

Final report of OTKA PD109644: Investigation of environmental factors in different perinatal injuries in animal models

During the course of the last years, we were investigating the possible positive effects of environmental stimuli to ameliorate the severe consequences of perinatal asphyxia, perinatal stress and retinal damage caused by risk factors of retinopathy of prematurity (ROP) in rat models. All these perinatal insults can cause long-term deficits both in rodent and in human. We primarily focused on early symptoms and signs of damage as well as recovery, since these early changes are least focused in the literature.

Three major questions were raised:

1. Can environmental enrichment prevent the retardation of somatic and neurologic development of asphyctic rats?
2. What effects does maternal stress have on early neurobehavioral development of rat offsprings? Can it be modified by environmental enrichment?
3. Can enriched environment alter the histologic outcome in the rat model of ROP? Does neonatal hyperglycaemia cause alterations similar to the classic OIR models, and if so, can it be ameliorated by environmental enrichment?

In the first year proposal we have finished the experiments and published our results regarding the first major topic: the protective effects of environmental enrichment against perinatal asphyxia were described. Environmental enrichment can significantly protect against asphyxia induced retardation of growth, and improve neurological signs, such as reflex performance and motor coordination in asphyctic injury. (Kiss P, Vadász Gy, Kiss-Illés B, Horváth G, Tamás A, Reglődi D, Koppán M: Environmental enrichment decreases asphyxia-induced neurobehavioral developmental delay in neonatal rats, INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 14:(11) pp. 22258-22273., 2013)

However, as we have started to get our results from the next series of experiments using the prenatal stress paradigm, we could not detect significant changes in neurobehavioral development. After many trials, we managed to evaluate the data, and published the results during the third year of the proposal (Tímea Kvarik, Barbara Mammel, Dora Reglodi, Marta C Antonelli, Jozsef Farkas, Andrea Tamas, Tibor Ertl, Tamas Atlasz, Greta Bodzai, Peter Kiss and Judit Gyarmati: Effects of Maternal Stress during Different Periods of Pregnancy on the Early Neurobehavioral Response of Rats, Journal of Neurology and Neuroscience, 2016).

In the meantime, we still came to the conclusion that the paradigm should be changed to provide better results, and to use a more clinically relevant method for prenatal stress than restraint of the pregnant rats. Thus, in the second year, we started to establish a paradigm for modeling the effects of maternal smoking in offspring. Unfortunately this experiment had to be postponed after the preliminary study, because the student research fellows who were trained to do the time-consuming testing did not join the research group.

Also, during the first and second year the retinopathy of prematurity model was adapted to our laboratory. We are able to produce retinal damage in newborn Sprague-Dawley rats using the oxycycler, with alternating hypoxia-hyperoxia paradigm. First results showed that retinal neovascularisation can be prevented by intravitreal administration of pituitary adenylate cyclase

activating polypeptide. These results were published during the third year of present OTKA (Kvarik T, Mammel B, Reglodi D, Kovacs K, Werling D, Bede B, Vaczy A, Fabian E, Toth G, Kiss P, Tamas A, Ertl T, Gyarmati J, Atlasz T: PACAP Is Protective in a Rat Model of Retinopathy of Prematurity, JOURNAL OF MOLECULAR NEUROSCIENCE 60:(2) pp. 179-185., 2016).

Because of above listed factors, I have requested a one-year prolongation of the proposal, which the committee has kindly supported.

During this last, fourth year we have finally managed to carry on with the prenatal stress paradigm using maternal smoking. However we don't have enough data so far to be published, the experiments are still ongoing.

The investigation of the ROP model was also continued. Here we had a problem with the oxycycler, which caused a major hiatus where we could not perform any successful experiments. Briefly, the O₂ sensor of the machine broke and provided false data, and since the machine itself does not have any self-checking function, we did not realize what the cause of the problem was for multiple months. As the experimental results were unexpectedly contradictory, we started to look for the possible issue, repeating every step multiple times, until we managed to figure out, that it was caused by the broken sensor. We were only able to continue the experiments recently, where we try to use environmental enrichment for improving visual function in ROP rats.

We are also in the final stage of investigating the effects of pituitary adenylate cyclase activating polypeptide (PACAP) in perinatal asphyxia. PACAP is well known for its effects on neurobehavioral development, and it is able to counteract glutamate toxicity as well as ischemic damage in rats in a similar way than environmental enrichment. PACAP is also influenced by enrichment as we have shown (Horvath G, Kiss P, Nemeth J, Lelesz B, Tamas A, Reglodi D: Environmental enrichment increases PACAP levels in the CNS of adult rats., NEUROENDOCRINOLOGY LETTERS 36:(2) pp. 143-147., 2015), thus we have conducted a series of experiments for the possible protective effects of PACAP in perinatal asphyxia.

To further investigate the role of PACAP, we also started to adapt our neurobehavioral testing method previously used in rats to mice. We have found that mice lacking endogenous PACAP (heterozygous and homozygous KO) have significantly slower weight gain and slower neurobehavioral development than wild type mice. We have published these results, and plan to do further experiments on mouse models (Jozsef Farkas, Balazs Sandor, Andrea Tamas, Peter Kiss, Hitoshi Hashimoto, Andras D. Nagy, Balazs D. Fulop, Tamas Juhasz, Sridharan Manavalan, Dora Reglodi. Early Neurobehavioral Development of Mice Lacking Endogenous PACAP Journal of Molecular Neuroscience April 2017, Volume 61, Issue 4, pp 468-478).

In summary, the OTKA PD 109644 has resulted in advances in the investigation of perinatal injuries and their relation to environmental stimuli. Altogether 6 peer-reviewed articles (IF: 10,205, 14 independent citations), and one book chapter were produced during this OTKA.