Examination of the neuronal network regulating satiety using refeeding as a satiety model

Ph.D. Thesis

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INTRODUCTION

The balance between the energy intake and energy expenditure is very tightly controlled by the central nervous system. The brain has to sense the amount and the composition of the consumed food, the actual condition of the adipose depots and the needs of other peripheral organs. For this precise regulatory process, the communication of the brain and the peripheral organs is inevitable. This peripheral organ-brain communication is primarily mediated via the bloodstream and by the visceral sensory nerves. Through the bloodstream, peripheral hormones and metabolites reach the central nervous system. The energy homeostasis-related hormones such as leptin and insulin carry information about the energy stores of the body, while the gastrointestinal hormones like cholecystokinin (CCK) and peptide YY (PYY) mediates information about the consumed food. The primary central target of these circulating signals is the hypothalamic arcuate nucleus (ARC), where two antagonistic neuron groups are located which have pivotal role in the regulation of energy homeostasis. One of these two cell types synthesizes anorexigenic peptides, the α-melanocyte-stimulating hormone (α-MSH), derived by the posttranslational processing of the pro-opiomelanocortin (POMC) prohormone, and the cocaine- and amphetamine-regulated transcript (CART) peptide. The other neuron group has orexigenic role, it produces agouti-related protein (AgRP) and neuropeptide Y (NPY). Both of these peptides stimulate food intake and inhibit energy expenditure. Neurons of the ARC integrate the blood derived information with neuronal signals and transmit this message toward the so called second-order neuronal groups. The melanocortin 4 receptor (MC4-R) plays critical role in the mediation of the effects of the feeding-related ARC neurons, because α-MSH serves as an agonist, while AgRP is an endogenous antagonist of the MC4-R. The mutation of MC4-R results in morbid obesity. The actual state of the mechano- and chemoreceptors located in the gastrointestinal tract and the local effects of gastrointestinal hormones are conveyed to the brain mainly through the vagus nerve. The fibers of the vagus nerve terminate in the nucleus tractus solitarii (NTS). After signal integration, the NTS relays this information toward the forebrain through the ascending brainstem pathways to influence feeding-related brain areas. For example, the activation of glutamatergic neurons of the NTS inhibits the food intake through the NTS parabrachial nucleus (PBN)- central nucleus of the amygdala (CEA) pathway.

The two main parameters of the energy homeostasis, the food intake and the energy expenditure generally regulated simultaneously by the central nervous system, for example, the decrease of food intake is accompanied by the increase the energy expenditure. However, there is a condition, the refeeding of animals after a period of fasting, when the regulation of these two processes are uncoupled. Two hours after the onset of feeding, the animals become sated, terminate the feeding. At this time the neuronal activation marker, the c-Fos protein, appears in the ARC POMC neurons indicating the activation of POMC neurons. However, the energy expenditure does not increase in this early phase of refeeding, it is increased only 24 hours after the start of food intake. Based on this observation, we hypothesized that examination of the activated neuronal groups in this early phase of refeeding helps the better understanding of the mechanisms that results in the development of satiety. Since the two energy homeostasis-related sensor areas of the brain, the ARC POMC neurons and NTS are activated at the same time during refeeding, and the vagus nerve-NTS pathway is known to play pivotal role in the determination of meal size, we hypothesized that the ARC POMC neurons are activated by the vagus nerve-NTS-ARC pathway.

AIMS

Due to the very high incidence of obesity in western countries and the lack of efficient and side effect free therapy, elucidation of the neuronal circuits regulating the food intake has crucial importance. Therefore, the goal of my PhD work was to better understand the neuronal network involved in the development of satiety in refed rats. To reach this goal, our specific aims were:

1. To map the refeeding-activated neuronal groups in the rat brain.
2. To determine whether refeeding-induced activation of POMC neurons in the arcuate nucleus is dependent upon the vagus nerve and/or ascending brainstem pathways.
3. To map the connections of parabrachial nucleus with other refeeding-activated neuronal groups.
4. To map the connections of central nucleus of amygdala with other refeeding-activated neuronal groups.
5. To elucidate the role of the subnuclei of the central nucleus of amygdala in the development of satiety during refeeding.

METHODS

Animals:

The experiments were carried out on adult male Wistar rats (TOXI-COOP KKT, Budapest, Hungary) weighing 270–310 g or Sprague–Dawley rats (230–250 g body weight) purchased from Charles River Hungary Ltd (Isaszeg, Hungary). All experimental protocols were reviewed and approved by the Animal Welfare Committee at the Institute of Experimental Medicine of the Hungarian Academy of Sciences.

Surgical procedures:

Retrograde and anterograde tract tracing

The retrograde tracer cholera toxin β subunit (CTB; List Biological Laboratories, Campbell, CA) and the anterograde tracer, *Phaseolus vulgaris* leuco-agglutinin (PHAL; Vector Laboratories Inc.) was injected by iontophoresis into the region of PBN and CEA. Rats were anesthetized and their head positioned in a stereotaxic apparatus with the bregma and lambda in the horizontal plane. Through a burr hole in the skull, a glass micropipette (17.5-20 μm outer tip diameter) filled with 0.5% CTB in 0.01M PB at pH 8.0 was lowered into the brain at stereotaxic coordinates corresponding to the PBN, and CEA based on the atlas of Paxinos and Watson. The CTB was deposited for 10 min (6 μA (in cases injection to the PBN) and 5 μA (CEA administration) positive current, pulsed on—off at 7s intervals) using a constant-current source. The transport time intervals were 7-10 days.

Vagotomy

Bilateral subdiapharagmatic vagotomy, and sham operation as control was performed on male Sprague–Dawley rats. After anesthesia, the animals were placed in a dorsal recumbent position and a laparotomy was performed to expose the stomach and lower oesophagus at the subdiaphragmatic level. Trunks of the vagus nerve were isolated on the surface of the subdiaphragmatic part of the oesophagus and removed with the connective tissue. Sham surgery was performed in a similar way, except that the vagal trunks were not cut and the connective tissue was not removed. One day after surgery, the vagotomized animals were divided into two groups and the sham-operated animals were divided into three groups. The first group was fasted for 40 hours, whereas the second group was fasted

for 40 hours and then given free access to food for 2 hours before perfusion. The third group of the sham-operated rats was fasted for 40 hours and then refed with the same amount of food that was consumed by the refed, vagotomized rats (pairfed group). At the end of the experiment the animals were transcardially perfused.

Transection of the ascending brainstem pathways

After the anesthesia, a 3mm-wide glass knife was lowered into the brain at the level of mesencephalon, parallel with the coronal plane under stereotaxic guidance. Control animals were operated the same way, although the glass knife was not lowered into the brain (sham-transected animals). After 2 weeks of survival, the animals with transection were divided into two groups: the first group was fasted for 40 hours, whereas the second group and the sham-transected animals were fasted for 40 hours and then given free access to food for 2 hours before perfusion. The effectiveness of the transections were examined by analysis the noradrenerg innervations of POMC neurons.

Chemogenetic activation of CEA subnuclei using hSyn-hM3D(Gq)-mCherry adeno associated virus (AAV) (DREADD virus)

hSyn-hM3D(Gq)-mCherry AAV virus was injected unilaterally into the subnuclei of CEA during stereotaxic surgery by a microinjector. After two weeks, the animals were fasted for 40 hours. 15 minutes before the refeeding the animals were injected ip with CNO (ligand of hM3D(Gq)) and the control animals were treated with saline. One week later, the experiment was repeated as follows: rats that had been injected with CNO in the first experiment received saline injection, and the saline treated rats received CNO injection after 40 hours fasting. The food intake was monitored using TSE PhenoMaster system. One week later, after the 40 hours fasting period, the first injection protocol was repeated with the difference that the animals did not receive food. Two hours after the treatment, the animals were deeply anesthetized and perfused transcardially.

Fasting and refeeding

The food was removed from the cages of rats for 40 hours. During this time, they had free access to water. After the fasting period, standard rodent food was reintroduced to the animals and the rats were allowed to eat *ad lib* for 2 h. At the completion of the refeeding interval, the animals were deeply anesthetized and perfused transcardially.

Immunocytochemistry

At the end of all experiments, the animals were anaesthetized and then perfused transcardially with 150 ml of 4% paraformaldehyde (PFA) in 0.1M phosphate buffer (PB, pH 7.4). The brains were rapidly removed and postfixed in the same fixative for 2 hours and cryoprotected by immersion in 30% sucrose in PBS overnight. Coronal, 25 μm thin sections were cut with a freezing microtome. Free-floating tissue sections were pretreated with 0.5% H_2O_2 and 0.5% Triton X-100 in PBS for 15 min to reduce the endogenous peroxidase activity and increase the permeability of cell membranes, respectively. To reduce nonspecific antibody binding, the sections were treated with 2.5% normal horse serum in PBS for 20 min. The antibodies were diluted in the same solution. The used antibodies, chromogens or fluorochromes are summarized in the Table 1.

Statistical analysis

For the statistical analysis the Statistica 8.0 software was used. The data were described as mean+SEM. T-test was used for comparison of two groups. The statistical examination of three or more groups was performed using one- or two-way ANOVA followed by Newman–Keuls or Tukey HSD *post hoc*, respectively.

Table 1: Summary of antibodies, reagents, fluorochromes and chromogens used for immunohistochemical studies

Detected Antigen	Primary antibody	Detection
c-Fos	rabbit antiserum against c-Fos (Oncogen; 1:10000)	biotinylated-anti-rabbit IgG (Jackson), ABC biotinylated tyramide amplification, Ni-DAB
		Nissl-staining
DBH/ POMC	mouse antiserum against DBH (oncogene; 1:1000)	Alexa 555-conjugated donkey anti-mouse IgG (Invitrogen)
	rabbit antiserum against POMC (Phoenix; 1:2000)	FITC-conjugated donkey anti-rabbit IgG (Jackson)
c-Fos/ POMC	rabbit antiserum against c-Fos (Oncogen; 1:10000)	biotinylated donkey anti-rabbit IgG (Jackson), ABC, biotinylated tyramide amplification, streptavidin-labeled FITC (Vector)
	rabbit antiserum against POMC (Phoenix; 1:2000)	Alexa 555-conjugated donkey anti-rabbit IgG (Jackson)
CTB	goat antiserum against CTB (Listlab; 1:10000)	biotinylated donkey anti-sheep IgG (Jackson), ABC, Ni-DAB
		Nissl-staining
PHAL	goat antiserum against PHAL (Vector; 1:10000)	biotinylated donkey anti-sheep IgG (Jackson), ABC, Ni-DAB
		Nissl-staining
CTB/ c-Fos	goat antiserum against CTB (Listlab; 1:5000)	biotinylated donkey anti-sheep IgG (Jackson), ABC, DAB
	rabbit antiserum against c-Fos (Oncogen; 1:10000)	biotinylated donkey anti-rabbit IgG (Jackson) ABC, Ni-DAB
CTB/ c-Fos/ POMC	goat antiserum against CTB (Listlab; 1:10000)	biotinylated-anti-sheep IgG (Jackson), ABC, biotinylated tyramide amplification, streptavidin-labeled FITC (Vector)
	rabbit antiserum against c-Fos (Oncogen; 1:2000)	FITC-conjugated donkey anti-rabbit IgG (Jackson)
	rabbit antiserum against POMC (Phoenix; 1:2000)	Cy5 donkey anti-rabbit IgG (Jackson)
PHAL/ c-Fos/ HuC/HuD	goat antiserum against PHAL (Vector; 1:5000)	biotinylated donkey anti-sheep IgG (Jackson), ABC, biotinylated tyramide amplification, streptavidin-labeled Alexa 555 (Vector)
	rabbit antiserum against c-Fos (Oncogen; 1:2000)	FITC-conjugated donkey anti-rabbit IgG (Jackson)
	mouse antiserum against HUC/D (Molecular probes; 1:500)	Cy5-anti-mouse IgG (Jackson)
c-Fos/ RFP/ HuC/HuD	rabbit antiserum against c-Fos (Oncogen; 1:10000)	biotinylated-anti-rabbit IgG (Jackson), ABC, biotinylated tyramide amplification, streptavidin-labeled FITC (Vector)
	rabbit anti-RFP (1:3000, Rockland)	Alexa 555-conjugated donkey anti-rabbit IgG (Jackson)
	mouse antiserum against HUC/D (Molecular probes; 1:500)	DyLight 649-conjugated anti-mouse IgG (Jackson)

RESULTS

Distribution of the refeeding-activated neuronal groups in the rat brain

In the forebrain, refeeding-induced the highest increase in the number of c-Fos-containing neurons in the prelimbic area (Prl), medial and laterocapsular subdivisions of the central nucleus of amygdala (CEAm, CEAlc), the bed nucleus of stria terminalis (BST), primarily the medial subdivision including anterior and ventral parts, dorsomedial hypothalamic nucleus (DMN), lateral preoptic area (LPO), the ventral and lateral parvocellular subdivisions of the PVN (PVNv, PVNl) and the parasubthalamic nucleus (PSTN). In the brainstem, a large number of c-Fos-IR neurons was observed in the medial and lateral parts of the PBN, in the medial, intermediate and commissural subdivisions of the NTS and the area postrema (AP). Moderate to weak neuronal activation was found in the somatosensory cortex representing the oral surface, jaw and upper lip region, medial orbital cortex (MOB), lateral olfactory tract, piriform-amygdalar area (Pir), olfactory tubercle, posterolateral cortical amygdaloid nucleus, agranular insular area and secondary motor cortex. Moderate neuronal activation was seen in the amygdaloid complex in the medial nucleus of amygdala and the cortical amygdaloid nucleus and in the diencephalon including the anterior hypothalamic area (AH), lateral hypothalamic area (LH), lateral and dorsomedial posterior arcuate nucleus (dmpARC), zona incerta (ZI), paraventricular thalamic nucleus (PVT) and paratenial thalamic nucleus. In the metencephalon there was also a redistribution in the pattern of c-Fos-containing neurons in the ventrolateral part of the periaqueductal grey (PAGvl). In the fasted rats, the c-Fos containing nuclei were concentrated in the lateral part of PAGvl, while the number of c-Fos-IR nuclei decreased in this region and a diffuse neuronal activation was apparent in the lateral and ventrolateral periaqueductal grey (PAGvl). Nevertheless, intense neuronal activation was seen in the fasted rats in the nucleus reuniens, midbrain reticular nucleus and pontine gray. Moderate to weak activation was found in fasted rats compared to the refed rats in the lateral septal nucleus.

Activation of anorexigenic pro-opiomelanocortin neurons during refeeding is independent of the inputs mediated by the vagus nerve and the ascending brainstem pathways

Food and water intake

Sham-vagotomized animals consumed significantly more food (Sham *vs.* Vagotomized 2-h food intake: 8.1 ± 0.7 g *vs.* 2.7 ± 0.6 g; $P < 0.001$) and water (Sham *vs.* Vagotomized 1-h water intake: 7.6 ± 0.9 g *vs.* 2.4 ± 0.6 g; $P < 0.001$; 2-h water intake: 13.2 ± 2.5 g *vs.* 6.5 ± 5.0 g; $P < 0.05$) than the vagotomized animals during the 2 h refeeding period after the fast. The sham-vagotomized, pairfed group consumed 2.7 g of food during the refeeding period. Animals with unilateral transection of ascending brainstem pathways ate the same amount of food as the sham-operated animals (Sham *vs.* Transected: 8.7 ± 0.9 g *vs.* 8.7 ± 0.6 g) during the 2-h refeeding period.

Effect of vagotomy on refeeding-induced activation of POMC neurons in the ARC

Only a few POMC-IR neurons containing c-Fos-immunoreactivity were seen in the ARC of fasting animals, without apparent differences between sham-operated and vagotomized rats. Refeeding of sham-operated animals led to a marked increase in the number of double-labelled neurons in the ARC. Refeeding also resulted in an increased number of double-labelled POMC neurons in vagotomized rats, although the increase was less pronounced. The number of double-labelled POMC neurons also increased in the pairfed sham-operated group compared to fasting controls, although it appeared to be lower than both the intact and vagotomized rats. Two-way ANOVA analysis of sham fasted, sham refed, vagotomized fasted and vagotomized refed groups revealed a significant main effect of both vagotomy and refeeding ($P < 0.01$). Only few POMC-IR neurons containing c-Fos immunoreactivity were seen in the ARC of fasting animals, without apparent differences between sham-operated and vagotomized rats. Refeeding of sham-operated animals led to a marked increase in the number of double-labelled neurons in the ARC. Refeeding also resulted in an increased number of double-labelled POMC neurons in vagotomized rats, although the increase was less pronounced. The number of double-labelled POMC neurons also increased in the pairfed sham-operated group compared to fasting controls, although it appeared to be lower than both the intact and vagotomized rats. By one-way ANOVA, sham pairfed animals showed a significant increase in the c-Fos response compared to sham fasted and vagotomized fasted animals, but also a significant

decrease compared to sham refed animals (percentage of c-Fos containing POMC neurons, sham-fasted *vs*. sham-refed *vs*. sham-pairfed: 2.1 ± 1.5% *vs*. 48.7 ± 3.7% *vs*. 13.5 ± 2.4%; $p < 0.05$; vagotomized fasted *vs*. vagotomized refed: 2.9 ± 0.7% *vs*. 27.6 ± 2.4%; $p < 0.001$).

Effect of transection of ascending brainstem pathways on refeeding-induced activation of POMC neurons in the ARC

The effectiveness of the unilateral transection of the ascending brainstem pathways were verified by the examination of the number of noradrenergic (dopamine β hydroxylase (DBH) containing fibers) axon varicosities on the surface of the ARC POMC neurons. On the transected side, the number of DBH-IR varicosities on the surface of POMC neurons in the ARC was reduced by a mean of 74.05 ± 3.55% compared to the intact side. In addition, quantitative analysis of the number of POMC neurons receiving contacts by DBH-IR varicosities showed a marked reduction on the transected side in both fasted and refed animal groups (intact side *vs*. transected side in fasted animals: 93.1 ± 3.1 *vs*. 50.8 ± 13.1; in refed animals 91.8 ± 2.9 *vs*. 56.2 ± 2.8; $P < 0.05$).

In fasted animals, only few c-Fos-IR POMC neurons were observed in both the intact and the transected sides of the ARC. Refeeding-induced a marked and significant increase in the number of c-Fos-labelled POMC neurons in the sham-operated animals and also on both sides of the ARC in the transected animals (percentage of double-labelled POMC neurons, intact side fasted *vs*. intact side refed: 6.5 ± 2.6% *vs*. 28.0 ± 3.4%; $p < 0.001$; transected side fasted *vs*. transected side refed: 7.4% ± 2.3 *vs*. 25.2 ± 3.8%; $p < 0.001$). Transection had no influence on the effect of refeeding on the number of double-labelled neurons ($p = 0.37$). The number of c-Fos-IR POMC neurons was similar in the sham operated refed and in either side of the refed transected animals (23.4 ± 4.6; sham *vs*. transected side refed, $p = 0.75$; sham *vs*. intact side refed, $p = 0.69$).

Connections of PBN with other refeeding-activated neuronal groups

Origins of the refeeding-activated neuronal inputs of the PBN

In refed, CTB-injected animals, the greatest number of refeeding-activated neurons that project to the PBN (CTB- and c-Fos-containing) were observed in the PVN, particularly in PVNv and PVNl, the PSTN and in the medial, intermediate and commissural subdivisions of the NTS. A moderate number of double-labeled neurons were observed in

the BST, primarily the medial part of this nucleus, the CEA, lateral hypothalamic area and area postrema. Scattered c-Fos and CTB-containing neurons were detected in the agranular insular cortex, anterior hypothalamus, ARC, hypothalamic dorsomedial nucleus and zona incerta.

Examination of the connection between the PBN and refeeding-activated ARC POMC neurons

Triple-labeling immunocytochemistry for c-Fos, CTB and POMC was used to determine whether the PBN receives direct or indirect refeeding-activated inputs from POMC neurons in the ARC. A large number of POMC neurons in the ARC contained c-Fos-immunoreactivity in their nuclei after refeeding, but only a very small portion of these cells contained also CTB-immunoreactivity (1 or 2 cells/section). However, POMC-IR axons were seen to heavily innervate refeeding-activated, PBN projecting neurons in the PVNv, PVNl and PSTN. These data suggest that PVN subdivisions and the PSTN mediate the effects of the activated POMC neurons on the PBN.

Identification of the refeeding-activated targets of the PBN

Large number of PHAL-IR fibers were found in refeeding-activated areas in close association with refeeding-activated neurons in the BST and CEA and the PSTN after the PHAL injection to the PBN of rats. Less intense innervation was observed in the area of anterior hypothalamus, PVNv and PVNl, ARC, DMN, LH, ZI, and AH. Only scattered PHAL-IR fibers were found in association with c-Fos containing neurons in the agranular insular area and NTS.

Connections of central nucleus of amygdala with other refeeding-activated neuronal groups

Origin of the refeeding-activated inputs of the CEA

After iontophoretic injection of CTB into the CEA, the majority of the double-labeled neurons containing both c-Fos and CTB were detected ipsilateral to the injection site. A large number of double-labeled cells were observed in the PVN, PSTN, PBN and in the PAGvl. Few CTB-c-Fos double-labeled neurons were detected in the prelimbic area, agranular insular area, visceral, piriform, primary and somatosensory cortex, BST, ARC, perifornical nucleus, LH, ZI and NTS.

Examination of the connection of CEA and refeeding-activated ARC POMC neurons

After iontophoretic injection of CTB into the CEA, only few refeeding-activated POMC-IR neurons were found to project to the CEA. However, in the PSTN, POMC-IR fibers frequently formed appositions on refeeding-activated neurons projecting to the CEA suggesting that the PSTN may relay the effect of the activated POMC neurons to the CEA.

Innervation of refeeding-activated neuronal populations by CEA neurons

On the ipsilateral side of tracer injection, numerous PHAL-IR fibers were detected on the surface of c-Fos-IR neurons in the BST, LH, posterior magnocellular part of the PVN, PSTN, PVT, PAG, NTS, and PBN. Only few PHAL-IR fibers were identified in the surface of neurons in the medial parvicellular part of the PVN.

Effect of chemogenetic activation of CEA on the food intake during refeeding

Activation of the CEAm neurons by ip CNO administration resulted in significant decrease of food intake, in the first 60 min of refeeding. Animals injected with CNO ate 15.32±3.69mg/g lean body weight whereas saline injected animals ate 23.32±5.1mg/g lean body weight, ($p=0.0035$). In the second hour, the CNO injected animals consumed significantly more food (10.51 ±5.03 mg/g lean body weight) than the saline injected animals (2.46±2.56 mg/g lean body weight) ($p=0.006$). In the CNO treated animals with CEAlc injection CNO treatment did not cause significant changes of food intake (Saline versus CNO in the first hour (g/lean body weight): CNO: 24.71±0.7 versus 20.88±1.19 $p= 0.24$; Saline versus CNO in the second hour (g/lean body weight): 2.33±0.79 versus 3.37±2.23 $p= 0.96$).

Brain areas activated by the activated CEA neurons

The chemogenetic activation of CEAm neurons induced significant increase of the number of c-Fos-IR nuclei in the BST, PSTN and PBN compared to virus injected saline treated animals. The activation of CEAlc did not influence the activation of BST and PSTN, but it resulted in significant increase of neuronal activation in the PBN. The increase in the number of c-Fos-IR nuclei in this brain region was less than that induced by the activation of CEAm.

CONCLUSIONS

Our studies provide detailed map of the pattern of refeeding-induced neuronal activation. This information contributes to the better understanding of the neural network involved in the development of satiety. These data verify the involvement of the well-known feeding-related nuclei such as the NTS, PBN and CEA in the regulation of refeeding-induced satiety. In addition, our data also suggest the role of other brain areas, like the PSTN and dmpARC, in the regulation of satiety. Further examination of the role of these brain regions in the development of satiety may contribute to the better understanding of the neuronal regulation of energy homeostasis.

We also demonstrated that the ARC POMC neurons are activated independently from the vagus nerve and from the ascending brainstem pathways. These data suggest that the POMC neurons are activated solely by direct effects of circulating hormones/metabolites during refeeding. We presume that the information reaching the brain via the ARC and the NTS is integrated at the level of the NTS by the influence of the ARC POMC neuron-PVNv-NTS pathway on the sensitivity of the NTS neurons for the vagus nerve derived inputs.

The mapping of the connectivity of PBN and CEA with refeeding-activated neuronal groups revealed that these areas have bidirectional connections with each other and with other refeeding-activated cell groups suggesting that the information flow is not unidirectional in the satiety network, but this network rather utilizes short feedback mechanisms to support the precise tuning of neuronal activity. The rich connectivity of the PSTN with the other refeeding-activated groups suggests that PSTN is an important node of this network that may have critical role in the integration of neuronal activity in the satiety network.

In addition, our tract tracing data together with the chemogenetic examination of the role of CEA subnuclei in the regulation of feeding indicate that a PBNm-CEAm pathway plays important role in the regulation of food intake during refeeding. This pathway is different than the PBNel-CEAlc pathway described by Palmiter's research laboratory which pathway is involved in the development of conditional taste aversion. Thus, we presume that parallel PBN-CEA pathways regulate different aspect of food intake.

PUBLICATIONS

Publications related to the thesis

Zséli G, Vida B, Martinez A, Lechan R M, Khan A M, Fekete C (2016) Elucidation of the Anatomy of a Satiety Network: Focus on Connectivity of the Parabrachial Nucleus in the Adult Rat. *J Comp Neurol* 524 (14), pp. 2803-27

Fekete C*, **Zséli G***, Singru PS, Kádár A, Wittmann G, Füzesi T, El-Bermani W, Lechan RM (2012) Activation of Anorexigenic Pro-Opiomelanocortin Neurones during Refeeding is Independent of Vagal and Brainstem Inputs. *J Neuroendocrinol* 24 (11), pp. 1423-1431
* these authors contributed equally to this work

Singru PS, Wittmann G, Farkas E, **Zséli G**, Fekete C, Lechan RM (2012) Refeeding-activated glutamatergic neurons in the hypothalamic paraventricular nucleus (PVN) mediate effects of melanocortin signaling in the nucleus tractus solitarius (NTS). *Endocrinology* 153 (8), pp. 3804-3814

Other publications

Sárvári A, Farkas E, Kádár A, **Zséli G**, Füzesi T, Lechan RM, Fekete C (2012) Thyrotropin-releasing hormone-containing axons innervate histaminergic neurons in the tuberomammillary nucleus. *Brain Res* 1488, pp. 72-80

Kiss L, Pintye A, **Zséli G**, Jankovics T, Szentiványi O, Hafez YM, Cook RTA (2010) Microcyclic conidiogenesis in powdery mildews and its association with intracellular parasitism by Ampelomyces. *Eur J Plant Pathol* 126 (4), pp. 445-451