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Effect of ketamine enantiomers and maternal deprivation on depressive-like behavior and plasma concentration of BDNF in rats

Efeito dos enantiômeros de cetamina e da privação materna no comportamento depressivo e na concentração plasmática de BDNF em ratos

Efecto de los enantiómeros de ketamina y la privación materna sobre el comportamiento depresivo y la concentración plasmática de BDNF en ratas

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Abstract

Background: Low doses of different ketamine enantiomers have demonstrated rapid behavioural and biological actions in animals with depressive-like behavior. However, some authors report different effects depending on model of depression and ketamine isomer used. Objective: Our primary aim was to evaluate the effect of ketamine enantiomers

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on depressive-like behavior, anhedonic-like behavior and locomotor activity of rats subjected to maternal deprivation (MD). Secondarily, we investigated the Brain-derived neurotrophic factor plasma concentration between experimental groups. Methods: Male rats (n=71) were randomized into seven experimental groups: one non-deprived with placebo and other six deprived split into control and intervention groups. Rats were treated with a single intraperitoneal dose of ketamine, esketamine, or arketamine. One hour after drug application, we performed experimental tests to evaluate the antidepressant-like behavior and locomotor activity. Furthermore, blood was collected to measure plasmatic BDNF levels. Results: We did not observe induction of depressive-like in rats subjected to MD. Furthermore, there was no change in BDNF (F (6,64) = 0.9664, p=0.455) between all experimental groups. Conclusion: Neither MD nor the different ketamine isomers were able to change depressive-like behavior or plasma BDNF levels.
Keywords: Ketamine; Depressive disorder, treatment-resistant; Maternal deprivation; Brain-derived neurotrophic factor.

Resumo

Introdução: Baixas doses de diferentes enantiômeros de cetamina demonstraram rápidas ações comportamentais e biológicas em animais com comportamento semelhante ao depressivo. No entanto, alguns autores relatam efeitos diferentes dependendo do modelo de depressão e do isômero de cetamina utilizado. Objetivo: Nosso objetivo principal foi avaliar o efeito dos enantiômeros de cetamina no comportamento tipo depressivo, comportamento tipo anedônico e atividade locomotora de ratos submetidos à privação materna (MD). Secundariamente, investigamos a concentração plasmática do fator neurotrófico derivado do cérebro entre grupos experimentais. Métodos: Ratos machos (n=71) foram randomizados em sete grupos experimentais: um não privado com placebo e outros seis privados divididos em grupos controle e intervenção. Os ratos foram tratados com uma dose intraperitoneal única de cetamina, escetamina ou arcetamina. Uma hora após a aplicação da droga, foram realizados testes experimentais para avaliar o comportamento antidepressivo e a atividade locomotora. Além disso, foi coletado sangue para dosagem dos níveis plasmáticos de BDNF. Resultados: Não observamos indução de sintomas depressivos em ratos submetidos à DM. Além disso, não houve alteração no BDNF (F (6,64) = 0,9664, p=0,455) entre todos os grupos experimentais. Conclusão: Nem a MD, nem os diferentes isômeros de cetamina foram capazes de alterar o comportamento depressivo ou os níveis plasmáticos de BDNF.
Palavras-chave: Cetamina; Transtorno depressivo resistente a tratamento; Privação materna; Fator neurotrófico derivado do encéfalo.

Resumen

Antecedentes: Dosis bajas de diferentes enantiómeros de ketamina han demostrado acciones conductuales y biológicas rápidas en animales con comportamiento similar a la depresión. Sin embargo, algunos autores informan efectos diferentes según el modelo de depresión y el isómero de ketamina utilizado. Objetivo: Nuestro objetivo principal fue evaluar el efecto de los enantiómeros de ketamina sobre el comportamiento depresivo, el comportamiento anhedónico y la actividad locomotora de ratas sometidas a privación materna (MD). En segundo lugar, investigamos la concentración plasmática del factor neurotrófico derivado del cerebro entre los grupos experimentales. Métodos: Se asignaron aleatoriamente ratas macho (n=71) a siete grupos experimentales: uno no privado con placebo y otros seis privados divididos en grupos de control y de intervención. Las ratas fueron tratadas con una dosis intraperitoneal única de ketamina, esketamina o arketamina. Una hora después de la aplicación del fármaco, realizamos pruebas experimentales para evaluar el comportamiento similar al antidepresivo y la actividad locomotora. Además, se recogió sangre para medir los niveles plasmáticos de BDNF. Resultados: No observamos inducción de síntomas de tipo depresivo en ratas sometidas a MD. Además, no hubo cambios en BDNF (F (6,64) = 0,9664, p = 0,455) entre todos los grupos experimentales. Conclusión: Ni la MD ni los diferentes isómeros de la ketamina fueron capaces de cambiar el comportamiento depresivo o los niveles plasmáticos de BDNF.
Palabras clave: Ketamina; Trastorno depresivo resistente al tratamiento; Privación materna; Factor neurotrófico derivado del encéfalo.

1. Introduction

Psychiatric disorders, such as depression and schizophrenia, are usually associated with subjective symptoms (e.g. sadness, anxiety, hallucinations, delusions), proper to the human species and difficult to measure in other animals (Salgado & Sandner, 2013). The poor understanding of the pathophysiology of psychiatric disorders hinders the development of animal models.

Scientific evidences have shown that adverse events early in life, including negligence and infancy trauma, could induce changes in the neurodevelopment, which lead to higher risk for neuropsychiatric conditions, such as anxiety, schizophrenia and major depressive disorders (MDD) (Ellenbroek & Riva, 2003; Réus et al., 2019). Nonetheless, systematic reviews recently

published point out that some inconsistencies are shown in maternal deprivation (MD) model results and this can be influenced by variations in experimental protocols (Tractenberg et al., 2016; Wang et al., 2020).

Ketamine is a racemic mixture consisting of two isomers (S)-ketamine (or esketamine) and (R)-ketamine (or arketamine). Its main mechanism of action is the noncompetitive antagonism of N-methyl-D-aspartate (NMDA) receptor. However, depending on the dose administered, ketamine can interact with different receptors in the central nervous system (dopaminergic, opioids, GABAergic, monoaminergic, cholinergic) and promote several pharmacological actions (anesthetic, analgesics, dissociative, anti-inflammatory and antidepressant) (Delfino et al., 2020; Zanos et al., 2018). In animal models, it is known that high doses of ketamine produce psychotomimetic behaviors and schizophrenic-like effects (Chan et al., 2012; Zugno et al., 2013) and low doses produced rapid antidepressant-like actions (Fukumoto et al., 2017; Zanos et al., 2016). These effects can also vary depending on the ketamine isomer applied (Yang et al., 2015).

Furthermore, some authors describe that ketamine is able to increase Brain-derived neurotrophic factor (BDNF) central levels and reverse cortical atrophy in laboratory animals subjected to different models of chronic stress including MD (Réus et al., 2011; Yang et al., 2015). But it is not clear if this central increase is related with peripheral concentrations of BDNF (Nedelec et al., 2018). We hypothesized that MD induces depressive and anhedonic behavior in rats and that these effects can be reversed by treatment with different ketamine enantiomers. Secondarily, we believe that this mechanism can be related to the increase in plasma BDNF levels.

Our main objective was to evaluate the effect of MD and the administration of different ketamine enantiomers on depressive, anhedonic-like behavior and locomotor activity of rats. Secondarily, we investigated the BDNF plasma concentration between different experimental groups.

2. Methodology

Animals and ethical concerns

Pregnant female Wistar rats weighing between 200 and 350g (60 days of life) were obtained from the Neuroscience Laboratory from Federal University of Bahia (Salvador, Brazil). All mothers and pups were kept in a controlled environment with temperature (22 ± 2◦C) and 12-hour light/dark cycle (7 a.m. to 7 p.m.) with ad libitum access to food and water. The study was approved by the local Ethic Committee of Using Animals in Research (CEUA-ICS-UFBA, IRB 1638100518), and was performed in accordance with EC Directive 86/609/EEC for animal experiments and ARRIVE Guidelines Checklist.

Maternal deprivation

MD was performed following the same protocol previously published (Réus et al., 2011, 2013, 2015; Valvassori et al., 2024; Zugno et al., 2013). The pups were deprived of the dam for 3 hours per day during the first 10 days. During the MD protocol, the dam was placed in a separate cage and carried to another room. The pups were maintained grouped in the nest in the presence of maternal odour. The dam was returned to their nest at the end of each 180min daily deprivation. Control (non-deprived) groups with the same birth week as pups in the deprived group, remained in the same room in their resident boxes together with their mothers throughout. On weaning day (D21), the mother and the females are removed from the nest and donated to Wild Animal Screening Center (CETAS-IBAMA).

Treatment and Experimental Design

A single dose of ketamine (Syntec) (10mg/kg), esketamine (Cristalia S.A.) (10mg/kg) or arketamine (Cristalia S.A.) (5, 10 or 20mg/kg) was injected intraperitoneally (i.p.) one hour before the experimental tests. Treatments were administrated in a

volume of 1 ml/kg and were performed in adulthood (at least 60 days after birth). After the last behavioral test, the animals were anesthetized with sodium pentobarbital 40mg/kg plus lidocaine 10mg/kg. Ketamine doses were selected based on the previous studies (Fukumoto et al., 2017; Nedelec et al., 2018; Réus et al., 2015). Males rats (n=71) were separated into groups of 4-5 per box until the day of the experiment (D60) and were randomized into seven experimental groups: (1) non-deprived + placebo (saline) (n=9); (2) deprived + saline (n=11); (3) deprived + ketamine (10mg/kg) (n=11); (4) deprived + esketamine (10mg/kg) (n=9); (5) deprived + arketamine (5mg/kg) (n=11); (6) deprived + arketamine (10mg/kg) (n=11) and (7) deprived + arketamine (20mg/kg) (n=9) (Figure 1A). The randomization was performed through an electronic randomization software platform (http://www.randomizer.org).

Open field test (OFT)

On day 60, rats were exposed to the open-field test one hour after acute treatment, in order to evaluate the possible effects of MD and/or drug treatment on exploratory activity. Animals were gently placed on the left rear quadrant of OFT arena (50 length x 50 height x 39 depth cm2, divided into 12 equal quadrants). On a period of 5 min, a blinded expert observer manually recorded the crossings (number of quadrants crossed) and rearing (the number of vertical lifts leaning on their two hind legs). A 10% alcohol solution was used to clean the open-field apparatus between each rat to remove potentially interfering odors.

Splash test (ST)

On day 60, ten minutes after the OFT session, the ST was performed on the same animals to assessing anhedonia and self-care behavior. Sucrose solution (10%) was spurted on the back of the rat while it was in their home cages. Then the animal was placed in the left quadrant of OFT arena facing the wall and allow for 5 minutes. The grooming duration (time spent cleaning themselves, licking their back, or passing their front legs on their muzzle to clean the solution that has been sprayed) was recorded to a blinded expert observer.

Forced swim test (FST)

The FST was performed immediately after the ST and was conducted according to previous reports (Réus et al., 2015; Slattery & Cryan, 2012). The forced swimming test was carried to evaluate antidepressant-like effects of ketamine and/or depressive-like behavior associated with MD and consisted of two sessions. The first session (day 59) was the pre-FST that lasted 15 minutes and aimed acclimates the animal to the swim test procedure. Twenty-four hours after the pre-FST (day 60), the experimental FST was performed, lasting 5 min. Rats were individually placed in an acrylic cylinder (30 diameter x 60 height cm) filled with 30 cm water (23 ± 1 °C). Three types of behavior were evaluated: a) swimming, time spent diving or performing other active movements of forelimbs or hind limbs around the tank; b) climbing, time spent vigorously moving hind legs or placing part of the body out of the water, usually against the walls of the tank; and c) immobility, time spent floating and performing the necessary movements to keep the muzzle above water. The antidepressant-like response is characterized by decreased immobility time and more swimming/struggling (Slattery & Cryan, 2012). All behavioral tests were conducted during the day (between 7 a.m. and 4 p.m.). Figure 1A illustrates a time-line diagram of experiments.

Measurement of plasma BDNF

Blood samples were collected, through intracardiac puncture, and stored in EDTA tubes. The samples were centrifuged, and the plasma was collected, aliquoted and stored at -20° C. The concentrations of BDNF were determined in plasma by ELISA (DuoSet; R&D Systems) following the manufacturer's instructions.

Statistical analysis

A sample size calculation was made a priori based on the Gpower 3.0 program. Effect size for detecting differences in immobility time was calculated from data obtained in Réus et al. (Réus et al., 2013) study and through the website (https://www.psychometrica.de/ effect_size.html) (f = 0.5993). Considering an alpha error of 0.05 and a power of 95%, the sample size n was 70 animals.

The primary experimental outcome was the depressive-like behavior (immobility time). We also evaluated the anhedonic-like behavior (grooming time) and the locomotor activity (number of crossings). Secondarily, we measured plasmatic levels of BDNF.

The statistical analysis was performed using IBM SPSS Statistics 20 software package and PAST Statistics version 4.03 (HAMMER, Oyvind Paleontogical) programs. The Shapiro-Wilk test was performed to test for the normality of data. Variables with non-normal distribution were analyzed by permutation ANOVA and permutation post-hoc. Variables with normal distribution were analyzed by One-way ANOVA followed by Tukey post-hoc test to evaluate differences among experimental groups. When the variances were unequal, we used Welch's correction. $P < 0.05$ were considered statistically significant. Data was presented as mean ± standard deviation (SD).

3. Results

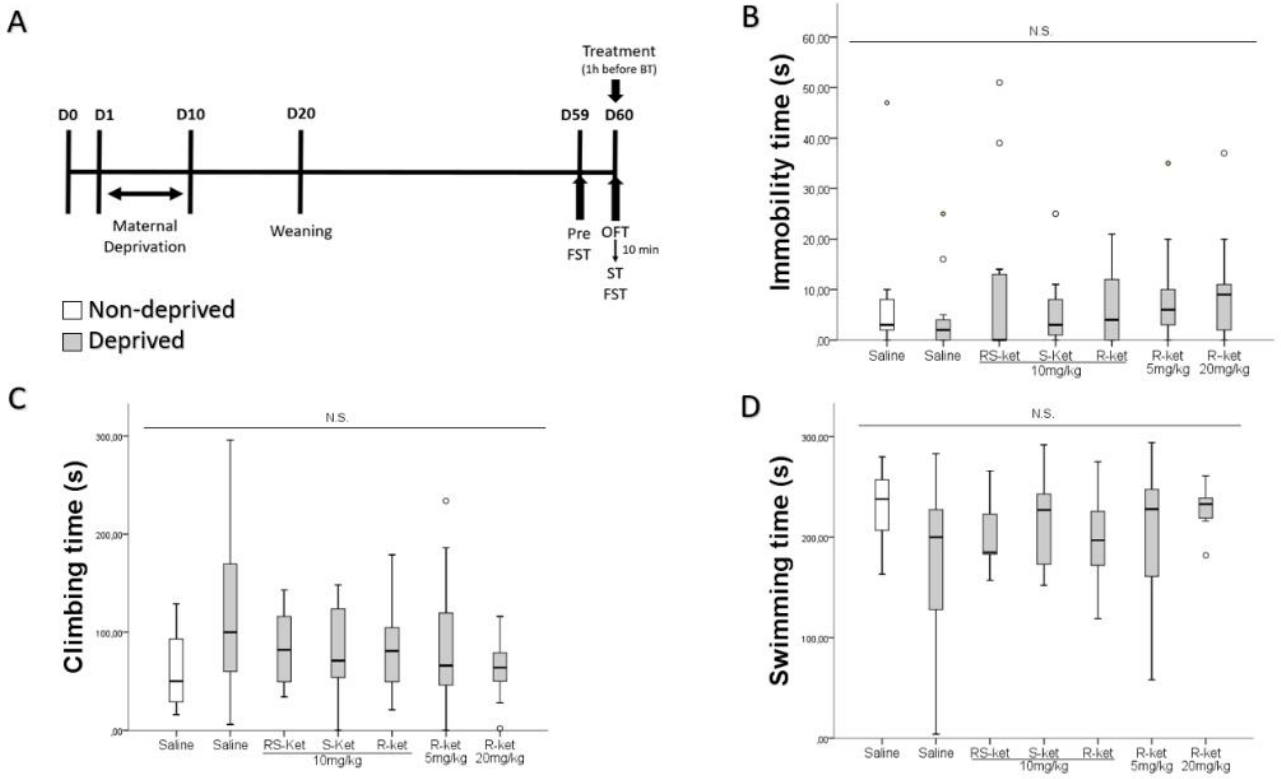
Regarding the forced swimming test, there was no statistically significant differences the immobility time ($F(6,64)= 0.4074$, Permutation $p=0.9234$) (Figure 1B), swimming time ($F(6,64)= 1.473$, Welch $p=0.2235$) or climbing time ($F(6,64)= 1.11$, Welch $p=0.3817$) between experimental groups. Despite that, we found a tendency for less immobility time in rats treated with saline and subjected to MD (4.82 ± 8.17) when compared to non-deprived controls (8.44 ± 14.85). This result shows that MD was not able to induce depressive-like behavior in the FST. We did not observe any statistically significant behavioral changes of the different ketamine enantiomers in FST.

We also did not observe induction of anhedonic-like behavior in rats subjected to MD. Although there seems to be a tendency to reduce grooming time in deprived rats treated with saline (28.35 ± 43.42) when compared to non-deprived controls (78.95 ± 30.75), there was no statistically significant difference between these groups. We also did not observe significant differences in the grooming time for any ketamine's isomers ($F(6,64)= 1.134$, $p=0.3532$).

In the open field test, we did not find any significant changes between the groups with regard the number of rearings ($F(6,64)=1.238$, $p=0.2988$); however there was significant changes in the number of crossings ($F(6,64)=3.08$, $p=0.01034$) (Figure 2A). There was an increase in locomotor activity in deprived control rats (101 ± 23, $p=0.012$) when compared to non-deprived (71 ± 22). We also found that this increase remained significant in deprived rats treated with racemic ketamine (97 ± 20, $p=0.41$) when compared to non-deprived. There were no statistically significant changes between the other groups (Figure 2A).

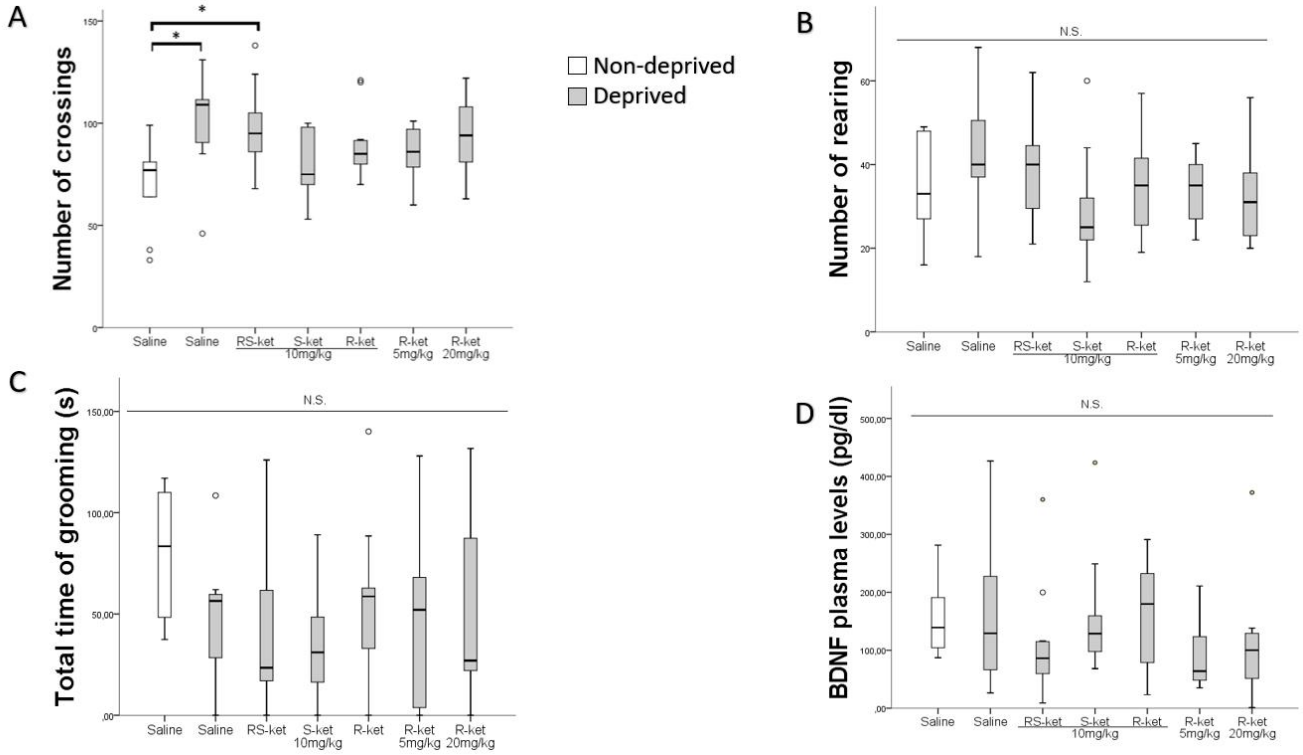
The effects of the MD and different ketamine isomers on BDNF plasma levels are showed in the Figure 2B. Neither MD nor ketamine treatment influenced plasma BDNF levels ($F(6,64)=0.9664$, $p=0.455$).

Figure 1 – Effects of (RS)-ketamine, (S)-ketamine and (R)-ketamine on antidepressant-like behaviour in rats subjected to maternal deprivation. (A): Schematic time-line diagram of MD model, treatment and behavioral tests. MD was performed from day 1 (D1) to day 10 (D10) after the first postnatal day (D0). Saline, (RS)-Ketamine, (S)-Ketamine or (R)-Ketamine in a single injection were administrated i.p. at 60th day, 1 hour before behavioral tests (BT). BT including forced swimming (FST), splash test (ST), and open-field (OFT) and were performed at 60th day. After the behavioral tests blood samples were assessed to the ELISA analysis. (B-D): Behaviors analyzed in the FST were computed in seconds (s), immobility time ($F(6,64)= 0.4074$, $p=0.9234$), climbing time ($F(6,64)= 1.11$, $p=0.3817$) or swimming time ($F(6,64)= 1.473$, $p=0.2235$) . All data are presented as mean ± SD (n=9-11). PERMANOVA for B; ANOVA with Welch's correction for C and D. *P < 0.05. N.S: Not significant.



Source: Authors.

Figure 2 – Effects of (RS)-ketamine, (S)-ketamine and (R)-ketamine on anhedonic-like behaviour, locomotor activity and BDNF plasma levels in rats subjected to maternal deprivation. (A-C): Behaviors analyzed in the open field test (OFT), number of rearings ($F(6,64)=1.238$, $p=0.2988$), number of crossings ($F(6,64)=3.08$, $p=0.01034$), grooming time in seconds ($F(6,64)= 1.134$, $p=0.3532$). (D) Plasma BDNF levels ($F(6,64)=0.9664$, $p=0.455$). All data are presented as mean ± SD (n=9-11). One way ANOVA for A, B and C; PERMANOVA for D. *P < 0.05. N.S.: Not significant.



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4. Discussion

In the present study, we evaluate the effect of MD and the administration of different ketamine enantiomers on depressive, anhedonic-like behaviors and locomotor activity of rats, as well as investigated the BDNF plasma concentration between experimental groups. Our findings did not confirm our hypothesis that MD is able to induce depressive-like behavior and reduce plasma concentration of BDNF. Due to the failure of MD to induce depressive phenotype, it was not possible to assess whether the different enantiomers of ketamine exhibit antidepressant-like effect. We also did not observe significant changes in plasma BDNF levels between deprived groups treated with different isomers of ketamine or saline.

Surprisingly, we found that rats submitted to MD showed increased locomotor activity in the OFT when compared to non-private rats, both treated with vehicle. The hypermobility condition was maintained in the deprived group treated with ketamine when compared to the control group but was not observed in animals exposed to other enantiomers.

Many previous studies have shown that MD, with the same or very similar parameters used in this study, is able to induce depressive-like behavior (Dimatelis et al., 2012; Réus et al., 2011, 2015) and reduced BDNF central levels (Lippmann et al., 2007; Réus et al., 2011, 2015), even when used alone, that is, not associated with other stress models or genetic alterations. It was observed that, in adulthood, animals exposed to this method showed increased immobility time in FST, reduced grooming time in ST and reduced BDNF concentration in brain tissue. It was also reported that MD does not cause changes in the locomotor activity, thus validating the FST results. This studies also evidenced that ketamine is able to reverse this alteration, causing antidepressant-like behaviour and increase in central BDNF (Réus et al., 2011, 2015; Wang et al., 2024).

However, other authors have shown that MD, performed very similarly to our study but with some peculiarities (specifically deprivation between D2 and D14 and maintenance of pups with controlled temperature during the separation time) was not able to induce depressive-like behavior when applied alone (Marais et al., 2008; Palareti et al., 2016; Vargas et al., 2016). There are reports that maternal separation increased susceptibility to stress but is not sufficient to generate depressive-like behavioural alterations (Marais et al., 2008; Murthy & Gould, 2018; Schmidt et al., 2011; Vargas et al., 2016). According to some studies, only when maternal separation was associated with genetic predisposition or other stressors in adulthood, there was an induction of depressive-like behavior (Marais et al., 2008; Murthy & Gould, 2018; Schmidt et al., 2011; Vargas et al., 2016).

These divergent results corroborate with findings of systematic reviews, meta-analysis and comprehensive reviews that evaluated the behavioral phenotype of early life stress (Bonapersona et al., 2019; Murthy & Gould, 2018; Schmidt et al., 2011; Wang et al., 2020). All of these studies discuss the high impact of variations in the paradigm (eg. postnatal day that begin and end the model, hours of separation, phase circadian cycle during which MD occurred, single versus group isolation, temperature of isolation), animal characteristics (eg. species, lineage, genetic variations) and control group (eg. early handling, non-handling and animal facility rearing) in the behavioral results of early life stress (Bonapersona et al., 2019; Murthy & Gould, 2018; Schmidt et al., 2011; Tractenberg et al., 2016; Wang et al., 2020). There were also noted that different results were observed between groups that performed the same model, with the same species, lineage and control group. Murthy and Gould (Murthy & Gould, 2018) argue that this may occur due to other factors such as laboratory management (eg. baseline housing and test conditions), additional stress in control and experimental group and natural variations in maternal response to stress.

Furthermore, it is important highlight that, although many studies show association between early life stress and depression (Ladd et al., 2000; LeMoult et al., 2020), according to the best of our knowledge no systematic review or meta-analysis has been able to confirm this association in rat models. Besides that, some authors report a high level of resilience in the majority of individuals exposed to early life stress (ELS) (Murthy & Gould, 2018; Rehan et al., 2017; Schmidt et al., 2011). According to Schmidt et al. (Schmidt et al., 2011) comprehensive review, when applied alone (out of the genetic and environmental context), none of the established models of early life stress can be considered as robust to induce depressive-like or anhedonic-like behavior. Some systematic reviews with meta-analysis suggest, however, that ELS may increase anxiety-like behavior in elevated plus maze, but not in OFT, and decreases social behavior (Bonapersona et al., 2019; Wang et al., 2020). These findings agree with our observations that MD was not capable to induce depressive-like behaviour in OFT, anxiogenic-like behaviour in OFT and anhedonic-like behavior in ST.

This study showed that MD induced a state of hypermobility (increased number of crossings in OFT). Private mice treated with racemic ketamine kept the state hyperlocomotion, while those treated with other enantiomers of ketamine had locomotion similar to the control group. Large part of studies with MD have not shown significant changes in locomotor activity in the OFT (Wang et al., 2020). Nonetheless, hyperlocomotion is reported in some studies that applied subanesthetic doses of ketamine in deprived rats (Réus et al., 2013; Zugno et al., 2013).

Hyperlocomotion in rodents is described as one of the phenotypes associated with psychotic or schizophrenic behaviors (Hunt et al., 2006; Yang et al., 2015; Zugno et al., 2013). Non-competitive antagonists of NMDA receptors induce a behavioral syndrome in rodents characterized by hyperlocomotion, stereotypes and impairments in social interaction and cognitive function (Hunt et al., 2006). Moreover, there are reports that MD can induce schizophrenic behavior in rodents (Ellenbroek & Riva, 2003). Ketamine-induced schizophrenia-like behaviors in rodents generally require the chronic application of high doses. However, there are reports of increased locomotor activity in rats even when ketamine was administered in a single subanesthetic dose (Hunt et al., 2006; Yang et al., 2015; Zugno et al., 2013).

Regarding the differential effect of ketamine's isomers on hyperlocomotion, according to Ryder et al. (Ryder et al., 1978), in equianalgesic doses, esketamine causes less stimulation of locomotor activity than arketamine. Yang et al. (Yang et al., 2015), on the other hand, argues that arketamine has less psychotomimetic effects (therefore less hyperlocomotion) than esketamine. In our study, we did not found differences in locomotion in rats treated with arketamine or esketamine, while those treated with the racemic form showed hyperlocomotion. These differences in the results may be due to different methods used to evaluate locomotion. In our study, we used the OFT for 5min, while Ryder et al. (Ryder et al., 1978) used a photocell-type electronic motility meter also for 5 min, and Yang et al. (Yang et al., 2015) used the animal movement analysis system SCANETMV- 40 for 60min.

In addition, we did not detect significant changes in BDNF plasmatic levels among any of the experimental groups in our study. A large body of evidence suggests that subanesthetic doses of ketamine increases brain concentration of BDNF in minutes or hours after administration in rodents subjected or not to different models of depression (Autry & Adachi, 2012; Dong et al., 2017; Getachew & Tizabi, 2019; Zhang et al., 2019). Some authors have demonstrated a reduction in the concentration of BDNF in the amygdala, hippocampus, striatum and frontal cortex in rats subjected to MD (Ellenbroek & Riva, 2003; Lippmann et al., 2007; Réus et al., 2011). On the other hand, studies have not observed a good correlation between the central and peripheral plasma levels (Nedelec et al., 2018).

In a previous study of our group (Caliman-Fontes et al., not published data), we demonstrated that the infusion of ketamine or esketamine in patients with treatment resistant depression is not able to modify serum levels of BDNF. Some authors defend that the assessment of BDNF serum levels produces more reproducible, stable and reliable results than plasmatic levels (Elfving et al., 2010; Polyakova et al., 2015). However, the evaluation of BDNF in serum allows to find concentrations 100 times higher than in plasma (Polyakova et al., 2015). Based on our previous clinical findings (Caliman-Fontes et al., not published data), we hypothesized that the measurement of plasma levels could be more sensitive to detect small acute changes in this neurotrophin, although in this first preclinical trial, we were unable to observe a significant effect of ketamine treatment on plasma BDNF levels. Our results suggest, therefore, that the evaluation of plasma BDNF levels are not a good tool to detect neither the effect of MD nor ketamine effect in rats.

As limitations of our work, we point out the non-assessment of the central levels of BDNF and the impossibility of including other experimental groups to verify whether the association of MD with other stressors later in life could induce a depressive phenotype. In contrast, we demonstrate negative results after the application of MD, which might help other research groups in choosing the experimental model of depression and will be important in future studies of meta-analysis. Recent meta-analysis on MD has shown a high risk of bias in the conclusions about the behavioral effects of MD (Bonapersona et al., 2019), which may occur mainly due to the non-publication of negative studies.

5. Conclusion

MD did not induce depressive-like or anhedonic-like behaviors in rats but increased locomotor activity. It is necessary to standardize the MD model to better understand the translational importance of this method in the study of psychiatric disorders. Ketamine and its enantiomers did not cause significant changes in depressive-like and anhedonic-like behaviors in rats subjected to MD. Neither MD nor the different ketamine isomers were able to alter plasma BDNF levels.

Based on the results of this study, future investigations could consider alternative animal models or clinical studies to validate and expand upon the findings observed, with the aim of enhancing the understanding of the therapeutic effects associated with the different ketamine enantiomers in the treatment of depression.

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