Chemico-Biological Interactions 342 (2021) 109476

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

A single administration of ascorbic acid rapidly reverses depressive-like behavior and hippocampal synaptic dysfunction induced by corticosterone in mice

Daiane B. Fraga [a], Anderson Camargo [a], Gislaine Olescowicz [a], Dayane Azevedo Padilha [a], Francielle Mina [b], Josiane Budni [b], Patricia S. Brocardo [c], Ana Lúcia S. Rodrigues [a,*]

[a] Department of Biochemistry, Center of Biological Sciences, Federal University of Santa Catarina, Florianopolis, SC, Brazil
[b] Laboratory of Experimental Neurology, Graduate Program in Health Sciences, University of Southern Santa Catarina, Criciúma, SC, Brazil
[c] Department of Morphological Sciences, Center of Biological Sciences, Federal University of Santa Catarina, Florianopolis, SC, Brazil

ARTICLE INFO

Keywords:
Ascorbic acid
Corticosterone
Dendrite spine
Depression
Ketamine

ABSTRACT

Ketamine is the prototype for glutamate-based fast-acting antidepressants. The establishment of ketamine-like drugs is still a challenge and ascorbic acid has emerged as a candidate. This study investigated the ascorbic acid's ability to induce a fast antidepressant-like response and to improve hippocampal synaptic markers in mice subjected to chronic corticosterone (CORT) administration. CORT was administered for 21 days, followed by a single administration of ascorbic acid (1 mg /Kg, p.o.), ketamine (1 mg /Kg, i.p.) or fluoxetine (10 mg /Kg, p.o.) in mice. Depressive-like behavior, hippocampal synaptic proteins immunocontent, dendrite spines density in the dentate gyrus (DG) were analyzed 24 h following treatments. The administration of ascorbic acid or ketamine, but not fluoxetine, counteracted CORT-induced depressive-like behavior in the tail suspension test (TST). CORT administration reduced PSD-95, GluA1, and synapsin (synaptic markers) immunocontent, and these alterations were reversed by ascorbic acid or ketamine, but only ketamine reversed the CORT-induced reduction on GluA1 immunocontent. In the ventral and dorsal DG, CORT decreased filopodia-, thin- and stubby-shaped spines, while ascorbic acid and ketamine abolished this alteration only in filopodia spines. Ascorbic acid and ketamine increased mushroom-shaped spines density in ventral and dorsal DG. Therefore, the results show that a single administration of ascorbic acid, in a way similar to ketamine, rapidly elicits an antidepressant-like response and reverses hippocampal synaptic deficits caused by CORT, an effect associated with increased levels of synaptic proteins and dendritic remodeling.

1. Introduction

Major depressive disorder (MDD) is a prevalent psychiatric disorder that causes a profound socioeconomic burden [1]. It has been reported that more than 50% of depressed patients have hyperactivity of the HPA axis, an alteration that may be normalized by chronic treatment with antidepressants [2]. Accordingly, the repeated administration of corticosterone (CORT) in rodents has been reported to cause depressive-like behavior and neurochemical alterations, which is sensitive only to chronic, but not acute, administration of conventional antidepressants [3–5]. However, it is sensitive to a single administration of ketamine, which resembles what occurs in the clinical situation [6,7]. Therefore, these features make the CORT model a useful tool for screening fast-acting antidepressant drugs.

Ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, produces a rapid and efficacious antidepressant response even in patients who have failed to respond to two or more typical antidepressants [8,9]. Preclinical studies have shown that the rapid antidepressant actions of ketamine are associated with stimulation of synaptic structural plasticity and synaptogenesis [10,11]. Particularly, ketamine antagonizes NMDA receptors in inhibitory GABAergic interneurons, resulting in a disinhibition of pyramidal cells of the hippocampus with the consequent release of glutamate that may preferentially activate α-amino-3-hydroxy-5-methyl-4isoxazolepropionic acid (AMPA) receptors [12]. These events subsequently lead to the brain-derived neurotrophic factor release that culminates in the activation of the mechanistic target of rapamycin complex 1 (mTORC1) signaling

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

https://doi.org/10.1016/j.cbi.2021.109476
Received 17 February 2021; Received in revised form 31 March 2021; Accepted 12 April 2021
Available online 17 April 2021

Abbreviations

AMPA	alpha-amino-3-hydroxy-methyl-5-4-isoxazole propionic acid
CORT	corticosterone
DG	dentate gyrus
GluA1	AMPA receptor subunit 1
HPA	hypothalamic-pituitary-adrenal
MDD	major depressive disorder
mTORC1	mechanistic target of rapamycin complex 1
NMDA	N-methyl-D-aspartate
PSD-95	postsynaptic density protein-95 kDa
TST	tail suspension test

pathway [13]. mTORC1 activation promotes the synthesis of proteins involved in spinogenesis and synaptogenesis such as postsynaptic density protein 95 (PSD-95), AMPA receptor subunits 1 (GluA1), and synapsin [10,14].

Despite the great potential of ketamine for the management of MDD, it has addictive properties and may cause dissociative effects and neurotoxicity upon repeated use. Therefore, identifying compounds that share with ketamine similar mechanisms of action is crucial for establishing novel fast-acting antidepressants [15]. In this context, ascorbic acid has emerged as a promising antidepressant strategy since it has been shown to elicit antidepressant responses in preclinical and clinical studies [16]. Ascorbic acid is highly concentrated in the brain, being considered a neuromodulator that may improve synaptic plasticity, dendrite development, and neuronal signaling [15,16]. In this regard, it was recently shown that a single administration of ascorbic acid is effective to increase the dendritic spine density in the ventral DG of the hippocampus in mice subjected to novelty-suppressed feeding test [17], a test is sensitive to the acute administration of ketamine but not to the conventional antidepressants [18,19], which resembles the effects of these drugs in human patients.

In this context, it is tempting to hypothesize that ascorbic acid elicits a fast-onset antidepressant response associated with the modulation of synaptic components in the hippocampal region. Here, we investigated the ability of a single administration of ascorbic acid to counteract CORT-induced depressive-like behavior and synaptic dysfunction (impairment on either synaptic proteins or spine architecture) in the hippocampus of mice, using ketamine as a positive control. To ascertain the specificity of the effects, fluoxetine, a conventional antidepressant drug that does not produce rapid antidepressant response was used.

2. Materials and methods

2.1. Animals

Adult female Swiss mice (55–60 days of age) were used in this study, considering that the prevalence of MDD is higher in women than in men [20] and that females are shown to be more susceptible to stress than males [21]. Mice were maintained at 20–22 °C with free access to water and food, under a 12 h/12 h light–dark cycle (lights on at 07:00 h) in groups of 10 animals per cage (41 × 34 × 16 cm). All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the Institution. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs and treatment

Mice were administered orally (p.o.) by gavage with vehicle or CORT (20 mg/Kg, p.o., dissolved in distilled water with 2% Tween 80 and 0.2% DMSO) for 21 days. Ascorbic acid, ketamine, or fluoxetine were administered at a single dose immediately after the last CORT administration. Vehicle, ascorbic acid (1 mg/Kg), and fluoxetine (10 mg/Kg) were administered orally (p.o.) whereas ketamine (1 mg/Kg) was administered intraperitoneally (i.p.). On the 22nd day, 24 h after the treatments, mice were submitted to the behavioral tests. All drugs (Sigma Chemical Co. (St. Louis, USA) were freshly prepared and administered at a volume of 10 mL/kg body weight. The doses of ketamine, fluoxetine, ascorbic acid, and CORT as well as the time points were chosen based on previous studies [4,22,23]. Two cohorts of animal were used in this study (n = 6–8). One cohort of mice was immediately euthanized by decapitation, and hippocampi were collected for Western blotting analysis, and the second cohort of animals was deeply anesthetized and submitted to transcardial perfusion with 0.9% NaCl for hippocampal morphological analyses.

2.3. Tail suspension test

The total duration of immobility in the tail suspension test was measured as previously proposed [24]. Visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period, and mice were considered immobile only when they hung passively and completely motionless.

2.4. Locomotor activity

The locomotor activity was scored using an activity monitor (Insight Equipment Laboratory) in a 40 × 60 × 50 cm box containing 16 infrared sensors. The distance traveled (cm) was registered in a 15-min session [25].

2.5. Western blotting analysis

Hippocampus was quickly dissected and snap-frozen with liquid nitrogen before storage at −80 °C until use. The samples were mechanically homogenized in 400 μl of TRIS 50 mM pH 7.0, EDTA 1 mM, NaF 100 mM, PMSF 0.1 mM, Na_3VO_4 2 mM, Triton X-100 1%, glycerol 10%, Sigma Protease Inhibitor Cocktail (P2714). Lysates were centrifuged (10000 g for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in TRIS 100 mM pH 6.8, EDTA 4 mM, SDS 8%, and boiled for 5 min. Thereafter, sample dilution (40% glycerol, TRIS 100 mM, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β-mercaptoethanol (final concentration 8%) were added to the samples [26]. Protein content was quantified using bovine serum albumin (BSA) as a standard [27]. The samples containing 60 μg protein/track were separated by SDS-PAGE (miniVE Vertical Electrophoresis SystemTM, GE Healthcare Life Sciences, Piscataway, NJ, USA) using 7–10% gel and the proteins were transferred to nitrocellulose membranes using a semi-dry blotting apparatus (1.2 mA/cm2; 1.5 h). Subsequently, membranes were blocked with 5% BSA in TBS (10 mM Tris, 150 mM NaCl, pH 7.5). The immunocontent of PSD-95 (#2507), GluA1 (#13185), synapsin (#2312), and β-actin (loading control, #4970), were detected using specific antibodies (all obtained from Cell Signaling Technology, 1:1000) diluted in TBS-T (10 mM Tris, 150 mM NaCl, 0.1% Tween-10, pH 7.5) containing 2.5% BSA and incubated overnight. Subsequently, the membranes were incubated with anti-rabbit antibody horseradish peroxidase-conjugated secondary antibody (Cell Signaling, 1:2500, #7074) for 60 min, and the immunoreactive bands were developed using a chemiluminescence kit (Amersham ECL Select, Piscataway, USA). All blocking and incubation steps were followed by three washes (5 min) of the membranes with TBS-T. The optical density (OD) of the bands was quantified using Image Lab Software® 4.1 (Bio-Rad Laboratories). The immunocontent of PSD-95, synapsin, and GluA1 were determined as a ratio of OD of PSD-95, synapsin, and GluA1 band over

the OD of the β-actin band. Results are expressed as compared to the control group 100%.

2.6. Golgi staining

Under deep anesthesia with isoflurane, mice were transcardially perfused with 0.9% NaCl solution. Brains were immediately removed, placed in vials containing 20 mL modified Golgi-Cox solution, and stored at room temperature, in the dark for 14 days [17,28,29]. Following this period, the brains were transferred to 30% sucrose solution. Coronal sections (200 μm) were generated throughout the length of the hippocampus using a vibratome (Vibratome Series 1000, St Louis, MO, USA). Sections were immediately mounted onto 2% gelatin-coated microscope slides, and then the sections were sequentially placed in distilled H_2O (1 min), ammonium hydroxide (30 min), distilled H_2O (1 min), Kodafix for the film (30 min), distilled H_2O (1 min), 50% ethanol (1 min), 70% ethanol (1 min), 95% ethanol (1 min), 100% ethanol (10 min) and 100% ethanol/xylene (1:1 for 20 min) [30]. After processing, all slides were covered and stored in the dark using a Confocal Leica DMI6000 B microscope (Leica Microsystem, Wetzlar, Germany). Z-sections of labeled neurons and dendritic segments were collected. The images were processed using bath deconvolution on a Confocal Leica DMI6000 B microscope, to optimize the image quality. Golgi-impregnated cells were selected if they fulfilled the following criteria: had consistent impregnation throughout the extent of the cell body and dendrites, were able to be distinguished from neighboring impregnated cells, and had intact dendritic trees.

2.7. Dendrite spine analysis

A subset of Golgi-impregnated cells was randomly chosen to evaluate spine density. Neurons were uniformly distributed throughout the dorsoventral axis of the DG. For this study, 8–10 ventral DG and 8–10 dorsal DG segments were analyzed per animal. Starting from the origin of the branch (as a reference point), and continuing away from the cell soma, spines were counted manually along with the selected dendrite segment. Only dendritic protrusions less than 3 μm in length were counted for this analysis [31]. The total number of dendritic spines and the number of each type of dendritic spine, normalized to 10 μm of the dendritic segment length, were counted, to ensure that each spine was counted only once, under 100x magnification. From each region, Z-stacks (stack depth varied depending on the dendrite segment) were obtained for each segment, and the number of spines per 10 μm was manually quantified using ImageJ software. Dendritic spines were traced as a full dendrite in the z-plane and inspected in the x–y plane for each individual z-step. A typical dendritic segment was approximately 16–22 μm long. Spines are commonly categorized into morphological subtypes according to size and the relative proportions of the spine head and neck. The spine types were classified based on the following dimensions: length, from the base of the dendrite to the tip of its head (L); the maximum neck diameter (nd); and the maximum head diameter (hd). Thus, individual spines were included in each category based on the specific ratios of L/nd and hd/nd [32]. Morphometric analyses were conducted for each dendritic spine, and measurements were used to categorize spines into filopodia, thin, stubby, and mushroom classes. Filopodium-shaped spines presented hd = nd with an L value < 3 μm, whereas thin-shaped spines presented hd = nd with an L value < 2 μm. Stubby-shaped spines presented an L similar to the nd and the hd, values, which tended to be < 1 μm. Mushroom-shaped spines presented a hd value much larger than the nd value, with an L value < 1 μm [33]. As previously reported, the filopodium-, thin- and stubby-shaped dendritic spines were classified as “immature” spines, while “mushroom”-shaped dendritic spines were considered “mature” spines [17].

2.8. Statistical analysis

The Kolmogorov-Smirnov test was used to assess data normality. Normally distributed data from the behavioral and neurochemical experiments were analyzed using a two-way ANOVA, followed by Newman-Keuls *post-hoc* test, and data are presented as mean +S.E.M. Since the total spine density relied on a normal distribution, the two-way ANOVA followed by Newman-Keuls *post-hoc* test was used. As filopodia, thin, stubby, and mushroom dendritic spines density did not follow a normal distribution, we used cumulative probability plots to measure shifts per 10 μm of the dendritic segment in the different experimental groups. Cumulative distribution probabilities were compared by Kruskal-Wallis followed by Dunn’s multiple comparisons test. The description of statistical data of the two-way ANOVA (all main effects, interactions, and p values) is listed in Suppl. Table 1, and the description of statistical data of the Kruskal-Wallis is listed in Suppl. Table 2. In all tests the significance was assigned when $p < 0.05$.

3. Results

3.1. Ascorbic acid and ketamine counteract depressive-like behavior induced by CORT administration

To test the ability of ascorbic acid and ketamine to elicit a fast antidepressant response, mice were subjected to chronic CORT administration. Fluoxetine was used as a negative control (Fig. 1A). The increased immobility time in the tail suspension test elicited by CORT ($p < 0.01$) was significantly diminished by a single administration of ascorbic acid or ketamine ($p < 0.01$; Fig. 1B), but not fluoxetine. The locomotor activity of mice was not altered by any treatment (Fig. 1C), ruling out nonspecific motor effects of CORT or treatments that could influence activity in the tail suspension test.

3.2. Ascorbic acid and ketamine, but not fluoxetine, rescued CORT-induced synaptic proteins impairment

To investigate the ability of ascorbic acid and ketamine to modulate synaptic proteins (PSD-95, GluA1, and synapsin) downstream to mTORC1 in the hippocampus, we performed the western blotting analysis (Fig. 1D and E). The administration of ascorbic acid *per se* significantly increased GluA1 immunocontent ($p < 0.01$) when compared with vehicle-treated mice, whereas ketamine *per se* increased only PSD-95 immunocontent ($p < 0.01$). Conversely, chronic CORT administration decreased PSD-95 ($p < 0.01$), GluA1 ($p < 0.01$), and synapsin ($p < 0.01$) immunocontent when compared to vehicle-treated mice. Ascorbic acid and ketamine completely prevented CORT-induced decrease on PSD-95 and synapsin immunocontent ($p < 0.01$), but only ketamine abolished CORT-induced GluA1 reduction ($p < 0.01$). Fluoxetine failed to affect any of the synaptic proteins investigated. Altogether, these results suggest that ascorbic acid and ketamine rescued hippocampal synaptic dysfunction induced by CORT.

3.3. Protective effects of ascorbic acid and ketamine, but not fluoxetine, on CORT-induced dendritic spine density and architecture impairments in hippocampal DG

To determine if ascorbic acid and ketamine are effective to rapidly increase dendritic spine density and remodeling in hippocampal DG, we next performed the morphological analysis in dorsal and ventral DG areas. The repeated administration of CORT significantly decreased the total spine density in the dorsal DG area ($p < 0.05$; Fig. 2A and B). Ascorbic acid *per se* significantly increased dendritic spine density when compared to the vehicle-treated group ($p < 0.05$) and abolished CORT-induced dendritic spine loss ($p < 0.05$), similar to ketamine ($p < 0.05$). However, fluoxetine administration did not cause significant effects on this parameter. Transitions in spine size and stability, from an immature

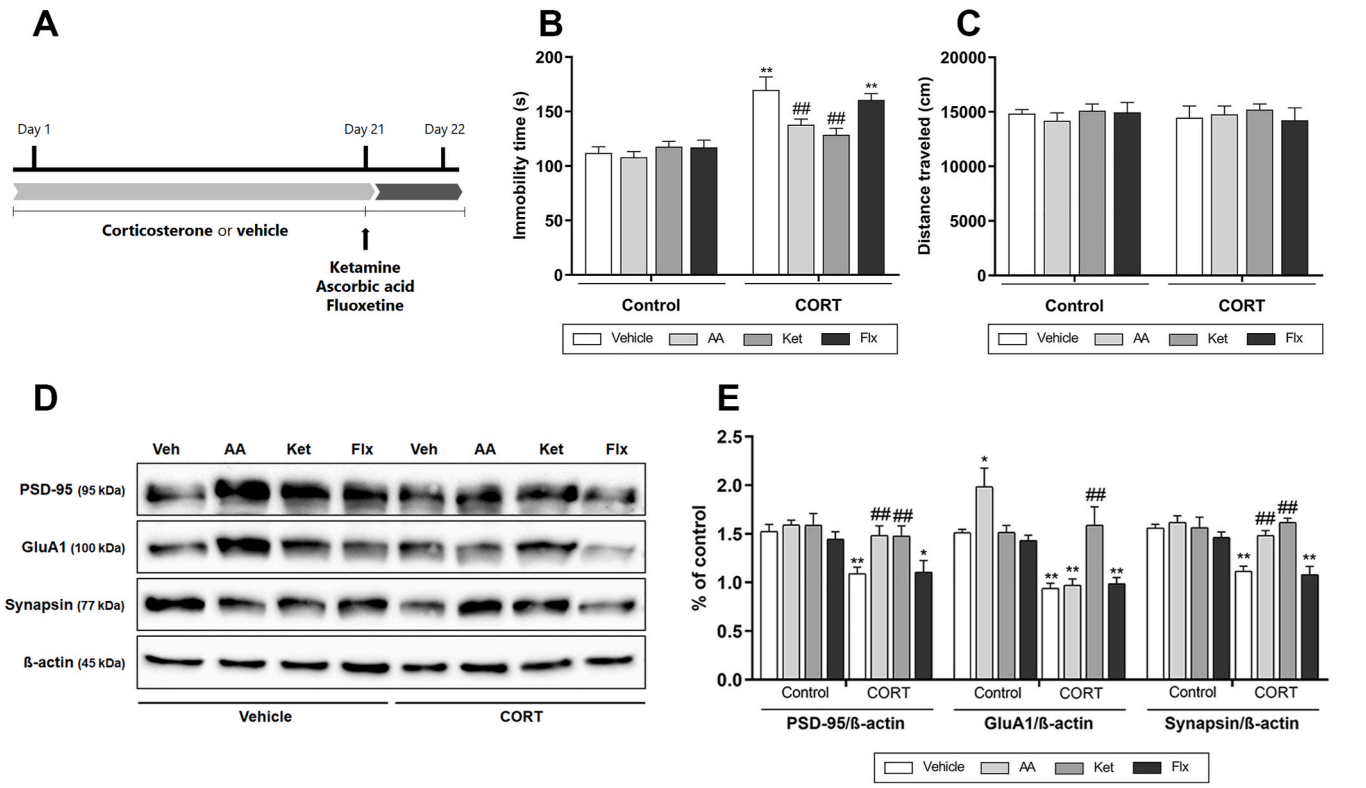


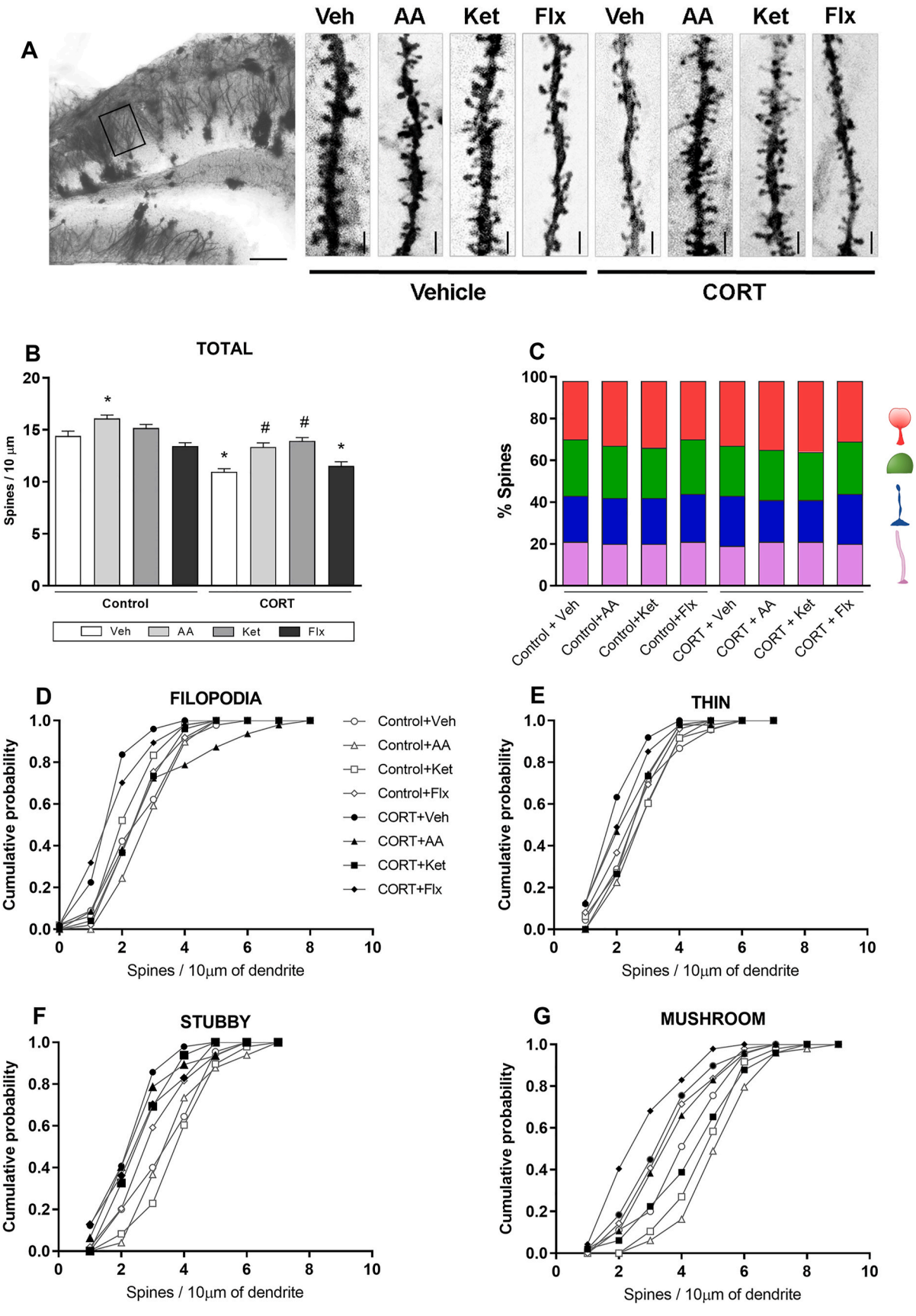
Fig. 1. A single administration of ascorbic acid (1 mg/Kg, p.o.), ketamine (1 mg/Kg, i.p.), but not fluoxetine (10 mg/kg, p.o.) reversed depressive-like behavior in the tail suspension test and protected against impairments on hippocampal synaptic proteins immunocontent induced by CORT (20 mg/Kg, p.o., 21 days). Experimental timeline (**A**). Immobility time in the tail suspension test (**B**). Locomotor activity (**C**). Representative western blots of PSD-95, GluA1, and synapsin (**D**). Quantification of PSD-95, GluA1, and synapsin immunocontent (**E**). Values are expressed as mean ± S.E.M (n = 6–8). *p < 0.05 and **p < 0.01 compared with the vehicle-treated control group; ##p < 0.01 as compared with the CORT-treated group (two-way ANOVA followed by Newman-Keuls *post-hoc* test). Veh: vehicle, AA: ascorbic acid, Ket: ketamine, Flx: fluoxetine.

unstable filopodium to a mature stable spine is an important aspect of maturation and strengthening of the synaptic contact and critical for rapid antidepressant-like effect [34]. Therefore, in a next step of the study the percentage of filopodium-, thin-, stubby- and mushroom-shaped spines following treatments in the dorsal DG area were determined (Fig. 2C). The analysis of cumulative probability distributions plotted for filopodia-shaped spine density revealed a significant decrease in CORT-treated mice ($p < 0.01$; Fig. 2D), and this alteration was reversed by both ascorbic acid and ketamine ($p < 0.01$). Furthermore, chronic administration of CORT significantly decreased thin-shaped spines density ($p < 0.01$; Fig. 2E), and ketamine treatment abolished this effect ($p < 0.01$). The density of stubby-shaped spines was also reduced in CORT-treated mice, regardless of treatment ($p < 0.05$; Fig. 2F). Regarding the density of mature dendritic spines (classified as mushroom-shaped spines), a significant increase in mice administered with ascorbic acid *per se*, as compared to control-vehicle mice was observed ($p < 0.05$; Fig. 2G). Ketamine treatment significantly increased mushroom-shaped spines density in CORT-treated mice ($p < 0.01$). No significant alterations on mushroom-shaped spines density were observed in CORT-vehicle or fluoxetine groups, as compared to the control group.

We next examined different dendritic segments in the ventral DG area for each experimental group (Fig. 3A). CORT treatment significantly decreased the total spine density in the ventral DG area when compared to the vehicle-treated group ($p < 0.05$; Fig. 3B). The administration of ascorbic acid and ketamine *per se* significantly increased the dendritic spine in this area as compared to the vehicle-treated group ($p < 0.01$). Both ascorbic acid and ketamine treatments ($p < 0.05$), but not fluoxetine, counteracted CORT-induced dendritic spine loss in the ventral DG area. Although fast-acting antidepressants increase dendritic spine density in the ventral hippocampus [17,35], it is unclear how these drugs affect dendritic spine subtypes and maturation under chronic stress conditions in this hippocampal area. Therefore, we next determined the percentage of filopodium-, thin-, stubby- and mushroom-shape spines following treatments in the ventral DG area (Fig. 3C). Cumulative probabilities plotted for filopodia-shaped spine density in the ventral DG area revealed a significant decrease in CORT-treated mice ($p < 0.01$; Fig. 3D), an alteration abolished by both ascorbic acid and ketamine ($p < 0.01$). Conversely, cumulative probability distributions for thin-shaped spines density demonstrated no significant effect of any treatment (Fig. 3E). Additionally, CORT administration significantly decreased stubby-shaped spines density as compared to the vehicle-treated group ($p < 0.01$; Fig. 3F), but this effect was not abolished by any treatment. Moreover, the administration of ascorbic acid and ketamine significantly increased the mushroom-shaped spine density in the ventral DG area (Fig. 3G) either in vehicle-treated mice ($p < 0.01$ and $p < 0.05$, respectively) and in CORT-treated mice ($p < 0.01$ for both). However, no changes in mushroom-shaped spines were observed in mice treated with CORT or fluoxetine.

4. Discussion

We demonstrate for the first time that a single administration of ascorbic acid counteracted the depressive-like behavior in the TST and most of the alterations on synaptic proteins and dendritic spine architecture in the hippocampus induced by the repeated CORT administration (a protocol that mimics chronic stress) in mice. The effects elicited by ascorbic acid were similar to those observed when mice were administered with ketamine. Conversely, the conventional



(caption on next page)

Fig. 2. Protective effects of ascorbic acid and ketamine, but not fluoxetine, on CORT-induced dendritic spine density and morphology impairments in the dorsal DG area. Panel **A** shows a representative image of the dorsal DG region analyzed (magnification of 10 × – scale bar = 200 μm), and representative images of different dendritic segments in the dorsal DG area for each experimental group (magnification of 100 × – scale bar = 2 μm). Effects of CORT (20 mg/Kg, p.o., for 21 days) treatment and a single administration of ascorbic acid (1 mg/Kg, p.o.), ketamine (1 mg/kg, i.p.) or fluoxetine (10 mg/Kg, p.o.) on total spine density in the dorsal DG area (**B**). The results are expressed as mean ± S.E.M (n = 6). *$p < 0.05$ compared with the vehicle-treated control group; #$p < 0.05$ as compared with the CORT-treated group (two-way ANOVA followed by Newman-Keuls *post-hoc* test). Percentage of different spine morphologies after CORT (20 mg/Kg, p.o., for 21 days) treatment and a single administration of ascorbic acid (1 mg/Kg, p.o.), ketamine (1 mg/Kg, i.p.) or fluoxetine (10 mg/Kg, p.o.) in the dorsal DG area (**C**). Cumulative probability of total spine density of filopodia (**D**), thin (**E**), stubby (**F**), mushroom (**G**) dendritic spines in the dorsal DG area (Kruskal-Wallis followed by Dunns *post-hoc* test; $p < 0.05$ compared with the vehicle-treated control group). Veh: vehicle, AA: ascorbic acid, Ket: ketamine, Flx: fluoxetine.

antidepressant fluoxetine did not afford any antidepressant or protective effects against CORT-induced alterations.

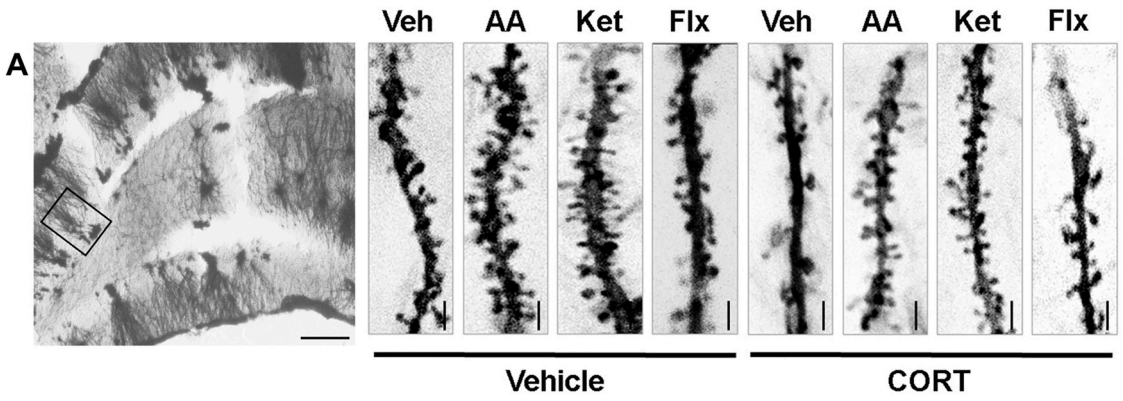
The conventional antidepressants have been used for the treatment of MDD since the 1960s, but a major flaw of the conventional therapy is related to their limited efficacy and long therapeutic time lag, highlighting the necessity for the development of new antidepressants with a faster onset and greater efficacy [36,37]. The most promising solution to overcome the limitations of conventional antidepressants was the discovery of ketamine, which has been shown to produce a fast antidepressant effect in depressive patients after a single administration, even in treatment-refractory patients [8,9,38,39]. Although ketamine has a great potential for the management of MDD, it has unwanted effects that limit its use and for this reason, there is a need for searching novel compounds with the ability to induce fast antidepressant effects [15]. In this study, we investigated whether ascorbic acid, a promising target for antidepressant responses, could rapidly elicit antidepressant and pro-synaptogenic effects in mice subjected to chronic administration of CORT. Several previous studies have indicated that this model produces behavioral and neurochemical alterations that resemble those observed in depressive individuals [4,40,41].

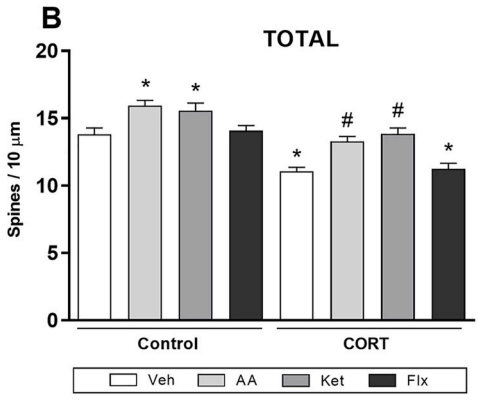
The first set of experiments provides evidence that a single administration of ascorbic acid effectively counteracted the depressive-like elicited by the chronic administration of corticosterone. This effect was not observed when mice received a single dose of fluoxetine, in agreement with previous studies [4,7]. Considering that one of the most critical limitations of the currently available antidepressants is that they produce a therapeutical response only after their chronic administration, ascorbic acid is as effective as ketamine to abolish the behavioral effect of CORT in the TST is a promising finding. Further clinical studies are welcome to investigate the ability of ascorbic acid as a strategy for the management of MDD. In line with our results, the acute administration of ascorbic acid produced an antidepressant-like effect in the tail suspension test mediated by mTORC1 stimulation [23] and in the novelty-suppressed feeding test [17], a behavioral test useful to screen drugs with fast-acting actions [18,19]. Moreover, a single combined administration with sub-effective doses of ketamine plus ascorbic acid was effective in counteracting the depressive-like behavior induced by chronic unpredictable stress [42].

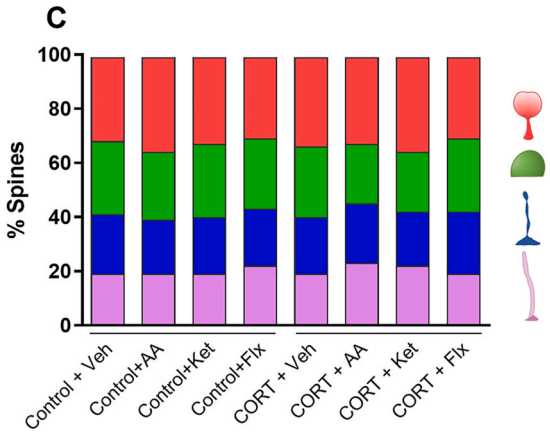
Considering that fast-acting antidepressants like ketamine have been shown to increase synaptic protein expression, such as PSD-95, GluA1, and synapsin immunocontent [10,43,44], and deficits in these synaptic proteins were reported in the hippocampus and prefrontal cortex of individuals with MDD [45–48], in the next step, we investigated the effects of ascorbic acid on pro-synaptogenic proteins in the hippocampus. Here, we show that ascorbic acid increased hippocampal GluA1 immunocontent. Moreover, CORT administration reduced the immunocontent of all the synaptic proteins evaluated, in agreement with studies that reported that chronic stress or corticosterone administration reduces the immunocontent of these proteins in the prefrontal cortex and hippocampus [4,11,49,50]. Noteworthy, ascorbic acid and ketamine counteracted CORT-induced decrease in PSD-95 and synapsin, while only ketamine abolished the reduction on GluA1 caused by CORT. Conversely, fluoxetine did not present any effect, as previously reported in the prefrontal cortex of rats [10]. In accordance with our results, ascorbic acid ameliorated the lead-induced damage in Purkinje cells through the increase in PSD-95 and synaptophysin contents in the cerebellum [51]. Importantly, ascorbic acid treatment has been associated with an increase on synapsin I and PSD-95 expression supporting the formation of new synapses [52]. These data suggest the ability of ascorbic acid, in a way similar to ketamine, to modulate the levels of proteins critical for the formation, maturation, and function of new spine synapses. The absence of effect of fluoxetine in any synaptic marker concurs to the hypothesis that ascorbic acid has an advantage over this conventional antidepressant due to its ability to elicit neurochemical alterations similar to ketamine. Considering that: a) increased synthesis of synaptic proteins and new synapse formation have been shown to be dependent on mTORC1 upregulation [11,53]; b) the previous demonstration of the role of mTORC1 stimulation for the antidepressant-like effect of ascorbic acid [23], our results suggest that mTORC1-dependent mechanisms may underlie the effects of ascorbic acid.

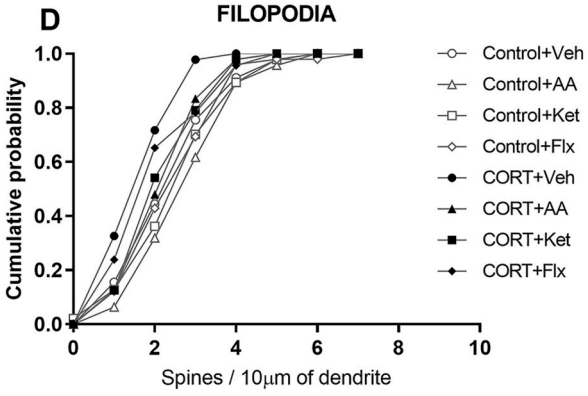
We next evaluated the ability of ascorbic acid to counteract the effects of CORT administration on hippocampal dendritic spine density. Importantly, clinical brain imaging and *postmortem* studies have demonstrated structural and functional abnormalities in limbic and cortical structures in MDD patients, suggesting that spine synapse connectivity is altered in this medical condition [54]. Likewise, structural and functional synaptic deficits have been shown in preclinical studies using rodent models of stress, an effect associated with depressive-like behavior [11,55]. Here we showed that CORT-treated mice presented a reduction in hippocampal dendritic spine density accompanied by a depressive-like behavior, in agreement with previous studies [55,56]. More importantly, we showed herein that a single administration of ascorbic abolished CORT-induced dendritic spine loss in the dorsal and ventral DG areas, similar to the result obtained when ketamine was acutely administered. In addition, we observed that ascorbic acid *per se* significantly increased the total density of dendritic spines. These results agree with a previous study that reported ascorbic acid's ability to increase dendritic spines density 1 h after its administration in the ventral DG of mice, in a way similar to ketamine [17], but it is the first evidence regarding the ability to ascorbic acid to counteract the synaptic impairment in mice subjected to a pharmacological model of stress. Of note, it has been reported that ketamine may induce spinogenesis and synaptogenesis in hippocampal DG [57], and these morphological alterations are likely implicated in its antidepressant effect. Fluoxetine administration did not cause any morphological alterations in dendritic spines in hippocampal DG, in agreement with the fact that it failed to afford an antidepressant-like effect in CORT-treated mice. Thus, given that ascorbic acid, in a similar fashion to ketamine, rapidly stimulates the dendritic spines formation in the hippocampal DG and considering the essential role of synaptic remodeling for the effects of rapid antidepressants [53,54], it is tempting to speculate that this effect could be associated with its rapid antidepressant-like response.

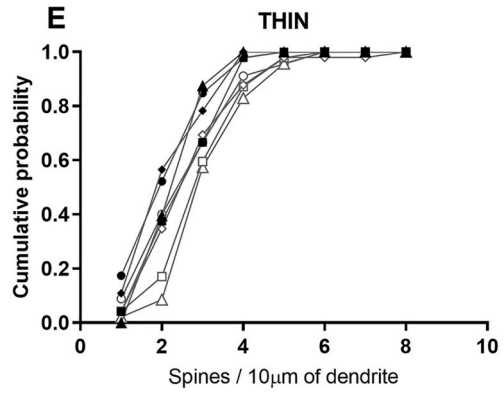
In the next set of experiments, we found that CORT administration induced a thin spine loss in the dorsal DG area as well as a stubby and filopodia spine loss in the dorsal and ventral DG, although it did not affect mushroom-shaped spines in both dorsal and ventral DG. In agreement with our results, chronic CORT exposure decreased thin and stubby, but not mushroom, spine density in the CA1, CA3, and DG areas, suggesting that mushroom spine is more stable and resistant to chronic CORT exposure [55]. Importantly, we provide the first evidence that a single administration of ascorbic acid increases mushroom and filopodia

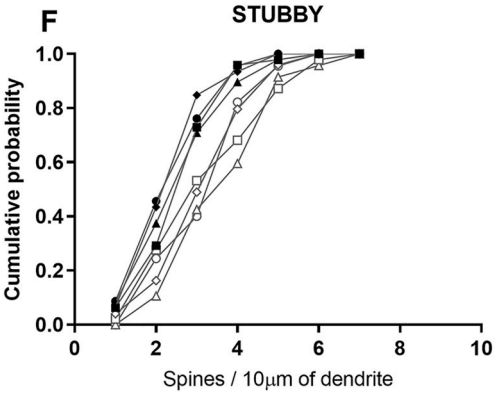


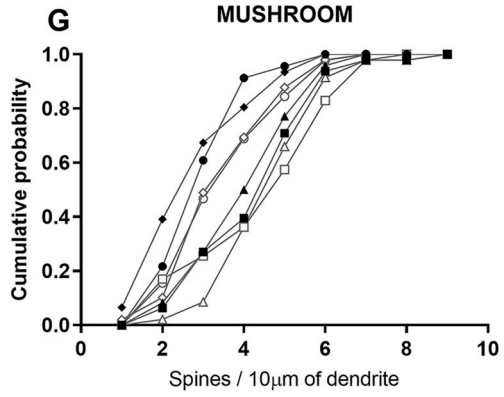












(caption on next page)

Fig. 3. Protective effects of ascorbic acid and ketamine, but not fluoxetine, on CORT-induced dendritic spine density and morphology impairments in the ventral DG area. Panel **A** shows a representative image of the ventral DG region analyzed (magnification of 10 × – scale bar = 200 μm), and representative images of different dendritic segments in the dorsal DG area for each experimental group (magnification of 100 × – scale bar = 2 μm). Effects of CORT (20 mg/Kg, p.o., for 21 days) treatment and a single administration of ascorbic acid (1 mg/Kg, p.o.), ketamine (1 mg/Kg, i.p.) or fluoxetine (10 mg/Kg, p.o.) on total spine density in the ventral DG area (**B**). The results are expressed as mean ± S.E.M (n = 6). *p < 0.05 compared with the vehicle-treated control group; #p < 0.05 as compared with the CORT-treated group (two-way ANOVA followed by Newman-Keuls *post-hoc* test). Percentage of different spines morphologies after CORT (20 mg/Kg, 21 days) treatment and a single administration of ascorbic acid (1 mg/Kg, p.o.), ketamine (1 mg/Kg, i.p.) or fluoxetine (10 mg/Kg, p.o.) in the ventral DG area (**C**). Cumulative probability of total spine density of filopodia (**D**), thin (**E**), stubby (**F**), mushroom (**G**) dendritic spines in the ventral DG area (Kruskal-Wallis followed by Dunns *post-hoc* test; p < 0.05 compared with the vehicle-treated control group). Veh: vehicle, AA: ascorbic acid, Ket: ketamine, Flx: fluoxetine.

spines in the dorsal and ventral DG areas in CORT-treated mice, in a way similar to ketamine. Furthermore, ascorbic acid increased mushroom-shaped spines in the dorsal DG area in vehicle-treated mice. These results are in line with ascorbic acid's ability to promote mushroom and filopodia spines formation in mice subjected to the novelty-suppressed feeding test [17]. Remarkably, alterations in mushroom spine densities may represent a target for rapid-acting antidepressant drugs since the maturation of the newly formed dendrite spine correlates with the behavioral improvement after antidepressant treatment [54]. Therefore, one may suppose that the remodeling in dendrite spine architecture in the hippocampal DG could be associated with the antidepressant-like effects of ascorbic acid, in a way similar to ketamine.

5. Conclusion

Collectively, our findings indicate that ascorbic acid is effective to reverse the depressive-like behavior and most of the neurochemical and morphological synaptic alterations induced by CORT. The beneficial effects of ascorbic acid are similar to those exerted by ketamine, reinforcing the hypothesis that ascorbic acid may be further investigated as a useful approach for the management of depression [15]. More importantly, the advantage of the use of ascorbic acid for the management of MDD is related to its safety even upon chronic use and its ability to exert antidepressant-like effects at low doses [16]. Although it remains questionable if the new dendrite spine and maturation associated with ascorbic acid treatment are indicative of functional synapses, our results raise the possibility that antidepressant-like effect mediated by ascorbic acid could be associated with the modulation of synaptic plasticity in the hippocampus. Further studies are also required to determine the functional importance of synaptic alterations mediated by ascorbic acid for its antidepressant-like effect. The promising results showed herein regarding the ascorbic acid's ability to elicit fast behavioral and synaptic alterations may have a therapeutic interest.

Funding

This study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, #449436/2014–4, #310113/2017–2), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). ALSR, JB, and PSB are recipients of CNPq Research Productivity Fellowship.

Ethics statement

All procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and the protocols were approved by the Institutional Ethics Committee.

Author contributions

Ana Lúcia S. Rodrigues: Conceptualization, Funding acquisition, Project administration, Resources, and Supervision. Daiane B. Fraga: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, and Writing the manuscript draft. Anderson Camargo: Data curation, Formal analysis, Investigation, Methodology, and Writing the manuscript draft. Gislaine Olescowicz: Data curation, Formal analysis, Investigation, and Methodology. Dayane Azevedo Padilha: Investigation, and Methodology. Francielle Mina: Investigation, and Methodology. Josiane Budni: Investigation, Methodology, and Writing the manuscript draft. Patricia S. Brocardo: Conceptualization, Investigation, Methodology, and Writing the manuscript draft.

Declaration of competing interest

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

Acknowledgments

The authors thank funding agencies CNPq and CAPES by the financial support, and the Laboratório Multiusuário de Estudos em Biologia (LAMEB) by technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbi.2021.109476.

References

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

[2] C.M. Pariante, H.A. Alhaj, V.E. Arulnathan, P. Gallagher, A. Hanson, E. Massey, R. H. McAllister-Williams, Central glucocorticoid receptor-mediated effects of the antidepressant, citalopram, in humans: a study using EEG and cognitive testing, Psychoneuroendocrinology 37 (2012) 618–628, https://doi.org/10.1016/j.psyneuen.2011.08.011.

[3] Y. Ago, K. Yano, R. Araki, N. Hiramatsu, Y. Kita, T. Kawasaki, H. Onoe, S. Chaki, A. Nakazato, H. Hashimoto, A. Baba, K. Takuma, T. Matsuda, Metabotropic glutamate 2/3 receptor antagonists improve behavioral and prefrontal dopaminergic alterations in the chronic corticosterone-induced depression model in mice, Neuropharmacology 65 (2013) 29–38, https://doi.org/10.1016/j.neuropharm.2012.09.008.

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

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

[6] H. Koike, M. Iijima, S. Chaki, Effects of ketamine and LY341495 on the depressive-like behavior of repeated corticosterone-injected rats, Pharmacol. Biochem. Behav. 107 (2013) 20–23, https://doi.org/10.1016/j.pbb.2013.03.017.

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

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

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

[10] N. Li, B. Lee, R.J. Liu, M. Banasr, J.M. Dwyer, M. Iwata, X.Y. Li, G. Aghajanian, R. S. Duman, mTOR-dependent synapse formation underlies the rapid antidepressant

effects of NMDA antagonists, Science 329 (2010) 959–964, https://doi.org/10.1126/science.1190287.
[11] N. Li, R.J. Liu, J.M. Dwyer, M. Banasr, B. Lee, H. Son, X.Y. Li, G. Aghajanian, R. S. Duman, Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure, Biol. Psychiatr. 69 (2011) 754–761, https://doi.org/10.1016/j.biopsych.2010.12.015.
[12] C.G. Abdallah, T.G. Adams, B. Kelmendi, I. Esterlis, G. Sanacora, J.H. Krystal, Ketamine's mechanism of action: a path to rapid-acting antidepressants, Depress. Anxiety 33 (2016) 689–697, https://doi.org/10.1002/da.22501.
[13] A.E. Lepack, M. Fuchikami, J.M. Dwyer, M. Banasr, R.S. Duman, BDNF release is required for the behavioral actions of ketamine, Int. J. Neuropsychopharmacol. 18 (2014) 1–6, https://doi.org/10.1093/ijnp/pyu033.
[14] R.S. Duman, B. Voleti, Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents, Trends Neurosci. 35 (2012) 47–56, https://doi.org/10.1016/j.tins.2011.11.004.
[15] A. Camargo, A.L.S. Rodrigues, Novel targets for fast antidepressant responses: possible role of endogenous neuromodulators, 247054701985808, Chronic Stress 3 (2019), https://doi.org/10.1177/2470547019858083.
[16] M. Moretti, D.B. Fraga, A.L.S. Rodrigues, Ascorbic acid to manage psychiatric disorders, CNS Drugs 31 (2017) 571–583, https://doi.org/10.1007/s40263-017-0446-8.
[17] D.B. Fraga, A.P. Costa, G. Olescowicz, A. Camargo, F.L. Pazini, A.E. Freitas, M. Moretti, P.S. Brocardo, A.L.S. Rodrigues, Ascorbic acid presents rapid behavioral and hippocampal synaptic plasticity effects, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 96 (2020) 109757, https://doi.org/10.1016/j.pnpbp.2019.109757.
[18] F.L. Pazini, J.M. Rosa, A. Camargo, D.B. Fraga, M. Moretti, A. Siteneski, A.L. S. Rodrigues, mTORC1-dependent signaling pathway underlies the rapid effect of creatine and ketamine in the novelty-suppressed feeding test, Chem. Biol. Interact. 332 (2020) 109281, https://doi.org/10.1016/j.cbi.2020.109281.
[19] A. Camargo, F.L. Pazini, J.M. Rosa, I.A.V. Wolin, M. Moretti, P.B. Rosa, V.B. Neis, A.L.S. Rodrigues, Augmentation effect of ketamine by guanosine in the novelty-suppressed feeding test is dependent on mTOR signaling pathway, J. Psychiatr. Res. 115 (2019), https://doi.org/10.1016/j.jpsychires.2019.05.017.
[20] World Health Organization, Depression and Other Common Mental Disorders: Global Health Estimates, World Health Organization, 2017, pp. 1–24. CC.BY-NC-SA.3.0.IGO.
[21] S. Yoshimura, S. Sakamoto, H. Kudo, S. Sassa, A. Kumai, R. Okamoto, Sex-differences in adrenocortical responsiveness during development in rats, Steroids 68 (2003) 439–445, https://doi.org/10.1016/S0039-128X(03)00045-X.
[22] M. Moretti, A.E. de Freitas, J. Budni, S.C.P. Fernandes, G. de O. Balen, A.L. S. Rodrigues, Involvement of nitric oxide-cGMP pathway in the antidepressant-like effect of ascorbic acid in the tail suspension test, Behav. Brain Res. 225 (2011) 328–333, https://doi.org/10.1016/j.bbr.2011.07.024.
[23] M. Moretti, J. Budni, A.E. Freitas, P.B. Rosa, A.L.S. Rodrigues, Antidepressant-like effect of ascorbic acid is associated with the modulation of mammalian target of rapamycin pathway, J. Psychiatr. Res. 48 (2014) 16–24, https://doi.org/10.1016/j.jpsychires.2013.10.014.
[24] L. Steru, R. Chermat, B. Thierry, P. Simon, The tail suspension test: a new method for screening antidepressants in mice, Psychopharmacology 85 (1985) 367–370, https://doi.org/10.1007/BF00428203.
[25] F.D. Pacheco, L. Canever, G. Antunes Mastella, P. Gomes Wessler, A. Kunz Godoi, I. Hubbe, A. da Costa Afonso, D. Celso, J. Quevedo, A. Ioppi Zugno, Effects of ketamine on prepubertal Wistar rats: implications on behavioral parameters for Childhood-Onset Schizophrenia, Int. J. Dev. Neurosci. 79 (2019) 49–53, https://doi.org/10.1016/j.ijdevneu.2019.10.006.
[26] A. Camargo, A.P. Dalmagro, A.L.B. Zeni, A.L.S. Rodrigues, Guanosine potentiates the antidepressant-like effect of subthreshold doses of ketamine: possible role of pro-synaptogenic signaling pathway, J. Affect. Disord. 271 (2020) 100–108, https://doi.org/10.1016/j.jad.2020.03.186.
[27] G.L. Peterson, A simplification of the protein assay method of Lowry et al. which is more generally applicable, Anal. Biochem. 83 (1977) 346–356, https://doi.org/10.1016/0003-2697(77)90043-4.
[28] A. Freitas, I. Heinrich, T. Moura, D. Fraga, A. Costa, D. Azevedo, P. Brocardo, M. Kaster, R. Leal, A.L.S. Rodrigues, Agmatine potentiates antidepressant and synaptic actions of ketamine: effects on dendritic arbors and spines architecture and Akt/S6 kinase signaling, Exp. Neurol. 333 (2020) 113398, https://doi.org/10.1016/j.expneurol.2020.113398.
[29] R. Gibb, B. Kolb, A method for vibratome sectioning of Golgi-Cox stained whole rat brain, J. Neurosci. Methods 79 (1998) 1–4, https://doi.org/10.1016/S0165-0270(97)00163-5.
[30] T.S. Kannangara, C.A. Bostrom, A. Ratzlaff, L. Thompson, R.M. Cater, J. Gil-Mohapel, B.R. Christie, Deletion of the NMDA receptor GluN2A subunit significantly decreases dendritic growth in maturing dentate granule neurons, PloS One 9 (2014) 1–10, https://doi.org/10.1371/journal.pone.0103155.
[31] G. Calfa, C.A. Chapleau, S. Campbell, T. Inoue, S.J. Morse, F.D. Lubin, L. Pozzo-Miller, HDAC activity is required for BDNF to increase quantal neurotransmitter release and dendritic spine density in CA1 pyramidal neurons, Hippocampus 22 (2012) 1493–1500, https://doi.org/10.1002/hipo.20990.
[32] I.Y.Y. Koh, W.B. Lindquist, K. Zito, E.A. Nimchinsky, K. Svoboda, An image analysis algorithm for dendritic spines, Neural Comput. 14 (2002) 1283–1310, https://doi.org/10.1162/089976602753712945.
[33] M. Giachero, G.D. Calfa, V.A. Molina, Hippocampal dendritic spines remodeling and fear memory are modulated by GABAergic signaling within the basolateral amygdala complex, Hippocampus 25 (2015) 545–555, https://doi.org/10.1002/hipo.22409.
[34] K.P. Berry, E. Nedivi, Spine dynamics: are they all the same? Neuron 96 (2017) 43–55, https://doi.org/10.1016/j.neuron.2017.08.008.
[35] J.D. Jett, A.M. Boley, M. Girotti, A. Shah, D.J. Lodge, D.A. Morilak, Antidepressant-like cognitive and behavioral effects of acute ketamine administration associated with plasticity in the ventral hippocampus to medial prefrontal cortex pathway, Psychopharmacology 232 (2015) 3123–3133, https://doi.org/10.1007/s00213-015-3957-3.
[36] M.P. Kaster, M. Moretti, M.P. Cunha, A.L.S. Rodrigues, Novel approaches for the management of depressive disorders, Eur. J. Pharmacol. 771 (2016) 236–240, https://doi.org/10.1016/j.ejphar.2015.12.029.
[37] G.I. Papakostas, D.F. Ionescu, Towards new mechanisms: an update on therapeutics for treatment-resistant major depressive disorder, Mol. Psychiatr. 20 (2015) 1142–1150, https://doi.org/10.1038/mp.2015.92.
[38] N. DiazGranados, L. Ibrahim, N. Brutsche, R. Ameli, L. Henter, D. Luckenbaugh, R. Machado-Vieira, C.A. Zarate, Rapid resolution of suicidal ideation after a single infusion of an NMDA antagonist in patients with treatment-resistant major depressive disorder, J. Clin. Psychiatr. 71 (2010) 1605–1611, https://doi.org/10.4088/JCP.09m05327blu.Rapid.
[39] R.B. Price, M.K. Nock, D.S. Charney, S.J. Mathew, Effects of intravenous ketamine on explicit and implicit measures of suicidality in treatment-resistant depression, Biol. Psychiatr. 66 (2009) 522–526, https://doi.org/10.1016/j.biopsych.2009.04.029.
[40] A.E. Freitas, J. Egea, I. Buendia, V. Gómez-Rangel, E. Parada, E. Navarro, A. I. Casas, A. Wojnicz, J.A. Ortiz, A. Cuadrado, A. Ruiz-Nuño, A.L.S. Rodrigues, M. G. Lopez, Agmatine, by improving neuroplasticity markers and inducing Nrf2, prevents corticosterone-induced depressive-like behavior in mice, Mol. Neurobiol. 53 (2016) 3030–3045, https://doi.org/10.1007/s12035-015-9182-6.
[41] P.B. Rosa, C.M. Ribeiro, L.E.B. Bettio, A. Colla, V. Lieberknecht, M. Moretti, A.L. S. Rodrigues, Folic acid prevents depressive-like behavior induced by chronic corticosterone treatment in mice, Pharmacol. Biochem. Behav. 127 (2014) 1–6, https://doi.org/10.1016/j.pbb.2014.10.003.
[42] M. Moretti, I. Werle, P.B. da Rosa, V.B. Neis, N. Platt, S.V.S. Souza, A.L. S. Rodrigues, A single coadministration of subeffective doses of ascorbic acid and ketamine reverses the depressive-like behavior induced by chronic unpredictable stress in mice, Pharmacol. Biochem. Behav. 187 (2019) 172800, https://doi.org/10.1016/j.pbb.2019.172800.
[43] M.V. Fogaça, K. Fukumoto, T. Franklin, R.J. Liu, C.H. Duman, O.V. Vitolo, R. S. Duman, N-Methyl-D-aspartate receptor antagonist *d*-methadone produces rapid, mTORC1-dependent antidepressant effects, Neuropsychopharmacology 44 (2019) 2230–2238, https://doi.org/10.1038/s41386-019-0501-x.
[44] V.B. Neis, M. Moretti, P.B. Rosa, Y. de O. Dalsenter, I. Werle, N. Platt, F. N. Kaufmann, A.F. Rosado, M.H. Besen, A.L.S. Rodrigues, The involvement of PI3K/Akt/mTOR/GSK3β signaling pathways in the antidepressant-like effect of AZD6765, Pharmacol. Biochem. Behav. 198 (2020) 173020, https://doi.org/10.1016/j.pbb.2020.173020.
[45] V. Duric, M. Banasr, C.A. Stockmeier, A.A. Simen, S.S. Newton, J.C. Overholser, G. J. Jurjus, L. Dieter, R.S. Duman, Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects, Int. J. Neuropsychopharmacol. 16 (2013) 69–82, https://doi.org/10.1017/S1461145712000016.
[46] A.M. Feyissa, A. Chandran, C.A. Stockmeier, B. Karolewicz, Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 33 (2009) 70–75, https://doi.org/10.1016/j.pnpbp.2008.10.005.
[47] H.J. Kang, B. Voleti, T. Hajszan, G. Rajkowska, C.A. Stockmeier, P. Licznerski, A. Lepack, M.S. Majik, L.S. Jeong, M. Banasr, H. Son, R.S. Duman, Decreased expression of synapse-related genes and loss of synapses in major depressive disorder, Nat. Med. 18 (2012) 1413–1417, https://doi.org/10.1038/nm.2886.
[48] A. Rafalo-Ulinska, J. Piotrowska, A. Kryczyk, M. Sowa-kucma, P. Misztak, G. Rajkowska, C.A. Stockmeier, W. Datka, G. Nowak, B. Szewczyk, Zinc Transporters Protein Level in Postmortem Brain of Depressed Subjects and Suicide Victims, vol. 83, 2016, pp. 220–229, https://doi.org/10.1016/j.jpsychires.2016.09.008.
[49] A.H. Iyo, A.M. Feyissa, A. Chandran, M.C. Austin, S. Regunathan, B. Karolewicz, Chronic corticosterone administration down-regulates metabotropic glutamate receptor 5 protein expression in the rat hippocampus, Neuroscience 169 (2010) 1567–1574, https://doi.org/10.1016/j.neuroscience.2010.06.023.
[50] A. Camargo, A.P. Dalmagro, M.M. de Souza, A.L.B. Zeni, A.L.S. Rodrigues, Ketamine, but not guanosine, as a prophylactic agent against corticosterone-induced depressive-like behavior: possible role of long-lasting pro-synaptogenic signaling pathway, Exp. Neurol. 334 (2020) 113459, https://doi.org/10.1016/j.expneurol.2020.113459.
[51] S.M. Nam, I.S. Cho, J.S. Seo, T.H. Go, J.H. Kim, S.S. Nahm, B.J. Chang, J.H. Lee, Ascorbic acid attenuates lead-induced alterations in the synapses in the developing rat cerebellum, Biol. Trace Elem. Res. 187 (2019) 142–150, https://doi.org/10.1007/s12011-018-1354-6.
[52] M. Cansev, M. Turkyilmaz, J.W.C. Sijben, C. Sevinc, L.M. Broersen, N. Van Wijk, Synaptic membrane synthesis in rats depends on dietary sufficiency of vitamin C, vitamin E, and selenium: relevance for alzheimer's disease, J. Alzheim. Dis. 59 (2017) 301–311, https://doi.org/10.3233/JAD-170081.
[53] N. Li, B. Lee, R.-J. Liu, M. Banasr, J.M. Dwyer, M. Iwata, X.-Y. Li, G. Aghajanian, R. S. Duman, mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists, Science 329 (2010) 959–965.
[54] C.H. Duman, R.S. Duman, Spine synapse remodeling in the pathophysiology and treatment of depression, Neurosci. Lett. 601 (2014) 20–29, https://doi.org/10.1016/j.neulet.2015.01.022.

[55] G. Wang, Y. Cheng, M. Gong, B. Liang, M. Zhang, Y. Chen, C. Zhang, X. Yuan, J. Xu, Systematic correlation between spine plasticity and the anxiety/depression-like phenotype induced by corticosterone in mice, Neuroreport 24 (2013) 682–687, https://doi.org/10.1097/WNR.0b013e32836384db.

[56] J.C. Morales-Medina, F. Sanchez, G. Flores, Y. Dumont, R. Quirion, Morphological reorganization after repeated corticosterone administration in the hippocampus, nucleus accumbens and amygdala in the rat, J. Chem. Neuroanat. 38 (2009) 266–272, https://doi.org/10.1016/j.jchemneu.2009.05.009.

[57] C. Dong, J.C. Zhang, W. Yao, Q. Ren, M. Ma, C. Yang, S. Chaki, K. Hashimoto, Rapid and sustained antidepressant action of the mGlu2/3 receptor antagonist MGS0039 in the social defeat stress model: comparison with ketamine, Int. J. Neuropsychopharmacol. 20 (2017) 228–236, https://doi.org/10.1093/ijnp/pyw089.