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Is Forced Swimming Immobility a Good Endpoint for Modeling Negative Symptoms of Schizophrenia? - Study of Sub-Anesthetic Ketamine Repeated Administration Effects

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ABSTRACT

Immobility time in the forced swimming has been described as analogous to emotional blunting or apathy and has been used for characterizing schizophrenia animal models. Several clinical studies support the use of NMDA receptor antagonists to model schizophrenia in rodents. Some works describe the effects of ketamine on immobility behavior but there is variability in the experimental design used leading to controversial results. In this study, we evaluated the effects of repeated administration of ketamine sub-anesthetic doses in forced swimming, locomotion in response to novelty and novel object recognition, aiming a broader evaluation of the usefulness of this experimental approach for modeling schizophrenia in mice. Ketamine (30 mg/kg/day i.p. for 14 days) induced a not persistent decrease in immobility time, detected 24h but not 72h after treatment. This same administration protocol induced a deficit in novel object recognition. No change was observed in mice locomotion. Our results confirm that repeated administration of sub-anesthetic doses of ketamine is useful in modeling schizophrenia-related behavioral changes in mice. However, the immobility time during forced swimming does not seem to be a good endpoint to evaluate the modeling of negative symptoms in NMDAR antagonist animal models of schizophrenia.

Key words: antidepressant-like effect, immobility behavior, locomotor activity, memory negative symptoms.

INTRODUCTION

Immobility time in the forced swimming was first described by Porsolt et al. (1977) as behavioral despair and originated one of the most recognized and useful animal tasks for screening potential antidepressant drugs. In addition to its association with depressive symptoms, an increase in immobility time has been also considered a successful strategy for dealing with stress. It has been

proposed that after repeated swimming animals learn that move is useless and become immobile faster and for a longer period for saving energy (Borsini et al. 1986, de Kloet and Molendijk 2016, Masuda et al. 2001, Parra et al. 1999, West 1990). However, studies showing a deleterious effect of the repeated forced swim on cognitive tasks, such as the Morris water maze (Abel and Hannigan 1992, Rates 1998), novel object recognition (Yuen et al. 2012), object location (Borsoi et al. 2015a) and prepulse inhibition tests (Borsoi et al. 2015a, b),

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contradict this successful strategy theory. On the other hand, immobility behavior in the forced swimming has been also related to schizophrenia negative symptoms, as apathy and lack of initiative (Langen et al. 2012, Noda et al. 1995, Porsolt et al. 2010).

One of the most studied pharmacological animal models of schizophrenia uses N-methyl-D-aspartate receptor (NMDAR) noncompetitive antagonists administration to rodents. These models are based on several pharmacological, genetic and *post-mortem* studies that strongly suggest an important role for NMDAR hypofunction in the pathophysiology of schizophrenia (Coyle 2012, Kantrowitz and Javitt 2012, Pilowsky et al. 2006). In healthy volunteers, the noncompetitive NMDAR antagonist ketamine induces effects similar to positive and negative schizophrenia symptoms as well as cognitive dysfunctions (Adler et al. 1999). Furthermore, schizophrenic patients treated with this drug present an exacerbation of psychosis (Lahti et al. 1995). These and several other evidence support the proposition of the hypoglutamatergic hypothesis of schizophrenia and the use of NMDAR antagonists to model this psychiatric disorder in animals.

The NMDAR antagonist most used to model schizophrenia in rodents is phencyclidine (PCP). Noda et al. (1995) reported that repeated administration of PCP (10 mg/kg/day s.c., 14 days) to mice was able to increase the immobility of animals when exposed to forced swimming until 21 days after stopping treatment. According to authors, this behavior can be associated to negative symptoms of schizophrenia, as lack of initiative, apathy or emotional blunting. Atypical antipsychotics, such clozapine and risperidone, are able to reverse the pro-immobility effect induced by PCP repeated administration (Corbett et al. 1999, Mouri et al. 2012, Murai et al. 2007, Noda et al. 1995, 1997). Similar results were described after repeated administration of another NMDAR antagonist, MK-801 (0.2 mg/kg/day for 15 days i.p.): an increase on the immobility time which was blocked by antipsychotics administration (Kawaura et al. 2015, 2016, Langen et al. 2012, Rundfeldt et al. 2000). These data lead to the use of the increase in

the immobility time in the forced swimming as a tool to evaluate affective flattening in schizophrenia animal models (Neill et al. 2014) and even for the development of new drug candidates (Porsolt et al. 2010).

Besides PCP and MK-801, ketamine has also been used to model schizophrenia symptoms in rodents (Adler et al. 1999, Bubenikova-Valesova et al. 2008, Large 2007). This drug is able to induce hyperlocomotion, stereotypy, as well as deficits in attentional and memory processes in rodents (Chan et al. 2008, Imre et al. 2006, Neves et al. 2013, Pietersen et al. 2007, Pitsikas et al. 2008, Rodvelt et al. 2008, Wang et al. 2006). Becker et al. (2003) and Becker and Grescsch (2004) showed that a sub-anesthetic dose of ketamine (30 mg/kg/day i.p.) administered for 5 days reduced the non-aggressive social behavior in rodents, a sign that can be associated to schizophrenia negative symptoms.

Few studies describe the effects of ketamine repeated administration on the immobility behavior in the forced swimming and the results are controversial. Some authors characterized an increase in the immobility as a behavior related to apathy (Chatterjee et al. 2011, Chindo et al. 2012, Hou et al. 2013), while others showed a decrease on this behavior (Akinfiresoye and Tizabi 2013, Garcia et al. 2008b, Owolabi et al. 2014, Parise et al. 2013, Popik et al. 2008, Tizabi et al. 2012) probably related to the described rapid antidepressant effect of this drug (Rot et al. 2010, Zarate et al. 2006). In a recent review, Neill et al. (2014) discuss this issue and highlight the necessity of more studies to achieve a better understanding of schizophrenia symptoms modeling by NMDAR antagonists using behavioral tasks associated to anhedonia and emotional blunting, especially in mice. Thus, in this work we investigated the effects of repeated administration of sub-anesthetic doses of ketamine in the forced swimming paradigm. Locomotion in response to novelty and novel object recognition (tasks related to positive and cognitive impairments presented by schizophrenia patients, respectively) were also performed in order to make a broader evaluation.

MATERIALS AND METHODS

ANIMALS

Adult CF1 male mice (25 – 35 g) from Fundação Estadual de Produção e Pesquisa em Saúde do Rio Grande do Sul breeding colony were used. Animals were housed in groups in plastic cages (17 x 28 x 13 cm, 6 mice per cage) with free access to food (Nuvital®) and water. Mice were kept at constant room temperature (22 ± 2 °C) and humidity (60%), under a 12h light-dark cycle (lights off at 7:00 pm) and were adapted to local conditions for at least 72 h before the experiments. All procedures were previously approved by the local Animal Care Ethical Committee (CEUA-UFRGS; approval number 2006541) and performed according to Brazilian guidelines (Brasil 2013) and to Directive of the European Parliament and of the Council of the European Union of 22 September 2010 (2010/63/EU).

DRUGS

S(+)-Ketamine hydrochloride (kindly supplied by Cristália®, São Paulo, Brazil) was used in this study. The drug was dissolved directly in saline (NaCl 0.9%) and was administered by intraperitoneal route in a volume of 1mL/100g body weight. Vehicle groups received saline. Ketamine doses are expressed as salt.

FORCED SWIMMING

The methodology used was based on that described by Noda et al. (1995, 1997) with some modifications according to the standard protocol used by our group (Viana et al. 2008, Borsoi et al. 2015a, b). Animals were habituated to laboratory conditions one hour before exposition to the forced swimming session. The room was maintained at 23 ± 2 °C, under artificial lighting. Mice were forced to swim for 3 min in transparent acrylic recipients (15 x 15 x 30 cm) containing water up to

15 cm depth at 21 ± 1 °C. At the end of the session, animals were removed from the water and gently dried. The measurement of animals' immobility (seconds) was performed by trained observers blind to the treatments. A mouse was considered immobile when it remained floating making only the movements necessary to keep its head above the water. All forced swimming sessions occurred between 10 a.m. and 3 p.m.

LOCOMOTOR ACTIVITY IN RESPONSE TO NOVELTY

Locomotor activity was measured in an rectangular acrylic arena (30 x 30 x 45 cm, transparent walls and black floor divided into 24 squares of equal area) completely novel to animals. Mice were allowed to freely explore the arena during 15 minutes. A trained observer unaware of the treatments recorded the total number of squares crossings, rearings and groomings during exploration period.

NOVEL OBJECT RECOGNITION

The novel object recognition task was performed in the same arena used for locomotion study. Animals were submitted to three experimental trials. Initially, mice were placed in the empty arena for 10 min (habituation session). In the acquisition trial (trial 1 – performed 24h after the habituation session), animals were replaced in the same arena containing two identical objects (objects A) for 10 min and left to freely explore them. Exploration was defined as the animal directing the nose to the object while looking at, sniffing or touching it. Ninety minutes later, a second trial was performed (trial 2 – short term memory test). Animals were placed back in the arena for 10 min and an unknown object (object B) replaced one of the objects presented in the first trial. The total time spent exploring each object was determined by an observer unaware of the treatments. The long term memory test (trial 3) was carried out 24h after the acquisition trial. In this last trial a third object was presented to animals as the novel object (object C) together with the familiar one (object A) and again the total time spent in exploration

of each object was determined. The recognition index in each session was calculated as: time exploring the novel object / time exploring the novel object + time exploring the familiar object. The objects were similar in size, color, made of the same material but different in shapes. The arena and the objects were cleaned between each trial using ethanol 10% to avoid odor trails. All procedures were done in a room dimly lit (15 lux).

EXPERIMENT 1: EFFECT OF DIFFERENT DOSES AND PERIODS OF KETAMINE TREATMENT IN THE FORCED SWIMMING

On the first day (day 1), mice were exposed to forced swim as described at subsection *Forced swimming*. On the following day (day 2), the animals were randomly distributed into three different groups: vehicle (treated with saline 1 mL/100g body weight i.p.), ketamine 30 (treated with ketamine 30 mg/kg/day i.p.) and ketamine 45 (treated with ketamine 45 mg/kg/day i.p.). Ketamine doses were selected based on literature data (Becker et al. 2003, Becker and Grescsch 2004). Treatments were given once a day from the 2nd to the 15th day. Twenty-four hours after the last treatment (16th day), animals were forced to swim once again.

In order to identify the best treatment length, a similar schedule was performed. On the first day, mice were forced to swim as described at subsection *Forced swimming*. On the following day, animals were randomly distributed into four different groups: vehicle (treated with saline 1 mL/100g body weight i.p. for 14 days), ketamine 1 day (treated with saline 1 mL/100g i.p. for 13 days and with ketamine 30 mg/kg i.p. for 1 day), ketamine 5 days (treated with saline 1 mL/100g i.p. for 9 days and with ketamine 30 mg/kg i.p. for 5 days) and ketamine 14 days (treated with ketamine 30 mg/kg/day i.p. for 14 days). Twenty-four hours after the last treatment (16th day), the animals were forced to swim once again.

Finally, the persistence of ketamine's effect on the forced swimming was evaluated as follow: mice were forced to swim as described at subsection

Forced swimming. On the following day, animals were randomly distributed into two different groups: vehicle (treated with saline 1 mL/100 g body weight i.p. for 14 days), ketamine (treated with ketamine 30 mg/kg/day i.p. for 14 days). After this period, animals were exposed to forced swimming again at the following days: 16th, 18th, 22nd, 29th and 36th, i.e. one, three, seven, fourteen and twenty-one days after treatment. A scheme of the experimental schedules described is presented in Figure 1a.

EXPERIMENT 2: KETAMINE REPEATED TREATMENT EFFECT ON LOCOMOTION AND NOVEL OBJECT RECOGNITION

On the first day, mice were exposed to forced swimming as described at subsection *Forced swimming*. On the following day, the animals were randomly distributed into three different groups: sham (mice only gently handled for 14 days), vehicle (treated with saline 1 mL/100g body weight i.p. for 14 days) and ketamine (treated with ketamine 30 mg/kg/day i.p. for 14 days). Twenty-four hours later (16th day), the animals spontaneous locomotion were evaluated as described at subsection *Locomotor activity in response to novelty* and immediately forced to swim for a second time. The novel object recognition procedure started 24h after the last swimming session and was carried out as described at subsection *Novel object recognition*. A scheme of the experimental schedule described above is presented in Figure 1b.

STATISTICAL ANALYSIS

Data are expressed as mean \pm S.E.M. Firstly, all datasets were tested for their normal distribution (Shapiro-Wilk test) and homogeneity of variances (Brown-Forsythe test). Thus, the following statistical tests were employed: Students t test (paired and unpaired), one way analysis of variance (ANOVA) followed by Student-Newman-Keuls *post hoc* test, two-way repeated-measures analysis

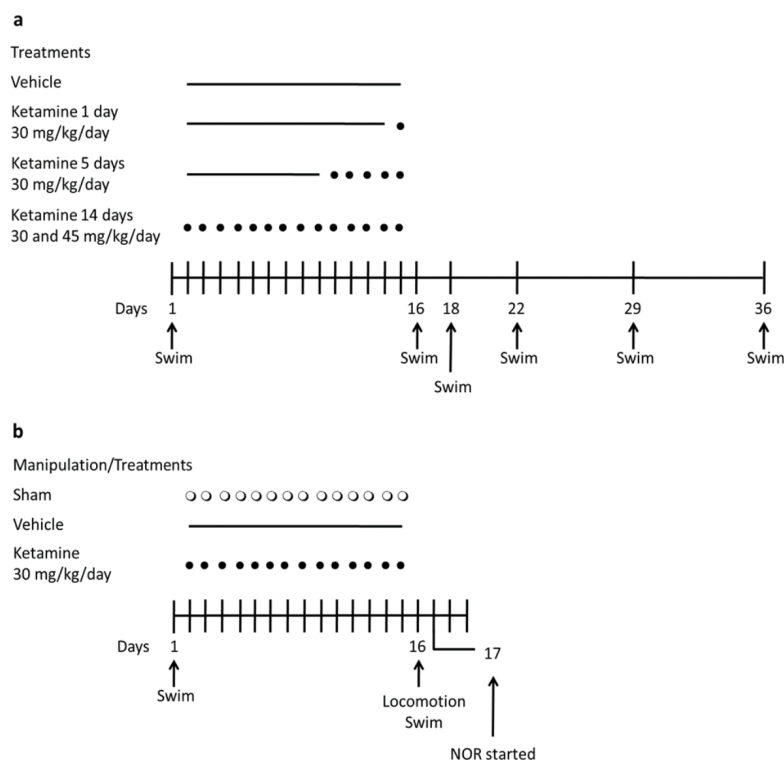


Figure 1 - Experimental schedules used in this work. **a)** Evaluation of the effect of different doses and periods of treatment with ketamine on the immobility of animals exposed to forced swim. **b)** Evaluation of ketamine repeated treatment on locomotion and novel object recognition task. ○ Gentle handling; - vehicle administration (saline 1 mL/100 g body weight i.p.); • ketamine administration (i.p.).

of variance ANOVA followed by Student-Newman-Keuls *post hoc* test. Tests were selected according to experimental design, the number of datasets and the number of factors to be analyzed. Analyses were performed using Sigma Stat 2.03 software (Jandel Scientific Corporation). Differences were considered statistically significant at $p < 0.05$.

RESULTS

EFFECT OF DIFFERENT DOSES AND PERIODS OF KETAMINE'S TREATMENT ON THE FORCED SWIMMING

At first, the effects of two different doses of ketamine on the forced swimming were evaluated:

30 and 45 mg/kg/day for 14 days. The two-way repeated-measures ANOVA revealed a significant day influence on immobility time and an interaction between treatment and day (treatment: $F_{(2, 29)} = 0.987$, $p = 0.385$; day: $F_{(1, 29)} = 24.435$, $p < 0.001$; treatment versus day interaction: $F_{(2, 29)} = 6.874$, $p = 0.004$). The experimental groups did not differ on the immobility behavior in the first experimental day, i.e., before starting any treatment (Student-Newman-Keuls *post-hoc* test, $p > 0.331$). The lowest ketamine dose evaluated induced a significant decrease in animals immobility time on the second exposition to the forced swimming procedure (Student-Newman-Keuls *post-hoc* test,

$p = 0.020$ compared to vehicle treated group). However, animals treated with vehicle (Student-Newman-Keuls *post-hoc* test, $p < 0.001$) and ketamine 45 mg/kg (Student-Newman-Keuls *post-hoc* test, $p = 0.040$) presented an increase in the immobility time between the first and second swimming session, which was not shown in the animals treated with ketamine 30 mg/kg (Student-Newman-Keuls *post-hoc* test, $p = 0.487$) (Figure 2). Given that ketamine 30 mg/kg dose was the only one that induced a statistically significant change in animals behavior, this dose was chosen for different periods of treatment evaluation.

Analyzing the data of animals treated with ketamine 30 mg/kg/day for 1, 5 or 14 days, ketamine induced a reduction on the immobility time of these animals in the second swimming session (16th day) depending on the duration of treatment. This reduction reaches statistical significance only on those animals treated for 14 days (One-way ANOVA, $F_{(3,45)} = 3.071$, $p = 0.038$, Student-Newman-Keuls *post-hoc* test, $p = 0.031$) (Figure 3). To investigate the persistence of the anti-immobility effect of ketamine, animals were subject to swimming sessions repeatedly after the end of the treatment. The results show that ketamine's effect is not persistent, since it is already absent three days after treatment interruption (18th day, Student's *t* test, $t = 0.271$, $p = 0.789$). The behavior of vehicle and ketamine treated animals is similar from 18th to 36th day (Student's *t* test, 22th day: $t = 0.128$, $p = 0.899$; 29th day: $t = 0.068$, $p = 0.946$; 36th day: $t = 0.615$, $p = 0.545$) (Figure 4).

KETAMINE REPEATED TREATMENT EFFECT ON LOCOMOTION IN RESPONSE TO NOVELTY

Hyperactivity due to exposition to a novel environment has been considered one of the few behaviors related to schizophrenia positive symptoms that can be assessed in animal models (Powell and Miyakawa 2006). Furthermore, the effect of drugs on the forced swimming can be

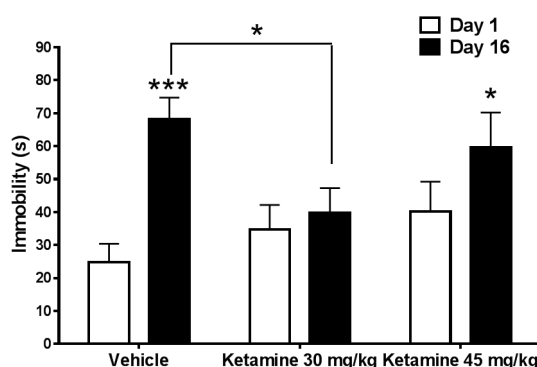


Figure 2 - Ketamine 30 mg/kg/day i.p. for 14 days decreases the immobility time of animals on the forced swimming test. Results are expressed as mean \pm S.E.M (n = 8-12). *** $p < 0.001$, * $p < 0.05$ on the post-hoc test.

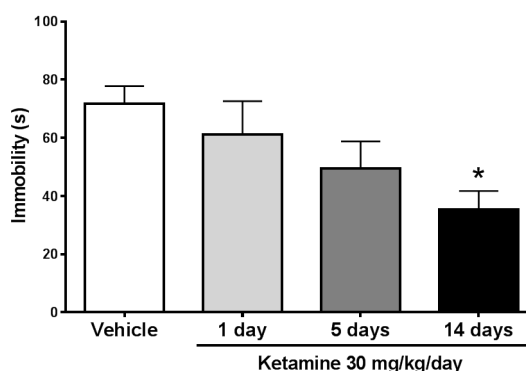


Figure 3 - Ketamine 30 mg/kg/day i.p. decreases the immobility time of animals on the forced swimming test only when administered for 14 days. Results are expressed as mean \pm S.E.M (n = 11-12). * $p < 0.05$ on the post-hoc test.

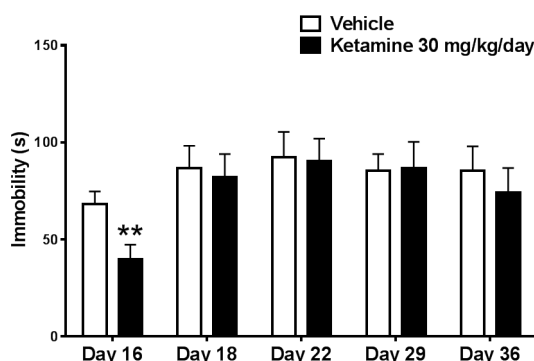


Figure 4 - Anti-immobility effect of ketamine 30 mg/kg/day i.p. (14 days) is not persistent. Results are expressed as mean \pm S.E.M (n = 12). ** $p < 0.01$ on Student's *t* test.

interpreted on different ways. Stimulants or central depressants drugs often alter the immobility time in a non-specific manner and can lead to a misinterpretation of the results. Thus, the effect of ketamine repeated treatment on animal's locomotion was also evaluated (Table I). A significant effect of animals manipulation was found in horizontal (One-way ANOVA, $F_{(2, 26)} = 5.507$, $p = 0.011$) and vertical exploration parameters (One-way ANOVA, $F_{(2, 26)} = 7.559$, $p = 0.003$), but not for groomings (One-way ANOVA, $F_{(2, 26)} = 0.828$, $p = 0.449$). A significant decrease on crossings (Student-Newman-Keuls *post-hoc* test, $p = 0.016$) and rearings (Student-Newman-Keuls *post-hoc* test, $p = 0.002$) was found in mice treated with ketamine when compared to sham group. However, it was also found a significant difference between vehicle and sham groups (Student-Newman-Keuls *post-hoc* test, crossings: $p = 0.012$, rearings: $p = 0.014$). This can be interpreted as a higher stress response to a new environment by the sham group since these animals were less stressed during the manipulation period when compared with injected animals. No significant differences on the evaluated parameters between saline and ketamine treated animals were found (Student-Newman-Keuls *post-hoc* test, crossings: $p = 0.535$, rearings: $p = 0.259$). Besides the differences in locomotion, immobility time in the forced swimming session on day 16 of the sham group did not differ from vehicle group (data not shown).

KETAMINE REPEATED TREATMENT EFFECT ON NOVEL OBJECT RECOGNITION

NMDA receptor antagonists are also used to induce cognitive impairments related to schizophrenia symptoms in rodents. Thus, the effect of ketamine repeated treatment on the novel object recognition task was also evaluated. In the acquisition trial, all animals explored equally the two identical objects (Paired Student's test, Sham: $t = 1.410$, $p = 0.201$; Vehicle: $t = 0.214$, $p = 0.816$; Ketamine:

TABLE I
Spontaneous locomotion evaluated before the second exposition to the forced swimming (16th day).

Parameter	Sham (n = 08)	Vehicle (n = 09)	Ketamine 30 mg/kg/day (n = 10)
Crossings	422 ± 103	260 ± 91*	291 ± 121*
Rearings	106 ± 34	69 ± 25*	54 ± 26**
Groomings	7 ± 4	10 ± 3	8 ± 4

Mean (± SD) of crossings, rearings and groomings. ** $p < 0.01$, * $p < 0.050$ on the post-hoc test when compared to Sham group.

$t = 0.272$, $p = 0.791$) (data not shown). Two intervals of memory retention were assessed on the same animals: short term memory (ninety minutes after the acquisition trial) and long term memory (24h after the acquisition trial, including a reconsolidation step). On the short term memory assessment, only the animals that were not subject to any treatment (sham group) retained the memory of the familiar object by spending more time exploring the new one (Paired Student's t test, $t = -3.463$, $p = 0.013$) (Figure 5a). One unexpected result was the impairment on short term memory observed in the vehicle group (Paired Student's t test, $t = 0.211$, $p = 0.839$) (Figure 5a). However, in the long term memory assessment, both sham (Paired Student's t test, $t = -3.855$, $p = 0.008$) and vehicle (Paired Student's t test, $t = -3.102$, $p = 0.015$) groups remembered the familiar object (Figure 5b). In this trial, the amnesic effect of ketamine becomes even more evident by the lack of difference on time exploring the novel and the familiar object (Paired Student's t test, $t = 0.191$, $p = 0.853$) (Figure 5b).

DISCUSSION

In this work we demonstrated that repeated treatment with ketamine (30 mg/kg/day i.p. for 14 days) induced a decrease in the immobility time of mice exposed to forced swim and that this effect is

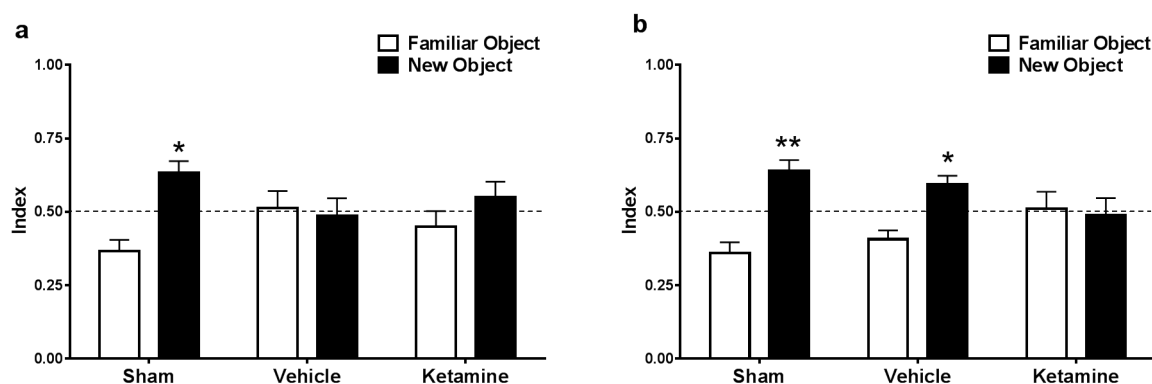


Figure 5 - Amnesic effect of ketamine (30 mg/kg/day i.p. 14 days) in the novel object recognition task. Results are expressed as mean \pm S.E.M (n = 7-10). **a)** Short term memory (90 min interval). **b)** Long term memory (24 h interval) * $p < 0.05$, ** $p < 0.01$ on Paired Student's t test.

not long lasting, since it is absent just three days after treatment interruption. Given that ketamine administration induced an anti-immobility effect without altering mice locomotion, we can assume that, in our experimental conditions, this drug induced an antidepressant-like effect.

The antidepressant effect of sub-anesthetic doses of ketamine has been demonstrated in several studies in mice (Maeng et al. 2008, Popik et al. 2008, Silva et al. 2010), rats (Akinfiresoye and Tizabi 2013, Fraga et al. 2013, Garcia et al. 2008a, b, 2009, Li et al. 2010, Parise et al. 2013, Popik et al. 2008, Reus et al. 2011, 2013, Tizabi et al. 2012, Yang et al. 2012) and humans (Maeng and Zarate 2007, Rot et al. 2010, 2012, Zarate et al. 2006), and the investigation of its mechanism of action or even the involvement of active metabolites is an active field of research (Li et al. 2010, Newport et al. 2015, Zanos et al. 2016). Contrary to our results, there are few works successfully describing the use of ketamine repeated treatment to mimic schizophrenia negative symptoms in the forced swimming. Chindo et al. (2012) demonstrated an increase in the immobility time of Wistar rats that received ketamine 30 or 50 mg/kg/day i.p. for 10 days and that this effect persisted until 21 days after treatment. This ketamine effect was inhibited by clozapine and risperidone, but not by haloperidol.

Using mice, other authors have demonstrated that repeated treatment with ketamine induces a persistent increase in the immobility time only when administered at a high (anesthetic) dose (100 mg/kg, i.p.) (Chatterjee et al. 2011, Hou et al. 2013, Moghaddam et al. 2014). However, there are studies conducted in rats that corroborate our findings. The first one used male Wistar rats and demonstrated that ketamine 50 mg/kg/day i.p. for 14 days induced a decrease on animals' immobility time, which is not persistent after treatment interruption (Popik et al. 2008). The second one showed an anti-immobility effect of ketamine 0.5 and 2.5 mg/kg/day i.p. for 10 days in female Wistar Kyoto rats, which is still detectable one week after treatment (Tizabi et al. 2012). Parise et al. (2013) also reported a decrease in the immobility behavior without effect on locomotion in animals treated with ketamine 20 mg/kg i.p. twice a day for 15 days. Furthermore, Li et al. (2010) demonstrated that acute administration of increasing doses of ketamine suppresses its own anti-immobility effect. A similar effect can be also seen in our results (absence of effect with 45 mg/kg/day i.p.).

Besides the nature of the effect (i.e., increase or decrease in the immobility time), controversial results can also be found concerning the duration of ketamine effect. Our results demonstrated a not persistent

antidepressant-like effect for this drug. However, some studies in mice report the long lasting effects of ketamine even after a single administration (Maeng et al. 2008, Tizabi et al. 2012). It's important to consider that in our work, the persistence of ketamine's effect was evaluated in the same animals repeatedly forced to swim. Using this experimental approach, we observed an increase in mice immobility throughout the days. Similar results were reported elsewhere (Borsini et al. 1986, Masuda et al. 2001, Parra et al. 1999, West 1990, de Kloet and Molendijk 2016) and lead to the proposition that, after repeated swimming, animals adapt to the forced swimming exposure and become immobile faster and for a longer period. Thus, the involvement of cognitive function (memory and learning) as well as strategies of coping with stress in the development of this behavior cannot be discharged. Although our results show mice adaptation to the forced swimming exposure, it is not likely that this phenomenon have masked the persistence of ketamine's effect since other groups successfully used similar experimental designs. For example, Tizabi et al. (2012) demonstrated the persistence of ketamine antidepressant-like effect after repeated administration in rats exposing the same animals repeatedly to forced swimming. Furthermore, in their first studies Noda et al. (1995) showed the persistence of phencyclidine pro-immobility effect using the same experimental design as our, and similar results were also described for MK-801 (Langen et al. 2012) and even for the high ketamine dose (Chatterjee et al. 2012).

Divergence between our results with ketamine and those first reported by Noda et al. (1995) for phencyclidine repeated administration may be explained by the different profiles of action of these drugs on the neurotransmitter systems most commonly associated with behavioral alterations in the forced swimming, as well as with the symptoms of major depression and schizophrenia. Despite the pharmacological similarity between these two compounds, some important differences are reported. Phencyclidine and ketamine act as non-competitive NMDAR antagonists, are D_2 receptors partial agonists and show dopamine reuptake

inhibitory activity. However, they differ in their affinity for several receptors, such as NMDAR, 5-HT_{2A} and D_2 , resulting in distinct selectivity profiles. Ketamine has similar affinity for D_2 and NMDAR ($K_i = 0.5 \mu\text{M}$), which are 30 fold higher than its affinity for 5-HT_{2A} receptors ($K_i = 15 \mu\text{M}$). In contrast, phencyclidine has higher affinity for NMDAR and 5-HT_{2A} receptors ($K_i = 2$ and $5 \mu\text{M}$, respectively) than for D_2 receptors ($K_i = 37 \mu\text{M}$) (Dersch et al. 1994, Kapur and Seeman 2002, Nishimura et al. 1998, Seeman et al. 2005). Animals treated during 14 days with phencyclidine (10 mg/kg/day s.c.), in addition to the increase in immobility time in forced swimming, showed an imbalance of dopaminergic and serotonergic systems in the frontal cortex characterized by a stimulation of the serotonergic neurotransmission and inhibition of the dopaminergic neurotransmission (Jentsch et al. 1998, Noda et al. 2000). The stimulation of serotonergic neurotransmission appears to have an important role in the pro-immobility effect induced by the repeated treatment with phencyclidine, since only second generation antipsychotics (with considerable affinity for 5-HT_{2A}) are able to reverse this effect (Noda et al. 2000). On the other hand, ketamine repeated treatment is able to induce an increase in serotonergic and dopaminergic neurotransmissions in rodents' frontal cortex (Lindefors et al. 1997) and striatum (Chatterjee et al. 2012). It is known that substances able to stimulate dopaminergic neurotransmission have significant anti-immobility effect in the forced swimming (Vaugois et al. 1996, Viana et al. 2005). Thus, this different pattern of action of sub-anesthetic doses of ketamine and PCP upon dopaminergic neurotransmission might be responsible for the opposite effects induced by these drugs in the forced swimming.

Although differences in the experimental protocols used cannot be ruled out, the nature and the persistence of ketamine repeated treatment effects on forced swimming seem to be dependent on dose and animal species used, generating controversial results. This dual effect of this drug has been discussed elsewhere (Fraga et al. 2013, Neill et al. 2014). Some authors propose that it can be

attributed to the blockade of NMDAR composed by different patterns of subunits (GluN2a vs. GluN2b) (Jiménez-Sánchez et al. 2014) while others attribute it to an increase in glutamate efflux in cortical areas and a consequent activation of AMPA receptors leading to modulation of non-classical signaling pathways such as the GSK3/ β -catenin (Maeng et al. 2008, Beurel et al. 2016). Thus, considering the use of the forced swimming in a drug development program aiming new antipsychotics development, this bias could compromise results interpretation. Therefore, taking into account the failure of ketamine to induce an increase in immobility time in our experimental conditions, we propose that the immobility time in the forced swimming is not a good endpoint for modeling negative symptoms in NMDAR antagonist animal models of schizophrenia.

Another test that is being used to characterize animal models of schizophrenia and to evaluate compounds aimed to improve cognitive deficits in schizophrenia is the novel object recognition task. This behavioral test has been considered to have higher translational and face validity for schizophrenia than the immobility in the forced swimming, since recognition memory is impaired in schizophrenic patients (Gabrovska et al. 2003, Neill et al. 2010). We demonstrated that repeated administration of a sub-anesthetic dose of ketamine caused impairment in this task, even after memory reconsolidation of the familiar object (presentation of the familiar object twice to the same animals). This is in line with other studies demonstrating that acute administration of low dose ketamine impairs the recognition of the novel object both in rats (Boutadakis and Pitsikas 2010, Georgiadou et al. 2014, Goulart et al. 2010, Nikiforuk et al. 2013, Pitsikas et al. 2008) and mice (Chan et al. 2008, 2012), as well as in other tasks of learning and memory (Imre et al. 2006, Moghaddam et al. 1997, Moosavi et al. 2012, Uchihashi et al. 1994, Wang et al. 2006). As far as we know, only two studies beside ours presented the results of ketamine repeated administration on this task in rodents. Jacklin et al. (2012) reported a deficit in novel

object recognition paradigm in Long Evans rats using a sub-anesthetic ketamine dose (30 mg/kg i.p. twice a day for 10 days) that corroborate our findings. In contrast, Hou et al. (2013) showed that repeated administration of a high dose of ketamine (100 mg/kg i.p. – the same that increases immobility time) did not alter novel object exploration index in Swiss-Kunming mice. Neill et al. (2014) already propose that the effects of NMDAR antagonists repeated exposure on tasks used to evaluate anhedonia and emotional blunting in rodents requires higher doses than those used to induce other behavioral impairments related to schizophrenia and our data seems to be in agreement with this assumption.

Interestingly, non-treated animals (sham) presented a higher stress-induced hyperlocomotion due to novel environment exposition when compared to vehicle or ketamine groups, which can be interpreted as a higher sensitivity to mild stressful conditions. Furthermore, we demonstrated an impairment on short term memory for the vehicle treated group, which indicates a deleterious effect induced by repeated stress that could be related to daily intraperitoneal injections or to the combination of the treatment schedule and the forced swimming in mice (Drude et al. 2011). Similar effect was observed with restraint stress (Bowman et al. 2009). However, these observations did not compromise the demonstration of the absence of hyperlocomotion and the presence of a cognitive deficit induced by ketamine, since after memory reconsolidation (long term memory evaluation) vehicle treated mice remembered the familiar object. Thus, our data confirm the usefulness of repeated sub-anesthetic ketamine to induce cognitive impairments relevant to model schizophrenia symptoms in rodents and corroborate the use of this pharmacological approach as a useful animal model to study some aspects of this disease (Becker et al. 2003, Neill et al. 2010). Moreover, memory tasks must be of higher relevance in drug development studies since they do not have the bias that underlies the forced swimming behavior.

In summary, ketamine (30 mg/kg/day i.p. for 14 days) induced a decrease in the immobility time of mice forced to swim that is not related to hyperactivity.

This confirms the antidepressant-like effect of ketamine in this behavioral task. Otherwise, impairment in the novel object recognition test was induced by the same treatment condition. Thus, in our experimental conditions ketamine induced antidepressant-like and amnesic-like effects. Considering this data, we can propose that the immobility time in the forced swimming test do not seems to be a good endpoint to evaluate the modeling of negative symptoms in NMDAR antagonist animal models of schizophrenia.

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