Research report, September 25, 2015. Dr. Ágota Ádám OTKA PD-102551

The principal results of the research project supported by OTKA (NKFI) PD-105251grant can be summarized as follows:

1. Investigating the effect of the designer drug MDPV (methylenedioxypyrovalerone) on neonatal mouse brain.

The primary purpose of the study was to evaluate the potentially harmful effect of MDPV on the developing brain, as evidenced by the appearance of the apoptotic effector enzyme caspase 3 (Casp3), as a marker of apoptotic cells. To this end, we used a mouse model which can be compared with the human fetus of third trimester, considering the developmental stage of the brain. Litters of 7-day-old C57BL/6J mice were treated either with i.p. injection of 10 mg/kg b.wt. of MDPV or vehicle (saline), and sacrificed after 24 h. Similar dose of MDPV enhanced locomotor activity of pups, investigated using open field test optimized for mice of that age. The brains were processed for anti-caspase 3 (Casp3) immunohistochemistry and the apoptotic cells were identified and counted. We found prominent increase in the number of apoptotic cells in the piriform cortex, retrosplenial area, hippocampus CA1 and nucleus accumbens, whereas the overall density of cells did not change significantly in these regions. The neurons of the nucleus accumbens appeared to be especially sensitive to MDPV: Casp3-immunoreactive cells marked out the core and shell regions of the accumbens. Highest percentage of apoptotic cells as compared to total cell density was also found in the nucleus accumbens. However, we did not observe the same effect on the brain of adult mice. Thus, MDPV did not seem to increase apoptosis in the mature nervous system. The results are in agreement with the assumption that synthetic drugs of the cathinone family might cause damage to a wide variety of neurons in the developing brain. The effective period appears to correspond to that of the third trimester of human fetus. Neurochemical characterization of the neurons undergoing apoptosis, as well as better definition of the sensitive time window will be the subject of further studies.

Our results were published in NeuroToxicology:

Ágota Ádám, László István Gerecsei, Nikolett Lepesi, András Csillag (2014) Apoptotic effects of the ‘designer drug’ methylenedioxypyrovalerone (MDPV) on the neonatal mouse brain. *NeuroToxicology,* Volume 44, Pages 231–236. *DOI: 10.1016/j.neuro.2014.07.004*

2. Investigating the behavioral effects of synthetic cathinones using avian models

In this study, the effects of three different abusive agents of the cathinone family, mephedrone, butylone and 3,4 methylene-dioxypyrovalerone (MDPV) were tested in young domestic chicks, following administration of single intraperitoneal injections (10 mg/bwt). Early maturing (precocial) birds are particularly suited for investigation of isolation stress-

related behavioral response and stereotypic or targeted pecking. Both mephedrone and MDPV increased the frequency of distress calls of socially isolated birds as measured over a period of 10 min. While this effect of mephedrone was only evident in the first half of observation period, an increase with MDPV was more lasting. Though increased non-distress vocalization, butylone failed to enhance distress calls probably due to a general adverse effect on muscle tone. Apart from its effect on distress vocalization, mephedrone did not alter the behavior of chicks. However, both butylone and MDPV showed prominent behavioral changes, which were examined in another set of long term experiments, over a period of 120 min. Butylone caused hyperventilation and a robust impairment of postural control, whereas neither the wakeful activity level, nor the pecking frequency was significantly affected. Conversely, no hyperventilation or postural disorder was observed with MDPV, however, both waking state and pecking were significantly enhanced. The results may be relevant to potentially different and specific effects of cathinone drugs under stress-related conditions, as well as on other physiological and behavioral parameters, even in case of closely related compounds.

Our results were published in Neuroscience Letters:

Csilla Karina Zsedényi, Gergely Zachar, András Csillag, Ágota Ádám (2014) Effect of synthetic cathinones: mephedrone, butylone and 3,4 methylene-dioxypyrovalerone (MDPV) on social separation induced distress vocalization, vigilance and postural control of young domestic chicks. *Neuroscience Letters*, Volume 580, pp. 88-93. *DOI information: 10.1016/j.neulet.2014.07.027*

3. Effects of prenatal exposure to 'designer drug' methylenedioxypyrovalerone (MDPV) on the behavior of neonatal and adolescent mice

The objective of our study was to determine the effect of MDPV (administered from the 8th to the 14th day of gestation) on the behavior of neonatal and adolescent mice. Pregnant mice were treated either with s.c.. injection of 10 mg/kg b.wt. MDPV or vehicle (saline) once a day. We measured maternal care (pup retrieval test), locomotor activity (open field test) and motor coordination (grip strength test) of dams. On pups we examined locomotor activity at postnatal day 7 (open field test, force plate actometry) and day 21 (open field test) and motor coordination on day 21 (grip strength test). We detected weaker maternal care among treated animals, whereas there was no difference between the results open field test in treated and control mothers. Locomotor activity of the pups showed an increase in the MDPV treated group both at 7 and 21 days of age, as measured in the open field test. By using force plate actometry no significant difference was measured in the total distance covered, however, the areal coverage increased in the MDPV-treated group of 7-day-old pups. Motor coordination of pups (grip strength test) was unaffected by MDPV treatment. The results clearly suggest that chronic systemic administration of the cathinone agent MDPV to pregnant mice can exert post partum effects on their offspring.

In our study, the time window for the MDPV injections corresponded to the second half of the first trimester in human development. Based on existing data, the drug was present in the pregnant female mouse at a time of the differentiation of dopaminergic (TH expressing) neurons but prior to the appearance of dopamine receptors D1 and D2. Thus, in so far as

dopaminergic mechanisms are involved in the observed changes, these likely affected the presynaptic, rather than the postsynaptic side. As a potential mechanism, neurodegeneration (enhanced apoptosis) in striatum and nucleus accumbens has been demonstrated by our group in young postnatal mouse pups following systemic bolus injection of MDPV (see section 1).

A significant setback of maternal care was apparent in the drug treated mothers, particularly when the latency for complete pup retrieval was compared. Moreover, the refractory period preceding the onset of pup retrieval also appeared to lengthen. In principle, this deficiency may have been due to negligence on the dam's side, possibly based on perturbation of dopaminergic mechanisms.

The results clearly demonstrate that chronic systemic administration of the cathinone agent MDPV to female mice at critical time periods of pregnancy can exert post partum effects on their offspring.

Our current publications on this topic:

Effects of the designer drug methylenedioxypyrovalerone on the developing brain in experimental animal model. Gerecsei László István, Ádám Ágota (2015) Orv Hetil. 156(30):1221-5. doi: 10.1556/650.2015.30202.

Submitted to Neuroscience letters (under review):

László István Gerecsei, András Csillag, Gergely Zachar, Lőrinc Gévai, László Simon, Ágota Ádám. Effects of prenatal exposure to 'designer drug' methylenedioxypyrovalerone (MDPV) on the behavior of neonatal and adolescent mice. *Submitted to journal*

Fringe benefits of our grant funded research

We adopted the force plate actometry as a method for observing motor activity in 7-day-old mice pups. Laboratory Animals' Motor Behavior Apparatus (LAMBA-1) was developed by the Laboratory of Sensorimotor Adaptation, Semmelweis University (Director: Dr. L. Simon) and was first adapted to human posturography for monitoring of positioning maneuvers of subjects. This method can yield to a more complex metric of locomotion than what could be deduced from open field tests alone in 7-day-old mice (lacking vision and with highly erratic motor capabilities).

As a university teacher, I find it really important to help undergraduate students in their first steps towards scientific research. I encouraged students to join my research project and find their own parts of the research where they can give their own contribution to the whole project. The diploma work of Nikolett Lepesi, MSc student of Szent István University was based on our work together on this project. She successfully defended her thesis in 2014. László Gerecsei (currently 6th year medical student of Semmelweis University), another undergraduate student under my supervision, has won a prize on the Student Research Conference ('TDK') at the Semmelweis University in 2014 and in 2015. In 2015 he also won a 2nd prize in the National Conference for Research Students ('OTDK'). There are two other undergraduate student working on the ongoing research projects at the moment: Krisztina

Horváth (Szent István University, MSc in Biology, 1st year) and Anett Bozsik (ELTE BSc in Biology, 3rd year).

With our publication in Orvosi Hetilap, László Gerecsei and I won the prize of "Dr. Fehér János Emlékére Alapítvány" in 2015 May.

Experiments in progress

Our previous experiments raised the possibility of a direct neurotoxic effect of cathinone drugs on the developing CNS. As a logical extension of this work, an attempt is made to test these agents (mainly the MDPV) in embryonic forebrain explants. Based partly on the introduction of a new tissue culture laboratory at our department (funded by the NAP Project; supervised by Dr. Alán Alpár) and in part on a collaborative project with Dr. Diego Echevarría Aza of the University of Alicante, Spain, an experimental neuroembryologist, we performed pilot experiments on stage ED 11-12 mouse embryos. Using Prof. Echevarria's dissecting method, telencephalic samples were harvested and the explants were incubated on tissue culture membranes for 24 hours, with or without adding the drug. The explants successfully survived and following fixation with paraformaldehyde they were frozen and cut with a cryostat. The apoptotic cells were detected by subsequent immunostaining against caspase 3. Currently we are working on optimization of the dissection technique and specific transcription factors allowing specific identification of appropriate subregions of the developing neural tube. This will likely enable us to establish if and which part of the developing prosencephalon show specific alterations in response to drug exposure. The figure below shows an example of caspase 3 immunoreactive elements in the developing neural tube following MDPV treatment on ED 11 embryos.