their key role as regulators of synaptic transmission and of the abnormal glutamate overexcitation implicated in both acute and chronic brain diseases. We have previously showed that activation of astrocytic A2AR reduce astrocytic glutamate uptake under physiological and pathological conditions, and that A2AR are aberrantly up-regulated upon multiple brain insults.

Methods: We incorporated EGFP reporter either alone or combined with either a small hairpin to down-regulate A2AR (shA2AR) or a control sequence (shCTR) into Mokola Lyssavirus (Mok-G) and Vesicular Stomatitis Virus (VSV-G) lentivectors and tested whether Mok-G-coated lentiviruses selectively and efficiently transduced astrocytes in primary culture or in mouse brain through stereotaxic administration of lentivectors into striatum [STR], hippocampus [HIPP] and prefrontal cortex [PFC] (compared to neurtotropic VSV-G-coated lentivirus as controls). Herein, we evaluated viral spreading and cell-type transduction through immunofluorescent colocalization of EGFP with glial (GFAP and vimentin) and neuronal (NeuN) markers.

Results: After 25 days post-infection, Mok-G,EGFP transduced 68% of cultured astrocytes (EGFP- and DAPI-positive, n=1); 100% of GFAP-positive cells colocalized with EGFP as well as 86% cells expressing Vimentin only and 47% expressing both Vimentin and GFAP. Mok-G,shA2AR lentiviruses robustly reduced A2AR immunoreactivity compared to Mok-G,shCTR in cultured astrocytes. At 4 weeks post-brain administration, Mok-G,EGFP was expressed mainly in astrocytes (GFAP-positive cells) in both STR and HIPP, and to a lower extent in the PFC, whereas VSV-G-coated lentivirus colocalized with NeuN marker and not with GFAP in any tested brain areas.

Conclusion: These data supports the ability of Mok-G lentivectors to efficiently transduce astrocytes to control A2AR density, paving the way for their application to control pathophysiological processes involving astrocytes.


References


PS077

Adenosine A1 receptor antagonism prevents DSI in hippocampal CA1 pyramidal cells

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Aim: How adenosine interfere with a short-term form of neuronal plasticity dependent on endocannabinoid, the depolarization-induced suppression of inhibition (DSI).

Introduction: The widely consumed psychoactive drug cannabis, containing cannabinoid compounds, and/or caffeine, with adenosinergic antagonizing proprieties, exert their central actions by affecting cognitive operations such as learning and memory. Indeed, endogenous adenosine and endocannabinoids (eCB) are known to interfere with physiological synaptic plasticity phenomena that represent the neuronal substrate of memory formation.

Methods: Whole-cell voltage-clamp recordings (Vh = −70 mV) were performed on hippocampal CA1 pyramidal cells of 3 to 5 weeks-old C57BL/6 mice. Slices (350 μm thick) were perfused with artificial cerebrospinal fluid (aCSF) supplemented with glutamate receptor antagonists (CNQX, 25 μM and DL-APV, 50 μM) to block glutamatergic transmission and isolate GABA-mediated responses. Inhibitory postsynaptic currents (IPSCs) were evoked every 3 s through a stimulation electrode placed in stratum radiatum. The recording electrode was filled with a CsCl-based intracellular solution and DSI was evoked through a 5 s voltage step of +80 mV. The magnitude of DSI was measured 9 s after the depolarizing step and DSI recovery was evaluated between 30 and 60 s after depolarization.

Results: When recording eCB-mediated DSI we observed a decrease in electrical-evoked IPSC amplitudes to 81.0 ± 5.4% of baseline (p < 0.01, n = 14) that fully recovered to 90.2 ± 5.4% after 30–60 s. The adenosine A1 receptor antagonist, DPCPX (100 nM), prevented DSI, recordings showing a non-significant change in IPSCs amplitude to 95.1 ± 12.0% of baseline (p = 0.3473, n = 10) that was maintained throughout the recovery period (87.1 ± 12.0%).

Conclusion: These results suggest that tonic adenosine A1 receptor activation is necessary for the occurrence of DSI. The mechanisms involved in this process remain unclear and need further investigation.

References


http://dx.doi.org/10.1016/j.pbj.2017.07.012

PS087

High-sucrose diet effects on the dendritic trees of developing neurons of the adolescent rat

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Aim: In the present study, we aimed to explore the effect of high-sucrose diets on the dendritic trees of immature granule cells of the adolescent male rats.

Introduction: Adolescence is a period of high susceptibility to exogenous factors as the rat brain is still developing. Evidence shows that high-sucrose diets may be more detrimental to adolescent rats, therefore we intended to study immature granule cells in the hippocampal formation of these animals. For that, we used
Looking for modulatory brain areas in the visual circuit related to freezing behaviour

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Aim: I have been studying a visual circuit that is known to trigger freezing: the connection between the Retina, the Superior Colliculus and the Parabigeminal Grey. The aim of this investigation is to look for cerebral nuclei that could be inputs of the SC and, therefore, regulate this behaviour. In other words, it is a search for modulatory brain areas in the circuit: Retina → SC → PBg.

Introduction: Information supplied by the retina initiates interactions in the brain that eventually lead to conscious perception of the visual scene, conventional reflexes such as adjusting the size of the pupil or triggering certain behaviours. Innate defensive behaviours evoked by threatening stimuli are essential to survival. When a danger suddenly appears, a mouse can either escape or freeze. I am interested in how the visual world causes freezing and why.

Methods: The tracing strategy used is based on two injections (stereotaxic surgery) with two different retrograde viruses. The first injection is in the PBg with a modified HSV (Herpes Simplex Virus) and the second one, 21 days later, with RVdG (Rabies Virus G-deleted) in the SC. The combined characteristics of these viruses allowed me to specifically follow the circuit. After perfusing the animals, slicing the brains and staining with specific antibodies attached to fluorochromes, I took images with a fluorescent confocal microscope.

Results: With a pertinent image processing and comparison with the brain atlas, I was able to identify which brain areas were mostly labelled: zona incerta, substantia nigra and L5 in V1 (visual cortex).

Conclusion: It is known that these three nuclei are involved in other visual pathways but this finding suggest that they also could have a role in freezing response to a visual stimulus. The current work is now focused on finding out how each one participates in modulating the behaviour.

Acknowledgements: This thesis is going to be evaluated by the University of Barcelona and it is supported by the KU Leuven, the experiments were performed at NERF (Neuro-Electronics Research Flanders) in the Karl Farrow’s Laboratory.

http://dx.doi.org/10.1016/j.pbj.2017.07.014

PS109

Cafeteria-diet effects on learning and memory, anxiety and fear response of the adolescent rat

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Aim: We aimed to explore the effect of high caloric diets on adolescent male rats to mimic the feeding behavior of human adolescents in the Western world.

Introduction: Age of high-caloric diet exposure is an important factor for the cognitive and anxiety outcomes as key processes of brain development and maturation occur during adolescence. Evidence shows high-caloric diets to affect differently learning and memory performance in an age-dependent way, being more detrimental to adolescent rats.

Methods: At 4 weeks of age, 30 adolescent male Wistar rats were randomly allocated to control, high-sugar (HS) and high-fat high-sugar (HFHS) diet groups during 4 weeks. After this period, behavioral tests were performed to study: (1) anxiety behavior in the elevated plus-maze (EPM) and open field tests, (2) learning and memory processes in the Morris water maze (MWM) and novel object recognition test, (3) fear response in fear conditioning tests and (4) depression state in forced swim test.

Results: Our results show that only HFHS-treated rats presented more anxiety than control rats, spending more time in the closed arms and less time in open arms of EPM. Moreover, HFHS-treated animals presented an impairment of spatial learning in the final phase of acquisition and an impairment of spatial memory, since these rats spend less time in the target quadrant of MWM and cross less times the former position of the platform. There were no differences between groups regarding locomotor activity, fear acquisition and memory, object novelty detection and exploration, and depression state.

Conclusion: In conclusion, anxiety behavior and spatial learning and memory are particularly affected by a cafeteria-type diet in young rats. This data confirms previous evidence reporting adolescence as a susceptible period of brain development to neural insults. Furthermore, the results show that there are different cognitive

doublecortin (DCX), a microtubule-associated protein expressed by neuronal precursor cells and immature neurons, which is used as a marker for neurogenesis.

Methods: At 4 weeks of age, adolescent male Wistar rats were randomly allocated to control group (n = 7) and to an high-sucrose (30% sucrose) diet group (n = 4, HS) during 4 weeks. After this period, rats were sacrificed and DCX immunocytochemistry was performed. The dendritic trees of the DCX-immunostained cells were drawn with the aid of a camera lucida. A metric analysis of the dendritic trees was performed, and the following parameters were quantified: total dendritic length, the total number of terminal segments, the total number of intermediate segments, mean length of terminal segments and mean length of intermediate segments.

Results: Our results show that the total dendritic length of HS adolescent rats was significantly reduced when compared with controls (p < 0.03). There were no other differences in the others parameters quantified.

Conclusion: In conclusion, the dendritic trees of immature neurons of the dentate gyrus of HS adolescent rats appear to be disturbed after the exposition of this diet. This data confirms previous evidence reporting adolescence as a susceptible period of the brain development with likely consequences in cognition. If that is so, and if the reported results can be extrapolated to man, public health interventions are necessary to advise adolescents concerning their diet.

Acknowledgements: This article was supported by ERDF through the operation POCI-01-0145-FEDER-007746 funded by the Programa Operacional Competitividade e Internacionalização – COMPETE2020 and by National Funds through FCT – Fundação para a Ciência e a Tecnologia within CINTESIS, R&D Unit (reference UID/IC/4255/2013).

http://dx.doi.org/10.1016/j.pbj.2017.07.013