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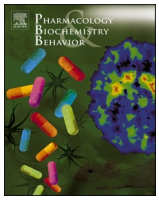

Sex differences in the antidepressant-like response and molecular events induced by the imidazoline-2 receptor agonist CR4056 in rats

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ABSTRACT

In searching for novel targets to design antidepressants, among the characterized imidazoline receptors (IR), I2 receptors are an innovative therapeutical approach since they are dysregulated in major depressive disorder and by classical antidepressant treatments. In fact, several I2 agonists have been characterized for their antidepressant-like potential, but the results in terms of efficacy were mixed and exclusively reported in male rodents. Since there are well-known sex differences in antidepressant-like efficacy, this study characterized the potential effects induced by two I2 drugs, CR4056 (i.e., most promising drug already in phase II clinical trial for its analgesic properties) and B06 (a compound from a new family of bicyclic α-iminophosphonates) under the stress of the forced-swim test in male and female rats exposed to early-life stress. Moreover, some hippocampal neuroplasticity markers related to the potential effects observed were also evaluated (i.e., FADD, p-ERK/ERK, mBDNF, cell proliferation: Ki-67 + cells). The main results replicated the only prior study reporting the efficacy of CR4056 in male rats, while providing new data on its efficacy in females, which was clearly dependent on prior early-life stress exposure. Moreover, B06 showed no antidepressant-like effects in male or female rats. Finally, CR4056 increased FADD content and decreased cell proliferation in hippocampus, without affecting p-ERK/t-ERK ratio and/or mBDNF content. Interestingly, these effects were exclusively observed in female rats, and independently of early-life conditions, suggesting some distinctive molecular underpinnings participating in the therapeutic response of CR4056 for both sexes. In conjunction, these results present CR4056 with an antidepressant-like potential, especially in female rats exposed to stress early in life, together with some neuronal correlates described in the context of these behavioral changes in females.

1. Introduction

The past, present, and future of imidazoline receptors (IRs) was recently reviewed by Bousquet et al. (2020), in the context of their possible involvement in different brain functions, especially the ones mediated through I2 receptors. In particular, the alteration of I2 receptors is not only linked with psychiatric disorders (i.e., major depressive disorders, García-Sevilla et al., 1996a, 1996b), but also I2 receptors are regulated by classical antidepressant treatments (García-Sevilla et al., 1999). The use of several I2 selective compounds has been key in describing the roles that this receptor plays in mediating some pharmacological effects. One recurrent discussion in the field has always been whether particular pharmacological effects induced by a specific I2 drug were truly mediated by I2 receptors (as discussed by Li, 2017). This was considered the case when the drug selectively bound to I2 receptors and the effects were blocked by idazoxan, the only known functional I2 receptor antagonist. In this context, several I2 selective agonists, which are capable of inducing analgesia in animal models of chronic pain

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(Ferrari et al., 2011; reviewed by Bousquet et al., 2020), inducing hypothermia and neuroprotection, while improving cognition in rodents (e.g., Griñán-Ferré et al., 2019; Abás et al., 2017, 2020; Vasilopoulou et al., 2020, 2021; Rodríguez-Arévalo et al., 2021), have shown mixed results in terms of their antidepressant-like potential in preclinical studies done in rodents under the stress of the forced-swim test. Some prior reports suggested the induction of antidepressant-like responses for several I2 agonists: 2-BFI in rats (Nutt et al., 1995; Hernández-Hernández and García-Fuster, 2021) and mice (Tonello et al., 2012), BU224 in rats (Finn et al., 2003), CR4056 in rats (Siemian et al., 2019), and MCR5 in a senescence-accelerated mouse model (Vasilopoulou et al., 2020). Contrarily, other studies reported negative data for several other I2 drugs: e.g., BU224, BU239, BDF 8082 in mice (O'Neill et al., 2001), 2-BFI or phenyzoline in rats (Siemian et al., 2019), LSL60101 (also known as Garsevil) in rats (Hernández-Hernández et al., 2021), tracizoline (known as LSL61122 or Valldemossine) in aged rats (Hernández-Hernández and García-Fuster, 2021). However, most of these prior studies, if not all, used only male rodents and did not consider sex as a biological variable (Miller et al., 2017; Beltz et al., 2019; Docherty et al., 2019) , which is extremely important, especially given the described sex differences in the efficacy of many antidepressants (e.g., LeGates et al., 2019; García-Cabrerizo et al., 2020; Ledesma-Corvi et al., 2022).

Therefore, in an attempt to gain knowledge on the potential sex differences in antidepressant-like responses induced by some I2 drugs, we further characterized the response of CR4056 (a compound that features an imidazoline ring), whose efficacy was already described for male rodents (see Siemian et al., 2019), since it is the most promising I2 agonist (i.e., phase II clinical trial for its analgesic properties, see for example Vellani et al., 2020). Moreover, this study also aimed at characterizing the potential antidepressant-like response induced by B06, a compound from a new family of bicyclic α-iminophosphonates recently depicted in terms of its structure (as compared to other I2 drugs including CR4056), its affinity for I2 receptors, its modulation over brain Fas-associated protein with death domain (FADD) and/or its agonistic beneficial effects in behavior and cognition in several murine models of Alzheimer's disease (see Abás et al., 2020; Vasilopoulou et al., 2021). These drugs were evaluated in the forced-swim test (Slattery and Cryan, 2012), both in naïve and early-life stressed rats as a mild model with the potential to mimic a negative impact at a preclinical level (see similar designs but for other antidepressants: García-Cabrerizo et al., 2020; Bis-Humbert et al., 2021a; Ledesma-Corvi et al., 2022).

In terms of the potential molecular markers behind the antidepressant-like effects of I2 drugs, and similarly to what was previously evaluated in the context of other I2 agonists (Garau et al., 2013; Griñán-Ferré et al., 2019; Abás et al., 2017, 2020; Hernández-Hernández et al., 2021; Rodríguez-Arévalo et al., 2021), the present study ascertained the regulation of FADD, a cell fate marker within the apoptotic pathway modulated by several classical antidepressant drugs (García-Fuster and García-Sevilla, 2016). Moreover, since members of the MAPK signaling (i.e., ERK1/2) showed a direct link in the regulation of FADD by certain pharmacological agents (e.g., García-Fuster et al., 2007), we

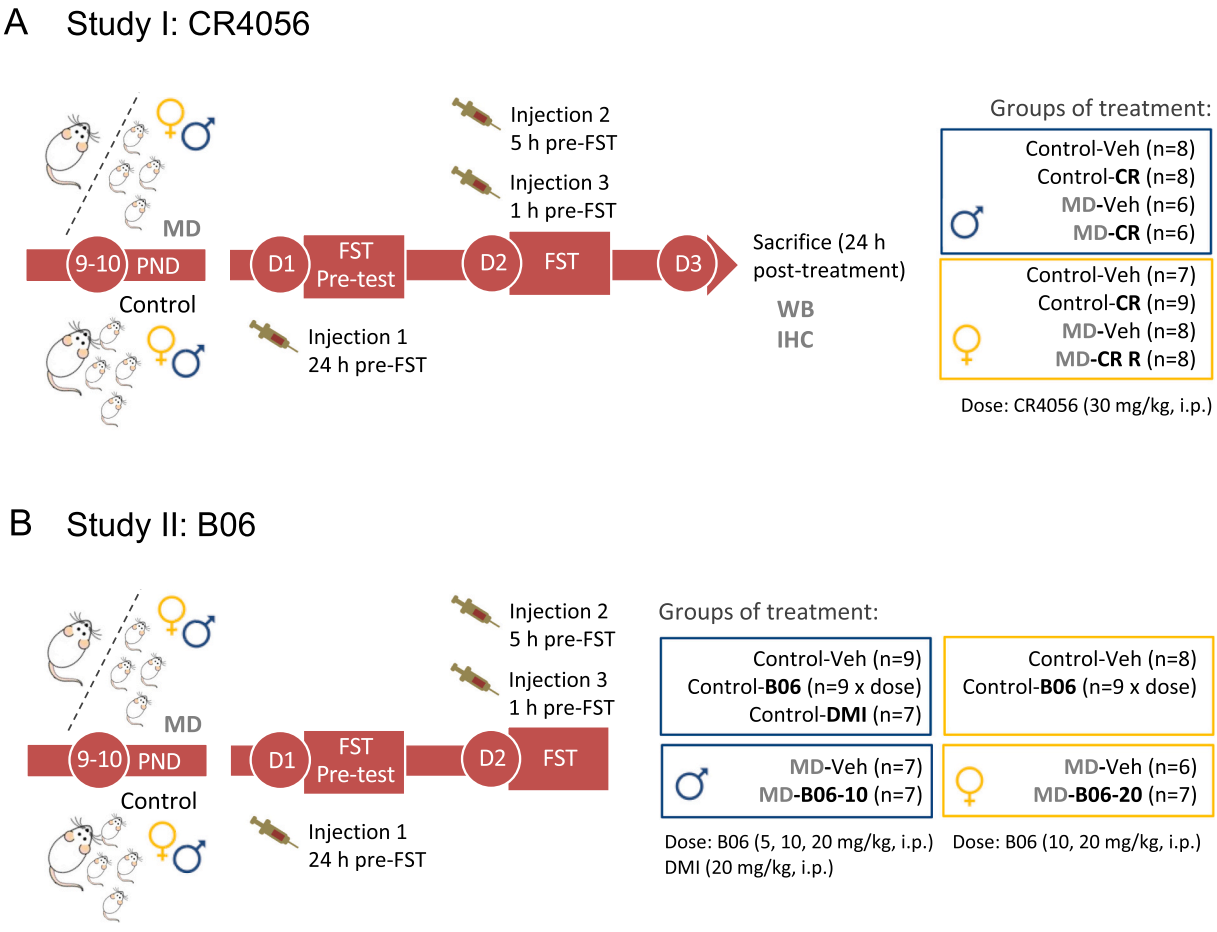

Fig. 1. Experimental design. **A** Study I: Characterization of the potential antidepressant-like effect of CR4056. **B** Study II: Characterization of the potential antidepressant-like effect of B06. For both studies, a total of 156 male and female rats were exposed in early-life to maternal deprivation (MD) or control condition. During adulthood, rats were drug- or vehicle-treated (3 pulses in 24 h, i.p.) and scored in the forced-swim test. Groups of treatment including the number of rats per group are shown for each drug and experimental condition under study. Abbreviations: CR, CR4056; D, day; DMI, desipramine; FST, forced-swim test; IHC, immunohistochemistry; MD, maternal deprivation; PND, post-natal day; Veh, vehicle; WB, Western blot.

also evaluated the regulation of p-ERK/t-ERK. Finally, mBDNF was evaluated as a key molecular marker mediating antidepressant-like actions (e.g., Diniz et al., 2018; Casarotto et al., 2021), together with the regulation of hippocampal cell proliferation (e.g., Numakawa et al., 2018), since hippocampus is a key region modulating affect (reviewed by Flores et al., 2022).

2. Material and methods

2.1. Animals

For this study, we utilized a total of 156 adult Sprague-Dawley rats (85 males and 71 females; Fig. 1) that were bred in the animal facility at the University of the Balearic Islands. Rats resided under standard housing conditions (12 h light/dark schedule, lights on at 8:00 AM; 22 °C, 70 % humidity) and unlimited access to a standard diet and water. Rats were given at least 1 week to acclimatize to the housing conditions and the handling prior to the actual experiments, which were performed during the light period. All procedures complied with the ARRIVE Guidelines (Percie du Sert et al., 2020), were approved by the competent authority responsible for ensuring compliance with the regulations governing the use of animals in scientific experiments as updated in 2010 (Directive 2010/63/EU) and the Spanish Royal Decree 53/2013 for animal experiments, and were awarded ethical approval by the Local Bioethical Committee and the Regional Government in the Balearic Islands (Spain). In an effort to avoid unnecessary stress in female rats during the experimental procedures, the specific stages of the estrous cycle were not examined. This was also justified by the fact that female rats are not more variable than their male counterparts in neuroscience research due to hormonal periodicity (e.g., Becker et al., 2016; Kaluve et al., 2022), but also because their cyclicity was not part of our research question (see Beltz et al., 2019).

2.2. Early-life conditions

As a way of inducing early-life stress conditions we relied on a well-defined paradigm of maternal separation that induces certain negative psychophysiological effects on rodents (Ellenbroek et al., 1998, 2005; Marco et al., 2009; also reviewed in Marco et al., 2015), but not necessarily a depressive-like phenotype (reviewed by Schmidt et al., 2011). Particularly, in our research group, we have reliably used this specific paradigm of maternal separation (24 h, post-natal day, PND, 9–10) with reproducibility among different waves of experiments and with hundreds of rats (García-Cabrerizo et al., 2020; Bis-Humbert et al., 2021a, 2021b; Bis-Humbert and García-Fuster, 2021; Ledesma-Corvi et al., 2022). In general, early-life stress modeled with maternal separation failed to reproduce baseline changes in affective-like behavior and locomotor activity, but proved differences in the effectiveness of selected antidepressant drug treatments (see Ledesma-Corvi et al., 2022), presenting itself as a suitable platform in which to characterize novel antidepressant options. Therefore, our experimental design exposed whole litters to a single episode of maternal deprivation (24 h, PND, 9–10), during which time pups were left in their home cage with no nutritional supplements, and the mother was placed in an adjacent cage. The weights of the pups were monitored right before and after maternal separation. Litters from the control groups received the same amount of handling, since they were weighed on PND 9 and 10, but were kept with the mother during the procedure. Rats were weaned at PND 22 and grouped by sex and early-life condition in cages of 2–4 rats.

2.3. Synthesis of the compounds CR4056 and B06

Compound CR4056 (6-(1*H*-imidazol-1-yl)-2-phenylquinazoline) was prepared following the synthetic sequence described in the patent by Giordani (2008). On the other hand, compound B06 (diethyl (1*RS*,3a*SR*,6a*SR*)-5-(3-chloro-4-fluorophenyl)-4,6-dioxo-1-phenyl-1,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrrole-1-phosphonate) was synthetized as described in Abás et al. (2020).

2.4. Pharmacological drug treatments

Study I aimed at characterizing the antidepressant-like response of CR4056 in control and maternally deprived male and female rats as described in Fig. 1A. The dose of CR4056 (30 mg/kg, i.p.) was selected based on a preliminary study from our group, in which this dose was capable of inducing hypothermia in male rats as measured 90 min post-treatment (data not shown), and from a prior published study (dose of 32 mg/kg: Thorn et al., 2012).

Study II, which aimed at characterizing the antidepressant-like response of another I2 drug, B06 (doses of 5, 10 or 20 mg/kg, i.p.; Abás et al., 2020) was performed in four independent experiments in which control or maternally deprived, male or female rats were evaluated separately (see groups of study in Fig. 1B). This was caused by animal availability and/or procedural logistics. Desipramine hydrochloride (DMI, 20 mg/kg, i.p.), was used as a positive control of the antidepressant-like response in either male (see García-Fuster and García-Sevilla, 2016) or both male and female rats (Ledesma-Corvi and García-Fuster, 2022). In an attempt to reduce the number of animal and/or experimental groups used, desipramine was not tested in maternally deprived rats, since it was not part of our research question. The goal was to use desipramine exclusively in male control rats as a positive control during the experiment, to ensure that a lack of response by the drugs under study was not due to something particular and/or methodological.

2.5. Forced-swim test

This test was first developed to assess acute antidepressant-like activity in rodents (Slattery and Cryan, 2012), and has been extensively used in our group to evaluate the antidepressant-like potential of several drugs (e.g., García-Fuster and García-Sevilla, 2016; Bis-Humbert et al., 2020, 2021a; Ledesma-Corvi et al., 2022; Ledesma-Corvi and García-Fuster, 2022), including I2 agonists (Hernández-Hernández et al., 2021; Hernández-Hernández and García-Fuster, 2021), as well as non-pharmacological treatment options (e.g., electroconvulsive treatment, see García-Cabrerizo et al., 2020), with reliable and reproducible results across time. Prior to any drug treatment, rats were individually forced to swim for 15 min (pre-test session, see Fig. 1) in cylinders (41 cm high × 32 cm diameter) filled with water (25 ± 1 °C) up to 25 cm in depth. Then, rats were treated with 3 pulses of the indicated drug and dose of study (CR4056, B06 or DMI; see Fig. 1) 24, 5 and 1 h before a 5 min test session that was videotaped (see Fig. 1). Rats were dried off with a paper towel at the end of each swimming session, before returning them to their cage. Later on, each video was analyzed with the software Behavioral Tracker (CA, USA) by an experimenter blind to the treatment conditions that scored the time spent (s) immobile vs. active (climbing or swimming) for each rat. It is important to remember that the forced-swim test is a reliable test to measure antidepressant-like responses, but not necessarily to phenotype a depressive-like response (reviewed by Armario, 2021), and thus it might not be reliable to detect basal differences in affective-like behavior when comparing maternally deprived vs. control rats.

2.6. Brain sample collection and neurochemical evaluations

Brains were collected for the experimental groups in which an antidepressant-like response was observed (i.e., CR4056) by rapid decapitation 24 h post-treatment (see Fig. 1A). The regulation of the selected neuroplasticity markers was evaluated by Western blot analysis in the right hippocampus, which was freshly dissected. To do so, total homogenates were prepared as previously described (e.g., García-Cabrerizo et al., 2015), and brain proteins (40 μg) were separated by

electrophoresis on 10–12 % SDS-PAGE mini-gels (Bio-Rad Laboratories, CA, USA). Membranes were incubated with the appropriate primary antibody: anti-FADD (H-181) (1:2500) and anti-mBDNF (1:2500) were both from Santa Cruz Biotechnology (CA, USA); anti-p-ERK1/2 (p44/p42) (1:1000) and anti-t-ERK1/2 (1:1000), from Cell Signaling (MA, USA); and anti-β-actin (clone AC-15) (1:10000; Sigma–Aldrich, MO, USA). The next day, membranes were incubated with the secondary antibody (anti-rabbit or -mouse IgG linked to horseradish peroxidase; 1:5000; Cell Signaling), and immunoreactivity was detected with ECL reagents (Amersham, Buckinghamshire, UK) in autoradiographic films (Amersham ECL Hyperfilm), which were then quantified by densitometric scanning (GS-800 Imaging Calibrated Densitometer, Bio-Rad). For each marker and rat, we calculated percent changes in immunoreactivity with respect to male-control-vehicle samples (100 %) in various gels, and the mean value was used as a final estimate. β-actin was used as a loading control as it did not change with the treatment conditions.

The left half-brain was snap-frozen with isopentane (−30 °C) and stored at −80 °C until hippocampal cell proliferation was quantified with Ki-67 antibody (1:20,000; provided by Drs. Huda Akil and Stanley J. Watson, University of Michigan, MI, USA) by immunohistochemistry (e.g., García-Fuster et al., 2010; Garcia-Fuster et al., 2017; García-Cabrerizo et al., 2020). Briefly, experiments were performed in 1 slide per rat containing 8 tissue-sections from the middle portion of hippocampus that were cryostat-cut (30 μm sections), slide-mounted and post-fixed in 4 % paraformaldehyde. The next steps included a series of sequential incubations, including that with a biotinylated anti-rabbit antibody (1:1000 respectively, Vector Laboratories, CA, USA), an Avidin/Biotin complex (Vectastain Elite ABC kit; Vector Laboratories), and the chromogen 3,3′-diaminobenzidine (DAB) for signal detection. Finally, tissue was counterstained in cresyl violet, dehydrated in graded alcohols and immersed in xylene before cover-slipping it with Permount®. Positive cells were quantified in 8 sections/rat by an experimenter blind to the treatment groups with a Leica DMR light microscope (63× objective lens) and focusing through the thickness of the tissue. The quantified area (mm^2) in each section was measured with a densitometer (GS-800 Imaging Calibrated Densitometer, BioRad) to correct the total number of proliferating cells (Ki-67+) in the dentate gyrus by the total area analyzed (mm^2) as previously described (e.g., García-Cabrerizo et al., 2015; Garcia-Fuster et al., 2017).

2.7. Data analysis

GraphPad Prism, Version 9.5 (GraphPad Software, USA) was used for data analysis and graph plotting was performed following the guidelines in experimental pharmacology for displaying data and statistical methods (e.g., Curtis et al., 2018; Michel et al., 2020). Results are reported in bar graphs as mean values ± standard error of the mean (SEM), with symbols shown for each individual rat. For Study I, and depending on the presence of sex differences, data was either analyzed with three-way ANOVAs (independent variables: Sex, Early-life Condition, Treatment) or two-way ANOVAs (independent variables: Early-life Condition, Treatment) for each sex separately followed by multiple comparisons tests to perform *post-hoc* pair-wise statistical evaluations when appropriate. For Study II, and given that each sex and early-life experimental condition was tested in separate experiments, statistical analysis were performed through one-way ANOVAs (followed by Dunnett's multiple comparisons test when appropriate) or unpaired two-tailed *t*-tests depending on the number of groups to compare. Significance was set at $p \leq 0.05$.

3. Results

3.1. Characterizing the potential antidepressant-like effect of CR4056 in rats (Study I)

When analyzing the results obtained in the forced-swim test through three-way ANOVAs (Fig. 2A–C), specific sex differences emerged that conditioned and/or justified the subsequent analysis for each sex separately. In particular, there were sex differences in immobility ($F_{1,52} = 27.11$, $###p < 0.001$; Fig. 2A) and climbing ($F_{1,52} = 27.66$, $###p < 0.001$) behavior, as measured 1 h after the last treatment injection. Female rats showed higher immobility (mean of 199 s vs. 133 s for males; Fig. 2A), lower climbing (mean of 79 s vs. 144 s for males; Fig. 2B), but no change in the time spent swimming (mean of 21 for both sexes; Fig. 2C).

Therefore, the antidepressant-like effects induced by CR4056 in the forced-swim test were evaluated 1 h after the administration of 3× pulses given in a 24-h period for each sex separately. In male rats, a two-way ANOVA showed a significant effect of Early-Life Condition ($F_{1,24} = 4.90$, $p = 0.037$) and of Treatment ($F_{1,24} = 9.11$, $p = 0.006$), but no interaction ($F_{1,24} = 0.01$, $p = 0.944$). In particular, rats exposed to maternal deprivation showed lower rates of immobility than controls (general drop of −42 ± 19 s, MD vs. Control groups, Fig. 2A), and CR4056 induced an antidepressant-like effect independently of early-life condition (drop in immobility of −57 ± 19 s vs. Veh-treated groups, Fig. 2A). Interestingly, in females, besides the significant effect of Early-Life Condition ($F_{1,28} = 5.70$, $p = 0.024$) and Treatment ($F_{1,28} = 27.56$, $p < 0.001$), there was a significant interaction between Early-Life Condition x Treatment ($F_{1,28} = 4.60$, $p = 0.041$). *Post-hoc* comparisons (Tukey's multiple test) revealed that CR4056 induced an antidepressant-like effect in maternally-deprived rats (−125 ± 24 s immobile, $***p < 0.001$ vs. MD-Veh-treated rats; Fig. 2A), but also when comparing CR4056 effects depending on the prior Early-Life Condition, since it showed a higher efficacy in rats previously exposed to maternal deprivation (−77 ± 23 s immobile, $\$p = 0.013$ vs. Control-CR; Fig. 2A).

We later evaluated what active behavior was behind the changes observed in immobility. In male rats, there was a significant effect of Treatment ($F_{1,24} = 7.99$, $p = 0.009$), but no effect of Early-Life Condition ($F_{1,24} = 4.08$, $p = 0.055$) or interaction ($F_{1,24} = 0.16$, $p = 0.690$). Therefore, the antidepressant-like effect of CR4056 in male rats was driven by an increase in climbing (+52 ± 18 s, CR vs. Veh-treated groups, Fig. 2B). In females, besides the significant effect of Treatment ($F_{1,28} = 26.67$, $p < 0.001$), there was a significant interaction between Early-Life Condition x Treatment ($F_{1,28} = 4.00$, $p = 0.05$), but no effect of Early-Life Condition ($F_{1,28} = 3.51$, $p = 0.071$). *Post-hoc* comparisons (Tukey's multiple test) revealed that CR4056 induced an antidepressant-like effect in maternally-deprived rats by increasing climbing (+121 ± 24 s, $***p < 0.001$ vs. MD-Veh-treated rats; Fig. 2B), but also when comparing CR4056 effects depending on the prior Early-Life Condition, since it showed a higher efficacy in rats previously exposed to maternal deprivation (+66 ± 23 s, $\$p = 0.040$ vs. Control-CR; Fig. 2B). Finally, no relevant changes were observed for swimming behavior (Fig. 2C).

3.2. Characterizing the potential antidepressant-like effect of B06 in rats (Study II)

The effects of B06 in the forced-swim test were evaluated, as mentioned earlier, in separate experiments (control rats separately than maternally-deprived, and male separately than female rats). Therefore, the potential effects were evaluated for each experiment separately. Particularly, B06 at the doses tested did not induce an antidepressant-like effect as measured through immobility time in male control (one-way ANOVA: $F_{3,32} = 1.28$, $p = 0.300$) or maternally-deprived ($t = 1.05$, $df = 12$, $p = 0.314$; Fig. 2D) rats. Similarly, no effects were observed for female control (one-way ANOVA: $F_{2,23} = 2.00$, $p = 0.158$) or maternally deprived ($t = 0.88$, $df = 11$, $p = 0.396$; Fig. 2D) rats. Moreover, when evaluating activity time, no significant changes were observed for climbing or swimming behaviors both for male or female rats (statistical analyses not shown; see Fig. 2E and F). As a positive control, we used desipramine at a dose known, in our experimental conditions, to induce a significant antidepressant-like effect for male (García-Fuster and García-Sevilla, 2016) or for both male and female rats (Ledesma-Corvi

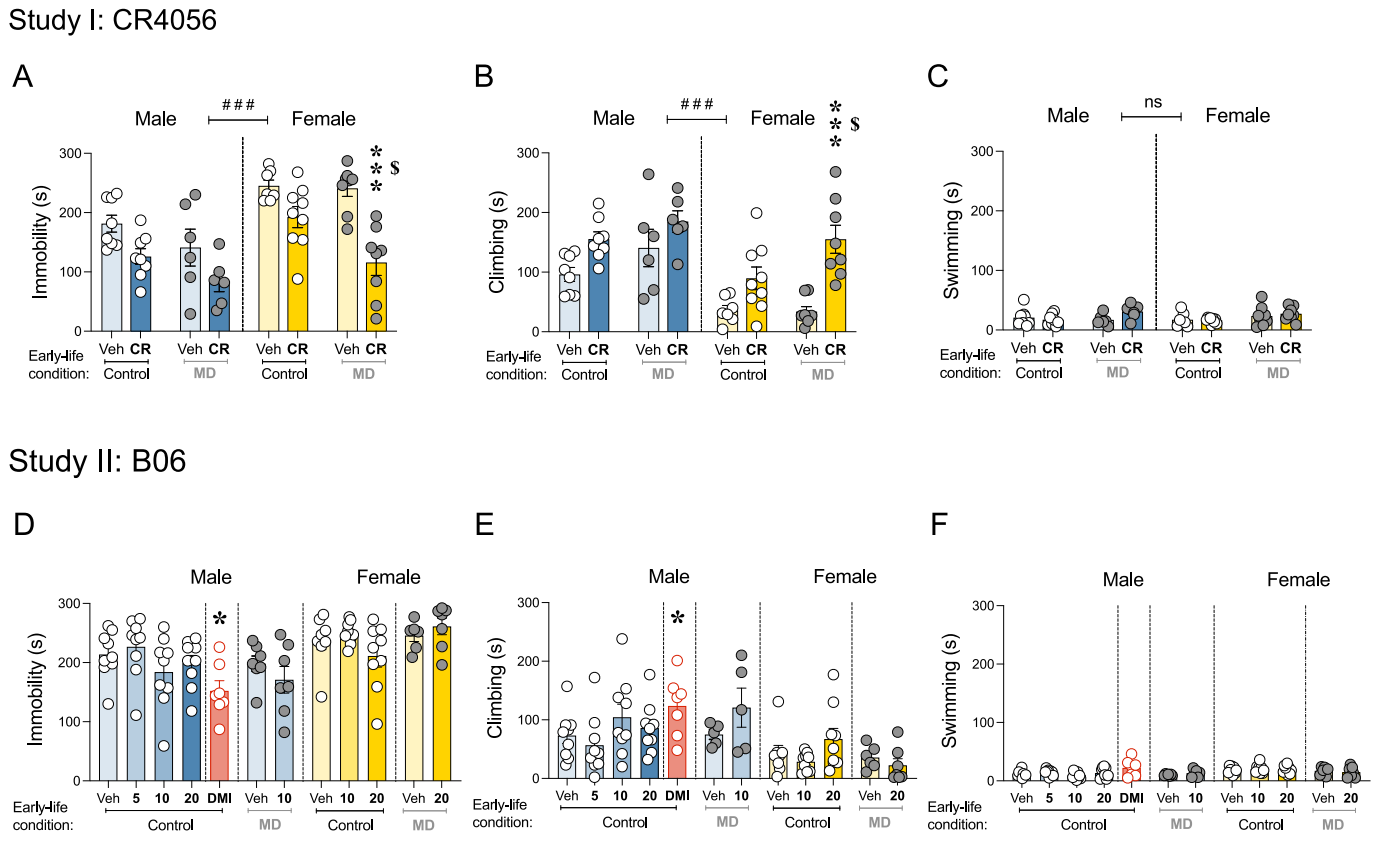

Fig. 2. **A–C** Study I: Characterization of the potential antidepressant-like effect of CR4056. **D–F** Study II: Characterization of the potential antidepressant-like effect of B06. Antidepressant-like responses were measured in the forced-swim test after treatment in male and female rats with or without early-life stress (maternal deprivation). Columns represent mean ± SEM of the time spent (*s*) **A, D** immobile, **B, E** climbing or **C, F** swimming (individual symbols are shown for each rat). Three-way ANOVAs were used to detect sex differences: $###p < 0.001$. Antidepressant-like effects were evaluated for each sex separately by two-way ANOVAs followed by Tukey's multiple comparisons test when appropriate: $*p < 0.05$ when compared with Control-Veh-treated rats, $***p < 0.001$ when compared with MD-Veh-treated rats, and $\$p < 0.05$ when compared with Control-CR-treated rats. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and García-Fuster, 2022). As expected desipramine induced a positive response by decreasing immobility ($t = 2.85$, $df = 14$, $*p = 0.013$ vs. Veh-treated rats; Fig. 2D) and increasing climbing ($t = 2.21$, $df = 14$, $*p = 0.045$ vs. Veh-treated rats; Fig. 2E), while no changes were induced on swimming behavior (Fig. 2F).

3.3. Characterizing the molecular effects of CR4056 in rats (Study I)

Since CR4056 showed a clear effect on antidepressant-like behaviors, we wanted to further study this effect on a molecular level. The analysis of the molecular events induced by CR4056 through three-way ANOVAs (Fig. 3A–E), disclosed basal sex differences in the hippocampal protein content of FADD ($F_{1,51} = 41.63$, $###p < 0.001$; Fig. 3A) and mBDNF ($F_{1,51} = 9.36$, $##p = 0.004$; Fig. 3C), but not of p-ERK/t-ERK ratio ($F_{1,39} = 0.28$, $p = 0.603$; Fig. 3B) nor for the rate of cell proliferation (Ki-67 + cells/mm^2: $F_{1,52} = 3.37$, $p = 0.072$; Fig. 3E). Based on some sex differences, the subsequent analyses were performed for each sex separately.

While in male rats no significant effects of Early-Life Condition or Treatment were observed for FADD modulation (statistical analyses not shown; see Fig. 3A), there was a significant effect of Treatment in female rats ($F_{1,27} = 7.37$, $p = 0.011$; Fig. 3A). In particular, CR4056 induced an increase in hippocampal FADD content in females independently of early-life condition (+21 ± 8 % vs. Veh-treated groups, Fig. 3A). When analyzing p-ERK/t-ERK ratio or mBDNF protein levels in hippocampus, no significant effects were observed by Early-Life Condition or Treatment both for male or female rats (statistical analyses not shown; see Fig. 3B and Fig. 3C). Similar to what was observed for FADD, when analyzing the number of Ki-67 + cells/area, there were no effects of Early-Life Condition or Treatment for male rats, but a significant effect of Treatment for female rats ($F_{1,28} = 8.45$, $p = 0.0071$); CR4056 induced a decrease in cell proliferation in females independently of early-life condition (−6.8 ± 2.3 + cells/mm^2 vs. Veh-treated groups, Fig. 3E). This experiment was validated with a second immunohistochemistry study that reported identical results.

4. Discussion

The main results of the study reinforced, along with prior literature, the importance of including both sexes when characterizing pharmacological agents at the preclinical level. Indeed, male and female rats differed basally in the response induced in some behavioral tests, conditioning and/or justifying the subsequent analysis for each sex separately. For example, in the forced-swim test, female rats were more immobility and less active (i.e., climbing) than their male counterparts. Some basal differences were also observed in the expression of certain neurochemical markers (i.e., females showed higher hippocampal FADD and mBDNF protein content). As for the potential antidepressant-like effects of the drugs tested in the forced-swim test, the results replicated the only prior study reporting the efficacy of CR4056 in male rats, while providing some new data on its efficacy in female rats, which was clearly dependent on prior early-life stress exposure. However, B06 showed no antidepressant-like effects in male or female rats. Finally, CR4056 increased levels of FADD protein content and decreased cell proliferation in hippocampus as measured 24 h post-treatment, without affecting p-ERK/t-ERK ratio and/or mBDNF content. Interestingly, these effects were exclusively observed in female rats, and independently of

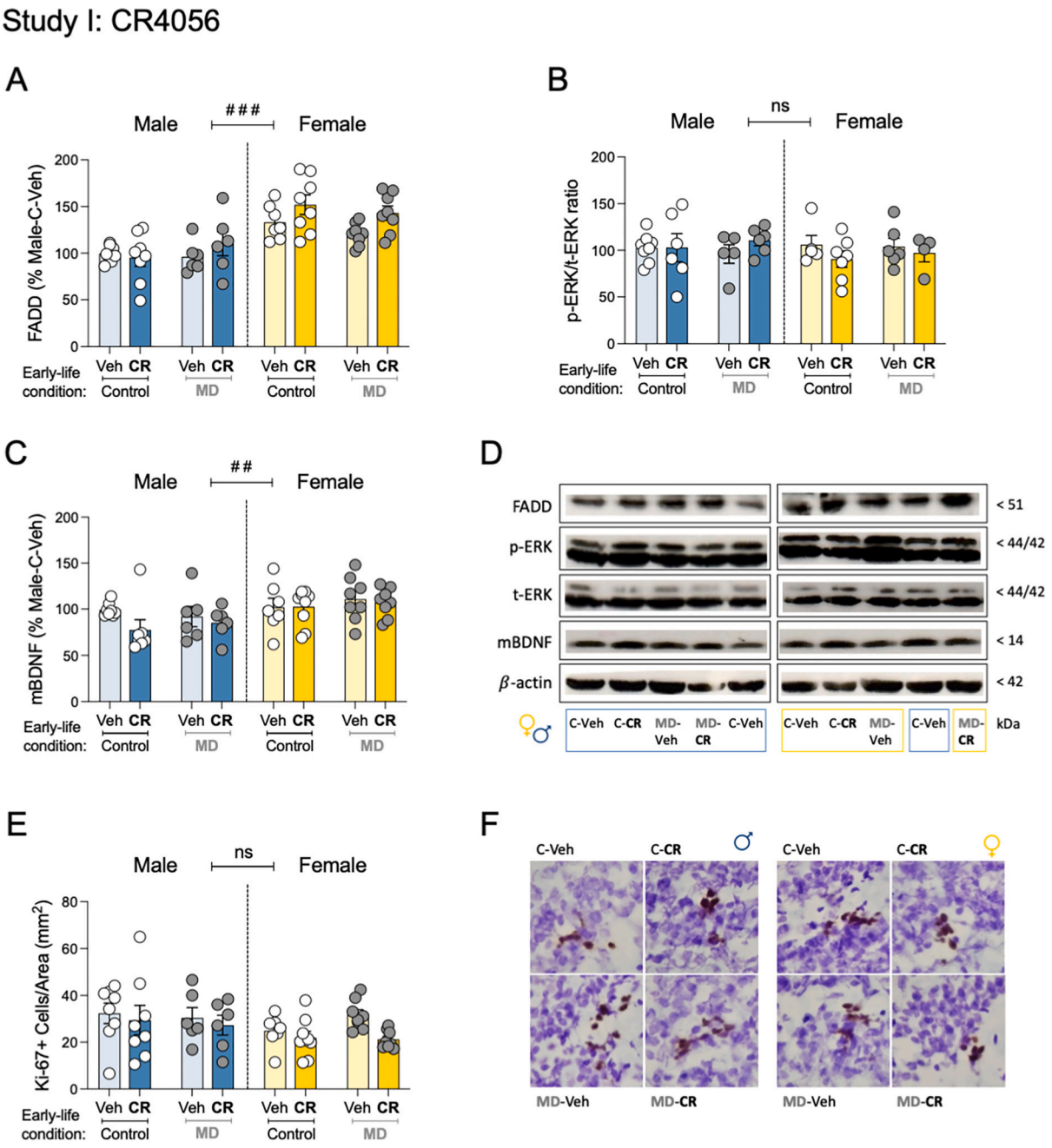

Fig. 3. Study I: Characterization of the molecular effects of CR4056. **A–D** Changes in the modulation of hippocampal proteins were measured by western blot 24 h after treatment in male and female rats with or without early-life stress (maternal deprivation). **A** FADD, **B** p-ERK/t-ERK ratio, and **C** mBDNF proteins. Columns represent mean ± SEM of n experiments per group and expressed as % Male-Control-Vehicle (individual values are shown for each rat in symbols). Three-way ANOVAs were used to detect sex differences $##p < 0.01$ and $###p < 0.001$. A two-way ANOVA detected a significant increase in FADD content for female rats treated with CR4056. No other changes were observed. **D** Representative immunoblots depicting labeling of FADD, p-ERK, t-ERK, mBDNF and the corresponding for β-actin as a loading control. **E–F** Changes in hippocampal cell proliferation were measured by immunohistochemistry 24 h after treatment in male and female rats with or without early-life stress (maternal deprivation). Columns represent mean ± SEM of Ki-67+ cells/area (mm^2) (individual symbols are shown for each rat). No statistical differences were detected. **F** Representative images showing individual Ki-67 + cells (brown labeling in the blue granular layer) taken with a light microscope (63× objective lens). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

early-life conditions, suggesting some distinctive molecular underpinnings taking place right after the therapeutic response of CR4056 for both sexes.

The antidepressant-like effects induced by CR4056 in the forced-swim test were evaluated (1 h after the administration of 3× pulses given in a 24-h period) for each sex separately, given the clear basal sex differences described in behavior in the present study and as previously reported (see for example Ledesma-Corvi et al., 2022 for our prior results). In male rats, CR4056 induced an antidepressant-like effect independently of early-life condition (i.e., decreased immobility and increased climbing). An improvement in the test through climbing behavior was previously reported for other I2 agonists (e.g., Hernández-Hernández and García-Fuster, 2021), in line with the expected increase in brain noradrenaline produced by these drugs known to mediate some of their responses (e.g., Ferrari et al., 2011; Siemian et al., 2018), and similarly to antidepressants that enhance noradrenaline

neurotransmission (see Detke et al., 1995, 1997; Rénéric et al., 2002). Although it was suggested that I2 receptor activation might stimulate locomotion activity in rats (Barrot et al., 2000), prior studies reported, at a dose-range that include the dose tested in the present study, either no changes in the locomotor response induced by CR4056 in the open field in Wistar rats (Ferrari et al., 2011) or 5xFAD mice (Mota et al., 2022), or even a decrease in locomotion in Sprague-Dawley rats (Thorn et al., 2012). These prior data ensured that the increased activity observed in the forced-swim test by CR4056 was not due to potential increases in locomotor activity. Interestingly, CR4056 also induced an antidepressant-like effect in female rats by decreasing immobility and increasing climbing in the forced-swim test. However, this beneficial effect was mainly driven by how it interacted with early-life stress exposure, since efficacy was higher in females with a prior exposure to maternal deprivation; the significant effect was observed both when comparing the response vs. maternally deprived vehicle-treated rats, or vs. control CR4056-treated rats. This interaction aligned with prior studies demonstrating that certain beneficial effects of I2 agonists needed a prior insult to be effective (e.g., CR4056 improved cognition only in 5xFAD but not in wild-type mice; Mota et al., 2022) and/or with the effects described for other antidepressant drugs (i.e., low dose of ketamine) only observed in female rats exposed to early-life stress (Ledesma-Corvi et al., 2022). Therefore, together with the prior study that characterized the effects induced by CR4056 in the forced-swim test, but exclusively in male reserpinized rats (dose of 32 as opposed to 10 mg/kg that was not efficacious; Siemian et al., 2019), our data validated the antidepressant-like potential for this drug (dose of 30 mg/kg), while including both sexes, and suggested a better efficacy for females, especially in the presence of a previous early-life stressor. The fact that CR4056 worked so well for females is of relevance, since most antidepressants previously tested failed to show efficacy in females when scored in the forced-swim test at doses that would otherwise work for males (e.g., García-Cabrerizo et al., 2020; Ledesma-Corvi et al., 2022), and given the lack of knowledge regarding the effects of I2 agonists on female rats.

Contrarily to the potential beneficial effects of CR4056, another I2 drug from a new structural family of bicyclic α-iminophosphonates (B06; see Abás et al., 2020 for its structural characterization, and also Fig. 4) did not induce signs of an antidepressant-like potential, at the doses tested, and as measured through immobility time in the forced-swim test in male or female rats (both for control or maternally-deprived conditions). Since no prior studies have been done attempting to characterize this family of new compounds in terms of their antidepressant-like potential, a positive control was evaluated in parallel. In particular, desipramine at a dose known to induce, in prior studies from our group and with equivalent experimental conditions, a significant antidepressant-like response of similar magnitude both in male (García-Fuster and García-Sevilla, 2016) and female rats (Ledesma-Corvi and García-Fuster, 2022), also showed decreased immobility in the forced-swim test paired with increased climbing. The present lack of antidepressant-like potential of B06 aligned with prior data, in terms that not all I2 drugs are capable of displaying antidepressant-like responses (e.g., O'Neill et al., 2001; Siemian et al., 2019; Hernández-Hernández et al., 2021). The differences in antidepressant-like responses observed for I2 agonists might rely, as previously discussed (Hernández-Hernández et al., 2021) in their chemical structures. For example, as shown in Fig. 4, CR4056 features the atom of the nitrogen of the imidazole linked to the rest of the heteroaromatic ring, while LSL60101 embodies a 2-imidazolebenzofuranyl, and 2-BFI a 2-imidazolinebenzofuranyl (imidazoline is a nucleus with one of the double bonds of the imidazole reduced) (Nutt et al., 1995; Tonello et al., 2012). However, B06 does not contain the imidazole/imidazoline nucleus (Fig. 4). As mentioned in a prior study (Abás et al., 2020), the structure of B06 differed from the restricted substitution pattern of I2-IR drugs, with its bicyclic-iminophosphonate structure being the first described non-imidazoline/non-imidazole- containing compound, showing outstanding affinity to I2-IR. In fact, a 3D-QSAR study revealed key structural parameters for the design of future promising structures, proposing a pharmacophore for certain standard agonists (i.e., LSL60101, tracizoline, idazoxan and BU99008; see Abás et al., 2020). A prior study reported that there are differences in efficacy among existing I2 receptor agonists (e.g., Qiu et al., 2015), suggesting that the limited efficacy induced by B06 on I2 receptors might be insufficient to produce an antidepressant-like response in this particular model. Interestingly, this lack of efficacy might not be generalized, since B06 has proven to cross the blood brain barrier in sufficient quantities to induce pharmacological effects, both behavioral and neurochemical (Abás et al., 2020). In fact, B06 levels correlated between plasma and the brain when the drug was administered at a dose of 10 mg/kg (Bagán et al., 2022), a dose within the range tested in the present study (5–20 mg/kg). Particularly, SAMP8 mice treated with 5 mg/kg of B06 during 3 weeks showed improved cognitive rates through a neuronal pathway (Vasilopoulou et al., 2021), and B06 was capable of inducing behavioral improvements at a dose of 5 mg/kg in a 5xFAD mouse model (Abás et al., 2020).

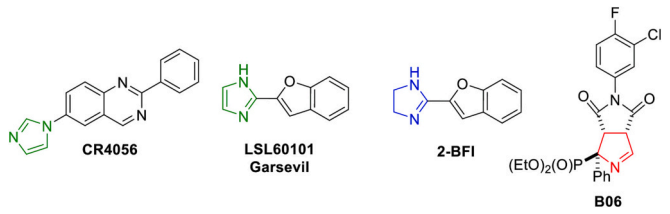

Fig. 4. Chemical structure of CR4056, LSL60101, 2-BFI and B06 (2-imidazole nucleus in green, 2-imidazoline in blue, and 3,4-dihydro-2*H*-pyrrole in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The molecular events induced by CR4056 as measured 24 h post-treatment also revealed sex differences in the basal content of some of the markers evaluated in hippocampus (i.e., FADD and mBDNF), while others showed similar contents for both sexes (i.e., p-ERK/t-ERK ratio, Ki-67 + cells). Interestingly, the only changes induced by CR4056 were observed exclusively in female rats, and independent of early-life conditions. Particularly, FADD was increased and cell proliferation was decreased in hippocampus 24 h after treatment. No changes were observed in p-ERK/t-ERK ratio or mBDNF protein levels in hippocampus by CR4056 in rats. Preliminary non-published data from our group proved that a single dose of 30 mg/kg of CR4056 induced hypothermia (90 min post-treatment) while reduced hippocampal FADD in male rats as measured 2 h post-treatment, similarly to most I2 agonists previously evaluated (e.g., Abás et al., 2017, 2020; Rodríguez-Arévalo et al., 2021). These acute effects reverted when measured 24 h post-treatment (Abás et al., 2020), in line to what we report here for CR4056 in male rats. The higher content of hippocampal FADD in female rats, as compared to males, was expected since it was previously reported (e.g., Ledesma-Corvi and García-Fuster, 2022; ; as well as other unpublished data from our group). However, this is the first time that we measured FADD in hippocampus of female rats following the administration of an I2 drug. Interestingly, the results demonstrated increased FADD levels 24 h post-treatment in adult female rats treated with CR4056; sex-specific effects that emerged after the observed antidepressant-like response. This pattern of FADD regulation was previously observed for other antidepressants (García-Fuster and García-Sevilla, 2016), including desipramine (Ledesma-Corvi and García-Fuster, 2022). It was suggested, as it could be speculated for CR4056, that following an acute antidepressant administration, certain neuroprotective actions might be initiated that cause a decrease in FADD (1 or 2-h post-treatment), to latter increase its content (1-day post-treatment, i.e., rebound effect after the acute initiation of an anti-apoptotic response, see prior similar results by García-Fuster et al., 2009), followed by a normalization of all levels 5-days post-treatment (e.g., Ledesma-Corvi and García-Fuster, 2022). Similarly,

earlier studies showed that while a single drug injection with other I2 drugs decreased FADD in hippocampus (Abás et al., 2017, 2020; Rodríguez-Arévalo et al., 2021), repeated (5 days) administration showed no changes on FADD (Abás et al., 2020), in line with the lack of effects observed for male rats in the present study (3 pulses in a 24 h period) and the ones observed in other brain regions (i.e., cerebral cortex, see Garau et al., 2013).

Finally, CR4056 decreased cell proliferation in hippocampal samples of female rats as measured 24 h post-treatment, in conjunction with FADD increases, and in line with a prior study that reported increased FADD (both at protein and mRNA levels) with impaired cell proliferation rates (Ki-67+ mitotic progenitor cells; García-Fuster et al., 2011). In that study, the decrease in cell proliferation was speculated to be associated with a neurotoxic effect induced by cocaine, although, in line with the prior discussion on FADD, it could also be justfied by an adaptive response following a putative speculated acute increase. In this context, our initial hypothesis was to find increased proliferation levels since most antidepressants increase neurogenesis (see reviews over the past 20 years by Duman et al., 2001 and Kot et al., 2022) but also mBDNF (e.g., Diniz et al., 2018; Casarotto et al., 2021), which was also unaltered. However, we did not collect samples when the antidepressant-like effect was observed (1 h post-treatment), which probably, in line with the previous literature, would show an acute increase in cell proliferation and mBDNF. Also, most of these markers were previously evaluated almost exclusively for male rodents, and thus the lack of detailed molecular data on female brains together with the discrepancies observed in the neurochemical events taking place for this sex might still justify some level of neuroplasticity associated and/or emerging after the positive behavioral response observed.

In conjunction, these results present CR4056, an I2 agonist, as a potential candidate to be further studied as an antidepressant, especially in female rats in which their condition might be stress-related. Moreover, some neuronal correlates were dysregulated right after these behavioral changes in females, which will require a deeper analysis in terms of their meaning and potential targeting for the development of future drugs.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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References

Abás, S., Erdozain, A.M., Keller, B., Rodríguez-Arévalo, S., Callado, L.F., García-Sevilla, J. A., Escolano, C., 2017. Neuroprotective effects of a structurally new family of high affinity imidazoline I2 receptor ligands. ACS Chem. Neurosci. 8, 737–742.

Abás, S., Rodríguez-Arévalo, S., Bagán, A., Griñán-Ferré, C., Vasilopoulou, F., Brocos-Mosquera, I., Muguruza, C., Pérez, B., Molins, E., Luque, F.J., Pérez-Lozano, P., de Jonghe, S., Daelemans, D., Naesens, L., Brea, J., Loza, M.I., Hernández-Hernández, E., García-Sevilla, J.A., García-Fuster, M.J., Radan, M., Djikic, T., Nikolic, K., Pallàs, M., Callado, L.F., Escolano, C., 2020. Bicyclic α-iminophosphonates as high affinity imidazoline I2 receptor ligands for Alzheimer'sdisease. J. Med. Chem. 63, 3610–3633.

Armario, A., 2021. The forced swim test: historical, conceptual and methodological considerations and its relationship with individual behavioral traits. Neurosci. Biobehav. Rev. 128, 74–86.

Bagán, A., Morales-García, J.A., Griñán-Ferré, C., Díaz, C., Pérez del Palacio, J., Ramos, M.C., Vicente, F., Pérez, B., Brea, J., Loza, M.I., Pallàs, M., Escolano, C., 2022. Insights into the pharmacokinetics and in vitro cell-based studies of the imidazoline I2 receptor ligand B06. Int. J. Mol. Sci. 23, 5408.

Barrot, M., Rettori, M.C., Guardiola-Lemaitre, B., Jarry, C., Le Moal, M., Piazza, P.V., 2000. Interactions between imidazoline binding sites and dopamine levels in the rat nucleus accumbens. Eur. J. Neurosci. 12, 4547–4551.

Becker, J.B., Prendergast, B.J., Liang, J.W., 2016. Female rats are not more variable than male rats: a meta-analysis of neuroscience studies. Biol. Sex Differ. 7, 34.

Beltz, A.M., Beery, A.K., Becker, J.B., 2019. Analysis of sex differences in pre-clinical and clinical data sets. Neuropsychopharmacology 44, 2155–2158.

Bis-Humbert, C., García-Fuster, M.J., 2021. Adolescent cocaine induced persistent negative affect in female rats exposed to early-life stress. Psychopharmacology 238, 3399–3410.

Bis-Humbert, C., García-Cabrerizo, R., García-Fuster, M.J., 2020. Decreased sensitivity in adolescent versus adult rats to the antidepressant-like effects of cannabidiol. Psychopharmacology 237, 1621–1631.

Bis-Humbert, C., García-Cabrerizo, R., García-Fuster, M.J., 2021a. Antidepressant-like effects of cannabidiol in a rat model of early-life stress with or without adolescent cocaine exposure. Pharmacol. Rep. 73, 1195–1202.

Bis-Humbert, C., García-Cabrerizo, R., García-Fuster, M.J., 2021b. Increased negative affect when combining early-life maternal deprivation with adolescent, but not adult, cocaine exposure in male rats: regulation of hippocampal FADD. Psychopharmacology 238, 411–420.

Bousquet, P., Hudson, A., García-Sevilla, J.A., Li, J.X., 2020. Imidazoline receptor system: the past, the present, and the future. Pharmacol. Rev. 72, 50–79.

Casarotto, P.C., Girych, M., Fred, S.M., Kovaleva, V., Moliner, R., Enkavi, G., Biojone, C., Cannarozzo, C., Sahu, M.P., Kaurinkoski, K., Brunello, C.A., Steinzeig, A., Winkel, F., Patil, S., Vestring, S., Serchov, T., Diniz, C.R.A.F., Laukkanen, L., Cardon, I., Antila, H., Rog, T., Piepponen, T.P., Bramham, C.R., Normann, C., Lauri, S.E., Saarma, M., Vattulainen, I., Castrén, E., 2021. Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. Cell 184, 1299–1313.

Curtis, M.J., Alexander, S., Cirino, G., Docherty, J.R., George, C.H., Giembycz, M.A., Hoyer, D., Insel, P.A., Izzo, A.A., Ji, Y., MacEwan, D.J., Sobey, C.G., Stanford, S.C., Teixeira, M.M., Wonnacott, S., Ahluwalia, A., 2018. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br. J. Pharmacol. 175, 987–993.

Detke, M.J., Rickels, M., Lucki, I., 1995. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology 21, 66–72.

Detke, M.J., Johnson, J., Lucki, I., 1997. Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. Exp. Clin. Psychopharmacol. 5, 107–112.

Diniz, C.R.A.F., Casarotto, P.C., Resstel, L., Joca, S.R.L., 2018. Beyond good and evil: a putative continuum-sorting hypothesis for the functional role of proBDNF/BDNF-propeptide/mBDNF in antidepressant treatment. Neurosci. Biobehav. Rev. 90, 70–83.

Docherty, J.R., Stanford, S.C., Panattieri, R.A., Alexander, S.P.H., Cirino, G., George, C. H., Hoyer, D., Izzo, A.A., Ji, Y., Lilley, E., Sobey, C.G., Stanley, P., Stefanska, B., Stephens, G., Teixeira, M., Ahluwalia, A., 2019. Sex: a change in our guidelines to authors to ensure that this is no longer an ignored experimental variable. Br. J. Pharmacol. 176, 4081–4086.

Duman, R.S., Nakagawa, S., Malberg, J., 2001. Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology 25, 836–844.

Ellenbroek, B.A., van den Kroonenberg, P.T., Cools, A.R., 1998. The effects of an early stressful life event on sensorimotor gating in adult rats. Schizophr. Res. 30, 251–260.

Ellenbroek, B.A., Derks, N., Park, H.J., 2005. Early maternal deprivation retards neurodevelopment in Wistar rats. Stress 8, 247–257.
Ferrari, F., Fiorentino, S., Mennuni, L., Garofalo, P., Letari, O., Mandelli, S., Giordani, A., Lanza, M., Caselli, G., 2011. Analgesic efficacy of CR4056, a novel imidazoline-2 receptor ligand, in rat models of inflammatory and neuropathic pain. J. Pain Res. 4, 111–125.
Finn, D.P., Martí, O., Harbuz, M.S., Vallès, A., Belda, X., Márquez, C., Jessop, D.S., Lalies, M.D., Armario, A., Nutt, D.J., Hudson, A.L., 2003. Behavioral, neuroendocrine and neurochemical effects of the imidazoline I2 receptor selective ligand BU224 in naive rats and rats exposed to the stress of the forced swim test. Psychopharmacology 167, 195–202.
Flores, A.D., Yu, W.S., Fung, M.L., Lim, L.W., 2022. Neuromodulation and hippocampal neurogenesis in depression: a scoping review. Brain Res. Bull. 188, 92–107.
Garau, C., Miralles, A., García-Sevilla, J.A., 2013. Chronic treatment with selective I2-imidazoline receptor ligands decreases the content of pro-apoptotic markers in rat brain. J. Psychopharmacol. 27, 123–134.
García-Cabrerizo, R., Keller, B., García-Fuster, M.J., 2015. Hippocampal cell fate regulation by chronic cocaine during periods of adolescence vulnerability: consequences of cocaine exposure during adolescence on behavioral despair in adulthood. Neuroscience 304, 302–315.
García-Cabrerizo, R., Ledesma-Corvi, S., Bis-Humbert, C., García-Fuster, M.J., 2020. Sex differences in the antidepressant-like potential of repeated electroconvulsive seizures in adolescent and adult rats: regulation of the early stages of hippocampal neurogenesis. Eur. Neuropsychopharmacol. 41, 132–145.
García-Fuster, M.J., García-Sevilla, J.A., 2016. Effects of anti-depressant treatments on FADD and p-FADD protein in rat brain cortex: enhanced anti-apoptotic p-FADD/FADD ratio after chronic desipramine and fluoxetine administration. Psychopharmacology 233, 2955–2971.
García-Fuster, M.J., Miralles, A., García-Sevilla, J.A., 2007. Effects of opiate drugs on Fas-associated protein with death domain (FADD) and effector caspases in the rat brain: regulation by the ERK1/2 MAP kinase pathway. Neuropsychopharmacology 32, 399–411.
García-Fuster, M.J., Clinton, S.M., Watson, S.J., Akil, H., 2009. Effect of cocaine on Fas-associated protein with death domain in the rat brain: individual differences in a model of differential vulnerability to drug abuse. Neuropsychopharmacology 34, 1123–1234.
García-Fuster, M.J., Perez, J.A., Clinton, S.M., Watson, S.J., Akil, H., 2010. Impact of cocaine on adult hippocampal neurogenesis in an animal model of differential propensity to drug abuse. Eur. J. Neurosci. 3, 79–89.
García-Fuster, M.J., Flagel, S.B., Mahmood, S.T., Mayo, L.M., Thompson, R.C., Watson, S. J., Akil, H., 2011. Decreased proliferation of adult hippocampal stem cells during cocaine withdrawal: possible role of the cell fate regulator FADD. Neuropsychopharmacology 36, 2303–2317.
Garcia-Fuster, M.J., Parsegian, A., Watson, S.J., Akil, H., Flagel, S.B., 2017. Adolescent cocaine exposure enhances goal-tracking behavior and impairs hippocampal cell genesis selectively in adult bred low-responder rats. Psychopharmacology 234, 1293–1305.
García-Sevilla, J.A., Escribá, P.V., Sastre, M., Walzer, C., Busquets, X., Jaquet, G., Reis, D. J., Guimón, J., 1996a. Immunodetection and quantitation of imidazoline receptor proteins in platelets of patients with major depression and in brains of suicide victims arch. Gen. Psychiatr. 53, 803–810.
García-Sevilla, J.A., Escribá, P.V., Busquets, X., Walzer, C., Guimón, J., 1996b. Platelet imidazoline receptors and regulatory G proteins in patients with major depression. NeuroReport 8, 169–172.
García-Sevilla, J.A., Escribá, P.V., Guimón, J., 1999. Imidazoline receptors and human brain disorders. Ann. N. Y. Acad. Sci. 881, 392–409.
Giordani S. Mandelli I. Verpilio S. Zanzola F. Tarchino G. Caselli T. Piepoli S. Mazzari F. Makovec L.C. Rovati, 2008. 6-1H-imidazoquinazoline and quinolines derivatives, new potent analgesics and anti-inflammatory agents. World Intellectual Property Organization. United States, US7994181 B2 2011-08-09. International application number PCT/EP2006/065013. International Publication date WO 2008/014822 Al.
Griñán-Ferré, C., Vasilopoulou, F., Abás, S., Rodríguez-Arévalo, S., Bagán, A., Sureda, F. X., Pérez, B., Callado, L.F., García-Sevilla, J.A., García-Fuster, M.J., Escolano, C., Pallàs, M., 2019. Behavioral and cognitive improvement induced by novel imidazoline I2 receptor ligands in female SAMP8 mice. Neurotherapeutics 16, 416–431.
Hernández-Hernández, E., García-Fuster, M.J., 2021. Evaluating the effects of 2-BFI and tracizoline, two potent I2-imidazoline receptor agonists, on cognitive performance and affect in middle-aged rats. Naunyn Schmiedeberg's Arch. Pharmacol. 394, 989–996.
Hernández-Hernández, E., García-Sevilla, J.A., García-Fuster, M.J., 2021. Exploring the antidepressant-like potential of the selective I2-imidazoline receptor ligand LSL60101 in adult male rats. Pharmacol. Rep. 73, 288–295.
Kaluve, A.M., Le, J.T., Graham, B.M., 2022. Female rodents are not more variable than male rodents: a meta-analysis of preclinical studies of fear and anxiety. Neurosci. Biobehav. Rev. 143, 104962.
Kot, M., Neglur, P.K., Pietraszewska, A., Buzanska, L., 2022. Boosting neurogenesis in the adult hippocampus using antidepressants and mesenchymal stem cells. Cells 11, 3234.
Ledesma-Corvi, S., García-Fuster, M.J., 2022. Revisiting the antidepressant-like effects of desipramine in male and female adult rats: sex disparities in neurochemical correlates. Pharmacol. Rep. 74, 626–636.
Ledesma-Corvi, S., Hernández-Hernández, E., García-Fuster, M.J., 2022. Exploring pharmacological options for adolescent depression: a preclinical evaluation with a sex perspective. Transl. Psychiatry 12, 220.
LeGates, T.A., Kvarta, M.D., Thompson, S.M., 2019. Sex differences in antidepressant efficacy. Neuropsychopharmacology 44, 140–154.
Li, J.X., 2017. Imidazoline I2 receptors: an update. Pharmacol. Ther. 178, 48–56.
Marco, E.M., Adriani, W., Llorente, R., Laviola, G., Viveros, M.P., 2009. Detrimental psychophysiological effects of early maternal deprivation in adolescent and adult rodents: altered responses to cannabinoid exposure. Neurosci. Biobehav. Rev. 33, 498–507.
Marco, E.M., Llorente, R., López-Gallardo, M., Mela, V., Llorente-Berzal, Á., Prada, C., Viveros, M.P., 2015. The maternal deprivation animal model revisited. Neurosci. Biobehav. Rev. 51, 151–163.
Michel, M.C., Murphy, T.J., Motulsky, H.J., 2020. New author guidelines for displaying data and reporting data analysis and statistical methods in experimental biology. Mol. Pharmacol. 97, 49–60.
Miller, L.R., Marks, C., Becker, J.B., Hurn, P.D., Chen, W.J., Woodruff, T., McCarthy, M. M., Sohrabji, F., Schiebinger, L., Wetherington, C.L., Makris, S., Arnold, A.P., Einstein, G., Miller, V.M., Sandberg, K., Maier, S., Cornelison, T.L., Clayton, J.A., 2017. Considering sex as a biological variable in preclinical research. FASEB J. 31, 29–34.
Mota, B.C., Ashburner, N., Abelleira-Hervas, L., Liu, L., Aleksynas, R., Rovati, L.C., Caselli, G., Sastre, M., 2022. I2-imidazoline ligand CR4056 improves memory, increases ApoE expression and reduces BBB leakage in 5xFAD mice. Int. J. Mol. Sci. 23, 7320.
Numakawa, T., Odaka, H., Adachi, N., 2018. Actions of brain-derived neurotrophin factor in the neurogenesis and neuronal function, and its involvement in the pathophysiology of brain diseases. Int. J. Mol. Sci. 19, 3650.
Nutt, D., French, N., Handley, S., Hudson, A., Husbands, S., Jackson, H., Jordan, S., Lalies, M.D., Lewis, J., Lione, L., et al., 1995. Functional studies of specific imidazoline-2 receptor ligands. Ann. N. Y. Acad. Sci. 763, 125–139.
O'Neill, M.F., Osborne, D.J., Woodhouse, S.M., Conway, M.W., 2001. Selective imidazoline I2 ligands do not show antidepressant-like activity in the forced swim test in mice. J. Psychopharmacol. 15, 18–22.
Percie du Sert, N., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Hurst, V., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E. S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 18, e3000411.
Qiu, Y., Zhang, Y., Li, J.X., 2015. Discriminative stimulus effects of the imidazoline I2 receptor ligands BU224 and phenyzoline in rats. Eur. J. Pharmacol. 749, 133–141.
Rénéric, J.P., Bouvard, M., Stinus, L., 2002. In the rat forced swimming test, chronic but not subacute administration of dual 5-HT/NA antidepressant treatments may produce greater effects than selective drugs. Behav. Brain Res. 136, 521–532.
Rodríguez-Arévalo, S., Bagán, A., Griñán-Ferré, C., Vasilopoulou, F., Pallàs, M., Brocos-Mosquera, I., Callado, L.F., Loza, M.I., Martínez, A.L., Brea, J., Pérez, B., Molins, E., de Jonghe, S., Daelemans, D., Radan, M., Djikicm, T., Nikolic, K., Hernández-Hernández, E., García-Fuster, M.J., García-Sevilla, J.A., Escolano, C., 2021. Benzofuranyl-2-imidazoles as imidazoline I2 receptor ligands for Alzheimer's disease. Eur. J. Med. Chem. 222, 113540.
Schmidt, M.V., Wang, X.D., Meijer, O.C., 2011. Early life stress paradigms in rodents: potential animal models of depression? Psychopharmacology 214, 131–140.
Siemian, J.N., Wang, K., Zhang, Y., Li, J.X., 2018. Mechanisms of imidazoline I2 receptor agonist-induced antinociception in rats: involvement of monoaminergic neurotransmission. Br. J. Pharmacol. 175, 1519–1534.
Siemian, J.N., Shang, L., Seaman Jr., R.W., Zhu, Q., Zhang, Y., Li, J.X., 2019. Effects of imidazoline I2 receptor agonists on reserpine-induced hyperalgesia and depressive-like behavior in rats. Behav. Pharmacol. 30, 429–434.
Slattery, D.A., Cryan, J.F., 2012. Using the rat forced swim test to assess antidepressant-like activity in rodents. Nat. Protoc. 7, 1009–1014.
Thorn, D.A., An, X.F., Zhang, Y., Pigini, M., Li, J.X., 2012. Characterization of the hypothermic effects of imidazoline I_2 receptor agonists in rats. Br. J. Pharmacol. 166, 1936–1945.
Tonello, R., Villarinho, J.G., da Silva Sant'Anna, G., Tamiozzo, L., Machado, P., Trevisan, G., Pinto Martins, M.A., Ferreira, J., Rubin, M.A., 2012. The potential antidepressant-like effect of imidazoline I2 ligand 2-BFI in mice. Prog. Neuropsychopharmacol. Biol. Psychiatr. 37, 15–21.
Vasilopoulou, F., Bagan, A., Rodriguez-Arévalo, S., Escolano, C., Griñán-Ferré, C., Pallàs, M., 2020. Amelioration of BPSD-like phenotype and cognitive decline in SAMP8 mice model accompanied by molecular changes after treatment with I2-imidazoline receptor ligand MCR5. Pharmaceutics 12, 475.
Vasilopoulou, F., Griñán-Ferré, C., Rodríguez-Arévalo, S., Bagán, A., Abás, S., Escolano, C., Pallàs, M., 2021. I2 imidazoline receptor modulation protects aged SAMP8 mice against cognitive decline by suppressing the calcineurin pathway. Geroscience 43, 965–983.
Vellani, V., Sabatini, C., Milia, C., Caselli, G., Lanza, M., Letari, O., Rovati, L.C., Giacomoni, C., 2020. CR4056, a powerful analgesic imidazoline-2 receptor ligand, inhibits the inflammation-induced PKCε phosphorylation and membrane translocation in sensory neurons. Br. J. Pharmacol. 177, 48–64.