KUTATÁS EREDMÉNYEIT ÖSSZEFOGLALÓ RÉSZLETES ZÁRÓJELENTÉS (FINAL REPORT ON THE RESULTS OF THE RESEARCH PROJECT)

Neurogenomic basis of parental behaviour: gene expressional differences in brain on phylogenetic and individual levels

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Recent studies of brain mechanisms of parental behaviours have focused on rodents, where the female is typically the sole care provider. In the framework of the proposal, we investigated brain centres of bird provisioning in both males and females, and compared the data with rodents. We investigated a passerine songbird, the zebra finch (*Taeniopygia guttata*) with biparental care of the offspring. To identify activated neurons, the immediate early gene product, Fos protein, was labelled. Parenting-related neuronal activation was induced by removing temporarily the nestlings first, then re-uniting the focal male or female parent with the nestlings (parental group), whereas control birds were not reunited with the nestlings. Parents showed an increased level of parental behaviour following reunion in both sexes. Offspring-induced parental behaviour-related neuronal activation was found in the preoptic, ventromedial (VMH), and paraventricular hypothalamic nuclei, and the bed nucleus of the stria terminalis. In addition, the number of Fos-ir neurons in the accumbens nucleus predicted the frequency of feeding behaviour. Although males showed an overall more intensive neuronal activation and we found sex-specific activation between different nuclei, these differences were independent of parenting. Our study identified a number of nuclei involved in parental care in birds, and implies similar regulatory mechanisms in males and females. The activated brain regions show similarities to rodents while differences were also identified. Further studies are necessary to establish the role of the avian-specific neuronal activation in the VMH of zebra finch parents (Fazekas et al., 2020).

Subsequently, a gene expressional study was performed using RNA sequencing. The brain regions of zebra finch identified in the previous activational study was dissected from social pairs without nestlings and parents taking care of the nestlings. Previously, we established that the zebra finch brain regions, which contain neurons activated in response exposure to nestlings are located in the hypothalamic and septal regions. Therefore, gene expression in brain tissue blocks containing these parts of the brain were dissected and investigated. Our aim was to separate the influence of lactation and behaviour-related gene expression changes, so we examined the non-lactating bird, zebra finch. We dissected these brain regions on the post hatching day 13 and RNA sequencing was used to perform transcriptomic study. We identified 31622 unigenes in our transcriptome. Among them, 213 genes were well annotated and differentially expressed between the two conditions. In comparison to social pairs, 13 genes were upregulated in breeding pairs with threshold log2fold change of 1.5, while 39 genes were downregulated. The DEGs of breeding pairs were enriched in choline and tyrosine monoxygenase transporter activity, social behaviour, acetylcholine and dopamine biosynthetic processes based on gene ontology and KEGG pathway analysis. The genes expression variation related to the cholinergic and dopaminergic systems suggest that these transmitters might be involved in the control of parental behaviour in the hypothalamic-septal region of zebra finches (Fazekas et al., 2019).

Next, the suckling-related activation pattern was identified in the rat. The c-fos method was applied to identify activated neurons. Fasted rat pups were returned to their mothers for suckling and sacrificed 2 hours later for Fos immunohistochemistry. Double labelling was also performed to characterize some of the activated neurons. For comparison, another group of fasted pups were given dry food before Fos mapping. After suckling, we found an increase

in the number of Fos-immunoreactive neurons in the insular and somatosensory cortices, central amygdaloid nucleus (CAm), paraventricular (PVN) and supraoptic hypothalamic nuclei, lateral parabrachial nucleus (LPB), nucleus of the solitary tract (NTS), and the area postrema. Double labelling experiments demonstrated the activation of calcitonin gene-related peptide-ir (CGRP-ir) neurons in the LPB, corticotropin-releasing hormone-ir (CRH-ir) but not oxytocin-ir neurons in the PVN, and noradrenergic neurons in the NTS. In the CAm, Fos-ir neurons did not contain CRH but were closely apposed to CGRP-ir fiber terminals. Refeeding with dry food-induced Fos activation in all brain areas activated by suckling. The degree of activation was higher following dry food consumption than suckling in the insular cortex, and lower in the supraoptic nucleus and the NTS. Furthermore, the accumbens, arcuate, and dorsomedial hypothalamic nuclei, and the lateral hypothalamic area, which were not activated by suckling, showed activation by dry food. Neurons in a number of brain areas are activated during suckling, and may participate in the signalling of satiety, taste perception, reward, food, and salt balance regulation (Barna et al., 2018).

Suckling contributes to hormonal adaptations to motherhood, which includes oxytocin release and consequent milk ejection. We identified evidence that the posterior intralaminar complex of the thalamus is a major relay nucleus conveying somatosensory information supporting maternal behaviour and oxytocin release in mothers, and may be involved more generally in social cue evoked oxytocin release, too (Dobolyi et al., 2018). The major endocrine hormone regulating parental behaviour is prolactin in both avian and mammalian species even though we identified some differences in its actions depending on the taxa. In particular, prolactin participates in the control of nest building while its behavioural actions in rats may restrict to the initiation of caring behaviour in the peripartum period (Dobolyi et al., 2020). Next, we wanted to separate the prolactin-mediated and the neuronally-mediated actions of nursing. Neurons directly affected by prolactin were visualized by using pSTAT5 immunohistochemistry in relation to Fos-expressing neurons in suckled mother mice. In response to pup exposure following 22-h pup deprivation, we found a markedly elevated number of pSTAT5-containing neurons in several brain regions, including the lateral septum, medial amygdaloid nucleus, subparafascicular area, caudal periaqueductal grey, dorsal raphe, lateral parabrachial nucleus, nucleus of the solitary tract, and the periventricular, medial preoptic, paraventricular, arcuate and ventromedial nuclei of the hypothalamus. Pup exposure also induced Fos expression in all of these brain regions except the arcuate and ventromedial hypothalamic nuclei. Bromocriptine treatment known to reduce prolactin levels eliminated pSTAT5 from most brain regions while it did not affect Fos activation following suckling. The degree of colocalization for pSTAT5 and Fos ranged from 8 to 80% in the different brain regions suggesting that most neurons responding to pup exposure in mother mice are driven either by prolactin or direct neuronal input from the pups, while the number of neurons affected by both types of inputs depends on the examined brain area. In addition, both pSTAT5 and Fos were also double-labelled with estrogen receptor alpha (ERα) in mother mice, which revealed a very high degree of colocalization between pSTAT5 and ERα with much less potential interaction between Fos- and ERα-containing neurons suggesting that estrogen-sensitive neurons are more likely to be affected by prolactin than by direct neuronal activation (Olah et al., 2018).

For the field studies, we purchased the necessary equipment and arranged and the journey and the save storage and transfer of samples. In the summer of 2016, we carried out an expedition for shorebird brain sample collection. Since the expedition of Taymir peninsula was prevented by the unavailability of Russian scientists, we collected birds, specifically lapwing (Vanellus vanellus) and redshank (Tringa totanus) individuals at Turov, Belarus near the Pripyat river in collaboration with scientists of the National academy of Sciences of Belarus (Dr. Natalya Korlianova and Dr. Pavel Pinchuk). Dr. Gergely Zachar participated in

the expedition between 02.06.2016 and 24.06.2016, for which we also gained supplementary travel support from a bilateral grant between the Hungarian and Belarussian Academies of Sciences. In the field, we identified the regions where the species are nesting, and the circumstances are logistically adequate for carry out the brain sampling. We also developed the optimal catching brain sampling and storing methods and adapted it to the local infrastructure. After finding the nests, individuals of both species were caught using mist nets or nest funnels as needed. After collecting the birds, photos were taken to assess plumage patterns and morphology measurements were made to assess sexual dimorphism. No more than 5 minutes after caught, the birds were decapitated, blood collected, and the brain dissected. We obtained good quality tissue, blood samples and precise data on the parental strategy of the observed species, so that they will be suitable for correlations between behavioural, morphological and hormonal variables. We also identified the climatic factors, which will predict the onset and quality of the following breeding season to collect more individuals during the forthcoming breeding seasons if needed.

To compare neurogenomics in birds with rodents, we next investigated the preoptic area with systems biological microarray tools as the major organizing centres of maternal behaviour and lactation are located in the hypothalamic medial preoptic area (MPOA). Insulin-like growth factor I (IGF-I) is an effector of the growth hormone axis; however, its function in the brain is largely unexplored. We identified increased maternal IGF binding protein-3 (IGFBP-3) expression in preoptic rat microarray data and confirmed it by RT-PCR (Leko et al., 2017a, Leko et al., 2017b). *In situ* hybridization histochemistry showed markedly elevated IGFBP-3 expression in the MPOA and the arcuate nucleus in rat dams. Prolonged intracerebroventricular injection of IGF-I or antagonism of brain IGFBP-3 with an inhibitor (NBI-31772) using osmotic minipumps increased pup retrieval time, suggesting reduced maternal motivation. Suckling-induced prolactin release and pup weight gain were also suppressed by IGF-I, suggesting reduced lactation. In addition, IGF-I-induced tyrosine hydroxylase expression and its specific phosphorylation in tuberoinfundibular dopaminergic neurons suppress prolactin secretion (Leko et al., 2017a, Leko et al., 2017b). Thus, IGF-I may inhibit both behavioural and lactational alterations in mothers. Neurons in the MPOA and arcuate nuclei express IGFBP-3 during the postpartum period to neutralize IGF-I effects. IGFBP-3 can prevent the blockade of maternal behaviour and lactation exerted by IGF-I, suggesting a novel modulatory mechanism underlying the behavioural and hormonal effects during central maternal adaptations (Dobolyi and Leko, 2019).

Since novel elements of the brain maternal network have been functionally identified as selective lesion of galanin neurons in the preoptic area of the hypothalamus eliminated, while their stimulation elicited care of the offspring, we hypothesized that molecular changes accompany the identified alterations of neuronal functions. These molecular adaptations were addressed using systems biological tools at the mRNA as well as the proteome level, which led to the identification of maternally altered genes in different parts of the rat brain. Thus, we identified a novel neuropeptide, amylin, which appears in the rodent brain only in the preoptic area of parenting rodents. In line with major differences between parental care systems between mammalian and avian species, amylin was found in several distinct regions of zebra finch brain showing sexually dimorphic distributional pattern. To establish the presence and role of amylin in the bird brain, we investigated the distribution of amylin in brains of adult male and female zebra finches in 3 different reproductive stages (i.e. paired without young, incubating eggs or provisioning nestlings) and in unpaired control birds living in same sex flocks. Amylin mRNA was identified in the hypothalamus of zebra finch by RT-PCR, which was also used to produce probes for *in situ* hybridization. Amylin showed a much wider brain distribution than in rodents. A strong and, in some regions, sexually dimorphic label was found in the striatum and several brain regions of the social behavioural network in both males and females. Many regions

responsible for the learning of birdsong also contained amylin-positive neurons, and some regions showed sex differences reflecting the fact that vocalisation is sexually dimorphic in the zebra finch: only males sing. Area X, a striatal song centre present only in males, was labelled in paired but not unpaired male. Area X. The wider distribution of amylin in birds as compared to rodents suggests a more general role of amylin in social or other behaviours in avian species than in mammals. Alternatively, parental care in birds may be a more complex behavioural trait involving a wider set of brain regions. The sex differences in song centres, and the changes with reproductive status suggest a participation of amylin in social behaviours and related changes in the singing of males (Zachar et al., 2020)

Transcriptome sequencing in rats identified further gene expressional differences related to maternal behaviours. Transcriptome sequencing was first applied in the preoptic region of rat dams in comparison to a control group of mothers whose pups were taken away immediately after parturition and did not exhibit caring behaviour 10 days later. Differentially expressed genes were found and validated by quantitative RT-PCR, among them NACHT and WD repeat domain containing 1 (Nwd1) is known to control androgen receptor (AR) protein levels. The distribution of Nwd1 mRNA and AR was similar in the preoptic area. Therefore, we focused on this steroid hormone receptor and found its reduced protein level in rat dams. To establish the function of AR in maternal behaviour, its antagonist was administered intracerebroventricularly into mother rats and increased pup-directed behaviour of the animals. AR levels are suppressed in the preoptic area of mothers possibly mediated by altered Nwd1 expression in order to allow sustained high level care for the pups. Thus, our study first implicated the AR in the control of maternal behaviours (Leko et al., 2021).

To establish the protein level changes in mothers, and to compare them to those of mRNA level changes, we applied proteomics to compare protein level changes associated with motherhood in the rat preoptic area. Using 2-dimensional fluorescence gel electrophoresis followed by identification of altered spots with mass spectrometry, 12 proteins were found with significantly increased, and 6 proteins with significantly reduced level in mothers. Gene ontological analysis suggested that most altered proteins are involved in glucose metabolism and neuroplasticity. These proteins may support the maintenance of increased neuronal activity in the preoptic area and morphological changes in preoptic neuronal circuits known to take place in mothers. Increase in the level of *alpha-crystallin B chain* (Cryab) was confirmed with Western blotting, too. This small heat shock protein may also contribute to maintaining the increased activity of preoptic neurons by stabilizing protein structures. Common regulator and common target analysis of the altered proteins suggested a role of prolactin in the molecular changes in the preoptic area. The results first identified the protein level changes in the maternal preoptic area. The altered proteins contribute to the maintenance of maternal behaviours and may also be relevant to *postpartum* depression, which can occur as a molecular level maladaptation to motherhood (Udvari et al., 2019).

In addition to whole tissue homogenates, synaptic proteins were also compared between mothers and control animals. We isolated synaptosome fractions from the hypothalamus of mother rats at the 11th *postpartum* day. Non-maternal dams deprived of their pups immediately after parturition were the control group. Proteomic analysis by two-dimensional differential gel electrophoresis combined with mass spectrometric protein identification established 26 significant proteins: 7 increasing and 19 decreasing protein levels were found in maternal dams in comparison with control animals. The altered proteins are mainly involved in energy homeostasis, protein folding, and metabolic processes suggesting the involvement of these cellular processes in maternal adaptations. Bioinformatical network analysis revealed that cytokines, growth factors, and protein kinases are common regulators of the altered proteins, which indicates a complex regulation of the proteome change in mothers. The results suggest that maternal responsiveness is associated with synaptic proteins level

changes in the hypothalamus, and, that growth factors and cytokines may govern these alterations (Udvari et al., 2017).

One of the proteins identified to have reduced level in the maternal hypothalamus is Complement component 1q subcomponent binding protein (C1qbp), a multifunctional protein involved in immune response, energy homeostasis of cells as a plasma membrane receptor, and a nuclear, cytoplasmic or mitochondrial protein. Therefore, we addressed to identify C1qbp in the rat brain using *in situ* hybridization histochemistry and immunolabelling at light and electron microscopic level. C1qbp has a topographical distribution in the brain established by the same pattern of C1qbp mRNA-expressing and protein-containing neurons. Double labelling of C1qbp with the neuronal marker NeuN, with the microglia marker Iba1, and the astrocyte marker S100 demonstrated the presence of C1qbp in neurons but not in glial cells in the normal brain, while C1qbp appeared in microglia following their activation induced by focal ischemic lesion. Only restricted neurons expressed C1qbp, for example, in the PVN, magnocellular neurons selectively contained C1qbp. Further double labelling by using the mitochondria marker Idh3a antibody suggested the mitochondrial localization of C1qbp in the brain, confirmed by correlated light and electron microscopy at 3 different brain regions. Post-embedding immunoelectron microscopy also suggested uneven C1qbp content of mitochondria in different brain areas but also heterogeneity within single neurons. These data suggest a specific function of C1qbp in the brain related to mitochondria, such as the regulation of local energy supply in neuronal cells (Barna et al., 2019).

The medial prefrontal cortex (mPFC) as the uppermost cortical centre of maternal control and the associated mood changes was also investigated in mothers using proteomics. Proteomic differences between rat dams and control mothers deprived of their pups immediately after delivery were examined. A 2-D DIGE minimal dye technique combined with LC-MS/MS identified 32 different proteins that showed significant changes in expression in the mPFC, of which, 25 were upregulated and 7 were downregulated in dams. The identity of one significantly increased protein, the small heat-shock protein alpha-crystallin B chain (Cryab), was confirmed via Western blot analysis. The elevation of its mRNA level in rat dams was also demonstrated via RT-PCR. Alpha-crystallin B chain was distributed in scattered cells in the mPFC, as demonstrated by immunohistochemistry. Using double labelling, it was found to be localized in parvalbumin-containing neurons, which, as fast-spiking interneurons, are associated with depression. The function of alpha-crystallin B chain should be further investigated to establish whether it can be used to identify drug targets for future drug development. The high number of protein-level alterations found between mothers taking care of their litter and those without pups indicates that pup nursing is associated with cortical protein-level changes. The functional classification of the altered proteins was conducted using the UniProt and Gene Ontology protein databases. The identified proteins predominantly participate in synaptic transport and plasticity, neuron development, oxidative stress and apoptosis, and cytoskeleton organization. Alterations in proteins participating in synaptic transport, plasticity and neuron development suggest neuroplastic changes in the maternal brain. A common regulator and target analysis of these proteins determined using the Elsevier Pathway Studio Platform suggests that protein level changes associated with pup nursing are driven by growth factors and cytokines, while the MAP kinase pathway was identified as a common target. A high proportion of the proteins that were found to be altered in the mPFC are associated with depression suggesting that the physiological effects of the protein-level alterations in the maternal mPFC could promote the incidence of postpartum depression (Volgyi et al., 2017).

Maternal behaviour appears in rodents in response to pup exposure even in the absence of hormones. Therefore, we addressed maternally activated neuronal inputs to the preoptic area and identified a posterior thalamic region, which may relay somatosensory information

from the pups to the mother to control maternal responsiveness (Cservenak et al., 2017a, Cservenak et al., 2017b). A neuropeptide, TIP39 is induced in the thalamic neurons of mother rats projecting to the hypothalamus. Synaptic innervation of oxytocin and galanin neurons by TIP39-containing nerve terminals has also been demonstrated suggesting that somatosensory neuronal input reaches these cells and may participate in the control of maternal behaviours (Cservenak et al., 2017a, Cservenak et al., 2017b). The preoptic area is known to be responsible to control maternal behaviours. We demonstrated that it is the GABAergic cells in this area, which is involved in control of caring behaviour (Dimén et al., 2019). Furthermore, increase of the activity of these neurons concomitantly increased depression-like behaviours as well, a painfully known problem in a too big (10-15%) portion of human mothers.

To validate molecular components of the parental network in the preoptic area, the neuromodulator system consisting of parathyroid hormone 2 receptor (PTH2R) and its ligand, tuberoinfundibular peptide of 39 residues (TIP39) was investigated as far as preoptic functions. Both receptor and ligand are abundant in preoptic and hypothalamic regions important in maternal behaviour and thermoregulation, and TIP39 also highly increases its gene expression level in mother rodents. Here, we addressed if the PTH2R contributes to the lactational hyperthermia and altered behaviours during motherhood. The core body temperature (T_c) of PTH2R knockout mice (KO) and their wild type (WT) littermates was continuously recorded via telemetric device in virgin, pregnant and lactating stages. During lactation we also conducted the following behavioural tests: pup retrieval to the nest, observation of undisturbed maternal behaviour, forced swimming, and open field test. T_c showed circadian rhythms in both the KO and WT at all stages. In virgin mice, we found no significant difference between genotypes in temperature or locomotion. Except for the virgin stage, we found significant differences between the T_c of KO and WT animals, WT temperature being higher despite the increased locomotor activity in KO mice. KO mothers spent more time out of the nest in a one hour undisturbed observation, and showed higher locomotor activity in the open field test, otherwise the maternal behaviours of the two groups did not differ. We found that in the forced swimming test, KO mothers engaged significantly more time with floating and less with struggling than WT animals. We concluded that the PTHR2R is an important contributor of elevated core body temperature during the lactation period. This finding is consistent with previous literature on the heat-inducing role of PTH2R in male mice and also with the marked increase in the gene expression level of TIP39 in the postpartum period in mothers. Furthermore, mice lacking the PTH2R demonstrate depression-like behaviours without major alteration in other behavioural tests suggesting the selective involvement of the PTH2R in postpartum depression (Gellen et al., 2017b).

Maternal neglect leads to abnormal behaviour of the offspring including depression-like behaviour in adulthood. As a model, neonatal rodents were treated with clomipramine and showed depression-like behaviour, which persisted throughout their adulthood. We described protein expression changes in the prefrontal cortices of neonatally clomipramine-treated adult rats correlating with behavioural abnormalities. Clomipramine was subcutaneously administered into rat pups between postnatal days 8-21, while controls received saline injections. Behavioural tests were made on 3 months old rats and animals were selected for the proteomic experiment according their behavioural performances. The proteomic study was conducted using two-dimensional differential gel electrophoresis (2-D DIGE). We found significant changes in 35 protein spots and 32 proteins were identified by mass spectrometry analysis. The altered proteins are related to biological functions, such as inflammation, transcription, cell metabolism and cytoskeleton. The macrophage migration inhibitory factor (MIF) showed the largest difference in expression, which was confirmed with Western blot. Subsequently, the localization of MIF was investigated in the forebrain of rats with immunohistochemistry. MIF showed a widespread distribution and was predominantly

expressed in astrocytes. We concluded that neonatal clomipramine exposure induces sustained modification in protein expression, which may form the molecular basis of the observed depression-like behaviour in adult rats. Also our data support the idea that the alteration of early development of the brain by drugs could result in sustained pathological changes in the cellular phenotype in the prefrontal cortex leading to behavioural symptoms (Gellen et al., 2017a).

A major symptom of depression is the disturbance of sleep in mothers. Therefore, sleep and local field potential characteristics were addressed during the reproductive cycle in female rats using long-term (60-70 days) recordings. Changes in homeostatic sleep regulation was tested by sleep deprivation (SDep). The effect of mother-pup separation on sleep was also investigated during the postpartum (PP) period. First half of the pregnancy and early PP period showed increased wakefulness (W) and higher arousal indicated by elevated beta and gamma activity. Slow wave sleep (SWS) recovery was suppressed while REM sleep replacement was complete after SDep in the PP period. Pup separation decreased maternal W during early-, but increased during middle PP. More W, less SWS, higher light phase beta activity but lower gamma activity was seen during the post-weaning oestrus cycle compared to the virgin one. Maternal sleep can be governed by the foetuses/pups needs and their presence, which elevate W of mothers. Complete REM sleep- and incomplete SWS replacement after SDep in the PP period may reflect the necessity of maternal REM sleep for the offspring while SWS increase may compete with W essential for maternal care. Maternal experience may cause sleep and LFP changes in the post-weaning oestrus cycle (Toth et al., 2020).

EEG abnormalities were sometimes recorded in mothers. To address this issue, we investigated epilepsy-prone mother rats. Absence epileptic activity was analysed during pregnancy, the postpartum period and after weaning to establish alterations of seizures throughout the reproductive cycle. Wistar Albino Glaxo Rijswijk (WAG/Rij) rats were used in the study as a model of absence epilepsy and because their seizures do not interfere with rearing offspring. The number of spike-wave discharges (SWDs) was gradually elevated from the 19th pregnancy day to delivery. Meanwhile, the characteristics of individual SWDs did not change suggesting that SWD generation remained the same. In the postpartum and postweaning periods, the number of SWDs was not increased in the absence of pups. However, returning the pups to mothers resulted in a markedly elevated number of SWDs for 1 h. If pups were taken away after 30 min, the number of SWDs dropped suggesting that the presence of pups increased the SWD number. The time mothers spent with the litter and in kyphosis suckling posture were in correlation with their SWD number further suggesting the importance of interaction with pups in SWD induction. Suckling elevates prolactin levels but surprisingly, its intracerebroventricular injection markedly reduced SWD number in suckled WAG/Rij mothers suggesting that the SWD-inducing effect of suckling is not mediated by prolactin. Rather, the elevated prolactin level may provide some protection against pro-epileptic effects of suckling. In conclusion, we first identified periods within the reproductive cycle with increased absence epileptic activity, implying that more attention should be devoted to epileptic activity changes in mothers (Lakatos et al., 2016, Kovacs et al., 2017).

Finally, the rewarding value of pups was addressed in mothers as a functional consequence of adaptation. Its central is located in the reward circuit including the ventral tegmental area and the accumbens nucleus. Deoxynivalenol (DON), a trichothecene mycotoxin was used as an experimental tool. Pup retrieval latencies were markedly increased by DON administration, and DON-treated mother rats spent less time with nursing suggesting reduced maternal motivation. In a supplementary control experiment, DON did not induce conditioned place preference arguing against its addictive or aversive actions. To address, which neurons can be affected by DON, the neuronal activation pattern following intraperitoneal injection of DON (1 mg/kg) was investigated in adult male rats and the results were confirmed in mice, too. DON-induced neuronal activation was assessed by c-Fos

immunohistochemistry. DON injection resulted in profound c-Fos activation in only the elements of the reward system, such as the accumbens nucleus, the medial prefrontal cortex, and the ventral tegmental area. Further double labelling studies suggested that GABAergic neurons were activated by DON treatment. The results imply that acute uptake of the DON can influence the reward circuit of the brain and exert inhibitory actions on goal-directed, reward-driven behaviours. In addition, the results also suggest that DON exposure of mothers may have specific implications (Csikos et al., 2020).

Publications of the Results of the Project

- All papers acknowledge NKFIH OTKA K116538
- The 24 publications consists of 19 original research articles, 3 review papers and 2 as yet unpublished conference abstracts
- The PI is corresponding first or last author in 22 out of 24 publications
- Among the 22 already published articles there are 10 D1, 9 Q1, and 3 Q2 papers.

1. Barna J, Dimen D, Puska G, Kovacs D, Csikos V, Olah S, Udvari EB, Pal G, Dobolyi A (2019) Complement component 1q subcomponent binding protein in the brain of the rat. Sci Rep 9:4597. (IF: 4.011, D1)
2. Barna J, Renner E, Arszovszki A, Cservenak M, Kovacs Z, Palkovits M, Dobolyi A (2018) Suckling induced activation pattern in the brain of rat pups. Nutr Neurosci 21:317-327. (IF: 3.950, D1)
3. Cservenak M, Keller D, Kis V, Fazekas EA, Ollos H, Leko AH, Szabo ER, Renner E, Usdin TB, Palkovits M, Dobolyi A (2017a) A Thalamo-Hypothalamic Pathway That Activates Oxytocin Neurons in Social Contexts in Female Rats. Endocrinology 158:335-348. (IF: 3.961, Q1)
4. Cservenak M, Kis V, Keller D, Dimen D, Menyhart L, Olah S, Szabo ER, Barna J, Renner E, Usdin TB, Dobolyi A (2017b) Maternally involved galanin neurons in the preoptic area of the rat. Brain Struct Funct 222:781-798. (IF: 4.231, D1)
5. Csikos V, Varro P, Bodi V, Olah S, Vilagi I, Dobolyi A (2020) The mycotoxin deoxynivalenol activates GABAergic neurons in the reward system and inhibits feeding and maternal behaviours. Arch Toxicol 94:3297-3313. (IF: 5.66, D1)
6. Dimén D, Puska G, Sipos E, Zelena D, Dobolyi A (2019) Chemogenetically influenced GABAergic neurons in the preoptic area of mice affect maternal behaviours. 16th Meeting of the Hungarian Neuroscience Society 77.
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10. Fazekas EA, Morvai B, Udvari EB, Maher K, Zachar G, Urrutia A, Székely T, Pogány A, Dobolyi A (2019) Parenting-related gene expressional changes in the brain of female zebra finches. 16th Meeting of the Hungarian neuroscience society Abstract Number:MITT-79.
11. Fazekas EA, Morvai B, Zachar G, Dora F, Szekely T, Pogany A, Dobolyi A (2020) Neuronal activation in zebra finch parents associated with reintroduction of nestlings. J Comp Neurol 528:363-379. (IF: 3.21, D1)

12. Gellen B, Volgyi K, Gyorffy BA, Darula Z, Hunyadi-Gulyas E, Baracskay P, Czurko A, Hernadi I, Juhasz G, Dobolyi A, Kekesi KA (2017a) Proteomic investigation of the prefrontal cortex in the rat clomipramine model of depression. J Proteomics 153:53-64. (IF:3.722, Q1)
13. Gellen B, Zelena D, Usdin TB, Dobolyi A (2017b) The parathyroid hormone 2 receptor participates in physiological and behavioral alterations of mother mice. Physiol Behav 181:51-58. (IF: 2.517, Q1)
14. Kovacs Z, Lakatos RK, Barna J, Dobolyi A (2017) Absence epileptic activity in Wistar Albino Glaxo Rijswijk rat mothers. Brain Res 1657:368-376. (IF: 3.125, Q1)
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