(PVT). Then, with immunohistochemistry, we assessed the MC3R dynamic response to metabolic challenges such as 16h fasting or acute high fat diet (HFD) exposures. The results reveal that MC3R neurons in the thalamus are activated by the metabolic changes and therefore implicated in regulating energy homeostasis. Finally, we studied the impact of MC3R deletion on AgRP and POMC neuronal projections in intra and extra-hypothalamic nuclei. For this purpose, we used MC3R-KO males and females adult mice and performed free floating immunohistochemistry with antibodies targeting the Agouti-related peptide (AgRP) and alpha-melanocortin stimulating hormone (α-MSH). These neuropeptides are synthetized and released by, in order, AgRP and POMC neurons. Image analysis of the fiber density revealed that AgPR and POMC neurons in the hypothalamus, but not in the thalamus, are affected when MC3R is not expressed. All confocal images collected from immunohistochemistry experiments were analyzed with ImageJ FJI software and one-way ANOVA statistical test. Altogether, our results suggest that thalamic MC3R neurons contribute to the regulation of energy homeostasis and that MC3R is a key factor to the development and the maintenance of AgRP and POMC neuronal projections to the hypothalamus.

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No conflict of interest

doi: https://doi.org/10.1016/j.nsa.2023.101039

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NEUROSCIENCE APPLIED 2 (2023) 101019 101040

MOLECULAR SIGNATURE OF KETAMINE RESPONSE NON-RESPONSE IN A PRECLINICAL MODEL OF DEPRESSION

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Abstract text

Introduction: Stress is considered a key risk factor in the onset of neuropsychiatric disorders, including depression 1. Accordingly, the Chronic Mild Stress (CMS) rodent model of depression is widely used in the preclinical setting to study both etiopathogenetic and antidepressant mechanisms 2. Different responses to stressful stimuli might lead to adaptive or maladaptive changes underlying resilience and vulnerability to stress. Recently, ketamine has emerged as the first rapid-acting antidepressant drug effective in patients with treatment resistant depression. However, the molecular mechanisms underlying ketamine response/non-response are still largely unknown.

Aims: The aim of this study is to evaluate molecular changes at transcriptional and protein levels in the hippocampus of CMS rats treated with a subanesthetic dose of ketamine, in order to identify the molecular signature of ketamine rapid antidepressant response/non-response.

Materials/Methods: CMS was applied for 5 weeks on male rats as previously described 3. Sucrose preference test was used to measure the anhedonic phenotype and acute subanesthetic ketamine (10 mg/Kg) was intraperitoneally injected 24 h before sacrifice. The hippocampus was collected to obtain RNA and proteins. Transcriptional changes in the hippocampus were evaluated by RNA-seq analysis followed by bioinformatic enrichment analysis. Western blot experiments were performed on different cellular compartments obtained by centrifugation (homogenate, cytosol, nuclear, and synaptic fractions) to measure changes in the expression of proteins involved in intracellular signaling pathways.

Results: Starting from 3 weeks of CMS, rats developed depressive-like behavior as demonstrated by the reduction of sucrose preference. Moreover, the sucrose preference test allowed us to classify the animals as resilient and vulnerable. Acute ketamine induced antidepressant-like behavior in most of the animals but some of them (about 30%) were non-responders remaining anhedonic. RNA-seq revealed several genes differently regulated among the groups (FDR$\leq$0.05). Enrichment analysis showed differences between control and vulnerable rats and between ketamine responders and non-responders in the expression of genes involved in pathways such as glutamatergic synapse, synaptic signaling and organization, modulation of neuronal and dendritic spine morphology.

Subcellular specific changes in protein expression were found in the experimental groups. One-way ANOVA followed by Tukey's post-hoc was applied to measure statistical significant differences among the experimental groups. In the hippocampus, pAkt was increased in resilient and ketamine-responder rats while vulnerable rats showed increased levels of pGsk3β and pEF2 that were completely restored in ketamine-responder rats. pERK1 and pERK2 were both significantly increased in the nucleus of ketamine responder rats.

Conclusions: The transcriptional profile highlighted major changes in the expression of genes belonging to synaptic- and glutamatergic-related pathways. At the same time, the changes in the expression of proteins involved in synaptic plasticity between ketamine responder and non-responder animals suggest specific differential activation of intracellular signaling response. These integrated results should help us to better elucidate ketamine's mechanism of action and resistance to this drug.

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No conflict of interest

doi: https://doi.org/10.1016/j.nsa.2023.101040

P.123

NEUROSCIENCE APPLIED 2 (2023) 101019 101041

DYSBINDIN, D3 RECEPTORS, AND THEIR GENETIC INTERACTION DIFFERENTLY REGULATE NEUROPLASTICITY GENES IN THE MOUSE BRAIN

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Abstract text

Background: Cognitive impairment in schizophrenia represents a clinically and pharmacologically unsolved challenge. Alteration in Dysbindin (DYS) and dopamine receptors D3 levels seem to contribute to the cognitive deficits common in this psychiatric disorder. In fact, reduced DYS gene expression and protein levels have been reported in the hippocampus (HIPP) and prefrontal cortex (PFC) of patients with schizophrenia, whereas reduced D3 levels have been associated with increased negative schizophrenic symptoms [1,2]. On the other hand, a concomitant reduction in D3 and DYS functionality correlates with improvement in memory capabilities in both rodent models and patients with schizophrenia 3. To date, the molecular mechanisms through which this epistatic interaction improves neuroplasticity have not been fully elucidated. Thus, in this study, by employing mutant mice bearing selective heterozygosis for D3 and/or

DYS, we aimed to better understand the functional interactions (single and synergic) between these schizophrenia-susceptibility genes and neuroplasticity-related genes in brain areas implicated in both cognitive functions and negative symptoms of schizophrenia. In particular, we focused our attention on two subtypes of the glutamate receptor (NMDA and AMPA) and the neurotrophin BDNF, which play a critical role in mediating cognitive functions impaired in schizophrenia 4.

Methods: The mRNA levels of ionotropic glutamate receptors NMDA (GRIN1, GRIN2A, and GRIN2B) and AMPA (GRIA1 and GRIA2), and those of BDNF were analyzed in the prefrontal cortex (PFC), and hippocampus (HIPP) of wild-type (WT– D3×DYS +/+), D3 single heterozygous (D3+/−), DYS single heterozygous (DYS+/−), and double D3 and DYS heterozygous (D3×DYS+/−) male adult mice (N=5 for each genotype). Data from single areas were analyzed with One-way ANOVA followed by Tuckey's post hoc test.

Results: The expression levels of GRIN1 were significantly downregulated in the PFC and HIPP of D3+/- (Tuckey: p=0.005 and p= 0.03) and DYS+/- (p=0.006 and p= 0.01) mice with respect to WT controls. These reduced GRIN1 levels were reversed to WT levels in D3×DYS+/− mice in both PFC (p<0.0001) and HIPP (vs D3+/-: p=0.02; vs DYS+/-: p=0.06). Similarly, double heterozygous mice showed a significant upregulation of GRIN2A and BDNF in both areas compared to D3+/- (GRIN2A - PFC: p=0.049 and HIPP: p=0.0008; BDNF – PFC: p=0.004 and HIPP:0.004) and DYS+/- (GRIN2A - PFC: p=0.049 and HIPP: p=0.0009; BDNF – PFC: p=0.005 and HIPP:0.003) mice. Furthermore, a significant down-regulation was observed of GRIA1 in the HIPP of D3+/- and DYS +/- mice compared to their D3×DYS+/− counterparts (p=0.008 and p=0.14, respectively). Animals presenting DYS hypofunction (DYS+/- and D3×DYS+/−), instead, showed a decrease in GRIN2B hippocampal mRNA levels compared to controls (p=0.002). No difference was observed for the expression levels of GRIA2 in both areas and of GRIA1 and GRIN2B in the PFC.

Conclusion: This study provides new insights into the effect of the D3/DYS interaction in regulating the transcription of neuroplasticity-related genes in two key brain areas involved in schizophrenia. Our results may help to clarify the genetic mechanisms and functional interactions involved in the development of negative symptoms of this psychiatric disorder and eventually lead to more specific and individual treatment options. (Supported by PRIN-MIUR Prot.2017K2NEF4)

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No conflict of interest

doi: https://doi.org/10.1016/j.nsa.2023.101041

P.124

NEUROSCIENCE APPLIED 2 (2023) 101019 101042

SEX AFFECTS THE TRANSCRIPTIONAL EFFECTS OF A NEUROINFLAMMATORY HIT EXPERIENCED IN DEVELOPMENT IN THE MOUSE HIPPOCAMPUS

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Abstract text

Background: Investigating the vulnerability of critical time windows, like adolescence, is essential to understand the impact of environmental changes, stress, and immune stimulation on the maturing brain and the risk to develop neuropsychiatric disorders later in life 1,2,3. Currently the role of sex is emerging as an important discriminant in defining the response to stress. In this context women seem to be more susceptible to develop brain pathologies but the underlying mechanisms related to their development are still not well understood 4,5.

Aim: In the current study, we have evaluated 1) the acute transcriptional effects of an immune challenge experienced during adolescence (PND35) 2) whether this immune challenge may affect the molecular sequelae of a second inflammatory hit experienced during adulthood in the hippocampus in male or female mice.

Methods: Male and female C57BL6J mice were injected with lipopolysaccharide (LPS) (0.1 mg/kg i.p.) or saline (as controls) at post natal day 35 (PND35) and either sacrificed 6 or 24 hours (h) later (n=7-8 per group) or left undisturbed until adulthood. At 12 weeks of age, these animals received an injection of either LPS (0.83 mg/kg i.p.) or saline (n=6-8) and were sacrificed 24h later. By means of qPCR, we performed gene expression analysis on the hippocampus. Data were analysed with a Three-way analysis of variance (ANOVA) followed by Tukey post-hoc test.

Results: We have focused our attention on two of the main pro-inflammatory cytokines: IL-1β and TNFα. In animals exposed to LPS at PND35, hippocampal mRNA expression levels of both cytokines were affected by time (6/24h), treatment (sal/LPS), and sex (male/female) (ANOVA; p<0.05). In particular, TNF-α and IL-1β mRNA levels were significantly increased 6h after LPS in females at PND35. This trend was observed also in their male counterparts but was less pronounced and was significant only for TNF-α. In general, the transcription of the targets returned to basal levels 24h after the immune challenge.

In adult animals (12 weeks old), TNF-α and IL-1β hippocampal mRNA levels were significantly affected by the treatment received in adulthood (sal/LPS) but not in adolescence (sal/LPS), while only IL-1β expression was influenced by sex (male/female), no main effect was observed for the adolescence treatment (ANOVA; p<0.05). An exposure to LPS in adulthood upregulated the expression of both cytokines in the hippocampus irrespective of the treatment the animals received in adolescence. This effect was generally lower in females than in males, especially for IL-1β.

Conclusions: Male mice appear to be less responsive than females toward an immune challenge experienced during puberty. This trend is reversed for adult animals. Taken together, our data suggest that sex modulates the transcriptional effects evoked by an acute immune challenge in the hippocampus of both adolescent and adult animals.

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Conflict of interest

Disclosure statement: Supported by PRIN-MIUR Prot. 201779W93T

doi: https://doi.org/10.1016/j.nsa.2023.101042

P.201

NEUROSCIENCE APPLIED 2 (2023) 101019 101043

A DYNAMICAL COMPUTATIONAL MODEL TO SUPPORT UNDERSTANDING OF SCHIZOPHRENIA SPECTRUM DISORDERS

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Introduction: The diagnostic severity of schizophrenia spectrum disorder is a major challenge in psychiatry. One of the most salient challenges faced by this disorder is raised by its complex dynamics: it can emerge quietly, most of the time in young people (e.g., with the category of "ultra-high risk of psychosis"), then appears above the threshold of clinical significance insidiously or suddenly, and finally evolves over a relatively short term by bursts, to finally leads to an involution of the general functioning of patients in the longer term [1]. Given the clinical significance of this disorder, this article seeks to provide a computational model capturing the heterogeneous individual evolutions of schizophrenia spectrum disorder.