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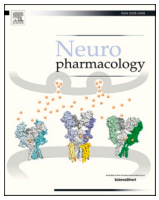

PPAR-α activation in a model of autism modulates social reward and endocannabinoid signaling selectively in male rats

S. Salviati [a], G. Braccagni [b], E. Corridori [a], G. Sabatini [c], F. Fanti [c], F. Guzzi [d], M. Parenti [d], N. Battista [c], S. Scheggi [a,*,1], C. Gambarana [a,1]

[a] Department of Molecular and Developmental Medicine, University of Siena, Siena, Italy
[b] Department of Cellular and Systems Pharmacology, College of Pharmacy, University of Florida, Gainesville, FL, USA
[c] Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy
[d] Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy

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ABSTRACT

Social difficulties in autism spectrum disorder (ASD), including reduced sensitivity and responsiveness to the rewarding value of social stimuli (social anhedonia), may arise from dysregulation of dopaminergic and endocannabinoid (eCB) pathways. We examined whether social reward processing was restored in male and female rats prenatally exposed to valproic acid (VPA) by PPARαs activation which modulates dopaminergic and eCB transmission. After 4-week administration of the PPARα agonist fenofibrate (FBR), social interaction and social reward sensitivity were assessed, and levels of eCBs and related markers measured in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). In VPA-exposed male rats, FBR administration reinstated social behavior, increased N-palmitoyl ethanolamine (PEA) levels in the NAc and normalized fatty-acid amide hydrolase (FAAH) levels in the NAc and mPFC. In contrast, VPA-exposed female rats showed very subtle ASD-like social deficits, different patterns of eCB alterations, with decreased N-oleoyl ethanolamine (OEA) levels in both regions, and divergent responses to FBR. Overall, these findings suggest that FBR modulation of the eCB system contribute to improving social behavior in VPA-exposed male rats. Moreover, differences in eCB signaling in male and female rats may play a role in the development of sex-divergent phenotype following prenatal VPA exposure.

1. Introduction

Autism spectrum disorder (ASD) encompasses a heterogeneous group of lifelong neurodevelopmental conditions characterized by persistent alterations in social interaction and communication, restricted interests, and repetitive behaviors (American Psychiatric Association, 2013). Increasing evidence suggests that social deficits in ASD, including social anhedonia, or impaired ability to experience social interactions as rewarding, may stem from dysregulation of dopaminergic (Kosillo and Bateup, 2021; Blum et al., 2024) and endocannabinoid (eCB) systems (Navarro et al., 2022; Friuli et al., 2025; Chakrabarti et al., 2015; Karhson et al., 2016). Through their modulation of prefrontal and subcortical circuits, these systems shape the maturation of neural networks from childhood through adolescence (Peters and Naneix, 2022) and regulate behavioral processes linked to reward, motivation, and learning (Zamberletti et al., 2017; Melancia et al., 2018). Disruptions of this developmental trajectory contribute to the framework proposed by the social motivation theory of ASD that impaired reward processing diminishes the salience of social cues, causing reduced social orienting, engagement, and social anhedonia. This cascade prevents the acquisition and refinement of socially appropriate behaviors in individuals with ASD (Chevallier et al., 2012; Clements et al., 2018; Bottini et al., 2018).

Fatty-acid-derived ligands, including the eCB anandamide (AEA) and the eCB-like N-oleoyl ethanolamine (OEA) and N-palmitoyl ethanolamine (PEA), are key regulators of emotional and social behaviors (Marco et al., 2013; Wei et al., 2015, 2017; Zamberletti et al., 2017; Servadio et al., 2016). OEA and PEA share AEA metabolic pathways, being synthetized by N-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) and degraded by fatty-acid amide hydrolase (FAAH) (Ueda

* Corresponding author.
E-mail address: simona.scheggi@unisi.it (S. Scheggi).
[1] Equal contribution.

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et al., 2010). In rodents, FAAH inhibition enhances social interaction (Cassano et al., 2011), social play (Trezza et al., 2012), and ASD-like behavioral abnormalities (Melancia et al., 2018). Elevated AEA levels within the nucleus accumbens (NAc) activate CB1 receptors, a mechanism necessary and sufficient for encoding the reinforcing properties of social stimuli in mice (Wei et al., 2015, 2017), whereas PEA administration reduces ASD-related behavioral impairments (Cristiano et al., 2018). Accordingly, ASD children show reduced circulating levels of AEA, PEA, and OEA (Karhson et al., 2018; Aran et al., 2019; De Pol and Kolla, 2021).

Activation of peroxisome proliferator-activated receptor-α (PPARα) by synthetic agonists such as fenofibrate (FBR) stimulates both eCB and dopaminergic systems (Melis et al., 2013b; Scheggi et al., 2016; Hempel et al., 2023). PPARα activation elevates PEA and OEA levels (Melis et al., 2013a), modulates the activity of midbrain dopamine neurons (Melis et al., 2010, 2013b), and enhances D1 receptor transmission in the NAc (Scheggi et al., 2016). Notably, we previously demonstrated that chronic FBR treatment alleviates social deficits, together with the blunted dopaminergic response to salient stimuli, in the valproic acid (VPA)-induced rat model of ASD (Scheggi et al., 2020). However, it remains unclear whether FBR-induced PPARα activation also restores eCB signaling in brain circuits involved in social reward processing, hence mitigating reward-related dysfunctions in rats prenatally exposed to VPA.

Using the VPA model, the present study aimed to determine whether (i) the social impairments observed in VPA-exposed rats reflect specific deficits in social reward processing, e.g. social anhedonia and (ii) early FBR administration rescues emerging ASD-related social deficits by reinstating the rewarding value of social stimuli. To address these aims, we assessed the responses to social novelty and quantified the reinforcing properties of social interaction through the conditioned place preference (CPP) protocol (Schiavi et al., 2019). Further, (iii) to test whether long-term PPARα stimulation modifies the biosynthesis of eCB and eCB-like mediators associated with social reward processing, we measured AEA, PEA, and OEA levels in the NAc and medial prefrontal cortex (mPFC), along with the expression of FAAH, NAPE-PLD, and CB1 receptors. Because eCBs are produced on demand from membrane phospholipids, their regional concentrations provide a reliable proxy for signaling activity (Piomelli, 2014).

ASD affects an estimated 1 in 31 U.S. children at age 8, with a male-to-female ratio of 3.4 (Shaw et al., 2022). This male predominance may partly reflect diagnostic tools and criteria developed primarily for male subjects, leading to under-recognition of ASD in females (Rippon, 2024). Importantly, the ASD phenotype in females is not merely a milder form of the male presentation; rather, girls often display a stronger intrinsic drive for social connection. In line with this sexual dimorphism, the current study included both male and female rats to capture potential sex-specific patterns in social anhedonia, its neurobiological underpinnings and outcomes of PPARα activation.

2. Material and methods

2.1. Animals

Experiments were performed using male Sprague Dawley rats (Charles River, Calco, Italy) housed in cages of 3-4 animals at constant temperature (22 ± 2 °C) and humidity (55 ± 10%) with a 12 h reverse light/dark cycle and free access to food and water. All procedures were performed in accordance with the European (EU Directive, 2010/63) and the Italian (D.L. 2014/26) legislations and animal use was approved by the Italian Ministry of Health (Authorization N. 70/2018-PR).

2.2. Generation of the VPA rat model of ASD

The ASD model was induced as described (Scheggi et al., 2020). At G12.5, pregnant females received a single intraperitoneal (i. p.) injection of 500 mg/kg VPA in 0.9% (w/v) sodium chloride (saline, 2 ml/kg body weight) (Rodier et al., 1996; Schneider and Przewlocki, 2005), or an equal volume of saline. Such treatment induces changes in neuroanatomy and neurogenesis and behavioral deficits in rat pups (Roullet et al., 2013), without miscarriage or fetal re-absorption.

2.3. Fenofibrate treatment

At weaning, male and female rats were separately housed and randomly assigned to four groups: saline-exposed rats fed with standard diet (Saline-SD), or FBR-enriched diet (Saline-FBR), and VPA-exposed rats fed with standard diet (VPA-SD), or FBR-enriched diet (VPA-FBR).

The SD (4RF21, Mucedola Srl, Settimo Milanese, Italy) was supplemented with FBR 0.2% (w/w) (Molport, Riga, Latvia) to obtain the FBR diet, or was re-pelleted to obtain pellets with appearance and consistency identical to FBR-enriched pellets (custom-made by Mucedola Srl). Feeding with the FBR diet resulted in an average daily intake of 200 mg/kg, a dose previously reported to reduce motivational and social anhedonia in animal models (Scheggi et al., 2016, 2020). Food consumption per cage and individual body weight were measured every other day to estimate food intake and general health (data not shown) and confirmed daily intake estimates obtained in previous studies (Scheggi et al., 2016, 2020). Thus, individual daily intake of FBR can only be estimated in this study, but the primary goal was to minimize stress exposure, particularly in weaned young pups.

2.4. Behavioral tests

2.4.1. Three-chamber tests for social behavior and social novelty

The three-chamber test was used to assess the deficits in social behavior as detailed in Scheggi et al. (2020). During the 10-min test, the time spent sniffing and exploring the social and non-social stimuli and the latency to initiate social interaction with the unfamiliar rat were measured. At the end of the test, each rat was tested in a second 10-min session to quantify the social motivation to interact with a new stranger animal (social novelty). An identical cage, containing an unfamiliar rat (stranger 2) was placed in the previous non-social stimulus chamber and the time that the test rat spent with stranger 1 and stranger 2 animals was measured.

2.4.2. Conditioned place preference test induced by a social stimulus

The conditioned place preference (CPP) protocol is based on the classical Pavlovian conditioning and allows to measure the rewarding properties of social interaction and social reward learning (Schiavi et al., 2019). Conditioning was performed in a rectangular plexiglass three-chamber apparatus following and adapting the protocol used by Trezza et al. (2009). The central (neutral) compartment had transparent floor and walls, while the two conditioning compartments were identical in size but visually distinct (black versus black-and-white stripes walls and floor). Compartments were separated by guillotine doors. Since the amount of social behavior displayed by adolescent rats is a function of the duration of the preceding social isolation period, rats were individually housed after the baseline preference session (day 1) and throughout the experiment (day 1-6, Fig. 1A). On day 1, each animal freely explored the CPP apparatus for 15 min. The time spent in each compartment was recorded to determine each animal's baseline side preference. We used a counterbalanced place conditioning design based on baseline preference (Tzschentke, 2007) in which social interaction was paired with the non-preferred chamber for 50% of the rats, and for the other 50% the preferred compartment was designated as the social-conditioning compartment. A control animal of similar age and body weight, fed with SD, was used as the social stimulus. From days 2-5, rats underwent two 15-min conditioning sessions per day (one social, one non-social), separated by 3-4 h; the order of the sessions was counterbalanced across animals to avoid potential order or time-of-day effects. During sessions, rats were confined either to the empty

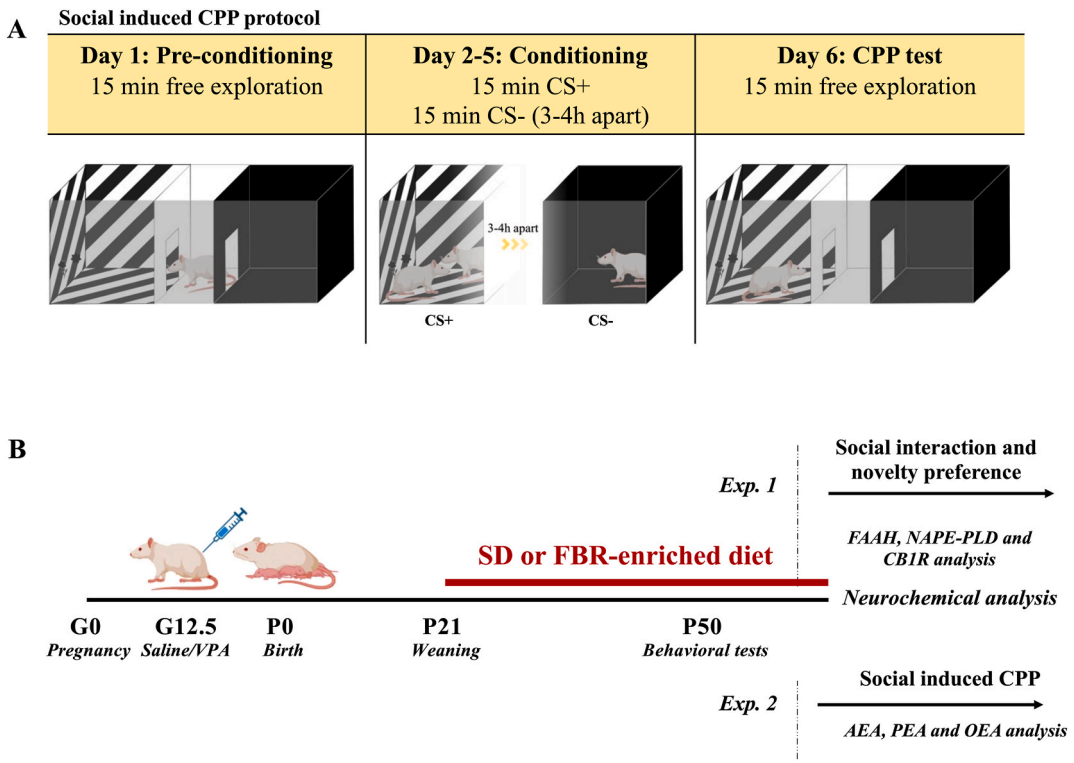

Fig. 1. CPP schematic protocol (A) and timeline of experimental set-up (B)
G: gestational day; P: postnatal day; SD: standard diet; FBR: fenofibrate; FAAH: *fatty-acid amide hydrolase*; NAPE-PLD: *N-acyl phosphatidylethanolamine phospholipase D*; CB1R: CB1 receptor; AEA: anandamide; OEA: Oleoyl ethanolamine; PEA: Palmitoyl ethanolamine; CPP: conditioned place preference. CS^{+}; social stimulus-paired compartment; CS^{-}: non-social stimulus-paired compartment. Created with BioRender.com.

compartment (non-social) or the compartment containing the social stimulus. On day 6, rats were placed in the neutral compartment with free access to the whole empty apparatus for 15 min. Time spent in each compartment was recorded to evaluate social CPP.

2.5. Neurochemical experiments

Three days after behavioral tests, rats were sacrificed by decapitation and the NAc and mPFC areas were dissected and identified using the Rat Brain Atlas of Paxinos and Watson (2007). Tissues were flash-frozen in liquid nitrogen and stored at −80 °C until assayed (Scheggi et al., 2016).

2.5.1. Quantification of AEA, OEA and PEA in rat brain tissues through UHPLC/MS-MS

eCBs content was determined according to Battista et al. (2023). Briefly, brain areas were weighed and homogenized in methanol/formic acid (200 mM) supplemented with deutered internal standards (IS) by using Precellys® Evolution homogenizer tubes prefilled with 1.4 mm ceramic beads (Bertin Technologies). Supernatants (50 μl) were collected, purified by micro-solid-phase extraction and eluates were analyzed by using a Waters UPLC Acquity H-Class system (Waters) coupled to a 4500 Qtrap tandem mass spectrometer (Sciex) equipped with an electrospray ionization (ESI) source. Analytes were separated on a Waters Acquity BEH C18 reversed-phase column. Data acquisition and processing were performed using Analyst 1.7.3 software, while peak integration and quantification were conducted with MultiQuant 3.0.3 (Sciex). Analyte concentrations were calculated by interpolation and normalized to respective deuterated IS using peak area ratios. Final values are reported as pmol/mg of tissue.

2.5.2. Immunoblotting

Samples of NAc and mPFC were separated on a 4-15% Criterion TGX Stain free Precast Gel (Bio-Rad Laboratories) as detailed in Scheggi et al. (2020). The following primary antibodies were used: anti-FAAH (Cell Signaling, #3829), anti-NAPE-PLD (Cayman Chemical, #10305), anti-CB1 receptors (Cell Signaling, #93815) and anti-β-actin (Santa Cruz Biotechnology, sc-517581). Band intensities were quantified using Image Lab software/Gel Doc XRS + system.

2.6. Drugs

Sodium valproate (PubChem CID 16760703) was purchased from Sigma-Aldrich, and FBR (PubMed CID 3339) was obtained from Molport (Riga, Latvia).

2.7. Experimental protocols

A total of 160 rats from 22 saline-treated dams and 25 VPA-treated dams were used during two different separate experiments as outlined in Fig. 1B. One male and one female offspring per litter was assigned to each experimental group to avoid litter effect.

The first experiment was designed to study social interaction and preference for social novelty. Male and female pups prenatally exposed to saline or VPA were fed with SD or FBR diets from PND 21 and tested for social interaction and social novelty preference at PND 50-53 (n = 12/group). Three days after behavioral tests, rats were sacrificed and the regions of interest dissected for the analysis of FAAH, NAPE-PLD, and CB1 receptor expression levels by immunoblotting.

The second experiment was designed to assess the rewarding value of social stimuli by examining the performance of saline- or VPA-exposed rats in a social stimulus-induced CPP protocol, and the impact of FBR administration. Male and female pups exposed to saline or VPA were treated with SD or FBR diets from PND 21. From PND 50 rats were exposed to CPP (male rats, n = 8/group; female rats, n = 7-8/group). Three days after behavioral tests, rats were sacrificed and the regions of interest dissected out for the determination of AEA, PEA, OEA and 2-AG

by UPLC/MS-MS.

2.8. Statistical analysis

All data are expressed as means ± SEM and analyzed using GraphPad Prism 10 (GraphPad, San Diego, CA, USA). Group sizes (*n*) for all experiments are provided and refer to independent single measurements. Behavioral and neurochemical data were analyzed by two-way ANOVA with VPA exposure and treatment (FBR-enriched or SD diet) as factors or three-way ANOVA with VPA exposure and FBR treatment as between factors and stimulus as within factor. *Post hoc* analysis was performed with the Bonferroni's multiple comparisons test. In the immunoblotting experiments, the values from treatment groups were normalized to the corresponding control values and expressed as percentages. All statistical details are shown in Table S1 in supplementary materials. Group size was determined by power analysis calculated using the variance estimates obtained from previous experiments. The experimenters were blind to the treatments and, whenever possible, to saline or VPA exposure (tail malformations or chromodacryorrhea sometimes revealed VPA exposure).

3. Results

3.1. Impairments in social interaction and effects of FBR treatment are more evident in VPA-exposed male rats

First, the three-chamber test was used to evaluate the preference for the social or non-social stimuli. In agreement with our previous results (Scheggi et al., 2020), the analysis of the time spent interacting with the social and non-social stimulus showed that VPA-exposure induced an impairment in the preference for the social stimulus, which was prevented by FBR administration. Specifically, only the VPA-SD group showed a reduced interest for the social stimulus relative to the non-social one. Male VPA-treated rats displayed marked social deficits,

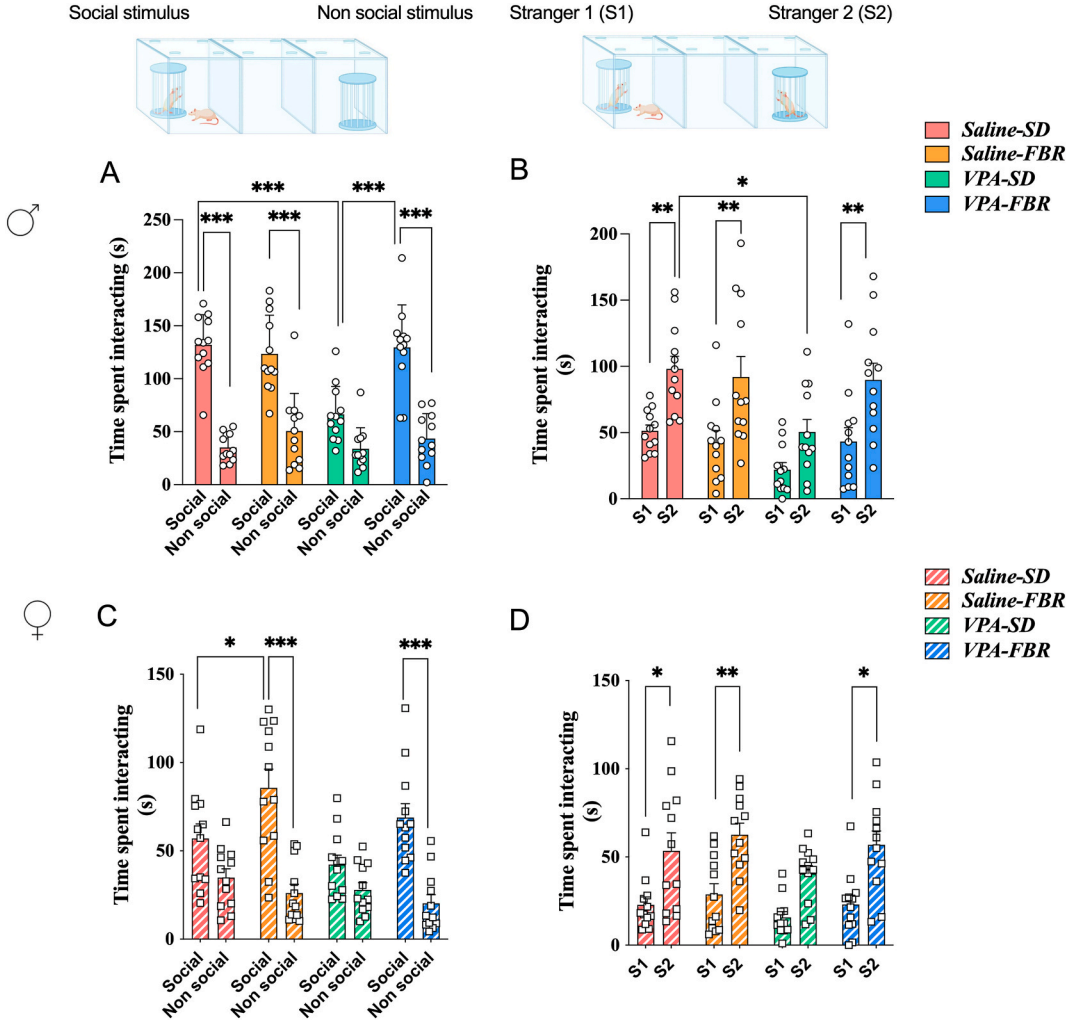

Fig. 2. FBR administration rescued the sex-dependent social deficits in VPA-exposed rats
Male (A-B) and female behavior (C-D).
(A) FBR administration rescued social deficit in VPA male rats (three-way ANOVA, VPA exposure: $F_{1,44} = 7.152$, $p = 0.0105$; FBR treatment: $F_{1,44} = 9.865$, $p = 0.0030$; Stimulus: $F_{1,44} = 169.9$, $p < 0.0001$; VPA x FBR = $F_{1,44} = 6.717$, $p = 0.0129$; VPA x FBR x stimulus = $F_{1,44} = 12.27$, $p = 0.0011$). The exploration of the non-social stimulus was unaffected. (B) Lack of preference for the unfamiliar rat (S2) in VPA-exposed males, an effect prevented by FBR treatment (three-way ANOVA, VPA exposure: $F_{1,\ 44} = 5.592$, $p = 0.0225$; S2 social stimulus: $F_{1,\ 44} = 55.38$, $p < 0.0001$; VPA x FBR: $F_{1,\ 44} = 5.102$, $p = 0.0289$). (C) Slight reduction in sociability, while FBR treatment per se increased social interaction in female rats (three-way ANOVA, VPA exposure: $F_{1,44} = 4.450$, $p = 0.0406$; Stimulus: $F_{1,44} = 75.75$, $p < 0.0001$; FBR x Stimulus: $F_{1,44} = 18.59$, $p < 0.0001$). (D) All groups of female rats discriminated between the two social stimuli and FBR treatment produced an overall modulatory influence on social behavior; the main effect of VPA exposure was not significant (three-way ANOVA, VPA exposure: $F_{1,44} = 2.824$, $p = 0.100$; Stimulus: $F_{1,44} = 50.35$, $p < 0.0001$; FBR: $F_{1,44} = 4.243$, $p = 0.0454$).
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ Bonferroni's multiple comparisons test.

spending significantly less time sniffing and exploring the conspecific compared with saline-exposed rats ($p < 0.001$). Notably, FBR administration restored social interaction to control levels ($p < 0.001$). Conversely, the time spent interacting with the non-social stimulus was not affected by either VPA exposure or FBR administration (Fig. 2 A). At the end of the 10-min test, the non-social stimulus was replaced by an unfamiliar rat (stranger 2, S2), while the original social stimulus now represented the familiar rat (stranger 1, S1). In male rats, the analysis of the time spent interacting with S1 and S2 showed that VPA-exposure induced an impairment in the preference for the novel social stimulus S2, which was prevented by FBR administration. Indeed, while the Saline-SD and Saline-FBR groups showed the expected clearcut preference for S2 ($p = 0.0023$ and $p = 0.0012$, respectively), the VPA-SD group did not exhibit any preference for S2 or S1 ($p = 0.2081$). However, under FBR administration, VPA-exposed male rats preferred the unfamiliar S2 rat ($p = 0.0024$, Fig. 2C).

Consistent with our previous results (Scheggi et al., 2020), female rats showed a generally lower tendency to interact with social stimuli. Exposure to VPA caused only a slight reduction in sociability, while FBR treatment per se increased social interaction. Indeed, analysis of the time spent exploring the social and non-social stimuli revealed that treatment enhanced the interaction of female rats with the social stimulus (Fig. 2C). Saline-FBR rats displayed higher social exploration compared to Saline-SD rats ($p = 0.0390$) and both Saline-FBR and VPA-FBR showed an increased interaction with the social compared to non-social stimulus ($p < 0.001$). In the social novelty preference test, female rats clearly discriminated between the two social stimuli and FBR treatment produced an overall modulatory influence on social behavior; in contrast the main effect of VPA exposure was not significant. Consistent with the main effect of S1-S2 stimuli, post hoc analyses showed a clear preference for the novel social stimulus in all groups, except in the in VPA-SD rats ($p = 0.0119$ in Saline-SD, $p = 0.0038$ in Saline-FBR, $p = 0.0039$ in VPA-FBR, $p = 0.0782$ in VPA-SD). Although this pattern points toward a possible reduction in social discrimination following VPA exposure in females, the absence of significant interaction effects indicates that this difference cannot be conclusively attributed to group-specific effects. Rather, it may reflect increased variability or more subtle behavioral alterations in VPA-exposed female rats (Fig. 2D). Together, these findings suggest that, while social stimulus discrimination is generally preserved across groups, VPA exposure may be associated with a weaker or less consistent expression of this behavior, whereas FBR treatment appears to exert a broader modulatory effect. Overall, female rats displayed a relatively low baseline level of social interaction in the three-chamber test, with VPA exposure causing mild impairment in general sociability. Treatment with FBR consistently enhanced social engagement, increasing exploration of social stimuli in both control and VPA-exposed animals.

3.2. VPA exposure led to a selective decrease in social reward in male rats, reversed by FBR treatment that restored responses comparable to controls

To study whether the deficits in social interaction observed in the VPA rats were related to the reduced reward value of social stimuli and whether FBR treatment restored social reward, we exposed to the CPP protocol the male and female litters prenatally exposed to saline or VPA.

A social stimulus-paired compartment (CS^+) was designated for each rat. Across the four experimental groups, we compared the time spent in the CS^+ and in the CS^- compartments during the baseline preference assessment (pre-test) and following the conditioning sessions (post-test). To evaluate conditioning-induced changes in preference for the CS + compartment while accounting for potential side biases, we calculated two measures. First, we quantified the change in time spent in the CS^+ compartment between the pre- and the post-test ($CS^+_{post} - CS^+_{pre}$), which reflects learning-dependent changes relative to each subject's baseline. Second, we computed a difference score based on the time spent in the CS^+ versus CS^- during post-conditioning test, corrected for baseline differences as described in Yates (2023): $[(CS^+ - CS^-)_{post} - (CS^+ - CS^-)_{pre}]$. At baseline, rats in all groups show no preference for either compartment or for the neutral one (Fig. 3A and D). In male rats, the analysis of the difference between the time spent in the CS^+ compartment before and after conditioning showed that the control groups (Saline-SD and Saline-FBR) developed preference for the CS^+ compartment compared to VPA-SD ($p = 0.0163$ and $p = 0.0393$, respectively). VPA rats displayed less interest towards the CS^+ compartment, developing an aversive behavior toward the environment associated with social stimulus, as evidenced by the shift from baseline preference. Remarkably, VPA-exposed male rats treated with FBR developed a preference for the CS^+ compartment, similar to the control groups and different from the VPA-SD group ($p = 0.0489$) (Fig. 3 B). Consistent results were obtained using the difference score: VPA-SD rats showed reduced preference for CS^+ compartment ($p = 0.022$ compared to Saline-SD) while FBR treatment restored this preference ($p = 0.0433$). The difference between the time spent in the CS^- compartment before and after conditioning was similar for rats in all experimental groups (Fig. S1 in Supplementary materials). Overall, these findings indicate that in male rats prenatal VPA exposure induced a decreased interest towards social stimuli, while FBR treatment rescued the rewarding value of social interaction in VPA-exposed rats, promoting the preference for the socially paired compartment.

Despite similar behavioral outcomes between male and female rats during preconditioning, the VPA-exposed female rats did not differ from the saline-exposed female rats in the CPP test. Specifically, female rats from all experimental groups developed similar preference for the CS^+ compartment as shown by the analysis of the difference between time spent in CS+ compartment before and after the conditioning (Fig. 3E) or the analysis of the difference score (Fig. 3F). The time spent in the CS^- compartment was similar before and after conditioning (Fig. S1 in Supplementary materials).

Overall, in the behavioral tests that we performed the VPA-exposed female rats showed only minimal deficits in the social domain compared to their saline-exposed control groups, further confirming that in the rat VPA model the typical ASD phenotype is only apparent in males (Scheggi et al., 2020).

3.3. Sex-dependent effects of prenatal VPA exposure on eCB levels and effect of FBR administration on PEA levels in the NAc

Next we tested whether (i) differences in levels of eCB and eCB-like ligands in the NAc and mPFC were present and potentially contributed to the different behavioral responses observed in male and female rats of different experimental groups, and (ii) the positive effect on responses to social stimuli induced by FBR administration in the male VPA rats showed any relationship with the levels of eCB and eCB-like ligands. To this end, we measured AEA, PEA, OEA and 2-AG in the mPFC and NAc of male and female litters exposed to saline or VPA.

In male rats, AEA levels were decreased by VPA exposure in the NAc and mPFC and were not restored to control levels upon FBR administration (Fig. 4A–D). VPA exposure and FBR administration did not modify OEA levels in the NAc (Fig. 4B), but remarkably, FBR administration increased PEA levels in the NAc, that were not affected by VPA exposure (Fig. 4C). VPA exposure and FBR administration did not modify OEA and PEA levels in the mPFC (Fig. 4E and F), nor 2-AG levels in both regions (Fig. S2 in Supplementary materials).

In female rats, prenatal VPA exposure was associated with decreased levels of AEA in the NAc but not in the mPFC, and FBR administration was ineffective (Fig. 5A–D). Differently from what observed in male rats, OEA levels in the NAc and mPFC were decreased by VPA exposure and were not restored to control levels by FBR administration (Fig. 5B–E). PEA (Fig. 5C–F) and 2-AG levels (Fig. S2 in Supplementary materials) were not modified by prenatal VPA exposure or FBR administration in both regions.

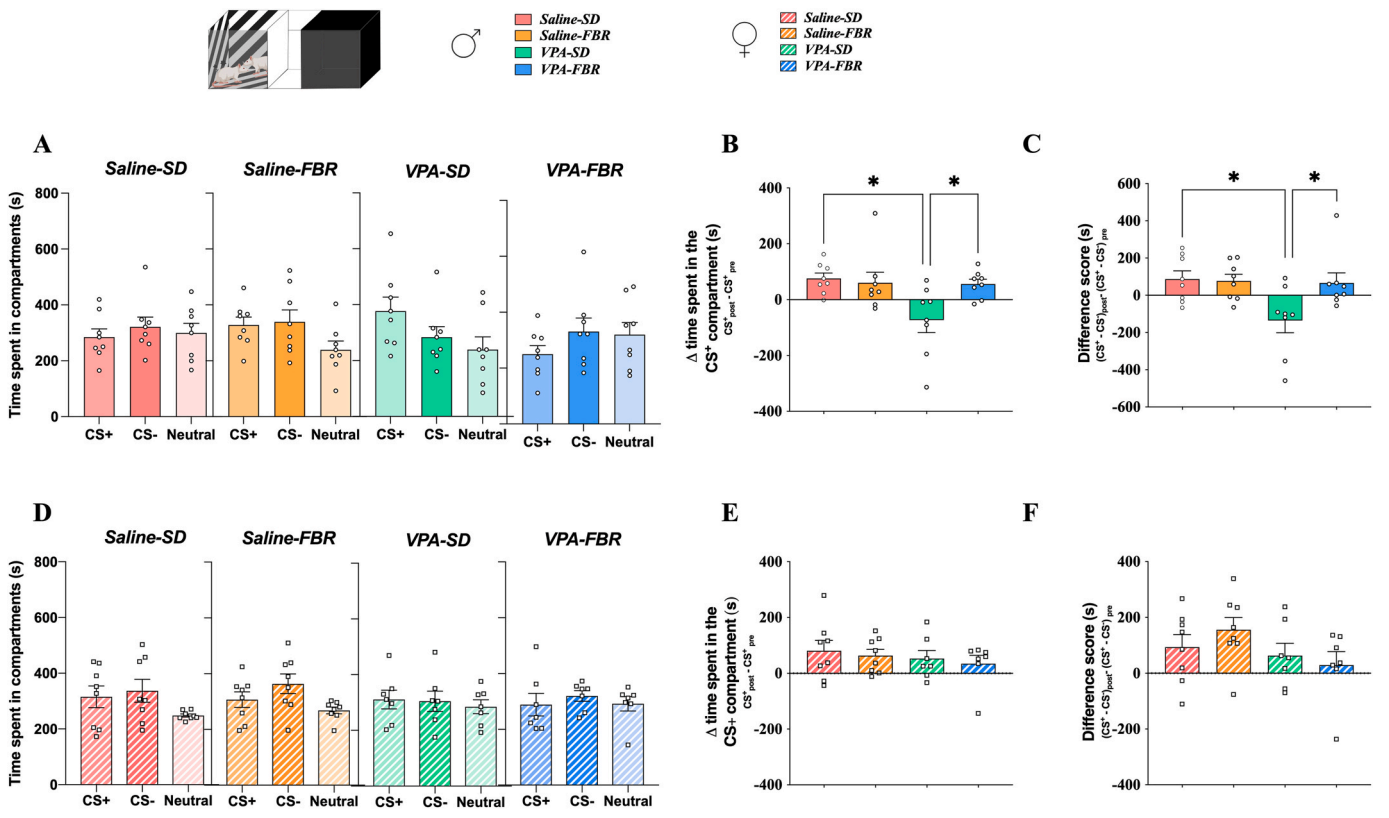

Fig. 3. FBR administration rescued sex-dependent social reward deficits in VPA-exposed rats

(A) Male and female rats (D) showed no baseline preference for any compartment. (B) Change in time spent in the CS^+ compartment ($CS^+_{post} - CS^+_{pre}$) revelead that Saline-SD and Saline-FBR rats developed CS^+ preference, while VPA-SD rats did not; FBR treatment restored to control levels CS^+ preference in VPA-exposed rats (two-way ANOVA: VPA exposure $F_{1, 28} = 5.71$, $p = 0.024$; FBR treatment: $F_{1, 28} = 3.12$, $p = 0.088$; interaction $F_{1, 28} = 5.11$, $p = 0.037$). (C) Post conditioning difference score ($CS^+ - CS^-$), corrected for baseline, confirmed that only VPA-SD rats failed to show CS^+ preference (two-way ANOVA: VPA exposure $F_{1, 28} = 5.237$, $p = 0.0299$; FBR treatment: $F_{1, 28} = 3.524$, $p = 0.071$; interaction $F_{1, 28} = 4.311$, $p = 0.0472$). (E, F) Female rats developed and maintained CS^+ preference regardless of treatment as shown by the raw difference $CS^+_{post} - CS^+_{pre}$ (two-way ANOVA, VPA exposure: $F_{1, 26} = 0.87$, $p = 0.35$; FBR treatment: $F_{1, 26} = 0.34$, $p = 0.56$; interaction: $F_{1, 26} = 0.0007$, $p = 0.97$) and normalized difference score (two-way ANOVA, VPA exposure: $F_{1, 26} = 3.034$, $p = 0.0934$; FBR treatment: $F_{1, 26} = 0.098$, $p = 0.7558$; interaction: $F_{1, 26} = 1.134$, $p = 0.2968$)

*$p < 0.05$ Bonferroni's multiple comparisons test.

Overall, these data confirm that VPA-exposed rats exhibited lower AEA levels compared to the Saline-SD group, although in the females such reduction was restricted to the NAc. The increased levels of PEA in the NAc of all the FBR treated male rats may be one of the elements contributing to the positive effect of FBR administration on social behavior, selectively observed in VPA-exposed male rats. In addition, these results show for the first time that VPA exposure induced in female rats a decrease in OEA levels, that were unchanged in males. Thus, sex-differences in levels of lipid mediators after prenatal VPA-exposure may contribute to the different behavioral phenotype exhibited by male and female rats.

3.4. Long-term stimulation of PPARα by FBR administration reduced the FAAH expression levels increased after prenatal VPA exposure

We then analyzed the expression levels of the enzymes mainly responsible for the synthesis (NAPE-PLD) and catabolism (FAAH) of AEA, OEA and PEA in the NAc and mPFC.

Male VPA-SD rats showed a clear increase in FAAH levels in both regions, which was prevented by FBR administration (Fig. 6A–D). Specifically, VPA-SD rats showed increased levels of FAAH ($p = 0.0091$ compared to Saline-SD rats) and FBR treatment reduced FAAH levels in VPA-FBR rats in both regions ($p < 0.0001$ compared to VPA-SD group, in both regions), but also in the NAc of Saline-FBR rats ($p = 0.012$ compared to Saline-SD rats).

No changes were observed in the levels of expression of NAPE-PLD (Fig. 6B–E) or CB1 receptors (Fig. 6C–F).

In female rats, VPA exposure increased FAAH levels in the NAc, an effect not reversed by FBR (Fig. 7A), while FAAH expression in the mPFC, as well as NAPE-PLD and CB1 receptor levels in both regions, remained unchanged across all experimental groups (Fig. 7B–D).

These results show that prenatal VPA exposure is associated with decreased AEA levels and increased FAAH levels in the NAc and mPFC of male rats (Figs. 4 and 6), and in the NAc of female rats (Figs. 5 and 7). Although these results suggest an enhanced degradation of AEA by FAAH, the restoration of FAAH levels in VPA-exposed rats by FBR administration was not accompanied by an increase in AEA levels (Figs. 4–7). However, in the NAc of male rats, long-term stimulation of PPARα by FBR administration increased the levels of PEA, another FAAH substrate, in parallel with the restoration of the sensitivity to the rewarding value of social stimuli in VPA-exposed male rats.

4. Discussion

This study provides novel neurobiological evidence that in the VPA model of ASD the PPARα agonist FBR modulates eCB and eCB-like mediators, particularly in the NAc, and suggests that these effects may play an auxiliary role in the mitigation of the ASD-like phenotype. Specifically, FBR administration restored in male rats the response to social stimuli impaired by in utero VPA exposure. These behavioral effects were accompanied by FBR-induced increased PEA levels in the NAc and normalization of the upregulated levels of FAAH in the NAc and mPFC of

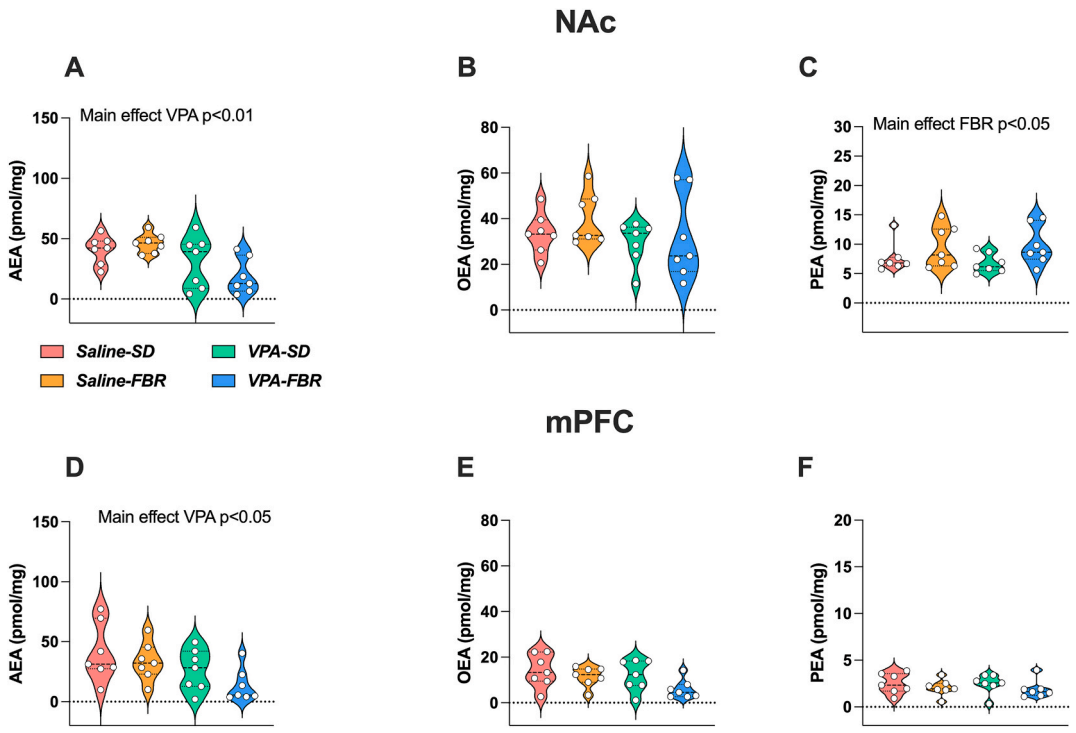

Fig. 4. In VPA-exposed male rats FBR administration did not restore AEA levels but increased PEA levels in the nucleus accumbens.
(A, D) Content of AEA (Two-way ANOVA, NAc: VPA exposure: $F_{1, 24} = 11.28$, $p = 0.0026$; FBR treatment: $F_{1, 24} = 0.41$, $p = 0.52$; interaction: $F_{1, 24} = 2.49$, $p = 0.12$; mPFC: VPA exposure: $F_{1, 24} = 6.27$, $p = 0.019$; FBR treatment: $F_{1, 24} = 2.16$, $p = 0.15$; interaction: $F_{1, 24} = 0.16$, $p = 0.69$). (B, E) Content of OEA (Two-way ANOVA, NAc: VPA exposure: $F_{1, 24} = 1.49$, $p = 0.23$; FBR treatment: $F_{1, 24} = 1.09$, $p = 0.31$; interaction: $F_{1, 24} = 0.20$, $p = 0.65$; mPFC: VPA exposure: $F_{1, 24} = 3.12$, $p = 0.089$; FBR treatment: $F_{1, 24} = 3.95$, $p = 0.058$; interaction: $F_{1, 24} = 0.65$, $p = 0.45$). (C, F) Content of PEA (Two-way ANOVA, NAc: VPA exposure: $F_{1, 24} = 0.089$, $p = 0.76$; FBR treatment: $F_{1, 24} = 5.21$, $p = 0.031$; interaction: $F_{1, 24} = 0.26$, $p = 0.61$; mPFC: VPA exposure: $F_{1, 24} = 0.0004$, $p = 0.98$; FBR treatment: $F_{1, 24} = 1.34$, $p = 0.25$; interaction: $F_{1, 24} = 0.17$, $p = 0.68$).

VPA-exposed male rats.

In female rats, a different pattern of changes in behavior and in eCBs levels after VPA exposure or FBR administration was observed. Thus, the results suggest that the observed sex differences in the ASD-like phenotype may be related, amongst other factors, to distinctive VPA-induced modifications in eCB levels in male and female rats.

Several studies have linked ASD to impairments in the processing of social rewards. Specifically, the social motivation hypothesis of ASD (Chevallier et al., 2012) assumes that deficits in social domains arises from a reduced sensitivity to social stimuli and social cues and their rewarding value, although difficulties in learning and dynamically adapting to novel complex environmental contingencies are also key determinants (Velikonja et al., 2019; Demetriou et al., 2018). Here we used the three chamber- and social novelty tests and the CPP protocol to investigate different constructs of social reward, namely social seeking/liking, social motivation, and social cognition. As shown in Figs. 2 and 3, VPA-exposed male rats exhibited impaired interest for social stimuli, compromised social recognition memory and a significant difficulty in properly processing social stimuli as reinforcers, as assessed through the diminished social interaction, the lack of preference for social novelty, and reduced time spent in the social conditioned compartment, whereas the value of non-social stimuli was not modified compared to control rats. These results suggest that deficits in reward processing in VPA-exposed male rats involved both the consummatory and the motivational aspects of the social function, further supporting the social motivation framework of ASD (Chevallier et al., 2012). In line with these results and the social motivation theory, VPA-exposed male rats show a blunted dopaminergic response to social stimuli in the NAc (Scheggi et al., 2020) and altered expression of dopaminergic receptors (Schiavi et al., 2019). Both conditions may impair the neural processing necessary to assign salience and hedonic value to social stimuli. Moreover, hypoactivation of the reward system has been implicated in ASD (Dichter and Adolphs, 2012; Dichter et al., 2012; Ernst et al., 1997; Pavăl, 2023). However, we cannot exclude that prenatal VPA exposure may also impair the cognitive processing of social stimuli during the conditioning phase of CPP, as proposed by Schiavi et al. (2019).

In recent years, PPARαs have been investigated with particular interest for their role in neurological and psychiatric disorders, but their physiological role in the central nervous system is still not entirely defined. PPARαs are highly expressed in the PFC, NAc, and midbrain neurons (Warden et al., 2016) and are involved in the modulation of VTA-NAc dopaminergic transmission (Melis et al., 2013a). In this mesolimbic circuit, the synthetic PPARα agonist FBR, by facilitating the switch between tonic and phasic activity of VTA dopaminergic neurons (Melis et al., 2013b; Scheggi et al., 2016), may increase dopamine release in the NAc in response to salient stimuli. Our previous studies suggest that FBR is endowed with pro-motivational properties and positively affects several different constructs of anhedonia, primarily manifested as reduced motivation to acquire rewarding palatable stimuli (Scheggi et al., 2016, 2022) or engage in social interaction (Scheggi et al., 2020). Thus, the modulation of firing of VTA dopaminergic neurons projecting to the NAc may contribute to the pro-motivational effect of FBR.

A second mechanism may be implicated in the pro-motivational properties of FBR. Indeed, improvements in social behaviors have been observed following treatments with natural or synthetic cannabinoids that activate PPARα. Accordingly, long term activation of PPARα by FBR positively regulates the biosynthesis of eCBs and eCB-like ligands (e.g., OEA and PEA) (Murru et al., 2022). Of note, the lipid derivatives OEA and PEA are critically involved in regulating the responses to social stimuli (see Friuli et al., 2025) and their dysregulation has been described in ASD, both in humans and animal models (De Pol and Kolla,

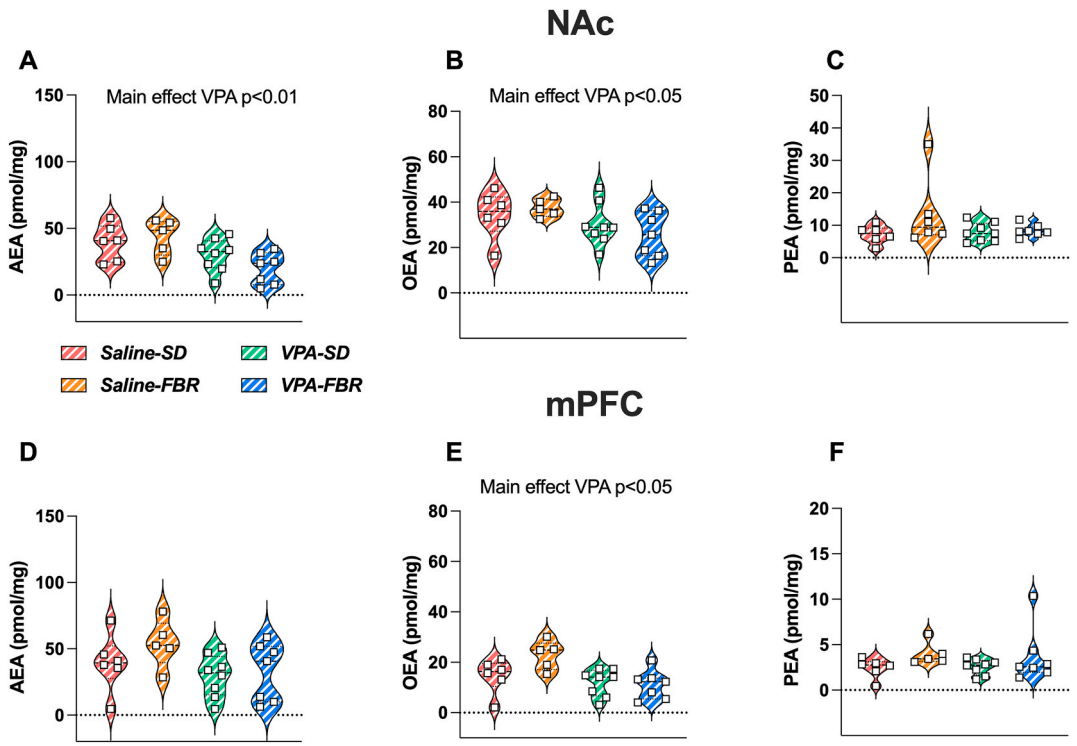

Fig. 5. In female rats VPA exposure decreased AEA and PEA levels and FBR administration did not affect AEA, OEA or PEA levels.
(A, D) Content of AEA (Two-way ANOVA, NAc, VPA exposure: $F_{1, 22} = 10.98$, $p = 0.0032$; FBR treatment: $F_{1, 22} = 0.33$, $p = 0.56$; interaction: $F_{1, 22} = 2.01$, $p = 0.17$; mPFC, VPA exposure: $F_{1, 22} = 4.04$, $p = 0.056$; FBR treatment: $F_{1, 22} = 1.31$, $p = 0.26$; interaction: $F_{1, 22} = 0.56$, $p = 0.46$). (B, E) Content of OEA (Two-way ANOVA, NAc, VPA exposure: $F_{1, 22} = 4.98$, $p = 0.036$; FBR treatment: $F_{1, 22} = 0.038$, $p = 0.84$; interaction: $F_{1, 22} = 1.11$, $p = 0.30$; mPFC, VPA exposure: $F_{1, 22} = 9.52$, $p = 0.0054$; FBR treatment: $F_{1, 22} = 2.38$, $p = 0.13$; interaction: $F_{1, 22} = 3.68$, $p = 0.067$). (C, F) Content of PEA (Two-way ANOVA, NAc, VPA exposure: $F_{1, 22} = 0.98$, $p = 0.33$; FBR treatment: $F_{1, 22} = 2.34$, $p = 0.13$; interaction: $F_{1, 22} = 1.60$, $p = 0.21$; mPFC, VPA exposure: $F_{1, 22} = 0.011$, $p = 0.91$; FBR treatment: $F_{1, 22} = 3.37$, $p = 0.080$; interaction: $F_{1, 22} = 0.077$, $p = 0.69$).

2021; Kerr et al., 2013). This observation led us to hypothesize that long term FBR administration relieves social deficits in the VPA-treated ASD rats also by raising the endogenous levels of eCBs and eCB-like ligands in the NAc and mPFC. Our results show that AEA levels were decreased in the NAc and mPFC of VPA-exposed male rats and selectively in the NAc of VPA-exposed female rats. However, FBR administration was not able to restore AEA levels to control values. At first glance, these findings appear to contrast with the well-established role of AEA in the processing of social stimuli. Numerous studies demonstrated a crucial involvement of AEA in the regulation of social and emotional behaviors (Marco et al., 2011, 2013). For instance, exposure to social stimuli per se increases AEA levels in brain regions engaged in the processing of salient natural rewards, including the striatum and NAc (Marco et al., 2011), and AEA-mediated activation of CB1 receptors is both sufficient and necessary for the reinforcing properties of social interaction within the NAc (Wei et al., 2015; reviewed in Wei et al., 2017). Nevertheless, it is plausible that FBR-induced improvements in social behavior are in part mediated by modifications in eCB signaling in brain regions not examined in this study, such as the amygdala and hippocampus, where eCBs critically regulate emotional salience, social memory, and stress responsiveness. FBR-induced modifications in these circuits may compensate for persistent AEA deficits within the NAc and mPFC, thereby contributing to the observed rescue of behavioral deficits.

On the other hand, although levels of PEA were not significantly reduced in VPA-exposed male and female rats, FBR treatment elicited a significant elevation of PEA in male rats, selectively in the NAc. Accordingly, several lines of research have linked PEA with autism. Lower plasma levels of AEA and PEA have been reported in ASD children in comparison to those of neurotypical unaffected children (Karhson et al., 2018; Aran et al., 2019) and PEA supplementation as monotherapy or as add-on therapy reduced overall autism symptoms severity (Antonucci et al., 2015; Bertolino et al., 2017; Khalaj et al., 2018). Of note, PEA administration reduced repetitive behavior and social deficits in different rodent models of ASD (Bertolino et al., 2017; Cristiano et al., 2018). In line with a possible role of PEA as a local modulator of dopaminergic transmission in the NAc (Piomelli, 2014; Murillo-Rodríguez et al., 2011), it is likely that FBR administration by activating PPARα and modifying fatty acids metabolism raised PEA levels (Murru et al., 2022). Thus, increased PEA levels may positively influence the encoding of reward associated stimuli in this brain region, by increasing the salience of social reward and ultimately strengthening the resultant stimulus-evoked reward responses dependent on the activation of eCB system that modulates synaptic activity of D1 dopaminergic neurons within the NAc (Bilbao et al., 2020).

The hypothesis that FBR would restore the social competence in the VPA model to some extent by modulating eCBs levels may also be rooted in changes in FAAH and NAPE-PLD activity or expression. Indeed, we show for the first time that levels of FAAH were increased in the NAc and mPFC of VPA-exposed male rats and in the NAc of VPA-exposed female rats, and that FBR administration restored FAAH expression to control values. Consistently, a decrease in NAPE-PLD and an increase in FAAH mRNA levels were previously reported in the whole brain of rats prenatally exposed to VPA (Servadio et al., 2016). Furthermore, in genetic ASD models based on *FMR1* deletion, FAAH inhibition and reinstatement of AEA levels attenuated social deficits (Wei et al., 2016; Schiavi et al., 2023; but see also contrasting data). However, FAAH is unlikely to be the only factor driving these effects, as additional pathways may contribute to degradation of AEA and other *N*-acylethanolamines, like PEA or OEA. For instance, N-acylethanolamine acid amidase (NAAA), another key enzyme involved in PEA degradation, may also play a role (Ueda et al., 2010). Moreover, an increased expression of cyclooxygenase and lipoxygenase, which metabolize AEA, has been reported

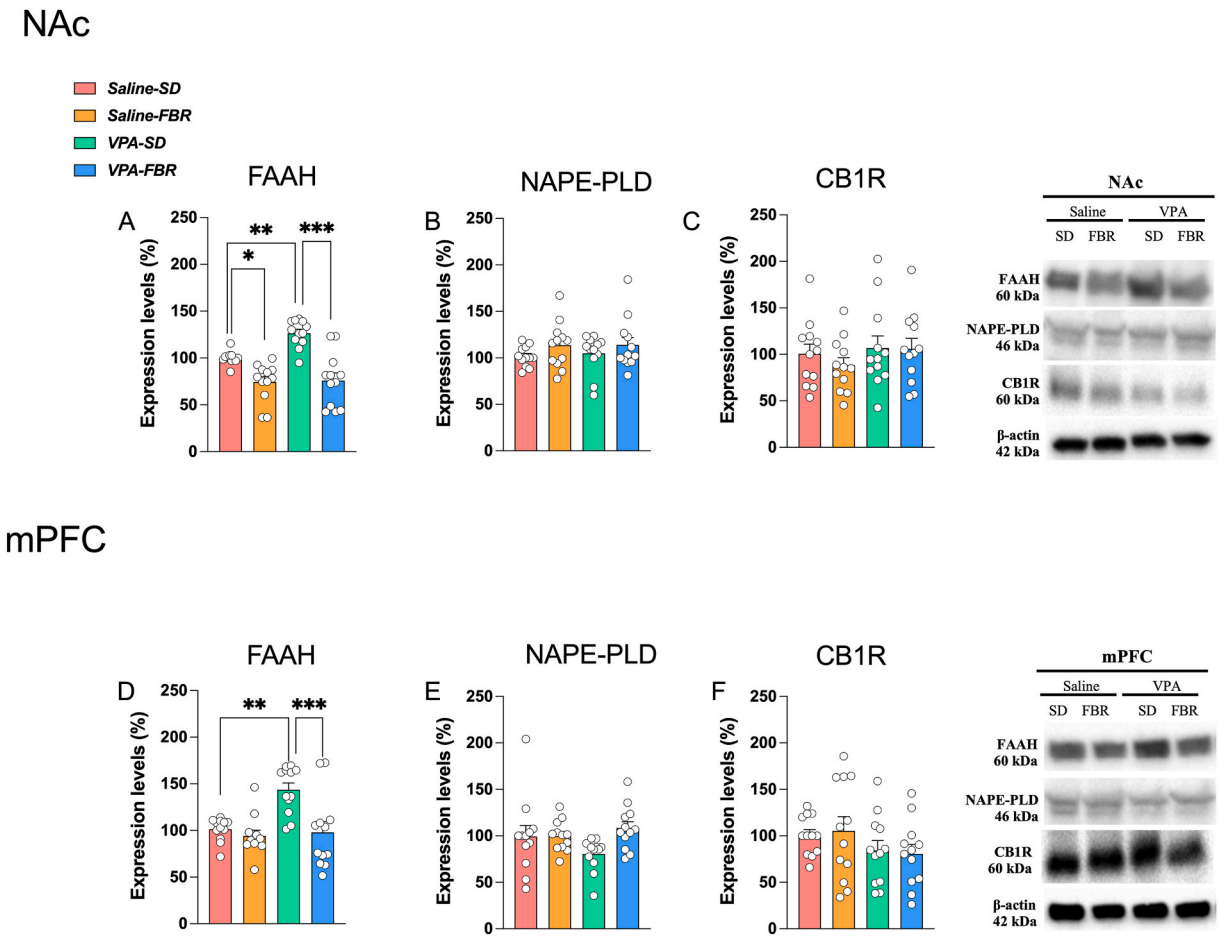

Fig. 6. Effects of VPA exposure and FBR treatment on endocannabinoid-related markers in male rats.
(A, D) FAAH protein levels (two-way ANOVA, NAc, VPA exposure: $F_{1,\ 44} = 6.41$, $p = 0.015$; FBR treatment: $F_{1,\ 44} = 47.49$, $p < 0.0001$; interaction: $F_{1,\ 44} = 5.07$, $p = 0.029$; mPFC, VPA exposure: $F_{1,\ 44} = 9.06$, $p = 0.0043$; FBR treatment: $F_{1,\ 44} = 11.78$, $p = 0.0013$; interaction: $F_{1,\ 44} = 6.31$, $p = 0.015$). (B, E) NAPE-PLD expression (two-way ANOVA; NAc: VPA: $F_{1,\ 44} = 0.079$, $p = 0.77$; FBR: $F_{1,\ 44} = 2.71$, $p = 0.10$; interaction: $F_{1,44} = 0.06$, $p = 0.80$; mPFC: VPA: $F_{1,44} = 0.47$, $p = 0.49$; FBR: $F_{1,\ 44} = 3.45$, $p = 0.069$; interaction: $F_{1,\ 44} = 3.24$, $p = 0.078$. (C, F) CB1 receptor expression (two-way ANOVA; NAc: VPA: $F_{1,\ 44} = 1.20$, $p = 0.27$; FBR: $F_{1,\ 44} = 0.37$, $p = 0.54$; interaction: $F_{1,\ 44} = 0.28$, $p = 0.59$; mPFC: VPA: $F_{1,\ 44} = 3.36$, $p = 0.073$; FBR: $F_{1,\ 44} = 0.001$, $p = 0.97$; interaction: $F_{1,\ 44} = 0.14$, $p = 0.70$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Bonferroni's multiple comparisons test. On the right, representative blots.

following VPA exposure (Yadav et al., 2017; Gassowska-Dobrowolska et al., 2020). Therefore, the exact mechanism of action of FBR remains unclear. In male rats exposed to VPA, FBR restored FAAH expression but did not normalize AEA levels, while significantly increasing PEA in the nucleus accumbens. This pattern suggests a selective modulation of lipid signaling rather than a broad restoration of endocannabinoid tone.

Female rats exposed to VPA did not show clear impairments of social behavior, as assessed in the social interaction, social novelty test and socially induced CPP. Females showed generally lower levels of social interaction overall, and VPA caused only a slight reduction in sociability without leading to marked impairments; in the CPP paradigm, which involves both reward processing and learning, VPA did not produce detectable deficits in female rats. Within this framework, FBR appears to enhance social interaction under normal conditions and to alleviate subtle VPA-induced social alterations, promoting more typical social behavior. These results are in agreement with previous reports of mild or very subtle ASD-like social deficits in female rats (Scheggi et al., 2020; Jeon et al., 2018; Mamali et al., 2025) and support that the male bias in the clinical definition of the ASD phenotype extends to preclinical models. Moreover, the characterization of the modifications induced in the female brain by prenatal VPA exposure has only been performed in a limited number of studies (e.g., Melancia et al., 2018). Of relevance in this context, we report for the first time decreased levels of OEA after prenatal VPA exposure in female rats, both in the NAc and mPFC, differently from what observed in the male littermates. Repeated FBR administration did not restore OEA levels to control values. Evidence shows that OEA administration is endowed with anti-stress and anti-depressant activities in different rodent models of depressive-like symptoms (Jin et al., 2015; Rani et al., 2021; Romano et al., 2020). Since OEA seems to be involved in the modulation of mood and anxiety (Yu et al., 2015; Romero-Sanchiz et al., 2019) more than in social behavior, where a prominent role is played by AEA and PEA, the sex-differences in levels of eCB and eCB-like mediators after prenatal VPA-exposure may contribute to the different behavioral phenotype in male and female rats. Thus, a further in-depth evaluation designed to detect atypical social behaviors, vulnerability to stress, and anxiety- and depressive-like responses in VPA-exposed female rats is warranted.

We would like to acknowledge some limitations of this study that can be overcome in future research. First, we used a single rodent model of ASD. Although the VPA model has been demonstrated to reliably reproduce in rodents the core behavioral traits of ASD in humans, we do not presume, given the complexity of ASD, that any rodent model can fully recapitulate the spectrum of behavioral and neural changes characteristic of ASD. Thus, the present findings may not be fully generalized to social anhedonia in ASD, even though some of the behavioral and neurochemical findings are in agreement with what reported in other rodent models. Moreover, the study relied exclusively on behavioral assays to infer constructs such as social motivation and social reward

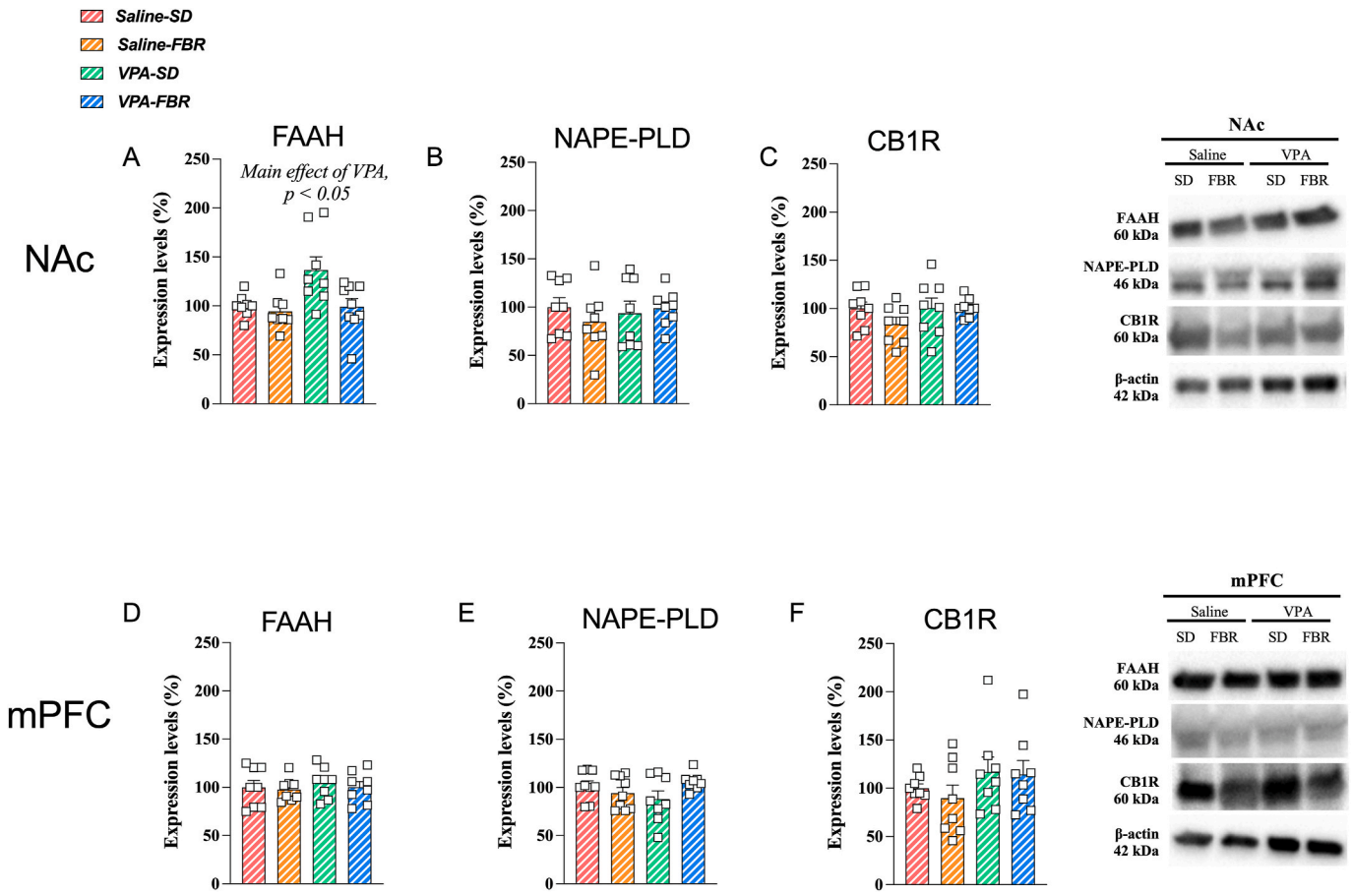

Fig. 7. Effects of VPA exposure and FBR treatment on endocannabinoid-related markers in female rats.
(A, D) FAAH protein levels (Two-way ANOVA, NAc: VPA exposure: $F_{1, 28} = 4.83$, $p = 0.036$; FBR treatment: $F_{1, 28} = 6.62$, $p = 0.0156$; interaction: $F_{1, 28} = 3.74$, $p = 0.0632$; mPFC: VPA exposure: $F_{1, 28} = 0.42$, $p = 0.520$; FBR treatment: $F_{1, 28} = 0.36$, $p = 0.54$; interaction: $F_{1, 28} = 0.041$, $p = 0.83$). (B, E) NAPE-PLD protein levels (NAc: VPA: $F_{1, 28} = 0.13$, $p = 0.71$; FBR: $F_{1, 28} = 0.23$, $p = 0.63$; interaction: $F_{1, 28} = 0.96$, $p = 0.33$; mPFC: VPA: $F_{1, 28} = 0.047$, $p = 0.82$; FBR: $F_{1, 28} = 1.44$, $p = 0.23$; interaction: $F_{1, 28} = 3.21$, $p = 0.082$). (C, F) CB1 receptor expression (NAc: VPA: $F_{1, 28} = 1.44$, $p = 0.23$; FBR: $F_{1, 28} = 1.16$, $p = 0.28$; interaction: $F_{1, 28} = 1.27$, $p = 0.2682$), mPFC: VPA: $F_{1, 28} = 2.65$, $p = 0.11$; FBR: $F_{1, 28} = 0.25$, $p = 0.61$; interaction: $F_{1, 28} = 0.092$, $p = 0.76$).
On the right, representative blots.

valuation; although validated, these tasks do not fully capture the complexity of human social functioning. The translational relevance of these findings may be limited by the dose of FBR used. We acknowledge that this dose exceeds clinically used human doses, which constrains direct translation; however, species-specific differences in FBR metabolism between humans and rats have been reported and should be considered when interpreting these findings. A further limitation is that FAAH and NAPE-PLD expression were measured at the protein level only, and complementary analyses of enzymatic activity, substrate turnover, or gene expression, which would provide a better definition of the mechanisms underlying the observed lipidomic changes, were not performed. Finally, the mechanistic hypothesis that this study proposes is largely based on associative results, and further investigation using pharmacological or genetic manipulation (e.g., PPARα antagonism, PEA administration, or FAAH inhibition) would strengthen the present findings. Moreover, since our results confirm a sexual dimorphism in the VPA model with the typical behavioral phenotype, neurochemical modifications and responses to FBR administration almost entirely restricted to male rats, only limited inferences to female rats can be drawn from the present study.

The results of this study may provide further relevant information to develop therapeutic interventions that when initiated during postnatal neurodevelopment may foster the responsiveness to social stimuli, thus improving the development and acquisition of social competences in ASD subjects. Moreover, they may contribute to repurpose fibrates as novel therapeutics in ASD management.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

CRediT authorship contribution statement

S. Salviati: Formal analysis, Investigation. **G. Braccagni:** Investigation. **E. Corridori:** Investigation. **G. Sabatini:** Data curation, Writing – review & editing. **F. Fanti:** Investigation. **F. Guzzi:** Investigation. **M. Parenti:** Conceptualization, Writing – review & editing. **N. Battista:** Data curation, Writing – original draft, Writing – review & editing. **S. Scheggi:** Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **C. Gambarana:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

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

Acknowledgments

This article is dedicated to the memory of Prof. M. Graziella De Montis, who recently passed away. We gratefully acknowledge her contribution to in vivo experiments and the lively, helpful discussions and critical comments on the drafts of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2026.110996.

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