

Investigation of the central regulation of taste perception and metabolism in human and animal experiments

Ph.D. thesis

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I. Introduction

The energy which is necessary for the proper function of the cells is stored in the high-energy chemical bonds of the nutriment (carbohydrates, fat, protein) of the outer milieu. The food intake (energy-uptake) is a periodic process, but the function of the cells needs constant energy supply. To fulfil this requirement, a part of the consumed nutrients is metabolized and supplies energy and functioning as building blocks, whereas the other part is stored in the liver and in the adipose tissue as glycogen and fat. The function of food intake is to maintain a constant level of energy stores and not to fulfill the momentary needs of the tissues. The urge for food intake is present, when the energy stores are decreased but are still capable to cover the energy needs. This necessity develops in the form of a complex psychophysiological state called hunger. Hunger is a central motivational state caused by an absolute or relative energy deficit. During this state such behavioral processes are pronounced which try to eliminate this energy deficiency. The first phase of food intake is the seeking of the nutrients, which phase remarkably depends on the actual activity and motivational state of the individual. In humans the regular dining time, the sight, the taste and smell of the food all have pronounced significance. In the animals besides the above mentioned factors, light is also essential because e.g. rats start to seek food only after getting dark. The next phase of food intake is the consummation of the given food which is accompanied by perceptual effects (e.g. oddly taste-experiences). On the one hand, this process, the eating is terminated by running out of the nourishment but prior to that by perceptual-motivational mechanisms, and the result will be a complex intrinsic state called satiety. Satiety develops much earlier than the repositories are filled practically in the preabsorptive phase in which tastes and certain intrinsic factors such as e.g. tension of the intestinal wall, neurochemicals and the central nervous system play a distinct role. Taste perception has a fundamental role because it is the first "gate" in the organism where chemical analysis happens which provides essential information regarding the positive or negative judgment of a given nutrient. It is well-known, that if consummation of a food or drink with a determined taste causes gastrointestinal discomfort, then the individual will avoid the given food or drink. This is known as the conditioned taste aversion (CTA) phenomenon which is fundamental in the survival of the animal and it has a role in distinguishing the eatable food from the poisonous material.

Numerous pathological processes are known where the energy balance is disturbed either on the side of the biochemical reactions or on the side of regulatory mechanisms. Diabetes mellitus (DM) -mainly the non-insulin dependent form-, which is endemic in our days, is rated among the dysfunction of both the peripheral and central regulatory mechanisms together with obesity which could cause the above mentioned DM because of the excessive food intake. The dysfunction of these regulatory processes could cause serious weight-loss such as in case of anorexia nervosa which could often be life-threatening.

Since the end of the 19th and the beginning of the 20th century the so called center

theories have emerged, and the most popular theory stated that antagonistic centers play role in the regulation of food intake. The stimulation of the so-called hunger-center, localized in the lateral hypothalamic area (LHA), causes complex food-searching and consummative responses, so the animal recognizes approaches and ingests the food. The consummative response has an imperative manner: it sustains until the stimulation is terminated and it is independent from the fullness state of the gastrointestinal tract. The lesion of the same region causes aphagia, adipsia and a rapid loss in body weight. The stimulation of the ventromedial nucleus of the hypothalamus (VMH) inhibits eating (the animal even drops the food from the mouth). However, the destruction of the VMH -because of the dominance of the neurons in the LHA - provokes rapid increase in appetite and an increment in body weight which leads to obesity.¹⁻³

During the last decades, several extrahypothalamic brain regions were found whose lesion causes similar symptoms to what was shown in the LHA or VMH syndrome. The amygdala⁴ and the globus pallidus⁵ have to be emphasized because of their fundamental significance. It has to be noted as well, that similar disturbances could be induced by the lesion of several brain regions such as the tegmentum⁶, substantia nigra⁷, nucleus accumbens⁸ or the temporal lobe.⁹ In the investigation of the regulation of feeding, the question emerges, which substance (plasma metabolite, humoral factor, etc.) functions as a signal of hunger or satiety: in the former, the lack of this substance induces feeding, whereas in the latter, the presence of this substance terminates feeding. It was obvious, that related materials of the three main nutrients (glucose, fat, amino acids) were held for responsible for this. So, the glucostatic,¹⁰ lipostatic¹¹, and aminostatic¹² models of the regulation of feeding were born. It was revealed by single-neuron recordings in the central nervous system, that the neurons could be divided in three groups based on the responses to glucose: glucose-sensitive (GS) neurons decrease the firing-rate to intravenously or microiontophoretically administered glucose; glucose-receptor (GR) neurons increase their activity to glucose; glucose insensitive (GIS) neurons do not respond to any changes in the glucose concentration (they only utilize glucose for metabolism). In the VMH, one-third of the neurons are GR, whereas in the LHA similar fraction of the neurons are of the GS type.^{2, 13}

The GS and GR neurons respond not only to glucose, but to several chemical stimuli of the inner and outer environment (free fatty acids, insulin, glucagon, neuropeptides, taste, smell etc.) and to other sensory signals (visual, acoustic) as well.² Because the responsive types of these neural elements could be found in several brain regions hereafter the term glucose-monitoring (GM) neurons will be used. The presence of these neurons was proven by our research group in the HT, AMY, GP and recently in the orbitofrontal- and mediodorsal prefrontal cortex and in the nucleus accumbens as well.¹⁴⁻²⁴ Based on the above, in this complex, hierarchically organized GM network, information converges from the inner and outer environment, creating the neural base of complex and diverse regulatory mechanisms.

Taste-perception is one of the most important and most delicate sensory-perceptual factor in feeding. Taste perception makes it possible to make a "quality-control"

among the given foods. It makes it possible to distinguish between edible from non-eatable, the nutritive food from the dangerous, poisonous object. Unlike smell, where thousands of different scents could be differentiated, in taste perception there are only five primary taste stimuli: salty, sweet, sour, bitter and the so called “umami”, which was discovered by Kikunae Ikeda in the early 20th century. Taste-stimuli not only have hedonic (pleasant or unpleasant) components, but they relay information about the quality of a given food or drink. The sweet and umami taste has information that the food is rich in energy. The bitter taste could refer to the presence of poisonous substance. The sour taste is the signal of organic acids, and it could indicate that a given food is uneatable, vicious. The salty taste provides information in the control of electrolyte and fluid homeostasis.

II. Aims and questions

In accordance with the research profile of our group, diverse human and animal experiments were conducted in multiple clinical collaboration by the means of modern imaging techniques.

Experiments were performed to answer the following questions:

1. is there any taste perception disturbance in patients with eating disorder?
2. is there any difference in the brain activity, observed by functional imaging methods, in eating and metabolic diseases (anorexia nervosa, obesity) compared to healthy individuals?
3. could be observed activity changes in the central nervous system (especially in the regions rich in glucose-monitoring neurons) following multiple intravenous glucose administration?

III. Experiments

A. Human clinical investigations

3. Anorexia nervosa

3.1. Introduction

Anorexia nervosa (AN) is a complex psychiatric disorder posing rapidly increasing burden on the modern societies.²⁵ It has an increasing frequency all over the world, and it has the highest death rate of any psychiatric diseases.²⁶ AN has two main types: restrictive and purgative ones. It develops overwhelmingly in young adolescent women.²⁷ AN is characterized by extreme dietary restriction, a relentless pursuit of thinness, an obsessive fear of becoming fat, loss of body weight, and a variety of metabolic and endocrine alterations, including primary or secondary amenorrhea.²⁸⁻³⁰ The persons suffering from AN exhibit a disturbed perception of their own body shape and size as well.

Despite the relative abundance of taste studies in eating disorder patients, in AN a par excellence taste reactivity study with the use of all the five primary taste qualities has not been performed yet. In the present experiments, therefore, taste reactivity

study was conducted in restrictive type AN patients, and their hedonic evaluations were compared to those of age-matched healthy control subjects.

3.2. Subjects and methods

Altogether 25 subjects volunteered initially in this study. Restrictive type AN subjects were diagnosed based on criteria of the DSM-IV.²⁷ Finally, after excluding three volunteers because of their uncertain diagnosis or unfitting morphometric or other examination data, 11 AN patients, ten women and one man (BMI: 16.7 ± 1.6 ; age: mean 23.3 years) and 11 age-matched healthy control subjects, nine women and two men (BMI: 22.8 ± 1.9 ; age: mean 24 years) participated in these experiments. All the volunteers were screened with the EAT-40 test and the EDI test has been additionally performed as well.

All subjects were free of salivary dysfunction, and histories of gastrointestinal or other diseases, and their serum zinc and amylase concentrations were in the physiological range (12-24 $\mu\text{mol/l}$ and 28-100 IU/l, respectively).

The sessions took place in a quiet, well-separated room in the Psychiatry and Psychotherapy Clinic of the Pécs University, Medical School. Written informed consent was obtained from all subjects. The protocol fully conformed to the provisions of the Declaration of Helsinki (1995; rev. Edinburgh, 2000). The project has been approved by the Ethics Committee of Pécs University, Medical School.

Gustatory functions were tested by presenting 5 ml liquid taste stimuli at room temperature in disposable plastic cups. Subjects, fasting for at least 6 hrs before the examination, were instructed to perform inter-stimulus distilled water (DW) rinses ad lib. Two concentrations of each tastants were used: 0.1 M and 0.5 M sucrose as sweet, 0.1 M and 0.5 M NaCl as salty, 0.1 M and 0.5 M monosodium glutamate (MSG) as umami, 0.003 M and 0.03 M HCl as sour, 0.3 mM and 3 mM quinine HCl (QHCl) as bitter, and 5 and 25% orange juice (OJ) as complex (pleasant) taste. The sip and spit method was employed.³¹ The testee had to swirl around the solution in the mouth and then had to spit it out. Between two taste solutions, DW rinses were performed to eliminate the taste from the subjects' oral cavity. After each taste solution, the subject had to put a single pencil mark on a 200 mm visual analogue scale (VAS) where the left side (-100 mm) meant the hedonically negative, whereas the right side (+100 mm) meant the hedonically positive tastes. The middle point of the scale, the 0, meant that the solution was neutral for the participant. For the VAS data, the distance between the 0 and any given pencil mark was measured to the accuracy of 1 mm. Both concentrations of sucrose, the lower concentration salt (0.1 M NaCl) and umami (0.1 M MSG), and both concentrations of orange juice were considered as the hedonically positive tastes, whereas the hedonically negative tastes were the stronger salt (0.5 M NaCl) and umami (0.5 M MSG), and both concentrations of the acid (0.003 M, 0.03 M HCl) and quinine solutions (0.3 mM, 3 mM QHCl).^{32, 33}

The sessions were videotaped for further analysis of the facial expressions regarding innate, discriminative motor reactions of the facial muscles to adequate stimulation of

the peripheral gustatory receptors.³⁴ Verbal commentaries of the subjects were also recorded.

For statistical analysis of data, the SPSS software package was used. Both individual and group VAS-score averages were calculated and independent samples t-tests were employed for averaged and normalized scores. Group comparisons were made by the Mann-Whitney U test, and Spearman rank correlation coefficients (Spearman's rho /Srho/) were calculated as well. Statistical differences were considered to be significant at $p < 0.05$ or less.

3.3. Results

In the present study, characteristic taste perception abnormalities were found in the AN patients. On the one hand, their general taste reactivity tended to be weaker compared to that of individuals in the control group ($t_{2,262}=1.945$; $p=0.053$). On the other hand, and the most characteristically, pronounced deficits were seen in the hedonic evaluation of gustatory stimuli. The hedonic ratings of the anorexic patients given for the pleasant tastes, in comparison to the controls, proved to be significantly lower ($t_{2,130}=2.714$; $p < 0.008$), whereas the ratings given for the unpleasant, aversive tastes were similar ($t_{2,130}=0.564$; N.S.).

The analysis of reactivity data of the individual stimuli also revealed characteristic gustatory perception deficit of the patients. Pleasantness ratings in the AN group, compared to controls, significantly decreased for the lower concentration of sucrose ($t_{1,20}=2.561$; $p < 0.02$), salt ($t_{1,20}=2.61$; $p < 0.02$), and umami ($t_{1,20}=3.812$; $p < 0.002$). Reactivity scores to the strong and either pleasant or unpleasant, robust taste sensation eliciting test solutions (higher concentration of sucrose, both concentrations of orange juice, as well as the stronger salt and umami, and both concentrations of HCl and QHCl) did not differ significantly in the AN patients and control subjects.

The group comparisons of BMI, EAT 40, and several of the EDI subscales (drive for thinness /dft/, body dissatisfaction /bd/, ineffectiveness /ie/, interoceptive awareness /ia/, maturity fears /mf/) revealed remarkable differences between the anorexic and control subjects (for both BMI and EAT 40, $p < 0.001$; for dft, $p < 0.01$, for bd, $p < 0.001$, and for ie, ia, and mf, $p < 0.01$, respectively). In addition, a clear relationship among these parameters and the taste reactivity scores was also clearly demonstrated (lower concentration umami vs. BMI, Srho: 0.529, $p < 0.01$; lower concentration sucrose vs. EAT 40, Srho: 0.448, $p < 0.05$; lower concentration salt vs. EAT 40, Srho: 0.434, $p < 0.05$; lower concentration umami vs. EAT 40, Srho: 0.557, $p < 0.01$). Of the EDI subscales, correlation of these data with taste reactivity scores was found especially high in case of drive for thinness (lower concentration sucrose vs dft, Srho: 0.432, $p < 0.05$; lower concentration salt vs. dft, Srho: 0.429, $p < 0.05$; lower concentration umami vs. dft, Srho: 0.467, $p < 0.05$;) and body dissatisfaction (lower concentration sucrose vs bd, Srho: 0.435, $p < 0.05$; lower concentration salt vs. bd, Srho: 0.421, $p < 0.05$; lower concentration umami vs. bd, Srho: 0.479, $p < 0.05$).

3.4. fMRI investigation of taste perception associated brain activity in AN

Our previous experiments and other published data raised the possibility of the presence of taste perception abnormalities in AN.³⁵⁻⁴³ Therefore, it was reasonable to clarify the central taste perception mechanisms by applying not only pleasant and unpleasant but high-calorie taste stimuli as well.

3.5. Materials and methods

3.5.1. Subjects

Ten AN patients and 10 healthy, age and gender matched control subjects participated in this study (age: AN: 30.3±4.21 years; control: 34.5±3.73 years; BMI: AN: 17.16±3.02; control: 21.75±2.12). All the subjects were right-handed and all of them signed an informed consent before the experiment. The patients were recruited by the DSM-IV criteria but those were excluded who either suffered in any other psychiatric disease (depression, schizophrenia) or used any kind of agents modifying taste perception (medications, alcohol consumption, smoking).

3.5.2 Taste stimulation

The fMRI session was scheduled 3-4 h after the subject consumed a standardized meal (465 kcal/100 g, rice with chicken) to avoid the confounding effect of hunger or satiety. By using a ten points arbitrary scale, the hunger ratings were recorded prior to scanning, and there was no significant difference between the two groups (5.1±0.4 vs. 4.8±0.3, respectively). Before the scanning session, the taste sensitivity of the subjects was roughly estimated by presenting them a low concentration solution of the five basic taste qualities. No sensitivity deficit was detected by this method in the subjects. Two polyvinyl (PVC) tubes with inner diameter of 1 mm were placed into the mouth of the volunteer. Two unimodal and one multimodal taste solution in three separate fMRI runs were used as stimuli, whereas distilled water (DW) served as rinse and a neutral stimulus in all runs. 0.1 M sucrose (sweet, unimodal) as pleasant, 0.03 mM quinine hydrochloride (bitter, unimodal) as unpleasant, and, a high-calorie (150 kcal/100 ml), vanilla flavoured nourishment solution (Nutridrink©) as a complex multimodal stimulus were delivered via the tubing. In each run only one taste solution was used, so there were a sucrose vs. DW, QHCl vs. DW, and Nutridrink vs. DW run. To minimize order effects the runs followed each other in a random order. Between each run, the subjects were allowed to have a rest for 3-5 min.

As it was described earlier, after the functional measurements, the subjects had to put a single pencil mark on a 200 mm visual analogue scale (VAS) where the left side (-100 mm) meant that the taste was hedonically negative, whereas the right side (+100 mm) meant that the taste was hedonically positive. The middle point of the scale, the 0 meant that the solution was neutral for the participant.

3.5.3. MR imaging

Subjects were laid into a Siemens Magnetom TIM Trio (Siemens AG., Erlangen, Germany) 3T clinical MR scanner in supine position with eyes closed. During all functional MR imaging runs, 360 volumes of T2*-weighted EPI image series with 23 axial slices were acquired (TR/TE: 2500/36 ms, FoV: 192 mm, matrix: 96*96, in-plane resolution: 2x2 mm, slice thickness: 4 mm, no gap, interleaved slice order to avoid crosstalk). The slices were positioned parallel to the AC-PC line. Following the functional scans, a high-resolution anatomical T1-weighted axial 3D-MPRAGE image (TR/TE/TI: 1900/3.41/900 ms; FA: 9°; FOV: 210 x 240 mm²; 224x256 matrix; slice thickness: 0.94 mm; 160 slices; voxel size: 0.94 x 0.94 x 0.94 mm³; 180 Hz/pixel receiver bandwidth) was acquired for later usage during the registration to a standard image in the MNI-space.

3.5.4. Experimental design

A block design was used, in which one block contained 12 active and 24 baseline (passive) scans, and the blocks were repeated ten times. Altogether 360 scans were acquired during one functional measurement. The solutions and the DW in 5 ml volume were delivered in 2-3 sec at the start of every active and passive phases, respectively, by using a pneumatic syringe pump. The subjects had to swirl around the solution in their mouth during all phases, and then, when instructed, had to swallow it.

3.5.5. fMRI data analysis

Pre-processing and statistical analysis were performed using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Pre-processing included brain extraction,⁴⁴ MCFLIRT motion correction,⁴⁵ spatial smoothing with 5mm full width at half maximum, and a high-pass temporal filter of 100 s. The temporal filtering applied to the data was used for the model as well. Whole brain general linear model (GLM) time-series statistical analysis of individual data sets was carried out using FILM (FMRIB's Improved Linear Model) with local autocorrelation correction.⁴⁶ The single-session data sets were registered into standard space using FLIRT in a two-step process.⁴⁵ An independent samples t-test was applied on the data sets to find any statistically significant differences between the two subject groups. Furthermore, individual contrasts were entered into a simple linear regression model with either the BMI or the VAS as the covariate of interest.

3.6. Results

3.6.1 Visual analogue scale

There was a significant difference between the two groups in the hedonic responses in the pleasant and the high-calorie stimuli, whereas in case of the unpleasant stimuli

no significant differences were observed (sucrose: 3.9 ± 5.76 AN vs. 35.1 ± 8.77 control, $p < 0.0001$; quinine: -95.6 ± 5.25 AN vs. -99.3 ± 1.63 control, N.S.; vanilla flavored nourishment (31.8 ± 9.02 AN vs. 58.8 ± 19.03 control, $p < 0.001$).

3.6.2. Taste stimulation induced brain activation

After stimulating with the hedonically positive sucrose solution, the controls showed significantly higher activation compared to AN in left and right anterior cingular cortex, left frontal-, central opercular cortex, in the left insular cortex, in the bilateral middle frontal gyrus, and in left and right caudate nucleus.

Stimulating with the hedonically unpleasant quinine the controls showed significantly higher activation in the frontal opercular cortex, in the left and the right insula, in the right parietal opercular cortex, in the left and right OBF, in the bilateral middle frontal gyrus, in the left and right pallidum, furthermore in the left and right caudate nucleus. In case of the high-calorie vanilla flavored nourishment the AN patients showed significantly higher activation compared to controls in the left and right anterior cingular cortex, in the left OBF, in the right middle frontal gyrus, in the left NAcc and in the left putamen.

When the BMI was added in a single regression model as a covariate, positive correlation was found in the sucrose condition in the left, right anterior cingular cortices; left, right central opercular cortices; left, right frontal opercular cortex; left, right parietal opercular cortex; left, right insular cortices; left, right middle frontal gyrus; left, right OBF; right amygdala; left, right putamen; left, right pallidum; left, right caudate nuclei and in the left, right thalamic nuclei (Pearson correlation.: 0.545; $p < 0.05$). In the quinine condition, the activation also positively correlated with BMI left, central opercular cortex; right insula; right middle frontal gyrus, and in the left and right thalamic nuclei (Pearson correlation.: 0.715; $p < 0.001$). In the high-calorie condition, the left, right anterior cingulate cortices; left, right frontal-; left, right central-; right parietal opercular cortices; left, right insula; left, right middle frontal gyri, left OBF; left, right pallidum; left, right putamen; left, right caudate nuclei and in the left and right thalamic nuclei showed activation positively correlating with BMI (Pearson correlation: 0.538; $p < 0.05$).

When the subjective VAS hedonic scores were entered into the regression model, in the sucrose vs. water condition the left and right anterior cingulate cortex; the left, right frontal- central and parietal opercular cortices; left insular cortex; the left and right middle frontal gyri; the left putamen and the left caudatum showed activation positively correlating with the VAS scores (Pearson correlation: 0.725; $p < 0.001$). In the quinine vs. water condition, the activation in the left parietal opercular cortex; right middle frontal gyrus; left amygdala; left pallidum; left putamen; and in the left thalamic nuclei showed activation negatively correlating with the hedonic scores (Pearson correlation: -0.744; $p < 0.001$). Finally, in the high-calorie vs. water condition, activation in the left and right NAcc and the right caudate showed positive correlation with the subjective ratings (Pearson correlation: 0.736; $p < 0.001$).

3.7. Investigating taste perception alteration in obesity by applying fMRI

3.7.1. Introduction

Elucidating the underlying neural mechanisms of the central control of feeding and metabolism is fundamental in the neurophysiological research because related diseases (obesity, type 2 diabetes mellitus, etc.) put enormous and increasing costs on the health care systems of the modern societies. The pathophysiological mechanisms of these illnesses and the central regulation of relevant functions even in healthy condition are not sufficiently understood yet. Although functional MR is broadly utilized to examine various cognitive functions, to date it was rarely employed in the investigation of brain mechanisms associated with taste perception deficits in eating and metabolic disorders. Despite a relative abundance of these feeding associated investigations, only a few similar studies focused on changes of taste information processing in obese patients. In the present series of experiments, therefore, our purpose was to compare gustatory stimulation elicited brain activity changes of obese and healthy control subjects in a condition when the intrinsic physiological state of hunger and satiety were kept on a constant level.

3.7.2 Materials and methods

3.7.2.1 Subjects

Twelve obese (BMI: 34.05 ± 3.35 , 9 women, 3 men) and twelve healthy, age (38.3 ± 4.2 vs. 37.1 ± 3.8) and gender matched subjects (BMI: 21.42 ± 2.53) participated in this study. The exclusion criteria were the following: 1) smoking, 2) medications influencing taste perception, 3) any psychiatric disorder in the history, 4) any kind of endocrinological disease in the history, 5) chronic alcohol consumption (more than 2 alcoholic beverages/day). All subjects tested were right-handed and none of them were on diet. Examinations were initiated after informed consent of the participants have been signed. The protocol was in full agreement with international, national and university regulations.

3.7.2.2. Methods

The experimental design, the measuring parameters and the data evaluation were the same as applied in the previously demonstrated experiments in anorexia nervosa (see sections 3.5.2.-3.5.5.).

3.7.3. Results

3.7.3.1.. Visual analogue scale

Significant differences were found between the two groups in the pleasantness ratings given for sucrose (62.5 ± 11.38 in obese vs. 27 ± 4.4 in controls; $p < 0.001$), for quinine (-92 ± 7.9 in obese vs. -67.5 ± 14.36 in controls; $p < 0.001$), and for vanilla (94.5 ± 5.4 in obese vs. 48.75 ± 11.89 in controls; $p < 0.001$), respectively.

3.7.3.2. Taste stimulation induced brain activation

In general, taste vs. distilled water (DW) stimulation induced brain activation was found to be significantly bigger in the obese patients compared to the control subjects.

In the sucrose vs. water condition, a significantly higher activation was found in the obese group compared to controls in the right central_operculum; right frontal operculum; left, right insula; right middle frontal gyrus; left OBF; left parietal operculum; right amygdala and in the left NAcc. In the quinine vs. water condition, there was significantly higher activation in the obese group in the left, right anterior cingulate cortices; left, right frontal, central and parietal opercular cortices; left, right insular cortices; left, right middle frontal gyri; left, right OBF; left, right amygdala; left, right NAcc; left, right pallidum; left, right putamen; left, right caudate nuclei and left and right thalamic nuclei. In the high-calorie vs. water condition, the obese group showed significantly higher activation compared to controls in the left central opercular cortex; left, right frontal opercular cortices; and left parietal opercular cortices; left, right insular cortices; the left, right middle frontal gyri; left, right OBF left amygdala; left NAcc; left pallidum, left putamen and in the left caudate nucleus. Responses of the control group were not found significantly greater than those of the obese group for any of the taste stimuli compared to DW. Furthermore, there was no significant difference in the deactivation patterns between the two groups in response to any taste stimulus.

When the BMI was added in a single regression model as a covariate, positive correlation was found in the sucrose vs. water condition in the; left, right central opercular cortices; right frontal opercular cortex; left parietal opercular cortex; left, right insular cortices; right middle frontal gyrus; left; right OBF; right amygdala; left, right caudate nuclei and in the left, right NAcc (Pearson correlation: 0.681; $p < 0.001$). In the quinine vs. water condition, the activation also positively correlated with BMI in the left, right anterior cingulate cortices; left, right frontal-;central-;parietal opercular cortices; left, right insula; left, right middle frontal gyri, left, right OBF; left, right amygdala; left, right NAcc; left, right pallidum; left, right putamen; left, right caudate nuclei and in the left and right thalamic nuclei (Pearson correlation: 0.717; $p < 0.001$). In the vanilla vs. water condition, the left, right frontal-; left central-;left parietal opercular cortices; left, right insula; left, right middle frontal gyri, left, right OBF; left, right amygdala; left, right NAcc; left, right pallidum; left, right putamen; left, right caudate nuclei and in the left and right thalamic nuclei showed activation positively correlating with BMI (Pearson correlation: 0.705; $p < 0.001$).

When the subjective VAS hedonic scores were entered into the regression model, in the sucrose vs. water condition the left anterior cingulate cortex; the left frontal and parietal opercular cortices; left insular cortex; left, right OBF, the left and right middle frontal gyri, and the left NAcc showed activation positively correlating with the VAS scores (Pearson correlation: 0.690; $p < 0.001$). In the quinine vs. water condition, the activation in the left, right anterior cingulate cortices; left, right frontal, central and

parietal opercular cortices; left, right insular cortices; left, right middle frontal gyri; left, right OBF; left, right amygdala; right NAcc; left, right pallidum; left, right putamen; right caudate nuclei and left and right thalamic nuclei showed activation negatively correlating with the hedonic scores (Pearson correlation: -0.691; $p < 0.001$). Finally, in the high-calorie vs. water condition, activation in the left central opercular cortex; left, right frontal opercular cortices, left parietal opercular cortex; the left, right OBF and left and right middle frontal gyri showed positive correlation with the subjective ratings (Pearson correlation: 0.624; $p < 0.001$).

B. Primate fMRI experiments

3.8.1 Introduction

The function of neural systems involved in the regulation of homeostasis was described in several studies. In this regulating circuit the hypothalamus has a distinct role, by controlling not only the vegetative and hormonal functions but the feeding associated behavior as well.⁴⁷ The glucose-monitoring neurons, the key elements of this regulatory circuit were first described in this region as well.¹³ It is known about these neurons that they respond not only to glucose but to other chemical-humoral and various many other stimuli.^{13, 48-50} Our recent animal experiments showed that the streptozotocin injected into the VMH, by selective destruction of these neurons, causes symptoms similar to those seen in the type 2 diabetes mellitus.⁵¹ Based on these studies, experiments were conducted on rhesus monkeys to elucidate whether repeated intravenous glucose administration could cause any activity changes in the central nervous system, especially in the regions rich in glucose monitoring neurons.

3.8.2. Methods

3.8.2.1. Subjects

Three adult rhesus monkeys (*Macaca mulatta*) from the colony of the PUMS Institute of Physiology were involved in the investigations (2 male, 1 female; age (years): 9 ± 2.48 ; body weight (kg): 7.5 ± 2.89). Obeying domestic and international regulations the animals were kept in separated, individual cages. In the animal room, 12-12 hours long light-dark cycle was employed and constant room temperature and humidity were also assured. Conduction of the experiments was officially approved (BA02/2000-8/2012).

3.8.2.2. Anesthesia

Prior to the scanning session a 12 hour food deprivation took place. On the day of the MR experiment, following an intramuscular premedication of ketamine (10mg/kg) (Calypsol[®], Richter Gedeon Rt.), intravenous cannulas were inserted in both arms. Total intravenous anesthesia was conducted during the fMRI measurement by administering 0.5% Propofol (flow rate: 0.6 ml/min; dose: 0.025 mg/min) by means of an infusion pump. Through one of the cannulas, Salsol infusion solution was administered and the glucose loads were conducted, and through the other cannula the Propofol was administered. (Propofol was chosen because there are data that

this agent does not affect blood glucose level⁵²)

3.8.2.3. MR protocol

The animals were laid on the right side during the measurement. Flex-coil was used for excitation and signal reception. During all functional MR imaging runs, 1300 volumes of T2*-weighted EPI image series with 20 axial slices were acquired (TR/TE: 3000/36 ms, FoV: 65 mm, matrix: 64*64, in-plane resolution: 1x1 mm, slice thickness: 1.9 mm) Following the functional scans, a high-resolution anatomical T1-weighted axial 3D-MPRAGE image (TR/TE/TI: 1900/3.78/900 ms; FA: 9°, FOV: 140 x 140 mm²; 245x256 matrix; slice thickness: 0.94 mm; voxel size: 0.94 x 0.94 x 0.94 mm) was acquired for later usage during the registration to the standard space.

3.8.2.4 Experimental design

The first five minutes (100 scans) of the functional measurement served as a baseline, then in the 5., 20., 35., and 50. minutes (100., 400., 700., 1000. scan) 10 ml 20% glucose solution was administered in 10 seconds. Then the solution was washed in by a high flow-rate of Salsol infusion for approximately 30 seconds. The blood samples were taken independently from the MR experiments. The protocol was the same as in the MR measurements, the glucose infusions appeared in the same times so those could be correlated with the data from the fMRI experiments.

3.8.2.5. Blood glucose and insulin measurements

The blood glucose level was determined from whole venous blood by means of cold-chemistry photometry (Spotchem, EZ SP4430, Arkray, Japan), whereas insulin levels were determined by ELISA (Human Insulin Kits, Alpco Immunoassays, USA, IEMS Reader MF, 140100-735, Inter Labsystems Kft, Labsystems). The blood samples were centrifuged on 4°C 1000 rpm for 20 minutes. The lipid particles of Propofol were removed from the plasma by LRA (Lipid Removal Agent, Sigma-Aldrich Co.)

3.8.2.6 fMRI data analysis

Pre-processing included brain extraction,⁴⁴ motion correction,⁴⁵ spatial smoothing with 1.5mm Gaussian filter, and a high-pass temporal filter of 840 s. Statistical evaluation of the data was carried out by applying MELODIC (Multivariate Exploratory Linear Optimized Decomposition into Independent Components) v3.01. The algorithm of MELODIC is capable to separate individual signal from a mixed noisy data set. The average blood glucose and insulin values were entered as regressors. First the blood glucose and insulin values were extrapolated to 1300 time points, then an average were calculated, a 840 s high-pass filter was applied, then the variance was normalized.

The program identified the regions whose signals correlated with the values of either the glucose or the insulin levels.

3.8.3 Results

During data evaluation, the algorithm found correlation with changes of the blood glucose and insulin levels and the signal intensity changes in hypothalamus, in both amygdala and the OBF. F-test was also conducted to clarify whether the signal intensity changes and the changes in the blood glucose and the insulin levels are related or not. In case of the glucose the result was $F=79.28$, $p<0.001$, whereas in case of the insulin it was: $F=82.67$, $p<0.001$.

IV. Discussion

Anorexia nervosa

A full-scale taste reactivity study, including all five primary gustatory qualities and a complex taste (orange juice), with immediate hedonic evaluation of the stimulus solutions has not been performed yet in AN. Our experiments elucidated a relative weakness in general taste reactivity of AN patients. As the most characteristic finding of the present study, however, the hedonic ratings of the restrictive type anorexic patients for the pleasant, but not for the aversive tastes proved to be significantly lower compared to those of control subjects. Furthermore, the correlation analysis substantiated the clear relationship of these results with the cognitive disturbances characterizing AN.

Hypogeusia and dysgeusia, mainly involving the sour, bitter and salty tastes have already been demonstrated in AN,^{39, 53-56} nevertheless, perceptual-motivational aspects of the findings so far received diverse interpretations. Earlier studies emphasized that a characteristic “carbohydrate phobia” exist in anorexic patients.^{28,}

³⁰ Later reports, however, suggested no alteration of sweet taste preference but a definite dislike of foods rich in fat in anorexic-restrictors.^{36, 40, 57} Sunday and Halmi also pointed out that in the patients (alike healthy subjects) the sweeter the solution perceived the more the solution liked.⁵⁷ Our findings only partly agree with the above data since in the present examinations hedonic ratings of the higher concentration sucrose solution in the AN patients did not differ from those in the control subjects.

The virtual contradiction between our results - no difference in sour and bitter responsiveness, and decreased hedonic ratings for the lower concentration sucrose solution - and data of literature can be resolved by taking into consideration the obvious heterogeneity of the methodologies employed. In fact, all the other studies used either food items (e.g. sucrose sweetened cheese, milk or cream) or “pure” gustatory stimuli but in different volume, concentration or delivery method, whereas, to date, our investigation appears to be the first to use in eating disorder patients a par excellence human taste reactivity test with immediate hedonic evaluation of the stimulus solutions.

Restrictive type anorexics displayed significantly lower hedonic ratings for the mild pleasant taste stimuli, indicating that these patients may experience a reduced pleasure in eating. Previously it has already been suggested that a hedonic monitor, biased by body weight and caloric intake, exists, and also that pleasure plays physiological role in regulating body weight in lean and obese subjects.⁵⁸ More specifically, taste preference profiles for sweet solutions were shown to be a

sensitive index of nutritional status of the organism.⁵⁹ Eiber and her co-workers observed a decrease in hedonic response of patients when sucrose solutions were swallowed compared to when they were spit.³⁷ This reflects to the fact that AN patients in addition to their decreased ability to experience pleasure, do have excessive fear of gaining weight.

Modern neuroimaging studies revealed that there are taste information processing deficits in the insula and ventral and dorsal striatum present even in recovered restrictive-type anorexics.⁶⁰ Disturbance of the relevant perceptual-motivational mechanisms in AN is also substantiated by the findings of Tóth and her co-workers who reported a lower omega complexity during taste exposure,⁴² and found a significantly higher proportion of theta and lower percentage of alpha1 activity in anorexic patients.⁴³ Our functional neuroimaging studies are in harmony with the above mentioned results. The AN patients showed reduced activation after stimulating with sucrose in the insula and in the opercular cortex, both are in the primary gustatory cortex and in the anterior cingulate cortex which is responsible for the hedonic evaluation of the given stimulus. When stimulating with quinine besides the elements of the primary gustatory cortex, and the OBF which is also involved in hedonic evaluation, with other forebrain structures (pallidum, putamen) the caudate showed decreased activation as well. Previous fMRI studies raised the possibility of a certain calorie-fear in the background of AN.³⁸ Our imaging investigations could supply a new theory. The AN patients showed decreased activation to “pure” taste stimuli, but for solutions with higher consistency, the eating disorder patients showed a significantly higher activation compared to healthy controls in cortical regions (OBF, middle frontal gyrus) and in limbic structures (putamen, NAcc). This could raise the possibility, that the calorie-content might not have an inhibiting effect, but the emotional-motivational effect of taste stimulus with a hedonic value. The patient could associate pleasant or unpleasant memories to taste stimuli, and similar to conditioned taste aversion, therefore it could reduce food intake. Our results showed evidence for the existence of taste-associated complex perceptual-motivational disturbance in restrictive type AN patients. To achieve a better understanding of the background of symptoms of this complex disease may also contribute to developing well-targeted and more effective therapeutic approaches.

Obesity

In the present study, gustatory stimulation induced differential fMRI brain activation patterns were revealed in obese patients compared to healthy control subjects. In general, to date, relatively few imaging studies aimed to identify brain areas involved in gustatory perception in relation to the ingestion of various taste solutions. Early PET studies demonstrated the crucial role of the insular and opercular cortices and that of the OBF in taste information processing.⁶¹⁻⁶⁴ Further fMRI and MEG experiments compared the brain activation effects of various pleasant and aversive taste stimuli in healthy volunteers.^{65, 66} In addition to demonstrating the activation of the insula and the opercular cortex in both studies, the fMRI investigations revealed that the OFC and the AMY get activated not only by a pleasant taste stimulus but by an aversive one as well.

Imaging studies in obesity so far mainly focused on recognizing differential responsiveness of patients to various food cues. In PET examinations, the rCBF was found higher in the right parietal and temporal cortices in the obese patients compared to control subjects during the presentation of food items vs. non-food items. In addition, in the obese women, the activation of the right parietal cortex was associated with an enhanced feeling of hunger when looking at food.⁶⁷ Similar results were assessed among obese children by using fMRI.⁶⁸ It was found that the obese group showed increased dorsolateral prefrontal cortex (dlPFC) activity for visual food stimuli, and furthermore, their heart rate deceleration showed a positive correlation with the activation in the ventrolateral PFC. In a recent study, increased PFC activity was observed in obese children to food pictures in a starving state, and no activity reduction during satiation in the PFC/OFC as well as in other reward processing regions such as the nucleus accumbens.⁶⁹

Our results are in agreement with these above findings. Compared to controls, in the obese patients, significantly higher activation was observed to the hedonically positive stimulus sucrose in the secondary gustatory cortex (OFC) and the cingulate cortex, both responsible for coding the reward value of a given taste.⁷⁰ Observations of Faurion et al showed that there can be a lateralized activation in the gustatory cortex (mainly in the inferior insula) for taste stimuli, which could be related to handedness.⁷¹ Our findings are in full harmony with these results. All of the participants were right-handed in the present study. Although we did not find lateralized activity in the primary gustatory cortex, but the secondary taste cortices showed lateralization. Brain areas with significantly higher activation to the sweet stimulus in the obese group were identified in the left OFC, and in case of the quinine, and the high calorie condition, the activation of the OFC was more prominent on the left side.

The within-group analysis of the quinine vs. water condition provided evidence for significantly increased activation of the primary (insular, opercular) and secondary (OFC) taste cortices, putamen, caudate and different limbic areas (AMY, NAcc, pallidum) in the obese compared to control subjects. This result, interpreted as the obese patients showed a pronounced finickiness to quinine, is in concordance with the classic data of Kennedy who demonstrated that hyperphagic rats in the static phase of obesity developed finickiness.⁷²

In case of stimulating by the vanilla flavoured nourishment as a high-calorie multimodal stimulus, significantly higher activation was found in the obese group in the OFC, limbic areas, the pallidum, putamen and caudate nucleus, structures involved in sensing fat content and the palatability of food.⁷³ Enhanced activation was also detected in the parietal operculum where the oral somatosensory cortex is localized. This result is in agreement with findings of another study demonstrating increased resting activity of the somatosensory cortex in obese patients.⁷⁴ The high activation of the OFC in the obese group in the vanilla condition is well substantiated by previous reports on the preference of sugar/fat mixtures by obese subjects.⁷⁵ The OFC also has been shown to exert liquid food stimulus related activity depending on the subjective pleasantness of the given food. In our present study, for the vanilla

flavoured high-calorie liquid food stimulus, the VAS hedonic scores also positively correlated with the activation in the OFC, opercular cortices, and in the middle frontal gyri as well.

Our results support an explanation for the motivational background of overeating in obesity. The patients, compared to controls, had an increased activity for sucrose in the OFC. This can be due to an enhanced motivation to eat more of the pleasant foods, because this region are responsible for „scoring” the reward value of a food or any other stimulus.⁷⁶ The higher the given intrinsic score the more palatable the given food or taste is, therefore, more and more will be consumed of it to feel more pleasure. This theory is supported by the findings of Schäfer and colleagues.⁷⁷ They have shown that the structural abnormality of the OFC could have a crucial role in binge eating disorder and bulimia nervosa by affecting the reward processing and self-regulatory mechanisms as well.

Results of previous neurophysiological experiments in the rat and rhesus monkey raise the possibility that malfunctioning of the network of the so called glucose-monitoring (GM) neurons could be in the background of the taste perception alterations of obese patients. These hierarchically organized special chemosensory neural cells are found in several brain regions (such as the hypothalamus,^{13, 78} amygdala⁷⁹ globus pallidus,^{17, 21} nucleus accumbens,²⁴ orbitofrontal and mediodorsal prefrontal cortices^{23, 80}), and are known to respond to various endogenous (e.g. neuromodulators, changes of the blood glucose level, insulin) and exogenous signals, among others, gustatory stimuli as well.^{13, 17, 21, 49, 78, 80} The selective destruction of these neurons reportedly elicit characteristic, type 2 diabetes mellitus like homeostatic disturbances and taste perception deficits.⁸⁰⁻⁸² In the present study, the obese patients displayed significantly enhanced activation to intraorally delivered stimuli of differential hedonic value and palatability, predominantly just in those limbic forebrain regions (OFC, AMY, NAcc, pallidum) where the above mentioned GM neurons have been localized. In obesity dysfunctions of the chemosensory cells in the AMY and NAcc, key structures of the reward circuitry, may have a distinguished role in overeating due to their altered responsiveness to tastes.

Primate experiments

Our imaging studies on primates also support the central role of the glucose-monitoring neurons in the regulation of metabolism. After multiple glucose loads, activity changes could be observed in the region of the hypothalamus, the AMY and the OBF. The increase in blood glucose also activated the relevant humoral-vegetative-visceral centers which contain GM neurons as well.⁸³ Our findings are in full harmony with previous investigations in which the presence of GM neurons were proven in the hypothalamus,^{13, 48-50} in AMY⁷⁹, in the OBF⁸¹, and in the mdPFC as well.^{19, 23}

The repeated i.v. glucose administration, which could be the model of the rapid, repeated food or carbohydrate intake, causes significant humoral-metabolic and generalized homeostatic changes (fluctuation in hormone and metabolite levels, cardiovascular-circulatory alterations). The coordinated appearance of compensatory processes to these changes, in order to maintain the balance of homeostasis, is of

distinguished significance, and this has become possible by the increased activation of brain regions demonstrated in the above investigations.^{13, 47, 49, 84-86}

Extending these primate studies may supply more data and help to understand the pathophysiological processes in obesity, eating disorders and metabolic diseases as well.

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Own publications

I. Papers

A. Publications related to the thesis

Csaba Szalay, Mihály Aradi, Attila Schwarcz, Gergely Orsi, Gábor Perlaki, Livia Németh, Sophia Hanna, Gábor Takács, István Szabó, László Bajnok, András Vereczkei, Tamás Dóczi, József Janszky, Sámuel Komoly, Péter Örs Horváth, László Lénárd and Zoltán Karádi: Gustatory perception alterations in obesity: an fMRI study. *Brain research* 1473:131-140, 2012 IF:2,728

Vereczkei András, Szalay Csaba, Aradi Mihály, Schwarcz Attila, Orsi Gergely, Perlaki Gábor, Karádi Zoltán, Németh Livia, Hanna Sophia, Takács Gábor, Szabó István, Bajnok László, Mohos Elemér, Lénárd László, Dóczi Tamás, Janszky József, Komoly Sámuel, Horváth Örs Péter: Functional MRI investigation of brain activity triggered by taste stimulation. *Magyar Sebészet* 2011; 64(6): 289–293

Csaba Szalay, Ildikó Ábrahám, Szilárd Papp, Gábor Takács, Balázs Lukács, Ágnes Gáti, Zoltán Karádi: Taste reactivity deficit in anorexia nervosa. *Psych. Clin. Neurosci.* 64(4):403-7 (2010) IF:1.559

B. Further publications

Takács G, Szalay C, Nagy B, Szabó I, Simon D, Berki T, Karádi Z Insulin and leptin plasma levels after the microinjection of interleukin-1 β into the nucleus accumbens of the rat. *Acta Physiol Hung.* 2012; 99(4):472-8. IF:0.75

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G. Orsi, G. Perlaki, N. Kovács, M. Aradi, Z. Papp, K. Karadi, C. Szalay, Z. Karadi, L. Lenard, T. Tenyi, E. Plozer, R. Gabriel, F. Nagy, T. Doczi, S. Komoly, H. Jokeit, A. Schwarcz, J. Janszky: Body weight and the reward system: the volume of the right amygdala may be associated with body mass index in young overweight men. *Brain imaging and behavior* 5:149-157 (2011) IF: 1.044

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M. Aradi, R. Steier, P. Bukovics, C. Szalay, G. Perlaki, G. Orsi, J. Pál, J. Janszky, T. Dóczi, A. Schwarcz: Quantitative proton MRI and MRS of the rat brain with a 3 T clinical MR scanner. *J Neuroradiol* 38(2):90-97 (2011), doi:10.1016/j.neurad.2009.11.003 IF: 2.1

Gábor Takács, Szilárd Papp, Balázs Lukáts, Csaba Szalay, Bernadett Nagy, Dimitrios Fotakos, Zoltán Karádi: Homeostatic alterations after IL-1b microinjection into the nucleus accumbens of the rat. *Appetite* 54 (2010) 354-362 IF: 2.341

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Zoltán Karádi, Balázs Lukáts, Szilárd Papp, Csaba Szalay, Róbert Egyed, László Lénárd and Gábor Takács (2005). Involvement of forebrain glucose-monitoring neurons in taste information processing: Electrophysiological and behavioral studies. *Chemical Senses* 30 Suppl 1:i168-i169 IF 2.691

II. Book section

Lukáts, B., Egyed, R., Papp, S., Takács, G., Szalay, Cs., Lénárd, L. & Karádi, Z. (2006). Involvement of the orbitofrontal cortical IL-1 β mechanisms in the central homeostatic control. In K. Ishii, K. Natsume & A. Hanazawa (Eds.), *International Congress Series*, (Vol. 1291, pp. 137-140): Elsevier.

III. Conference abstracts

Gálosi, R., Szalay, Cs., Aradi, M., Pál, J., Perlaki, G., Schwarcz, A., Lénárd, L., and Karádi, Z.: Examination of MnCl₂ toxicity to develop a useful method for activity-induced MEMRI in 3T clinical scanner. 14th Conference of the Hungarian Neuroscience Society (MITT), 2013. január 17-19. Budapest, Hungary

Szalay, C, Aradi, M, Schwarcz, A, Orsi, G, Perlaki, G, Németh, L, Takács, G, Gáti, Á,

Lénárd, L and Karádi, Z: Taste perception disturbances in anorexia nervosa: fMRI investigations. IBRO International Workshop, 2012. január 19-22, Szeged, Hungary

Gálosi, R, Szalay, C, Aradi, M, Pál, J, Perlaki, G, Schwarcz, A, Lénárd, L and Karádi, Z: Mapping of brain structures involved in complex behavioural processes: Application of manganese-enhanced magnetic resonance imaging in rats., IBRO International Workshop, 2012. január 19-22, Szeged, Hungary

Szalay C, Aradi M, Auer T, Orsi G, Schwarcz A, Hanna S, Nemeth L, Nagy B, Takács G, Lénárd L and Karadi Z (2009). Human and Monkey fMRI Pilot Experiments in the Understanding of Central Regulatory Disturbances of Feeding and Metabolism. *Frontiers in Systems Neuroscience*. Conference Abstract: 12th Meeting of the Hungarian Neuroscience Society. doi: 10.3389/conf.neuro.01.2009.04.020

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Szalay Cs., Aradi M., Auer T., Schwarcz A., Hanna S., Németh L., Nagy B., Takács G., Lénárd L. és Karádi Z.: A funkcionális MR alkalmazása táplálkozási és metabolikus betegségek központi szabályozási zavarainak megértésében: bevezető kísérletek A Magyar Neuroradiológus Társaság 17. Konferenciája Pécs 2008. november 6-8.

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