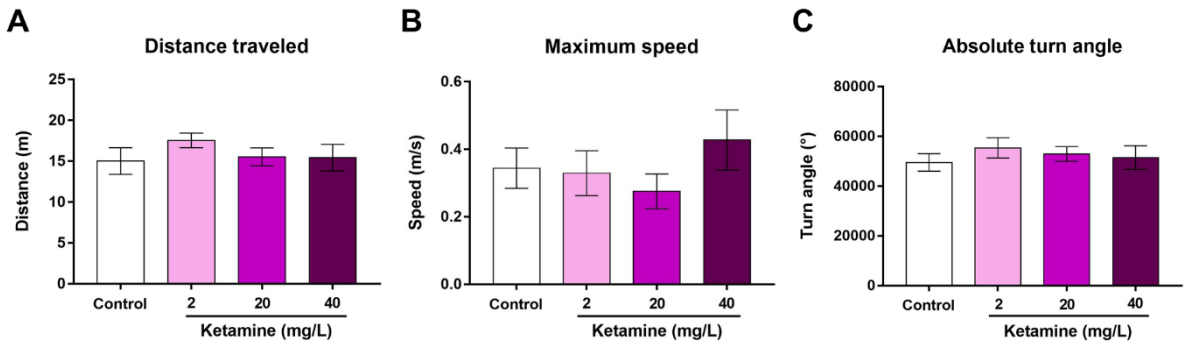


Figure 4

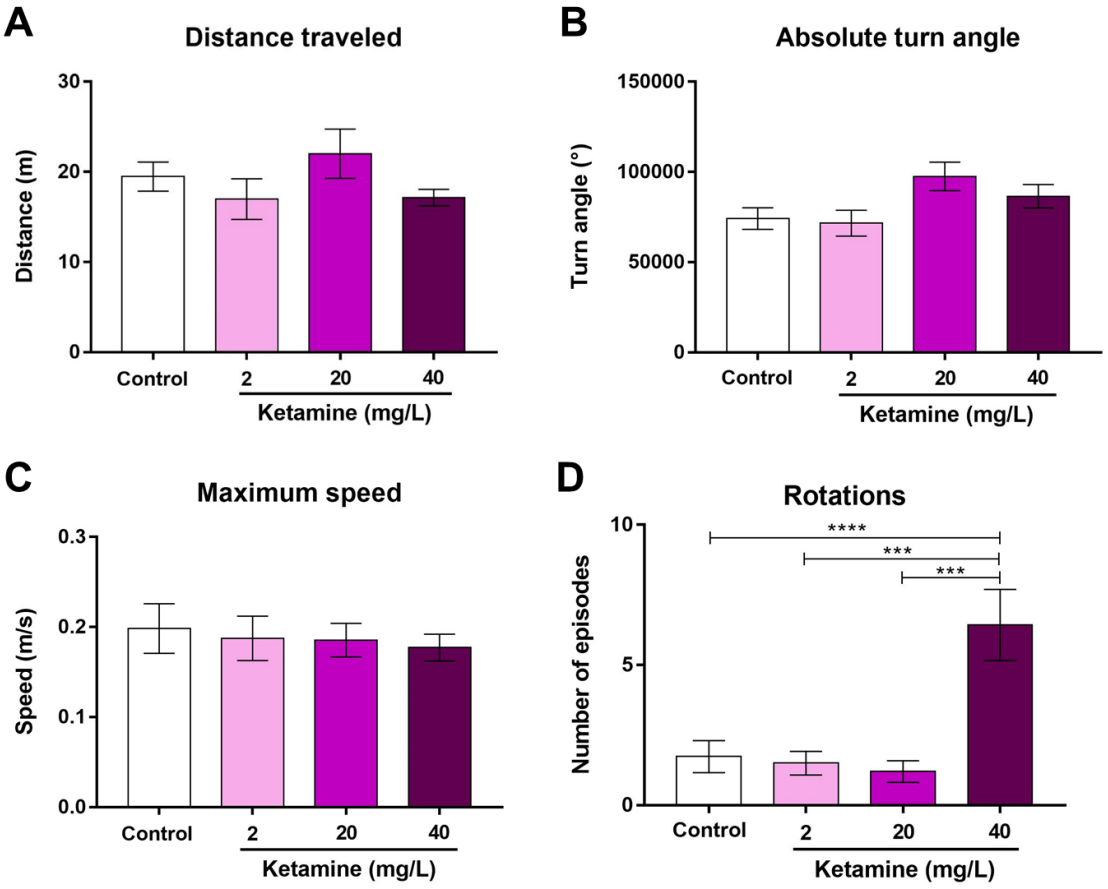


Figure 5

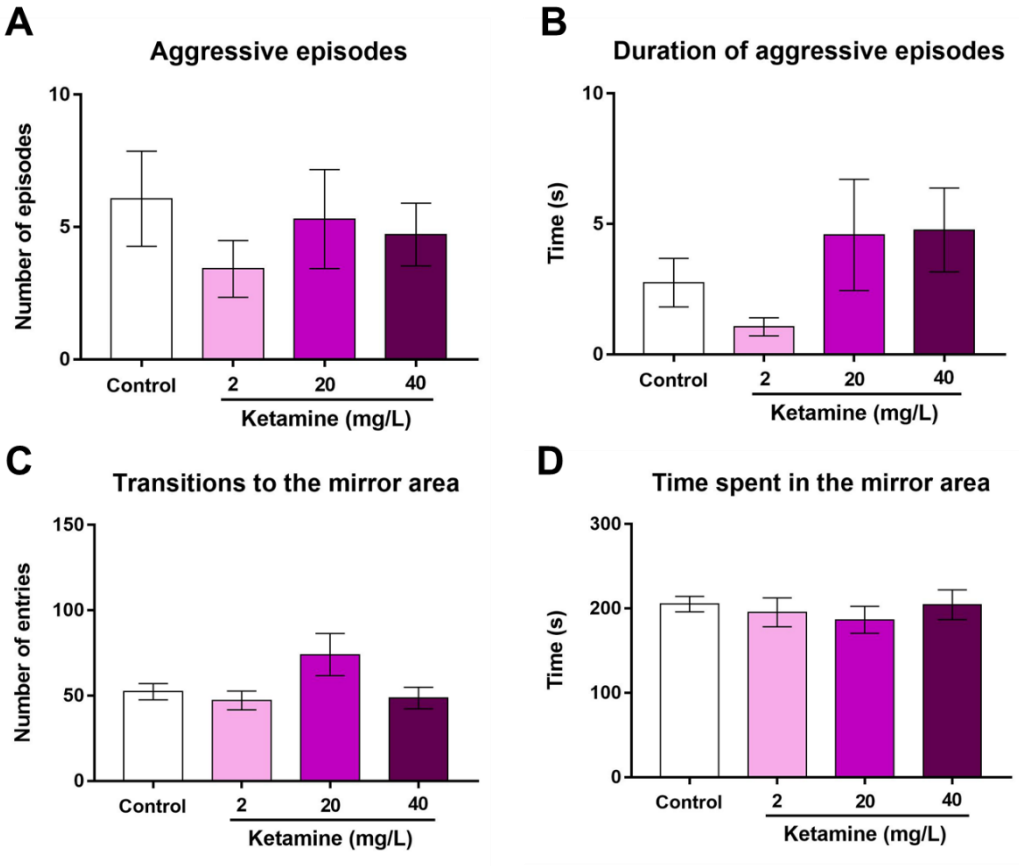


Figure 6

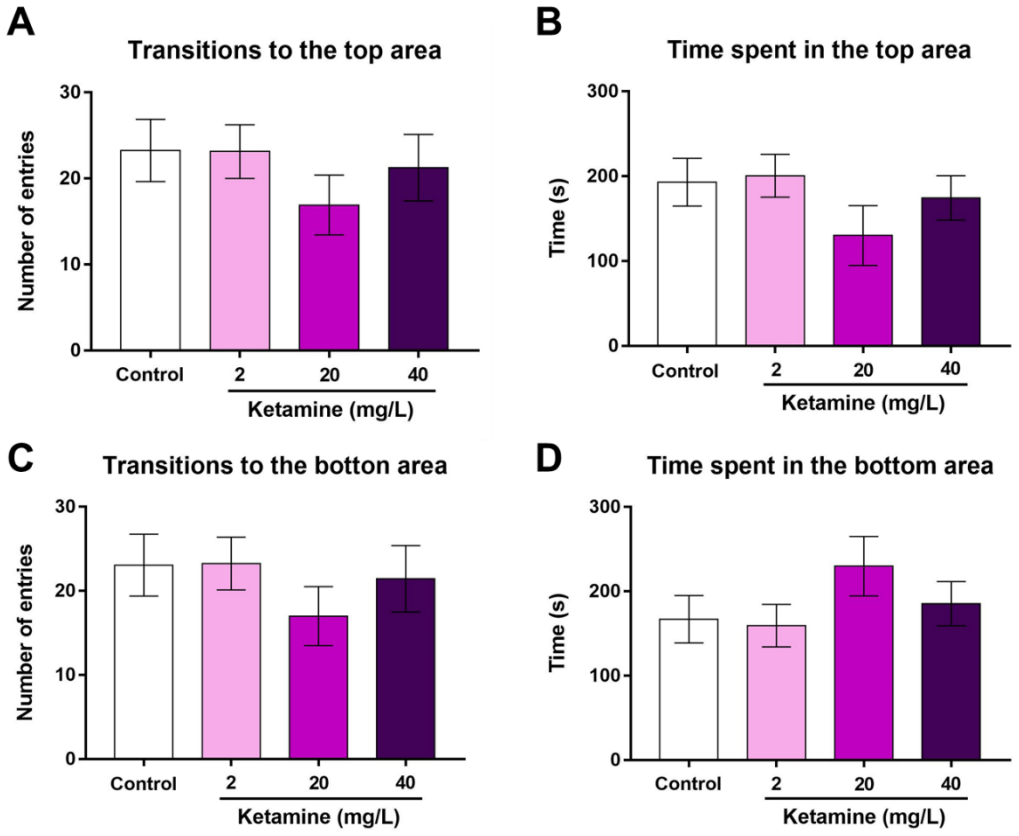
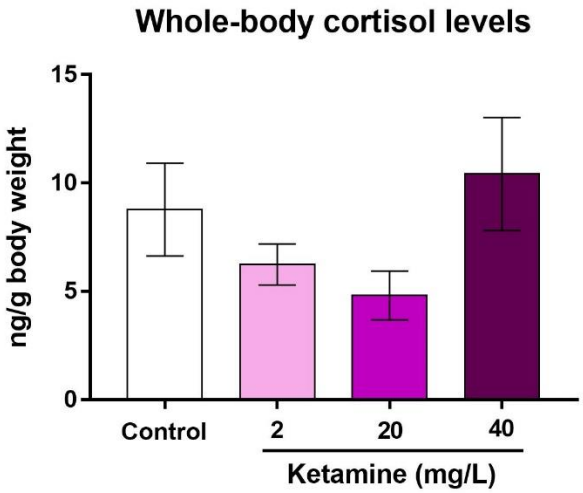


Figure 7


Whole-body cortisol levels
ng/g body weight
15
10
5
0
Control
2
20
40
Ketamine (mg/L)

4.3 ATIVIDADE DA ACETILCOLINESTERASE

4.3.1 Metodologia

Imediatamente após a exposição aguda e 24 h após a exposição repetida a cetamina os peixes foram anestesiados em água fria (4 °C) e eutanasiados por decapitação para a remoção do encéfalo. Cada encéfalo foi homogeneizado em 300 µL de tampão fosfato de potássio (TFK) 100 mM pH 7,4. Após a homogeneização, as amostras foram centrifugadas à 1.000 x *g* por 10 minutos a 4 ºC. A análise da atividade enzimática foi realizada utilizando o sobrenadante.

A atividade da AChE foi quantificada colorimetricamente utilizando o método cinético de Ellman et al (1961) como modificado por Pereira et al (2004). Este método é baseado na hidrólise da acetiltiocolina, onde a tiocolina reage com ácido 5,5'-ditiobis-2-nitrobenzoico (DTNB) formando o tiolato de coloração amarela. Aproximadamente 5 µg de proteína (sobrenadante) foi pré-incubada em um meio contendo TFK 100 mM, DTNB 1 mM em pH 7,3 à 25 °C por um período de 1 minuto. A reação enzimática inicia-se pela adição de 0,8 mM de acetiltiocolina. As leituras das amostras foram realizadas em espectrofotômetro à 412 nm de 30 em 30 segundos por um período de 2 min. Todas as amostras foram testadas em duplicata. A atividade enzimática foi expressa como nmol de acetiltiocolina hidrolisada por hora por mg de proteína. O teor de proteínas foi quantificado pelo método de Bradford (1976) usando albumina de soro bovino como padrão.

4.3.2 Resultados

Na figura 1 estão apresentados as atividades da AChE de encéfalos de peixes-zebra expostos a cetamina de forma aguda – 1 exposição de 20 min ou de forma repetida – 7 dias (exposição) de 20 min. A exposição a cetamina não alterou a atividade da AChE em nenhuma das concentrações testadas, tanto para a exposição aguda como repetida.

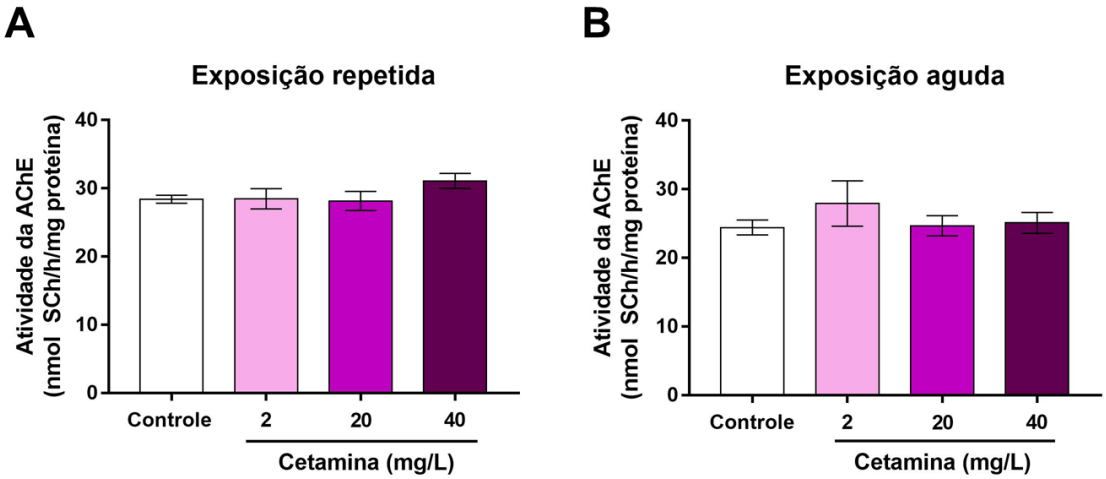


Figura 1 – Atividade da AChE de encéfalo de peixes-zebra expostos a cetamina: exposição aguda (A), repetida (B). Os dados são apresentados como média ± erro padrão da media e analisados por ANOVA de uma via (*n* = 4 - 8 animais por grupo).

5 DISCUSSÃO

Nesta dissertação foi investigado os efeitos comportamentais e bioquímicos de concentrações subanestésicas de cetamina, um fármaco amplamente usado na clínica, e aplicado em um modelo experimental alternativo, o peixe-zebra.

No modelo de exposição aguda (uma exposição por 20 min) verificou-se que os animais submetidos à concentração de 2 mg/L apresentaram um aumento no comportamento agressivo (número de episódios e duração) enquanto que aqueles expostos à 20 e 40 mg/L apresentaram uma diminuição, ou seja, efeito oposto. Observou-se também hiperlocomoção induzida pelas concentrações de 20 e 40 mg/L, devido ao aumento da distância percorrida, estereotipia, devido ao aumento nas rotações na concentração 40 mg/L, e prejuízo na consolidação da memória em animais expostos à concentração de 40 mg/L.

Dados da literatura mostram que peixes-zebra expostos agudamente por 15 min a MK-801 na concentração de 5 μM (1,1 mg/L) apresentam diminuição no comportamento agressivo (ZIMMERMANN et al., 2016) e quando expostos a concentração de 20 μM (4,4 mg/L) por 15, 30 e 60 min apresentam hiperlocomoção (SEIBT et al., 2010). Hiperlocomoção também foi verificada em camundongos tratados com cetamina (10 mg/kg) e MK-801 (0,3 mg/kg) administrados i.p. (HOLUBOVA et al., 2014). Estes dados corroboram com os obtidos em nosso estudo mostrando que a cetamina, um antagonista glutamatérgico, induz efeitos similares ao MK-801 e que estes efeitos são dependentes da dose utilizada.

Um estudo recente do nosso laboratório mostra que MK-801 (2 mg/kg, i.p.) induz efeito amnésico robusto em peixe-zebra (FRANSCESCON et al., 2020) utilizando a mesma tarefa comportamental que nós usamos neste estudo, a esquiva inibitória, embora aqui obtivemos um efeito amnésico bastante tênue. Mas, tomando os dois estudos podemos confirmar o envolvimento do receptor NMDA na consolidação da memória, uma vez que ambos os compostos, cetamina e MK-801, são antagonistas deste receptor (LIAO et al., 2018; SEIBT et al., 2011). Já em roedores, os efeitos da cetamina na memória são controversos. Um estudo mostra que doses subanestésicas de 50 e 100 mg/kg de cetamina injetadas por via i.p. não alteram a memória em ratos (CHROBAK; HINMAN; SABOLEK, 2008). Outro estudo apresenta comprometimento da consolidação da memória em ratos com doses de 20 ou 100 mg/kg (i.p.)

(BOULTADAKIS; PITSIKAS, 2011). Em camundongos, doses subanestésicas (5, 10, 20 e 50 mg/kg, i.p.) também induziram interrupções na reconsolidação de memória (WANG et al., 2006). Adicionalmente, foi relatada diminuição da memória de trabalho (ADLER et al., 1998) e déficit na formação da memória (UMBRICHT et al., 2000) quando a cetamina é administrada por via intravenosa em humanos.

No modelo de exposição repetida à cetamina (7 exposições de 20 min) verificou-se ausência de alteração na locomoção, comportamento do tipo agressividade e exploração vertical (comportamento do tipo ansiedade) nas concentrações testadas. Em contrapartida, animais expostos à concentração de 40 mg/L apresentaram aumento no comportamento estereotipado (rotações) (ver discussão abaixo).

Poucos estudos abordam esses comportamentos em exposição repetida. Quanto ao comportamento do tipo ansiedade, efeito ansiolítico foi obtido em um estudo com ratos tratados com MK-801 (0,25 mg/kg, i.p.) 2 vezes ao dia por 4 dias (KOCAHAN et al., 2013) e em peixes-zebra expostos agudamente por 20 min a concentrações de cetamina de 40 e 60 mg/L (DE CAMPOS; BRUNI; DE MARTINIS, 2015). Estes resultados diferem dos obtidos em nosso estudo provavelmente devido a diferença das doses e do protocolo experimental utilizado.

Em ambos os protocolos de exposição, aguda ou repetida à cetamina, observou-se o comportamento do tipo estereotipado (nado circular). Este comportamento já havia sido verificado anteriormente, tanto para a exposição aguda quanto repetida à cetamina (ZAKHARY et al., 2011); entretanto, a comparação de efetividade de dose/concentração fica em aberto porque não está claro neste estudo a concentração utilizada, talvez por problema de editoração. O nado circular de maneira repetitiva, é característico de estereotipia em peixes. Esse fenótipo de comportamento anormal é semelhante ao evidenciado em humanos voluntários saudáveis tratados com cetamina (KRYSTAL et al., 1994; LAHTI et al., 2001) e em camundongos com características clínicas de esquizofrenia (TORRES et al., 2004, 2005). Sugere-se que, devido a sensibilidade do peixe-zebra aos efeitos psicotomiméticos da cetamina, o receptor NMDA de peixes pode ter função homóloga ao de cérebro de mamíferos (ZAKHARY et al., 2011).

A exposição repetida a cetamina não interferiu nos níveis de cortisol. O cortisol é um hormônio liberado em situação de estresse coordenado pelo eixo HPI no peixe-zebra (TRAN; CHATTERJEE; GERLAI, 2014). Poucos estudos relatam sobre os efeitos de drogas antagonistas glutamatérgicas sobre os níveis deste hormônio. Estudos mostram que peixes-zebra exposto a cetamina agudamente (de 20 e 40 mg/L) apresentam

diminuição nos níveis de cortisol (RIEHL et al., 2011) ou aumento dos níveis do hormônio quando os peixes-zebra são expostos a cetamina na concentração de 20 mg/L por 2 semanas (PITTMAN; HYLTON, 2015). Em nosso estudo vimos que a ausência de efeito da cetamina sobre os níveis de cortisol acontece em paralelo a ausência de efeitos sobre a agressividade e ansiedade.

A atividade da AChE foi analisada em encéfalo de peixes-zebra nos dois protocolos de exposição: aguda e repetida. Nenhuma das concentrações em qualquer um dos protocolos causou alteração na atividade da enzima, mostrando que o sistema colinérgico, no que tange a enzima que hidrolisa o neurotransmissor na fenda, não é sensível a cetamina neste modelo. Um estudo relata que ratos tratados com cetamina (25 mg/kg, i.p.) por 7 dias apresentaram aumento na atividade da AChE no córtex pré-frontal, hipocampo e estriado (ZUGNO et al., 2015). Outro estudo mostra que camundongos tratados agudamente com cetamina (100 mg/kg, i.p.) apresentam um aumento na atividade da AChE no córtex, estriado e hipocampo. Essa mesma dose também aumenta a atividade enzimática apenas no córtex e no hipocampo em tratamento de 10 dias (CHATTERJEE et al., 2012). Em larvas de peixes medaka, há um aumento de 14% na atividade da AChE quando as mesmas são expostas a 0,004 μM (0,00095 mg/L) de cetamina por 14 dias (LIAO et al., 2018). Embora neste estudo, a atividade da AChE não tenha sido alterada, a modulação de outros sistemas de neurotransmissão pode estar envolvida nos efeitos neurocomportamentais promovidos pela cetamina no peixe-zebra.

Os resultados obtidos nesta dissertação fortalecem a aplicabilidade do peixe-zebra em estudos comportamentais e fisiológicos com a cetamina, auxiliando no entendimento dos efeitos da cetamina, uma droga complexa, sobre comportamentos como a agressão, ansiedade, locomoção e comportamento estereotipado. Assim, destacamos o uso deste organismo modelo em uma perspectiva de pesquisa translacional como um instrumento para explorar demais envolvimentos da cetamina sobre comportamentos e mecanismos neurobiológicos. Além disso, ser útil no auxílio de uma possível aplicação terapêutica da cetamina além de um anestésico.

6 CONCLUSÕES

A partir dos resultados apresentados nesta dissertação podemos concluir que:

- Peixes-zebra expostos agudamente a cetamina apresentam alteração no comportamento agressivo (aumento na concentração de 2 mg/L e diminuição na concentração de 40 mg/L), hiperlocomoção (20 e 40 mg/L), comportamento estereotipado e prejuízo na consolidação da memória (40 mg/L);

- Peixes-zebra expostos repetidamente a cetamina não apresentam comportamento agressivo, alteração na locomoção nem comportamento do tipo ansiedade, porém apresentam comportamento estereotipado (40 mg/L);

- Peixes-zebra expostos aguda e repetidamente a cetamina não apresentam alteração na atividade da AChE, mostrando que o sistema colinérgico, no que tange a enzima que hidrolisa o neurotransmissor na fenda, não é sensível a cetamina;

- A cetamina não interferiu nos níveis de cortisol de corpo inteiro provavelmente devido à ausência de efeitos sobre comportamentos do tipo agressivo e ansiedade.

REFERÊNCIAS

ADLER, C. M. et al. Effects of ketamine on thought disorder, working memory, and semantic memory in healthy volunteers. **Biological Psychiatry**, v. 43, n. 11, p. 811–816, 1 jun. 1998.

AVDESH, A. et al. Regular care and maintenance of a zebrafish (Danio rerio) laboratory: an introduction. **Journal of Visualized Experiments: JoVE**, n. 69, p. e4196, 18 nov. 2012.

BERTONCELLO, K. T. et al. Taurine prevents memory consolidation deficits in a novel alcohol-induced blackout model in zebrafish. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 93, p. 39–45, 14 mar. 2019.

BLANCHARD, D. C.; BLANCHARD, R. J. Ethoexperimental approaches to the biology of emotion. **Annual Review of Psychology**, v. 39, p. 43–68, 1988.

BLANK, M. et al. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. **Neurobiology of Learning and Memory**, v. 92, n. 4, p. 529–534, nov. 2009.

BLASER, R. E.; CHADWICK, L.; MCGINNIS, G. C. Behavioral measures of anxiety in zebrafish (Danio rerio). **Behavioural Brain Research**, v. 208, n. 1, p. 56–62, 17 mar. 2010.

BOULTADAKIS, A.; PITSIKAS, N. Anesthetic ketamine impairs rats' recall of previous information: the nitric oxide synthase inhibitor N-nitro-L-arginine methylester antagonizes this ketamine-induced recognition memory deficit. **Anesthesiology**, v. 114, n. 6, p. 1345–1353, jun. 2011.

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, p. 248–254, 7 maio 1976.

BRAGA, M. M. et al. Evaluation of spontaneous recovery of behavioral and brain injury profiles in zebrafish after hypoxia. **Behavioural Brain Research**, v. 253, p. 145–151, 15 set. 2013.

BURT, T. Donepezil and related cholinesterase inhibitors as mood and behavioral controlling agents. **Current Psychiatry Reports**, v. 2, n. 6, p. 473–478, dez. 2000.

CACHAT, J. et al. Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. **Nature Protocols**, v. 5, n. 11, p. 1786–1799, nov. 2010.

CANEVER, L. et al. A rodent model of schizophrenia reveals increase in creatine kinase activity with associated behavior changes. **Oxidative Medicine and Cellular Longevity**, v. 3, n. 6, p. 421–427, dez. 2010.

CHATTERJEE, M. et al. Neurochemical and molecular characterization of ketamine-induced experimental psychosis model in mice. **Neuropharmacology**, v. 63, n. 6, p. 1161–1171, nov. 2012.

CHEN, J.-T. et al. Regulation of cytochrome P450 gene expression by ketamine: a review. **Expert Opinion on Drug Metabolism & Toxicology**, v. 14, n. 7, p. 709–720, jul. 2018.

CHROBAK, J. J.; HINMAN, J. R.; SABOLEK, H. R. Revealing past memories: proactive interference and ketamine-induced memory deficits. **The Journal of Neuroscience: The Official Journal of the Society for Neuroscience**, v. 28, n. 17, p. 4512–4520, 23 abr. 2008.

COMAI, S. et al. The psychopharmacology of aggressive behavior: a translational approach: part 2: clinical studies using atypical antipsychotics, anticonvulsants, and lithium. **Journal of Clinical Psychopharmacology**, v. 32, n. 2, p. 237–260, abr. 2012.

COYLE, J. T. et al. Glutamatergic synaptic dysregulation in schizophrenia: therapeutic implications. **Handbook of Experimental Pharmacology**, n. 213, p. 267–295, 2012.

CRISP, T. et al. The local monoaminergic dependency of spinal ketamine. **European Journal of Pharmacology**, v. 194, n. 2–3, p. 167–172, 5 mar. 1991.

DAHM, R.; GEISLER, R. Learning from small fry: the zebrafish as a genetic model organism for aquaculture fish species. **Marine Biotechnology (New York, N.Y.)**, v. 8, n. 4, p. 329–345, ago. 2006.

DAS, S. et al. Aggression as an independent entity even in psychosis - The role of cortisol. **Psychiatry Research**, v. 259, p. 405–411, jan. 2018.

DE CAMPOS, E. G.; BRUNI, A. T.; DE MARTINIS, B. S. Ketamine induces anxiolytic effects in adult zebrafish: A multivariate statistics approach. **Behavioural Brain Research**, v. 292, p. 537–546, 1 out. 2015.

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

DICKERSON, D. et al. Ethanol-like effects of thiopental and ketamine in healthy humans. **Journal of Psychopharmacology (Oxford, England)**, v. 24, n. 2, p. 203–211, fev. 2010.

DOMINO, E. F. Taming the ketamine tiger. 1965. **Anesthesiology**, v. 113, n. 3, p. 678–684, set. 2010.

DOMINO, E. F.; CHODOFF, P.; CORSSEN, G. PHARMACOLOGIC EFFECTS OF CI-581, A NEW DISSOCIATIVE ANESTHETIC, IN MAN. **Clinical Pharmacology and Therapeutics**, v. 6, p. 279–291, jun. 1965.

DOMINO, E. F.; LUBY, E. D. Phencyclidine/schizophrenia: one view toward the past, the other to the future. **Schizophrenia Bulletin**, v. 38, n. 5, p. 914–919, set. 2012.

DULAWA, S. C.; JANOWSKY, D. S. Cholinergic regulation of mood: from basic and clinical studies to emerging therapeutics. **Molecular Psychiatry**, v. 24, n. 5, p. 694–709, 2019.

DURIEUX, M. E. Inhibition by ketamine of muscarinic acetylcholine receptor function. **Anesthesia and Analgesia**, v. 81, n. 1, p. 57–62, jul. 1995.

EDWARDS, J. G.; MICHEL, W. C. Odor-stimulated glutamatergic neurotransmission in the zebrafish olfactory bulb. **The Journal of Comparative Neurology**, v. 454, n. 3, p. 294–309, 16 dez. 2002.

EGAN, R. J. et al. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. **Behavioural Brain Research**, v. 205, n. 1, p. 38–44, 14 dez. 2009.

EIDE, K. et al. Continuous subcutaneous administration of the N-methyl-D-aspartic acid (NMDA) receptor antagonist ketamine in the treatment of post-herpetic neuralgia. **Pain**, v. 61, n. 2, p. 221–228, maio 1995.

ELLMAN, G. L. et al. A new and rapid colorimetric determination of acetylcholinesterase activity. **Biochemical Pharmacology**, v. 7, p. 88–95, jul. 1961.

ENGIN, E.; TREIT, D.; DICKSON, C. T. Anxiolytic- and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. **Neuroscience**, v. 161, n. 2, p. 359–369, 30 jun. 2009.

FEATHERSTONE, D. E. Intercellular glutamate signaling in the nervous system and beyond. **ACS chemical neuroscience**, v. 1, n. 1, p. 4–12, 20 jan. 2010.

FONTANA, B. D. et al. Modulatory action of taurine on ethanol-induced aggressive behavior in zebrafish. **Pharmacology, Biochemistry, and Behavior**, v. 141, p. 18–27, fev. 2016.

FONTANA, B. D. et al. The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review. **Experimental Neurology**, v. 299, n. Pt A, p. 157–171, jan. 2018.

FRAGA, D. B. et al. Anxiolytic effects of ascorbic acid and ketamine in mice. **Journal of Psychiatric Research**, v. 100, p. 16–23, 2018.

FRANSCESCON, F. et al. Neuroprotective role of taurine on MK-801-induced memory impairment and hyperlocomotion in zebrafish. **Neurochemistry International**, v. 135, p. 104710, 2020.

GERLAI, R. Zebra fish: an uncharted behavior genetic model. **Behavior Genetics**, v. 33, n. 5, p. 461–468, set. 2003.

GERLAI, R. Zebrafish antipredatory responses: a future for translational research? **Behavioural Brain Research**, v. 207, n. 2, p. 223–231, 5 mar. 2010.

GERLAI, R. A small fish with a big future: zebrafish in behavioral neuroscience. **Reviews in the Neurosciences**, v. 22, n. 1, p. 3–4, 2011.

GODAR, S. C. et al. The role of monoamine oxidase A in aggression: Current translational developments and future challenges. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 69, p. 90–100, 01 2016.

GRAVEN-NIELSEN, T. et al. Ketamine reduces muscle pain, temporal summation, and referred pain in fibromyalgia patients. **Pain**, v. 85, n. 3, p. 483–491, abr. 2000.

GREIFENSTEIN, F. E. et al. A study of a 1-aryl cyclo hexyl amine for anesthesia. **Anesthesia and Analgesia**, v. 37, n. 5, p. 283–294, out. 1958.

HOLUBOVA, K. et al. Pregnanolone Glutamate, a Novel Use-Dependent NMDA Receptor Inhibitor, Exerts Antidepressant-Like Properties in Animal Models. **Frontiers in Behavioral Neuroscience**, v. 8, p. 130, 2014.

HOPTMAN, M. J. Impulsivity and aggression in schizophrenia: a neural circuitry perspective with implications for treatment. **CNS spectrums**, v. 20, n. 3, p. 280–286, jun. 2015.

HOWE, K. et al. The zebrafish reference genome sequence and its relationship to the human genome. **Nature**, v. 496, n. 7446, p. 498–503, 25 abr. 2013.

HUSTVEIT, O.; MAURSET, A.; OYE, I. Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. **Pharmacology & Toxicology**, v. 77, n. 6, p. 355–359, dez. 1995.

IRIFUNE, M. et al. Evidence for GABA(A) receptor agonistic properties of ketamine: convulsive and anesthetic behavioral models in mice. **Anesthesia and Analgesia**, v. 91, n. 1, p. 230–236, jul. 2000.

JAVITT, D. C. Twenty-five years of glutamate in schizophrenia: are we there yet? **Schizophrenia Bulletin**, v. 38, n. 5, p. 911–913, set. 2012.

JOHNSTONE, M.; EVANS, V.; BAIGEL, S. Sernyl (CI-395) in clinical anaesthesia. **British Journal of Anaesthesia**, v. 31, p. 433–439, out. 1959.

JONES, L. J.; NORTON, W. H. J. Using zebrafish to uncover the genetic and neural basis of aggression, a frequent comorbid symptom of psychiatric disorders. **Behavioural Brain Research**, v. 276, p. 171–180, 1 jan. 2015.

KALUEFF, A. V. et al. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. **Zebrafish**, v. 10, n. 1, p. 70–86, mar. 2013.

KHARASCH, E. D.; LABROO, R. Metabolism of ketamine stereoisomers by human liver microsomes. **Anesthesiology**, v. 77, n. 6, p. 1201–1207, dez. 1992.

KIEFER, R.-T. et al. Efficacy of ketamine in anesthetic dosage for the treatment of refractory complex regional pain syndrome: an open-label phase II study. **Pain Medicine (Malden, Mass.)**, v. 9, n. 8, p. 1173–1201, nov. 2008.

KOCAHAN, S. et al. The effects of N-Methyl-D-Aspartate receptor blockade during the early neurodevelopmental period on emotional behaviors and cognitive functions of adolescent Wistar rats. **Neurochemical Research**, v. 38, n. 5, p. 989–996, maio 2013.

KRYSTAL, J. H. et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. **Archives of General Psychiatry**, v. 51, n. 3, p. 199–214, mar. 1994.

KRYSTAL, J. H. et al. Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function. **Archives of General Psychiatry**, v. 62, n. 9, p. 985–994, set. 2005.

KYSIL, E. V. et al. Comparative Analyses of Zebrafish Anxiety-Like Behavior Using Conflict-Based Novelty Tests. **Zebrafish**, v. 14, n. 3, p. 197–208, jun. 2017.

KYZAR, E. J. et al. Effects of hallucinogenic agents mescaline and phencyclidine on zebrafish behavior and physiology. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 37, n. 1, p. 194–202, 27 abr. 2012.

LAHTI, A. C. et al. Effects of ketamine in normal and schizophrenic volunteers. **Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology**, v. 25, n. 4, p. 455–467, out. 2001.

LAULIN, J.-P. et al. The role of ketamine in preventing fentanyl-induced hyperalgesia and subsequent acute morphine tolerance. **Anesthesia and Analgesia**, v. 94, n. 5, p. 1263–1269, table of contents, maio 2002.

LELE, Z.; KRONE, P. H. The zebrafish as a model system in developmental, toxicological and transgenic research. **Biotechnology Advances**, v. 14, n. 1, p. 57–72, 1996.

LI, L.; VLISIDES, P. E. Ketamine: 50 Years of Modulating the Mind. **Frontiers in Human Neuroscience**, v. 10, p. 612, 2016.

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, 15 abr. 2011.

LIAO, P.-H. et al. Illicit drug ketamine induces adverse effects from behavioral alterations and oxidative stress to p53-regulated apoptosis in medaka fish under environmentally relevant exposures. **Environmental Pollution (Barking, Essex: 1987)**, v. 237, p. 1062–1071, jun. 2018.

LIESCHKE, G. J.; CURRIE, P. D. Animal models of human disease: zebrafish swim into view. **Nature Reviews. Genetics**, v. 8, n. 5, p. 353–367, maio 2007.

LUGINBÜHL, M. et al. Modulation of remifentanil-induced analgesia, hyperalgesia, and tolerance by small-dose ketamine in humans. **Anesthesia and Analgesia**, v. 96, n. 3, p. 726–732, table of contents, mar. 2003.

MAXIMINO, C. et al. Scototaxis as anxiety-like behavior in fish. **Nature Protocols**, v. 5, n. 2, p. 209–216, fev. 2010a.

MAXIMINO, C. et al. Measuring anxiety in zebrafish: a critical review. **Behavioural Brain Research**, v. 214, n. 2, p. 157–171, 25 dez. 2010b.

MERCADANTE, S. et al. Analgesic effect of intravenous ketamine in cancer patients on morphine therapy: a randomized, controlled, double-blind, crossover, double-dose study. **Journal of Pain and Symptom Management**, v. 20, n. 4, p. 246–252, out. 2000.

MORGAN, C. J. A. et al. Acute effects of ketamine on memory systems and psychotic symptoms in healthy volunteers. **Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology**, v. 29, n. 1, p. 208–218, jan. 2004.

NESHER, N. et al. Morphine with adjuvant ketamine vs higher dose of morphine alone for immediate postthoracotomy analgesia. **Chest**, v. 136, n. 1, p. 245–252, jul. 2009.

NEUMANN, I. D.; VEENEMA, A. H.; BEIDERBECK, D. I. Aggression and anxiety: social context and neurobiological links. **Frontiers in Behavioral Neuroscience**, v. 4, p. 12, 2010.

NEWCOMER, J. W. et al. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. **Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology**, v. 20, n. 2, p. 106–118, fev. 1999.

NG, M.-C. et al. Effect of MK-801-induced impairment of inhibitory avoidance learning in zebrafish via inactivation of extracellular signal-regulated kinase (ERK) in telencephalon. **Fish Physiology and Biochemistry**, v. 38, n. 4, p. 1099–1106, ago. 2012.

NOWACKA, A.; BORCZYK, M. Ketamine applications beyond anesthesia - A literature review. **European Journal of Pharmacology**, v. 860, p. 172547, 5 out. 2019.

O'DELL, T. J. et al. Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. **Proceedings of the National Academy of Sciences of the United States of America**, v. 88, n. 24, p. 11285–11289, 15 dez. 1991.

OLIVEIRA, T. A. et al. Alcohol impairs predation risk response and communication in zebrafish. **PloS One**, v. 8, n. 10, p. e75780, 2013.

OLIVEIRA, T. A. et al. Death-associated odors induce stress in zebrafish. **Hormones and Behavior**, v. 65, n. 4, p. 340–344, abr. 2014.

OLNEY, J. W.; FARBER, N. B. NMDA antagonists as neurotherapeutic drugs, psychotogens, neurotoxins, and research tools for studying schizophrenia. **Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology**, v. 13, n. 4, p. 335–345, dez. 1995.

PANULA, P. et al. Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases. **Zebrafish**, v. 3, n. 2, p. 235–247, 2006.

PAPP, M. et al. Antidepressant, anxiolytic and procognitive effects of rivastigmine and donepezil in the chronic mild stress model in rats. **Psychopharmacology**, v. 233, n. 7, p. 1235–1243, abr. 2016.

PEREIRA, M. E.; ADAMS, A. I. H.; SILVA, N. S. 2,5-Hexanedione inhibits rat brain acetylcholinesterase activity in vitro. **Toxicology Letters**, v. 146, n. 3, p. 269–274, 2 fev. 2004.

PIATO, A. L. et al. Acute restraint stress in zebrafish: behavioral parameters and purinergic signaling. **Neurochemical Research**, v. 36, n. 10, p. 1876–1886, out. 2011.

PITTMAN, J.; HYLTON, A. Behavioral, endocrine, and neuronal alterations in zebrafish (Danio rerio) following sub-chronic coadministration of fluoxetine and ketamine. **Pharmacology, Biochemistry, and Behavior**, v. 139 Pt B, p. 158–162, dez. 2015.

RAMBO, C. L. et al. Gender differences in aggression and cortisol levels in zebrafish subjected to unpredictable chronic stress. **Physiology & Behavior**, v. 171, p. 50–54, 15 mar. 2017.

RICO, E. P. et al. Zebrafish neurotransmitter systems as potential pharmacological and toxicological targets. **Neurotoxicology and Teratology**, v. 33, n. 6, p. 608–617, dez. 2011.

RIEHL, R. et al. Behavioral and physiological effects of acute ketamine exposure in adult zebrafish. **Neurotoxicology and Teratology**, v. 33, n. 6, p. 658–667, dez. 2011.

SEIBT, K. J. et al. Antipsychotic drugs prevent the motor hyperactivity induced by psychotomimetic MK-801 in zebrafish (Danio rerio). **Behavioural Brain Research**, v. 214, n. 2, p. 417–422, 25 dez. 2010.

SEIBT, K. J. et al. Antipsychotic drugs reverse MK-801-induced cognitive and social interaction deficits in zebrafish (Danio rerio). **Behavioural Brain Research**, v. 224, n. 1, p. 135–139, 10 out. 2011.

SHIN, S. S.; DIXON, C. E. Alterations in Cholinergic Pathways and Therapeutic Strategies Targeting Cholinergic System after Traumatic Brain Injury. **Journal of Neurotrauma**, v. 32, n. 19, p. 1429–1440, 1 out. 2015.

SHIN, S. Y. et al. Chronic administration of ketamine ameliorates the anxiety- and aggressive-like behavior in adolescent mice induced by neonatal maternal separation. **The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology**, v. 23, n. 1, p. 81–87, jan. 2019.

SINNER, B.; GRAF, B. M. Ketamine. **Handbook of Experimental Pharmacology**, n. 182, p. 313–333, 2008.

SISON, M.; GERLAI, R. Behavioral performance altering effects of MK-801 in zebrafish (Danio rerio). **Behavioural Brain Research**, v. 220, n. 2, p. 331–337, 7 jul. 2011.

SMITH, D. J.; WESTFALL, D. P.; ADAMS, J. D. Assessment of the potential agonistic and antagonistic properties of ketamine at opiate receptors in the guinea-pig ileum. **Neuropharmacology**, v. 21, n. 7, p. 605–611, jul. 1982.

SPIDEL, A. et al. Early psychosis and aggression: predictors and prevalence of violent behaviour amongst individuals with early onset psychosis. **International Journal of Law and Psychiatry**, v. 33, n. 3, p. 171–176, jun. 2010.

STEIMER, T. The biology of fear- and anxiety-related behaviors. **Dialogues in Clinical Neuroscience**, v. 4, n. 3, p. 231–249, set. 2002.

THEODORIDI, A.; TSALAFOUTA, A.; PAVLIDIS, M. Acute Exposure to Fluoxetine Alters Aggressive Behavior of Zebrafish and Expression of Genes Involved in Serotonergic System Regulation. **Frontiers in Neuroscience**, v. 11, p. 223, 2017.

TOMEK, S. E. et al. NMDA Receptor Modulators in the Treatment of Drug Addiction. **Pharmaceuticals (Basel, Switzerland)**, v. 6, n. 2, p. 251–268, 6 fev. 2013.

TORRES, G. et al. A neurobehavioral screening of the ckr mouse mutant: implications for an animal model of schizophrenia. **Brain Research Bulletin**, v. 62, n. 4, p. 315–326, 15 jan. 2004.

TORRES, G. et al. Ventricular size mapping in a transgenic model of schizophrenia. **Brain Research. Developmental Brain Research**, v. 154, n. 1, p. 35–44, 1 jan. 2005.

TRAN, S.; CHATTERJEE, D.; GERLAI, R. Acute net stressor increases whole-body cortisol levels without altering whole-brain monoamines in zebrafish. **Behavioral Neuroscience**, v. 128, n. 5, p. 621–624, out. 2014.

UMBRICHT, D. et al. Ketamine-induced deficits in auditory and visual context-dependent processing in healthy volunteers: implications for models of cognitive deficits in schizophrenia. **Archives of General Psychiatry**, v. 57, n. 12, p. 1139–1147, dez. 2000.

WANG, J. H. et al. Ketamine affects memory consolidation: differential effects in T-maze and passive avoidance paradigms in mice. **Neuroscience**, v. 140, n. 3, p. 993–1002, 7 jul. 2006.

WAY, G. P. et al. A comparison of methodologies to test aggression in zebrafish. **Zebrafish**, v. 12, n. 2, p. 144–151, abr. 2015.

WHITE, P. F. et al. Comparative pharmacology of the ketamine isomers. Studies in volunteers. **British Journal of Anaesthesia**, v. 57, n. 2, p. 197–203, fev. 1985.

WHITE, P. F.; WAY, W. L.; TREVOR, A. J. Ketamine--its pharmacology and therapeutic uses. **Anesthesiology**, v. 56, n. 2, p. 119–136, fev. 1982.

WHITLOCK, K. E.; WESTERFIELD, M. The olfactory placodes of the zebrafish form by convergence of cellular fields at the edge of the neural plate. **Development (Cambridge, England)**, v. 127, n. 17, p. 3645–3653, set. 2000.

WILKINSON, S. T. et al. The Effect of a Single Dose of Intravenous Ketamine on Suicidal Ideation: A Systematic Review and Individual Participant Data Meta-Analysis. **The American Journal of Psychiatry**, v. 175, n. 2, p. 150–158, 01 2018.

WILSON, C. et al. Naloxone increases ketamine-induced hyperactivity in the open field in female rats. **Pharmacology, Biochemistry, and Behavior**, v. 81, n. 3, p. 530–534, jul. 2005.

ZAKHARY, S. M. et al. A behavioral and molecular analysis of ketamine in zebrafish. **Synapse (New York, N.Y.)**, v. 65, n. 2, p. 160–167, fev. 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, ago. 2006.

ZHANG, C.; ZHOU, P.; YUAN, T. The cholinergic system in the cerebellum: from structure to function. **Reviews in the Neurosciences**, v. 27, n. 8, p. 769–776, 01 2016.

ZIMMERMANN, F. F. et al. Oxytocin reversed MK-801-induced social interaction and aggression deficits in zebrafish. **Behavioural Brain Research**, v. 311, p. 368–374, 15 2016.

ZUGNO, A. I. et al. Rivastigmine reverses cognitive deficit and acetylcholinesterase activity induced by ketamine in an animal model of schizophrenia. **Metabolic Brain Disease**, v. 28, n. 3, p. 501–508, set. 2013.

ZUGNO, A. I. et al. Omega-3 fatty acids prevent the ketamine-induced increase in acetylcholinesterase activity in an animal model of schizophrenia. **Life Sciences**, v. 121, p. 65–69, 15 jan. 2015.