9.5). SI was performed during 8 weeks in the peripubertal period. Four experimental groups (MIA, SI, double-hit and control) (n=12/sex/arm) were tested for social withdrawal—Social Preference Test (SPT)—and cognitive status—Novel Object Recognition Test (NORT). Locomotor response to acute amphetamine (5 mg/kg i.p.) was also evaluated (n=6/sex/arm).

Cortical samples (n=8/sex/arm) were processed for gene and protein expression assessment. NF-κB, IκBα and HDACs gene expression was determined by RT-qPCR. Cytoplasmic IκBα and nuclear NF-κB protein expression were measured by Western blot. Plasmatic cytokine expression (IL-1β, IL-2, IL-6, IL-10, IL-17A, IFN-γ and TNFα) was quantified using a magnetic bead-based multiple immunoassay. Data were analysed using non-repeated or repeated measures two-way or three-way ANOVAs as appropriate.

Results: The SPT Social Index was significantly reduced by MIA ($F[1,83]=7.919;p<0.01$) in both sexes. The NORT Discrimination Index was significantly impaired by MIA ($F[1,85]=10.93;p<0.001$) and SI ($F[1,85]=7.46;p<0.01$) in both sexes. Interestingly, double-hit groups showed worse scores in Social and Discrimination Index compared to single-hit groups. The hyperlocomotion induced by amphetamine was significantly increased by SI in female ($F[1,19]=5.36;p<0.05$) but not in male mice.

In male mice, MIA was significantly associated to increased NF-κB gene expression levels ($F[1,28]=4.702;p<0.05$). In female mice, MIA was significantly associated to decreased IκBα gene expression levels ($F[1,28]=7.074;p<0.05$) and SI showed a significant opposite effect ($F[1,28]=4.869;p<0.05$). Western Blot experiments showed no differences in IκBα and NF-κB protein expression. MIA was associated to a significant decrease of HDAC2 gene expression in male and female mice ($F[1,56]=5.953;p<0.05$), and decreased HDAC4 ($F[1,56]=5.525;p<0.05$) and HDAC8 ($F[1,56]=6.813;p<0.05$) gene expression was associated to SI in both sexes. Finally, a significant increase of the plasmatic concentration of the proinflammatory cytokines IL-6 ($F[1,35]=4.818;p<0.05$) and IFN-γ ($F[1,34]=13.95;p<0.001$) was associated to MIA in both sexes.

Conclusion: These results showed a significant impact induced by MIA and SI on schizophrenia related behaviours at adulthood in both sexes. This model also presents sex-dependent alterations in the gene expression of neuroinflammatory signalling proteins, although this appears not to be related to changes in the protein expression. Both sexes showed same altered gene expression of different HDACs, and altered peripheral inflammatory signalling induced by MIA. These data support the double-hit animal model as a valuable translational tool in schizophrenia research.

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Conflict of interest

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A REGULATOR GENE WITH AN IMPACT: RBFOX1 AND ITS ROLE IN NEUROPSYCHIATRIC DISORDERS

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Background: The gene encoding for the neuron-specific splicing and translation regulator RNA-binding protein fox-1 homolog-1 (*RBFOX1*) has been identified as a risk factor for multiple psychiatric disorders [1]. Beyond pleiotropic effects of common variants, rare genetic variants have causal relationship with autism spectrum disorders (ASD) and cytoplamatic deletion has been associated with synaptic and ASD genes' dysregulation [2,3]. Furthermore, *RBFOX1* has been linked to neuropsychiatric traits and disorders by neuroimaging and animal studies [4,5].

Objective: Here, we aim to explore the genetic landscape of *RBFOX1*, combining existing and new human study data with investigations in an *Rbfox1* knockout mouse model, to gain more insights into psychiatric disorders.

Methods: Large-scale datasets on psychiatric disorders and traits were data-mined for associations with common *RBFOX1* variants, using published genome-wide association studies. Burden analysis for rare copy number variants (CNVs) was performed in publicly available data. Target genes enrichment was assessed by hypergeometric tests and *RBFOX1* expression was explored in transcriptomic datasets of ASD patients and controls. Protein expression, according to on an aggression-linked single-nucleotide polymorphism (SNP) *rs6500744*, was examined in post-mortem prefrontal cortices (PFC: 31) via immunoblotting. Functional magnetic resonance imaging (fMRI) data from healthy adults during executive functioning and implicit emotion processing (324 and 313, respectively), as well as during fear learning in 47 patients with panic disorder, was compared between SNP allele carrier groups. Neuron-specific *Rbfox1* knockout mice and controls (adult males >7/group) were assessed for exploratory, anxiety-like, and social behaviours, as well as aggression, learning and memory, among others. Statistical analyses with a significance threshold of $p<0.05$ were performed as pertinent, including a chi-squared test, independent t-tests, and ANOVA.

Results: Gene-based and genome-wide association of *RBFOX1* ($p<0.001$) was determined for major depressive disorder (38 SNPs), cross-disorder meta-analysis (42 SNPs), risk tolerance (4 SNPs), and schizophrenia (8 SNPs), while respective associated genes were significantly enriched for RBFOX1 targets ($p<0.05$). CNVs were revealed as more frequent in ASD cases than in controls (5:1 ratio). *RBFOX1* expression was decreased in post-mortem frontal and temporal cortical transcriptome of individuals with ASD ($p<0.02$, FDR<0.1). Performed brain fMRI studies demonstrated that carriers of a risk allele of the SNP *rs6500744* displayed reduced PFC processing during cognitive control ($p=0.04$), increased reactivity to emotional stimuli ($p=0.01$) and enhanced fear expression after conditioning ($p=0.014$) in the anterior cingulate cortex. Analysis of PFC tissue did not reveal significant protein level changes based on SNP genotype but hinted at increased *RBFOX1* abundance in risk carriers. Investigating *Rbfox1* knockout mice revealed pronounced hyperactivity ($p<0.001$), stereotypical behaviour ($p<0.01$), impaired fear acquisition and extinction ($p<0.01$), and reduced aggression and social interest ($p<0.05$), reinforcing it a valid animal model of ASD.

Conclusion: This study's convergent evidence shows that common variants in *RBFOX1* are associated with a variety of psychiatric traits and disorders, while rare genetic variation seemingly exposes to early-onset neurodevelopmental psychiatric disorders (NDDs). Thus, the investigation of *RBFOX1* might lead to an improved understanding of psychiatric disorder aetiology. Ongoing studies are assessing the effects of *Rbfox1* overexpression and environmental factors in relation to NDDs.

Statement: AYY and AOL contributed equally and share joint first authorship.

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INVESTIGATING OF THEORY OF MIND ABILITIES AND ITS RELATIONSHIP WITH METABOLIC PARAMETERS IN BIPOLAR PATIENTS

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Background: Inflammation impairs the ability to interpret the mental state of another person. It has been shown that bipolar patients may have deficits in social cognition. However, experimental studies showed that bipolar disorder may be associated with the inflammation process. In this study we aimed to investigate the relationship between reading the mind in the eyes test (RMET), functionality levels and, metabolic and inflammation parameters in patients with bipolar disorder during the remission period.

Methods: The study included sixty nine patients with bipolar disorder in remission period and forty five healthy controls. Sociodemographic Form, Reading the mind in the eyes test (RMET), Morisky Medication Adherence Scale, Functioning Assesment Short Test were applied to the participants. Plasma Triglycerid**,** HDL, LDL, fasting glucose, insulin, C-reactive protein, sedimentation levels, HOMA scores, Body mass index were measured to index inflammation.

Results: A total of 69 patients 30 female (%42,9) and 39 male (%57,1) were included in our study, and the mean age of the participants was 38,94 (±11,90) and the mean age of health controls are (36±12). There was no significantly statistically difference between bipolar patient group and healthy control groups in terms of age. The disease duration of the patients participating in our study was between 1 and 35 and they had a history of manic (minimum:1-maximum:10) and depressive episode and the median of the both manic and depressive episode was 3. The performance of the bipolar patient group in the reading the eyes in the mind test statistically lower than the control group (p: 0,04). A significant correlation was found between the results of the participants' reading the mind in the eyes test (RMET), high education level (r:0,473, p:0,001), and height (r:0,339, p:0,017) and weight (r:0,282, p:0,049). However, a statistically significant negative correlation was found between the number of previous manic episodes and the results of RMET (r:-0,281, p: 0,001). A statistically significant negative relationship was found between RMET test scores and insulin levels (r:0,-406, p:0,004), fasting blood glucose (r:0,-401, p:0,004), triglyceride levels (r:-0,368, p:0,009). A statistically significant relationship was found between RMET scores and drug compliance levels. (r:0,343, p:0,016). No significant correlation was found between the mood stabilizers and Bipolar disorder functionality scores, Morisky drug adherence scores.

Conclusions: The present study showed that bipolar patients had lower ability to interpret the mental state of another person than healthy controls. Number of manic episodes and insuline, glucose and triglyceride levels are correlated with a lower ability to infer the mental states of others in bipolar patients which means that bipolar disorder can be effectively conceptualized as a multisistemic inflammatory diseases and number of manic episodes may indicate an increase of cognitive deficits. Thus, more treatment strategies should be developed on inflammation process of bipolar disorder

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ANTIDEPRESSANT-LIKE EFFECTS OF CANNABIDIOL IN A RAT MODEL OF EARLY-LIFE STRESS: SEX- AND AGE-DEPENDENT EFFICACY

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Purpose: Although there are well-known age and sex differences in the prevalence of major depression and in antidepressant efficacy (being females the most vulnerable ones and adolescence a less responsive period than adulthood), studies investigating depression-related behaviors and changes in antidepressant-like efficacy that include both variables are scarce [1,2]. In this context, cannabidiol is a non-psychoactive phytocannabinoid with great therapeutic potential in diverse psychiatric disorders [3], however its antidepressant-like potential has been mainly ascertained in male adult rodents, since there is not much evidence reported in the literature evaluating sex- and age-specific aspects of this treatment. Therefore, the present study compared the antidepressant-like effects of cannabidiol in a rat model of early-life stress in adolescent and adult rats and with a sex perspective.

Methods: Sprague-Dawley pups (8 litters, n=67) were exposed (PND 9) to early-life stress (maternal deprivation, MD) or used as controls (C), and separated by sex at weaning. Animals were treated (i.p.) with cannabidiol (10 mg/kg/day, n=18-17) or vehicle (0.9% NaCl, 1 ml/kg/day, n=17-15) for 7 days during adolescence (PND 49-56) rendering 8 experimental groups: C-male-vehicle (n=8), C-male-cannabidiol (n=9), MD-male-vehicle (n=9), MD-male-cannabidiol (n=9), C-female-vehicle (n=7), C-female-cannabidiol (n=8), MD-female-vehicle (n=8), MD-female-cannabidiol (n=9). Indicatives of the antidepressant-like potential of cannabidiol were evaluated during adolescence as: (1) decreased immobility time in the forced-swim test (FST) (acute effects, PND 49; repeated effects, PND 56); (2) increased feeding time in the novelty-suppressed feeding test (NSF; PND 58); and (3) increased 1% sucrose preference in the two-bottle choice test (PND 60-63). Rats were left undisturbed until adulthood when they were tested in the FST (PND 84; to ensure no persistent effects due to the adolescent treatment), before re-exposing them to the same treatment regimen (PND 89-95) to evaluate cannabidiol's antidepressant-like effects in adulthood (FST on PND 89 and 96; NSF on PND 98). Brains were collected on PND 99 for evaluating neurogenesis markers in the hippocampus.

Results: The main results demonstrated an impact of sex, age (adolescence vs. adulthood) and prior early-life stress exposure when evaluating the antidepressant-like potential of cannabidiol in rats. Cannabidiol was only efficient in the forced-swim test when administered in C-male rats, both during adolescence (FST: -46±16 sec spent immobile) and adulthood (FST: -70±27 sec spent immobile). No significant effects were observed for MD-male rats, nor for C-female or MD-female rats. Moreover, no changes were observed in the preference for sucrose or in the parameters analyzed in the novelty suppressed feeding test for any treatment groups. The neurogenesis analysis is still under evaluation.

Conclusions: The present results prove clear differences in the pharmacological actions exerted by cannabidiol by sex and by prior-early life exposure (antidepressant-like response only in C-male rats). Given that prior studies only focused on adult male subjects, the disparities observed in the current study reinforce the importance of including female rats in preclinical assays to ensure drug efficacy when later translating these results into the clinic. Future studies will aim at evaluating the possible mechanism behind these disparities.

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