Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
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Cite this: *Food Funct.*, 2025, **16**, 7283

Creatine differently prevents chronic colitis-induced motor deficits, anxiety and depressive behaviors, neuroinflammation, and microglial activation in male and female rats

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Creatine has been reported to exhibit neuroprotective and anti-inflammatory properties and alleviate symptoms of affective disorders. In this study, we used a chronic colitis model in male and female rats, using a dextran sulfate sodium treatment that mimics the remitting–relapsing phases of human ulcerative colitis. The results showed that in rats with colitis, oral creatine supplementation reduced the severity of colitis symptoms and prevented motor deficits, anxiety, and depression-like behaviors. These effects of creatine were consistent throughout the development of chronic colitis, remaining independent of remitting–relapsing periods. In response to creatine supplementation, colon and brain creatine levels increased in rats with chronic colitis without sex differences. Furthermore, at the end of the treatment, when chronic colitis was established, creatine ameliorated injury of colonic surface epithelial and prevented chronic colitis-associated neuroinflammation, evidenced by a decrease in the pro-inflammatory cytokines IL-1β, IL-6, and TNF mRNA, as well as microglial activation in the prefrontal and motor cortices of rats of both sexes. In general, creatine supplementation was more effective in females. In conclusion, creatine supplementation had sex-specific effects and could serve as a nutritional strategy to reduce the severity of colitis and its associated motor and mood disturbances, neuroinflammation, and microglial activation.

Received 3rd April 2025,
Accepted 18th August 2025

DOI: 10.1039/d5fo01620g

rsc.li/food-function

Introduction

Ulcerative colitis (UC), a type of inflammatory bowel disease, is marked by chronic inflammation of the intestinal tract with phases of relapse and remission, causing severe symptoms such as diarrhea, rectal bleeding, and weight loss.[1] Chronicity and recurrence of this disease reduce the quality of life of these patients and can lead to intestinal and extraintestinal manifestations such as neuropsychiatric disorders, depression, and anxiety that exhibit a higher incidence in women.[2–4] There are some reports showing that the symptoms of these disorders intensify during active disease phases, and others that they persist even during remission periods.[3,5] Previous studies, conducted by our research group and others, reported that rodents with experimental colitis exhibited, in addition to intestinal symptoms, anxiety- and depressive-like behaviors, and motor deficits that could be related to colitis-induced neuroinflammation in different regions of the brain.[6–9] The strong link between colitis and neuroinflammation established through the gut–brain axis is recognized as an important contributor to the pathogenesis and development of neuropsychiatric disorders.[6] In a rat model of chronic colitis, we have recently shown mood and motor disturbances throughout the disease progression, regardless of disease activity but influenced by sex, and that at least in part could be due to colitis-induced neuroinflammation in the motor and prefrontal cortices.[9] This neuroinflammation may be produced by activated microglia that release pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor (TNF).[6] These pro-inflammatory cytokines can then modify neuronal functions resulting in behavioral abnormalities. Therefore, neuroinflammation, potentially driven by activated microglia, is considered one of the mechanisms underlying neuropsychiatric disorders.[10] Furthermore, inhibition of microglia appears to mitigate neuroinflammation, emotional, and behavioral disorders.[11,12] Few studies, including ours conducted in rat models of colitis, linked high expression of pro-inflammatory cytokines with behavioral alterations.[8,9,12–14] Like other authors, we suggest that colitis-induced neuroin-

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flammation in specific brain regions induced by colitis may result from microglial activation in these regions.[6,15]

Conventional treatments for UC, such as aminosalicylates and corticosteroids in mild to moderate disease, or immunosuppressants and biological drugs in moderate to severe disease, alleviate symptoms, neither prevent disease progression nor mitigate psychiatric comorbidities. Therefore, scientists are exploring new therapeutic strategies, including nutritional supplements as adjunctive therapy. Few studies have investigated nutritional intervention strategies to promote and maintain pathological remission, improve clinical outcomes, avoid extraintestinal manifestations, such as neuropsychiatric symptoms of UC, and promote mental health.[16]

Creatine (Cr) (methylguanidine-acetic acid) is an amino acid derivative, one of the most popular nutritional supplements among athletes because it increases muscle mass and strength. Creatine can be obtained from the diet or by endogenous synthesis in a two-step pathway in the kidneys and liver through the enzymes amidinotransferase (AGAT) and guanidinoacetate *N*-methyltransferase (GAMT).[17] Other tissues can also synthesize it, although in a more limited manner, including the brain, which is one of the main users of creatine.[17] Creatine reaches the bloodstream after intestinal absorption through the apical creatine transporter (CrT), first described by our group,[18] and can cross the brain barriers and be taken up by neurons and glial cells.[19] Creatine can improve brain energy metabolism, cell resilience and survival, and modulate neurotransmitter systems. It also has antioxidant, antiapoptotic, and anti-inflammatory properties.[17] The combination of these effects could explain its neuroprotective effect and suggest that it could alleviate mood disorders, since maintaining energy balance in the brain is crucial for mental health and that alteration of brain bioenergetics and neuroinflammation may contribute to the symptoms of these disorders.[10,20] In fact, preclinical and clinical research indicates that creatine supplementation reduces depression symptoms, and although there is less research on anxiety, some results suggest that it may also have anxiolytic effects.[20–22] The beneficial effect of creatine may be to restore brain energy levels, influence the functioning of linked neurotransmitter systems that regulate mood, or exert anti-inflammatory effects on the brain. In addition, creatine supplementation appears to have sex-specific effects on mood. Some studies in animal models showed that creatine yielded more improvements in symptoms of depression in females.[20,22] Furthermore, human trials suggest that creatine could be more effective in depression in women, highlighting the importance of considering sex in studies of mood disorders.[20,22–24]

In addition to the brain, skeletal muscle is another tissue with high energy demand in which creatine plays a critical role in the recycling of ATP. Creatine supplementation can provide benefits to muscle performance even without exercise and, in general, improve motor activity.[25] Evidence indicates that creatine also has beneficial effects in patients with muscular dystrophies.[24] Additionally, in animal models of neurodegenerative diseases that present locomotion impairments, creatine supplementation slowed its progression and improved motor performance.[20,24,26,27]

In this work, we used a model of chronic colitis induced by dextran sulfate sodium (DSS) in male and female rats, as previously.[9] Our objective was to investigate whether creatine supplementation mitigates or prevents chronic colitis-induced behavior impairments in male and female rats during the relapsing and remitting phases of the disease. Specifically, we evaluated the effects of creatine on: (i) motor deficits, (ii) anxiety and depression-like behaviors, (iii) elevated expression levels of pro-inflammatory cytokines in the prefrontal and motor cortices, and (iv) microglial activation in the prefrontal and motor cortices. Furthermore, we examine whether these effects varied in a sex-dependent manner.

To our knowledge, no studies have investigated the use of creatine supplementation to alleviate or prevent chronic colitis-induced motor and mood impairments throughout its development and while examining the presence of potential sex differences.

Materials and methods

Chemicals

Dextran sulfate sodium (DSS) (M_W = 40 kDa) was purchased from PanReac AppliChem (Spain). Creatine monohydrate was obtained from Best Medical Diet S. L. (Sevilla, Spain). Paraformaldehyde (PFA), salts for the preparation of phosphate-buffered saline (PBS), sodium citrate, Triton X-100, bovine serum albumin (BSA), and paraffin were purchased from PanReac AppliChem (Spain). Normal goat serum was purchased from Chemicon International, Inc. (California, USA), ionized calcium binding adapter molecule 1 (Iba1) antibody (ab178846) was obtained from AbCam (Cambridge, UK) and Alexa Fluor-546 secondary antibody from Thermo Fisher Scientific (Massachusetts, USA).

Animals

Male and female Wistar rats (45 day-old) were used. Each experimental group, depending on the experiment, comprised between 5 and 7 or between 6 and 12 rats of each sex. They were humanely handled, and efforts were made to minimize the number of animals used and their suffering. The procedures concerning the protection of experimental animals followed the guidelines of the European Union Council (Directive 2010/63/UE) and the Spanish Royal Decree (BOE 34/11370, 2013). The research protocol was approved by the Animal Ethics Committee of the University of Seville and Junta de Andalucía (number of approval project: CEEA-US2021-14, 21/03/2022/051). The rats were obtained from the 'Centro de experimentación animal de la Universidad de Sevilla'. The animals were anesthetized by intraperitoneal injection of ketamine (50 mg kg^{-1}) plus xylazine (10 mg kg^{-1}) and sacrificed.

Experimental design: induction and evaluation of experimental chronic colitis and creatine supplementation

Rats with similar body weight values were randomized into one of the following experimental groups: control groups that included rats that received water (water) or water with creatine (water-creatine) and treated groups that included rats treated with dextran sulfate sodium (DSS) and rats treated with DSS and supplemented with creatine (DSS-creatine) (Fig. 1A). Control rats drank normal water. Chronic colitis was induced by administering three cycles of DSS.[28] The daily dose of DSS was 5.5 g per kilogram of body weight, administered in drinking water for 7 days. This dose is within the range of commonly used DSS percentages but was adjusted based on body weight, not allowing *ad libitum* drinking, to ensure that rats in the DSS groups received the same dose. Before DSS administration, water consumption and body weight were monitored to determine the average daily intake, allowing appropriate dosage adjustments throughout the treatment. Between the DSS cycles, the rats received normal water for 7 days, except after the third DSS cycle, when they received water for only 2 days (Fig. 1A). This treatment regimen mimics the relapsing–remitting inflammation periods characteristic of human ulcerative colitis (UC).

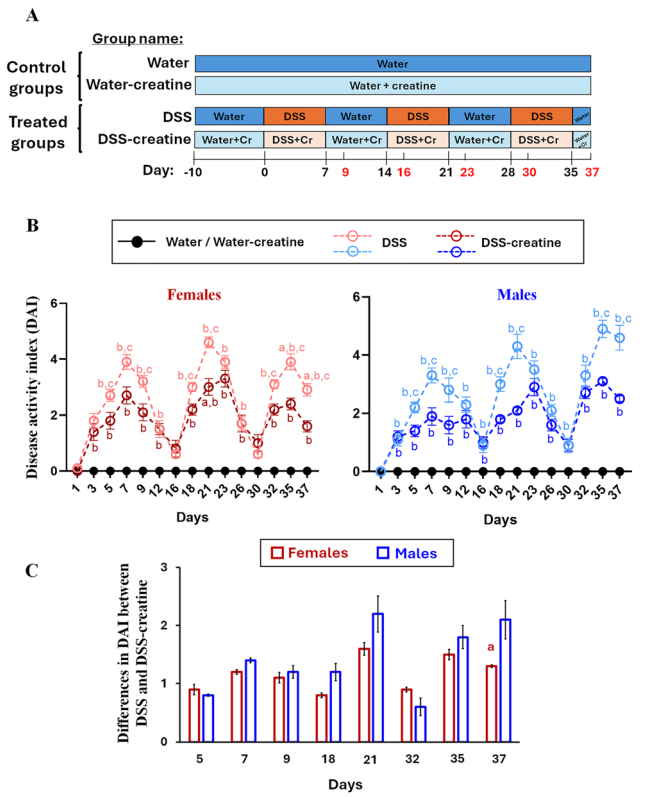

Fig. 1 Effect of creatine on chronic colitis in control and DSS-treated female and male rats. (A) Experimental design. The rats were randomized into control groups that included rats that received water (water) or water with creatine (water-creatine) and treated groups that included those who received DSS or DSS and creatine (DSS-creatine). Treatment consisted of administering 3 cycles of DSS in their drinking water for 7 days, with other 7 days between cycles. Behavioral tasks were conducted on days: 0 (pretest), 9, 16, 23, 30 and 37 (red). Creatine (cr) supplementation started 10 days before DSS treatment and was maintained uninterrupted throughout treatment in the water-creatine or DSS-creatine groups. (B) Disease activity index (DAI) assessed every 2–3 days. Data are means ± SEM (n = 6–12 males per group, n = 6–12 females per group). (C) Differences in DAI between the DSS and DSS-creatine groups on the days with higher scores. ANOVA followed by the Tukey *post hoc* test; [a] $p < 0.05$ females *vs.* males, [b] $p < 0.05$ DSS *vs.* control groups, and [c] $p < 0.05$ DSS *vs.* DSS-creatine.

Induction and progression of colon inflammation were evaluated by observing clinical symptoms using the disease activity index (DAI) as previously.[29] Every 2 days during DSS treatment, the animals were monitored for changes in body weight, stool consistency, and the presence of blood in their feces. Each parameter was scored on a scale of 0 to 3, and these scores were combined to obtain a cumulative total.

Creatine monohydrate was administered in drinking water to rats of both sexes at a dose of 1 g per kilogram of body weight per day. As with DSS, the dose was adjusted based on daily water consumption. Before starting the DSS treatment, the groups of rats with creatine supplementation consumed a preload of this same dose for 10 days, then continued uninterruptedly until the end of treatment (Fig. 1A). The dose was based on normalization of the body surface area between rat and human species.[30]

Biological preparations

For histological analysis, after the sacrifice of rats, distal colon samples were collected, fixed by overnight incubation with phosphate buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4; pH 7.4) containing 4% PFA, embedded in paraffin, and stored at 4 °C until use. Colon sections (5 μm-thick) were cut using a microtome, applied to adhesive-coated glass slides, and stained with hematoxylin and eosin. For PCR studies, after the sacrifice of rats, their motor and prefrontal cortices were removed, frozen in liquid nitrogen, and stored at −80 °C for the subsequent extraction of RNA and PCR measurements. For immunofluorescence assays, rats were anesthetized and transcardially perfused with 4% paraformaldehyde (PFA) in PBS. Subsequently, the brains were removed, postfixed with 4% PFA in PBS overnight, embedded in paraffin, and stored at 4 °C until use. Coronal sections of the brain, including the prefrontal cortex or motor cortex (10 μm-thick) were cut using a microtome and applied to adhesive-coated glass slides.

Analysis of surface epithelial injury

Histopathological scoring of surface epithelial injury was performed according to a protocol previously described.[31] This feature was defined as the damaged or lost surface epithelium with underlying inflammation, where the epithelial defect crossed the basement membrane and presented itself mostly as focal. It was graded on a scale from 0 to 4 based on the extent of epithelial destruction: a score of 0 was assigned for normal tissue without detectable epithelial damage; 1, corresponded to a slight injury with 1–25% epithelial loss; 2, mild injury involving 26–50% epithelial loss; 3, moderate injury

with 51–75% epithelial loss; and 4, severe injury with 76–100% epithelial loss.

Measurement of tissue creatine content

Creatine concentrations were determined in the distal colon and cerebral cortex of female and male rats from all experimental groups. Measurements were performed using a colorimetric creatine assay kit (Abnova, Taiwan), following the manufacturer's instructions. Briefly, tissues were homogenized in distilled water and then, centrifugated and the supernatant collected. A calibration curve was generated using the creatine standards provided in the kit and absorbance was measured at 570 nm. Creatine levels were expressed in micromolar and normalized to the weight of the tissue.

Relative quantification of real-time PCR

Real-time PCR was performed as previously described.[32] Briefly, total RNA was extracted from the prefrontal cortex and primary motor cortex of control rats (water and water-creatine) and treated rats (DSS and DSS-creatine) using the RNeasy® kit (Qiagen, Germany). cDNA was synthesized from 1 μg of total RNA with the QuantiTect® reverse transcription kit (Qiagen, Germany), following the manufacturer's instructions. The primers used are listed in Table S1. Real-time PCR was carried out using NZYSupreme qPCR Green Master Mix® (NZYtech, Portugal). β-Actin was used as the reference gene to normalize the samples. The comparative Ct method was used to determine the relative expression of mRNA, with the lowest value measured for each gene set at 1. All these measurements were taken from samples obtained on day 37 from all experimental groups.

Behavioral test

To assess motor activity and the emotional state of the rats, the behavioral test used in this study was the open field test, as previously described.[9] It was administered for the first time on day 0 (pretest) to all animals before randomizing them into control (water and water-creatine) and treated (DSS and DSS-creatine) groups. Then, as indicated in Fig. 1A, the test was performed on days 9, 16, 23, 30, and 37, which is 2 days after each period, in the case of DSS-treated groups to avoid coinciding with sudden changes in inflammation peaks. The control groups were evaluated in parallel. The following parameters were manually counted: percentage of time spent in the center of the cage, percentage of immobility time, number of crossings, number of rearings, and number of grooming episodes. Furthermore, DeepLabCut software was used to automatically measure the mean speed and traveled distance in the entire cage and in the center of the cage, the number of transitions from the periphery to the center of the cage, the total time spent in the corners, and the immobility time spent in the corners following the methodology as previously.[33–36]

Microglial activation

Immunofluorescence assays were used to assess microglial activation. Coronal brain sections that included the prefrontal cortex and primary motor cortex of control groups (water and water-creatine) or treated rats (DSS and DSS-creatine) of both sexes were used. Briefly, 10 μm-thick paraffin-embedded sections applied to adhesive-coated glass slides were washed with PBS, boiled in sodium citrate buffer (10 mM; pH 6) for 10 min and blocked with 5% bovine serum albumin (BSA), 5% normal goat serum and 0.1% Triton X-100 in PBS for 1 h at room temperature. The sections were then incubated overnight at 4 °C with the microglial marker Iba1 antibody diluted in blocking solution (1 : 200). Anti-Iba1 antibody binding was visualized with a Alexa Fluor-546 secondary antibody. Negative controls were performed in parallel without the primary antibody. The slides were mounted with antifade reagent (Vector, California, USA), and tissues were observed with a Zeiss Axioskop 40 fluorescence microscope and photographed using Zeiss Zen software. To evaluate activated microglia, we measured the Iba1 immunostaining area of both the microglial cell body and the entire cell to assess microglial morphological changes, following the method described by Hovens *et al.*,[37] as previously described.[8] Morphological modifications are reported as the ratio of the microglial cell body size to the total cell size and expressed as a percentage. We analyzed 3 immunolabeled sections per rat in 8–10 fields per section, covering a representative area of the prefrontal or primary motor cortex. An average of all measurements for each animal was calculated.

Statistical analysis

Data were expressed as the mean ± standard error of the mean (SEM). Statistical analyses were performed separately for male rats and female rats to investigate sex-specific effects. Evenly, analyses were performed with data pooled from both sexes. Sample size (n) is indicated in the figure legends. Comparisons between experimental groups were analyzed using two-way ANOVA followed by Tukey *post hoc* test, conducted with the GraphPad Prism software version 8.0. Differences were considered significant at $p < 0.05$. Principal component analysis (PCA) was performed using R version 4.3.2 (RStudio Team, 2023, Boston, MA, USA), with graphics created using the plotly package version 4.10.4. The proportion of variance explained is shown in each 3D diagram. Statistical significance of group separation was evaluated using PERMANOVA (permutational multivariate analysis of variance) with 999 permutations, based on Euclidean distances, implemented in Rstudio.

Results

Creatine reduces disease activity throughout the development of chronic colitis in male and female rats

First, we wanted to find out whether creatine supplementation prevents or reduces the symptoms of chronic colitis throughout its development and whether this effect was influenced by sex. For that, 10 days before and during DSS-induced chronic colitis, male and female rats received creatine in drinking water. Rats separated by sex were randomized into four groups, two control groups: rats received normal water (water) or water containing creatine (water-creatine) and two treated groups: rats

received DSS (DSS) or DSS and creatine (DSS-creatine) (Fig. 1A). We administered three DSS cycles followed each cycle by a water period to mimic relapsing–remitting episodes such as those occurring in human UC. To assess clinical manifestations throughout the development of the disease, we used the disease activity index (DAI) score, which includes recording body weight gain, stool consistency, and rectal bleeding. In both sexes, DSS-treated rats reached maximum DAI values on days 7, 21, and 35 and minimum values on days 16 and 30 (Fig. 1B). As we previously reported,[9] male rats compared to females exhibited slightly more severe symptoms of colitis, as manifested by higher DAI values at the end of DSS treatment, days 35 and 37 (Fig. 1B). Creatine supplementation significantly decreased DAI values in females on days 5, 7, 9, 18, 21, 32, 35, and 37, and in the case of males on days 5, 7, 9, 18, 21, 35, and 37 (Fig. 1B). Although creatine attenuated the clinical symptoms of colitis, they did not completely disappear during the relapsing periods. Additionally, to determine whether the effect of creatine on DAI was different depending on sex, we calculated the differences in DAI between the DSS and DSS-creatine groups and compared them between sexes. We found that only on day 37, when chronic colitis is established, the difference was significantly higher in the males (Fig. 1C). These data reveal that oral creatine supplementation ameliorated the severity of chronic colitis in a similar way in both sexes, except on day 37 where males exhibited a greater effect of creatine on colitis.

Creatine decreases surface epithelium injury in the colon of male and female rats with chronic colitis

Surface epithelial cell layer is a primary component of the colon's barrier. In UC, this epithelium undergoes significant injury, which strongly impacts barrier function which contributes to the pathogenesis of this disease.[1] We wanted to study whether creatine supplementation prevents or reduces this injury by promoting the integrity of the intestinal barrier. For that, we performed a histopathological analysis using hematoxylin–eosin staining colon sections and scored the surface epithelial injury on day 37 of treatment in all experimental groups. Representative images showed damaged or lost surface epithelium with focal location in DSS-treated rats of both sexes (Fig. 2A). The scoring revealed surface epithelium injury which was significantly less severe in females than in males (Fig. 2B). Creatine supplementation significantly reduced this injury only in males (Fig. 2B). The difference between the DSS and DSS-creatine groups and the comparison between sexes was significantly greater in males (Fig. 2C). These results indicate that oral creatine supplementation improved the integrity of the colonic barrier altered with chronic colitis and with males exhibiting a greater effect of creatine.

Effect of creatine supplementation on the creatine content of the colon and cerebral cortex in male and female rats

We addressed whether oral creatine supplementation modifies creatine content in the colon and cerebral cortex and whether there were sex differences. The results of creatine content are

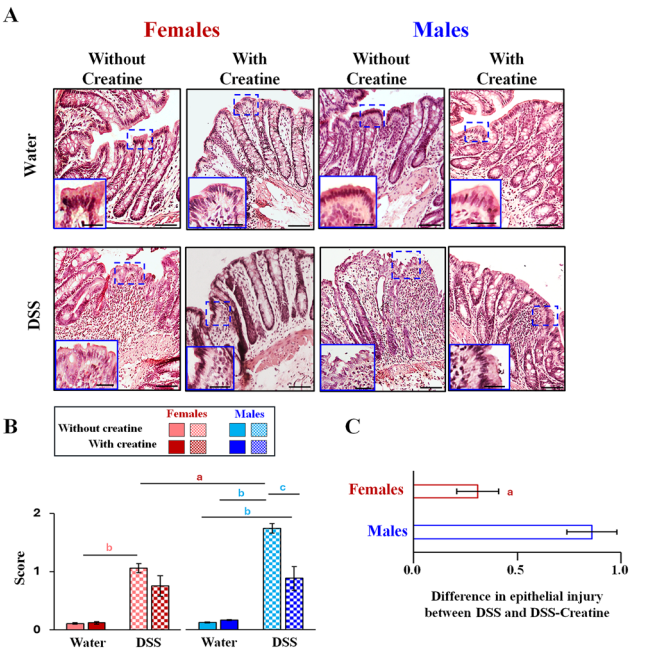

Fig. 2 Effect of creatine on surface epithelial injury in the colon of control and DSS-treated female and male rats. (A) Representative images of the hematoxylin/eosin-stained distal colon sections of the four experimental groups. The square in blue is amplified in an insert. The scale bar represents 100 μm in low magnification images and 50 μm in amplified images. (B) Scoring of surface epithelial injury in the colon. Data are means ± SEM (n = 5–7 males per group, n = 5–7 females per group). (C) Difference in epithelial injury between the DSS and DSS-creatine groups on day 37. ANOVA followed by the Tukey *post hoc* test; $^{a}p < 0.05$ females *vs.* males, $^{b}p < 0.05$ DSS *vs.* control groups, and $^{c}p < 0.05$ DSS *vs.* DSS-creatine.

shown in Fig. 3. We first observed that basal creatine concentrations in the colon and cortex of DSS-treated rats, except for female cortex, were lower than those in the control groups, although there were only significant differences compared to the water-creatine groups. In response to creatine supplementation, creatine levels increased in the colon and cerebral cortex of DSS-treated rats of both sexes, whereas no significant differences were observed in the water groups there were. Furthermore, comparisons between the two tissues showed that creatine concentrations in the cerebral cortex were significantly higher ($p < 0.001$) than those in the colon in all experimental groups. In DSS-creatine groups, the increase in creatine levels in the cortex was less than in the colon (about 20–30% in cortex *vs.* about 40–50% in colon). Regarding sex differences in creatine concentrations, we did not observe any in either the colon or the cortex.

Creatine differently prevents motor deficits caused throughout the development of chronic colitis in male and female rats

Next, we examined the effect of creatine supplementation on altered motor activity due to the development of chronic colitis and whether there were sex differences. Motor activity in male and female rats was analyzed using the open field test and the

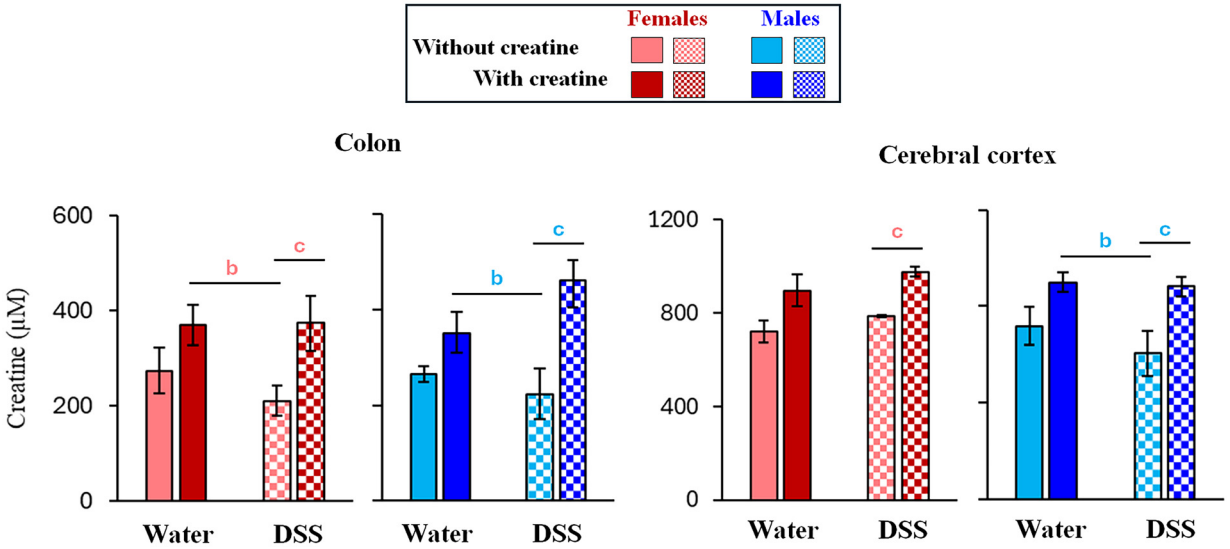

Fig. 3 Creatine content in the colon and cortex of control and DSS-treated female and male rats, with and without creatine supplementation. Creatine concentrations were measured in each experimental group on day 37 and are given as means ± SEM (n = 5–7 males per group, n = 5–7 females per group). ANOVA followed by the Tukey *post hoc* test; $^{b}p < 0.05$ DSS *vs.* control groups, and $^{c}p < 0.05$ DSS *vs.* DSS-creatine.

results are summarized in Fig. 4. The measured parameters were rearing, crossings, traveled distance, and average speed, as indicated in the Methods section. We performed a Principal component analysis (PCA) to summarize and visualize the information obtained from these motor activity parameters measured on day 37 of DSS treatment, when chronic colitis was established. This analysis showed that the grouping of rats depended on (i) the presence of chronic colitis (Fig. 4A), (ii) creatine supplementation (Fig. 4B) and (iii) sex (Fig. 4C). These PCA models also showed that the traveled distance was the most relevant variable in the data. We have previously observed that rats of both sexes with chronic colitis exhibited motor deficits that persisted throughout the development of the disease and the only motor parameter that showed to differ by sex was the traveled distance in which females exhibited lower values.[9] Here, first, we observed that the control groups (water and water-creatine) did not show differences between them or compared to day zero (pretest) to other time points in any of the motor activity parameters evaluated in females or males. The DSS groups showed reduced motor activity and the DSS-creatine groups, in both sexes, showed values almost identical to those of the control groups in all motor parameters and at all the time points evaluated. These results demonstrate that the impairments in locomotion induced by colitis, as manifested by decreased rearings, crossings, traveled distance, and average speed, were prevented by creatine supplementation from the beginning of treatment and throughout the course of chronic colitis (Fig. 4D). Representative images of the movement tracks of the rats in the open field of each experimental group on day 37 are illustrated in Fig. 4E.

Since in our previous study only the traveled distance exhibited sex differences,[9] we calculated the differences in this parameter between the DSS and DSS-creatine groups throughout the development of colitis (Table 1) and compared them between sexes. These differences were significantly higher in females than in males. In addition, in this parameter, the females in the DSS-creatine group reached values as control groups that were similar to those of the males (Fig. 4D).

In summary, creatine was able to maintain normal motor abilities in both sexes, preventing alterations in locomotion induced by chronic colitis, and with the females experiencing a greater beneficial effect of creatine on the traveled distance.

Creatine differently prevents anxiety-like behaviors caused throughout the development of chronic colitis in male and female rats

In our previous work, we also demonstrated that from the onset of chronic colitis and throughout its development, without any changes related to the remitting–relapsing phases, male and female rats exhibited anxiety-like behaviors, with the females exhibiting a more anxious profile.[9] Therefore, we wanted to investigate whether creatine supplementation ameliorates or prevents these behavioral alterations induced by colitis. To do this, we use the open field test and analyze anxiety-related parameters measured in the center (time spent, transitions, traveled distance, and average speed) and in the corners (time spent and still time) of the cage. As with motor activity, none of the parameters evaluated showed differences between the water and water-creatine groups or between day zero and other time points in the control groups. We also examined the data using a PCA and found that, as before, the rats were grouped according to (i) the presence of colitis (Fig. 5A), (ii) creatine supplementation (Fig. 5B), and (iii) sex (Fig. 5C). In these PCA models, the most relevant variable was the time spent in the corners. Furthermore, analysis of each anxiety-related parameter showed that, during the develop-

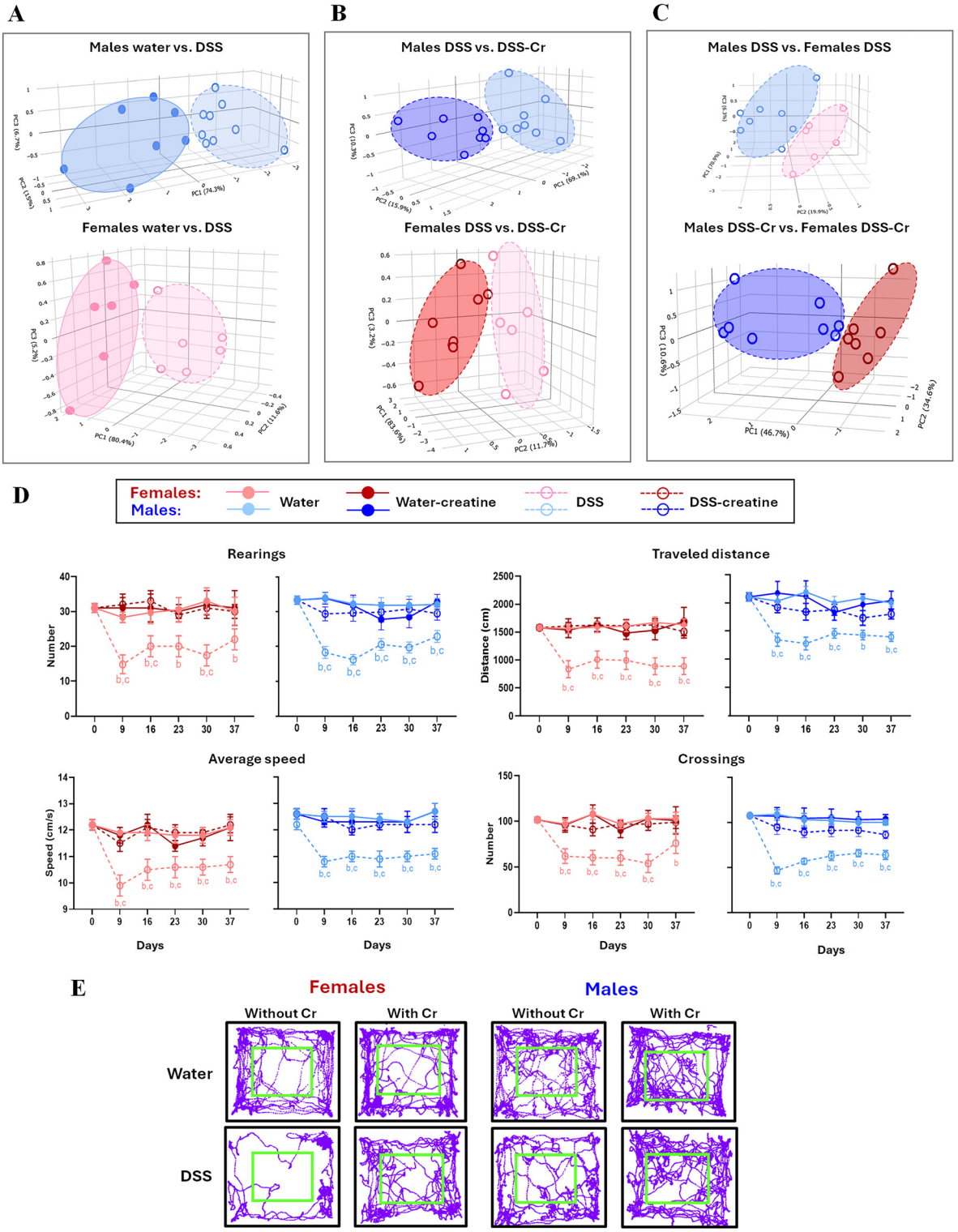

Fig. 4 Effect of creatine on motor activity in control and DSS-treated female and male rats. (A–C) Tridimensional principal component analysis (PCA) diagrams performed with motor activity parameters in the water, DSS and DSS-creatine (cr) groups of male and female rats on day 37. The proportion of variance explained is shown in each diagram. Statistical analysis using PERMANOVA showed significant group separations ($p < 0.05$). (D) Parameters related to motor activity measured using the open field test. (E) Representative images of the movement tracks of rats on day 37. Each purple spot in the image represents 1 second. Data are means ± SEM (n = 6–12 males per group, n = 6–12 females per group). ANOVA followed by the Tukey *post hoc* test; $^{b}p < 0.05$ DSS *vs.* control groups and $^{c}p < 0.05$ DSS *vs.* DSS-creatine.

Table 1 Differences in behavior parameters between the DSS and DSS-creatine groups in male and female rats

Day	Group	Traveled distance (cm)	Time in the center (%)	Transitions to the center (number)	Traveled distance in the center (cm)	Average speed in the center (cm s^{-1})	Time in the corners (%)	Still time in corners (s)	Immobility time (%)	Grooming (number)
9	Females	774** ± 24	1.1 ± 0.2	7.1 ± 0.7	64** ± 7	5.5* ± 0.7	18.3 ± 0.9	71* ± 5	20** ± 1	0.9 ± 0.1
	Males	577 ± 18	1.1 ± 0.01	10.1 ± 1.9	110 ± 7.1	2.2 ± 0.2	23 ± 2.5	55 ± 8	13.4 ± 0.08	0.8 ± 0.2
16	Females	619 ± 28	1.2 ± 0.3	8 ± 0.6	89 ± 7	4.9* ± 0.6	19.3* ± 2.3	53 ± 4	16.2 ± 1	0.4 ± 0.3
	Males	564 ± 36	1.5 ± 0.05	10.3 ± 1.9	82 ± 13.4	2.2 ± 0.1	11.9 ± 1.4	67.6 ± 0.3	17.2 ± 1	1 ± 0.1
23	Females	617** ± 54	1.7 ± 0.3	7 ± 0.5	78* ± 12	5.7* ± 0.9	22 ± 1.2	69** ± 0.5	15.4 ± 1.5	0.7 ± 0.2
	Males	418 ± 12	1.7 ± 0.01	8.9 ± 0.2	58 ± 0.3	2.3 ± 0.3	17.2 ± 0.2	44 ± 5.7	12.2 ± 1.2	0.7 ± 0.1
30	Females	741** ± 39	2.4** ± 0.2	12.7* ± 1	97* ± 6	6.7** ± 1.1	27.1** ± 3	64* ± 0.5	18** ± 1.6	0.9 ± 0.1
	Males	303 ± 46	1.4 ± 0.02	8 ± 0.3	70 ± 6.5	2.6 ± 0.1	11.9 ± 0.1	50 ± 3.6	12.2 ± 1	0.6 ± 0.2
37	Females	619** ± 36	2.2 ± 0.3	12.4** ± 1.1	68 ± 1.5	3.9* ± 0.4	24.9** ± 3	50 ± 3	18.3** ± 1	0.9 ± 0.1
	Males	405 ± 8.5	1.7 ± 0.02	6 ± 0.3	66 ± 1.5	1.9 ± 0.1	14.4 ± 0.6	56.6 ± 4	12 ± 0.9	0.6 ± 0.2

Data are expressed as means ± SEM (n = 6–12 animals per group). ANOVA analysis found significant differences between groups. Tukey test: *$p < 0.05$ and **$p < 0.001$ females *vs.* males.

ment of colitis, the male and female rats exhibited a reduction in the values of the parameters measured in the center of the cage (Fig. 5D) and increased those measured in the corners of the cage (Fig. 5E). Creatine maintained the basal values of all these parameters keeping them equal to controls throughout the development of colitis, without differences related to the remitting–relapsing periods (Fig. 5D). Thus, creatine supplementation enhanced the willingness of DSS-treated rats to stay in the center zone, an effect that persisted throughout the treatment. In Fig. 4E, the different activity tracking of male and female rats can be appreciated in the center and corners of the cage of each experimental group on day 37. Regarding sex differences, we calculated the differences in anxiety-related parameters between the DSS and DSS-creatine groups throughout the development of colitis (Table 1) and compared them between sexes. Most of the differences were significantly higher in females, suggesting a greater effect of creatine in females than in their male counterparts. It should be noted that, at the end of the treatment, there were no significant differences in the parameters measured in the center of the cage between the sexes in the DSS-creatine groups. This indicates that the females in this group reached control-like values that were similar to those of the males (Fig. 5D).

All these findings demonstrate that, in both sexes, creatine supplementation prevented anxiety-like behaviors triggered by chronic colitis throughout its development, although with a greater beneficial effect of creatine on females.

Creatine differently avoids depression-like behaviors caused throughout the development of chronic colitis in male and female rats

In addition to anxiety, we previously discovered that depression-related behaviors also occurred in both male and female rats along the development of chronic colitis, although without sex differences.[9] Here, we examined the impact of creatine supplementation on depressive-like behaviors induced over the course of chronic colitis in both sexes. We used once more the open field test to measure two parameters related to depression, the number of grooming episodes, and the immobility time, which refers to the period when the rats remain completely still. As in the other parameters, we did not observe differences between the water and water-creatine groups or between day zero and the other time points in the control groups (Fig. 6). The DSS groups of both sexes exhibited depression-like behaviors as evidenced by a significantly longer immobility time and a decreased grooming frequency at all evaluated time points. In contrast, in the DSS-creatine groups, the immobility time decreased, while the number of groomings increased, with both parameters reaching similar values to control groups. Therefore, creatine prevented the development of depression-related manifestations throughout the progression of chronic colitis (Fig. 6). We also calculated the differences in these parameters between the DSS and DSS-creatine groups throughout the development of chronic colitis (Table 1) and compared them between sexes. At various time points in DSS treatment, differences in immobility time were

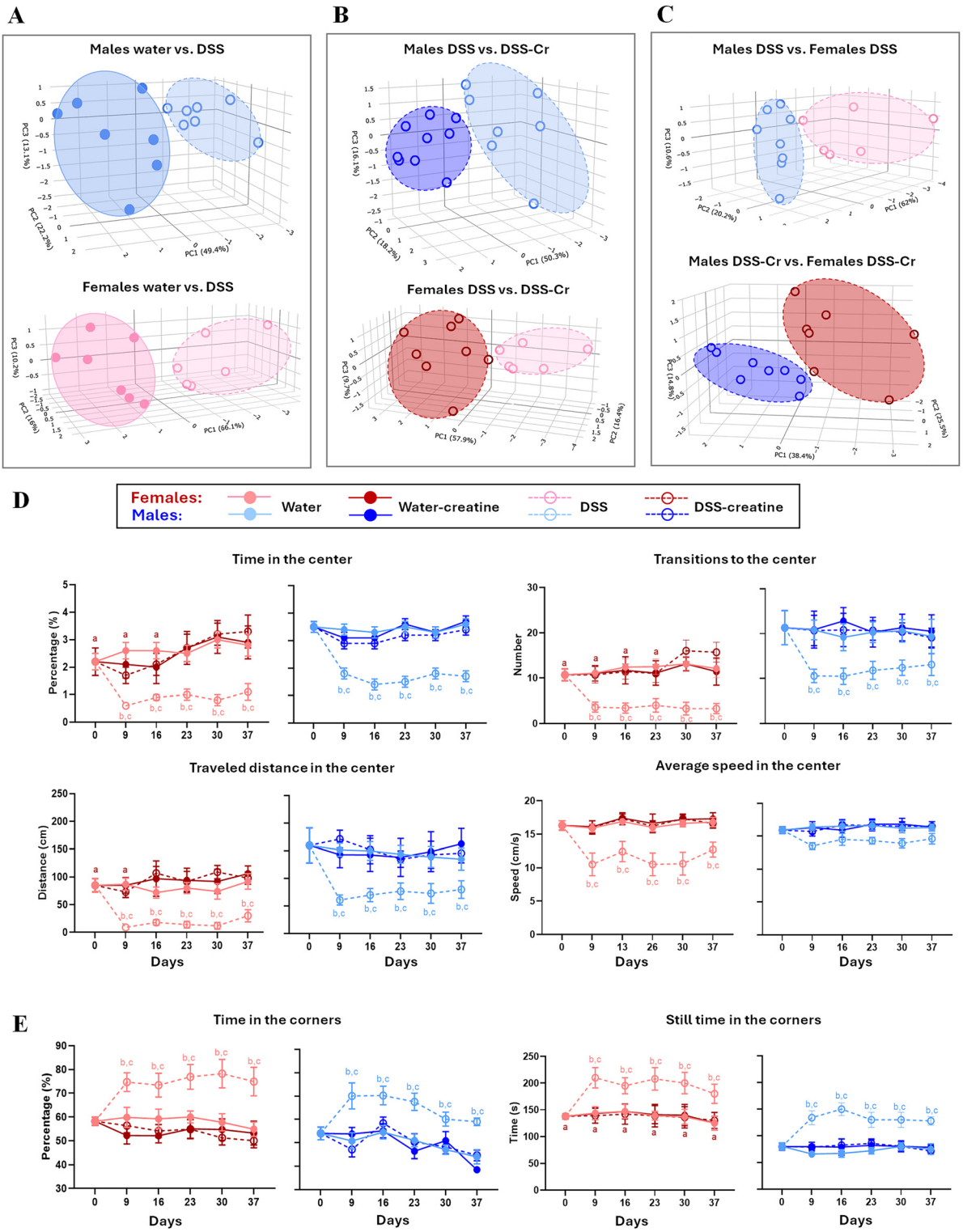

Fig. 5 Effect of creatine on anxiety-like behaviors in control and DSS-treated female and male rats. (A–C) Tridimensional principal component analysis (PCA) diagrams performed with anxiety-related parameters in the water, DSS and DSS-creatine (cr) groups of male and female rats on day 37. The proportion of variance explained is shown in each diagram. Statistical analysis using PERMANOVA showed significant group separations ($p < 0.05$). (D and E) Parameters associated with anxiety-related behaviors measured using the open field test in the center of the cage or in the corners of the cage. Data are means ± SEM (n = 6–12 males per group, n = 6–12 females per group). ANOVA followed by the Tukey *post hoc* test; a $p < 0.05$ females *vs.* males, ^{b} $p < 0.05$ DSS *vs.* control groups, and ^{c} $p < 0.05$ DSS *vs.* DSS-creatine.

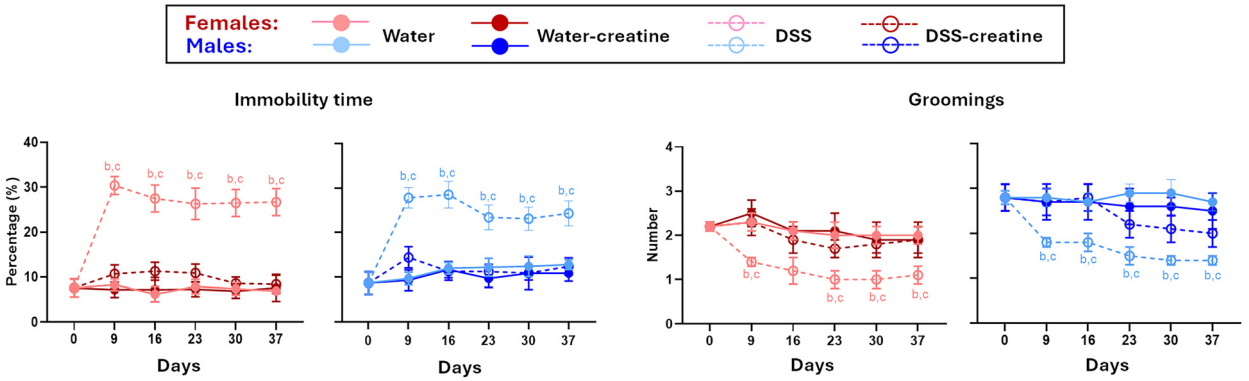

Fig. 6 Effect of creatine on depression-like behaviors in control and DSS-treated female and male rats. Parameters associated with depression-related behaviors measured using the open field test. Data are means ± SEM (n = 6–12 males per group, n = 6–12 females per group). ANOVA followed by the Tukey *post hoc* test; $^{b}p < 0.05$ DSS *vs.* control groups and $^{c}p < 0.05$ DSS *vs.* DSS-creatine.

significantly higher in females, suggesting a greater effect of creatine in females than in males. Furthermore, the values obtained by the females in the DSS-creatine group in immobility time reached control-like values that were equal to those of the males (Fig. 6).

Creatine differently prevents chronic colitis-induced neuroinflammation in the motor and prefrontal cortices in male and female rats

We have previously demonstrated that chronic colitis caused neuroinflammation in the prefrontal and motor cortices, varying the inflammatory profile according to sex, and this neuroinflammation could be responsible, at least in part, for the motor and mood impairments observed in colitis.[9] In this work, we wondered whether the normal mood and motor activity observed in the DSS-creatine groups in both sexes were due to the abolition or, at least, to the reduction in neuroinflammation induced by chronic colitis. For this purpose, we determined the mRNA relative abundance of the pro-inflammatory cytokines IL-1β, IL-6, and TNF by real-time PCR on day 37 in all experimental groups in male and female rats. To examine the effect of creatine, we analyzed data from both sexes combined or separated by sex obtained from control groups (water and water-creatine) and treated groups (DSS and DSS-creatine), in the motor and prefrontal cortices. As we had previously seen, in female and male rats with chronic colitis compared to controls, mRNA expression levels of these three cytokines were significantly up-regulated in the prefrontal and motor cortices (Fig. 7A and B). Then, analyzing the data combined or separately by sex, we observed that in the DSS-creatine groups the mRNA levels of the three cytokines decreased to control values, in the prefrontal and motor cortices of male and female rats (Fig. 7A and B).

Sex differences found in our previous study showing higher basal levels of TNF and IL-1β mRNA in the prefrontal cortex in females than in male rats who received water,[9] were also observed in those rats who received water with creatine (Fig. 7A and B). In the case of rats with chronic colitis, compared to their respective controls, we had observed higher levels of IL-6 in the prefrontal and motor cortices in males, and higher TNF in the motor cortex in females. Therefore, to determine the effect of creatine supplementation according to sex, we calculated the differences in mRNA abundance of IL-1β, IL-6 and TNF between the DSS and DSS-creatine groups in males and females. Comparison of these values revealed greater differences in both cortices, in IL-6 in males, whereas IL-1β differences were more pronounced in females (Fig. 7C and D), suggesting a greater effect of creatine on IL-6 in males and on IL-1β in females.

Therefore, our results point to the fact that creatine supplementation, with slight differences between males and females, prevented the chronic colitis-induced neuroinflammation in the prefrontal and motor cortices.

Creatine differently avoids chronic colitis-induced microglial activation in the motor and prefrontal cortices in male and female rats

Finally, we wondered whether chronic colitis triggers microglial activation in the motor and prefrontal cortices that could result in an increase in pro-inflammatory cytokines, such as those observed in IL-1β, IL-6, and TNF in DSS-treated rats. Then we examined whether this activation is prevented or reduced by creatine supplementation. Microglial activation was evaluated in all experimental groups using immunofluorescence assays that detected the marker Iba1 in the motor and prefrontal cortices of rats of both sexes on day 37. We determined the ratio between cell body size and total cell size of microglia, since this value increases with microglial activation. We analyzed data from combined or separated sexes obtained from control groups (water and water-creatine) and treated groups (DSS and DSS-creatine). The results revealed that DSS-induced chronic colitis increased the ratio between cell body size and total cell size in both cortices in male and female rats, indicating irregular shape and microglial activation (Fig. 8A–D). Furthermore, creatine supplementation avoided this colitis-induced microglial activation in the prefrontal and

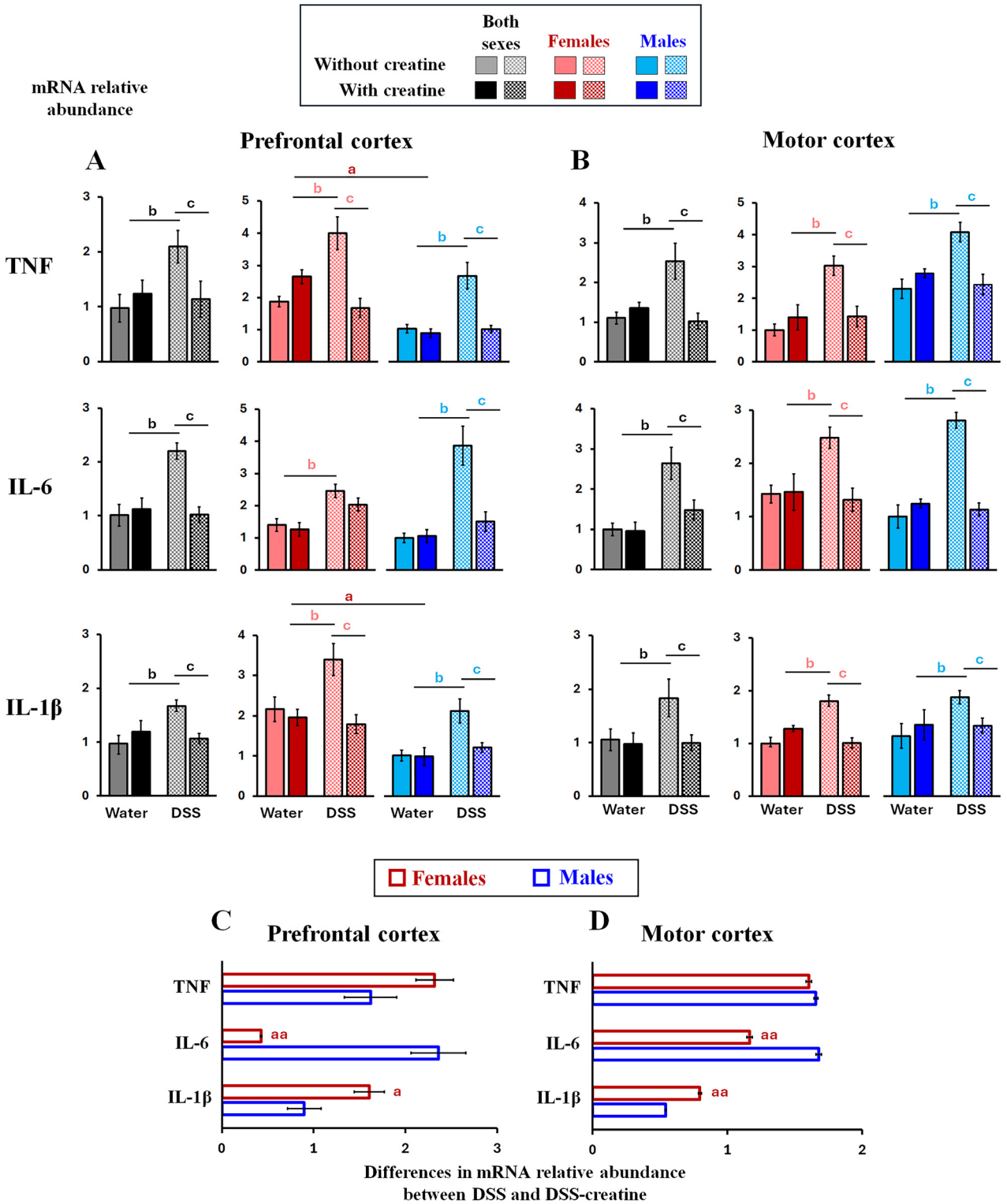

Fig. 7 Effect of creatine on mRNA expression of pro-inflammatory cytokines in the prefrontal and motor cortices of control and DSS-treated female and male rats. (A and B) mRNA levels as a relative abundance in each experimental group on day 37. Analyses were performed using combined measures from both female and male rats or separated by sex. (C and D) Differences in mRNA relative abundance between the DSS and DSS-creatine groups. Data are means ± SEM (n = 10–14 animals per group for data pooled from both sexes and n = 5–7 males per group, n = 5–7 females per group for data separated by sex). ANOVA followed by the Tukey *post hoc* test; $^{a}p < 0.05$ and aa $p < 0.001$ females *vs.* males, $^{b}p < 0.05$ DSS *vs.* control groups, and $^{c}p < 0.05$ DSS *vs.* DSS-creatine.

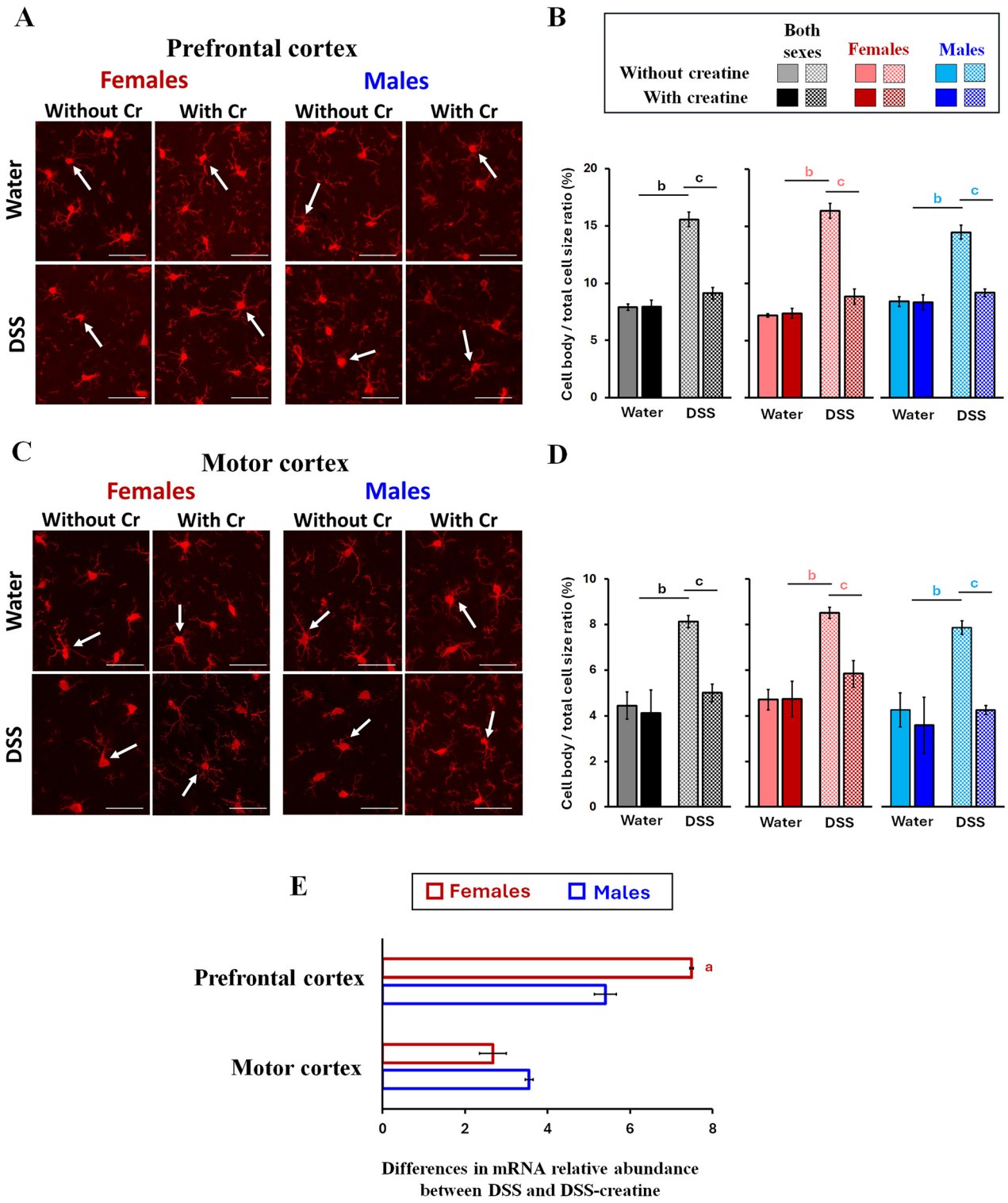

Fig. 8 Effect of creatine on microglial activation of the prefrontal and motor cortices of control and DSS-treated female and male rats. (A and C) Representative images of the prefrontal cortex and motor cortex of the four experimental groups with the iba1 immunostaining signal (red). Arrows indicate a representative cell. The scale bar represents 20 μm. (B and D) Quantification of microglial activation of the prefrontal or motor cortices as a cell body size to total cell size ratio expressed as a percentage on day 37. Analyses were performed using combined measures from both female and male rats or separated by sex. (E) Differences in microglia activation between the DSS and DSS-creatine (cr) groups. Data are means ± SEM (n = 10–14 animals per group for data pooled from both sexes and n = 5–7 males per group, n = 5–7 females per group for data separated by sex). ANOVA followed by the Tukey *post hoc* test; [a] $p < 0.05$ females *vs.* males, [b] $p < 0.05$ DSS *vs.* control groups, and [c] $p < 0.05$ DSS *vs.* DSS-creatine.

motor cortices and in both sexes. We calculated the differences in this activation between the DSS and DSS-creatine groups, and compared them between the sexes, we found a greater effect of creatine supplementation in the prefrontal cortex of females (Fig. 8E).

These findings demonstrate that chronic colitis triggered microglial activation in the motor and prefrontal cortices in male and female rats and that creatine supplementation prevented this activation more effectively in the prefrontal cortex of females.

Discussion

Accumulating evidence points out that creatine supplementation has beneficial effects for the management of neuropsychiatric disorders.[20,24] In the current work, we provide novel evidence that oral creatine supplementation mitigates the severity of chronic colitis symptoms in male and female rats. Importantly, we demonstrate for the first time that creatine prevents motor and mood disturbances induced during disease progression while also avoiding neuroinflammation and microglial activation in the prefrontal and motor cortices that occurred once chronic colitis was established. Furthermore, our findings reveal sex-specific differences in the effects of creatine and highlight its potential as a nutritional strategy to prevent these alterations.

We used a DSS chronic colitis model in male and female rats that exhibited relapsing–remitting periods, resembling the clinical course in human UC, as previously described.[9] Our previous research revealed sex differences in DSS-induced chronic colitis, with its severity slightly higher in males, as evidenced by some of their higher DAI values than in females.[9] In this study, we found that oral creatine supplementation reduced the severity of chronic colitis symptoms throughout its development in male and female rats, as demonstrated by the lower DAI values. It should be noted that the reduction in DAI occurred during the flare-up phases of the disease, although the symptoms do not completely disappear. This decrease in DAI indicates that creatine attenuated the manifestations associated with colitis, such as diarrhea, rectal bleeding, and weight loss, which in turn are a consequence of inflammatory events that occur within the colon. Creatine also reduced the injury in the surface epithelium of the colon improving the integrity of the intestinal barrier. In addition, we observed that males showed a greater effect of creatine on DAI and epithelial injury on day 37 when chronic colitis is established. The higher values of these parameters in males than in females on day 37 may explain why males have a greater effect of creatine at this time point. In line with our work, the role of creatine in alleviating the severity of the disease has already been demonstrated in murine models of acute colitis in females[38] or of chronic colitis in males,[39] and in patients with mild to moderate UC in a pilot clinical trial.[40] Moreover, these studies strengthen the idea that creatine regulates the energy balance of intestinal epithelium and promotes the functional integrity of the barrier. According to this, we have previously reported preliminary results as a meeting abstract showing that creatine supplementation enhanced its metabolism and reduced inflammation as well as alterations in tight junction proteins of the surface epithelial cells in the colon of rats with chronic colitis.[41] In summary, in this work we show for the first time a protective effect of creatine during the development of chronic colitis throughout the relapsing–remitting phases of the disease and that is influenced by sex when chronic colitis has developed.

Patients with UC have a higher prevalence of psychiatric disorders, such as depression and anxiety.[3–5] A significant number of studies, including ours, have shown that rodents with experimental colitis exhibited, in addition to intestinal symptoms, mood impairments resembling those of patients with UC.[6,7,9] Additionally, we and others have reported motor deficits in rodent colitis models.[8,9,42–45] Research in patients with UC has shown a reduction in muscle mass and strength[46] and impaired muscle function in women with UC.[47] Then we performed the open field test on male and female rats to investigate whether, in addition to improving the symptoms of colitis, creatine could reduce or prevent motor and mood alterations triggered throughout the development of chronic colitis.

Regarding motor activity, we discovered that creatine supplementation avoided the negative impact of chronic colitis on locomotion in both sexes and in females undergoing a greater effect. First, we observed that creatine had no effect on motor activity in those rats without DSS treatment (control groups), which agrees with a study carried out in female mice.[48] Then, in chronic inflammation of the colon, we found that creatine prevented impairments in locomotor function as evidenced by values in motor parameters, crossings, rearings, traveled distance and speed similar to control groups reached with the supplementation in male and female rats. This creatine effect was observed from the beginning of DSS treatment and was maintained throughout the development of the disease, regardless of the relapsing–remitting phases. Although it was quite similar in both sexes, females experienced a greater effect on the traveled distance, this parameter being shown to be the most relevant in the PCA analysis. To our knowledge, this is the first study to demonstrate an effect in preventing motor deficits linked to the development of chronic colitis. In other pathologies with motor dysfunction, such as some neurodegenerative diseases, animal studies showed that creatine supplementation improved motor performance or slowed the development of motor impairments.[20,24,26,27] However, these studies were conducted mainly in males or without comparing the effect of creatine between sexes. On the other hand, patients and animal models of creatine transporter (CrT) deficiency, which is a condition that affects mainly the brain while sparing other tissues, such as skeletal muscle, exhibit motor dysfunctions in addition to cognitive disabilities and behavioral disorders.[49] The pathogenesis of this cerebral creatine deficiency reveals the importance of creatine in the functions of different regions of the brain.

We have also investigated the effect of creatine supplementation on mood-related disorders associated with the development of chronic colitis. We have previously reported that as chronic colitis developed, both male and female rats displayed anxiety-like behaviors that were maintained regardless of the relapsing–remitting phases of the disease. Furthermore, as in women,[4,50] we observed that female rats had more anxiety than males under control and colitis conditions.[9] Here, we demonstrate that, throughout the development of chronic colitis, creatine supplementation maintained normal values of all the measured parameters related to anxiety. These findings indicated that creatine prevented anxiety-like behaviors triggered by chronic colitis. Interestingly, this effect was greater in females than in males, and although females became more anxious at the end of DSS treatment, rats of both sexes supplemented with creatine exhibited a similar tendency to stay in the center of the cage as controls. Although growing evidence suggests that creatine may have anxiolytic effects, to our knowledge, there is only one study showing the effect of creatine supplementation on anxiety. In this study, creatine attenuated anxiety-like behaviors found in a mouse model of epilepsy without indicating sex.[51] Research has also shown that low creatine concentrations[52,53] or a higher ratio of brain metabolite *N*-acetylaspartate/creatine levels[54,55] in the prefrontal cortex could be related to anxiety disorders. Based on these data, increasing creatine levels in this region of the brain would alleviate some symptoms of this disorder.[24]

Unlike anxiety, a significant number of preclinical and clinical studies have shown that creatine supplementation reduces the symptoms of depression and that it could be an adjunctive therapy for the management of this disorder.[21,22] Initially, it was believed that the low creatine concentrations observed in the prefrontal cortex of some patients with depression could explain this disorder, suggesting that restoring normal creatine levels could improve the symptoms. However, subsequent studies did not find significant changes in brain creatine levels in male or female subjects with depression[21,56] Our study reports for the first time the beneficial effect of creatine supplementation on depression-like behaviors associated with chronic colitis in rats. Previously, we demonstrated that chronic colitis elicits depression-like behaviors throughout its development regardless of relapsing–remitting periods and similarly in both sexes.[9] Here, we show that with creatine supplementation the measured parameters exhibited values similar to those of the controls, indicating that creatine avoided depressive-associated behaviors throughout the development of chronic colitis in both sexes. We also observed a sex-dependent impact of creatine supplementation, finding that this creatine antidepressant effect was greater in females than in males. Several studies carried out in different animal models of depression have shown that creatine exhibits an antidepressant-like effect.[51,57–62] Two of these studies that compared between sexes agreed with our results observed in females.[57,58,61] Furthermore, clinical studies revealed this beneficial effect of creatine on depression, including in female patients. Although these studies did not show whether the improvements were more significant than in male subjects, it was postulated that creatine might be more effective in women.[21–23] This was based on possible sex-specific differences in creatine levels or metabolism in the brain that could contribute to differing responses in women.[24] Early studies showed that women had lower levels of creatine in the brain, particularly in the frontal lobe, thus supplementation could be more effective for females; however, several subsequent studies did not report significant differences sex-dependent in creatine concentration within different parts of the brain, such as the prefrontal or motor cortices.[24] We also did not find sex differences in creatine concentrations in the colon and cerebral cortex of rats. We observed higher levels of creatine in the cortex compared to the colon. In response to oral creatine supplementation, creatine content increased in both the colon and cortex of male and female rats with chronic colitis; however, we did not observe significant differences in the water groups. The fact that the effect of creatine supplementation on its content occurs only in the DSS groups could be due to changes in the intestinal and brain barriers with inflammation. Previous studies conducted in humans and animal models and using different methods showed that creatine supplementation increased brain creatine.[20,63] The increase in cortical creatine concentrations observed in our study was consistent with the findings reported in these studies. To our knowledge, creatine levels have not been studied in the colon of rats with chronic colitis. A study reported elevated brain creatine levels in rats under basal conditions or with supplementation compared to other tissues but did not include the intestinal track.[64] Another study showed that acute colitis in mice decreased creatine levels in the colon and hippocampus but did not compare the levels between these tissues.[65] We did not find significant decreases in brain creatine content in rats with chronic colitis, suggesting that the behavior alterations observed in this study are not due to changes in creatine concentrations. This would agree with the studies in subjects with depression who did not exhibit changes in brain creatine levels.[21,56]

Several molecular mechanisms have been proposed underlying the effects of creatine on behavioral states.[21,24] We suggest a potential mechanism by which creatine prevents motor and mood alterations resulting from DSS-induced chronic colitis in rats of both sexes. As we and others have proposed, in patients with UC or in animal models of colitis, neuroinflammation could be responsible, at least in part, for the motor and mood impairments observed in this pathology.[6–9,12,13] Our results, as we showed previously, revealed that, in male and female rats, chronic colitis caused neuroinflammation in the prefrontal and motor cortices, evidenced by the up-regulation of pro-inflammatory cytokine mRNA levels, TNF, IL-1β, and IL-6.[9] This neuroinflammation exhibited different profiles between sexes, while females had higher baseline cytokine levels, males reached greater neuroinflammation.[9] Clinical studies and animal models suggest that sex differences in neuroinflammatory response could contribute to sexual dimorphism seen in some neuropsychiatric dis-

orders.[66] Creatine supplementation, in male or female rats with chronic colitis, remained the mRNA levels of the three cytokines equal to controls in the motor and prefrontal cortices. This indicated that creatine prevented chronic colitis-associated neuroinflammation in both cortices. We also found some sex differences in the effect of creatine on the expression of pro-inflammatory cytokines, a greater effect, in both cortices, on IL-6 in males and on IL-1β in females. Previous studies showed that creatine, in addition to reducing locomotion alterations, decreased neuroinflammation in the substantia nigra of male mice in a model of Parkinson's disease[26] and prevented IL-6 overexpression in the hippocampus of male rats in a model of cerebral palsy, a chronic motor disability.[27] However, in another study conducted in female rats, creatine failed to reduce lipopolysaccharide-induced neuroinflammation in the dentate gyrus.[67]

Affective disorders are accompanied by neuroinflammation that involves microglial activation.[10,66] As other authors, we suggest that the neuroinflammation induced by chronic colitis could be due to microglial activation.[7,15] We observed microglial activation in the prefrontal and motor cortices of DSS-treated male and female rats, and creatine supplementation avoided this activation. This effect of creatine was greater in the prefrontal cortex of females. To our knowledge, only two previous studies, where creatine decreased neuroinflammation, also provided an impact of creatine on brain microglial activation, one showing a decrease in the substantia nigra of male mice in a model of Parkinson's disease[26] and another in which creatine had no effect on the rat hippocampus in a model of cerebral palsy.[27] Then, the mechanism by which creatine prevents motor and mood alterations may be to avoid neuroinflammation in the areas of the brain responsible for these behaviors. This effect on neuroinflammation could, in turn, be a consequence of reduced microglial activation. Regarding sex differences, the variations in the effects of creatine on behaviors may be partially explained by its differing impacts depending on the sex on neuroinflammation and microglial activation. More research is needed to examine the underlying mechanisms.

In conclusion, oral creatine supplementation reduced the clinical symptoms of chronic colitis and injury of colonic surface epithelial, and, for the first time, our study provides evidence supporting that creatine was particularly effective in preventing motor deficits and anxiety and depressive-like behaviors that were produced throughout the development of chronic colitis. We also show that this effect was maintained uninterruptedly regardless of the remitting–relapsing phases of the disease and was different in males and females. In response to oral creatine supplementation, creatine levels increased in the colon and cerebral cortex of rats with chronic colitis. Creatine could have improved this behavioral state by directly or indirectly inducing an anti-inflammatory effect in the brain. Neuroinflammation and microglial activation in the motor and prefrontal cortices prevented by creatine may be the mechanism underlying the disappearance of motor and mood alterations. Therefore, creatine can be used as adjuvant therapy with drugs for the management of comorbid ulcerative colitis conditions. We propose its use to protect against motor and mood-related disorders linked to colitis and considering sex differences in therapeutic strategies.

Author contributions

G. Sotelo-Parrilla and A. Ruiz-Calero performed the animal treatments and assessment of experimental colitis, and behavioral tests; P. García-Miranda performed PCR experiments and analyzed the data; M. L. Calonge analyzed the data and contributed to the preparation of the figures; M. D. Vázquez-Carretero analyzed the data, prepared the figures, contributed to the work design, and manuscript preparation. M. J. Peral analyzed the data, conceived, designed and supervised the work, and prepared the manuscript. All authors were involved in the drafting and revision of the manuscript and have read and agreed to the final version of the manuscript.

Conflicts of interest

The authors declare no competing interest.

Data availability

The datasets generated and/or analyzed during the current study will be made available in the institutional repository of the University of Seville (**https://hdl.handle.net/11441/32574**). In the meantime, they will be available from the corresponding author upon reasonable request.

SI: Oligonucleotides sequences used for reverse transcription-polymerase chain reaction assays. See DOI: **https://doi.org/10.1039/d5fo01620g**.

Acknowledgements

This work was supported by the I + D + I project PID2019-105632RB-I00, funded by MCIN/AEI/10.13039/501100011033 granted to M. J. Peral. Authors also thank for the financial support provided by 'VII Plan Propio de Investigación y Transferencia de la Universidad de Sevilla', Spain.

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