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1. Introduction

The hypothalamus, the highest center and main cross road of numerous reproductive and homeostatic regulating processes, is located at the basal part of the central nervous system (CNS). The properties through which the hypothalamus is able to orchestrate all these functions make it an anatomically “overcrowded” brain structure. It has been known for higher brain areas that the two sides (hemispheres) usually have distinct physiological functions providing a solution for the “ergonomic” use of brain resources. Although the hypothalamus is also a morphologically symmetric brain structure, so far it has been considered as an unpaired midline structure in which the two sides regulate the same biological functions with an equal share.

One of the most important, at the same time most complicated, role of the hypothalamus is the control of cyclic female reproductive functions. Pathways controlling female reproductive life converge to the left and right hypothalamic sides. Interestingly, both sides, like in case of other hypothalamic regulatory processes like-named neuron populations (e.g.: gonadotrophin releasing hormone, kisspeptin, neuropeptide Y containing neurons) that integrate the same peripheral and central reproductive signals.

The main neuronal populations regulating food-intake and energy homeostasis are also located in the hypothalamus. The orexigenic (increasing hunger and food-intake) and anorexigenic (decreasing hunger and food-intake) neurons on both hypothalamic sides form complex circuitries, known as the melanocortin system. The identical neurons of the melanocortin system on both hypothalamic sides are believed to be able to react to the same peripheral metabolic signals.

The structure described above renders the hypothalamus a morphologically symmetric brain area, where both sides are equipped with the same sets of receptors, therefore they are able to regulate exactly the same biological functions

It has been established for some time that the left and right sides of the nervous system are specialized to the regulation of certain specific, but distinct functions. Along with these findings, asymmetry of the neuroendocrine hypothalamus has also been indicated. The first studies were published more than 40 years ago; however, those studies seem to have been discontinued and hence, the exact nature and function of this phenomenon is still unknown.

Most of these early studies examined the asymmetry of female reproductive processes. Based on the multiple roles of estrogen (main reproductive hormone, anorexigenic signal, trophic hormone), and the identified existence of unilateral feeding pathways that connect hypothalamic structures to brain areas with asymmetric functions, the hypothalamic regulation of food-intake may be just as lateralized as the regulation of reproductive functions.

Hypothalamus-driven homeostatic functions are considerably energy-dependent and therefore rely on mitochondrial ATP-production: mitochondrial ATP production is crucial in the supply of the hypothalamic energy needs and plays a permissive role in the regulation of the intensity of all energy consuming cellular processes. The regulated mitochondrial respiration correlating with the actual cellular energy consumption offers the method of measuring mitochondrial respiration rates to directly indicate the intensity (and changes in intensity) of overall functions in hypothalamic regions that are involved in the regulation of homeostatic processes.

2. Significance and aims of the study

In our modern life, a considerable part of human population is affected by certain kinds of reproductive or metabolic disorders. The hypothalamus, as the main integrator of peripheral and central reproductive and metabolic signals, plays a crucial role in keeping the physiological homeostasis that, if disturbed, results in the above disorders. Therefore, understanding the hypothalamic functions are of utmost importance that will help us develop better medical approaches in the above diseases.

We hypothesize that, in the morphologically symmetric hypothalamus, the like-named nuclei on the left and right sides have distinct roles. The dominance of one of the hypothalamic sides over the other could provide a much more precise and adequate adjustment to the peripheral signals by determining one single set-point in the regulation, furthermore, it could provide a solution for a more “ergonomic” use of hypothalamic resources.

Aims of this work were:

Ad1 To investigate whether there is a detectable metabolic asymmetry in the hypothalamic regulation of female reproduction (1).

Ad2-3 If **Ad1** is correct, to investigate whether the hypothalamic asymmetry related to female reproduction is estrogen-related (2), and whether this asymmetry also applies to those functions that are partly or entirely involved in the hypothalamic regulation of feeding (3)**.**

Ad4-5 To investigate whether there is a gonad-dependent asymmetry in the hypothalamic regulation of reproduction (4) and food-intake (5) in male rats.

Ad6-7 To analyze the differences in the hypothalamic regulation of reproduction and food-intake between male and female rats (6), and investigate gender-related energetics of hypothalamic mitochondria (7).

3. Materials and methods

Intact and gonadectomized, Wistar rats (*Rattus norvegicus*, breed: Crl:[WI]BR) were used to examine the effects of satiety states and presence or absence of gonadal steroids.

In experiment 1, we examined normal cycling female rats. The estrous phase of animals was determined immediately after sacrifice by vaginal smears in order to avoid hormonal influence caused by mechanical stimuli of the cervix.

In experiment 2, gonadectomized females were divided into estrogen-injected, or sham injected groups. Since the most prominent effect on mitochondrial metabolism in the hypothalamus was registered between 8-10 hours after estrogen treatment, subcutaneous injections were performed 10 hours before sacrifice. Both groups were further divided into *ad libitum* fed and fasted (24 hour long food-deprivation) subgroups.

In experiment 3, intact and castrated male animals were used to examine the effect of testosterone. Before the experiment, the animals were randomly separated into two subgroups, one of them remained *ad libitum* fed, while the other was fasted for 24 hours before sacrifice with constant water supply.

In all experimental animals, mitochondrial fractions (containing both perikaryal and synaptosomal mitochondria) were obtained from the separated left and right hypothalamic sides, then mitochondrial oxygen-consumption was measured. Results separately gained form the left and right hypothalamic sides of individuals were compared to each other. After comparison, we also identified the more active side of the individual (i.e. left or right sided metabolic dominance). As statistical analyses, Fisher's exact test was applied to evaluate sidedness, and two-way ANOVA with Bonferroni posttests to compare degree of asymmetry between groups.

4. Results and Discussion

The first findings that indicated hypothalamic sidedness were published more than 40 years ago; however, those studies seem to have been discontinued and hence, the exact nature and function of this phenomenon is still unknown.

Asymmetry in female hypothalami

Here, we demonstrate new results indicating a complex mechanism by which the left and right hypothalamic sides are able to regulate different homeostatic and reproductive processes in an asymmetric manner. In intact, normal cycling rats (experiment 1) and *ovx* rats (experiment 2), we found that an estrous phase- and estrogen-dependent metabolic lateralization exists in the cyclic regulation of female reproduction. This lateralization shows a right sided dominance, and according to the literature it might be related to the asymmetric production of gonadotrophin releasing hormone, and therefore to the regulation of the ovulation.

Furthermore, we could also describe a lateralized functioning of the hypothalamic feeding centers, and that this lateralization is influenced by estrogen. This finding, together with the existence of unilateral feeding pathways connecting hypothalamic structures to asymmetric brain areas, further support the idea that the food-intake related hypothalamic functions show a lateralized distribution between the two hypothalamic hemispheres in female rats, but the effects are less remarkable than the changes caused by the estrous cycle and estrogen.

Asymmetry in male hypothalami

In male animals (experiment 3), we also examined the left and right hypothalamic sides separately in order to further analyze reproduction- and food-intake related metabolic activities and, at the same time, to compare it with the results from female animals. We found that the function of hypothalamic feeding centers in males is also lateralized, however changes in the reproductive state of the animal did not result in notable alteration in the ratio of sided and not sided individuals.

In *ad libitum* conditions the right hypothalamic hemisphere was metabolically more active, while after 24 hours of food deprivation the left side takes over the metabolic dominance in almost half of the individuals. This is fully in line with our findings in female rats, where fasting balanced the proportion of left and right sided dominance as well, even if satiety state exerted less impact on the overall hypothalamic metabolism compared to the effect of gonadectomy. Fasting was able to abolish the right-sided dominance (at least in case of the applied condition), likely by activating the orexigenic neuronal populations on the contralateral hypothalamic side.

Gender-related differences in hypothalamic metabolism

The last and most interesting aspect of this study is the comparison of the results gained from female versus male animals. This comparison revealed fundamental gender-related differences in hypothalamic metabolic profiles. Hypothalamic asymmetry of females seems to be strongly estrogen-dependent, while male gonadal steroids do not seem to be strong determinants of the hypothalamic lateralization. In contrast to this, in males, it is the regulation of food-intake that elicits higher levels in metabolic changes in the hypothalamus, and influences sided metabolic alterations compared to the effects of heavy changes in gonadal factors; nevertheless, food deprivation resulted in a detectable decrease in the fold difference between the two hypothalamic hemispheres also in estrogen-treated females.

Besides the differences mentioned above between male and female hypothalami, our experiments revealed some other diverse characteristics: estrogen could increase the metabolism unilaterally, but the higher mitochondrial activity was not followed by elevated downregulation of biochemical processes. In contrast to female animals, males showed a further elevated downregulation of biochemical processes in our experiments suggesting that in lack of estrogen the mitochondrion has to adapt itself to the elevated ATP producing activity.

5. Conclusions

The hypothalamus is an anatomically and functionally “overcrowded” brain structure that regulates reproduction and food-intake among many other homeostatic processes. Although it is a morphologically symmetric brain structure, it has been referred to as an unpaired midline structure with the two sides equally and simultaneously regulating the exact same biological functions.

In this study, we demonstrated that the like-named nuclei on the left and right sides of the hypothalamus might have different roles, as it has been discovered and accepted long ago with regard to higher brain areas (cortex, thalamus). It seems that the left and right hypothalamic sides, even though they are able to regulate the same functions, might act on different activity levels to react to homeostatic stimuli that results in a side-linked dominance. Our results suggest that the functions of CNS from the spinal cord to the cerebral cortex are more and more specified to certain functions, and functions show lateralization to different degrees. This evolutionary process of lateralization would provide a much more effective use of brain resources. Based on the functional lateralization that we presented here it seems to be rightful to re-name the hypothalamic sides to hypothalamic hemispheres.

This study changes our current view on the regulation of female reproduction and food-intake, and provides new perspectives for the better understanding of these hypothalamus-driven physiological processes. Also, disturbances of lateralized functions may take part in the pathogenesis of hypothalamus-linked health conditions (infertility, obesity, anorexia nervosa, *etc*.), as it is already indicated in the case of other brain areas.

6. New scientific results

Ad1-2 A predetermined functional sidedness exists in the hypothalamic regulation of female reproduction. This lateralization is showing a right sided dominance, it is strongly estrogen-dependent, and it could be detected by our metabolic screening method.

Ad3 Food-intake related hypothalamic functions are also lateralized in female rats, although it has a milder impact on the hypothalamic sidedness than the reproductive functions.

Ad4 Testosterone (or lack of testosterone) in male animals is not contributing significantly to the hypothalamic asymmetry.

Ad5 Male animal show a strong lateralization in food-intake related hypothalamic regulatory functions. Right side seem to dominate in satiety, while left side dominates in food-deprived (fasting) stages.

Ad6 Female and male hypothalamic regulation of reproduction and food-intake (and probably other functions) show fundamental gender-related differences. Hypothalamic lateralization in males is mostly food-intake related, while in females, reproductive processes seem to have higher impact on the hypothalamic asymmetry, at least under the present experimental conditions

Ad7 Mitochondrial energetics of female and male rats differ radically: in females, estrogen induced elevated mitochondrial activity is not followed by elevated downregulating mechanisms (St4), however, in males, the downregulating processes are further increased after mitochondrial activation.

7. The author's scientific publications

Publications related to the topic of the present dissertation

Full text papers in peer-reviewed journal

Toth I, Kiss DS, Jocsak G, Somogyi V, Toronyi E, Bartha T, Frenyo LV, Horvath TL, Zsarnovszky A (2015) Estrogen- and Satiety State-Dependent Metabolic Lateralization in the Hypothalamus of Female Rats PLOS One 10(9):e0137462 doi: 10.1371/journal.pone.0137462

Toth I, Kiss DS, Goszleth G, Bartha T, Frenyo LV, Naftolin F, Horvath TL, Zsarnovszky A (2014) Hypothalamic Sidedness in Mitochondrial Metabolism: New Perspectives. Reprod Sci 21:1492–1498.

Toth I, Goszleth G, Frenyo LV (2012) A táplálékfölvétel fő szabályzó molekulái: a grelin, a leptin és azok kölcsönhatása: Irodalmi összefoglaló Magyar Állatorvosok Lapja 134:(8) 504-512

Poster and oral presentations on international conferences:

Toth I, Szabo Cs, Jocsak G, Somogyi V, Kiss DS, Bartha T, Frenyo LV, Zsarnovszky A (2015) Impact of satiety state and testosterone on the metabolic lateralization in the male rat hypothalamus - MITT (2015) Budapest (poster presentation)

Toth I, Kiss DS, Jocsak G, Bartha T, Frenyo LV, Zsarnovszky A (2014) A possible way to decrease "crowdedness" through functional asymmetry in the hypothalamus FEPS Budapest (poster presentation)

Toth I, Kiss DS, Jocsak G, Bartha T, Frenyo LV, Zsarnovszky A (2014) Metabolic lateralization in the hypothalamus: possible mechanism for cramming multiple functions into a small place FENS Milano (poster presentation)

Kiss DS, **Toth I**, Jocsak G, Bartha T, Frenyo LV, Zsarnovszky A (2014) Mitochondrial metabolic lateralization in the rat hypothalamus MITT-IBRO Debrecen (poster presentation)

Zsarnovszky A, **Toth I**, Scalise TJ, Somogyi V, Kiss DS, Gyorffy A, Goszleth G, Bartha T, Frenyo LV (2012) Possible hypothalamic laterality in the central regulation of GnRH release: thoughts that might lead to a novel approach in hypothalamic studies IBRO Szeged (poster presentation)

Oral presentations on Hungarian national conferences:

Kiss DS, **Toth I**, Jocsak G, Goszleth G, Bartha T, Frenyo LV, Zsarnovszky A (2015) A jóllakottság és tesztoszteron hatása a hypothalamus metabolikus féloldaliságára hím patkányban Akadémiai beszámoló (oral presentation)

Toth I, Kiss DS, Bartha T, Frenyo LV, Zsarnovszky A (2014) Tápláltság-függő mitokondriális metabolikus aszimmetria patkányok hypothalamus-féltekéinek sejtjeiben. Akadémiai beszámoló (oral presentation)

Toth I, Somogyi V, Gyorffy A, Goszleth G, Zsarnovszky A (2013) Biszfenol A, arzén és zearalenon hatása fejlődő patkány kisagysejtjeinek ösztrogén- és pajzsmirigyhormon receptor mRNS-ének expressziójára. Akadémiai beszámoló(oral presentation)

Kiss DS, **Toth I**, Goszleth G, Somogyi V, Bartha T, Frenyo LV, Zsarnovszky A (2013) A mitokondriális metabolizmus hypothalamicus aszimmetriája. Akadémiai beszámoló (oral presentation)

Toth I, Kiss DS, Somogyi V, Goszleth G, Bartha T, Frenyo LV, Zsarnovszky A (2012) A hipotalamikus GnRH szekréció unilaterális szabályozása. Akadémiai beszámoló (oral presentation)

Publications not related to the topic of the present dissertation

Somogyi V, Horvath TL, **Toth I**, Bartha T, Frenyo LV, Kiss DS, Jocsak G, Kerti A, Naftolin F, Zsarnovszky A (submitted) Influence of bisphenol A on thyroid hormone receptors in rat cerebellar cell culture. J Expo Sci Environ Epidemiol

Somogyi V, Horvath TL, **Toth I**, Bartha T, Frenyo LV, Kiss DS, Jocsak G, Kerti A, Zsarnovszky A (2015) Glial modulation of estrogen receptor beta mRNA expression is influenced by individual and combined effects of bisphenol A, zearalenone, arsenic and camphor in the developing rat cerebellum. - MITT (2015) Budapest (poster)

Somogyi V, Jocsak G, **Toth I**, Kiss DS, Goszleth G, Bartha T, Frenyo LV, Zsarnovszky A, Sterczer A (2015) A hepaticus encephalopathia hatása a fejlődő idegrendszerre: az ösztrogén- és pajzsmirigyhormon-receptorok mrns expressziójának vizsgálata kisagyi sejttenyészeten Akadémiai beszámoló (oral presentation)

Mandoki M, Jocsak G, Somogyi V, Kiss DS, **Toth I,** Bartha T (2014) Use of virtual patients in teaching veterinary physiology at the Faculty of Veterinary Science, Szent István University, Budapest FEPS Budapest (oral presentation)

Jocsak G, **Toth I**, Somogyi V, Kiss DS, Bartha T, Frenyo LV, Zsarnovszky A (2014) Identification of a likely mechanism for endocrine disruption: Effects of bisphenol A on the expression level of estrogen- and thyroid hormone receptors in the developing cerebellum FENS (2014) Milano (poster presentation)

Toth I, Kiss DS, Jocsak G, Bartha T, Frenyo LV, Zsarnovszky A (2014) Effects of Bisphenol A on the regulation of estrogen- and thyroid hormone receptor expression in presence of estrogen and/or thyroid hormones on the developing cerebellum IBRO Budapest – (poster presentation)

Scalise TJ, Gyorffy A, **Toth I**, Kiss DS, Somogyi V, Goszleth G, Bartha T, Frenyo LV, Zsarnovszky A (2012) Ligand-induced changes in oestrogen and thyroid hormone receptor expression in the developing rat cerebellum: a comparative quantitative PCR and Western blot study. Acta Veterinaria Hungarica 60:(2) 263-284. (Peer reviewed article)

Zsarnovszky A, **Toth I**, Scalise TJ, Somogyi V, Gyorffy A, Kiss DS, Goszleth G, Bartha T, Frenyo LV (2012) Analysis of ligand-dependent changes in estrogen receptor- and thyroid hormone receptor mRNA and protein expression in the developing rat cerebellum MÉT (2011) Pecs (poster presentation)

Toth I, Scalise TJ, Gyorffy A, Kiss DS, Somogyi V, Goszleth G, Bartha T, Frenyo LV, Zsarnovszky A (2011) Comparative analysis and functional implications of ligand dependent changes in estrogen- and thyroid hormone receptor expression in the developing cerebellum IBRO, Firenze (poster presentation)

Supervising of DVM theses

Knyihar V: Kisméretű agyszövet-minták mitokondriális metabolizmusának vizsgálati módszere és annak gyakorlati jelentősége, TDK dolgozat, 2012, Supervisors: Kiss DS, **Toth I**

Nyitrai Berdin B.: A hypothalamus funkcionális aszimmetriája, TDK dolgozat, scheduled time: 2015, Supervisors: **Toth I**, Kiss DS

Pope H: Functional asymmetry of the hypothalamus, a literature review, DVM thesis, scheduled time: 2015, Kiss DS, **Toth I**