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Striatal dopamine D_2, adenosine A_{2A} and cannabinoid CB_1 receptors balance as a target against non-cognitive symptoms in a mouse model of Alzheimer's disease

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ABSTRACT

Behavioral and psychological symptoms of dementia are almost ubiquitous in Alzheimer's disease (AD) but current therapies are not fully effective and safe. In this study, we aim to evaluate the role played by the interplay among striatal D_2, adenosine A_{2A} ($A_{2A}R$) and cannabinoid CB_1 (CB_1R) receptors in some of these non-cognitive impairments in a well-established animal model of AD, the double transgenic APP/PS1 mice. Our results reveal that the alterations existing in the ratios between these three receptors significantly correlate with the sensorimotor gating and the social interaction impairments occurring in APP/PS1 mice at 12 months of age. Moreover, the pharmacological stimulation of $A_{2A}R$ and CB_1R blunted the sensorimotor gating deficiencies in APP/PS1 mice. To note, we observed some age-dependent differences among male and female mice. In conclusion, the present study provides evidence for the contribution of an altered interplay between dopaminergic, adenosinergic and endocannabinoid systems in the sensorimotor gating deficits and social withdrawal occurring in AD and points to $A_{2A}R$ and CB_1R as a potential target to reverse these non-cognitive symptoms in AD patients.

1. Introduction

Behavioral and psychological symptoms of dementia (BPSD) are almost ubiquitous in Alzheimer's disease (AD) patients and are associated with poor outcomes in terms of function, quality of life, disease course, mortality and economic cost (Peters et al., 2015; Devanand et al., 2022). These non-cognitive symptoms include impairments in motivation, social behavior and awareness, mood disorders, anxiety, agitation, impulsivity and psychosis, all of which often require clinical intervention. Among them, psychotic symptoms, including hallucinations and delusions, are some of the most clinically relevant because they are associated with a more rapid progression to severe dementia and earlier death, confer greater risk of institutionalization and substantially increase caregiver burden (Ismail et al., 2022). Psychotic symptoms are estimated to occur in 41 % of people with AD and emerge at a rapid rate during progression from mild cognitive impairment to early and middle stages of disease (Ropacki and Jeste, 2005; Ballard et al., 2020). Antipsychotic drugs used to mitigate these symptoms are mainly dopamine (DA) D_2 receptors (D_2R) antagonists. These antipsychotic drugs are not fully effective and are associated with substantial side effects including somnolence, extrapyramidal symptoms, gait abnormalities and, more importantly, increased mortality in AD patients (Schneider et al., 2006; Ballard et al., 2020). Therefore, there exists a significant need to develop safer and more effective antipsychotic treatments for AD. However, a better understanding of the neurobiological bases of these non-cognitive symptoms in AD is essential to achieve this goal.

In this sense, the present study aims to evaluate the role played by the interplay among striatal D_2, adenosine A_{2A} ($A_{2A}R$) and cannabinoid CB_1 (CB_1R) receptors in two of the BPSD symptoms occurring in AD that are also present in psychotic disorders such as schizophrenia (*i.e.* sensorimotor gating and social interaction deficits). For doing so, we use double transgenic APP/PS1 mice, a well-established animal model of AD (Borchelt et al., 1997). We have focused on the striatum since this brain area has been demonstrated to be a key substrate for these symptoms in

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schizophrenia (Millan et al., 2014; Swerdlow and Light, 2018). Our interest in D_2R, $A_{2A}R$ and CB_1R is sustained by three main reasons. First, it is well accepted that psychotic symptoms such as hallucinations and delusions are sustained by hyperactivity of the mesolimbic dopaminergic pathway, which correlates with increased activity of D_2R in striatum and sustains the efficacy of current antipsychotic drugs (Seeman, 2013; Simpson et al., 2022). Second, increasing evidence suggest that glutamatergic and dopaminergic signaling are altered during psychosis as a consequence of adenosine hypofunction (Boison et al., 2012; Pasquini et al., 2022). The adenosinergic control of the striatal dopaminergic system largely depends on the strong antagonistic interaction between adenosine $A_{2A}R$ and dopamine D_2R at a behavioral and cellular level (Ferré et al., 2018). In fact, genetic deletion of $A_{2A}R$ induced a psychotic-like phenotype in mice (Moscoso-Castro et al., 2016). Moreover, some previous evidence points to the involvement of adenosinergic and dopaminergic systems in the development of non-cognitive symptoms in preclinical models of AD (Abad et al., 2016; Liu et al., 2020). Interestingly, β-amyloid-treated mice showed increased spontaneous locomotion, increased response to amphetamine and a blunted response to caffeine when compared to their respective control. This could mean that β-amyloid peptide predisposes to psychotic symptoms of AD by increasing the dopaminergic activity, likely decreasing adenosinergic inhibitory tone (Dall'Igna et al., 2004). Third, CB_1R is known to be expressed in mesolimbic areas, which project to dopaminergic neurons of the ventral tegmental area (VTA), and participates in the control of dopaminergic activity (Manzanares et al., 2018). CB_1R and D_2R have been demonstrated to reciprocally exert a negative allosteric modulation in the striatum (Marcellino et al., 2008), as it also occurs between CB_1R and $A_{2A}R$ (Tebano et al., 2012). Additional evidence support a crucial role for CB_1R in the progression of AD: (i) alterations in the expression or functionality of CB_1R have been described both in AD patients' brains and animal models (Aso et al., 2012; Manuel et al., 2014); (ii) the pharmacological stimulation of CB_1R led to a reduction of the neurotoxic effect of Aß peptide in some *in vitro* models and to a reversion of Aß-induced memory impairment in different *in vivo* models (reviewed in Aso and Ferrer, 2014); and (iii) the genetic deletion of CB_1R in APP/PS1 mice drastically reduced their survival and accelerated their memory impairment (Aso et al., 2018). Similarly, a relevant role in cognitive decline and synaptic loss in AD has been also described for $A_{2A}R$ (Horgusluoglu-Moloch et al., 2017; Temido-Ferreira et al., 2020; Viana da Silva et al., 2016).

In summary, previous reports suggest that alterations in $A_{2A}R$ and CB_1R occur during AD progression and that a complex interplay between D_2R, $A_{2A}R$ and CB_1R finely regulate dopaminergic activity and, therefore, could play a role on the sensorimotor gating and social interaction deficits associated to AD. The present study supports this hypothesis and for the first time evaluates the complex balance between dopaminergic, adenosinergic and endocannabinoid systems as potential substrate for non-cognitive symptoms in AD, in contrast to previous reports addressing the role of each specific neurotransmitter system or the interaction between only two of them. The consecution of our aims could help to optimize a potential therapy against these non-cognitive symptoms in AD based on targeting D_2R, $A_{2A}R$ and CB_1R interplay.

2. Experimental procedures

2.1. Animals

Male and female 4 and 12 month-age APP/PS1 mice and *wild-type* littermates (WT, C57BL/6 J background) were used for the study. The generation of mice expressing the human mutated APPswe and PS1dE9 has been described elsewhere (Borchelt et al., 1997). Animals were housed 3–4 per cage and maintained under standard animal housing conditions in a 12-h dark-light cycle with free access to food and water. Mice were randomly assigned to treatment groups and the experiments were conducted under blind experimental conditions. All animal procedures were carried out by following the guidelines of the European Communities Council Directive 2010/63/EU and with the approval of the local ethical committee of the University of Barcelona.

2.2. Pharmacological treatment

The CB_1R agonist ACEA and the selective $A_{2A}R$ agonist CGS-21680 were purchased from Sigma-Aldrich Química SL (Madrid, Spain) and Abcam (Cambridge, UK), respectively. CGS-21680 (0.5 mg/kg) (Sills et al., 2001) was dissolved in 1 % DMSO and ACEA (1.5 mg/kg) (Aso et al., 2012) in 5 % ethanol, 5 % Tween and 90 % saline for intraperitoneal (i.p.) injection. The drugs were administered 10 min before the behavioral test. The administered volume was 10 mL/kg of body weight.

2.3. Behavioral evaluation and sample collection

2.3.1. Three-chamber test

Social interaction and social memory of mice were evaluated in the three-chamber test (Kaidanovich-Beilin et al., 2011). The apparatus consists of a box divided into three chambers that are separated by two removable walls. First, the subject mouse was placed at the center of the middle chamber for 5 min as a habituation phase. Next, the session 1 (sociability test) consisted on positioning a novel mouse (hereinafter referred to as mouse 1) inside a wire cage in one of the lateral chambers and a novel object (identical but empty wire cage) in the opposite one. The time spent exploring the novel mouse (mouse 1, T_M) or the novel object (T_O), was analyzed during 10 min. The social memory and novelty test (session 2) began immediately after the sociability test. In this test, the previous mouse (mouse 1) remained in its wire cage (now called the familiar mouse) and a novel mouse (mouse 2) was placed in the wire cage in the opposite side. Again, time spent exploring the familiar mouse (mouse 1, T_F) or the novel mouse (mouse 2, T_N), was manually recorded during 10 min. Then, Sociability Index (SI) and Social Memory Index (SMI) were respectively calculated for the first and the second sessions: $SI = (T_M\text{-}T_O)/(T_M + T_O)$; $SMI = (T_N\text{-}T_F)/(T_N + T_F)$. At the end of the behavioral testing, the animals were sacrificed and their brains rapidly dissected, frozen, and stored at −80 °C until use.

2.3.2. Prepulse inhibition test (PPI)

The PPI was performed to evaluate the sensorimotor gating in mice, which is known to be impaired in psychotic conditions (Valle-León et al., 2021). The animals were submitted to a 5-minute session in the startle chamber with 65 dB white noise (acclimation phase) followed by a trial pulse basal consisting of 10 pulse-alone trials sessions of 120 dB and 20 ms (habituation phase). Subsequently, 5 randomly distributed blocks with 10 trials were presented. The blocks consist on i) trial without stimulation, background noise only (65 dB); ii) trial pulse with a startle stimulus (120 dB, 8000 Hz, 40 ms); iii) trial with 70 dB prepulse (70 dB, 10,000 Hz, 20 ms) + pulse (120 dB, 8000 Hz, 40 ms); iv) 75 dB prepulse (75 dB, 10,000 Hz, 20 ms) + pulse (120 dB, 8000 Hz, 40 ms); v) 80 dB prepulse (80 dB, 10,000 Hz, 20 ms) + pulse (120 dB, 8000 Hz, 40 ms). Inter-trial intervals were 30 s long and a background noise level of 65 dB was maintained. Startle amplitude after prepulse (70, 75 and 80 dB) and pulse (120 dB) were measured. The percentage of prepulse inhibition (% PPI) for each prepulse intensity, was calculated as: %PPI = (startle amplitude on pulse alone – startle amplitude on prepulse trial)/(startle amplitude on pulse alone) x 100.

2.4. Gel electrophoresis and immunoblotting

Striatal samples from mice were homogenized in ice-cold 10 mM Tris HCl (pH 7.4), 1 mM EDTA and 300 mM KCl buffer containing a protease inhibitor cocktail (Roche Molecular Systems, USA). The homogenates were centrifuged for 10 min at 1000 ×*g*. The resulting supernatants were centrifuged for 30 min at 12,000 ×*g* and the pellets were resuspended in 50 mM Tris HCl (pH 7.4) and 10 mM MgCl solution. Protein

concentration was determined using the BCA protein assay kit (Thermo Fisher Scientific, Inc., Rockford, IL, USA) and equal amounts of protein (10 μg) for each sample were loaded and separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (10 %) gels. Proteins were transferred onto Hybond®-LFP polyvinylidene difluoride (PVDF) membranes (GE Healthcare, Chicago, IL, USA) using a Trans-Blot® SD Semi-Dry Transfer Cell (Bio-Rad, Hercules, CA, USA). Then, PVDF membranes were blocked with 5 % non-fat milk in phosphate buffered saline (PBS) buffer containing 0.05 % Tween-20 (PBS-T) during 45 min. After washing, membranes were incubated overnight at 4 °C with rabbit polyclonal anti-D_2R (1:1500; Frontier Institute Co. Ltd., Shinko-nishi, Ishikari, Hokkaido, Japan; AB_2571596), mouse monoclonal anti-$A_{2A}R$ (1:1000, Santa Cruz Biotechnology, Dallas, TX, USA, sc-32261), rabbit polyclonal anti-CB_1R (1:1500; Abcam; ab23703), goat polyclonal anti-DAT (1:1000; Santa Cruz, sc-1433), mouse monoclonal anti-ENT1 (1:250; Santa Cruz, sc-377283) and rabbit polyclonal anti-β-tubulin (1:10,000, Abcam, Cambridge, UK; ab21058) antibodies in blocking solution overnight at 4 °C. PVDF membranes were washed with PBS-T three times (5 min each) before incubation with either a horseradish peroxidase (HRP)-conjugated goat antirabbit IgG (1/30,000; Pierce Biotechnology, Rockford, IL, USA), HRP-conjugated rabbit anti-goat IgG (1/10,000; Pierce Biotechnology) or HRP-conjugated goat anti-mouse (Thermo Fisher Scientific) in blocking solution at room temperature during 2 h. After washing the PVDF membranes with PBS-T 20 three times (5 min each), the immunoreactive bands were developed using a chemiluminescent detection kit (Thermo Fisher Scientific) and were detected with an Amersham Imager 600 (GE Healthcare Europe GmbH, Barcelona, Spain). When needed, membranes were stripped to remove the primary and secondary antibodies. The stripping efficacy was routinely tested by incubating again the secondary antibody to confirm the absence of signal before incubating additional primary antibodies, except on those cases when the MW of the expected bands was very different (*i.e.* D_2R and ENT-1). The sequence of primary antibodies incubated in each membrane is included in Supplementary material (uncropped blots). Densitometric quantification was carried out with Image J (NIH, US). Protein loading on the gel was normalized by β-tubulin detection. Six animals per group were analyzed.

2.5. Colocalization study by immunofluorescence and confocal imaging

Fixed brain samples were cut with a vibratome in 25 μm thick coronal sections. Sections were blocked with 4 % normal donkey serum solution and then incubated at 4 °C overnight with the rabbit polyclonal anti-D_2R (1:200; Frontier Institute Co. Ltd., AB_2571596), goat polyclonal anti-$A_{2A}R$ (1:200, Santa Cruz Biotechnology, Dallas, TX, USA, sc-7504) and mouse monoclonal anti-CB_1R (1:500; Synaptic Systems, Göttingen, Germany, 258011). Negative control sections were incubated with blocking solution instead of primary antibodies After washing, the sections were incubated with Alexa647 (1:1000, Thermo Fisher Scientific), Cy2 or Cy3 (1:1000, Jackson ImmunoResearch, Ely, United Kingdom) fluorescence secondary antibodies against the corresponding host species. After washing, the sections were mounted in *In Situ* Mounting Medium with DAPI (Sigma-Aldrich), sealed, and dried overnight. Sections were examined with a Carl Zeiss LSM880 confocal microscope at 40×. Manders split coefficients were calculated by using ImageJ software.

2.6. Statistical analyses

Statistical analysis was performed with GraphPad Prism 9 (RRID: SCR_002798; San Diego, CA, USA). Statistical difference was set at $P < 0.05$. The number of samples/animals (n) in each group is indicated in the corresponding figure legend. Data normality was assessed by the Shapiro–Wilk test. Univariate outliers were assessed by the Grubbs' test, while multivariate outliers were detected by Mahalanobis distance method. Comparisons among experimental groups were performed by three-way ANOVA with genotype, age, and sex as between factors (behavioral test results, immunoblotting quantifications), followed by Tukey's *post hoc* when required. When sex factor was not significant, data were analyzed with two-way ANOVA with genotype and age as between factors, followed by Tukey's *post hoc* when required. Pearson's or Spearman's correlation coefficients were calculated for parametric and non-parametric data, respectively.

3. Results

3.1. APP/PS1 mice exhibit non-cognitive symptoms at 12 months of age

Psychosis and impairments in social behaviors occur in most AD patients throughout the course of their illness. Our aim was to investigate whether a classic preclinical model of AD, the APP/PS1 mouse, recapitulates these common symptoms. Therefore, we evaluated social interaction, social memory, and sensorimotor gating in APP/PS1 mice in early (4 months) and advanced (12 months of age) stages of the neurodegenerative process. First, we observed that APP/PS1 mice at 12 months, but not at 4 months of age, exhibited impairments in social interaction ($P < 0.001$) and social memory ($P < 0.05$) when compared to WT littermates (Fig. 1A and B) in the three-chamber test. No significant differences were observed due to the sex in this behavioral test (*see* Supplementary Table 1 for statistical details). Next, the sensorimotor gating responses of these animals were evaluated using the PPI test. Interestingly, APP/PS1 mice at 12 months of age, but not at 4 months of age, showed sensorimotor gating impairment (Fig. 1C). A three-way ANOVA (genotype x age x sex) confirmed a significant effect of genotype, age, and sex on the percentage of prepulse inhibition (%PPI) at all prepulse intensities tested, except for genotype at 75 dB (*see* Supplementary Table 1 for statistical details). Tukey's *post hoc* test demonstrated a significant reduction in the % PPI in male APP/PS1 mice with respect to WT littermates at 12 months ($P < 0.01$ at 70 dB) and in male APP/PS1 mice at 12 months with respect to APP/PS1 mice at 4 months ($P < 0.01$ at 70 dB; $P < 0.001$ at 75 dB; $P < 0.05$ at 80 dB; Fig. 1C). Similarly, a significant decrease in % PPI was observed in female APP/PS1 mice at 12 months *versus* female APP/PS1 mice at 4 months ($P < 0.05$ at 70 dB; $P < 0.01$ at 75 dB; $P < 0.001$ at 80 dB; Fig. 1C). Interestingly, no differences between genotypes were observed in female mice aged 12 months in any of the prepulse intensities tested, likely due to an age-dependent decrease in %PPI in WT mice compared to 4 months WT animals ($P = 0.06$ at 70 dB; $P < 0.01$ at 75 dB; $P < 0.001$ at 80 dB; Fig. 1C).

3.2. Colocalization of the D_2R $A_{2A}R$, CB_1R in the striatum of mice

Previous findings demonstrated that the D_2R, $A_{2A}R$, and CB_1R are expressed in striatum and that they can colocalize in the same neurons, being able to form even heteromeric complexes (Bonaventura et al., 2014; Moreno et al., 2018). We performed a study based on immunofluorescence techniques and confocal imaging to determine the degree of colocalization between each pair of receptors in the striatum of control mice (WT mice at 4 months of age). The mean ± SEM ($n = 14$) for the Manders split coefficients, which reveal the proportional to the amount of fluorescence of the colocalizing pixels in each colour channel (Manders et al., 1993), were the following: 0.790 ± 0.024 for $A_{2A}R$-D_2R, 0.382 ± 0.035 for $A_{2A}R$- CB_1R and 0.381 ± 0.058 for D_2R- CB_1R correlations. Thus, our results confirm that D_2R, $A_{2A}R$, and CB_1R colocalize in the striatum but that a higher colocalization occurs between D_2R and $A_{2A}R$ than between CB_1R and the other two receptors (Fig. 2).

3.3. The density of the $A_{2A}R$, CB_1R, DAT and ENT1 is reduced in the striatum of aged male APP/PS1 mice

We aimed to evaluate the density of key components within the

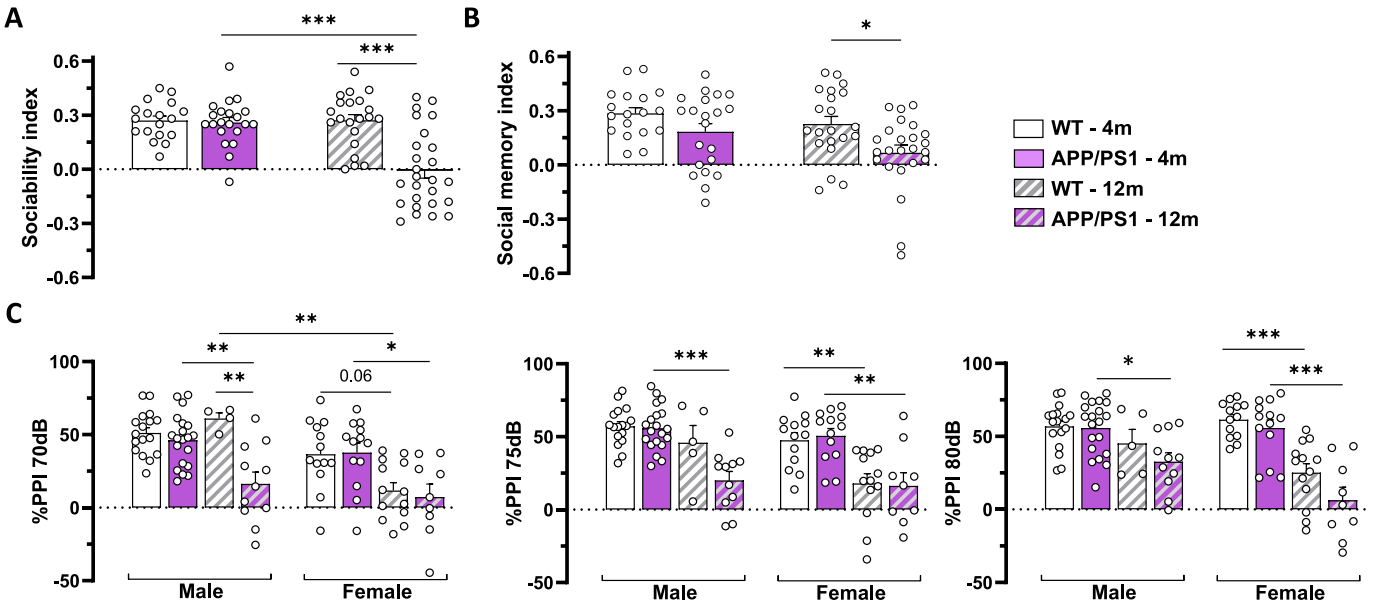

Fig. 1. Behavioral evaluation of non-cognitive symptoms in young and aged APP/PS1 mice. Social interaction and sensorimotor gating impairment in APP/PS1 mice at 12 months of age. (A) Sociability index and (B) Social memory index evaluated in the three-chamber test revealed a social interaction impairment in APP/PS1 mice at 12 months of age. (C) The percentage of the prepulse inhibition (%PPI) of the acoustic startle response was reduced in 12-months-old male APP/PS1 mice at all the acoustic prepulse intensities tested (left: 70 dB; middle: 75 dB; right: 80 dB). A significant reduction in the %PPI was observed in wild-type (WT) female mice at 12 months of age compared to 4-months-old female WT mice. Data are expressed as the mean ± SEM (n = 5–17). As sex factor was not significant for sociability and social memory, data were analyzed by two-way ANOVA with genotype and age as between factors, followed by Tukey's *post hoc* test. PPI test was analyzed by three-way ANOVA with Tukey's *post hoc* test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Tukey's *post hoc* test.

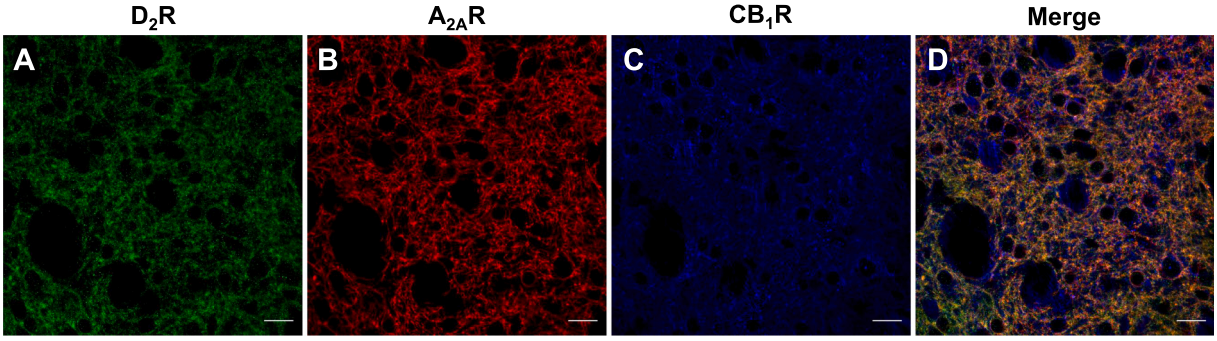

Fig. 2. Immunoblot detection of D_2R, $A_{2A}R$, CB_1R, DAT and ENT1 transporter in the striatum of young and aged APP/PS1 mice. (A) Representative immunoblots showing the density of D_2R, $A_{2A}R$, CB_1R, DAT and ENT1 transporter in striatal membranes from WT and APP/PS1 mice at 4 and 12 months of age. Striatal membranes were analyzed by SDS-PAGE (10 μg of protein/lane) and immunoblotted using rabbit anti-D_2R, mouse anti-$A_{2A}R$, rabbit anti-CB_1R, goat anti-DAT, mouse anti-ENT1, and rabbit anti-α-actinin antibodies (*see* Methods). (B) Relative quantification of D_2R, $A_{2A}R$, CB_1R, DAT and ENT1 transporter. The immunoblot protein bands corresponding to D_2R, $A_{2A}R$, CB_1R, DAT and ENT1 transporter from WT and APP/PS1 mice at 4 and 12 months of age (n = 5–6) were quantified by densitometric scanning. Values were normalized to the respective amount of β-tubulin in each lane to correct for protein loading. Results are expressed as percentage (mean ± SEM) and referred to WT mice at 4 months of age. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, three-way ANOVA with Tukey's *post hoc* test.

dopaminergic, adenosinergic and endocannabinoid systems in striatal samples of APP/PS1 mice at 4 and 12 months of age. To this end, we conducted immunoblot experiments on striatal membrane extracts from these animals. The density of D_2R, $A_{2A}R$, and CB_1R was established by the presence of protein bands of molecular weight ~70–80 kDa, ~50 kDa, and ~60 kDa, respectively (Fig. 3A), as previously described (Carriba et al., 2007; Valle-León et al., 2021). Similarly, the density of the DAT and ENT1 transporter was assessed by detecting a protein band of ~75 kDa, and ~50 kDa, respectively (Fig. 3A), as previously described (Song et al., 2017; Valle-León et al., 2021). A three-way ANOVA (genotype x age x sex) confirmed a significant main effect of genotype ($A_{2A}R$, CB_1R and ENT_1), age (CB_1R) and sex ($A_{2A}R$) as well as interaction between some of the factors for all proteins tested, except ENT_1 (*see* Supplementary Table 2 for statistical details). Thus, Tukey's *post hoc* test revealed that male APP/PS1 mice at 12 months, but not at 4 months of age, exhibited decreased levels of all the transmission components studied, except D_2R, compared to male WT mice (Fig. 3B). Interestingly, no genotype differences were observed between female mice of any age. It should be noted that female WT mice showed a significant reduction in the density of D_2R ($P < 0.05$), $A_{2A}R$ ($P < 0.01$) and DAT ($P < 0.05$), compared to male WT littermates of 12 months of age (Fig. 3B). In general, an age- and sex-dependent density reduction of the $A_{2A}R$, CB_1R, DAT and ENT1 transporter was demonstrated in APP/PS1 mice, while D_2R levels remained constant. To contextualize these alterations within the well-known functional and molecular interaction between dopaminergic, adenosinergic and endocannabinoid systems in the striatum (Carriba et al., 2007; Moreno et al., 2018), we determined the density ratios for D_2R, $A_{2A}R$ and CB_1R to assess potential

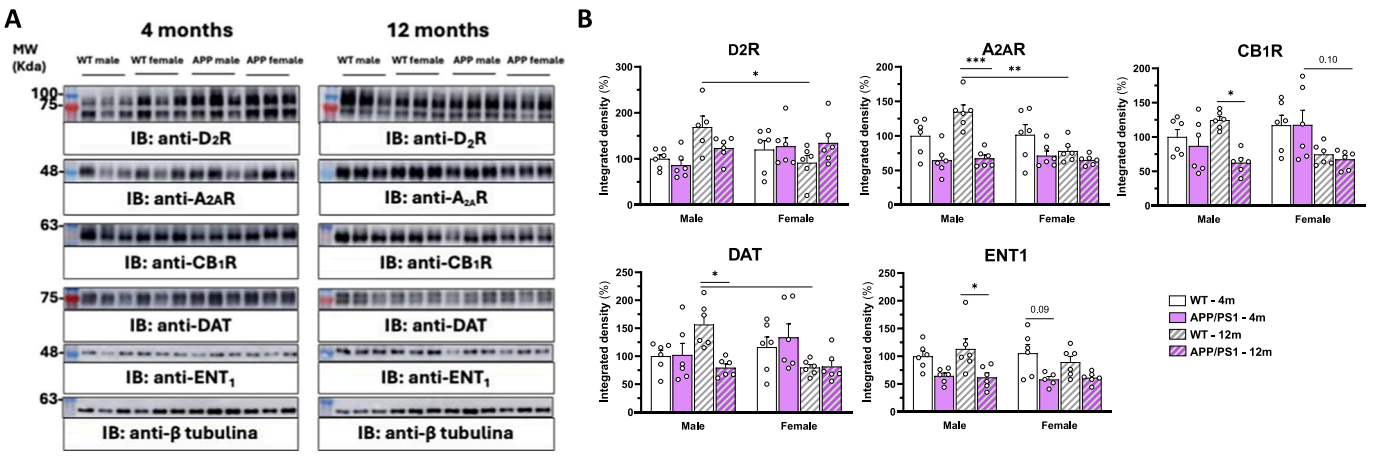

Fig. 3. Colocalization of D_2R, $A_{2A}R$ and CB_1R in the striatum of mice detected by immunofluorescence techniques and confocal imaging. Representative images of D_2R (A, green), $A_{2A}R$ (B, red) and CB_1R (C, blue) immunoreactivity in striatal sections of control mice (*see* Methods). D panel shows the merge of all three channels. Scale bar represents 20 μm. Calculated Manders split coefficients revealed that a higher colocalization occurs between D_2R and $A_{2A}R$ than between CB_1R and the other two receptors ($n = 14$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

stoichiometric alterations. A three-way ANOVA (genotype x age x sex) revealed a significant effect of genotype and age, but not sex, on the $D_2R/A_{2A}R$, D_2R/CB_1R and $CB_1R/A_{2A}R$ ratios. Consequently, a two-way ANOVA (genotype x age) confirmed a significant effect of genotype and age in the three evaluated ratios, as well as the interaction between factors in the D_2R/CB_1R ratio (Fig. 4). Tukey's *post hoc* tests revealed that at 12 months of age, APP/PS1 mice exhibited a significant increase in the $D_2R/A_{2A}R$ ($P < 0.001$) and the D_2R/CB_1R ($P < 0.01$) ratios (Fig. 4A and B), but not in the $CB_1R/A_{2A}R$ ratio (Fig. 4C). Interestingly, the $CB_1R/A_{2A}R$ ratio increased significantly ($P < 0.05$) at 4 months of age (Fig. 4C), mirroring the increase observed in the $D_2R/A_{2A}R$ ratio ($P < 0.05$; Fig. 4A). Finally, a significant age-dependent increase in the $D_2R/A_{2A}R$ ($P < 0.01$) and D_2R/CB_1R ($P < 0.001$) ratios was observed in APP/PS1 mice, while the $CB_1R/A_{2A}R$ ratio was reduced with age ($P < 0.01$; Fig. 4C). In summary, these findings revealed alterations on the relative densities of D_2R, $A_{2A}R$, and CB_1R across age in APP/PS1 mice, although they do not show a significant correlation with sex.

3.4. *Multiple cross-correlations of D_2R, $A_{2A}R$ and CB_1R densities and non-cognitive symptoms*

An accurate balance of D_2R, $A_{2A}R$, and CB_1R expression is assumed to be instrumental for their precise neurochemical interaction under physiological conditions. Therefore, we assessed potential correlations between receptors densities in the striatum and between these receptor densities and non-cognitive symptoms. First, we found a positive correlation between the density of D_2R and $A_{2A}R$ ($P < 0.001$, $n = 46$), D_2R and CB_1R ($p < 0.05$, n = 46), as well as $A_{2A}R$ and CB_1R ($p < 0.001$, n = 46), in the striatal samples from all mice analyzed (Supplementary Fig. 1). Later, we calculated these correlations for each experimental group. Importantly, these correlations were not observed in APP/PS1 mice, independently of age, except the correlation of D_2R and $A_{2A}R$, which was preserved in APP/PS1 mice at 4 but not at 12 months of age (Supplementary Fig. 1A). Next, we evaluated whether the observed alterations in D_2R, $A_{2A}R$, and CB_1R density (Fig. 3) and receptor ratios (Fig. 4) could correlate with the non-cognitive symptoms observed in aged APP/PS1 mice (Fig. 1). Interestingly, our results revealed a positive correlation between SI and %PPI at 80 dB with the levels of CB_1R (SI: $P < 0.05$; %PPI: $P < 0.01$; Fig. 5C and D) and the $CB_1R/A_{2A}R$ ratio (SI: $P < 0.05$; %PPI: $P < 0.05$; Fig. 5E and F), but a negative correlation between SI and %PPI at 80 dB with $D_2R/A_{2A}R$ ratio (SI: $p < 0.01$; %PPI: $p < 0.05$; Fig. 5A and B) and the D_2R/CB_1R ratios (SI: $p < 0.001$; %PPI: $p < 0.01$; Fig. 5C and D). In summary, this analysis suggested that increased relative density of D_2R respect to $A_{2A}R$ or CB_1R (*i.e.*, increased density ratio) is associated to SI and PPI impairments, whereas increased striatal CB_1R density or $CB_1R/A_{2A}R$ ratio is associated to better performance in SI an PPI tests.

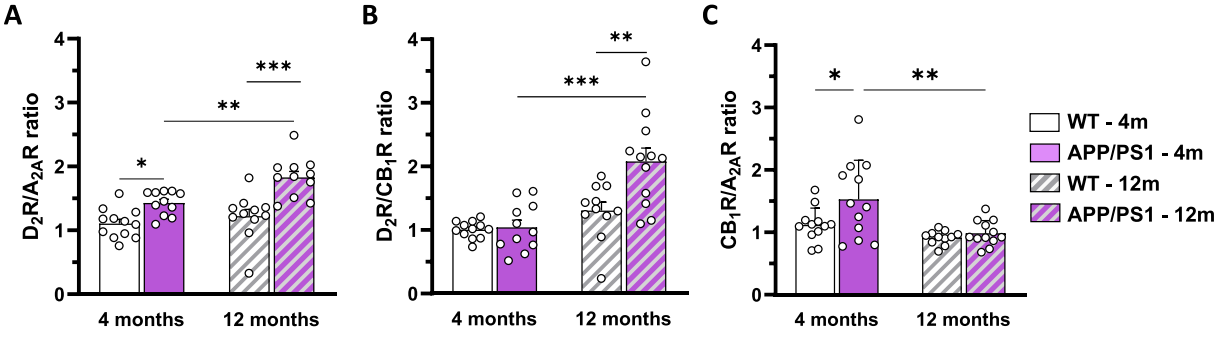

Fig. 4. Ratio between the expression of receptors evaluated by immunoblot in striatal samples from APP/PS1 and WT mice at 4 and 12 months of age. APP/PS1 mice exhibited higher $D_2R/A_{2A}R$ ratio at 4 and 12 months of age (A), higher D_2R/CB_1R ratio at 12 months of age (B) and higher $CB_1R/A_{2A}R$ ratio at 4 months of age (C) than their respective age-matched WT littermates. In contrast to the age-induced increase in the $D_2R/A_{2A}R$ (A) and D_2R/CB_1R (B) ratios in APP/PS1 mice, the $CB_1R/A_{2A}R$ ratio (C) was decreased in transgenic mice at 12 months respect to 4 months of age. Data are expressed as the mean ± SEM ($n = 11$–12 per group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-way ANOVA with genotype and age as between factors, followed by Tukey's *post hoc* test.

3.5. Activation of $A_{2A}R$ and CB_1R reversed the sensorimotor gating deficits of aged APP/PS1 mice

Based on our findings, we interrogated whether the activation of $A_{2A}R$ and CB_1R, to hypothetically counteract D_2R function, could potentially ameliorate any of the non-cognitive symptoms observed in aged APP/PS1 mice. To this end, we administered an acute dose of the $A_{2A}R$ agonist CGS21680 (0.5 mg/kg, i.p) and the CB_1R agonist ACEA (1.5 mg/kg, i.p.) to APP/PS1 mice at 12 months of age before assessing their behavioral performance using the three-chamber and the PPI test. Importantly, animals administered with CGS 21680 (0.5 mg/kg, i.p.) showed a significant reduction in locomotor activity. This decrease in locomotion hindered the testing of animals for social interaction and social memory, as their exploratory capacity was significantly impaired. However, this well-known hypolocomotion effect of CGS 21680 (Janusz and Berman, 1992) did not affect any of the acoustic startle reflex assayed in the PPI test. Interestingly, CGS 21680 (0.5 mg/kg, i.p) and, to a lesser extent, ACEA (1.5 mg/kg, i.p.), were able to reverse the sensorimotor gating deficit shown by male APP/PS1 at 12 months ($P < 0.05$; Fig. 6). However, the $A_{2A}R$ agonist also induced a decrease in the %PPI at 80 dB in female WT animals ($P < 0.05$ *vs.* vehicle, Fig. 6). Supplementary Table 3 includes all the statistical details. In general, these results are indicative that the selective activation of $A_{2A}R$ and CB_1R may have therapeutic potential to alleviate non-cognitive symptoms in AD.

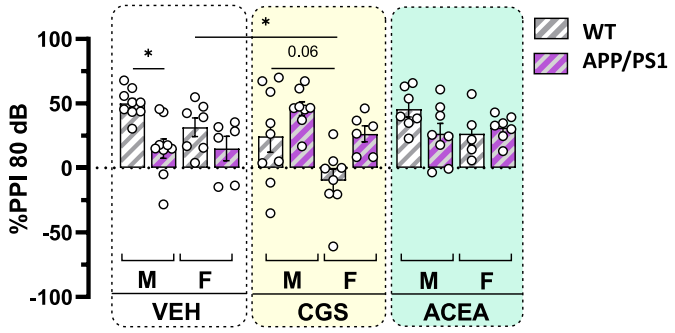

Fig. 6. Sensorimotor gating performance of aged APP/PS1 mice acutely treated with $A_{2A}R$ and CB_1R agonists. WT and APP/PS1 mice at 12 months of age were treated with an acute dose of a selective $A_{2A}R$ agonist (CGS 21680 0.5 mg/kg, i.p., CGS), a synthetic CB_1R agonist (ACEA 1.5 mg/kg, i.p.) or vehicle and evaluated in the PPI test 10 min later. Vehicle (VEH)-treated male APP/PS1 at 12 months of age exhibited a sensorimotor gating impairment characterized by a reduced %PPI at 80 dB prepulse intensity, which was not observed in CGS- and ACEA-treated APP/PS1 mice. Data are expressed as the mean ± SEM (n = 5–9). * $p < 0.05$, three-way ANOVA with Tukey's *post hoc* test.

4. Discussion

In the present study, we show compelling evidence that some non-cognitive symptoms associated with AD correlate with a reduction and an imbalance in the density of key components within the striatal dopaminergic, adenosinergic and endocannabinoid systems of aged APP/PS1 mice. Specifically, we report that sensorimotor gating deficits and social behavior impairments observed in aged APP/PS1 mice correlate with an age- and sex-dependent reduction in the density of the $A_{2A}R$, CB_1R, DAT and ENT1 transporters in the striatum, while D_2R levels remained constant. Importantly, the increase in the $D_2R/A_{2A}R$ and D_2R/CB_1R ratios was associated with a worsening of those non-cognitive symptoms, while increased striatal CB_1R density and $CB_1R/A_{2A}R$ ratio was associated to better performance in the three-chamber and PPI tests. Finally, the selective activation of $A_{2A}R$ and CB_1R alleviated sensorimotor gating deficits in aged male APP/PS1 mice, thus highlighting potential therapeutic use against this AD-associated BPSD.

APP/PS1 mice have been extensively used in preclinical studies to investigate AD pathogenesis and potential therapeutic interventions. Interestingly, the non-cognitive symptoms of dementia here observed in APP/PS1 mice are comparable to those previously described (Krivinko et al., 2020), and partially replicate those observed in AD patients (Jafari et al., 2020; Porcelli et al., 2019), thus supporting the validity of this animal model as a valuable tool for investigating new therapeutic strategies against BPSD in AD. Importantly, we observed some sex-

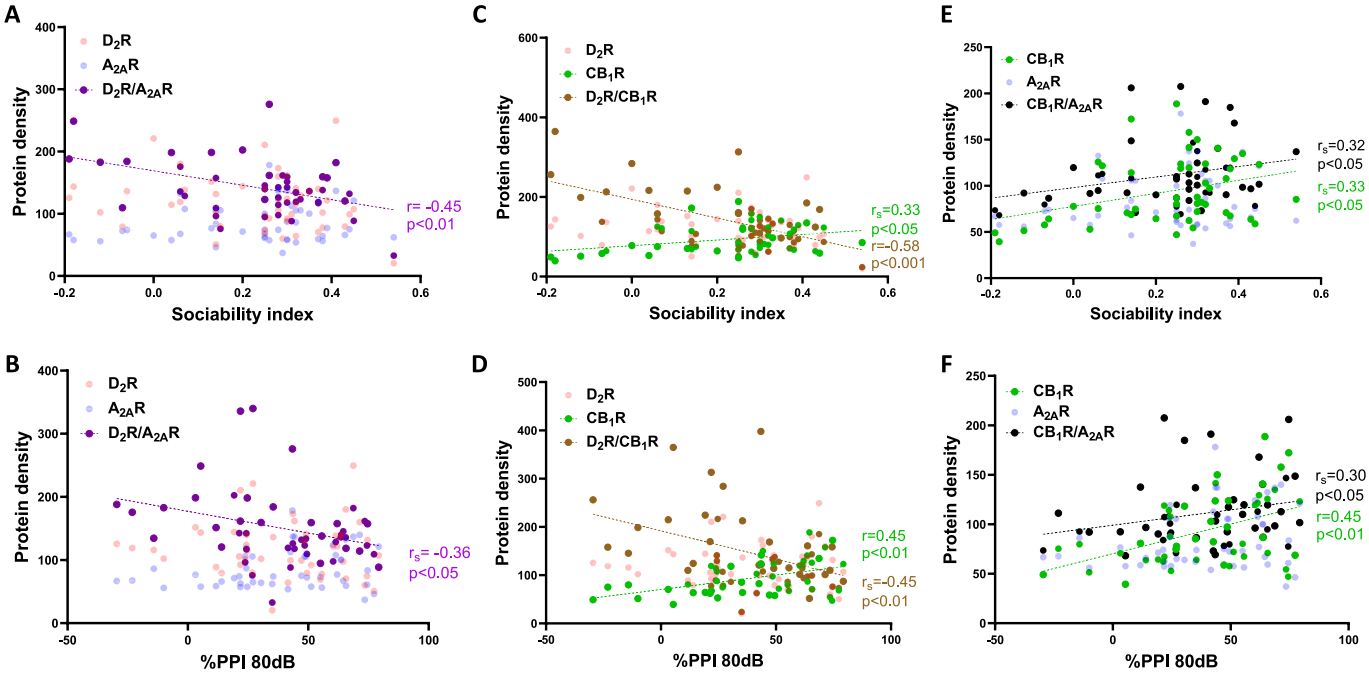

Fig. 5. Correlations between the D_2R, $A_{2A}R$ and CB_1R densities and their ratios in the striatum and non-cognitive symptoms of young and aged APP/PS1 mice. A negative correlation exists between the sociability index and percentage of the prepulse inhibition of the acoustic startle response at 80 dB prepulse intensity (%PPI 80 dB) with the $D_2R/A_{2A}R$ (A-B) and D_2R/CB_1R (C-D) ratios. In contrast, positive correlations between the sociability index and %PPI 80 dB respect (C-F) CB_1R levels and (E-F) $CB_1R/A_{2A}R$ were observed. All correlation coefficients are Pearson's, unless indicated otherwise (Spearman's; r_s). $N = 46–48$.

dependent differences among the experimental groups during our behavioral evaluations. Of particular interest was the observation that female WT mice at 12 months, but not at 4 months of age, exhibited a deficit in PPI compared to their male littermates, what limited the possibilities to assess specific sensorimotor gating impairment in aged female APP/PS1 mice. To our knowledge, this is the first time a similar age-dependent deficit in sensorimotor gating has been reported for female mice with a C57Bl6/J background. A previous study showed opposite results in female mice from a slightly different strain (C57Bl6/N), but only at 2 months of age (Zhang et al., 2015). Interestingly, this PPI deficit in female WT mice at 12 months of age was correlated with a decrease in the density of striatal D_2R, $A_{2A}R$ and DAT, highlighting the importance of these key signaling components in regulating sensorimotor gating also under physiological aging conditions.

Dopaminergic dysfunctions have been previously postulated to contribute to AD progression, although the nature of this contribution is still a matter of debate (Martorana and Koch, 2014). Remarkably, our findings suggest for the first time that an imbalance in the relative density of D_2R with respect to $A_{2A}R$ and CB_1R within the striatum may contribute to a putative hyperdopaminergic state, which was suggested previously by the increased DA levels found in the striatum of APP/PS1 mice at earlier stages (Abad et al., 2016). That hyperdopaminergic activity in the striatum could lead to the manifestation of psychotic-like symptoms (*i.e.* sensorimotor gating and social interaction impairments) in this animal model of AD. The decrease in the density of DAT observed in the striatum of aged APP/PS1 mice might theoretically contribute also to this hyperdopaminergic state by reducing the DA reuptake from the synaptic cleft. However, this assumption should be taken with caution considering the heterogenous results about DAT levels observed in schizophrenia. On one hand, [^{3}H]mazindol *in situ* radioligand binding and autoradiography in gray matter necropsies of schizophrenic patients revealed that DAT levels in the dorsal striatum and in the cortex were reduced and increased, respectively (Sekiguchi et al., 2019). In contrast, neuroimaging studies investigating DA function support the opposite, thus demonstrating increased DAT availability detected by PET and selective DAT radioligand in the midbrain, striatal, and limbic regions in those patients with chronic disease and long-term antipsychotic exposure (Artiges et al., 2017). These findings align with an increase in presynaptic DA function in patients with schizophrenia, underscoring the contribution of both striatal and extrastriatal DA dysfunction to positive psychotic symptoms (Artiges et al., 2017). Nevertheless, a recent meta-analysis of variance demonstrated a significant heterogeneity of the striatal DA function in schizophrenia, with no differences in the mean DAT availability between control and schizophrenic patients because of this heterogeneity, which could indicate that altered DAT availabilities may occur only in a subgroup of patients (Brugger et al., 2020). Thus, the increased ratio of $D_2R/A_{2A}R$ and D_2R/CB_1R will be compatible with an enhanced dopaminergic signaling in aged APP/PS1 mice, while the observed decrease in striatal DAT density would be indicative of neuroanatomical affectations of dopaminergic neurotransmission in these animals. Therefore, unlike to other psychotic disorders such as schizophrenia, this hyperdopaminergic activity in aged APP/PS1 mice would not rely on increased striatal D_2R levels rather than on decreased levels of $A_{2A}R$ and CB_1R, two GPCRs known to negatively control D_2R activity (Ferré et al., 2018; Marcellino et al., 2008). In fact, the diminished expression of $A_{2A}R$ and ENT_1 observed in APP/PS1 mice at 12 months of age is compatible with the hypoadenosinergic state that characterizes other psychotic disorders (Boison et al., 2012). Thus, a reduction in the antagonistic effect of $A_{2A}R$ on D_2R could lead to enhanced DA signaling through this receptor in the striatum. Similarly, decreased activity of CB_1R could facilitate D_2R activity, leading to exacerbated DA signaling in the mesolimbic dopaminergic system. Consistent with this hypothesis (summarized in Fig. 7), previous studies have found that decreased CB_1R activity is associated with psychosis in animal models (Ortega-Álvaro et al., 2015) and schizophrenic patients (Borgan et al., 2019).

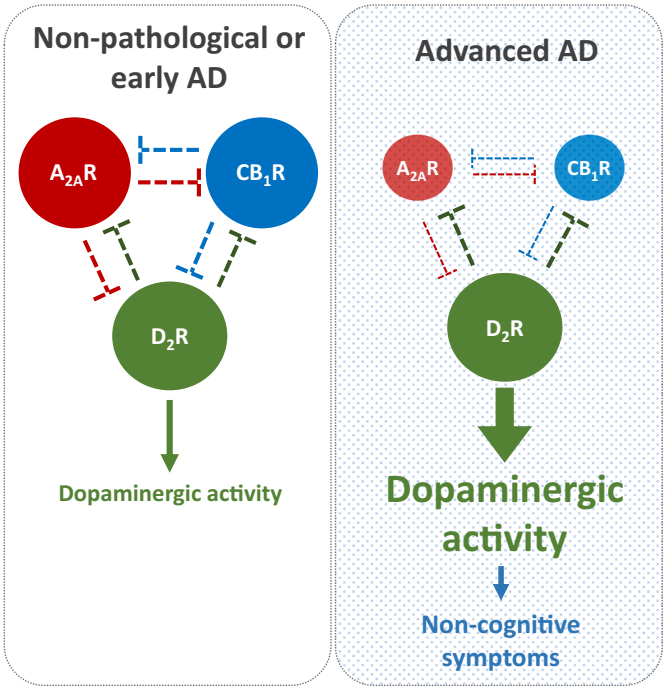

Fig. 7. Graphical summary of the results and hypothesis of the study. Under non-pathological conditions or at early AD stages (left panel), an equilibrium between the activity of D_2R (green), $A_{2A}R$ (red) and CB_1R (blue) exists, contributing to finely regulate dopaminergic activity in the striatum. At advanced AD stages (right panel), an hyperdopaminergic activity can occur due to the reduction in the antagonistic effect of $A_{2A}R$ and CB_1R on D_2R, leading to non-cognitive symptoms such as sensorimotor gating impairment and sociability deficits. Thus, increasing $A_{2A}R$ and CB_1R activity could help to mitigate the exacerbated DA signaling in the mesolimbic brain areas and, in consequence, to reduce these non-cognitive symptoms in AD. The size of the circles represents the amount of receptors expressed in each condition, whereas the width of the lines is proportional to the intensity of each receptor effects. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Collectively, these findings suggested that increasing $A_{2A}R$ and CB_1R activity could reduce psychotic symptoms in APP/PS1 mice. To test this possibility, we administered an acute dose of the selective $A_{2A}R$ agonist CGS 21680 and the CB_1R agonist ACEA to aged APP/PS1 mice. Our results revealed that male APP/PS1 mice treated with CGS 21680 and ACEA did not show the decreased %PPI at 80 dB prepulse intensity observed in vehicle-treated littermates, suggesting an improvement of the sensorimotor gating deficits shown by these AD-like animals.

In summary, the present study provides evidence supporting the role of an altered interaction among the dopaminergic, adenosinergic, and endocannabinoid systems in the sensorimotor gating deficits and social withdrawal observed in AD and points toward $A_{2A}R$ and CB_1R as a potential target to reverse these non-cognitive symptoms in AD patients.

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CRediT authorship contribution statement

Laura Gómez-Acero: Writing – original draft, Formal analysis, Data curation, Conceptualization, Investigation. **Nuria Sánchez-Fernández:** Investigation. **Paula Subirana:** Investigation. **Francisco Ciruela:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Ester Aso:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

None of the authors declare any conflict of interest.

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Data availability

Data will be made available on request.

References

Abad, S., Ramon, C., Pubill, D., Camarasa, J., Camins, A., Escubedo, E., 2016. Adolescent exposure to MDMA induces DAergic toxicity in substantia nigra and potentiates the amyloid plaque deposition in the striatum of APPswe/PS1dE9 mice. Biochim. Biophys. Acta 1862, 1815–1826.

Artiges, E., Leroy, C., Dubol, M., Prat, M., Pepin, A., Mabondo, A., De Beaurepaire, R., Beaufils, B., Korwin, J.P., Galinowski, A., D'Albis, M.A., Santiago-Ribeiro, M.J., Granger, B., Tzavara, E.T., Martinot, J.L., Trichard, C., 2017. Striatal and extrastriatal dopamine transporter availability in schizophrenia and its clinical correlates: a voxel-based and high-resolution PET study. Schizoph. Bull. 43, 1134–1142.

Aso, E., Ferrer, I., 2014. Cannabinoids for treatment of Alzheimer's disease: moving toward the clinic. Front. Pharmacol. 5, 37.

Aso, E., Palomer, E., Juvés, S., Maldonado, R., Muñoz, F.J., Ferrer, I., 2012. CB_1 agonist ACEA protects neurons and reduces the cognitive impairment of AβPP/PS1 mice. J. Alzheimers Dis. 30, 439–459.

Aso, E., Andrés-Benito, P., Ferrer, I., 2018. Genetic deletion of CB(1) cannabinoid receptors exacerbates the Alzheimer-like symptoms in a transgenic animal model. Biochem. Pharmacol. 157, 210–216.

Ballard, C., Kales, H.C., Lyketsos, C., Aarsland, D., Creese, B., Mills, R., Williams, H., Sweet, R.A., 2020. Psychosis in Alzheimer's disease. Curr. Neurol. Neurosci. Rep. 20, 57.

Boison, D., Singer, P., Shen, H.Y., Feldon, J., Yee, B.K., 2012. Adenosine hypothesis of schizophrenia - opportunities for pharmacotherapy. Neuropharmacology 62, 1527–1543.

Bonaventura, J., Rico, A.J., Moreno, E., Sierra, S., Sánchez, M., Luquin, N., Farré, D., Müller, C.E., Martínez-Pinilla, E., Cortés, A., Mallol, J., Armentero, M.T., Pinna, A., Canela, E.I., Lluís, C., McCormick, P.J., Lanciego, J.L., Casadó, V., Franco, R., 2014. L-DOPA-treatment in primates disrupts the expression of A(2A) adenosine-CB(1) cannabinoid-D(2) dopamine receptor heteromers in the caudate nucleus. Neuropharmacology 79, 90–100.

Borchelt, D.R., Ratovitski, T., van Lare, J., Lee, M.K., Gonzales, V., Jenkins, N.A., Copeland, N.G., Price, D.L., Sisodia, S.S., 1997. Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. Neuron 19, 939–945.

Borgan, F., Laurikainen, H., Veronese, M., Marques, T.R., Haaparanta-Solin, M., Solin, O., Dahoun, T., Rogdaki, M., Salokangas, R.K., Karukivi, M., Di Forti, M., Turkheimer, F., Hietala, J., Howes, O., METSY Group, 2019. In vivo availability of cannabinoid 1 receptor levels in patients with first-episode psychosis. JAMA Psychiatry 76, 1074–1084.

Brugger, S.P., Angelescu, I., Abi-Dargham, A., Mizrahi, R., Shahrezaei, V., Howes, O.D., 2020. Heterogeneity of striatal dopamine function in schizophrenia: meta-analysis of variance. Biol. Psychiatry 87, 215–224.

Carriba, P., Ortiz, O., Patkar, K., Justinova, Z., Stroik, J., Themann, A., Muller, C., Woods, A.S., Hope, B.T., Ciruela, F., Casado, V., Canela, E.I., Lluis, C., Goldberg, S.R., Moratalla, R., Franco, R., Ferre, S., 2007. Striatal adenosine A_{2A} and cannabinoid CB_1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. Neuropsychopharmacology 32, 2249–2259.

Dall'Igna, O.P., Hoffmann, A., da Silva, A.L., Souza, D.O., Lara, D.R., 2004. Beta-amyloid treatment sensitizes mice to amphetamine-induced locomotion but reduces response to caffeine. Neurodegener Dis 1, 38–43.

Devanand, D.P., Lee, S., Huey, E.D., Goldberg, T.E., 2022. Associations between neuropsychiatric symptoms and neuropathological diagnoses of Alzheimer disease and related dementias. JAMA Psychiatry 79, 359–367.

Ferré, S., Bonaventura, J., Zhu, W., Hatcher-Solis, C., Taura, J., Quiroz, C., Cai, N.S., Moreno, E., Casadó-Anguera, V., Kravitz, A.V., Thompson, K.R., Tomasi, D.G., Navarro, G., Cordomí, A., Pardo, L., Lluís, C., Dessauer, C.W., Volkow, N.D., Casadó, V., Ciruela, F., Logothetis, D.E., Zwilling, D., 2018. Essential control of the function of the striatopallidal neuron by pre-coupled complexes of adenosine A_{2A}-dopamine D_2 receptor heterotetramers and adenylyl cyclase. Front. Pharmacol. 9, 243.

Horgusluoglu-Moloch, E., Nho, K., Risacher, S.L., Kim, S., Foroud, T., Shaw, L.M., Trojanowski, J.Q., Aisen, P.S., Petersen, R.C., Jack Jr., C.R., Lovestone, S., Simmons, A., Weiner, M.W., Saykin, A.J., Alzheimer's Disease Neuroimaging Initiative (ADNI), 2017. Targeted neurogenesis pathway-based gene analysis identifies ADORA2A associated with hippocampal volume in mild cognitive impairment and Alzheimer's disease. Neurobiol. Aging 60, 92–103.

Ismail, Z., Creese, B., Aarsland, D., Kales, H.C., Lyketsos, C.G., Sweet, R.A., Ballard, C., 2022. Psychosis in Alzheimer disease - mechanisms, genetics and therapeutic opportunities. Nat. Rev. Neurol. 18, 131–144.

Jafari, Z., Kolb, B.E., Mohajerani, M.H., 2020. Prepulse inhibition of the acoustic startle reflex and P50 gating in aging and alzheimer's disease. Ageing Res. Rev. 59, 101028.

Janusz, C.A., Berman, R.F., 1992. The A2-selective adenosine analog, CGS 21680, depresses locomotor activity but does not block amygdala kindled seizures in rats. Neurosci. Lett. 141, 247–250.

Kaidanovich-Beilin, O., Lipina, T., Vukobradovic, I., Roder, J., Woodgett, J.R., 2011. Assessment of social interaction behaviors. J. Vis. Exp. 48, e2473.

Krivinko, J.M., Koppel, J., Savonenko, A., Sweet, R.A., 2020. Animal models of psychosis in Alzheimer disease. Am. J. Geriatr. Psychiatry 28, 1–19.

Liu, Y., Xu, Y., Li, M., Pan, D., Li, Y., Wang, Y., Wang, L., Wu, Q., Yang, M., 2020. Multi-target PET evaluation in APP/PS1/tau mouse model of Alzheimer's disease. Neurosci. Lett. 728, 134938.

Manders, E.M.M., Verbeek, F.J., Aten, J.A., 1993. Measurement of co-localization of objects in dual-colour confocal images. J. Microsc. 169, 375–382.

Manuel, I., de San, González, Román, E., Giralt, M.T., Ferrer, I., Rodríguez-Puertas, R., 2014. Type-1 cannabinoid receptor activity during Alzheimer's disease progression. J. Alzheimers Dis. 42, 761–766.

Manzanares, J., Cabañero, D., Puente, N., García-Gutiérrez, M.S., Grandes, P., Maldonado, R., 2018. Role of the endocannabinoid system in drug addiction. Biochem. Pharmacol. 157, 108–121.

Marcellino, D., Carriba, P., Filip, M., Borgkvist, A., Frankowska, M., Bellido, I., Tanganelli, S., Müller, C.E., Fisone, G., Lluis, C., Agnati, L.F., Franco, R., Fuxe, K., 2008. Antagonistic cannabinoid CB_1/dopamine D_2 receptor interactions in striatal CB_1/D_2 heteromers. A combined neurochemical and behavioral analysis. Neuropharmacology 54, 815–823.

Martorana, A., Koch, G., 2014. Is dopamine involved in Alzheimer's disease? Front. Aging Neurosci. 6, 252.

Millan, M.J., Fone, K., Steckler, T., Horan, W.P., 2014. Negative symptoms of schizophrenia: clinical characteristics, pathophysiological substrates, experimental models and prospects for improved treatment. Eur. Neuropsychopharmacol. 24, 645–692.

Moreno, E., Chiarlone, A., Medrano, M., Puigdellívol, M., Bibic, L., Howell, L.A., Resel, E., Puente, N., Casarejos, M.J., Perucho, J., Botta, J., Suelves, N., Ciruela, F., Ginés, S., Galve-Roperh, I., Casadó, V., Grandes, P., Lutz, B., Monory, K., Canela, E.I., Lluís, C., McCormick, P.J., Guzmán, M., 2018. Singular location and signaling profile of adenosine A_{2A}-cannabinoid CB_1 receptor heteromers in the dorsal striatum. Neuropsychopharmacology 43, 964–977.

Moscoso-Castro, M., Gracia-Rubio, I., Ciruela, F., Valverde, O., 2016. Genetic blockade of adenosine A2A receptors induces cognitive impairments and anatomical changes related to psychotic symptoms in mice. Eur. Neuropsychopharmacol. 26, 1227–1240.

Ortega-Álvaro, A., Navarrete, F., Aracil-Fernández, A., Navarro, D., Berbel, P., Manzanares, J., 2015. Differential pharmacological regulation of sensorimotor gating deficit in CB_1 knockout mice and associated neurochemical and histological alterations. Neuropsychopharmacology 40, 2639–2647.

Pasquini, S., Contri, C., Merighi, S., Gessi, S., Borea, P.A., Varani, K., Vincenzi, F., 2022. Adenosine receptors in neuropsychiatric disorders: fine regulators of neurotransmission and potential therapeutic targets. Int. J. Mol. Sci. 23, 1219.

Peters, M.E., Schwartz, S., Han, D., Rabins, P.V., Steinberg, M., Tschanz, J.T., Lyketsos, C. G., 2015. Neuropsychiatric symptoms as predictors of progression to severe Alzheimer's dementia and death: the Cache County dementia progression study. Am. J. Psychiatry 172, 460–465.

Porcelli, S., Van Der Wee, N., van der Werff, S., Aghajani, M., Glennon, J.C., van Heukelum, S., Mogavero, F., Lobo, A., Olivera, F.J., Lobo, E., Posadas, M., Dukart, J., Kozak, R., Arce, E., Ikram, A., Vorstman, J., Bilderbeck, A., Saris, I., Kas, M.J.,

Serretti, A., 2019. Social brain, social dysfunction and social withdrawal. Neurosci. Biobehav. Rev. 97, 10–33.
Ropacki, S.A., Jeste, D.V., 2005. Epidemiology of and risk factors for psychosis of Alzheimer's disease: a review of 55 studies published from 1990 to 2003. Am. J. Psychiatry 162, 2022–2030.
Schneider, L.S., Dagerman, K., Insel, P.S., 2006. Efficacy and adverse effects of atypical antipsychotics for dementia: meta-analysis of randomized, placebo-controlled trials. Am. J. Geriatr. Psychiatry 14, 191–210.
Seeman, P., 2013. Schizophrenia and dopamine receptors. Eur. Neuropsychopharmacology 23, 999–1009.
Sekiguchi, H., Pavey, G., Dean, B., 2019. Altered levels of dopamine transporter in the frontal pole and dorsal striatum in schizophrenia. NPJ Schizophr. 5, 20.
Sills, T.L., Azampanah, A., Fletcher, P.J., 2001. The adenosine A_{2A} agonist CGS 21680 reverses the reduction in prepulse inhibition of the acoustic startle response induced by phencyclidine, but not by apomorphine and amphetamine. Psychopharmacology (Berl) 156, 187–193.
Simpson, E.H., Gallo, E.F., Balsam, P.D., Javitch, J.A., Kellendonk, C., 2022. How changes in dopamine D2 receptor levels alter striatal circuit function and motivation. Mol. Psychiatry 27, 436–444.
Song, A., Zhang, Y., Han, L., Yegutkin, G.G., Liu, H., Sun, K., D'Alessandro, A., Li, J., Karmouty-Quintana, H., Iriyama, T., Weng, T., Zhao, S., Wang, W., Wu, H., Nemkov, T., Subudhi, A.W., Jameson-Van Houten, S., Julian, C.G., Lovering, A.T., Hansen, K.C., Zhang, H., Bogdanov, M., Dowhan, W., Jin, J., Kellems, R.E., Eltzschig, H.K., Blackburn, M., Roach, R.C., Xia, Y., 2017. Erythrocytes retain hypoxic adenosine response for faster acclimatization upon re-ascent. Nat. Commun. 8, 14108.
Swerdlow, N.R., Light, G.A., 2018. Sensorimotor gating deficits in schizophrenia: advancing our understanding of the phenotype, its neural circuitry and genetic substrates. Schizophr. Res. 198, 1–5.
Tebano, M.T., Martire, A., Popoli, P., 2012. Adenosine $A_{(2A)}$-cannabinoid $CB_{(1)}$ receptor interaction: an integrative mechanism in striatal glutamatergic neurotransmission. Brain Res. 1476, 108–118.
Temido-Ferreira, M., Ferreira, D.G., Batalha, V.L., Marques-Morgado, I., Coelho, J.E., Pereira, P., Gomes, R., Pinto, A., Carvalho, S., Canas, P.M., Cuvelier, L., Buée-Scherrer, V., Faivre, E., Baqi, Y., Müller, C.E., Pimentel, J., Schiffmann, S.N., Buée, L., Bader, M., Outeiro, T.F., Blum, D., Cunha, R.A., Marie, H., Pousinha, P.A., Lopes, L.V., 2020. Age-related shift in LTD is dependent on neuronal adenosine A_{2A} receptors interplay with mGluR5 and NMDA receptors. Mol. Psychiatry 25, 1876–1900.
Valle-León, M., Callado, L.F., Aso, E., Cajiao-Manrique, M.M., Sahlholm, K., López-Cano, M., Soler, C., Altafaj, X., Watanabe, M., Ferré, S., Fernández-Dueñas, V., Menchón, J.M., Ciruela, F., 2021. Decreased striatal adenosine A_{2A}-dopamine D_2 receptor heteromerization in schizophrenia. Neuropsychopharmacology 46, 665–672.
Viana da Silva, S., Haberl, M.G., Zhang, P., Bethge, P., Lemos, C., Gonçalves, N., Gorlewicz, A., Malezieux, M., Gonçalves, F.Q., Grosjean, N., Blanchet, C., Frick, A., Nägerl, U.V., Cunha, R.A., Mulle, C., 2016. Early synaptic deficits in the APP/PS1 mouse model of Alzheimer's disease involve neuronal adenosine A_{2A} receptors. Nat. Commun. 7, 11915.
Zhang, X., Li, Q., Wong, N., Zhang, M., Wang, W., Bu, B., McAlonan, G.M., 2015. Behaviour and prefrontal protein differences in C57BL/6N and 129 X1/SvJ mice. Brain Res. Bull. 116, 16–24.