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 \( (n = 4, \text{HS}) \) 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

**PS109**

**Looking for modulatory brain areas in the visual circuit related to freezing behaviour**

Maria Roa

NERF (Neuro-Electronics Research Flanders), Belgium

E-mail address: meriroa@hotmail.com.

**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 visual circuit 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 cause 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

**PS178**

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

André Ferreira 1,2,*, João Paulo Castro 1,3, José P. Andrade 1,2, Armando Cardoso 1,2

1 Department of Biomedicine – Unit of Anatomy, Faculty of Medicine, University of Porto, Alameda Prof. Hermnã Monteiro, 4200-319 Porto, Portugal
2 Center of Health Technology and Services Research (CINTESIS), Faculty of Medicine, University of Porto, Rua Dr. Plácido da Costa, 4200-450 Porto, Portugal
3 Physical Medicine and Rehabilitation Department, Centro Hospitalar Vila Nova de Gaia/Espinho, Vila Nova de Gaia, Portugal

E-mail address: andre.ferreira@live.com.pt (A. Ferreira).

**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
and emotional behavioral consequences between HS and HFHS diets.

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.2017.07.015

Oncology & Molecular Biology Parallel Oral Session
Friday, September 15th, 14h00

PS081

GE11 positive exosomes as a potential RNAi delivery system in clear cell Renal Cell Carcinoma
B. Adem1,∗, A.L. Teixeira2,∗, F. Dias2,∗, C. Ruivo1, R. Medeiros2,∗, C. Ruivo2,∗, S.A. Melo1,∗
1 Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal (IPS), 4200 Porto, Portugal, Institute of Pathology and Molecular Immunology of the University of Porto (IPATIMUP), 4200 Porto, Portugal
2 Molecular Oncology and Viral Pathology Group, IPO-Porto Research Center (CI-IPOP), Portuguese Institute of Oncology of Porto (IPO-Porto), 4200-072 Porto, Portugal
3 LPCC, Research Department Portuguese League Against Cancer (Liga Portuguesa Contra o Câncer-Núcleo Regional do Norte), 4200-177 Porto, Portugal
4 Health Sciences Faculty, Fernando Pessoa University, 4249-004, Porto, Portugal
5 FMUP, Medical Faculty of the University of Porto, Portugal
E-mail address: badem@ipatimup.pt (B. Adem).

Aim: Use GE11 positive (GE11+) exosomes as a targeted delivery system to EGFR overexpressing cells for the treatment of clear cell Renal Cell Carcinoma (ccRCC).

Introduction: ccRCC is the most prevalent subtype of renal cancer and the most lethal urologic tumor. Generally, it is radiochemotherapy resistant, and frequently associated with relapse after 5–11 months upon targeted therapy treatment, which highlight the need to develop new therapeutic strategies. Exosomes, extracellular vesicles of 40–150 nm that mediate intercellular communication, have emerged as promising therapeutic tools due to their engineering potential and ability to evade the immune system.

Methods: EGFR is known to be overexpressed in ccRCC thus, the expression of GE11, a peptide that binds to EGFR, in exosomes membrane enable a targeted delivery of therapeutic molecules to EGFR overexpressing cells. Exosomes derived from HEK293T were engineered to express the GE11 peptide on their surface and incubated with normal or tumor renal cell lines.

Results: Our results revealed EGFR overexpression at the mRNA and protein levels in a ccRCC cell line, compared to a normal renal cell line. Furthermore, tumor cells presented increased protein levels of phosphorylated EGFR when compared to normal cells. These results support the hypothesis of using an EGFR-based exosomes delivery model, the GE11+ exosomes. A higher percentage of tumor cells internalized GE11+ exosomes compared to exosomes derived from HEK293T cells transfected with control condition. Additionally, tumor cells exhibited an increased mean of fluorescence intensity compared to the control, suggesting that each cell uptakes more GE11+ exosomes in an EGFR-dependent manner. Importantly, GE11+ exosomes were internalized in a greater proportion by tumor cells rather than normal renal cell lines.

Conclusion: Overall, the use of GE11+ exosomes as a new delivery system is a promising therapeutic strategy for ccRCC treatment. Ultimately, these exosomes can be loaded with RNAi-based drugs to target deregulated genes in ccRCC.

http://dx.doi.org/10.1016/j.pbj.2017.07.016

PS145

Evaluation of combined cytoplasmic AR in tumour cells expression and tumour CD3 T-cells infiltrate as a prognostic score for patients with prostate cancer
V. Constâncio1,∗, M. McAllister2, S. Patek2, M. Underwood3,∗, H. Leung4, J. Edwards2
1 Biology Department, University of Aveiro, Portugal
2 Institute of Cancer Sciences, Wolfson Wohl Cancer Research Centre, University of Glasgow, United Kingdom
3 Department of Urology, Queen Elizabeth University Hospital, Glasgow, United Kingdom
4 Beatson Institute of Cancer Research, United Kingdom
E-mail address: veraconstancio@ua.pt (V. Constâncio).

Aim: We aimed to assess the prognostic value of using a cumulative score evaluating the expression of Androgen Receptor (AR) and the presence tumour inflammatory infiltrate as a prognostic marker for prostate cancer (PCa).

Introduction: PCa is the most common male cancer, in Europe. Currently, at diagnosis, only tumour-based factors, including clinical stage, tumour grade and circulating concentrations of Prostate-Specific Antigen (PSA) are used to predict PCa outcome. However, this can vary within patients sharing the same clinical conditions, leading to patient’s over/under treatment. It is now recognized that cancer progression is also dependent on tumour’s interaction with its microenvironment, specifically with immune cells. Therefore, the development of predictive biomarkers, capable of combining these two factors should be considered.

Methods: Immunohistochemistry for AR expression and CD3 T-cells was performed on biopsies from a cohort of 361 patients diagnosed with PCa. Semi-quantitative weighted histoscore and quantitative assessments were used.

Results: High cytoplasmic AR expression in tumour cells and high CD3 T-cells presence were associated with reduced overall survival (p = 0.000055, and p = 0.004, respectively), with strong association (p = 0.001) on X2 analysis. When patients were grouped as having: both markers low or one low and low/moderate and one high, and both high, this cumulative prognostic score was strongly associated with overall survival (p = 0.000001), being the mean overall survivals: 7.1 years (95% CI 6.5–7.6), 6.0 years (95% CI 5.4–6.6) and 3.8 years (95% CI 2.4–5.0), respectively. Moreover, on multivariate analysis, it was considered a significant independent predictor of overall survival (HR 1.982, 95% CI 1.018–3.859, p = 0.044).

Conclusion: These results confirm the clinical utility of assessing both tumour and microenvironment characteristics when predicting patients’ outcome, and suggest that the presence