Description and potential role in rodents' maternal care of amylin expressed in the central nervous system

Ph.D. thesis

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Budapest

2016

I. INTRODUCTION

Amylin, also known islet amyloid polypeptide (IAPP), is a 37-amino acid peptide belongs to the calcitonin peptide family. Amylin and its co-secretion with insulin from the pancreas was identified in 1986. The most established function of serum amylin is the inhibition of food intake stimulated by an increase in blood glucose levels. Pramlintide, an amylin analog, is used as a supplement to insulin in the treatment of both type 1 and type 2 diabetes mellitus. In addition to its role in glucose metabolism, clinical studies in energy metabolism and obesity have shown that amylin agonists could also induce weight loss. Other hormonal actions have been demonstrated both on the cardiovascular system and bone metabolism. The anorectic effect of peripheral amylin acts principally in the circumventricular organs of the central nervous system. Nevertheless, the amylin receptors belongs to the superfamily of G protein-coupled receptors class B known to be present in brain regions which may not available for amylin that has been released in the periphery. The expression of amylin in the central nervous system was described previously in the preoptic area of the hypothalamus of rat dams in our laboratory. According to the distribution of amylin mRNA-expressing neurons within the preoptic area and circumstances of its appearance, the central amylin - contrary to the amylin expressed in the periphery - might be involved in the physiological regulation of some aspects of maternal adaptations.

The preoptic area (POA), which has been named after its relative position to the decussation of retinal axons is situated above the optic chiasma. The sexual size difference in one of its nuclei suggested first that the POA may be involved in the control of reproductive behavior. Later, the male dimorphism in the number and size in rats' preoptic neurons was verified on human samples: this part of the preoptic area in young males is twice as large and contains twice as many cells compared to females. This structurally dimorphic structure is called the 'sexually dimorphic nucleus of the preoptic area' (SDN-POA). The high diversity of neurotransmitters, receptors and neuromodulators expressed in this region suggest that the preoptic area may play an essential role in numerous other control mechanisms. The POA also participates in the control of sexual behavior, in the regulation of the exhibition and maintenance of parental care, and in the neuroendocrine control of reproduction in mammals (except human and primates) through its gonadotropin releasing hormone expression. It also

takes part in thermoregulation, sleep-wake regulation, fluid and electrolyte balance, and in the neural control of urination.

The bed nucleus of the stria terminalis (BNST) – which is densely connected to the preoptic area - is located at the junction of hypothalamus, septum and amygdala, medial to striatum. This area plays a crutial part in sexual behavior. One of its nuclei is sexually dimorphic, similarly to the preoptic area. The BNST has further functions in inter-male aggression, in certain types of anxiety and stress repsonses, and in the control of maternal behavior mediated through the preoptic area.

Maternal behavior is a term for responses or behaviors displayed by the female that specifically support the development and growth of her offsprings. This set of responses is vital for the survival of the species. The expression of maternal behavior in mammals is regulated by the developmental and experiential event. In recent years because of human relevance the maternal care has received considerable research attention: postpartum mood disorders and postpartum depression may have a long-term negative effect on mothers' and off-springs' health and well-being. The relationships between the endocrine and neural systems play key roles in developmental and experiential processes of maternal behavior that affects both the establishment and maintenance of maternal care. Whereas it is commonly considered that the control of maternal behavior has a neuroendocrine basis, a number of hormones and neurochemicals together with sensory cues (olfactory, sensory and tactile inputs from pups) and cortical inputs converge on this neural network to maintain and modulate the expression of maternal care. Several similarities can be observed between human and non-human species regarding the circumstances (hormonal and sensory inputs) and features developing maternal behavior. Maternal behaviors have been extensively studied in the rat for decades, however recent methodological advancements in mice warrant an increasing use of this species in the study of maternal responsiveness. Maternal responsiveness can actually develop in response to pup exposure even in the absence of hormonal stimulation. This process called maternal sensitization results in the onset of sensitized females' maternal-like behavior toward pups. Among several types of tests that measure maternal motivation the conditioned place preference test (CPP) is most useful, while emotional changes in mothers can be well described by using forced swimming test (FST).

II. OBJECTIVES

2.1. Description of the time course and distribution of amylin in mother rats and mice according to the points below:

1. Do amylin mRNA levels change during pregnancy and lactation in the central nervous system of mother rats?
2. Does amylin induction occur in the hypothalamus of mice?
3. Can we also detect amylin immunoreactivity in the preoptic area of mother rats?
4. Is there any change in the level of amylin immunoreactivity in the periphery?

2.2. Investigation of the effects that lead to the induction of amylin in the central nervous system:

1. Is there any effect of ovariectomy and maternal sensitization following ovariectomy on amylin levels in the preoptic area?
2. How do maternal sensitization and fasting influence the central amylin mRNA expression?
3. Does maternal sensitization evoke amylin immunoreactivity in the preoptic area?

2.3. Investigation of activation of amylin neurons in mother rats. We addressed the further questions:

1. Do the amylin immunoreactive neurons show Fos activation following pup exposure?
2. Can the pup exposure activate the amylin mRNA-expressing neurons?

2.4. Examination of the relationship between amylin neurons and TIP39-PTH2R neuromodulator system:

1. How to describe the distribution of amylin-immunoreactive neurons and TIP39-fibers in the preoptic area?
2. Where are amylin-mRNA expressing neurons located compared to PTH2R-positive cells in the preoptic area?
3. Does the lack of PTH2R affect amylin mRNA level?

2.5. How do delivery and maternal sensitization influence maternal motivation and depression-like behavior in mice?

1. Does maternal motivation change during parturition or maternal sensitization?

2. Do delivery or sensitization procedure impact on activation (depression-like behavior) of mice measured by forced swimming test?

2.6. How does the absence of amylin affect the behavior of mother and sensitizated mice?

1. Is there any significant difference in place preference between wild type and amylin knockout mother mice?

2. Does the absence of amylin influence the behavior of mother mice measured by forced swimming test?

III. METHODS

Experiments were carried out according to protocols approved by the Animal Examination Ethical Council of the Animal Protection Advisory Board at the Semmelweis University, Budapest, and meet the guidelines of the Animal Hygiene and Food Control Department, Ministry of Agriculture, Hungary.

Animal experiments and histochemical studies were performed in the Department of Anatomy, Histology and Embryology of the Semmelweis University.

To describe the time course and distribution of amylin induction in the peripartum and postpartum periods in rats and mice we used *in situ* hybridization histochemistry (ISH). We examined the appearance of amylin peptide in the preoptic area and the pancreas using immunohistochemistry.

We used *in situ* hybridization histochemistry, real-time polymerase chain reaction and immunohistochemistry to describe the effect of ovariectomy, maternal sensitization and fasting on amylin induction and amylin immunoreactivity.

Topographical and functional relationship between amylin neurons and TIP39-PTH2R neuromodulator system were investigated using amylin-TIP39 double-label fluorescent immunohistochemistry, X-Gal histochemistry, and the change of amylin mRNA level in the lack of amylin were measured by *in situ* hybridization histochemistry.

We investigated the impact of parturition and maternal sensitization on behavior using conditioned place preference test and forced swimming test.

The maternal sensitization procedure was carried out by exposing female mice to young pups. During the sensitization period, the female rats were exposed to pups for 24 h each day, and female mice were exposed to pups for 2 h on 4 consecutive days.

IV. RESULTS

1. Time course and distribution of amylin induction in mother rats and mice

The elevated in situ hybridization signal of amylin during the postnatal period in rat dams, as well as in mother mice, suggest an elevated expression level of amylin. Our data also provide information on the time course of amylin induction. Amylin mRNA is not induced 1 day before parturition (on the 21st day of pregnancy) but is already present one day after delivery, and becomes even higher by the 9th and 23rd postpartum days. Amylin immunoreactivity also appears in the postpartum but not in the prepartum period. This confirms the specificity of the amylin signal and suggests that the elevation of amylin mRNA is translated into an increased synthesis of amylin peptide. The distribution of amylin mRNA-expressing and amylin-ir neurons was the same in the preoptic area. Therefore, we refer to these cells as amylin neurons. The distribution of amylin neurons was widespread within some but not all parts of the preoptic area. Amylin neurons were confined to the MPN, MPA, and BNSTv, where they were relatively evenly distributed among other types of neurons.

The amylin mRNA was detected not only in mother rats but also in lactating mother mice. The distribution of amylin mRNA expressing neurons and the circumstances leading to amylin induction in mice is the same as for mother rats: induction of amylin expression was observed only after parturition and these amylin-expressing neurons were situated in the MPN, parts of the MPA, and BNSTv. Comparing the maternal behaviour of rats and mice several similarities can be found, which suggest that amylin inducing in homologue areas in similar circumstances has identical role in both species.

The peripheral amylin is known to be stored and released from vesicles in the pancreatic islets of Langerhans, therefore, it is likely that amylin also possesses a regulated vesicular release from terminals of preoptic neurons. Amylin has been shown to be co-expressed with insulin, the most established function of serum amylin is the inhibition of food intake as a satiating hormone. The intensity of amylin immunoreactivity in pancreatic islets appeared similar in lactating mothers and mothers deprived of their pups immediately after parturition, so peripheral amylin may not participate in the control of maternal behavior, in contrast with the preoptic amylin expressed in mother rats, in which case its mRNA level remains elevated as long as the pups are not removed from the dams.

Nevertheless, some peptides (prolactin, oxytocin, and vasopressin) released from fibers terminating in the preoptic area may affect maternal behaviors, other neuropeptides, such as opioids, tachykinins, and corticotropin-releasing hormone are expressed in the preoptic area and however, in a number of additional brain regions, too. Thus, the restricted distribution of amylin expression in the preoptic area is unique among neuropeptides.

2. Effect of ovariectomy, maternal sensitization and fasting on amylin levels in the preoptic area

Since in rats and mice amylin appears in a region taking part in maternal adaptations and in a specific term with endocrine changes namely in postpartum period, there is a suspected role of sexual steroid hormones in the induction of amylin expression. While ovariectomy did not induce amylin mRNA in any part of the preoptic area, maternal sensitization following ovariectomy resulted in an elevated level of amylin mRNA in this region, suggesting the importance of the presence of pups in the induction of amylin and arguing against a role of sexual steroid hormone. These results using by ISH was supported by the quantitative RT-PCR data, which confirmed the induction of amylin mRNA in maternally behaving rats. The distribution pattern of amylin-expressing neurons in maternally sensitized rats was the same as that in mother rats.

As amylin has anorexigenic effect and was previously described as an adiposity hormone, we investigated the effects of maternal sensitization and fasting on amylin mRNA levels in the preoptic area. Fasting had no effect on the amylin mRNA level, in contrast, a significantly increased amylin mRNA level was found by quantitative real-time RT-PCR in maternally sensitized virgin female rats as compared to age-matched control female rats. Examination of the peripheral amylin level of mother rats did not show any difference in the quantity of amylin expression in the islets of Langerhans, no matter if the pups were present or in the absence of them. Therefore, the mechanism leading to amylin induction evolves in two different ways: the presence of amylin expressed in the periphery depends on food intake and the following changes in pancreatic exocrine secretion, while in the preoptic area the pup-exposure occurs the amylin induction.

The elevated amylin expression in maternally sensitized non-lactating nulliparous female rats was shown by RT-PCR. It was also investigated by immunohistochemistry, which demonstrated that the elevation of amylin mRNA is translated into an increased synthesis of

amylin peptide. Furthermore, not only pup-exposure but maternal care toward pups need to be present for the appearance of amylin, as amylin-ir neurons were not detected in the preoptic area of nulliparous rats that show aversive, not maternal behavior despite having been exposed to foster pups. Amylin-ir neurons are also absent in the preoptic area of nulliparous control female rats not exposed to foster pups similarly to female rats not sensitized despite the sensitization procedure.

These expressional data and correlative functional evidence (amylin induction after parturition as well as following maternal sensitization) suggest that amylin is a neuropeptide, which might be involved in the physiological regulation of some aspects of maternal adaptations.

3. Maternal activation of amylin-expressing neurons in the preoptic area

During the investigation of the maternal activation of preoptic amylin neurons c-Fos, an immediate early gene product expressed in activated cells, which was applied as a marker of neuronal activity. Removal of the pups from the mothers for 22 hours resulted in a considerable decrease in the number of Fos-positive neurons in the maternal brain. Fos-ir neurons appeared in response to pup exposure in a number of brain regions, including parts of the preoptic area. Within the preoptic area, Fos-immunoreactive neurons appeared in the medial preoptic nucleus, in parts of the medial preoptic area and in the ventral portion of the bed nucleus of the stria terminalis when the dams were exposed to their pups for 2 h following 22 h of separation. This finding confirmed previously reported expression and distribution patterns of Fos in the preoptic area of mother rats, moreover the distribution of neurons activated by pup exposure was very similar to the distribution of amylin-expressing neurons. Furthermore, double labeling of Fos with amylin immunoreactivity has demonstrated that the most of amylin neurons are activated by pup exposure. The percentage of Fos-positive amylin neurons did not differ between the MPN, MPA, and BNSTv. The co-expression of amylin and c-fos genes in the preoptic area of pup exposed mother rats was also evident based on the double labeling of amylin mRNA and Fos immunoreactivity: intensive Fos immunoreactivity in the preoptic area following pup-exposure was present in almost all of these amylin-expressing neurons, indicating an elevated activity of amylin neurons in postpartum rat dams in this area.

Neurons in the medial preoptic nucleus, and surrounding regions that activated by pup exposure project to different brain regions, including the lateral septum, the bed nucleus of the stria terminalis, the substantia innominata, the amygdala, and several different parts of the hypothalamus including the periparaventricular zone, the ventromedial and arcuate nuclei, and the lateral hypothalamic area, as well as the periaqueductal gray. Some of these projections may contain amylin in maternally behaving rats, which could activate amylin receptors present in these target areas. Therefore, amylin, together with its receptors, is a candidate to form a peptide neuromodulator system in the maternal brain.

The type of pup-related stimulus that activates amylin neurons remains undetermined. The pup-related suckling stimulus, as a major driver of maternal adaptations is a leading candidate, and the input related to the suckling stimulus may also activate the amylin neurons. There is evidence that TIP39 neurons in the posterior intralaminar complex of the thalamus relay the suckling information toward medial hypothalamic sites that regulate prolactin release. This thalamic region projects to the preoptic area, therefore, activation of amylin neurons could occur via posterior thalamic TIP39 neurons relaying suckling information.

4. Topographical and functional relationship between amylin neurons and TIP39-PTH2R neuromodulator system

TIP93 neurons in the intralaminar complex of the thalamus constitute a major input to the medial preoptic area as it was demonstrated by tract-tracing studies. TIP39 neurons in the PIL may affect maternal motivation via projections to the preoptic area. According to induction and appearance in the central nervous system TIP39 is very similar to amylin: TIP39-expressing cells in the brain are restricted to some nuclei and activated by pup-exposure. TIP39 is the only known endogenous high-affinity ligand of the PTH2 receptor. Besides its direct projections to MPA, the presence of its receptor, PTH2R was also described within preoptic region.

To investigate anatomical relationship between amylin-ir neurons and TIP39 fibers arising from PIL double immunohistochemistry was applied in preoptic area of rat dams. The distribution of amylin neurons and TIP39 fibers are very similar within preoptic region: TIP39 fibers - like amylin neurons - are most abundant in MPN, MPA, and the ventral part of BNST. Furthermore, TIP39 terminals closely apposed amylin neurons suggesting their innervation by TIP39 neurons.

The existence of a relationship between amylin and TIP39-PTH2R neuromodulator system is supported by the similar distribution of amylin neurons as ISH and immunohistochemisty and the presence of PTH2R expressing cells suggest. Amylin expression was investigated in mice lacking PTH2 receptor to reveal a functional relationship between amylin and TIP39 usingquantitative *in situ* hybridization histochemistry. The maternal induction of amylin levels was reduced significantly in the absence of PTH2 receptor. Relationship between posterior thalamic neurons containing TIP39- have been suggested to convey suckling information from the spinal cord towards limbic and hypothalamic maternal centers supports the amylin effect on maternal behavior innervated by TIP39 expressing neurons.

5. Effects of parturition and maternal sensitization on behavior in mice

Endocrine and emotional adaptations take place in mothers for a limited period of time to support the offspring. These changes following parturition evoke behavioral changes.

Two tests (conditioned place preference test, forced swimming test) are used to investigate maternal behavior in control (virgin) female, lactating mother, sensitized female mice, and - to exclude the potential effect of the estrous cycle - ovariectomized mice were also examined.

In our CPP experiment, measuring maternal motivation, examined animals could freely choose between a pup-associated cage and a control cage that was not related to any specific conditioned stimului. Mothers spent significantly more time in the pup-associated cage than control females. Moreover, the time mothers spent in the pup-associated cage was also significantly greater than the time they spent in the control cage. Meanwhile, control females had a tendency to spend more time in the control cage than in the pup-associated one. Ovariectomized mice behaved similarly to control females suggested that maternal motivation is dependent on pup experience rather than on ovarian hormones. Sensitized mice spent significantly more time in the pup-associated cage than in the control cage, while the amount of time they spent in the pup-associated cage did not differ from that of the mother mice. Thus, the degree of the preference was similar to the preference observed in mother mice, suggesting that maternal motivation is similarly elevated in sensitized nulliparous female mice.

Another characteristic of mother rodents is reduced anxiety in the postpartum period. Because the effect of motherhood on depression-like behaviors is crucially important but has not been specifically addressed in mice, we have performed a comparison using the forced swim test, the most widely used test of depression-like behavior in animals. Mice were allowed to swim in an open cylindrical container filled with water for 6 minutes, and the amount of time spent in active (struggling, swimming) and passive (immobility, floating) behaviors were measured. Mothers on 8 postpartum day spent significantly more time engaged in active behaviors than female controls, and the time mothers spent in active behaviors was also significantly greater than the time they spent immobile. To investigate the behavior of maternally sensitized mice we observed before that the time spent in active behaviors was not significantly different from the time spent in passive behaviors. In addition, the amount of time spent engaged in each of the different behaviors differed significantly between sensitized females and mothers. In contrast, the behavioral pattern of sensitized mice also similar to that of control females. When mice were ovariectomized to eliminate the potential effects of estrous cycle, a reduced active behavior (compared to passive behavior) was found. However, significance was not as high compared to control and sensitized females.

We made the first report of a preference for the pup-associated cage as opposed to a control cage in mice dams and sensitized female mice, and also we were the ones who provided the first demonstration that motherhood may have an anti-depressant effect. To conclude the results received from using CPP and FST the increases in maternal motivation and behaviors are separate from the constant depression-like behaviors in sensitized mice, suggesting that the sensitization process does not change the emotions of the female mice in the same way as they are altered in mouse dams.

6. Behavioral changes in mother and sensitized mice lacking the gene of amylin

The use of transgenic mouse lines allows gene-level discoveries regarding disturbances of maternal affect, maternal behaviors and increased maternal resiliency. Since our studies suggested a role of amylin in the control of maternal care,, we addressed maternal behavior in transgenic mice that do not express amylin.

The results of CPP tests showed that there was no significant difference between reproductive status (control female *vs.* mother mice) and genotype: the amount of time nulliparous females - naïve to pups - spent in familiar pup-associated cage habituated without pup-exposure did not differ from that of the amylin KO female mice, which did not take the half of the whole test period suggesting no preference for this cage. Furthermore, the time mothers spent in the pup-associated cage, where they were kept with pups for a 4-day-long habituation period, was also significantly greater than the time they spent in the control cage. The preference for pup-associated cage in wild type mother mice was not damaged in lactating mice lacking the gene of amylin, though amylin KO mother mice, similarly to wild type ones, spent significantly more time in the pup-associated cage than genotype-matched KO female mice differ only in reproductive status.

To investigate the effect of the lack of amylin on postmartum emotional alterations, forced swimming test was applied. According to our previous study, it is possible that peripartum hormonal changes or actual suckling is necessary for the reduction of depression-like behaviors, and these emotional changes can manifest in increased activity in FST. Maternally sensitized, nulliparous female mice behaved similarly to the control non-sensitized females: they spent more time engaged in passive behaviors, i.e. control female and sensitized mice showed depression-like behavior. The amount of time spent in passive and active behavior differed significantly between the 3 experimental groups. Amylin KO virgin female mice behaved similarly to the wild type control female: the time spent in passive behaviors was higher than the time spent in active behaviors, so elevated depression-like behavior was found. The absence of amylin had no effect on passive behavior of sensitized female mice in FST either. Moreover amylin KO lactating mother mice, in contrast to wild type ones, exhibited reduced activity, namely, they showed depression-like behavior.

Amylin, induced in the postpartum period, could be involved in the control of emotional and endocrine alterations in mothers. Consequently, it represents a new therapeutic direction to treat dysfunctions associated with motherhood, including postnatal depression.

V. CONCLUSIONS

Our most important novel findings are as follows:

1. An increased expression of amylin is reported in the preoptic area of postpartum rats and mice. Amylin is not induced 1 day before parturition but is already present one day after delivery, and becomes even higher by the 9th postpartum day. Amylin has a restricted distribution in the preoptic area: amylin neurons are distributed only in the medial preoptic nucleus (MPN), dorsolateral to the MPN in parts of the medial preoptic area (MPA) and further dorsolateral to this cell group in the ventral part of the bed nucleus of the stria terminalis. Amylin immunoreactivity also appears in the postpartum but not in the prepartum period, which confirms the specificity of the amylin signal and suggests that the elevation of amylin mRNA is translated into an increased synthesis of amylin peptide.

2. Amylin expression was also elevated in maternally sensitized, non-lactating, nulliparous female rats, but not in response to fasting or ovariectomy arguing against a role of starvation or sexual steroid hormones in the induction of amylin but suggesting the importance of the presence of pups in appearance of amylin.

3. Removal of the pups from the mothers for 22 hours resulted in a considerable decrease in the number of Fos-positive neurons in the preoptic area of mother rats. Upon returning the pups, high density of Fos-immunoreactive neurons was present in the preoptic area in lactating dams. Double immunolabeling revealed that the majority of amylin-ir neurons in the preoptic area were Fos-positive, so amylin neurons were activated in response to pup exposure.

4. Topographical and functional relationship exist between amylin neuron and TIP39-PTH2R neuromodulator systemThe distribution of amylin-containing neurons of mother mice is similar to the distribution of TIP39 fibers and PTH2 receptor expressing neurons in the preoptic area. Furthermore, the level of amylin mRNA in the preoptic area significantly decreased in mother mice lacking PTH2 receptor suggesting a role of the PTH2 receptor in the induction of amylin in the postpartum period.

5. The results of the conditioned place preference test suggest that maternal motivation is increased in postpartum mothers as well as in maternally sensitized female mice. We also

used the forced swim test, the most frequently applied behavioral test in rodents to describe the anti-depression-like behaviors of maternal mice. Anti-depression-like behavior was observed in postpartum mother mice but not in maternally sensitized and control virgin females.

6. The preoptic area induced in postpartum period did not influence maternal motivation measured by CPP, both wild type and amylin knockout mother mice prefered the pup-associated cage. However, amylin knockout mothers display depression-like symptoms in the forced swimming test - similar to control and senzitized mice.

VI. LIST OF PUBLICATIONS

1. Publications related to the thesis

1. **Szabó ER**, Cservenák M, Dobolyi A. (2012) Amylin is a novel neuropeptide with potential maternal functions in the rat. FASEB J, 26: 272-281. **IF: 5,704**

2. **Szabó ER**, Cservenák M, Lutz TA, Gévai L, Endrényi M, Simon L, Dobolyi A. (2015) Behavioral changes in mothers and maternally sensitized female mice. BEHAVIOR, Accepted **IF: 1,230**

2. Abstracts related to the thesis

1. **Szabó ER**, Cservenák M, Dobolyi A (2012) Amylin is a novel neuropeptide activated in the brain of mother rats. IBRO International Workshop 2012, Szeged. Clinical Neuroscience 2012, Volume 65, Supplement 2: P31.

2. **Szabó ÉR**, Cservenák M, Dobolyi Á (2012) Identification of amylin as a novel neuropeptide activated in the brain of mother rats. Joint Meeting of the Hungarian Biophysical Society, Hungarian Physiological Society, Hungarian Society of Anatomists and Hungarian Society of Microcirculation & Vascular Biology, Debrecen. Acta Physiologica 2012, Volume 205, Supplement 690: P43.

3. **Szabó ÉR**, Cservenák M, Domokos D, Dobolyi Á (2012) Identification of amylin as a novel neuropeptide activated in the brain of mother rats. XXI. International Semmelweis Symposium, Budapest. Orvosképzés 2012, Volume 87: 327.

4. Dobolyi A, Cservenak M, Lutz T, Usdin TB, **Szabo ER** (2014) Amylin, induced by suckling in the preoptic area of mothers, may be involved in the control of maternal motivation. 9th FENS Forum of Neuroscience, Milan, Italy. Abstract Number: FENS-1828.

5. **Szabó ÉR**, Cservenak M, Udvari E, Dobolyi Á (2014) Induction of amylin in the preoptic area of lactating dams depends on TIP39-containing posterior thalamic neurons. Meeting of

the Federation of European Physiological Societies (FEPS), Meeting Abstract: P10.10. Acta Physiologica 2014, Volume 211 Supplement 697: 144-145.

6. **Szabó ÉR**, Cservenák M, Lutz TA, Gévai L, Endrényi M, László Simon L, Dobolyi Á (2015) Behavioral changes in mother and maternally sensitized female mice. XV. Biannual Conference of the Hungarian Neuroscience Society (MITT), Budapest, Hungary, Abstract Number: P1/93

7. **Szabó ÉR**, Cservenák M, Udvari E, Dobolyi Á (2015) Induction of amylin in the preoptic area of lactating dams depends on TIP39-containing posterior thalamic neurons. Magyar Anatómus Társaság XIX. Kongresszusa, Szeged, Hungary, Abstract Number: P50.

3. Publication not related to the thesis

1. Vincze C, Pál G, Wappler EA, **Szabó ER**, Nagy ZG, Lovas G, Dobolyi A. (2010) Distribution of mRNAs encoding transforming growth factors-beta1, -2, and -3 in the intact rat brain and after experimentally induced focal ischemia. J Comp Neurol. 18: 3752-70. **IF: 3,774**

2. Cservenák M, **Szabó ÉR**, Bodnár I, Lékó A, Palkovits M, Nagy GM, Usdin TB, Dobolyi A. (2013) Thalamic neuropeptide mediating the effects of nursing on lactation and maternal motivation. Psychoneuroendocrino, 12: 3070-84. **IF: 5,591**

3. Helfferich F, Lourmet G, Szabó ÉR, Boldogkői Zs, Palkovits M. (2016) Facial virus inoculations infect vestibular and auditory neurons in rats. Clin Neurosci, 69: E001-E004. **IF: 0,386**

VII. ACKNOWLEDGEMENTS

First and foremost I'd like to express my utmost gratitude to my supervisor, Dr. Árpád Dobolyi, whose constant support, encouragement and advisement was vital in the accomplishment of this Thesis. I wish to thank his patience and his willingness for help anytime, anywhere. Special thanks for Prof. Miklós Palkovits, my first boss, whose tenacity, precision and love towards neuroscience and research is exemplatory for me to this very day. I'd like to thank associate professor Dr. Alán Alpár for his excellent in-house review of my Thesis, doing so with the highest precision and helpful constructivity.

Special thanks to vice chairman of the Depratment of Anatomy, Histology and Embryology, Dr. Gábor Gerber who kindly let me focus on my research by alleviating my workload at the University for a whole year.

I wish to thank the joyful team of Neuromorphology Laboratory for all their help, support and for cheerful days of work. I am especially grateful to Dr. Melinda Vitéz-Cservenák, Dr. Éva Dobolyiné Renner and Dr. Gabriella Pál for their professional help, to Nikolett Hanák, Szilvia Deák, and Viktória Dellaszéga-Lábas for their technical support, and to Magdolna Toronyay-Kasztner for the administrative assistance. I thank them being not only my collegues, but my friends as well.

Last, but not least I wish to thank my husband, my parents and my siblings for their ever present love and support which I could always rely on.