T21-005C

Comparing the efficiency of microglia depletion strategies during adulthood and development

B. Nagy, S. Siegert

Institute of Science and Technology Austria, Klosterneuburg, AT

Sensory perception and behavioral outcome require accurate neuronal circuit wiring. Microglia, the tissue-resident macrophages, have been shown to remove synapses, thus fine-tuning neuronal circuits. Here, we are interested to identify what impact the absence of microglia has on circuit formation and function using the retina as a model system. We are particularly focused on the physiological maturation of retinal ganglion cells during postnatal development in mice.
First, we had to find a sufficient approach to deplete microglia. We started to deplete microglia in adulthood using the tamoxifen-inducible mouse line Cx3cr1-CreERT2, crossed with either the Rosa26DTR or Rosa26DTA lines. Injection of tamoxifen is expected to lead to microlgia cell death upon a secondary injection of diptheria toxin in the Rosa26DTR-crossed line; or endogenously due to the diphteria toxi A-chain expression in the Rosa26DTA-crossed mice. Surprisingly, the depletion failed for the Rosa26DTR model. For the Rosa26DTA line, the depletion efficiency was below the previously reported percentages. Therefore, we investigated PLX5622, a CSF1R inhibitor, supplemented in chow, for which we confirmed complete microglial depletion in adult mice.
Next, we applied Rosa26DTA and the PLX5622 depletion strategies to newborn animals. The Rosa26DTA pups showed an incomplete depletion with 30-70% of retinal microglia remaining. In the PLX5622 model, nursed pups showed a depletion efficiency of 50%. Once the pups start to feed independently microglia are fully depleted.
In conclusion, the PLX5622 and the Rosa26DTA models show efficient depletion sufficiency; therefore these models allow us now to investigate the impact of microglia depletion efficiency and its timing on retinal circuit formation and function.

T21-006C

Effects of repetitive transcranial magnetic stimulation on brain metabolism and on glial cells

C. Zorzo[1], S.G. Higarza[1], M. Méndez[1], A.M. Pernía[2], J.A. Martínez-Esteban[2], J.L. Arias[1]

[1]Psychology, University of Oviedo. Instituto de Neurociencias del Principado de Asturias (INEUROPA)., Oviedo, ES
[2]Electronic Technology Area, University of Oviedo, Gijón, ES

Repetitive transcranial magnetic stimulation (rTMS) is a noninvasive neuromodulation technique. rTMS is capable to produce changes in the electrical potential of neurons and allows modifying cortical excitability outside the skull. Nowadays, there is an increase in rTMS appliance both in clinical practice and as a neurophysiological tool. However, the exact cellular and molecular mechanisms underlying rTMS-based therapies are not completely elucidated. In addition, although rTMS has the potential to affect a great variety of cell types, glial cells have been less studied. Our aim was to investigate the effects of three days high-frequency rTMS in neuronal metabolism and its effect on glial cells. Firstly, we assessed neuronal metabolic activity by performing quantitative cytochrome oxidase histochemistry (COx). Then, we wanted to know the safety of rTMS examining glial cells. For that purpose, we explored Glial Fibrillary Acidic Protein immunoreactive cells (GFAP-IR) and Iba1 immunoreactive cells (Iba1-IR). All administrations were performed with the stimulation coil located in the upper part of the skull, bregma -3.96 mm, where retrosplenial cortex is located. The animals were randomly split into two groups: a stimulated group (TMS) and a control group (CO). CO group was exposed to

the same conditions as the experimental group but without receiving real stimulation. Our results showed an enhancement of metabolic activity in TMS group compared to CO in cortical and subcortical areas: retrosplenial cortex, parietal cortex, CA1 and CA3 subfields of the hippocampus. Finally, we did not find changes between groups in astrocytic and microglial density in any of the immunostained regions. In the light of our experimental results, we can assume that three days of high-frequency rTMS applied in healthy rats does not alter neither astroglia reactivity nor inflammatory responses, such as microglia proliferation. Due to we have shown an upregulation of neuronal metabolic activity in cortical structures proximal to the stimulation focus and in inner structures such as the hippocampus, our work provides a novel insight into the effectiveness and safety of rTMS as a brain modulation therapy. Our results sum experimental evidence about the positive effect of rTMS in brain stimulation.

Acknowledgement

Gobierno de España PSI2017-83893-R, MINECO PSI2015-73111-EXP and PSI2017-90806-REDT, Programa "Severo Ochoa" de Ayudas Predoctorales de la CONSEJERÍA DE CULTURA Y DEPORTE del Principado de Asturias BP17-11 to C.Z.

T21-007C

Partial deletion of mGluR5 affects M1 and M2 phenotypes in microglia acutely isolated from SOD1^{G93A} mice during disease progression

M. Balbi[1], T. Bonifacino[1], M. Milanese[1,2], G. Bonanno[1,2,3]

[1]Department of Pharmacy, University of Genoa, Genova, IT
[2]Centre of Excellence for Biomedical Research, University of Genoa, Genova, IT
[3]IRCCS San Martino Polyclinic Hospital, Genova, IT

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the selective death of upper and lower motor neurons (MNs). Several mechanisms concur to the pathogenesis and progression of the disease, including glutamate(Glu)-mediated excitotoxicity and neural inflammation. In this frame, several pieces of evidence support the role of mGluR5 in ALS. In our previous studies we demonstrated that double mutant mice carrying the SOD1^{G93A} mutation and a partial mGluR5 deletion (SOD1^{G93A}mGluR5$^{+/-}$) displayed a delay of the pathology onset and an amelioration on survival probability and clinical symptoms. Although motor neuron death is the ultimate cause of the clinical output, other cells, such as astrocytes and microglia, play an important role in motor neuron damage. Moreover, how mGluR5s influence the different cell type contribution to the disease is not completely known.
The aim of this study was to investigate the effect of the partial deletion of mGluR5 in microglia cells prepared from mutant mice. Microglia was acutely purified from mouse brain, motor cortex and spinal cord and it was analyzed by flow cytometry and Western blot. We investigated the balance between the pro-inflammatory M1 and the protective M2 microglia phenotype in SOD1^{G93A} mice at different stages of ALS (120, 90 and 30 day-old mice, that represent late-, early- and pre-symptomatic phases) and in age-matched WT mice.
No alterations of the balance between the M1 and M2 phenotype were observed in SOD1^{G93A} vs. WT mice from brain and motor cortex, even at the late stage of the disease. On the contrary, M1 polarization prevailed in microglia isolated from 120 day-old SOD1^{G93A} mouse spinal cord. No significant modifications were observed in spinal cord at 90 and 30 day-old SOD1^{G93A} vs. WT mice. To verify the impact of the reduction of mGluR5 on the microglia phenotype, the differences between WT, SOD1^{G93A} and SOD1^{G93A}mGluR5$^{+/-}$ mice were analyzed. The results showed that M1 phenotype was greatly increased in the spinal cord of 120 day-old late symptomatic SOD1^{G93A}mGluR5$^{+/-}$ mice respect to both WT and SOD1^{G93A}; whereas, no differences were present in total brain and motor cortex.