FINAL REPORT NKFI K 115422

We focus our research on studying the physiological roles of nesfatin-1, an anorexigenic peptide originating from a precursor protein called nucleobindin-2 (NUCB2). NUCB2 can be post translationally cleaved into the N-terminal nesfatin-1, nesfatin-2 and the C-terminal nesfatin-3 by theory (Oh, Shimizu et al. 2006). Nesfatin-1 is responsible for the known biological actions, whereas functions of nesfatin-2 and -3 still remained unknown (Taché and Stengel 2013). During the work we faced several difficulties (e.g.: infection in the animal facility, personal changes in the project, Covid-19 pandemic). Despite all this, we made all effort to complete our objectives planned originally, and were also opened to expand new ideas that emerged during the work and promised meaningful results The following report contains a summary and a detailed description of our work.

Summary of the research

1. We prepared new animal models for research; nesfatin/loxP knock-in mouse, nesfatin-1 KO mouse and Tg(Pomc1-Cre)/nesfatin-loxP mouse strains.

2. We prepared new research tools; AAVs allowing neuronal specific silencing of NUCB2 expression (shRNA), or overexpression of nesfatin-1in rats.

3. We revealed a cooperation of nesfatin-1, prolactin-releasing peptide (PrRP) and noradrenaline that are coexpressed in the brainstem, in the adaptation to chronic hypernatremia.

4. We established that central nesfatin-1 resistance underlies development of type-2 diabetes in intrauterine undernourished rats. Using the same animal model, we identified a cell population in the accumbens nucleus that are probably responsible for stopping reward-related food intake. Malfunctioning of these cells can be linked to hyperphagia induced obesity.

5. We successfully depleted nesfatin-1 expression by shRNA in the rat supraoptic nucleus (SON) as well as in the arcuate nucleus (ARC), and overexpressed nesfatin-1 in the ARC. Based on these experiments, we connect function of nesfatin-1 in the SON to dehydration induced anorexia and in the ARC in the central regulation of the glucose homeostasis.

6. We proved that nesfatin-1 is involved in regulation of the thyroid hormone access in the brain via a *non-neuronal way*, which is a new mechanism of nesfatin-1's action. Ongoing work includes transgenic mice.

7. We revealed a new circuitry between PrRP neurons and MCH-nesfatin-1 cells that determines vulnerability to depression.

8. Publication capacity was also slowed down by Covid disease of colleagues and family. We produced/contributed to 7 papers and several conference abstracts. One additional manuscript is under the reviewing process. We have unpublished results to prepare 2-3 further manuscripts.

Detailed description of the research in the order in which the original objectives were described in the research plan

Aim 1. To investigate the various physiological effects of nesfatin-1 we plan to generate a mouse strain in which NUCB2 expression can be deleted by cell-type specific manner.

We generated the nesfatin/loxP knock-in mouse in a way that Cre exertion of the nesfatin-1 fragment having the reported biological effects, leaves the nesfatin-2 and 3 fragments (with unknown function) of the NUCB2 prohormone in an open frame, therefore the transcription of these fragments is unaffected by the genomic manipulation. Sequence polymorphisms in the NUCB2 gene were revealed within the genome. Several constructs and techniques were used by Dr Ferenc Erdélyi (KOKI) to solve the problem. The difficulties caused years of delay in the generation of the strain. The strain is on FVB/*Ant* background and

the back-crossing to c57BL/6 background is in process. The general development, food, water intakes, as well as the reproductivity of the strain is normal. We crossed the nesfatin-loxP mouse with a GT(ROSA)26Sor/Cre mouse (developed by Artemis Pharmaceuticals,now Taconic) and Tg(Pomc1-cre)16Lowl/J mouse (originated from JAX Stock #005965, The Jackson Laboratory, USA). These crossings resulted general nesfatin-1 knock-out and POMC cell specific nesfatin-1 knock-out lines, respectively. Nesfatin-1 immunohistochemistry confirmed the lack of nesfatin-1 in the general nesfatin-1 KO mouse. The Tg(Pomc1-Cre)/nesfatin-loxP mouse lacks nesfatin-1 in 60% of the POMC – nesfatin-1 cells in the arcuate nucleus. Preliminary data suggest that fasting induced body weight loss is reduced in the Tg(Pomc1-Cre)/nesfatin-loxP females, but not in males. We presented these preliminary data on the IBRO Workshop 2020. Back-crossing to c57BL/6 background and generation of knock-out mice lacking cre expression to avoid measuring the theoretical effect of Cre enzyme expression is under process.

Colocalization of nesfatin-1 with other neuropeptides in the mouse hypothalamus. Klaudia Sípos, Rege Sugárka Papp, Katalin Könczöl, Dorina Guba, Csaba Fekete, Ferenc Erdélyi, Máté Durst, ***Zsuzsanna E Tóth.*** *IBRO Workshop 2020, Szeged, Hungary*

As the progress of our transgenic mouse project was very slow, we designed and produced shRNA and scrambled RNA expressing AAV vectors to silence NUCB2 production *in vivo* in rats. We also produced different AAVs to be able to overexpress nesfatin-1 under synapsin, vasopressin and oxytocin promoters. Appropriate control viruses were prepared too. The viruses were cleaned and the titers determined. The shRNA and the synapsin-nesfatin-1constructs worked well in the tests 3 weeks after the injections. Unfortunately the oxytocin and vasopressin promoters did not work. These AAVs allow as to manipulate NUCB2 expression in the different brain nuclei of rats, opening new research projects for the future.

Aim 2. Examine the participation of tanycytes and astrocytes in conveying nesfatin-1's actions.

To see if nesfatin-1 influences action of T3 hormone in the brain we administered nesfatin-1 (Sigma) intracerebroventricularly (icv) in a series of experiments in different doses. Type 2 deiodinase (D2, converts T4 into active T3 by tanycytes and astrocytes in the hypothalamus) mRNA expression was measured by quantitative *in situ* hybridization (ISH) in the ependymal lining of the 3rd ventricle in the mediobasal hypothalamus and in the choroid plexus. Enzymatic activity of D2 was also determined by RIA at four different timepoints in mediobasal hypothalamic tissue samples following nesfatin-1 administrations. We found a significant increase in the D2 mRNA expression 2 h after treatments, and also in the enzymatic activity at 2 h and 4 h after treatments in the mediobasal hypothalamus (Fig.1), but not in the choroid plexus, where D2 is also expressed abundantly. Thyroid hormone transporter mRNA expression (OATPC1 and MCT8) was downregulated by nesfatin-1, while TSH-beta mRNA levels remained unchanged in the MBH (Fig.2.)

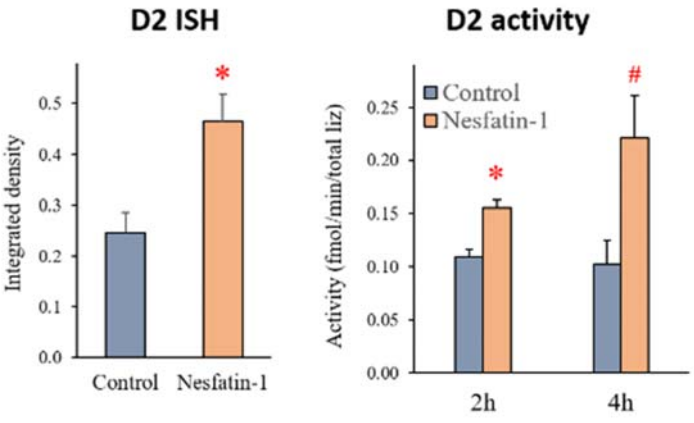

Figure 1. Effect of icv nesfatin-1 (100 pmol) on the mRNA expression and activity of D2 enzyme in the ependymal lining of the 3rd ventricle and in the mediobasal hypothalamus, respectively. Control rats received saline as vechicle. For ISH tissue was harvested 2h after treatments. Student's t-test, $p^{*}<0.05$ *vs*. control 2h, $p^{\#} < 0.05$ *vs*. control 4 h, means ± SEM, n =6.

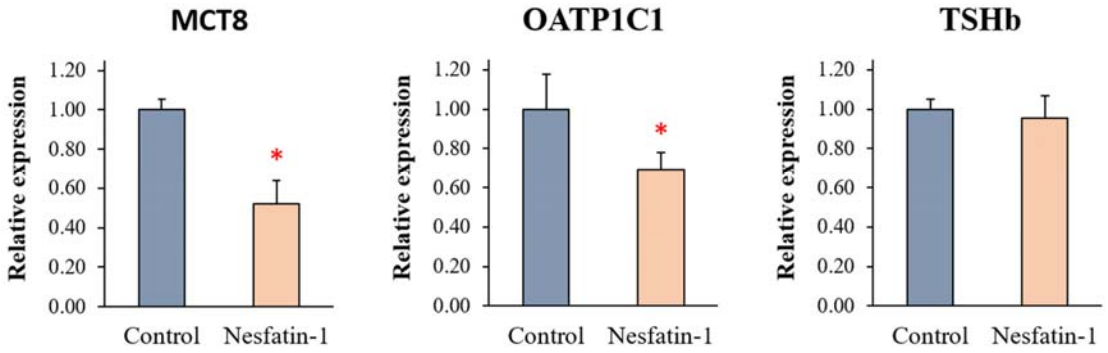

Figure 2. Effect of icv nesfatin-1 (100 pmol) on the mRNA expression of thyroid hormone transporters MTC8 and OATPC1 as well as that of TSH beta in the mediobasal hypothalamus. Data were measured by RTPCR from tissue samples harvested 2 h after rats were treated with nesfatin-1 or vehicle. Student's t-test, $p^{*}<0.05$, means ± SEM, n =5.

To investigate the relationship between D2 activity and the biological functions of nesfatin-1, we depleted the activity of D2 by reverse T3 injected icv, before the central injection of nesfatin-1, and measured the nocturnal food intake of rats. Both nesfatin-1 but not rT3 alone decreased food intake 4h after administration. Previous rT3 administration resulted in a relatively greater inhibition of food intake compared to the effect of rT3 or nesfatin-1 alone. As D2 activity can be increased by lipopolysaccharide (LPS) induced inflammation we started to investigate the role of nesfatin-1 in inflammation. LPS increased NUCB2 expression measured by *in situ* hybridization after 6 h in the hypothalamus.

To complete these experiments we treated homo- and heterozygous nesfatin-1 KO as well as wild type mice with LPS and to measure D2, IL6B and TNF-alpha mRNA levels by RT-PCR in the mediobasal hypothalamus. We revealed that LPS caused similar level of inflammation in both strains based on the detection of inflammatory cytokines. D2 levels were twice as high in the KO than in WT mice, however D2 levels failed to elevate in response to LPS in the KO mice. We are going to complete our experiments on transgenic mice prepare a manuscript on the new mechanism of nesfatin-1"s action acting on non-neuronal cells.

According to the original research plan, we also examined whether nesfatin-1 released into the CSF may be undergoing endocytosis by tanycytes. We injected biotin-labeled nesfatin-1 icv, and monitored its fate in time (30 min, 2 h, 4 h, 12 h, 24 h). We have used two different dose of nesfatin-1, and see no sign of uptake by the tanycytes by immunohistochemistry.

Additionally, we investigated the *in vivo* cleavage of the NUCB2 prohormone and how this is affected by LPS induced inflammation. Cleavage of NUCB2 peptide into nesfatin-1 and nesfatin-2,3 was detected in many organs, except the kidney and liver. A 55 kDa product was detectable in certain organs with both antibodies indicating a possible posttranslational modification of NUCB2. LPS treatment affected NUCB2 expression in the kidney, lung and testis. The results were presented at the 16th Annual Conference of the Hungarian Neuroscience Society.

Expression of NUCB2 and its fragments in intact rat and in inflammatory rat model. Dorina Guba, Máté Durst, Rita Matuska, ***Zsuzsanna E Tóth****. Poster ID: 64.16th Annual Conference of the Hungarian Neuroscience Society, Hungarian Academy of Sciences, January 17-18, 2019, Debrecen, Hungary*

In collaboration with Zita Puskar (SE Department of Anatomy, Histology and Embryology) we subjected rats also to carrageenan-induced inflammation and investigated the spinal cord. Here, NUCB2 expression was unaltered by the treatment. However, we detected and measured dipeptidylpeptidase 4 (DPP4) mRNA in the dorsal horn of L4-L6 segments of the experimental groups. These data were published in the Scientific Reports.

Glial cell type-specific changes in spinal dipeptidyl peptidase 4 expression and effects of its inhibitors in inflammatory and neuropatic pain. Király K, Kozsurek M, Lukácsi E, Barta B, Alpár A, Balázsa T, Fekete C, Szabon J, Helyes Z, Bölcskei K, Tékus V, ***Tóth ZE****, Pap K, Gerber G, Puskár Z. Sci Rep. 2018 Feb 22;8(1):3490. doi: 10.1038/s41598-018-21799-8. SJR indicator: D1*

As I indicated in the interim report, a series of reverse T3 experiments become useless, due to the mislabeling and delivering a wrong the product by Sigma that they revealed and replaced 9 month later. This event delayed this topic by almost a year, since each experiments took approximately a month each, as rats had to adapt to a reverse light-dark cycle for 3 weeks, in order to measure nocturnal food intakes during workdays. Later the animal facility become infected, which we noticed from the high mortality of the experimental animals and the inconsistent experimental data received in repeated experiments. Disinfection and reconstruction of the facility took 5 month, during this time all animal experiments were stopped.

At this time we turned to our previous work, in which we demonstrated by EEG that nesfatin-1 reduces the time spent in REM sleep and MCH-nesfatin-1 coexpressing neurons in the lateral hypothalamus attenuate NUCB2 expression during sleep deprivation (Vas, Ádori et al. 2013). As the MCH cell population represents a main hub in both sleep and mood regulations (Torterolo, Scorza et al. 2015), and treatment resistance to conventional monoaminergic antidepressant medications is frequent, we extended our investigations to identify new factors that may be important in regulation of MCH-nesfatin-1 neurons. With the help of many collaborators we found in male rats that both prolactin-releasing peptide (PrRP) expressing cells in the dorsomedial nucleus of the hypothalamus and PrRP-noradrenaline cells in the medulla oblongata innervate the MCH neurons. Sleep-deprivation upregulates PrRP expression, while centrally given PrRP inhibits the activation of MCH neurons elicited by sleep rebound following sleep deprivation. Ex vivo, PrRP hyperpolarilazes the MCH neurons, moreover, doubles the hyperpolarizing effect of noradrenaline on the MCH neurons. PrRP neurons in the medulla oblongata are among the first cell populations that respond to the vagus-mediated peripheral inflammation. We also demonstrated using learned helplessness- and peripheral inflammation-induced depression rat models that susceptibility to depression-like behavior is associated with overactivation of the PrRP system that leads to PrRP depletion, downregulation of PrRP receptors in the lateral hypothalamus and dysregulation of MCH expression. However, rats with normal PrRP receptor expression in the DLH remain resistant against developing depression-like behavior. PrRP-receptors are downregulated in the DLH of suicidal human subjects highlighting the clinical relevance of the involvement of PrRP in the pathophysiology of depression. Our findings may uncover a fundamental mechanism by which PrRP may enchance the effect of noradrenaline antidepressants on MCH neurons. Preliminary data were presented in form of lecture at the MITT 2019. We have recently submitted a manuscript ‘entitled “*The stress hormone, prolactin-releasing peptide affects sleep and mood via melanin-concentrating hormone neurons in rats*’ to the Journal of Neurosience. New experiments were asked, now we are working on this and prepare the revised version.

Stress, Sleep and Depression: New factors in an Old Circuit. ***Zsuzsanna E. Tóth****, 16th Annual Conference of the Hungarian Neuroscience Society Hungarian Academy of Sciences, January 17-18, 2019, Debrecen, Hungary*

According to the original goals, effect of nesfatin-1 on communication of astrocytes and tanycytes was investigated *in vitro* on astrocytic cell cultures prepared from 10 days old rats. We performed several Ca2+ imaging measurements that did not supported the idea of nesfatin-1's direct effect on tanycytes at least not via Ca2+, as a second messenger that is considered as a main communication form in these cells.

We also examined the intracellular signaling pathway routes initiated by nesfatin-1 stimulation along with appropriate positive controls. We used BRET based sensors to measure intracellular Ca2+, IP3, cAMP, PIP2, PIP3. We also measured activation of the MAP kinase pathway using Western blot approach (pERK and p38 detection). Using this screen, we could detect activation of Gq-, Gi- and Gs-coupled 7TM receptors, and thyrosin–kinase receptors, as well. Despite using different concentartions of nesfatin-1, we did not detect any change of these signaling effects in the examined cells including Neuro2A, GT1, 3T3-L1 cell lines, astrocytic and neuronal primary cell cultures from P0 mice. Since in a patent documentation GPR12 was identified as possible receptor of nesfatin-1, we purchased a clone contains the sequence of the mouse GPR12, and created constructs that allow expression of both untagged and GFP-tagged GPR12 in mammalian cells. Unfortunately, stimulation of these cells (COS-7, HEK293, Neuro2A) with nesfatin-1 did not resulted in the activation of the examined signaling pathways. As a final attempt, we purchased nesfatin-1 from different companies (Sigma, Phoenix, ProteoGenix) and ontained M30 (middle segment of nesfatin-1 that is considered as the domain responsible for the biological activity), but none of them worked in the above mentioned assays.

According to the newest publication in which intracellular effects of nesfatin-1 was investigated, we tested whether nesfatin-1 and its effective region the M30 midpeptide were able to inhibit the Kir2.1 voltage-dependent potassium channel. Unfortunately, none of the peptides was acting on the channels expressed either in HEK 293 cells or in Xenopus oocytes. To examine intracellular processing of the NUCB2 gene we purchased both mouse and rat cDNA and cloned the protein into a mammalian expression vector. In order to detect the proteins HA or mRFP (or both) tags were inserted between the signal peptide and the nesfatin-1. Proteins were transiently expressed in Neuro 2A cells and the products of NUCB2 gene were detected either in the cell lysates or in the cell supernatants by Western blot with antibodies against nesfatin-1 or the HA-tag. The fluorescence protein-tagged NUCB2 fragments were also examined by confocal microscopy. By this approach, we detected the proteins in the endoplasmic reticulum, but there were no any sign of Golgi localization or their presence in the secretion granules. Using the Western blot approach, we were able to detect the full-length form of the expressed protein in the supernatant, and in addition to the full length many degradation products in the cell lysate. To identify these protein products we generated various plasmids encoding the truncated products of NUCB2 that correspond to the expected cleavage sites between nesfatin 1-2 and 2-3. Surprisingly, while the mRFP-tagged constructs showed the expected expression in the mammalian cells, cellular appearance of the HA-tagged and wild type NUCB2 could not be revealed. Measurement of the mRNA transcribed from the exogenous DNA did not explained the difference of the protein levels. To increase the expression level of the wild type proteins we included the 5'-untranslated region (exon 1 and 2) into the plasmids, but there was no change of the expression. We presented these data in the 11th FENS Forum of Neuroscience.

Examination of the cellular effects and processing of NUCB2/Nesfatin-1 protein. Rita Matuska, Katalin Könczöl, Péter Várnai, ***Zsuzsanna E Tóth.*** *P148-E.01.a Neuroendocrine: Cellular signalling Abstract: 3314. 11th FENS Forum of Neuroscience, Berlin 2018, 7-11 July, 2018, Berlin, Germany.*

Aim 3. Examine whether nesfatin-1 acts on plastic mobility of the tanycytes and astrocytes.

We mostly planned *in vitro* experiments in this section. As all of our efforts to demonstrate any effects of nesfatin-1 *in vitro* has failed, we skipped these experiments. However, we started *in vivo* experiments to see the effect of salt loading on neuronal and astrocytic plasticity. Using Wistar and vasopressin deficient Brattleboro rats, we found that nesfatin-1, prolactin-releasing peptide and noradrenaline that are coexpressed in the medulla oblongata, work cooperatively in the adaptational responses to chronic hypernatremia. We published these data in the Brain Structure and Function.

Colocalized neurotransmitters in the hindbrain cooperate in adaptation to chronic hypernatremia. Rita Matuska, Dóra Zelena, Katalin Könczöl, Rege Sugárka Papp, Máté Durst, Dorina Guba, Bibiana Török, Peter Varnai, ***Zsuzsanna E. Tóth****. Brain Struct Funct. 2020; 225(3): 969–984. Published online 2020. Mar 21. doi: 10.1007/s00429-020-02049-y.* *SJR indicator: Q1*

We also started to perform salt loading experiments using nesfatin-1 KO mice and rats lacking nesfatin-1 expression in the supraoptic nucleus (SON), due to gene silencing by nesfatin-1 sh RNA injected into the SON. Our data suggest that lack of nesfatin-1 in the SON enhances the dehydration induced anorexia, and modifies the plastic response of the SON (size of the nucleus and the ventral glial astrocytic lamina) to salt loading. Our preliminary were presented in the IBRO 2022 Workshop.

Examination the role of nesfatin-1 in the supraoptic nucleus of rats. Klaudia Sípos, Rege S. Papp, Máté Durst, Miklós Geiszt and ***Zsuzsanna E. Tóth. I****nternational Neuroscience Meeting, Budapest 2022 – IBRO Workshop, P4.13 pp:108. Budapest, Hungary*

<u>Aim 4. Investigate whether nesfatin-1 affects neurogenesis.</u>

The postnatal first month is crucial for development of food intake-regulatory pathways in the hypothalamus in rats. Therefore, we started a developmental study on nesfatin-1's expression. A strong general expression of NUCB2/nesfatin-1 mRNA was found by in situ hybridization at the first week. By the 21st day, the expression of the mRNA decreases at several places, but remains strong in the main hypothalamic nuclei related to food intake regulation. Additionally, there was a transient mRNA expression in the ventromedial nucleus of the hypothalamus. The transient expression between the 10th and 18th day in the ventromedial nucleus was confirmed at protein level too. Data support the role of NUCB2/Nesfatin-1 in the early postnatal development. Additionally we prepared in situ hybridization probe for necdin, which was shown to regulate release of NUCB2 in vitro. Necdin mRNA expression was very strong in all neurons at birth and showed similar tendencies like the NUCB2 expression changes with age. The results were introduced at the FENS Regional Meeting 2017.

Necdin, a nesfatin-1/NUCB2 binding protein in the rat hypothalamus. Ocskay K, Könczöl K, Balázsa T, Durst M, Matuska R, ***Tóth ZE.*** *FENS Regional Meeting, 2017 09. 20-23, Pécs, Hungary.*

Due to the Covid-19 pandemic we could not continue this part of the project as we originally planned. However, we had brain tissue stored from earlier experiments, in which we birth dated cells by BrDU during the embryonic development in normal and intrauterine protein restricted embryos. We established that the number of nesfatin-1 positive cells was similar between the groups in the adults, however, there was a delay in the generation of the nesfatin-1 cells in the hypothalamus due to the poor intrauterine conditions. We found that this developmental abnormality may contribute to the central nesfatin-1 resistance that we detected in these rats. Intrauterine undernourishment by protein restriction (PR) is a widely used rodent model in the type 2 diabetes research field. It mimics well the consequences of the intrauterine growth restriction (IUGR) in humans, a common complication of pregnancy (Martin-Gronert and Ozanne 2007, Saleem, Sajjad et al. 2011, Pinney 2013), giving a great significance of our findings. Completing this study, we showed that PR rats develop fat preference, reduced glucose tolerance and insulin sensitivity parallel

with central nesfatin-1 resistance. Acute central nesfatin-1 injection inhibits food and intake, as well as fasting-induced neuronal activation in the arcuate nucleus (the primary center of food intake regulation in the brain) in normal, but not in PR rats. Chronic central nesfatin-1 infusion reduces bodyweight gain, as well as improves glucose tolerance and insulin sensitivity of normal rats only. Our data suggest that hypothalamic nesfatin-1-resistance may be decisive in development of type 2 diabetes in IUGR. We presented preliminary new research data on this topic at several conferences and prepared a manuscript, which has recently been accepted in the Frontiers of Neuroscience.

Nesfatin-1 resistance may underlie development of type 2 diabetes mellitus in maternally undernourished non-obese rats Máté Durst, Katalin Könczöl, Klementina Ocskay, Klaudia Sípos, Anett Szilvásy-Szabó, Péter Várnai, Csaba Fekete and ***Zsuzsanna E. Tóth****. Frontiers of Neuroscience in press. (https://www.frontiersin.org/articles/10.3389/fnins.2022.828571/abstract).*

To understand better the role of nesfatin-1 in the glucose homeostasis, we injected our recently prepared AAV constructs into the ARC of rats, and silenced NUCB2 expression or overexpressed nesfatin-1 in the neurons. Nesfatin-1 overexpression in the ARC improved glucose tolerance measured using intraperitoneal glucose tolerance test, while silencing of NUCB2 expression reduced it. Additionally, the icv insulin elicited phosphoAkt signaling was impaired in the ARC cells after treatment with NUCB2 shRNA. We presented preliminary data on this topic on the IBRO Workshop 2020, and are doing further experiments in order collect more data to prepare a manuscript.

Involvement of nesfatin-1/NUCB2 in the arcuate nucleus in the glucose homeostasis. Máté Durst, Rita Matuska, Péter Várnai, Eszter Soltész-Katona, Miklós Geiszt, ***Zsuzsanna E. Tóth****. IBRO Workshop 2020, Szeged, Hungary.*

We also showed that adult PR rats were hyperphagic, and had higher motivation to eat palatable food then controls. This was interesting for us, since according to an earlier report dopamine 1 receptor (D1R) bearing neurons are responsible to stop food intake through the lateral hypothalamus (O'Connor, Kremer et al. 2015), where MCH and nesfatin-1 neurons colocalize (Vas, Ádori et al. 2013). In further experiments we identified a group of food reward representing dopamine-1 receptor+ (D1R) neurons in the medial shell of the accumbens nucleus. Activation (Fos+) of these neurons was highly proportional to the consumed palatable food. D1R agonist treatment attenuated the intake of palatable food and diminished the number of reward-activated (Fos+) cells in the lateral hypothalamus that is the target area of accumbal D1R neurons. The lateral hypothalamic Fos + cells were non-MCH, non-nesfatin-1/NUCB2, non-orexin neurons. We published our final data in the International Journal of obesity.

Reward-representing D1-type neurons in the medial shell of the accumbens nucleus regulate palatable food intake. Durst M, Könczöl K, Balázsa T, Eyre MD, ***Tóth ZE****. Int J Obes (Lond). 2019 Apr;43(4):917-927. doi: 10.1038/s41366-018-0133-y. Epub 2018 Jun 15. SJR indicator: D1.*

Other related works:

We work in collaboration with many laboratories, trying to find new directions in nesfatin-1 research and using the techniques developed in our laboratory. In collaboration with Prof. Alán Alpár (SE Department of Anatomy, Histology and Embryology) the role of secretagogin, a Ca2+-sensor protein in the brain was investigated. As the C terminal part of the NUCB2 protein also contains Ca2+ binding domains we investigated the morphological basis of an interaction in the hypothalamus. We found only occasional co-expression of secretagogin and nesfatin-1. However, we contributed to a publication on the role of secretagogin in the signaling cascade that provokes structural remodeling of the rostral migratory stream. Our other works/collaborations also resulted several publications in well recognized international journals:

Secretagogin-dependent matrix metalloprotease-2 release from neurons regulates neuroblast migration. Hanics J, Szodorai E, Tortoriello G, Malenczyk K, Keimpema E, Lubec G, Hevesi Z, Lutz MI, Kozsurek M, Puskár Z, ***Tóth ZE****, Wagner L, Kovács GG, Hökfelt TG, Harkany T, Alpár A. Proc Natl Acad Sci U S A. 2017 Mar 7;114(10):E2006-E2015. doi: 10.1073/pnas.1700662114. Epub 2017 Feb 21.*

SJR indicator: D1

Zana M, Péterfi Z, Kovács HA, ***Tóth ZE****, Enyedi B, Morel F, Paclet MH, Donkó Á, Morand S, Leto TL, Geiszt M. Interaction between p22phox and Nox4 in the endoplasmic reticulum suggests a unique mechanism of NADPH oxidase complex formation. Free Radic Biol Med. 2018 Feb 20;116:41-49. doi: 10.1016/j.freeradbiomed.2017.12.031. Epub 2017 Dec 24.* *SJR indicator: Q1*

Disruption of the NOX5 Gene Aggravates Atherosclerosis in Rabbits. Petheő GL, Kerekes A, Mihálffy M, Donkó Á, Bodrogi L, Skoda G, Baráth M, Hoffmann OI, Szeles Z, Balázs B, Sirokmány G, Fábián JR, ***Tóth ZE****, Baksa I, Kacskovics I, Hunyady L, Hiripi L, Bősze Z, Geiszt M. Circ Res. 2021 Apr 30;128(9):1320-1322. doi: 10.1161/CIRCRESAHA.120.318611. Epub 2021 Mar 17.* *SJR indicator: D1*

Angiotensin II-Induced Cardiac Effects Are Modulated by Endocannabinoid-Mediated CB1 Receptor Activation. Zsuzsanna Miklós, Dina Wafa, György L. Nádasy, ***Zsuzsanna E. Tóth****, Balázs Besztercei, Gabriella Dörnyei, Zsófia Laska, Zoltán Benyó, Tamás Ivanics, László Hunyady and Mária Szekeres.* Cells *2021,* 10*(4), 724; https://doi.org/10.3390/cells10040724* *SJR indicator: Q1*

The Same Magnocellular Neurons Send Axon Collaterals to the Posterior Pituitary and Retina or to the Posterior Pituitary and Autonomic Preganglionic Centers of the Eye in Rats. Ágnes Csáki, Katalin Köves, Zsolt Boldogkői, Dóra Tombácz, ***Zsuzsanna E. Tóth****.* NeuroSci *2021, 2(1), 27-44; https://doi.org/10.3390/neurosci2010002*

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Saleem, T., N. Sajjad, S. Fatima, N. Habib, S. R. Ali and M. Qadir (2011). "Intrauterine growth retardation--small events, big consequences." Ital J Pediatr **37**: 41.

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Torterolo, P., C. Scorza, P. Lagos, J. Urbanavicius, L. Benedetto, C. Pascovich, X. López-Hill, M. H. Chase and J. M. Monti (2015). "Melanin-Concentrating Hormone (MCH): Role in REM Sleep and Depression." Front Neurosci **9**: 475.

Vas, S., C. Ádori, K. Könczöl, Z. Kátai, D. Pap, R. S. Papp, G. Bagdy, M. Palkovits and Z. E. Tóth (2013). "Nesfatin-1/NUCB2 as a potential new element of sleep regulation in rats." PLoS One **8**(4): e59809.