



Fast-acting antidepressant-like effects of ketamine in aged male rats

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Abstract

Background The aging process causes anatomical and physiological changes that predispose to the development of late-life depression while reduces the efficacy of classical antidepressants. Novel fast-acting antidepressants such as ketamine might be good candidates to be explored in the context of aging, especially given the lack of previous research on its efficacy for this age period. Thus, the aim of the present study was to characterize ketamine's effects in older rats.

Methods The fast-acting (30 min) and repeated (7 days) antidepressant-like effects of ketamine (5 mg/kg, *ip*) were evaluated in 14-month-old single-housed rats through the forced-swim and novelty-suppressed feeding tests. In parallel, the modulation of neurotrophic-related proteins (i.e., mBDNF, mTOR, GSK3) was assessed in brain regions affected by the aging process, prefrontal cortex and hippocampus, as well as possible changes in hippocampal cell proliferation.

Results Acute ketamine induced a fast-acting antidepressant-like response in male aged rats, as observed by a reduced immobility in the forced-swim test, in parallel with a region-specific increase in mBDNF protein content in prefrontal cortex. However, repeated ketamine failed to induce antidepressant-like efficacy, but decreased mBDNF protein content in prefrontal cortex. The rate of hippocampal cell proliferation and/or other markers evaluated was not modulated by either paradigm of ketamine.

Conclusions These results complement prior data supporting a fast-acting antidepressant-like effect of ketamine in rats, to further extend its efficacy to older ages. Future studies are needed to further clarify the lack of response after the repeated treatment as well as its potential adverse effects in aging.

Keywords Ketamine · Fast-acting antidepressant · Aging · BDNF

Introduction

Major depressive disorder is one of the most common and debilitating mental illnesses and a major public health problem worldwide. After anxiety disorders, depression is the most common mental health disorder among older adults, affecting 5–7% of the elder population [1], with suicide rates

also increasing between the ages of 60 and 90 [2]. Changes during aging predispose to the development of the so-called late-life depression [3], for which pharmacotherapy, psychotherapy, and electroconvulsive therapy are the treatments of choice [4]. However, classical antidepressants are known to change in efficacy with age [4–6], with reduced response rates ranging from 54% at age 54 to 42% at age 73 [6]. Despite these age-related differences, and in the context of our increasingly aging society, research focused on characterizing classical or novel therapeutic options for this age-group is really scarce (see some prior studies attempting to amend this [7–9]).

In this regard, the discovery of the rapid antidepressant effects of ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, is considered one of the major breakthroughs in the treatment of depression in the past decades, opening the door to a new class of fast-acting antidepressant options [10]. While a single administration of ketamine has demonstrated rapid and potent reductions in

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depressive symptomatology both in humans [11–14] and in animal models of depression [15–20], repeated doses seemed to sustain the observed short-term responses [21–23]. Indeed, esketamine, the S-enantiomer of ketamine, was first approved by the FDA in US in 2019 and then by EMA in Europe for the treatment of patients with resistant depression [24–25]. Although efficacy in adult patients seemed quite satisfactory, the potential effects of ketamine to treat late-life depression has been poorly investigated. In particular, and as reviewed by [26–27], only two randomized controlled trials have been conducted in an aging population (60 years and older) [28–29], with mixed results. While one trial found a positive effect of ketamine administration both on response and remission rates [28], the other one found no significant differences [29]; although some beneficial effects were observed (i.e., a trend towards an improvement as measured by the Montgomery-Asberg Depression Rating Scale). Additionally, a recent analysis of an open-label clinical trial showed indications of a lower antidepressant response (37.1% vs. 57.8%) and remission rates (15.8% vs. 47.4%) in older vs. younger depressed patients [30]. These promising but clearly insufficient analyses highlight the need for increased research on the potential antidepressant-like effects of ketamine in the aging population, including its characterization at the preclinical level.

In this context and to the best of our knowledge, the evaluation of ketamine as an antidepressant in aged animals is limited to a single study conducted in mice evaluating the effects of a single administration [9], and another one evaluating its prophylactic effects against stress-induced behaviors [31]. However, neither of these studies [9, 31] induced efficacy in aged animals using a dosing regimen proven effective in adolescent and adult rodents (e.g., [19–20]). Against this background the present study will further characterize the potential antidepressant-like response of ketamine in aging single-housed rats (e.g., [32]), since this stress paradigm in rodents (i.e., social isolation due to size requirements for the number of animals per cage) reproduced several behavioral features mimicking late-life depression (see our own studies phenotyping rats from middle-age and onward [33–34, 8]). Moreover, the present study will evaluate biomarkers of the antidepressant-like response such as the activation of brain neurotrophic factor (BDNF) (e.g., [10, 35]) and associated signaling partners (e.g., mTOR and GSK-3; [36–38]) in several brain regions (e.g., prefrontal cortex and hippocampus) impacted by aging (e.g., [39]) and/or an increase in hippocampal neurogenesis [40–42]; see a more recent study by [43]. A preliminary report of a portion of this work was presented at the 34th European College of Neuropsychopharmacology (ECNP) Congress Hybrid [44].

Materials and methods

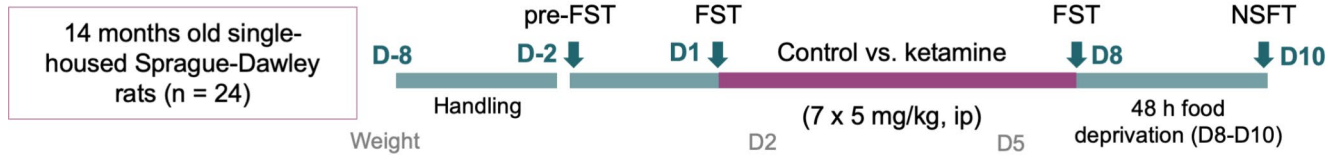
Animals

For the present study, a total of 48 male Sprague-Dawley rats were used when they reached 14-month-old. Rats were bred in the animal facility at the University of the Balearic Islands and were housed under standard vivarium conditions (22 °C, 70% humidity, 12-h light/dark cycle, lights on at 8:00 AM) with access to a standard diet and unlimited tap water (except when otherwise specified, see Fig. 1a for food deprivation prior to novelty-suppressed feeding test). Following animal housing regulations regarding the number of animals allowed per cage in terms of size and weight, 14-month-old rats were single-housed in standard cages for several months before testing started, which is a great model of chronic stress. All procedures were performed during the light period (between 8:00 AM and 3:00 PM), complied with the ARRIVE guidelines [45], the EU Directive 2010/63/EU for animal experiments, and the Spanish Royal Decree 53/2013, and thus were approved by the Local Bioethical Committee (CEEA 58/04/16) and the Regional Government (2016/08/AEXP). All efforts were made to minimize the number of rats used and their suffering. Unfortunately, since no aged female rats were available at the time when this experiment was performed, only male rats were included in the present study, and therefore the conclusions are limited to only one sex.

Behavioral procedures

All rats were handled several days prior to any behavioral or administration procedure. For this experiment, a total of 24 rats were used for the behavioral characterization of ketamine (Fig. 1a). Rats were randomly allocated into two groups and were treated daily for 7 consecutive days with ketamine (7×5 mg/kg, *ip*, $n = 12$; Anesketin: 100 mg/ml of ketamine from Dechra Pharmaceuticals, Northwich, United Kingdom) or saline (7×1 ml/kg of 0.9% NaCl, *ip*, $n = 12$). The dose of ketamine was chosen based on previous antidepressant results reported in the literature (e.g., [46–47]) and on our own results [19–20]. Antidepressant-like responses were ascertained by diverse tests previously validated in the field. We first measured behavioral despair under the stress of the forced-swim test (e.g., [48]), 30 min after the first treatment-dose (D1, acute effects) and 24 h after the last treatment dose (D8, repeated effects; see Fig. 1a) as earlier described by our group [33–34, 19]. Briefly, on pre-test day (D-2, see Fig. 1a), rats were placed for 15 min in single tanks (32 cm diameter x 41 cm high) filled with water (25 cm of depth, 25 ± 1 °C). Then, on test days (D1 and D8) rats were exposed for 5 min to the same

a Behavioral procedures



b Neurochemical procedures

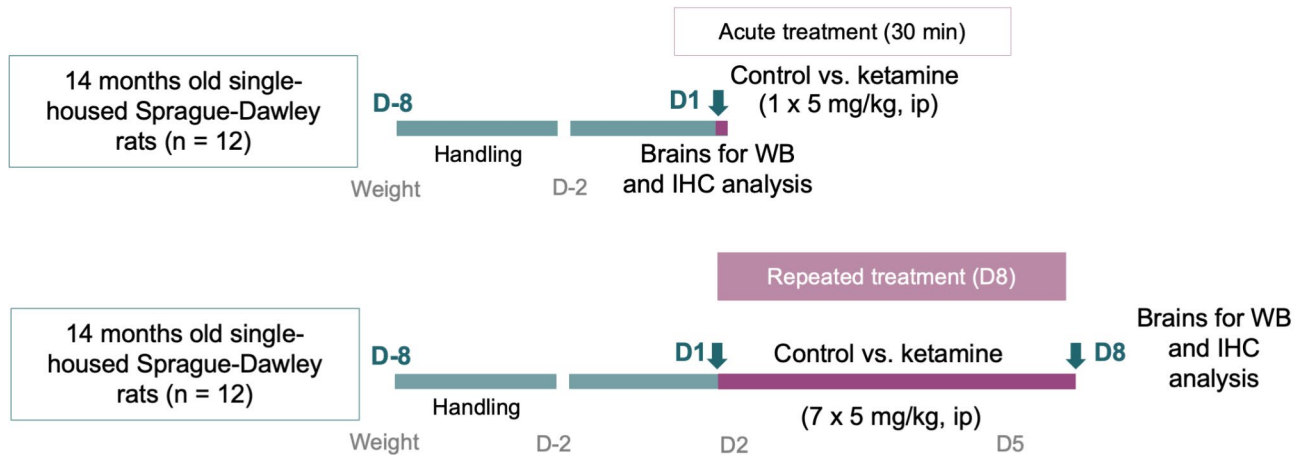


Fig. 1 Experimental design. (a) Behavioral procedures in single-housed rats (14 months old) after an acute (1 dose of 5 mg/kg, *ip*) or a repeated (7 doses of 5 mg/kg, *ip*, 1 dose per day) treatment with ketamine. Changes were evaluated 30-min (day, D1) or 24 h post-treatment (D8) in the forced-swim test (FST) and 3 days post-treatment

conditions, and their performances were videotaped. Videos were analyzed by an experimenter blind to the specific treatment groups with Behavioral Tracker software (CA, USA) to ascertain the time each rat spent (s) immobile (i.e., an indicative of despair) vs. active (i.e., escaping-like behaviors such as swimming or climbing). Moreover, the potential repeated antidepressant-like responses of ketamine were also evaluated in the novelty-suppressed feeding test 2 days after the last dose (D10). Following standard procedures [19, 49], rats were food-deprived for 48 h (D8-D10) since motivation for food is required for this particular test. During test day, each rat was placed for 5 min in a square open arena (60 cm x 60 cm, and 40 cm in high) under housing lighting conditions with three food pellets in the center [19]. Sessions were recorded and the parameters latency to center (s), time in center (s), latency to food (s), feeding time (s), and distance traveled (cm) were blindly scored using the ANY-maze software (version 7.37, Stoelting Co. Dublin, Ireland). Body weight was monitored through the treatment process and showed no changes between experimental groups and/or across

(D10) in the novelty-suppressed feeding test (NSFT). (b) Neurochemical procedures aimed at collecting brains at the same time points (acute, D1, or repeated, D8) to evaluate molecular markers by western blot (WB) and immunohistochemistry (IHC) experiments

time (data not shown). Notably, brains from the behavioral procedures were not collected for neurochemical studies.

Neurochemical procedures

For evaluating the neurochemical effects of ketamine, we utilized a separate group of 24 aged rats who were randomly assigned to the acute (1 dose; $n = 12$) or repeated (7 doses; $n = 12$) treatment groups (Fig. 1b). Similarly, to the previous experiment, each rat received the corresponding daily doses of saline (1 or 7×1 ml/kg of 0.9% NaCl, *ip*, $n = 6$ per group) or ketamine (1 or 7×5 mg/kg *ip*, $n = 6$ per group). Rats were then sacrificed without anesthesia by rapid decapitation with a large rodent guillotine following standard procedures in our group [8, 33] 30 min after the acute treatment or 24 h after last dose for the repeated treatment paradigm (D8). These time-points matched the particular times when forced-swim tests were performed so direct comparisons could be done between the potential antidepressant-like vs. neurochemical effects of ketamine in aged rats.

Once brains were rapidly extracted, the whole prefrontal cortex and the right hippocampus were freshly dissected, fast frozen in liquid nitrogen, and saved at $-80\text{ }^{\circ}\text{C}$ until further processing to evaluate target proteins by Western blot analysis. The left hemisphere was frozen in $-30\text{ }^{\circ}\text{C}$ isopentane (#143501,1611, Biolinea SL, Palma, Spain), and kept at $-80\text{ }^{\circ}\text{C}$ until the entire hippocampal extent (-1.72 to -6.80 mm from Bregma) was cryostat-cut in $30\text{ }\mu\text{m}$ sections. Consecutive sections were slide mounted in 24 slides per animal with 8 tissue-sections per slide, divided in 3 series (8 slides per series), covering the most anterior part of the hippocampus, the middle part and the most posterior part of it. The rate of cell proliferation (Ki-67 + cells) was then evaluated by immunohistochemical analysis as previously performed [50–52] in a representative sample of the whole hippocampus following a stereological procedure that counts every 8-th section taken (1 slide from each series containing the anterior, middle and posterior part of hippocampus).

Western blot analysis

Total homogenates of brain regions (prefrontal cortex or hippocampus) were prepared with minor modifications as previously described [53–54]. Each sample ($40\text{ }\mu\text{g}$ of total protein) was loaded in 7.5–14% SDS-PAGE minigels (Bio-Rad Laboratories, Hercules, CA, USA) that were resolved by electrophoresis and then processed following standard immunoblotting procedures [33, 55]. Membranes ($0.2\text{ }\mu\text{m}$: #10600001 or $0.45\text{ }\mu\text{m}$: #10600002, Merck SL, Barcelona, Spain) were incubated overnight at $4\text{ }^{\circ}\text{C}$ in blocking solution containing the specific primary antibodies: anti-BDNF (N-20) (dilution 1:10000; AB108319; Abcam, Cambridge, United Kingdom) for identifying the mature form of BDNF (mBDNF) [56]; anti-pS²⁴⁴⁸mTOR (dilution 1:1000; #2971) and anti-mTOR (dilution 1:1000; #2972) (Cell Signaling, MA, USA); anti-pS^{21/9}-GSK3 (dilution 1:1000; # 9331; Cell Signaling) for detecting inhibitory phosphorylation and anti-GSK3 (dilution 1:1000; 4G-1E; 05-412; Millipore) for total protein. Membranes were then incubated with an anti-rabbit (#7074S) or anti-mouse (#7076S) horseradish peroxidase-conjugated secondary antibody (1:5000; Cell Signaling, MA, USA) and the immunoreactivity of target proteins was displayed on autoradiographic films (#28906837, Amersham ECL Hyperfilm) using an ECL detection system (#17652005, Amersham, Buckinghamshire, United Kingdom). Autoradiograms were quantified by densitometric scanning (GS-800 Imaging Calibrated Densitometer, Bio-Rad). All samples were loaded at least 2–3 times in different gels, and percent changes

in each gel were calculated for each rat as compared to control-treated samples. For loading control, low quantities of total homogenates ($15\text{ }\mu\text{g}$) were run to detect β -actin (dilution 1:10000; AC-15; Sigma-Aldrich, MO, USA), since its content was not altered by the treatment followed.

Immunohistochemical analysis

Cell proliferation was labeled in the hippocampus with Ki-67 antibody (1:20,000; from Prof. Huda Akil and Stanley J. Watson, University of Michigan, MI, USA) as previously described [50–52]. Experiments were performed on 3 cryostat-cut sections ($30\text{ }\mu\text{m}$), one from the front, middle or posterior parts of the hippocampus, and containing 8 tissue-sections per slide. Briefly, slide mounted sections were post-fixed in 4% paraformaldehyde and processed through several steps including a series of sequential incubation, with biotinylated anti-rabbit antibody (1:1000, BA-100, Vector Laboratories, CA, USA), Avidin/Biotin complex (PK-6100, Vectastain Elite ABC kit; Vector Laboratories, CA, USA) and the chromogen 3,3'-diaminobenzidine (H54000, Thermo Fischer, MA, USA) for signal detection. Finally, tissue was counterstained in cresyl-violet (405760100, Thermo Fischer, MA, USA), dehydrated in graded alcohols, submersed in xylene and cover-slipped with Permount[®] (SP15-500, Fisher Chemical, NH, USA). The number of positive cells was counted by an experimenter blind to the treatment groups with a Leica DMR light microscope (63x objective lens) in a total of 3 slides per rat (8 sections per slide; total of 24 tissue sections per rat) and focusing through the depth of the tissue. The total number of cells was then multiplied by the sampling factor 8 to provide an overall estimate of the total number of Ki-67 + cells per rat in the left hippocampus.

Data and statistical analysis

All data were analyzed and plotted with GraphPad Prism, Version 10 (GraphPad Software, Inc, San Diego, CA, USA). Results are expressed as mean values \pm standard error of the mean (SEM); symbols represent individual values for each rat, following recommendations for displaying data and statistical results in pharmacology [57–58]. Potential changes induced by the acute or repeated treatments with ketamine for each behavioral feature and neurochemical marker analyzed were performed through two tailed Student's *t*-tests. All sets of data reported followed a normal distribution according to Shapiro-Wilk normality test. The level of significance was set at $p \leq 0.05$.

Results

Ketamine induced a rapid antidepressant-like response after an acute dose in aged rats: lack of efficacy following a repeated treatment

When evaluating the acute antidepressant-like effects of ketamine under the stress of the forced-swim test, a two-tailed Student's *t*-test revealed a significant reduction in immobility as observed 30 min post-ketamine administration (~13% reduction: -40 ± 15 s, $t=2.62$, $df=21$, $*p=0.016$ vs. control rats; Fig. 2a), which paralleled an increase in swimming behavior (~8% increase: $+25 \pm 6$ s, $t_{21}=4.29$, $***p<0.001$ vs. control rats; Fig. 2a). Acute ketamine did not induce changes in climbing behavior ($t_{21}=0.87$, $p=0.394$; Fig. 2a).

Interestingly, following the repeated treatment with ketamine (7 days of a daily 5 mg/kg injection), no significant changes were observed in the forced-swim test (immobility: $t_{22}=1.14$, $p=0.266$; swimming: $t_{22}=1.20$, $p=0.244$; climbing: $t_{22}=0.50$, $p=0.620$) and/or the novelty-suppressed feeding test (latency to center: $t_{22}=0.17$, $p=0.870$; time in center: $t_{22}=0.77$, $p=0.452$; latency to food: $t_{22}=0.33$, $p=0.743$), denoting the loss of antidepressant-like potential (Fig. 2b). Moreover, it is worth mentioning that none of the rats evaluated spend any feeding time (despite prior food deprivation) and no changes were observed in distance travelled among treatment groups (data not shown).

Region-specific neurochemical effects after acute or repeated ketamine administration in aged rats

Acute ketamine induced a region-specific modulation of mBDNF protein content (Fig. 3a); while it increased mBDNF in prefrontal cortex ($+42 \pm 13\%$, $t_9=3.23$,

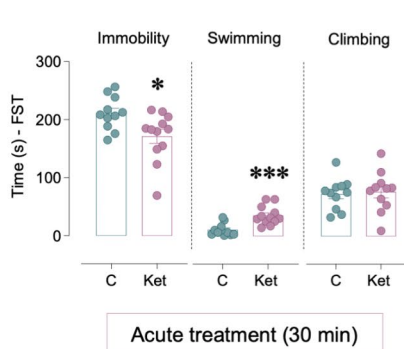
$*p=0.011$ vs. control rats), no changes were observed in hippocampus ($t_{10}=1.07$, $p=0.310$). When analyzing the regulation of its downstream signaling partners (i.e., active ratio of p-mTOR/mTOR and inhibitory ratio of p-GSK3/GSK3), no changes were observed in prefrontal cortex or hippocampus (Fig. 3a). Moreover, acute ketamine did not change the rate of hippocampal cell proliferation ($t_9=1.17$, $p=0.273$).

The effects of a repeated paradigm of ketamine on the neurochemical markers under study are shown in Fig. 3b. The data showed that repeated ketamine in aged rats decreased the protein content of mBDNF in prefrontal cortex ($-21 \pm 9\%$, $t_{10}=2.35$, $*p=0.041$ vs. control rats), but not in hippocampus ($t_9=1.26$, $p=0.239$). Similar to what was observed following an acute injection, no changes were observed following a repeated paradigm in p-mTOR/mTOR or p-GSK3/GSK3 in both regions analyzed (Fig. 3b). Finally, repeated ketamine did not change the rate of hippocampal cell proliferation ($t_{10}=2.06$, $p=0.067$). Representative images of selected western blot and immunohistochemistry experiments are shown in Fig. 3c.

Discussion

The present study investigated the acute and repeated antidepressant-like potential of ketamine in aged male rats following the same administration paradigms previously proven effective in adolescent and adult rats [19–20]. The main results showed a rapid fast-acting antidepressant-like effect of ketamine (observed 30 min post-treatment), as reported by a decrease in immobility and an increase in swimming behaviors in the forced-swim test, paired with an increase in mBDNF protein content in prefrontal cortex.

a Fast-acting acute effects



b Lack of efficacy following a repeated treatment

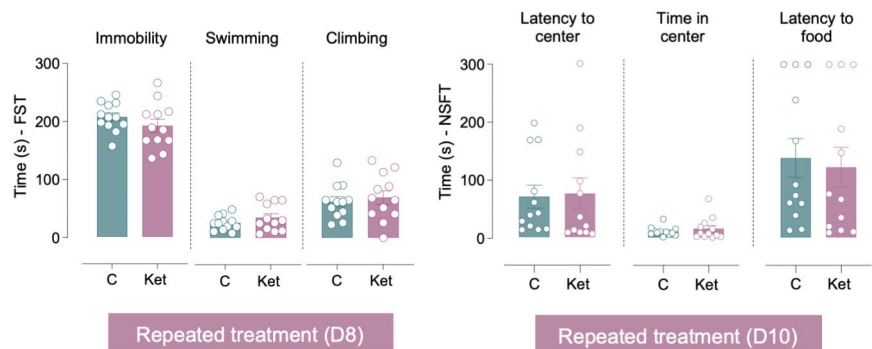
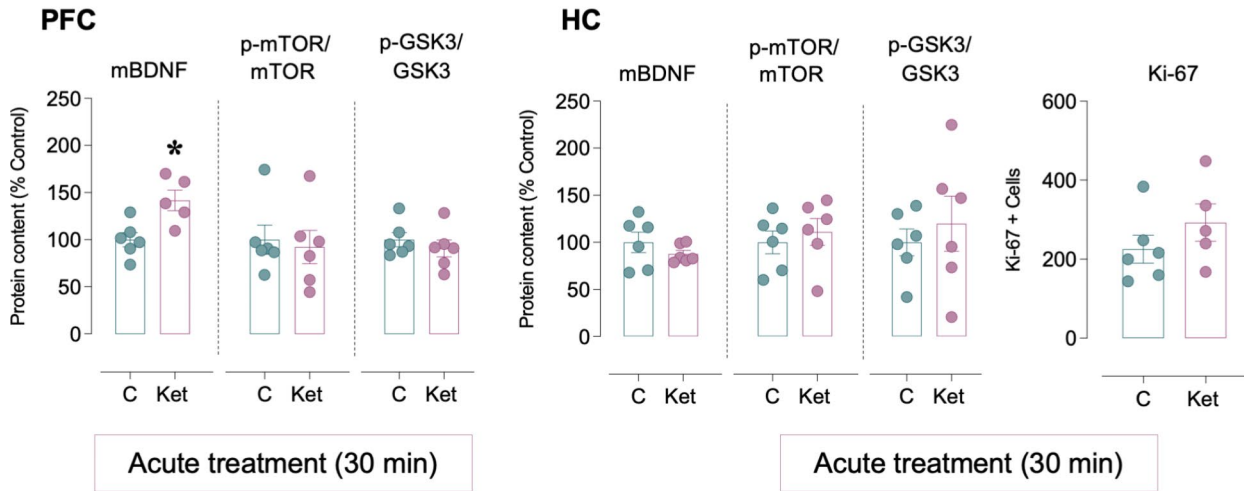


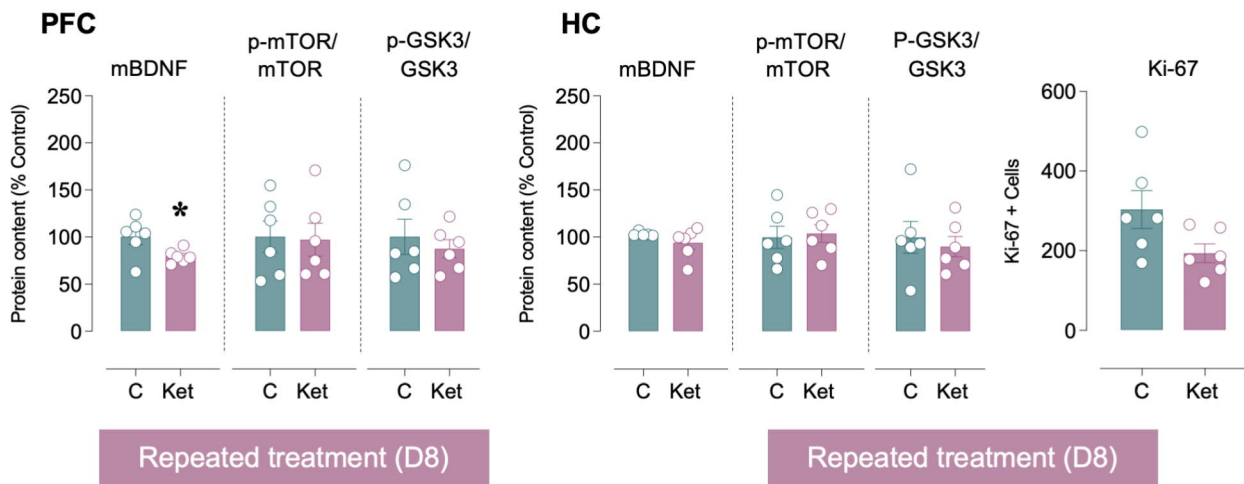
Fig. 2 Exploring the antidepressant-like effects of ketamine in aged rats. **(a)** Fast-acting acute effects of ketamine (1 single dose of 5 mg/kg, *ip*, D1) as measured in the forced-swim test (FST) 30 min post-treatment. **(b)** Lack of efficacy following a repeated treatment with ketamine (5 mg/kg, *ip*, 7 days, D1–D7) as evaluated in the FST 24 h post-treatment (D8), and in the novelty-suppressed feeding test

(NSFT) 3 days post-treatment (D10). Columns represent mean \pm SEM of time spent in each behavior. Individual values are shown in symbols for each rat. $***p<0.001$, $*p<0.05$ when comparing ketamine-treated rats (Ket) vs. control-treated rats (C) through two-tailed Student's *t*-tests

a Acute neurochemical effects



b Repeated neurochemical effects



c Representative images of selected experiments

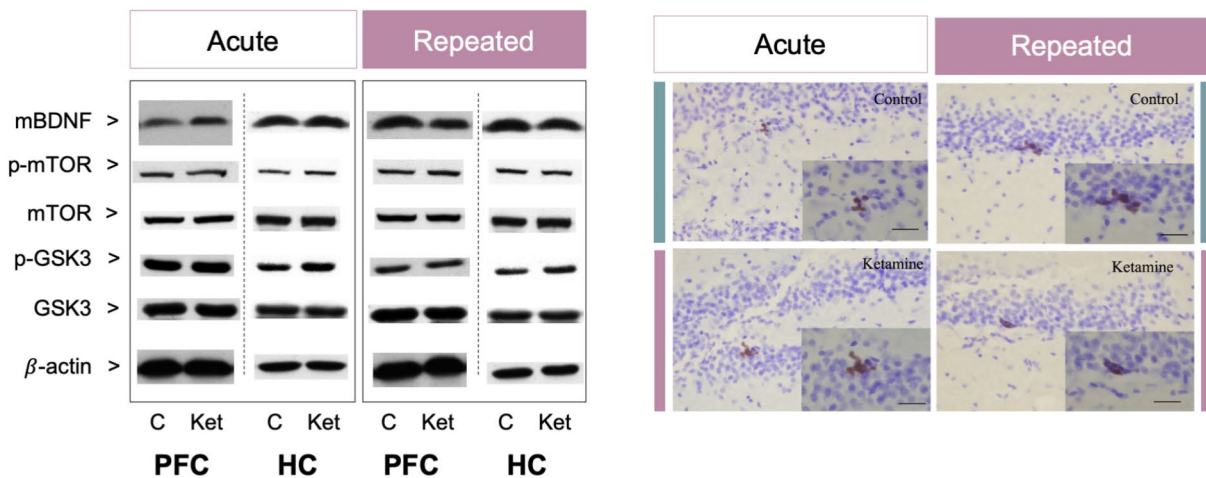


Fig. 3 Exploring the neurochemical effects of ketamine in aged rats. (a) Fast-acting acute (30 min post-treatment) or (b) repeated (24 h post-treatment, D8) effects of ketamine in prefrontal cortex (PFC) and hippocampus (HC) of the selected protein markers evaluated by western blot (mBDNF, p-mTOR/mTOR and pGSK3/GSK3) or immunohistochemistry (Ki-67 + cells). Columns represent mean \pm SEM of protein content (% Control) or Ki-67 + cells per group. Individual values are shown in symbols for each rat. * $p < 0.05$ when comparing ketamine-treated rats (Ket) vs. control-treated rats (c) through two-tailed Student's *t*-tests. (c) Representative images of selected western blot or immunohistochemistry experiments. Left panels: immunoblots depicting the labeling of each protein and loading control β -actin. For unprocessed full western blot images check Supplementary Figures S1–S6. Right panels: representative images of Ki-67 + cells (brown labeling in the blue granular layer) taken with a light microscope using a 40x objective lens. A magnified window is shown at 63x. Scale bar: 30 μ m. For other representative images check Supplementary Figures S7–S10

However, these acute effects were no longer observed after a repeated treatment paradigm, as measured by a lack of antidepressant-like response in two independent behavioral tests, combined with a decrease in mBDNF in prefrontal cortex. These results proved an acute fast-acting antidepressant-like response for ketamine in aged male rats (observed both behaviorally and at the neurochemical level), while suggested that its repeated administration might lead to molecular adaptive changes preventing its efficacy.

Ketamine induced a rapid antidepressant-like response, observed 30 min after a single administration in aged rats, consistent with previous studies [15–16]. Interestingly our prior studies which utilized the same acute dose of ketamine (5 mg/kg) showed differences in antidepressant-like efficacy depending on the age of animals, the biological sex and prior stress exposure [19–20]. Particularly, acute ketamine induced an antidepressant-like response in adolescent rats in the forced-swim test, an effect that was observed for both sexes, but that depended on prior stress exposure (see further details in [19]). However, acute ketamine in adult rats was inefficacious for both sexes and only showed efficacy when male rats were priority pretreated with letrozol (an aromatase inhibitor that blocks the biosynthesis of estrogens; [20]). Overall, these results suggest that there may be some similarities in the antidepressant-like response between adolescent and aged rats, with a similar dose needed in aged [8] and adolescent rats, but with the need for a higher dose to induce efficacy in adult rats [59]. Remarkably, it is worth mentioning that the parallelisms observed for adolescent and aged rats were under different sources of stress (i.e., early maternal separation for the adolescent study [19] vs. social isolation due to size and cage requirements in the present study with aged rats), suggesting that ketamine proved good efficacy for stress-related conditions. In particular, the present experimental paradigm of physiologically aged rats, individually housed for several months, and with an expected phenotype mimicking depressive-like manifestations (see characterization at [33]) proves to be a great

model in which to validate the fast-acting acute effects of ketamine administration.

Contrarily to prior reports demonstrating that under stressful situations repeated ketamine induced antidepressant-like effects in adolescent [19] and/or adult rats [20, 23, 60–64], the present results showed a lack of response in aged male rats. Disparities in the type of stressor and/or differences in ketamine pharmacokinetics due to age might be behind these discrepancies, as studies with anesthetic doses of ketamine showed a significant increase in half-life, drug availability, and duration of anesthesia in aged Sprague-Dawley rats compared to young rats [62]. Although test repetition might have been behind the lack of effects observed following the repeated paradigm, this seems unlikely, since we followed a standard procedure previously used in other studies from our group that had proven effects across time in the forced-swim test (e.g., [19–20, 52]). Moreover, similar to the low dose we tested (5 mg/kg), the same dose also rendered inefficacious in another study [61], suggesting dose-dependent effects as mentioned in some of the other reports, and the potential need for a much higher dose and/or an increasing-dosage regimen to observe a beneficial response after a repeated treatment, and overcome potential adaptive mechanisms. For example, pharmacodynamic responses caused by the daily repeated administration might be playing a role in this lack of efficacy (i.e., tachyphylaxis). In this context, we aimed at exploring the differences in the molecular responses elicited after an acute or repeated dosing paradigm in an attempt to further understand the behavioral results. Particularly, we explored markers of antidepressant-like responses (i.e., mBDNF and associated partners, as well as the first stage of hippocampal neurogenesis) in key brain areas mediating affective-like responses and impacted by the aging process (i.e., prefrontal cortex and/or hippocampus) (see [63–64] and references therein).

Concurrent with the observed fast-acting antidepressant-like response, a single administration of ketamine increased mBDNF expression in the prefrontal cortex of aged male rats. This effect was not observed in hippocampus, suggesting a region-specific role for prefrontal cortex in the molecular actions behind ketamine's response, and in line with prior results (reviewed by [35] and recently by [10]). BDNF-mediated activation of tropomyosin receptor kinase B (TrkB) induces the activation of several signaling pathways, including the inhibitory phosphorylation of the glycogen synthase kinase-3 (GSK-3) [36–37], which ultimately activates the mechanistic target of rapamycin complex 1 (mTORC1) [38]. However, no changes were detected in mBDNF-associated downstream partners in either brain region under evaluation (i.e., p-mTOR/mTOR and p-GSK3/GSK3 ratios). Interestingly, and in line with the present results, increased BDNF expression in the prefrontal cortex

has been shown to be necessary for the acute antidepressant-like effects of ketamine [10, 65]). However, during aging, changes in TrkB expression may be involved in the lack of downstream signaling [66]. Also, since ketamine activates mTOR signaling within 1 h in the prefrontal cortex [67], another possible explanation for these results could be that the chosen time point of study might be too early to observe changes in the TrkB downstream pathway.

Curiously, repeated ketamine treatment reduced mBDNF expression in the prefrontal cortex as evaluated 24 h after the last daily dose, without altering its content in hippocampus and/or its downstream partners and hippocampal cell proliferation. These effects could be related to the lack of antidepressant-like efficacy after repeated ketamine administration and might imply certain adaptive molecular changes to the acute increase in mBDNF and caused by the repeated administration of the drug. Moreover, since some studies in the literature described that the sustained antidepressant ketamine response seemed to require hippocampal progenitor differentiation through a TrkB-dependent mechanism [68], the observed lack of BDNF modulation in hippocampus after repeated ketamine treatment may predict its lack of effect on cell proliferation. Moreover, previous studies have shown that aging abolishes the neurogenic effect of classical (e.g., fluoxetine; [69]) and novel antidepressants (e.g., cannabidiol [8]), possibly due to changes in the neurogenic niche during aging [70].

A limitation of the present study is the fact that it was conducted exclusively in male rats, especially since depression is twice as common in women as in men [71] and given that ketamine has demonstrated sex-dependent antidepressant-like effects in rats of different ages [46, 19–20]. Unfortunately, the logistical burden of individually housing animals as they age for several months severely hampered the inclusion of sex as a biological variable in the present study. Therefore, future studies should ascertain sex differences when characterizing the potential antidepressant-like effects of ketamine in aging, as preclinical animal models must be truly representative of the aging population. Of special relevance would be to ascertain how potential sex differences regarding the functioning of the glutamate system (e.g., reviewed by [72]), and especially the NMDA receptor [73] might be affecting the antidepressant-like response of ketamine in aged male vs. female rats.

In conclusion, our study contributes by increasing the existing body of knowledge on the role of ketamine as an antidepressant through its effects in aged rats. Overall, acute ketamine administration showed a fast-acting antidepressant-like response in aged rats (behavioral and biomarker responses). Future studies are needed to clarify the lack of response after the repeated treatment as well as its potential adverse effects. Moreover, other aspects to further study when trying to find a safe and effective treatment for

age-related depression would include characterizing the duration of the acute antidepressant-like response, evaluating other doses and/or administration regimens, as well as including sex as a biological variable. In any case, ketamine seems like a great novel fast-acting option to be further explored for our aged population in which classical antidepressants showed reduced efficacy.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s43440-024-00636-y>.

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Author contributions EH-H and MJG-F were responsible for the study concept and design. EH-H, SL-C and JJ-P conducted the experiments and analyzed the behavioral and molecular data. EH-H wrote the first draft of the manuscript, and MJG-F edited it to its final version. All authors approved the final version of the manuscript.

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Data availability Data will be made available upon request.

Declarations

Competing interests The authors declare no competing interests.

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