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THE ROLE OF LACTATE ON METABOLIC REPROGRAMMING IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most malignant type of primary brain tumor in humans and it is often associated with a poor prognosis. The Warburg effect is a dominant phenotype of most cancers, which in responsible of excessive conversion of glucose to lactate, and most tumor cells use glycolysis rather than oxidative phosphorylation (OXPHOS) as the main energy metabolic pathway to produce ATP. Although glycolysis is far less efficient than OXPHOS for ATP generation, tumor cells display abnormally high glycolytic rates in order to preserve high ATP levels. Within the glioblastoma tumor microenvironment (TME), tumor cells, stromal cells, and infiltrating immune cells continuously interact and exchange signals through various secreted factors including cytokines, chemokines, growth factors, and metabolites. Glioma cells in the TME transform immune cells to suppress anti-tumor immune cells and evade immune surveillance. In a number of malignancies such as glioma, myeloid-derived suppressor cells (MDSCs) have been shown to infiltrate malignant tissues having critical role in the network.
The aim of the present study was to evaluated the role of Lactate on metabolic reprogramming in an *in vitro* model of glioblastoma multiforme. Our results suggested that Lactate (5 mM) induces a significant increase in cell proliferation, migration and invasion and was able to regulate positively mitochondrial biogenesis and increased OXPHOS genes, showing that it is involved in metabolic switch of GMB cell line.
In addition, we observed that Lactate induce a significant expansion of Treg and M-MDSCs in Healthy control PBMCs, confirming that it is involved in immune-escape mechanisms. In conclusion, the Lactate pathway may be a therapeutic target in Glioblastoma.

FOURTH SESSION: SEXUAL BEHAVIOR AND DIMORPHISM

SEXUALLY DIMORPHIC EFFECT OF GENISTEIN ON HYPOTHALAMIC NEURONAL DIFFERENTIATION *IN VITRO*

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Developmental actions of estradiol in the hypothalamus are well characterized. This hormone generates sex differences in the development of hypothalamic neuronal circuits controlling neuroendocrine events, feeding, growth, reproduction and behavior. *In vitro*, estradiol promotes sexually dimorphic effects on hypothalamic neuritogenesis. Previous studies have shown that developmental actions of the phytoestrogen genistein result in permanent sexually dimorphic effects in some behaviors and neural circuits *in vivo*. In the present study, we have explored if genistein, like estradiol, affects neuritogenesis in primary hypothalamic neurons and investigated the estrogen receptors implicated in this action. Hypothalamic neuronal cultures, obtained from male or female embryonic day 14 (E14) CD1 mice, were treated with genistein (0.1 μM, 0.5 μM or 1 μM) or vehicle. Under basal conditions, female neurons had longer primary neurites, higher number of secondary neurites and higher neuritic arborization compared to male neurons. The treatment with genistein increased neuritic arborization and the number of primary neurites and decreased the number of secondary neurites in female neurons, but not in male neurons. In contrast, genistein resulted in a significant increase in primary neuritic length in male neurons, but not in female neurons. The use of selective estrogen receptor antagonists suggests that estrogen receptor α, estrogen receptor β and G-protein-coupled estrogen receptors are involved in the neuritogenic action of genistein. In summary, these findings indicate that genistein exerts sexually dimorphicactions on the development of hypothalamic neurons, altering the normal pattern of sex differences in neuritogenesis.

TRIBUTYLTIN ALTERS THE DEVELOPMENT OF BRAIN CIRCUITS CONTROLLING FOOD INTAKE

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Spreading of metabolic syndrome is a raising concern. Recent findings highlight the possible involvement of environmental metabolic disruptors or obesogens (*i.e.*, compounds which may interfere with neuroendocrine system impairing the control of energetic balance) in this multifactorial disease. Organotins, as

tributyltin (TBT), are highly diffused environmental pollutants, acting as obesogens. In a recent study performed in adult mice chronically exposed to TBT we demonstrated alterations of the hypothalamic neuropeptide Y (NPY) expression in the paraventricular (PVN), in the arcuate and in the dorsomedial nuclei of males, whereas no changes have been observed in females. Also the pro-opio-melanocortin is affected but only in females. In the present study, we tested different doses of TBT (0.25-2.5-25 μg/Kg body weight/day) diluted in olive oil, administered orally to C57/BL6 dams from gestational day 8 to postnatal day 21, and we evaluated the long term effects in the adult offspring (33 male and 41 females perfused at 2 months of age). The selected doses are particularly interesting because the higher one corresponds to the "no observed adverse effect level" (NOEL) and the lower one to the "tolerable daily intake" (TDI). We have observed that indirect TBT exposure permanently alters feed efficiency, in particular at the intermediate dose in male and at the lower dose in females. Immunohistochemical analysis showed significant changes in NPY expression in females only in the PVN, but not in other hypothalamic areas, at all the tested doses. These results confirm that the NPY system is particularly vulnerable to the action of TBT, even if the effects are different depending on the period of exposure. Alarmingly, TBT doses defined as TDI had a deep and sex specific persistent effect.

MATERNAL SEPARATION IN ABA RATS PROMOTES CELL PROLIFERATION IN THE DENTATE GYRUS OF THE HIPPOCAMPUS

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Anorexia nervosa (AN) is a serious eating disorder characterized by self-starvation and excessive weight loss. Several studies support the idea that altered maternal care during the postnatal period could play a pivotal role in the pathogenesis of AN, highlighting a multifactorial etiology for this disorder. The activity-based anorexia (ABA) animal model mimics core features of the psychiatric disorder, including severe food restriction, weight loss, and hyperactivity. Previous results in rodents from our lab, obtained through the ABA model, showed that Maternal Separation (MS) induces behavioral changes in anorexic rats in a sexually dimorphic way: in females, the MS promoted hyperactivity and a less anxious-like phenotype in ABA animals; in males, instead, the MS attenuated the anxiolytic effect of the ABA protocol. These results led us to investigate the effect of the MS on brain areas involved in the control of the anxiety-like behavior. We focused our attention on the hippocampal neurogenesis, a process involved in the response to environmental stimuli and stressful condition. We analyzed the volume of the whole hippocampus and the proliferation rate in the dentate gyrus (DG), by quantifying Ki67 density and characterizing neuronal phenotype cells (DCX) and glial cells (GFAP) with double-fluorescence technique. Results obtained showed that only in maternally separated anorexic rats there is an increase of proliferation in DG, underlying the presence of a synergic effect of MS and ABA, that promoted the proliferation of new neurons and glia progenitors in the DG in a more evident way in females in comparison to males.

MARKERS OF NEURAL PLASTICITY AND ACTIVATION IN THE HIPPOCAMPUS OF MALE ROMAN HIGH- AND LOW-AVOIDANCE RATS THAT SHOW DIFFERENCES IN SEXUAL BEHAVIOR

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**Equal contribution*

The Roman High- (RHA) and Low-Avoidance (RLA) rat lines, displaying divergent biobehavioral traits and significant differences in sexual behavior (RHA rats exhibit higher sexual motivation and better copulatory performance than RLA rats), have been used in this study to characterize the neural plasticity processes induced by the sexual experience and underlying the adaptive modifications of behavior. These differences are very evident in sexually naïve rats, and persist, though reduced, after five copulatory tests, when sexual experience has been acquired. Since sexual activity is a natural reward that induces a wide range of neuroplastic changes in the limbic brain, we extended our previous data by studying whether the differences in sexual activity between the Roman lines are related to changes in the expression of Brain-Derived Neurotrophic Factor (BDNF) and its tyrosine kinase receptor B (trkB), c-Fos, FosB, and Activity regulated cytoskeleton-associated (Arc) protein in the dorsal (dHC) and ventral hippocampus (vHC) of sexually naïve and experienced RHA and RLA rats by Western Blot and/or immunohistochemistry. The results showed that, after sexual activity, the selected markers changed differentially in the dHC *vs* vHC of RHA and RLA rats. In both Roman lines, the changes were usually more evident in naïve rats, diminished in experienced rats and were higher in RHA than RLA rats. Our findings confirm that sexual activity induces a different neural activation in the dHC *vs* vHC, hippocampal divisions respectively involved in the processing of sensory signals into memories and in the emotional salience of memories, and leads to changes in synaptic plasticity with sexual experience acquisition, that depend upon the animals' genotypic/phenotypic characteristics.