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TIME-SPECIFIC KCNQ1 KNOCKOUT IN THE BRAIN LEADS TO COGNITIVE AND METABOLIC ALTERATIONS IN MICE

E. Pisa [1], A.M. Ottomana [1], C. Tonelli [1], E. Patriarca [1], S. Macrì [1]. [1] *Istituto Superiore di Sanità, Centre for Behavioural Sciences and Mental Health, Rome, Italy*

Introduction: Insulin signaling, a well-established pathway involved in maintaining metabolic homeostasis through its glucose-lowering activity, has recently been implicated in brain function. This emerging role is supported by the high expression of **insulin** receptors in the central nervous system, including the Insulin Receptor and Insulin-like Growth Factor 1 Receptor. Recent research highlights insulin's multifaceted role in the **brain**, affecting neuronal patterns, memory formation, and neurogenesis [1,2]. Consequently, abnormal insulin levels have been observed in **neurodegenerative** conditions such as Alzheimer's Disease [3] and **psychiatric disorders** like obsessive-compulsive disorder. Among several factors, **KCNQ1** is emerging as a key modulator of insulin signaling cascades in the central nervous system. KCNQ1 is a voltage-dependent potassium channel capable of modulating local insulin secretion by neurons. *Kcnq1* constitutive knock-out (KO) mice model exhibited a diabetic phenotype accompanied by repetitive behaviors and reduced cognitive flexibility. Dysregulated central insulin signaling may underlie obsessive-compulsive traits, behavioral rigidity, and deficits in memory and learning. Our aim is to distinguish the mechanisms of central and peripheral insulin dysregulation by investigating the role of KCNQ1 in the brain as a candidate molecule influencing insulin signaling. To achieve this, we capitalized on a conditional KO mice model for *kcnq1* in the brain, which inhibits the expression of KCNQ1 only within the brain and during specific time windows (adolescence and adulthood).
Methods: Male and female KCNQ1fl/fl NesCreERT2+ and KCNQ1fl/fl NesCreERT2- mice received Tamoxifen (62 mg/Kg) or Vehicle injections for five consecutive days, one week before testing. We assessed metabolic parameters via automated metabolic cages (general locomotion, fluid intake, lipid vs. carbohydrate metabolism, and energy expenditure). Glucose tolerance and insulin sensitivity tests were conducted to assess hyperglycemia and insulin resistance, respectively. Cognitive domains were evaluated through T-maze (spontaneous alternation), attentional set-shifting task (asst, cognitive flexibility), Barnes maze (spatial memory), marble burying (repetitive and compulsive-like behavior), and elevated zero-maze (anxiety-like behavior). The test battery was conducted in two separate cohorts, one for each age group (adolescence and adulthood). Data were analyzed using ANOVA with Genotype (KCNQ1fl/flNesCreERT2+ vs. KCNQ1fl/flNesCreERT2-), Treatment (Tamoxifen vs. Vehicle), and time blocks as within-subjects repeated measures. Tukey's test was used for post hoc comparisons.
Results: The absence of brain KCNQ1 impacted lipid metabolism during adolescence ($p<.05$) but did not affect metabolism in adulthood. Notably, conditional knockout mice exhibited attention deficits (with a higher number of trials and errors in the ASST compared to controls, $p<.05$) and increased compulsive behavior (as evidenced by reduced spontaneous alternation in the T-maze compared to controls, $p<.05$) at both ages.
Conclusion: Brain-specific deficiency of KCNQ1 impacted metabolic regulation solely during adolescence, yet manifested **impaired attention** and increased **compulsive behavior** across both adolescence and adulthood. These findings underscore KCNQ1's pivotal role in central insulin signaling modulation, shedding light on potential mechanisms underlying neurobehavioral disorders. The identification of potential biomarkers may enhance early diagnoses, allowing for preventive measures against insulin-related comorbidities.
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DORSAL HIPPOCAMPUS OREXIN 1 RECEPTORS MODULATE THE ORGANISATION AND EXPRESSION OF CONTEXTUAL CONDITIONED FEAR

G.M. Suzuki [1], G.V.M.O. Oliveira [1], P.M. Hernandes [2], N.C. Coimbra [2], C.T. Wotjak [3], R.C. Almada [4]. [1] *São Paulo State University UNESP- School of Sciences- Humanities- and Languages, Department of Biological Sciences, Assis- São Paulo, Brazil;* [2] *University of São Paulo- Ribeirão Preto Medical School FMRP-USP, Department of Pharmacology, Ribeirão Preto- São Paulo, Brazil;* [3] *Boehringer Ingelheim Pharma GmbH & Co. KG, Central Nervous System Diseases Research, Biberach an der Riss, Germany;* [4] *São Paulo State University Unesp- School of Sciences- Humanities and Languages, Department of Biological Sciences, Assis- São Paulo, Brazil*

Background: The dorsal hippocampus (DH) has been the subject of studies in neuroscience, especially given the evident participation of that encephalic structure in mnemonic processes and the control of fear and anxiety [1,2]. The hypothalamus is also implicated in the regulation of defensive behaviors, and the hypothalamic neuropeptide orexin (hypocretin) appears to be involved in processing stress, fear, anxiety, and panic [3,4]. The orexin system is composed of two neuropeptides, orexin-A and B (OXA and OXB), and two orexin receptors (OXRs), type 1 (OX1R) and type 2 (OX2R) [3]. Although recent studies have correlated the orexinergic system with the neurobiology of emotions, little is known about the role of orexinergic mechanisms in DH during the organization and expression of behavioral responses associated with learned fear. Herein, we investigated the hypothesis that orexinergic mechanisms in DH play a modulatory role in the organization and expression of contextual conditioned fear.
Methods: All experimental procedures were approved by the Animal Experiment and Use Committee of UNESP - Assis (002/2023) and carried out following the National Council for Animal Experimentation Control guidelines. Experiments were performed using male C57BL/6N mice (8-12 weeks). First, guide cannulae were implanted in the DH. After the surgery recovery, mice were placed into the conditioning chamber for 90 s before giving five foot shocks (0.7 mA, 1 s) at 90 s intervals. Independent groups of mice received injections of the OX1R antagonist SB 334867 (3, 30, and 300 nM/0.1 μL) into the DH either before the training or test sessions of contextual fear conditioning. The test session was performed 24 hours after conditioning and consisted of a 5-min re-exposure to the conditioning chamber without shock delivery. Freezing serves as a measure of contextual fear. Normality and homogeneity in the sampled distributions were confirmed with Shapiro-Wilk's test of normality and Bartlett`s test of homogeneity of variances. Data were submitted to one-way analyses of variance (ANOVA), followed by Tukey's post-hoc test, and statistical significance was set at $P \leq 0.05$.

Results: The selective blockade of OX1R (300 nM) in the DH significantly decreased the expression of contextual fear during the test session, either in mice treated before training ($F_{3,25} = 4.525$; $P < 0.01$; compared to the vehicle group, $P < 0.05$) or in the test session itself ($F_{3,28} = 3.674$; $P < 0.01$; compared to the vehicle group, $P < 0.05$). Mice treated in HD with SB 334867 both before conditioning and before the test session exhibited decreased freezing responses during the test session

Conclusions: Orexinergic mechanisms in HD, via OX1R, seem to be critical for the acquisition and expression of defensive responses associated with contextual conditioned fear. Therefore, our findings indicate that OX1R plays a crucial role in eliciting defensive behavior within the DH, and its antagonists seem promising in reducing excessive fear-related responses.

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CUTANEOUS PAIN SENSITIVITY IN MOUSE MODELS OF INTELLECTUAL DISABILITY AND AUTISM SPECTRUM DISORDER: INVOLVEMENT OF CAMP AND CGMP

M. Jarjat [1], A. Baron [2], N. Gilbert [1], A. Boulksibat [3], B. Bardoni [1]. [1] *IPMC, cnrs umr7275 inserm 1323, Valbonne, France;* [2] *IPMC, cnrs umr7275 inserm1323, Valbonne, France;* [3] *IPMC, cnrs umr 7275 inserm 1323, Valbonne, France*

Fragile X syndrome (FXS) is the most common form of inherited intellectual disability (ID) and autism spectrum disorder (ASD). It is due to the absence of expression of the Fragile X RiboMessengerProtein (FMRP). FXS is also associated with numerous sensory disorders. Indeed, disturbances in skin and pain sensitivity have been described in male patients as well as in Fmr1-KO mice, model of the disease, particularly in cases of inflammatory and chronic pain [1]. Phosphodiesterase 2A (PDE2A) is an enzyme that degrades cyclic AMP (cAMP) and cyclic GMP (cGMP), two second messengers modulating signaling pathways essential for brain development and, in adulthood, for memory and cognition. In addition, these pathways are involved in inflammatory and chronic pain hypersensitivity in the spinal cord [2]. PDE2A is expressed in all regions of the brain and in particular in the cortex, hippocampus and striatum and, as well as FMRP, in spinal cord neurons. The mRNA encoding PDE2A is one of the major targets of FMRP, which negatively modulates its expression. Thus, in the absence of FMRP (in FXS), the increase in the expression and therefore activity of PDE2A leads to a decrease in the intracellular levels of cAMP and cGMP [3]. Mutations in the PDE2A gene have been found in patients affected by ID and ASD. Recently, we have characterized $Pde2a^{+/-}$ mouse showing that these animals, where the levels of cAMP and cGMP are increased compared to WT mice, represent a new model of neurodevelopmental disorder characterized by socio-cognitive deficits [4].It is known that activation of the cAMP pathway in the central nervous system produces hyperalgesia and inhibition of this pathway reduces hyperalgesia in inflammatory, non-inflammatory and neuropathic pain models [5][6]. For example, increased phosphorylation by PKA of the transcription factor CREB, which could initiate gene transcription of "pain genes", has been described in cases of inflammatory and chronic hyperalgesia [2].To better understand the links between cognitive capacity disorders and pain, we decided to study pain sensitivity in mouse models of ID by hypothesizing that the altered levels of the second messengers (reduction in FXS and increase when PDE2A is mutated) play a key role at the molecular level for the two phenotypes: socio-cognitive and painful sensitivity. We will present here our most recent results comparing pain sensitivity behavior in Fmr1-KO and $Pde2a^{+/-}$ mice of both sexes. Furthermore, we will present data showing correlation of behaviour studied in these mice with the morphology of dendritic spines and with the PDE2A-dependent molecular pathways that we characterized in spinal cord neurons of both animal models during different steps of development. We think that our results will provide a strong insight in the identification of cAMP and cGMP-based therapy for chronic pain sensitivity by modulating the level of phosphodiesterases and in particular of PDE2A.

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TRANSCRIPTOMIC HORMONAL FINGERPRINT IN THE FEMALE OFFSPRING OF A SCHIZOPHRENIA-LIKE DOUBLE-HIT MURINE MODEL

E. Olabarrieta Lopez [1], P. Unzueta-Larrinaga [1,2], L.F. Callado [1,2,3], L. Urigüen [1,2,3]. [1] *Department of Pharmacology- University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain;* [2] *BioBizkaia Health Research Institute, Barakaldo, Bizkaia, Spain;* [3] *Centro de Investigación Biomédica en Red de Salud Mental, Cibersam, Madrid, Spain*

Background: In recent years, it has been suggested that sex differences in neuropsychiatric disorders may be related to estrogens, especially in mental diseases such as schizophrenia. Thus, it has been reported a lower incidence of schizophrenia in women of childbearing age and a symptom improvement in pregnant women, coinciding with a high level of estrogens. However, the etiopathogenesis of this disease remains to be elucidated. While the symptomatic onset of schizophrenia typically happens during late adolescence and early adulthood, the disorder stems from genetic and/or environmental factors that are present before the onset of the disease. One of these factors is cannabis consumption in early adolescence, which coincides with a period of hormonal imbalance, especially in women, with the onset of menarche.

Methods: Therefore, the aim of this study was to investigate the molecular mechanisms underlying the interplay between cannabis use, schizophrenia, and the dysregulation of female sexual hormones. To achieve this, we conducted a transcriptomic assessment of a "double-hit" mice model of schizophrenia-like symptoms. This model involved inducing a prenatal neurodevelopmental deficit through maternal immune activation (MIA) during pregnancy, followed by administration of THC during puberty. Specifically, female mice were administered poly (I:C) (PIC) (5 mg/ kg i.p) or its vehicle on gestation day 9.5. After weaning (PND21), the female offspring were treated with THC (10 mg/kg/day i.p) or its vehicle for 30 days. Following a 5-day washout period, female mice were euthanized, and RNA from the brain cortex was extracted for sequencing library preparation using the NEBNext Ultra II Directional RNA Library Prep kit for Illumina. Subsequently, samples were sequenced on Novaseq6000 (Illumina) using single-read sequencing with a read length of 101 nucleotides. Differential expression analysis was performed using the CUFFDIFF tool, while Metascape tool was employed to analyze the enrichment of specific gene ontologies for each experimental group.

Results: Transcriptomic results revealed that several group of genes were differentially expressed in every experimental group, most of them involved in the regulation of behavior, learning and synaptic transmission. However, in those animals treated with PIC and chronic THC during puberty, gene ontology affected showed that there was an altered expression of hormone-regulating genes and reproduction related genes in the female offspring. In this case, the volcano plot analysis revealed that the double-hit animal model exhibited 236 genes differentially expressed, with 171 upregulated and 65 downregulated.

Conclusions: In summary, the double-hit mice model showed alterations in the expression pattern of hormone regulation related genes, as well as reproductive genes in female mice. These findings support the idea that the hormone system, specially the estrogen signaling pathways, plays an important role in the etiopathogenesis of schizophrenia, proposing itself as the main cause of the differences found between sexes in psychiatric pathology.

References

Conflict of interest

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