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Abstracts

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Acute modulation of glucagon-like peptide-1 (GLP-1) signaling is not involved in the control of energy expenditure after Roux-en-Y gastric bypass (RYGB) surgery in rats

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Compared to traditional weight loss strategies, the compensatory decrease in energy expenditure (EE) in response to body weight loss is attenuated after RYGB surgery. Because basal and postprandial GLP-1 levels are increased after RYGB surgery, and because GLP-1 has also been shown to increase EE, we investigated if increased GLP-1 levels are involved in the alterations in EE after RYGB. Adult male Wistar rats were randomized for RYGB (n = 8) or sham surgery (n = 17). Part of the sham operated rats were food restricted and body weight-matched (BWm, n = 8) to the RYGB animals. The effects of acute subcutaneous administration of the GLP-1 antagonist exendin-9 (30 mg/kg) and the GLP-1 agonist exendin-4 (5 mg/kg) on EE were tested using indirect calorimetry. Rats were fasted during the light cycle before injections with exendin-9 (ex-9) and exendin-4 (ex-4), respectively. EE was measured in the fasted state for 1 h and during ad libitum access to food, and food intake (FI) was recorded. Ex-9 increased FI only in RYGB rats. EE was lower in RYGB and BWm compared to sham operated, ad libitum fed rats, but significantly higher in RYGB compared to BWm. There was no effect of ex-9 treatment on EE in either group of animals. Similarly, ex-4 decreased FI more in RYGB than in sham rats but did not modulate EE. We conclude that acute modulation of GLP-1 signaling is not directly involved in the altered EE after RYGB surgery in rats.

Roux-en-Y gastric bypass (RYGB) surgery leads to reduced bone mineral density (BMD) and metabolic acidosis in rats

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A decrease in BMD has been reported after RYGB surgery, potentially associated with vitamin D (VD) and calcium (Ca) malabsorption and consequential secondary hyperparathyroidism. However, because these conditions are frequently present in the obese population, it is unclear if they are the primary cause for bone changes after RYGB. We investigated changes in bone metabolism and Ca balance in rats after RYGB compared to a body-weight matched (BWm) control group. Adult male Wistar rats were randomized for RYGB (n = 15) or sham (n = 17) surgery. Part of the sham rats were food restricted and BWm (n = 8) to RYGB. Pre-op and at 2, 7 and 14 weeks post-op, BMD was measured by micro computed tomography (CT). Twenty-four hours Ca intake and fecal and urinary Ca loss were measured and blood sampling was performed for blood gas analysis and to determine Ca, VD, parathormone (PTH) and osteocalcin (OC) levels. BMD progressively decreased in RYGB in the first two post-op CT scans but then remained stable until week 14. Fecal Ca loss was only increased in RYGB rats 2 weeks after surgery, while urinary Ca loss remained elevated. RYGB rats had a lower blood pH, lower bicarbonate levels and higher lactate levels. Ca, PTH and OC levels were unchanged, but active VD was significantly increased. We conclude that RYGB rats are in metabolic acidosis, which may contribute to increased bone resorption. Potentially due to higher VD activation, Ca malabsorption was only present early after surgery and seems not to be the only cause of the bone loss.

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Effects of soluble dietary fibre on appetite, adiposity and gut satiety hormone secretion in rats

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Dietary fibre is thought to impact on satiety and may thereby contribute to healthy weight management. This experiment aimed to establish the effects on food intake, body weight, adiposity and gut satiety hormone secretion of increased levels of three major groups of soluble dietary fibres (complex carbohydrates), namely beta-glucan, pectin and fructooligosaccharide. The isocaloric diets, based on a standard purified rat diet with 5% cellulose (C) as an insoluble dietary fibre source, contained 10% cellulose (L) or 10% (approx. 26 mg/kcal) soluble fibre as oat beta-glucan (G), apple pectin (P), or fructooligosaccharide (F). Individually-housed healthy adult male Sprague Dawley rats (12 weeks old, 467 ± 6 g) were offered the pelleted diets *ad libitum* for 4 weeks (n = 10/group). Daily voluntary food intake and twice-weekly body weight measurements were taken, initial and final MRI scans performed, and final (trunk) blood samples analysed (by RIA kits) for satiety hormones GLP-1 and PYY. Cumulative food intake was decreased by 20% ($P < 0.001$), body weight gain decreased by 60% ($P < 0.001$), body fat content was 30% lower ($P < 0.001$), and plasma concentrations of total GLP-1 and PYY were elevated three-fold (both $P < 0.001$) in G, P and F groups compared with C and L groups. Therefore, increased soluble fibre of three different types in the diet decreased appetite, body weight gain and body fat content, producing a healthier long-term phenotype, and the data were consistent with the gut hormones GLP-1 and PYY playing a mediating role. Study supported by: Scottish Government RESAS Department.

How hunger affects VTA neuronal activity associated with reward-related behaviour and food choice

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Feeding is regulated by several defined neural circuits that control various aspects of feeding-behaviour such as satiety and the anticipation to food. We here addressed how the mesolimbic dopamine (mesDA) system, which signals reward-related information such as reward size and value, is implicated in feeding behaviour. In vivo electrophysiological recordings were made of (putative) DA neurons in the ventral tegmental area (VTA). Recordings were made during the execution of a behavioural task in which animals, when presented with a cue, were able to obtain food rewards that differed in reward value. Cue-onset, reward delivery and subsequent consumption were related to neuronal activity. We identified neurons that specifically increased their firing towards preferred versus non-preferred food. The modulation of VTA neuronal activity as well as behavioural performance following food restriction, leptin and ghrelin administration, are subsequently assessed in combination with optogenetics. Elucidation of the role of leptin and ghrelin signaling on mesDA neurons in relation to feeding behaviour might provide important insights into the role of this neural circuit in obesity and anorexia nervosa.

Liking for dairy and meat products and vegan substitutes. Influence of cognition

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Motivated by the increased availability of vegan substitutes for animal products, this study investigated liking for the products themselves and how much the idea of a products' being vegan affects liking for it. Two groups of subjects (40 subjects/group) tasted and rated how much they liked the taste of either vegan or animal-based dairy (chocolate milk and macaroni&cheese) and meat (meatballs and chicken tenders) products. Half of each group was told that the products were of animal origin and the other half was told that the products were vegan substitutes prior to tasting and rating. A 2 (foodtype – animal vs. vegan) by 2 (told – animal vs. vegan) by 4 (food – chocolate milk, macaroni&cheese, meatball, chicken tender) mixed ANOVA found a significant food × foodtype interaction [F (3, 228) = 13.26, $p < 0.001$] with subjects liking the animal-based chocolate milk more than the vegan version. A significant main effect [F (1, 76) = 6.03, $p < 0.02$] indicated that subjects who were told that the products were vegan liked them more than did subjects who were told they were animal-based. Thus, thinking a food is vegan increases liking for that product. The results are discussed in relation to expectations, familiarity, willingness to try the foods, and disgust. Supported by: Gardein and Amy's.

The control of food intake by mNTS leptin-receptor expressing neurons may involve monosynaptic communication with hypothalamic and mesolimbic nuclei

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Leptin receptors (LepRb) in the medial nucleus tractus solitarius (mNTS) are essential for the regulation of energy balance; however, the behavioral, molecular, and neuroanatomical mechanism(s) that mediate the intake-suppressive effects of mNTS LepRb activation are not yet defined. Here, we begin to test the hypothesis that direct signaling from mNTS LepRb neurons to hypothalamic/mesolimbic nuclei is necessary for the control of *appetitive feeding behavior*. Meal pattern analysis following unilateral mNTS leptin (0.1, 0.3, 0.6 mg/100 nl) revealed significant dose-dependent decreases in cumulative chow intake and in meal size from 2–24 h, and a decrease in meal number at 12 and 24 h post injection ($p < 0.05$). Double IHC was performed in the caudal brainstem for visualization of the monosynaptic retrograde tracer Fluorogold (2% in 300 nl; injected in LH, VTA, or NAc shell) and 4icv leptin-induced pSTAT3, a marker of LepRb signaling. Preliminary IHC data indicate that approximately 9.2, 6.8, and 10.5% of LepRb-expressing mNTS neurons project to LH, VTA, and NAc shell, respectively. Collectively, data provide behavioral and anatomical evidence for a mNTS LepRb-mediated reduction in meal size and number that may involve the engagement of higher-order appetitive or reward processing via direct connections to hypothalamic and mesolimbic nuclei. Supported by: NIH-DK21397 & -DK085435.

Role of the mTORC1 pathway in determining the weight-loss and neuro-proliferative effects of the ciliary neurotrophic factor in mice

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The mammalian target of rapamycin complex 1 (mTORC1) pathway regulates cellular responses to fuel status in the hypothalamus. Hypothalamic mTORC1 signaling is required for the acute appetite-suppressant action of the cytokine ciliary neurotrophic factor (CNTF), whilst induction of hypothalamic neurogenesis seems to explain the maintenance of the CNTF-induced body weight loss observed beyond treatment cessation. Thus, here we studied the role of the mTORC1 pathway in mediating the long-term effects CNTF on energy balance and hypothalamic cell proliferation. Two-weeks icv administration of the CNTF analogue axokine ($CNTF_{Ax}$) did not bear any effect on mice fed a standard chow, while it significantly reduced body weight and fat mass of high-fat fed wild-type (WT) mice but not of mice lacking S6 Kinase 1 ($S6K1^{-/-}$), a downstream mTORC1 target. In WT mice only, $CNTF_{Ax}$ treatment significantly decreased respiratory quotient and increased lipolysis while inhibiting fatty acid synthesis in the white adipose tissue. Finally, neuroanatomical analysis showed that $CNTF_{Ax}$ significantly increased the number of cells positive for the mTORC1 target phospho-S6 and the number of proliferating cells in the hypothalamus of WT but not $S6K1^{-/-}$ mice. Thus, the results obtained so far suggest that the effects of CNTF on energy balance and cell proliferation require a functional mTORC1 pathway. Supported by: ANR, INSERM, FP7-Marie Curie IRG.

Intraperitoneal (IP) glucagon-like peptide-1 (GLP-1) injections and meals in rats increase intestinal lymphatic GLP-1 similarly

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GLP-1 is released into the lamina propria of the intestinal mucosa and accumulates in intestinal lymph (IL). IP administration of GLP-1 also increases IL GLP-1 and may mimic a paracrine satiating effect of endogenous GLP-1 on intestinal vagal afferents. We therefore implanted chronic IL duct cannulas in adult rats and tested the effect of IP administered GLP-1 on IL concentration of free GLP-1 and on eating in rats after subdiaphragmatic vagal deafferentation (SDA) or sham operation. Confirming previous findings of ours (Rüttimann et al., Endocrinology 150, 1174–1181, 2009) 10 nmol/kg body weight GLP-1 reduced meal size in Sham but not in SDA rats, indicating that intact vagal afferents are necessary for the satiating effect of IP administered GLP-1. In response to 10 nmol/kg GLP-1 (n = 8) injected prior to a 2.0 g high fat meal IL GLP-1 increased from 3 pmol/L (0 min) to 19 ± 3 pmol/L (mean ± SEM) at 40 min (p < 0.05 vs. 10 ± 1 pmol/L for vehicle) and remained elevated (7 ± 2 pmol/L) until 2 h after meal onset. In response to 1 nmol/kg GLP-1 (n = 7) prior to the test meal IL GLP-1 increased to 14 ± 3 pmol/L at 20 min (p > 0.05 vs. 16 ± 4 pmol/L for vehicle) and returned to baseline at 2 h. These data suggest that increases in interstitial fluid GLP-1 concentration within the physiological range are sufficient to inhibit eating through intestinal vagal afferent signaling. This is consistent with a paracrine satiating effect of endogenous GLP-1.

Influence of the food selection criteria on the attitude towards food

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Asakawa and Okano (2009) extracted the following five factors from Japanese consumers by using the Food Choice Questionnaire (FCQ; Steptoe, Pollard, and Wardle, 1995): (1) mood, (2) safety, (3) convenience, (4) weight control, and (5) price. In this study, we examined the relationship between the food choice criteria and the attitude towards various foods. A total of 720 subjects answered 36 items in the FCQ and rated the following foods according to their likes and dislikes (like = 3, neutral = 2, dislike = 1): High-class chocolate, real coffee, apple, Big Mac, banana, cup ramen noodles, chocolate with a lot of polyphenols, pork cutlet, plastic bottles of green tea with a lot of catechin, fermented soybeans, domestic vegetables, and imported vegetables. We used structural equation modeling to examine relationships among the five factors and the subjects' rating of each food. Pollard, Steptoe, and Wardle (1998) reported that those who consider ''health,'' ''natural content,'' and ''weight control'' important tend to choose foods that are good for health. In this study, those who considered ''weight control'' important tended to like healthy foods such as plastic bottles of green tea with a lot of catechin and chocolate with a lot of polyphenols. Moreover, those who considered ''safety'' important tended to prefer domestic vegetables and foods made from high-quality ingredients, even though their prices were high. Further, they tended to avoid foods with many additives, such as cup ramen noodles or a Big Mac, even though such foods were easy to eat.

Estradiol (E2) increases body-weight loss and gut-peptide satiation after Roux-en-Y gastric bypass (RYGB) in ovariectomized (OVX) rats

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Although ~85% bariatric surgeries are performed in women and estrogens potently affect eating and weight regulation, the effects of the reproductive-axis function on bariatric-surgery outcome are unclear. Thus, we investigated the effects of RYGB on body weight and GLP-1 and CCK satiation in OVX rats with or without physiological E2 replacement. Rats were fed high-fat diet and chow for 3 wk, then underwent OVX and either RYGB or sham surgery (d 0). E2 (2 μg E2 benzoate/rat-4 d, sc) or oil-vehicle treatment (100 microl/rat) was begun on d 12. Starting on d 29, rats were fed Ensure Plus and chow until d 40. E2 significantly increased the effects of RYGB on eating and body weight (d 40: RYGB-oil rats lost 60 g vs. sham-oil rats, RYGB-E2 rats lost 110 g, P < 0.01). Daily energy intakes were similarly changed. Starting d 41, we tested the acute effects of Exendin (9–39) (100 μg/kg, IP) and Devazepide (1 mg/kg, IP) on Ensure test meals. E2 increased the eating-stimulatory effects of GLP-1 and CCK antagonism similarly in RYGB and sham rats, indicating that both peptides contribute to the control of eating after RYGB, but suggesting that RYGB does not affect their potencies. Consistent with this, E2 increased the numbers of ileal-mucosal cells immunopositive for GLP-1 but RYGB did not influence this effect. E2 increases the efficacy of RYGB, but the mechanisms remain unclear. Supported by: RO1-DK092608.

Estradiol (E2) increases meal-induced hindbrain c-Fos expression after Roux-en-Y gastric bypass (RYGB) in ovariectomized (OVX) rats

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Bariatric surgery is the most effective treatment for obesity. Eighty-five percentages of bariatric operations are done in women, but the effects of the reproductive-axis function on bariatric surgery outcome remain unclear. Here we tested the effects of RYGB and E2 on eating-induced hindbrain neural activity using c-Fos immunocytochemistry. Rats were fed high-fat diet and chow for 3 wk, then underwent OVX and either RYGB or sham surgery (S; d 0). E2 (2 μg E2 benzoate/rat-4 d, sc) or oil-vehicle treatment (100 microl/rat) was begun on d 12. Starting on d 29, rats were fed Ensure Plus and chow. Sixty minutes Ensure intake was measured in non-deprived rats at dark onset. On d 42, rats were food deprived 3 h before dark onset, fed 2.5 ml Ensure at dark onset, killed 90 min later, and c-Fos-positive cells/section evaluated in the subpostremal (sp) NTS and dorsal raphe (DR). Data for S-oil, S-E2, RYGB-oil, RYGB-E2, respectively, were: (1) 60-min intake (ml): 8 ± 1, 5 ± 0, 4 ± 0, 3 ± 0; (2) spNTS c-Fos: 2 ± 1, 16 ± 1, 92 ± 8, 185 ± 10; (3) DR c-Fos: 2 ± 1, 9 ± 0, 22 ± 2, 64 ± 5. That E2 decreased eating in RYGB rats despite their low control values parallels the exaggerated reductions in daily energy intake and weight loss in RYGB-E2 rats (not shown). The c-Fos data suggest that increased activity in NTS and DR pathways contribute to E2's effects on eating and weight regulation after RYGB and provide a platform for mechanistic studies. Supported by: RO1-DK092608.

Involvement of central cholinergic mechanisms on fluid intake induced by deactivation of the lateral parabrachial nucleus

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In the present study, we investigated the effects of atropine (muscarinic cholinergic antagonist) injected into the lateral ventricle (LV) on 0.3 M NaCl and water intake produced by muscimol ($GABA_A$ agonist) or by moxonidine (α_2-adrenoceptor/imidazoline agonist) injected into the lateral parabrachial nucleus (LPBN), respectively, in fluid-replete rats or in rats treated with furosemide + captopril subcutaneously (FURO + CAP). Male Holtzman rats (290–310 g, *n* = 7–8/group) with guide-cannulas implanted into the LV and bilaterally into the LPBN were used. In fluid-replete rats, bilateral injections of muscimol (0.5 nmol/0.2 μL) into the LPBN induced 0.3 M NaCl (32.2 ± 9.9, vs. saline: 0.4 ± 0.2 mL/4 h) and water intake (11.4 ± 4.4, vs. saline: 0.8 ± 0.4 mL/4 h), an effect reduced by atropine (20 nmol/1 μL) injected into the LV (0.3 M NaCl: 13.5 ± 5.0 mL/4 h; water: 2.9 ± 1.6 mL/4 h). Moxonidine (0.5 nmol) injected into LPBN increased FURO + CAP-induced 0.3 M NaCl intake (36.5 ± 9.8, vs. vehicle: 5.6 ± 2.0 mL/2 h) without changing water intake (15.6 ± 4.8, vs. vehicle: 9.1 ± 2.6 mL/2 h). Atropine injected into the LV did not modify FURO + CAP-induced 0.3 M NaCl (26.8 ± 6.2 mL/2 h) or water intake (14.4 ± 2.5 mL/2 h) in rats treated with moxonidine into LPBN. The results suggest that 0.3 M NaCl and water intake induced by the deactivation of the inhibitory mechanisms with muscimol injected into the LPBN is facilitated by central cholinergic mechanisms. Financial Support: FAPESP, CNPq. Supported by: FAPESP, CNPq.

Sexually dimorphic BOLD signaling and functional neural connectivity via fMRI in response to high vs. low energy-dense food cues in obese people

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There is evidence of sexually dimorphic behavioral and biological bases of human eating. Here we investigated sex-based differences in blood oxygen level-dependent (BOLD) signal via functional magnetic resonance imaging (fMRI) in response to high energy-dense (high-ED) vs. low-ED visual and auditory food cues in obese men (*n* = 17) vs. women (*n* = 14) in fed and fasted states. In response to high-ED (vs. low-ED) cues, obese men (vs. women) had higher BOLD signaling in areas related to attention and cognitive control, whereas obese women (vs. men) had higher BOLD signaling in emotion-related regions. Functional neural connectivity via psychophysiological interaction analysis was conducted using the ventral striatum and ventral anterior cingulate (key reward areas), and amygdala (key emotion processing area) as "seeds". Gender-based differences were explored with group level analyses. When fasted, obese men had higher functional connectivity in motor planning/execution and memory-related areas with seeds, whereas for obese women when fasted, there was higher functional connectivity in cognitive processing areas with seeds. When fed, obese men had higher functional connectivity in reward-related decision-making regions with seeds, whereas for obese women there was higher functional connectivity in cognitive processing/action planning areas with seeds. These findings may help facilitate new gender-specific treatment and prevention strategies for obesity. Supported by: NIH.

Gustatory cortex lesions do not disrupt perithreshold taste sensitivity to sucrose in rats

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Damage to gustatory cortex (GC) has been reported to interfere with conditioned taste aversion (CTA) learning, but taste sensitivity has never been psychophysically assessed. Here, after ingestion of 0.1 M NaCl (15 min), 22 rats were injected (2 mEq/kg, IP) with LiCl and 22 rats with NaCl. An equal number of rats from each injection group then received ibotenic acid lesions targeting GC (*n* = 30) and the rest were given PBS vehicle infusions into GC (*n* = 14). After postsurgical testing for CTA retention, the rats were trained in a gustometer in an operant 2-response taste detection task and psychometric functions for sucrose were measured. Rats were histologically divided blindly into groups based on the quality of their lesions. Three LiCl- and six NaCl-injected rats were deemed to have extensive bilateral damage to GC. Of these, one LiCl-injected rat retained, one partially retained, and one did not retain the CTA as assessed in a 30 min 1-bottle test. Interestingly, three excluded LiCl-injected rats with small lesions that were more posterior, including areas caudal to GC, showed deficits in expressing the CTA. Regardless of quality, the lesions did not lead to deficits in the EC50 of the psychometric function for sucrose (all rats mean = −1.99 ± 0.05 log M). These data suggest that GC is not necessary to maintain normal perithreshold taste sensitivity to sucrose in rats. More posterior regions of insular cortex, possibly outside of GC, could play a role in CTA retention. We are currently conducting a more quantitative assessment of lesion volume and location to refine our analysis. Supported by: NIH R01-DC-DC009821.

Neural systems that mediate food seeking and the influence of predicted and experienced value on choice

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Recent studies point to a number of learning and modulatory processes that contribute to food-seeking. Distinct learning processes mediate the acquisition of goal-directed and habitual actions, subserved by parallel circuits involving the dorsomedial and dorsolateral striatum, respectively. In addition, food can function as an incentive to reward actions, and as a reinforcer to strengthen habits. Evidence suggests that two forms of incentive process affect food seeking: (i) the experienced value of a particular food based on consummatory experience and (ii) the predicted value of a particular action based on cues that predict food delivery. Although incentive theories generally assume that these processes are controlled by a common associative mechanism, a number of recent findings suggest that they are dissociable behaviourally, anatomically and neurochemically. The latter predictive learning process may also play a role in habitual food-seeking, particularly in the function of the reinforcement signal, long ascribed to the dopaminergic input to dorsolateral striatum, that we have found is heavily regulated by the central amygdala. Although the complexity of food seeking provides a hurdle for the treatment of eating disorders, the suggestion that these apparently disparate determinants are functionally integrated within larger neural systems may provide novel approaches to these problems.

Dorsomedial hypothalamic NPY affects cholecystokinin-induced satiety via modulation of brainstem catecholamine neuronal signaling

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Dysregulation of NPY expression in the dorsomedial hypothalamus (DMH) has been shown to cause hyperphagia, but the pathway underlying this effect remains less clear. Hypothalamic neural systems play a key role in the control of food intake, in part, by modulating the effects of meal-related signals, such as cholecystokinin (CCK). An increase in DMH NPY expression decreases CCK-induced anorexia. Since activation of catecholamine neurons within the nucleus of solitary tract (NTS) contributes to the feeding effects of CCK, we hypothesized that DMH NPY modulates these NTS neural signals to affect food intake. We used an adeno-associated virus system to manipulate DMH NPY expression bi-directionally in rats to examine this pathway. Viral-mediated hrGFP anterograde tracing revealed that DMH NPY neurons projected to the NTS; the projections were in close proximity to catecholamine neurons as determined by tyrosine hydroxylase (TH) positive staining. Viral-mediated DMH NPY overexpression resulted in an increase in NPY contents in the NTS, a decrease in NTS TH expression and reduced exogenous CCK-induced satiety. Knockdown of DMH NPY expression produced the opposite effects. In addition, NPY administration into the 4th ventricle of intact rats limited CCK-induced satiety and overall TH phosphorylation. Together, these results demonstrate that DMH NPY descending signals affect CCK-induced satiety, at least in part, via modulation of NTS catecholamine neuronal signaling to regulate food intake. Supported by: NIH R01DK074269 and R01DK087888.

Circadian Genes of Behavior to Bioeanergetics

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Circadian systems are programmed by a transcription-translation feedback loop that generates daily cycles of energy storage and utilization in synchrony with the 24 h rotation of the Earth. In mammals, master pacemaker neurons located within the suprachiasmatic nucleus (SCN) are entrained to light, and these in turn generate 24 h oscillations in extra-SCN neurons and nearly all peripheral tissues, including those involved in glucose and lipid metabolism. During wakefulness and feeding, the molecular clock promotes glucose stimulated insulin secretion within ?-cells of the Islets of Langerhans. Clock ablation limited to pancreas results in hypoinsulinemic diabetes, indicating a primary function of peripheral clocks in glucose homeostasis. In contrast, ablation of the liver clock impairs oxidative metabolism, indicating that peripheral tissue clocks exert opposing effects in response to feeding and fasting. Our work focuses on how coordination of central and peripheral clocks contributes to long-term energy and glucose homeostasis across alternating behavioral (sleep-wake) and nutrient (fasting-feeding) states.

Fluid and electrolyte disturbances in the melanocortin-4 receptor deficient-rat

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Central melanocortin-4 receptor (MC4R) signaling has been extensively studied with regard to energy balance. Although ablation of MC4R increases food intake and decreases sympathetic output, the effect on fluid balance has not been well studied. Basal water intake in $MC4R^{-/-}$ rats was lower relative to that of $MC4R^{+/+}$ rats, and there was a slight reduction in basal sodium appetite. Water intake of $MC4R^{+/+}$ rats increased proportionally with food intake, whereas water intake was not correlated with food intake in $MC4R^{-/-}$ rats. Water intake in response to peripheral angiotensin-converting enzyme (ACE) inhibition was increased in both genotypes, but was significantly greater in $MC4R^{+/+}$ rats, indicating involvement of the renin-angiotensin system. Following 22 h fluid deprivation, $MC4R^{-/-}$ rats had low fluid intake and, unlike $MC4R^{+/+}$ rats, failed to regain fluid losses within 24 h. When gavaged with 2 M saline (5 mL), both $MC4R^{+/+}$ and $MC4R^{-/-}$ rats had significantly increased fluid intake, implying no disruption to osmoregulatory function. Additionally, urine output in response to gavaged normal saline (10 mL) was not reduced, suggesting no change in renal function. Overall, the data reveal a previously unreported role for the MC4R in thirst and fluid balance.

Feeding suppression and cardiovascular alterations of nisoxetine, a selective norepinephrine reuptake inhibitor

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Central noradrenergic pathways are involved in mediating feeding and cardiovascular control. One obstacle for safe and effective noradrenergic-acting obesity pharmacotherapy is developing a drug that reduces food intake and body weight with minimal cardiovascular impact. The purpose of these experiments was to determine the feeding inhibition of a selective noradrenergic reuptake inhibitor, nisoxetine. Acute administration of nisoxetine (3–10 mg/kg in saline; IP) dose dependently reduced food intake in male Sprague Dawley fed standard chow (Purina 5012). Nisoxetine produced a feeding suppression from saline baseline of 1.7% (3 mg/kg), 16.1% (10 mg/kg), and 34.5% (30 mg/kg) of 24 h intake. Tail-cuff volume pressure recording revealed only the 30 mg/kg dose of nisoxetine decreased mean blood pressure over the 24 h feeding period ($p < 0.01$). In order to determine the effects of diet-induced obesity on the feeding suppression of nisoxetine, male Sprague Dawley rats fed a high fat diet (60% fat; Research Diet 12492) or control diet (10% fat; Research Diet 12450B) were switch to a standard chow for 24 h. Nisoxetine (3 mg/kg) resulted in a 13.1% feeding suppression of standard chow in obese animals with a high fat diet exposure ($p < 0.05$) and only a 3.8% feeding suppression of standard chow in lean animals with control diet exposure (n.s.). Such data indicate alterations in the noradrenergic controls of feeding as a result diet and obesity. They also provide a basis for distinguishing how noradrenergic-acting compounds alter feeding behavior with minimal cardiovascular alterations.

Imitation of palatable food intake among normal-weight and overweight children

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We investigated whether children imitate the food intake of a normal-weight peer and assessed the role of the child's own weight status in this interaction. The participants ($N = 68$; 50% boys; age $M = 8.55$; 27.9% overweight) were asked to solve a puzzle with a same-sex normal-weight confederate who was instructed to take a chocolate-peanut (eating cue) when he/she was covertly signaled by a buzzer device by the experimenter. Each session (duration of 10 min) was videotaped. The total number of times the children picked food and the exact time at which each child picked food were coded and tested by means of a paired sample *t*-test (cue related picking ratios with non-cue related picking ratios) and multilevel proportional hazard models in a survival analysis framework. Findings showed that normal-weight as well as overweight children were more likely to eat in direct response to the confederates' eating than without such an eating cue. More specifically, normal-weight children were more likely to imitate the peer's food intake in the first 5-min compared to the second 5-min of the experimental session whereas overweight children were almost twice as likely to imitate the peer's intake in the second half compared to the first half of the session. This suggests that normal-weight children became less sensitive to eating cues of a peer whereas overweight children became more sensitive to these cues over time. Supported by: The present study was supported by a grant of the Behavioural Science Institute of the Radboud University Nijmegen, The Netherlands.

Role of dorsomedial hypothalamic NPY in energy balance control

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Within the hypothalamus, the orexigenic peptide NPY is primarily expressed in the arcuate nucleus (ARC) and the dorsomedial hypothalamus (DMH). While the actions of ARC NPY in the control of energy balance have been well studied, the importance of DMH NPY in maintaining energy balance is less understood. To determine such importance or potential roles for DMH NPY, we have generated recombinant vectors of adeno-associated virus (AAV)-mediated expression of NPY (AAVNPY) or knockdown of NPY expression (AAVshNPY) and examined whether AAV-mediated manipulation of NPY expression in the DMH affects food intake and/or energy expenditure in rats. We found that while NPY overexpression in the DMH results in increased food intake and body weight, knockdown of NPY expression in the DMH decreases food intake and body weight. These feeding effects of DMH NPY are specifically through affecting dark-period meal size. We further found that DMH NPY serves as an important modulator of energy expenditure, likely through affecting brown adipocyte-thermogenesis and physical activity. NPY knockdown in the DMH promotes white into brown adipocyte transformation and influences lipid mobilization. Finally, DMH NPY overexpression exacerbated high fat diet-induced increases in food intake and body weight, whereas DMH NPY knockdown attenuates these diet-induced hyperphagia and obesity. Together, our results demonstrate that DMH NPY plays an important role in the control of energy balance. Supported by: NIH R01DK074269 and R01DK087888.

Gastrointestinal vagal afferent innervation and meal patterns in mice with peripheral BDNF knockout

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The vagus nerve transmits information regarding the nutritional status of the gastrointestinal (GI) tract to the brain. Brain-derived neurotrophic factor (BDNF) is expressed in the developing GI tract and may shape vagal development or function. To study the long-term effects of BDNF on vagal development, targeted smooth muscle BDNF knockout (BDNF SM KO) mice were generated. Vagal afferent innervation and analysis of feeding behavior in BDNF SM KO mice were compared to controls. To label vagal afferent innervation in the GI tract, the nerve tracer horseradish peroxidase conjugated to wheatgerm agglutinin was injected into the nodose ganglion. This method labels mechanoreceptors called intraganglionic laminar endings (IGLEs) located in smooth muscle of the GI tract. Meal patterns were collected at 3–4 mo of age to study changes in feeding behavior. Preliminary results indicate BDNF SM KO mice showed significantly increased IGLEs in the intestine (54%) compared to controls, whereas those supplying the stomach exhibited normal density. Total daily food intake was not different between the groups. However, trends toward smaller meal size and increased intermeal interval in BDNF SM KO resulted in significantly increased satiety ratio compared to controls. During meal pattern collection, there were no body weight differences between the groups, although at time of nodose ganglion injection (5–6 mo), BDNF SM KO mice displayed a reduction in body weight compared to controls (13%). These results suggest peripheral BDNF may normally suppress or limit the development of intestinal IGLEs. Supported by: NIH.

Effects of insulin on phenotypically-identified neurons of the nucleus tractus solitarius

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In addition to regulating glucose utilization peripherally, insulin crosses the blood brain barrier, is transported into the brainstem, and affects whole-body metabolism through central mechanisms. Brainstem nuclei, specifically the dorsal motor nucleus of the vagus (DMV) and nucleus tractus solitarius (NTS), critically regulate visceral function. The NTS receives viscerosensory vagal input, and projects heavily to the DMV, which supplies parasympathetic vagal motor output to the abdominal viscera. Pathologies in which insulin is dysregulated, including diabetes, can disrupt this circuit, leading to gastric and other autonomic dysfunction. In the DMV, insulin significantly reduces glutamate release and neuronal excitability, with no effect on inhibition, suggesting a presynaptic effect on glutamatergic input, likely from NTS neurons. We used whole-cell patch-clamp recordings in brainstem slices of transgenic mice (FVB-TgN, GAD-GFP) to identify effects of insulin on synaptic properties of putative glutamatergic NTS neurons. Preliminary data suggests that insulin inhibits sEPSCs and action potentials in non-GFP neurons, but not in GFP+ cells. Neuronal phenotype of the recorded neurons will be verified using single-cell PCR. These experiments are currently being conducted in diabetic (streptozotocin-treated) mice. Such regulation may influence glucose utilization in the dorsal vagal complex and thus, autonomic visceral regulation. Supported by: NIH R01 DK056132 and F32 DK089717.

Food ingestion, brown adipose tissue (BAT) thermogenesis, and the ultradian basic rest-activity cycle

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Rats become active in an ultradian pattern every 1–2 h, with associated highly patterned increases in autonomic parameters, including body/brain temperature and arterial pressure and heart rate (Blessing et al., 2012), a pattern referred to as the basic rest-activity cycle (BRAC). Thermogenesis in brown adipose tissue (BAT) contributes to the increases in body/brain temperature (Blessing et al., 2012). When food is freely available, eating begins approximately 15 min after the onset of BAT thermogenesis. Even when the food container is empty, the rat disturbs the container at the same BRAC time point. Active BRAC phases occur in a stochastic manner, in apparent conflict with what might be predicted from a narrowly conceived idea of nutritional homeostasis. BRAC patterning is principally brain-programmed. Peripheral factors affect the BRAC, but, in general agreement with the ideas of Smith (Smith, 2000), when the rat is leading a sedentary existence in a controlled laboratory environment, with food freely available, initiation of eating is part of the central program regulating the BRAC. A peripheral trigger is not required. Woods and Strubbe (3) proposed that the "anticipatory" increase in temperature prepares the animal for the process of ingestion. We suggest that increases in brain temperature facilitate the complex synaptic processing that organizes the animal's interaction with the external environment, including the search for food and its subsequent ingestion. Supported by: NHMRC.

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Intrinsic and extrinsic influences on children's acceptance of new foods

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Both intrinsic (e.g. neophobia, sensory processing), and extrinsic (e.g. breastfeeding, age at weaning, parental feeding practices) factors influence young children's acceptance of new foods. The feeding practices that parents use when introducing new foods to their children have not been well described. A review of what is known about the factors that predict successful introduction of new foods to children is presented, followed by a summary of our recent study examining predictors of children's acceptance of novel fruits. In this study, we aimed to establish which feeding strategies parents use when introducing a novel fruit (NF) to their preschoolers and assessed the effectiveness of these strategies. We also explored the role of neophobia and sensory sensitivity. Twenty-five parents and their 2–4 year old children were observed eating a standardized lunch and a novel fruit. Parents reported on their children's neophobia and sensory processing. Acceptance of the novel fruit was predicted by the child's age at introduction of solids and the number of physical prompts displayed by parents. The number of taste exposures to the NF was predicted by the child's age of introduction to solids and parental use of rewards/bargaining. Neither child neophobia nor sensory processing predicted acceptance. Prompting and using rewards may help to facilitate children's novel food consumption. Early introduction of solids is also associated with greater willingness to consume a NF. Children's acceptance of new foods may be better predicted by extrinsic than intrinsic factors.

Coping style and prenatal stress interact in the predisposition to metabolic disorders

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We hypothesize that interactions between epigenetic factors and the stress coping style of an individual are crucial for the risk on metabolic disorders in later life. We have tested this hypothesis by assessing metabolic risk and behavioral coping style in male rats exposed to prenatal stress (PNS). Dams were stressed during days 14 till 21 of their pregnancy (variable stress). Offspring's coping style was determined by a defensive burying test. Passive coping rats are characterized by greater freezing behavior, whereas proactive rats display active burying behavior. We found that rats from a PNS rat population showed more extreme coping styles as compared to rats from a control population. There were no significant differences in body weight or food intake between PNS and control rats, neither on a chow nor a high fat diet (HFD). However, during an oral glucose tolerance test, PNS rats on a HFD showed a higher glucose and insulin responses than chow-fed rats or HFD-fed control rats. This effect was most pronounced in the passively-coping HFD PNS rats. During a restraint stress test, the PNS stress rats showed a lower corticosterone response. This effect was only observed in the proactive individuals. PNS stress may modulate the stress coping style in the male offspring, and this could influence the metabolic profile of the rats. Furthermore, our study indicates that PNS has an adverse effect on glucose tolerance in passive rats. In contrast, PNS may have protective properties during stress in proactive individuals. Supported by: NWO Rubicon grant.

Both the number of bites and the oral residence duration increase the oral sensory exposure to food and reduce ad libitum food intake

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Eating rate, largely influenced by bite size, is positively related to food intake. Bite size affects both the number of bites and the oral residence duration per food unit. The separate role of these two aspects on satiation and on orosensory exposure needs further clarification. The objective was to investigate contributions of the number of bites (bites/g) and oral residence duration (s/g) on, first, ad libitum intake of soup, and second, on the orosensory exposure per food unit. In this 2 × 2 crossover study, 56 healthy male subjects consumed ad libitum soup where the number of bites and the oral residence duration differed by a factor three, respectively: 6.7 bites/100 g vs. 20 bites/100 g, and 20 s/100 g vs. 60 s/100 g. All conditions had equal eating rate: 60 g/min. Effects on orosensory exposure to 30 g soup consumption in all conditions were measured by time intensity functions by 22 different healthy subjects. A higher number of bites and a longer oral residence duration reduced ad libitum intake by respectively ~22% and ~8% (P = 0.007), and both increased the orosensory exposure per food unit (P P = 0.003).A relatively higher number of bites and longer oral residence duration led to an increased orosensory exposure and reduced food intake. Designing foods that will be consumed with a relative high number of bites and long oral residence duration may be effective to reduce energy intake. Supported by: Science and Technology Foundation of the Netherlands Organization for Scientific Research (NWO-STW), with co-financers: Unilever, Danone Nederland, Royal FrieslandCampina, and Top Institute Food and N.

The activation of 5-HT_4 receptors in the Nucleus Accumbens Shel in rats submitted to a binge-eating protocol

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The Nucleus Accumbens (NAc) is involved in the ingestive behavior of palatable foods. The modulation of various serotonin receptor subtypes in the NAcc influences food intake. 5-HT_4 receptor activation in the NAc may cause, on the one hand, a decrease of food intake in both food-deprived and fed mice (Jean et al., 2007) and an increase in high fat sucrose consumption in fed rats (Pratt et al., 2009). In order to investigate the role of the 5-HT_4 receptors in compulsive eating behavior, we examined the effects of bilateral NAc shell infusions of 5-HT_4 receptor agonist BIMU-8 (0; 0.1 or 5 μg/02 μL/side) in fed rats subjected to an adaptation of the intermittent-access binge-eating protocol proposed by Corwin. We kept the animals in trios and only isolated them to receive sweet vegetal shortening (SVS) for 1 h 3 days a week, during 4 weeks versus a daily control group. The results showed that the intra infusion in the lowest dose slightly increased the ingestion of the SVS while in the highest dose decreased their intake in a statistically (One way ANOVA) significant manner. Both doses decreased chow intake during the binge-eating test period. Serotonin pathways have already been tied food intake, while more recently reward pathways were related to high fat food. The present study showed links between 5-HT_4 activation on the NAc and binge-eating behavior. Further studies are needed to better establish the relationship between these pathways and compulsive eating and hedonic consumption more generally. Supported by: CAPES.

Involvement of the area postrema in cancer-induced anorexia and body weight loss

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The cancer-anorexia-cachexia syndrome (CACS) has a negative impact on mortality and therapy success in cancer patients. Using a rat tumor model, we examined the role of the area postrema (AP) in the CACS. Buffalo rats were AP lesioned (APX) or sham operated (SHAM). Cultured Morris7777 hepatoma cells were inoculated to induce subcutaneous tumors. The effect of tumor growth on mean daily food intake (FI) per week and weekly body weight (BW) gain was measured for 4 weeks (wk 1–4) following tumor induction. In two groups of non-operated BW-matched rats the effect of tumor growth on body composition was analyzed by CT scans. Tumor bearing rats had lower fat and less lean tissue than controls (16.6 ± 0.8 cm^3 vs. 28.1 ± 1.4; 103.7 ± 2.3 vs. 131.9 ± 2.3). After 4 weeks tumor weight did not differ between APX and SHAM rats (9.6 ± 0.7g vs. 7.7 ± 2.3). In SHAM animals mean daily FI decreased (wk 2 = 22.4 ± 0.6 g, wk 3 = 18.2 ± 0.6, wk 4 = 13.6 ± 0.9), resulting in a mean FI reduction of -38.4 ± 5.4% between wk 2 and wk 4, which is consistent with the previously reported anorectic response for this tumor model. No significant FI changes in APX animals were observed (wk 2 = 17.5 ± 1 g, wk 3 = 17.8 ± 0.7, wk 4 = 15.9 ± 0.9). SHAM rats showed a significant decline in BW gain leading to a net body weight loss in wk 4 (wk 2 = 18 ± 1.6 g, wk 3 = 0.76 ± 2.4, wk 4 = -7.2 ± 1.7). Weekly BW gain in APX rats only decreased in wk 4, but did not reach a negative value (wk 2 = 10.9 ± 1.6 g, wk 3 = 8.5 ± 1.6 and wk 4 = 0.36 ± 3.2). The current findings substantiate the importance of the AP in the mediation of CACS. Supported by: SNSF and Krebsliga Zürich.

TRPV channels and osmoreception in the organum vasculosum lamina terminalis

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Osmosensitive neurons in the organum vasculosum lamina terminalis (OVLT) transduce changes in plasma osmolality into proportional changes in action potential firing rate to modulate downstream effector neurons that command adaptive changes in thirst. Recent studies have indicated that transient receptor potential vanilloid type 1 (TRPV1) and type 4 (TRPV4) ion channels are important for osmosensory transduction in OVLT osmoreceptor neurons but how these proteins regulate firing is not clear. Combined cellular imaging and steady state current voltage analysis during whole cell patch clamp recordings from isolated neurons has shown that decreases in cell volume caused by either fluid hypertonicity or pipette suction lead to the activation of a non-selective cation current. This current, but not the volume change, can be blocked by ruthenium red, a generic inhibitor of TRPV channels, as well as by SB366791 (an inhibitor of TRPV1) but not by HC067047 (a TRPV4 inhibitor). Analogously, OVLT neurons acutely isolated from TRPV1 knockout mice lacked osmosensory responses, but those obtained from TRPV4 knockout mice respond normally to hypertonic stimuli. OVLT neurons recorded in situ in hypothalamic explants from TRPV1 knockout animals are inhibited by hypotonicity and this effect is lost in double TRPV1/TRPV4 knockout animals. These data indicate that a product of the Trpv1 gene is required for cell-autonomous hypertonicity-sensing whereas TRPV4 mediates non cell-autonomous hypotonicity detection by OVLT neurons.

Amylin loses its satiating effect under hypoglycemic conditions

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The effect of amylin to inhibit gastric emptying and glucagon secretion in rats is reduced at low glucose levels. Because both effects, like amylin's anorectic effect, are mediated by the area postrema (AP), it is plausible that these phenomena are based on the co-sensitivity of AP neurons to amylin and glucose. Using hyperinsulinemic glucose clamps in free-feeding rats, we tested whether amylin's anorectic action is also reduced by hypoglycemia (HYPO). Following an 18 h fast, rats were infused with insulin and glucose for 45 min to clamp blood glucose at 6 mmol/l. HYPO (3–3.5 mmol/l) was induced between 45 and 60 min. Rats were injected with amylin (20 μg/kg SC) or saline and offered normal chow at 85 min. Control hyperinsulinemic/euglycemic (EU) rats were maintained at 8 mmol/l before and after amylin injection. Terminal experiments tested the effect of amylin on pERK expression, a marker of amylin action in the AP. Amylin significantly reduced 60 min food intake in EU (saline 7.1 ± 0.5 g vs. amylin 3.9 ± 0.7; $p < 0.01$; $n = 7$) but not HYPO rats (6.4 ± 0.4 vs. 4.5 ± 0.4; n.s.; $n = 10$). Glucose infusion rate had to be reduced at meal onset in saline but not in amylin-treated EU or HYPO rats; this suggests that endogenous glucose appearance was inhibited by amylin. Finally, amylin induced a similar pERK response in the AP in EU and HYPO rats. We conclude that amylin's action to decrease eating is blunted in hypoglycemia; the effect seems to be distal to amylin-induced pERK in AP neurons. The interaction between amylin and glucose is clinically relevant because amylin would not prevent eating at times when energy supply is needed. Supported by: Swiss National Science Foundation.

Bitter taste. The ubiquitous paradox from toxins in our food

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Our genus has been foraging plants and scavenging meats for millions of years. Our diet has changed with climatic cycles and with our movements among the continents. Most recently, perhaps 8–12 thousand years ago, we started to farm. Throughout the evolutionary history of our far reaching quest for nutrients there has been a constant threat that required biological vigilance: poisoning. Anti-nutrients, or toxins, may not only make us sick if ingested, they may kill us. In response to this threat we have evolved a vast array of bitter taste receptors with a large set of alleles that allow the population as a whole to detect most toxins in the diet. Even a single allele of a gene that codes for a sensitive bitter receptor can determine who perceives a particular food as bitter and who does not. But it is virtually impossible to eat without ingesting toxins, so we have also developed tolerances or even preferences for low level bitterness. Nevertheless, high level bitterness can be severely punishing and strong bitter taste alone may induce nausea. So our bitter taste receptor repertoire continues to evolve and we learn to cope with toxins in our foods by cooking and using salt to modify the bitter taste.

The development and cause of ghrelin resistance in NPY/AgRP neurons during high-fat diet feeding

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Ghrelin is an orexigenic hormone that promotes feeding via activation of arcuate NPY/AgRP neurons. Diet-induced obesity (DIO) from 12 weeks of high-fat diet (HFD)-feeding causes ghrelin resistance in these neurons (Briggs et al., 2010). Here, we investigate the timecourse over which this occurs. Ghrelin resistance occurs in NPY/AgRP neurons after 3 weeks of HFD-feeding and is preceded by impaired glucose tolerance. We hypothesized that HFD-induced hyperinsulinemia suppresses plasma ghrelin and ghrelin responsiveness. We confirmed that insulin suppresses ghrelin-induced NPY secretion and cell firing in lean mice, but 3 weeks of HFD-feeding caused hypothalamic insulin resistance as shown by decreased insulin-induced phosphorylation of Akt. Further, insulin pre-treatment did not suppress ghrelin's orexigenic effects in ghrelin-sensitive ob/ob mice. Thus, hyperinsulinemia does not cause NPY/AgRP neuronal dysfunction. Leptin also suppresses ghrelin-induced food intake (Kohno et al., 2006) and may contribute to ghrelin resistance in NPY/AgRP neurons. Three weeks of HFD-feeding increases plasma leptin, and in leptin-deficient ob/ob mice ghrelin's orexigenic effects persist in spite of obesity to a similar degree as ghrelin-resistant DIO mice. Ghrelin's effects were completely abolished by leptin pre-treatment. This suggests that hyperleptinemia in DIO mice contributes to ghrelin resistance in NPY/AgRP neurons. Further work is required to assess if leptin signaling is intact in DIO mice.

Effects on body weight, body composition, metabolism and insulin sensitivity in TRPM5 KO-mice on high fat diet

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The ion channel TRPM5 has a key role in taste signaling, both on the tongue and in the gut. Here we investigate the effects of TRPM5 gene knock out in mice on body weight, composition, insulin sensitivity and other metabolic parameters in diet induced obesity. Male TRPM5 KO (KO) and wild-type (WT) littermates were fed a 60% high fat diet (HFD) for 12 weeks ($n = 8$). Assessment of insulin sensitivity (oral glucose tolerance test, OGTT), body composition (DEXA) and energy expenditure (EE, Oxymax system) were done before and after 11, 9 and 10 weeks on HFD. Both groups became obese, but KO mice gained less body weight although no significant difference in food intake could be demonstrated. KO and WT mice did not differ in lean or fat mass when calculated as % of body weight. The TRPM5 KO mice showed increased insulin sensitivity in OGTT, largely dependent of body weight. Before starting on HFD the WT mice showed significantly higher activity as well as reduced EE compared to KO mice, but after the HFD there were no detectable differences. At termination the KO mice had significantly reduced brown adipose and liver weight, but no difference in triglycerides. An interesting observation is that the increased insulin sensitivity in the KO mice mainly was a result of their lower body weight. This is in contrast to our earlier study of TRPM5 KO mice on a fat and sugar rich cafeteria diet, where the improvement in insulin sensitivity was to a large extent independent of body weight. This suggests that the magnitude of protection against diabetes in TRPM5 KO mice is dependent on the sugar contents of the diet. Supported by: AstraZeneca.

TRPM5 KO-mice lack preference for sweet palatable food, but retains energy content driven food intake

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We have shown that TRPM5-deficient (KO) mice gain less weight on cafeteria diet and become less glucose intolerant compared to wild-type (WT) mice. Here, we investigated the effects of TRPM5 ablation on taste preference and caloric intake. In a lickometer assay, TRPM5 KO mice did not show an acute preference for either sucrose or the artificial sweetener sucralose as observed with WT littermates. After 3 days, the KO mice developed a preference for sucrose but not sucralose, indicating a caloric driven intake detached from taste. In another experiment, mice were fed either a 60% HFD or a combination of HFD and sugar-rich chocolate for 11 days. On HFD only, KO mice consumed 13 kcal/24 h/mouse while WT consumed 16 kcal/24 h/mouse. With HFD plus chocolate, KO mice still consumed 13 kcal/24 h/mouse while WT mice consumed 18 kcal/24 h/mouse. KO mice consumed significantly less of the chocolate (3 kcal/24 h/mouse) compared to WT mice (9 kcal/24 h/mouse). On both diets, the difference in food intake was reflected in decreased body weight gain in KO compared to WT mice. The reduced consumption of chocolate in TRPM5 KO mice is presumably related to their inability to recognise sweet taste. Since the KO mice consumed the same amount of calories independent of diet, their food intake seems to mainly be driven by the energy content of the food compared to WT mice that also have a hedonic drive for sweet taste. The lack of sweet taste preference observed in TRPM5 KO mice might explain why these mice become less obese and glucose intolerant on cafeteria diet. Supported by: Astrazeneca.

Examining food choice with fake foods. Encouraging consumers to make healthier food choices trough food positioning

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Experimental research involving food is often limited by practical issues. In the present study we assess the validity of a new experimental method using food replicas. The Fake Food Buffet (FFB) may be used to assess the influence of environmental cues such as variety and portion sizes on food choice and meal composition under well-controlled conditions. Experiments examining the influence of serving order on meal composition are presented to illustrate the applicability of the FFB method. In a test-retest reliability study, 57 students served themselves twice a meal from the FFB with replica carrots, beans, pasta and chicken, with an interval of 2 weeks. In the validity study, 49 students served themselves twice a meal from the FFB or a real food buffet (RFB), in random order within a 2-week interval. Two hundred and eighty-one persons participated in three FFB serving order studies, where buffet conditions were manipulated. Two-week test-retest reliability correlations (FFB) ranged from r = 0.79 for carrots and beans to r = 0.89 for pasta. The validity correlations between the FFB meal and the RFB meal ranged from r = 0.82 for pasta to r = 0.87 for beans. These high correlations indicate the FFB as a valuable tool for experimental nutrition research. Further, the serving order studies show that food positioning influences consumer's choices. Significant gender–condition interactions were observed. Position effects in buffet settings maybe used to encourage consumers to make healthier food choices.

Sensing of dietary composition by sleep and reward-related neurons

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Diet is a key determinant of brain function, but the underlying mechanisms are unclear. We found that hypothalamic orx/hcrt neurons, widely-projecting cells that promote wakefulness and reward-seeking in mammals, display distinct responses to different dietary macronutrients. Orx/hcrt cells were stimulated by nutritionally-relevant mixtures of dietary amino acids, both in vitro and during central or peripheral administration of amino acids in vivo. Typical dietary mixtures of amino acids directly stimulated orx/hcrt neurons through a dual mechanism involving inhibition of KATP channels and activation of system-A amino acid transporters. Furthermore, physiological mixtures of amino acids opposed the inhibition of orx/hcrt cells by glucose, indicating that orx/hcrt cells sense macronutrient balance, rather than net energy value, of extracellular fuels. There results demonstrate a new cell and nutrient-specific mechanism for dietary tuning of neural circuits that control brain state and vital behaviors.

Neuroprotective effect of polyunsaturated fatty acids in the hypothalamic cell lines

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Background: Previously, high fat diet (HFD) for 11-week induced neurodegeneration (NDG) in the hypothalamus. The HFD pair-fed rats at same energy intake as the control diet rats developed NDG, suggesting dietary fat and not excess calories contributes to the NDG. We also found HFD increased hypothalamic ceramides, lipid metabolites of palmitic acid (PA). Ceramides induce insulin resistance, cytokine production, activation of the caspases, and apoptosis. Increased dietary PUFAs are linked to prevention of NDG disorders by improving insulin sensitivity and oxidative damage, but the PUFA's contributions to hypothalamic integrity, which is important for energy balance, have yet to be evaluated. We hypothesized that PUFA protect against PA-induced NDG. *Methods*: Adult mouse and embryonic hypothalamic cell lines were pre-incubated with w-6 FA linoleic acid (LA), w-3 FA eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or soy oil (SO), followed by PA treatment. Cell viability and caspase-3 activity was measured at the end. Data were analyzed by one factor ANOVA. *Results*: PA at 0.3 mM increased cell death and caspase-3 activity in the hypothalamic cells. Addition of LA, DHA, EPA, or SO reduced PA-induced NDG and caspase-3 activity. *Conclusion*: These in vitro experiments support the hypothesis that PUFAs are neuroprotective against PA-induced NDG. Further studies will determine the neuroprotective effect of PUFAs in vivo and the mechanisms underlying the effect.

Inhibition of ghrelin O-acyltransferase or acyl-ghrelin differentially regulates central and peripheral energy balance

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Ghrelin O-acyltransferase (GOAT) catalyzes the octanyl acylation of ghrelin, an essential posttranslational modification required for the orexigenic actions of acyl-ghrelin. We characterized the central effects of GOAT and acyl-ghrelin inhibition for the first time. Administration of GOAT-specific chemical inhibitor (GOCoA-TaT) via a jugular catheter decreased metabolic parameters without altering body weight or food intake, over 5 days. However, central administration of GOAT inhibitor via ICV injection decreased body weight without altering food intake and only secondarily altered metabolic parameters in mice. These data are in contrast to blocking the action of acyl-ghrelin in the CNS. Endogenous levels of acyl-ghrelin are increased in 24-h fasted mice. Central administration of an acyl-ghrelin blocking antibody via ICV injection significantly diminished food intake (~30%) relative to mice injected with the control antibody. Collectively, our data suggest distinct peripheral and central actions of ghrelin in regulating whole-body energy balance. Supported by: NIH.

Hindbrain MC4 receptor participation in CCK-induced MAPK signaling and control of food intake is "upstream" of vagal afferent and NTS neuronal NMDA receptors

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CCK-induced reduction of food intake and increased NTS ERK phosphorylation (pERK) in the nucleus of the solitary tract (NTS) are attenuated by MC4 receptor antagonism, suggesting that NTS MC4 receptors contribute to control of food intake by CCK. However, NMDA receptor (NMDAR) activation also is required for CCK-induced reduction of food intake and ERK phosphorylation in vagal afferent terminals and NTS neurons. In addition, pERK-catalyzed synapsin 1 phosphorylation in vagal terminals also is abolished by NMDAR antagonism. We postulated that NMDAR participation in NTS ERK and synapsin phosphorylation are functionally downstream from MC4R activation. In support of this hypothesis, we find that both MC4R and NMDAR antagonists block CCK-induced pERK in NTS neurons and vagal terminals. However, MC4R agonist triggers pERK only in NTS neurons, and does not trigger pERK or synapsin phosphorylation in vagal terminals. These results indicate that "upstream" MC4R activation releases glutamate to bind at vagal afferent NMDARs. However, vagal afferent NMDAR activation and consequent phosphorylation of ERK and synapsin in vagal terminals cannot occur without coincident CCK-induced depolarization of the vagal terminals. Together with other findings, our observations also suggest that NTS NMDAR may integrate multiple signals for control of food intake by modulating vagal afferent signaling and synaptic function. Supported by: NIH grants DK-52849 and NS-20561.

NMDA receptor blockade prevents CCK-induced reduction of food intake and ERK-mediated synapsin phosphorylation in NTS vagal afferent terminals

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CCK-induced reduction of food intake requires activation of NMDA receptors (NMDAR) in the nucleus of the solitary tract (NTS) with consequent phosphorylation of extracellular signal-related kinase1/2 (pERK) in NTS neurons and/or NTS vagal afferent terminals. The mechanism by which NMDAR-mediated ERK1/2 phosphorylation enables CCK-induced reduction of food intake is obscure. However, pERK catalyzes phosphorylation of synapsin in axon terminals, resulting in increased transmitter release. We postulated that CCK-evoked activation of vagal afferent NMDAR results in pERK-catalyzed phosphorylation of synapsin in vagal afferent terminals, leading to increased synaptic strength and reduced food intake. We found that CCK triggers synapsin phosphorylation in NTS vagal terminals. IP CCK1 receptor antagonist or fourth ventricle NMDAR antagonist prevents CCK-induced pERK in NTS neurons and vagal terminals and abolishes synapsin phosphorylation in vagal terminals. Destruction of vagal afferent C-fibers with capsaicin attenuates CCK-induced reduction of food intake and abolishes pERK and synapsin phosphorylation in vagal afferent endings, but does not reduce pERK in NTS neurons. Our results support the hypothesis that reduction of food intake by CCK depends upon NMDAR-mediated pERK in vagal afferent terminals, and suggest that phosphorylation of synapsin may constitute a mechanism coupling CCK-induced reduction of food intake to vagal afferent NMDAR activation via pERK. Supported by: NIH grants DK-52849 and NS-20561.

Gut microbiota, low grade inflammation and metabolism

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Over the last 15 years, our work has been devoted to examine the way by which the bacteria present in the gut interact with nutrients and host biology to control obesity and associated disorders, including diabetes, inflammation and liver diseases. Recently, we discovered that the gut microbiota contribute to the development of the insulin resistance and the low grade inflammation characterizing obesity. We described the concept of metabolic endotoxemia (increase in plasma LPS levels) as triggering factor in the development of the metabolic alterations associated with obesity. Following this discovery, we found that the major factor involved in the development of metabolic endotoxemia observed upon obesity is related to the gut barrier function. For instance, we found that both nutritional and genetic obesity are associated with an increased gut permeability leading to the leakage of LPS and possibly other microbiota derived factors. Although the clear mechanisms involved in the bacteria–host interactions are still under investigation, we found that the gut microbiota control enteroendocrine functions such as L-cells (producing GLP-1 and GLP-2) number and differentiation, the endocannabinoid system tone but also leptin sensitivity. We found that selective changes in the gut microbiota composition by using prebiotics or specific "novel bacteria" such *Akkermansia muciniphila* might be a useful tool. Taken together, the compelling data currently published suggest that specific changes in the gut microbiota could be promoted to counteract fat mass development, diabetes and the low levels of inflammation associated with obesity.

Effects of food neophobia and food neophilia on diet and metabolic processing

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Past research reveals that food neophobic individuals (those individuals unwilling to try new foods) have significantly lower body weight compared to food neophilic individuals (those individuals overtly willing to try new foods) and average individuals. It is a basic tenet in dietetics that dietary variety reduces the risk of nutrient deficiency, which is also positively correlated with body weight. If neophobics have a more restrictive diet, they may be at increased nutritional risk. In the present study, the reliability and dietary basis of this body weight difference is explored by collecting dietary information. Participants completed a food diary for three random days during a random seven-day period, and completed questionnaires related to eating habits and body satisfaction. On average, there was a statistical difference between food-neophobics, food-neophilics, and an average group related to consumption of overall nutrients and calories with age, height, weight, and sex taken as covariates. The three groups were found to differ significantly on dietary intake of 20 specific nutritional and caloric items, with food-neophobics typically having the lowest intake of specific nutrients and calories overall. This lackluster level of nutritional consumption is seen as a sign of decreased nutritional health and may affect food-neophobics overall health.

Appetitive traits in infancy, childhood and adolescence. A multi-method approach to exploring individual differences

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We come into the world endowed with enduring predispositions towards food which interact with the environment to influence our eating behaviors and weight trajectories. But our fates are far from sealed – by understanding more about this process we can identify targets for biobehavioral interventions and modify our environments to lessen the impact of obesogenic traits. To do this requires robust, reliable assessments of appetite that can be used at different developmental stages, and several candidates now exist. These include behavioral measures such as tests of eating in the absence of hunger, eating rate, caloric compensation, and food intake patterns within multi-item meals, but also psychometric measures directly assessing traits such as food cue responsiveness and satiety sensitivity (e.g. Child Eating Behavior Questionnaire), and objective biomarkers such as brain responses to food cues measured using fMRI. The research base is still growing, but evidence from infants, children, and adolescents suggests that many of these appetite measures (a) differ with weight and adiposity, (b) differ according to familiarly-defined (genetic and environmental) obesity risk, and (c) differ according to genetic factors (e.g. number of FTO risk alleles) and environmental factors (e.g. parental feeding style). The next challenge is to combine appetitive trait measures with relevant genetic and environmental measures in the context of long-term prospective studies that can help unpack the complex interactive relationships underlying obesity development.

Role of orexin in conditioned saccharin-seeking

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Previous studies in our laboratory demonstrated that signaling at the orexin 1 receptor (OxR1) is involved in operant responding for sucrose and cue-induced reinstatement of extinguished sucrose-seeking. Interestingly, these effects were only seen in food-restricted rats and lead us to consider that conditioned activation of orexin neurons may increase motivation for food reward during food restriction. This study examined the involvement of the orexin system in operant responding for saccharin, a non-caloric sweet reward, and in cue-induced reinstatement of extinguished saccharin-seeking, using the OxR1 antagonist SB-334867 (SB). Ad libitum or food-restricted male Sprague Dawley rats were trained to self-administer saccharin, and the effects of pretreatment with SB (10–30 mg/kg) on established fixed ratio (FR) responding was tested. Reinstatement of saccharin-seeking was elicited by presentation of tone + light cues previously paired with saccharin reward. Results showed that SB decreased the number of reinforcers earned during FR and decreased cue-induced reinstatement in food-restricted rats and ad libitum fed rats. These results are in contrast to the effects of SB found only in food-restricted rats during sucrose reinforcement and seeking and indicate that signaling at the OxR1 is involved in saccharin reinforcement and necessary for reinstatement of saccharin-seeking elicited by saccharin-paired cues regardless of food restriction. Moreover, our results suggest that orexin increases motivation for sweet rewards in general but for caloric food rewards only during food restriction. Supported by: DA 023354, DA 017289, RR 016461.

Repeated exposure is sufficient to increase acceptance of a novel vegetable in pre-school children

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To compare the effectiveness of different learning strategies and individual differences in the acquisition of acceptance for a novel food, 72 pre-school children (23.6 ± 0.9 m) were assigned to one of 3 conditions. All children had repeated exposure (10 times) to a target vegetable – artichoke. In one condition the target was plain (n = 22); in another the target was sweetened (flavour–flavour learning: n = 25); in the third the target had added energy (flavour–nutrient learning; n = 25). Parents (n = 35) completed a food frequency questionnaire (FFQ) to assess vegetable consumption at home; the Child Eating Behaviour Questionnaire to measure food fussiness (FF) and enjoyment of food (EF); a Child Neophobia scale (CN) and child BMI z-scores were calculated by measuring heights and weights. Intake was measured pre-, during and three times post-exposure. Acceptance of the target increased across all conditions relative to a control (carrot; $p < 0.05$). This effect persisted for up to 5 weeks post-exposure ($p < 0.01$). Intake of the target was associated with EF ($p < 0.01$) and inversely with CN ($p < 0.01$) and FF ($p < 0.05$). Children who consumed fewer vegetables at home had higher scores on FF and CN (both $p < 0.05$), however, frequent vegetable intake at home did not predict intake of the target during exposure. Characteristics of the child (EF, FF, CN) appear to be better predictors of intake than what is eaten at home. Repeated exposure was sufficient to increase acceptance of a novel vegetable, with no additional benefit to learning accorded by either adding flavour or energy to the target. Supported by: European Community's Seventh Framework Programme (FP7/2007-2013) under the grant agreement n° 245012-HabEat.

Pathways to obesity. Contribution of common gene polymorphisms to child eating behaviour

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Not all individuals become overweight or obese in a permissive (obesogenic) environment. Susceptibility to obesity is heterogeneous, suggesting that differences in genetic pre-disposition interact with the environment to determine the expressed phenotype. Heritability estimates for appetite and eating behaviours associated with susceptibility to weight gain are high. Thus, weak satiety, enhanced responsiveness to food and hyperphagic eating traits such as eating in the absence of hunger (EAH) and increased eating rate are associated with influencing an increased BMI in twin and family studies. Robustly characterised common gene variants which contribute to variance in paediatric BMI and confer risk of obesity also appear to contribute to individual variation observed in appetite responses and eating behaviour, through specific eating patterns rather than through energy expenditure. These include variants of the fat mass and obesity associated (FTO), peroxisome proliferator-activated receptor (PPARG) and melanocortin 4 receptor (MC4R) genes, which have been implicated in energy intake, satiety responsiveness and specific eating behaviour phenotypes associated with the tendency to overeat. This talk will evaluate the current evidence for common gene polymorphisms in contributing to individual differences in child eating behaviour.

Ghrelin and bariatric surgery. Fat or fiction?

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Ghrelin, a potent orexigen primarily secreted from the stomach, circulates in two forms: *acyl* and *desacyl*. Altered ghrelin levels have been hypothesized to mediate part, or all, of the benefits produced by vertical sleeve gastrectomy (VSG) and Roux-en-y gastric bypass (RYGB) surgery. However, inconsistencies in the literature regarding the surgical consequences of bariatric surgery on ghrelin secretion have made the primacy of this hypothesis unclear. Thus, we measured circulating acyl and desacyl ghrelin levels using ELISA and mass spectrometry techniques in bariatric rats and compared them with *ad lib* fed sham operated rats or sham rats that were pair-fed to the RYGB treatment group. Circulating levels of acyl and desacyl ghrelin were significantly reduced after VSG, but were unaffected by RYGB. We then used a loss of function model to determine if altered ghrelin signaling was responsible for VSG's effects by assessing surgical outcomes in ghrelin deficient and wild-type mice. Ghrelin deficiency did not alter the surgery's effectiveness in terms of weight loss, food preference, or oral glucose tolerance. In all cases, differences between genotypes were non-significant after VSG, and greatly improved relative to outcomes observed in sham operated mice. We conclude that the metabolic effects of RYGB and VSG on these parameters do not result from the surgical removal of ghrelin producing cells, or altered ghrelin levels after surgery. Supported by: Ethicon Endosurgery, CIHR.

Site specific activation of lateral hypothalamic mGluR1 and R5 receptors elicits feeding in rats

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While substantial evidence implicates lateral hypothalamic (LH) ionotropic glutamate receptors in the stimulation of food intake, there is scant evidence on the contributions of metabotropic glutamate receptors (mGluR). The goal of the current study was to specify the brain site where mGluR stimulation elicits eating. To this end, we first tested whether unilateral LH injection of the selective mGluR group I agonist (*S*)-3,5-Dihydroxyphenylglycine (DHPG) would elicit feeding in satiated adult male Sprague-Dawley rats stereotaxically implanted with an indwelling guide cannula terminating in the LH. We found that DHPG reliably produced a dose-dependent stimulation of eating with doses of 1.0 nmol and 25 nmol eliciting mean 1 h postinjection intakes of 2.6 g (SEM ± 0.6) and 4.0 g (SEM ± 0.5), respectively. To validate the LH as the site of action, we conducted a mapping study comparing DHPG effects in the LH to surrounding regions (n = 5–9 subjects/brain site). We determined that 1.0 nmol elicited food intake specifically within the LH. These findings suggest roles for mGluR1 and/or mGluR5 in lateral hypothalamic circuits capable of stimulating feeding behavior.

Effect of *Hypericum perforatum* extract in an experimental model of binge eating in female rats

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Episodes of binge eating (BE) are characterized by compulsive, non-homeostatic consumption of an unusually large quantity of highly palatable food (HPF) in a short period of time. The present study evaluated the effect of *Hypericum perforatum* dry extract in an experimental model of BE. BE for HPF was evoked in female rats by three 8-day cycles of food restriction/refeeding and acute stress on the test day. Stress was induced by preventing access to HPF for 15 min, while rats were able to see and smell it. *Hypericum perforatum* dry extract (0.1% hypericin and 3.8% hyperforin) was given by gavage 1 h before access to HPF. Only rats exposed to both food restrictions and stress exhibited BE in the first 15–60 min after the stressful procedure. The dose of 250 mg/kg of *H. perforatum* extract significantly reduced and 500 mg/kg completely abolished the BE episode, while 125 mg/kg was ineffective. The same doses did not affect HPF intake in the absence of BE. The dose of 250 mg/kg did not significantly modify stress-induced increase in serum corticosterone levels, suggesting that the effect on BE is not due to suppression of the stress response. The combined administration of 125 mg/kg of *H. perforatum* together with salidroside, active principle of *Rhodiola rosea,* produced a synergic effect on BE. The present results indicate for the first time that *H. perforatum* extracts may have therapeutic properties in bingeing-related eating disorders.

The effect of food labels on the selection of foods purchased in a university dining hall

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Weekly commissary sales data for all pre-packaged food items sold in the Dining Units of Cornell Dining were analyzed for three semester prior to and three semesters after the introduction of food labels in the Spring of 2008. The mean total calories and total fat content were calculated for each food item using food composition data provided by the university dietician. Following the introduction of the food labels, a significant decrease in mean total calories ($P < 0.001$) and mean total fat ($P < 0.001$) purchased per week was observed. The labeled foods were further categorized into high and low calorie foods as well as high and low fat foods. The introduction of the food labels resulted in a significant increase in the purchase of low fat ($p < 0.001$) and low calorie ($p < 0.001$) foods with a corresponding reduction in the purchase of the higher fat, higher calorie items. These results support the idea that nutrition labels may be an effective intervention to facilitate more positive, healthful food purchasing behaviors. Supported by: Division of Nutritional Sciences, Cornell University.

Expression of Pavlovian appetitive conditioning recruits orexin neurons in the medial region of the lateral hypothalamus in the rat

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The aim of this study was to examine the involvement of the hypothalamic neuropeptide orexin in the acquisition and expression of Pavlovian appetitive conditioning. Food deprived male rats received either a tone which co-terminated with delivery of food pellets distinct from their regular chow diet, or presentations of the tone alone in the conditioning chamber followed by food delivery in their home cage. Animals were perfused after either a conditioning session of tone-food pairings (Pair), a conditioning session with tone only presentations (Tone) or after food delivery in the home cage (Food). Half of the animals were perfused after day 1 of training to examine the acquisition of Pavlovian appetitive conditioning (D1 Pair, $n = 8$; D1 Tone, $n = 7$; D1 Food, $n = 8$), while the other half were perfused after day 10 of training to allow for examination of the expression of appetitive conditioning (D10 Pair, $n = 8$; D10 Tone, $n = 8$; D10 Food, $n = 8$). D1 Pair animals (but not Tone or Food) showed learning during day 1 ($p < 0.05$), while D10 Pair animals (but not Tone or Food) showed robust expression of appetitive conditioning during day 10 ($p < 0.001$). Brains were processed for combined detection of Fos and orexin neurons with double immunohistochemistry. We found greater Fos induction in orexin neurons in D10 Pair compared with D10 Tone and D10 Food ($p < 0.05$) in the medial region of the lateral hypothalamus. These results indicate that the expression of Pavlovian appetitive conditioning recruits orexin neurons in the hypothalamus. Supported by: NIH grant DK085721 to GDP.

The flexibility of olfactory preferences. Do decision-making processes matter in the long run?

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Empirical studies have demonstrated that olfactory preferences can be modulated by cognitive processes related to decision-making. It is, however, still unclear whether such postchoice preference change persists over long time spans. This question was addressed here. In a first session, participants were asked to evaluate the pleasantness of 20 odors. We then gave them a budget of coins, each worth 5 CHF (~5 US $). Participants were instructed that they would have to spend ten of these coins during the experiment. On each of the 20 subsequent trials, they were asked to choose whether they wanted to spend 5 CHF on the next presented odor. They were then delivered with it. After these choice trials, they were asked to evaluate the pleasantness of the 20 odors again. In a second session one week later, participants were asked to do so one more time. We expected that spending money on an odor that turned out to be unpleasant would lead to cognitive dissonance. We consequently hypothesized that monetary choices would lead to a preference modulation for unpleasant odors only. Results confirmed this hypothesis: unpleasant odors were evaluated as more pleasant if their delivery was preceded by participants spending 5 CHF than if they were preceded by no spending. This choice-induced preference modulation was still present after 1 week. Therefore, although olfactory preferences appear to be flexible because they can be modulated by choices, this modulation also appears to be stable over time. Supported by: This research was supported by the National Center of Competence in Research for the Affective Sciences, financed by a grant from the Swiss National Science Foundation (51NF40-104897), hosted by the U.

Egg-based breakfasts enhance satiety and cognitive function in young adults

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It is well accepted that breakfast intake can have a positive effect on cognitive performance and mood. The present study examined whether the nutrient content of breakfast plays a role in determining the effects of the meal on behavior. Using a within subjects design, the effects of an egg-based breakfast (2 eggs, 2 slices of reduced-calorie whole-wheat toast, and 4 oz of 1% milk), an isocaloric cereal-based breakfast (2 cereal bars and 4 oz 1% milk), and no breakfast on blood glucose levels, mood and cognitive behavior were examined in male and female college students. Thirty, 60, or 120 mins after eating breakfast, blood glucose levels, hunger, mood, vigilance and spatial and short-term memory were measured. Blood glucose levels were significantly greater at all time points after a breakfast meal than after no meal, and were significantly higher in the cereal-bar condition relative to eggs or no breakfast at 30 and 60 mins post-meal. In all conditions, blood glucose levels had returned to fasting levels by 120 mins. At all time points, hunger ratings were significantly lower after intake of a meal than after no meal. At 60 mins, hunger ratings were significantly lower in the egg condition relative to the cereal-bar or the no-meal condition. At 30 and 120 mins, performance on a short-term memory task was better after intake of eggs than after intake of cereal or no meal. These findings indicate that in well-nourished young adults, the effects of different meal types have subtle, but significant effects on satiety and cognition. Supported by: The Egg Nutrition Center.

Changes in gut morphology and gut hormone gene expression following Roux-en-Y gastric bypass

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Background: Gastric by-pass (GB) surgery is presently the only effective anti-obesity treatment available. However, GB surgery also exerts potent anti-diabetic effects and it is speculated that unidentified gut peptides are involved. Here we assess gene expression and morphological changes throughout the gastrointestinal (GI) tract in Roux-en-Y gastric bypass (RYGB) operated rats. *Methods*: Three groups of rats (n = 5) were included: sham, sham weight-matched or RYGB. Five months post surgery the GI tract was dissected and divided into the biliopancreatic limb, alimentary limb, common channel and colon. From each region, 8–10 transverse biopsies were obtained for stereological analyses (volume and epithelial surface area, total L- and endocrine cell number) and qPCR analyses against a number of gut hormones. *Results*: The stereological analyses revealed a near 100% increase in epithelial volume of the alimentary limb following RYGB, coupled with an increase in epithelial surface area and cell number. Gene expression analyses revealed a marked regional effect on preproglucagon, CCK and PYY expression being nearly 3-fold when including gut hypertrophy. *Conclusions*: We provide a complete quantitative assessment of cellular changes in the rat GI tract with a corresponding regional assessment of gene expression differences following RYGB surgery. The study provides novel insight about gut endocrinology and important information about potential GI hot spots in human RYGB patients. Supported by: Gubra.

Evaluating the potential for rostral diffusion in the cerebral ventricles using angiotensin II-induced drinking in rats

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In spite of much evidence to the contrary, there is often concern that fourth ventricle (4V) injections may access forebrain structures, calling into question the validity of using these injections to evaluate the role of hindbrain structures. For example, injection of AngII into the lateral ventricle (LV) increases water intake, but a similar response is not observed after injection into the 4V. This alone suggests the requirement of forebrain structures, but the potential for an anti-dipsogenic pressor response to hindbrain AngII application allows for lingering concern of competing effects of AngII. In the present studies, we used a double cannulation approach (LV and 4V) to evaluate the effect of the AngII receptor antagonist, losartan, on the drinking response to AngII injected into the LV. Injections of losartan into the LV blocked the dipsogenic response to AngII given 5 min later into the LV. There was no effect, however, when losartan was injected into 4V, even when we used doses of losartan that were 25 times that needed when injected into the LV. We did, however, find that a high enough dose of losartan into the 4V, given 15 min before AngII application to the LV, caused a mild reduction in water intake, indicating that with a high enough dose and enough time, substances injected into the hindbrain ventricle may access forebrain structures. Collectively, these experiments suggest that substances injected into the 4V may access forebrain structures, but with proper controls and timing, the concerns regarding this approach should be minimal. Supported by: NIH HL-91911.

Genetic evidence that food addiction reflects an enhanced dopamine signal in brain reward pathways

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Our recent study (Appetite, 2011) indicated that food addiction (FA) is a classifiable condition with clinical symptomatology and psychobehavioural traits similar to conventional drug-abuse disorders, providing human support for the preclinical evidence of sugar/fat addiction in rats. The current study expands our investigation by testing whether genetic markers associated with elevated dopamine signaling are linked to FA, and its associated sub-phenotypes. A sample (n = 135) of overweight adults were classified with or without FA by the Yale Food Addiction Scale, which is based on the established criteria for substance dependence. Using blood DNA we calculated multilocus genetic profile scores (MGP) based on 5 dopamine polymorphisms. This method reflects the cumulative effect of multiple polymorphic loci – of known functionality – on a specific signaling mechanism. Our neural target was responsiveness of the ventral striatum, which regulates appetitive behaviours. Increased dopamine signaling would imply greater sensitivity to reward. Results indicated a stronger signal in those with FA (p = 0.015), suggesting a greater hedonic response to food compared to their non-FA counterparts. We also found that MGP scores correlated positively with binge-eating severity, food cravings, and emotional eating (p = 0.05–0.002). This represents the first genetic study of FA and supports the view that this syndrome reflects a hyper-sensitivity to rewarding stimuli – what we might describe as a Reward Surfeit Syndrome. Supported by: Canadian Institute of Health Research.

Roux en Y gastric bypass increases ethanol intake in the rat

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The Roux en y gastric bypass (RYGB) surgery is a commonly used procedure to treat diabetes and obesity. In addition to weight loss and resolution of metabolic syndromes, such as diabetes, the RYGB procedure has been reported to increase ethanol consumption in humans. Using an outbred rodent model, we demonstrate that RYGB increases post-surgical ethanol consumption. Specifically, we examined ethanol consumption in 23 Long Evans rats that underwent RYGB (n = 5) or sham (n = 10) surgery along with non-operated (n = 5) and weight matched controls (n = 8) using a two bottle choice paradigm consisting of 10% ethanol and water. Interestingly, RYGB rats consumed twice as much ethanol compared to controls, an effect that cannot be explained solely by post-surgical weight loss and that it is independent of pre-surgical body weight or dietary composition. Altered ethanol metabolism and post-surgical shifts in release of ghrelin were also unable to account for changes in ethanol intake. Further investigation of the potential physiological factors underlying this behavioral effect identified altered patterns of gene expression in brain regions associated with reward following RYGB surgery. These findings have important clinical implications as they demonstrate that RYGB surgery leads directly to increased ethanol intake and induction of neurobiological changes in brain circuits that mediate consummatory behavior. Supported by: Ethicon EndoSurgery Inc.

Overweight and obese humans are less active at, but not away from, home

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The built environment has been implicated in the development of the obesity epidemic in part by its influences on activity levels. We developed analytical methods to discriminate bouts of activity and inactivity from continuous triaxial accelerometer records and applied them to investigate differences in activity occurring at home vs. other locations for normal and overweight/obese individuals. Eighty-one male (49–58 y, 25–42 kg/m^2) and 84 female (49–58 y, 25–42 kg/m^2) subjects wore triaxial accelerometers to measure activity and GPS Loggers to record their location continuously for 7 consecutive days. Reasonable thresholds were identified for the discrimination of activity bouts as 15 min minimum duration, 15 min minimum interval, and 400 VMcpm minimum size, and for inactivity bouts of 15 min minimum duration, 15 min minimum interval, and 200 VMcpm maximum activity amount. Applying these thresholds, normal weight participants were found to be more active at home than away from home while overweight/obese participants were relatively inactive in both locations. Overweight/obese individuals appear to be less responsive to environmental cues for activity particularly at home. This suggests that to understand the development of obesity we must be careful not to just look at overall behavior but to investigate behaviors in specific environments. Supported by: National Institutes of Health – NIEHS. RFA-ES-04-0.

Food intake reductions and increases in energetic responses by hindbrain leptin and melanotan II are enhanced in mice with POMC-specific PTP1B deficiency

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Leptin regulates energy balance in part through catabolic effects via action on proopiomelanocortin (POMC) neurons. POMC neuron-specific deletion of protein tyrosine phosphatase 1B (PTP1B) (*Ptpn1*$^{loxP/loxP}$ POMC-Cre), a negative regulator of leptin signaling, results in resistance to diet-induced obesity and improved peripheral leptin sensitivity in mice, establishing PTP1B as an important component of POMC neuron regulation of energy balance. POMC neurons are expressed almost exclusively in the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract in the hindbrain, however, it is unknown how each population contributes to the phenotype of POMC-Ptp1b$^{-/-}$ mice, or whether improved leptin sensitivity in POMC-Ptp1b$^{-/-}$ mice involves altered hindbrain melanocortin receptor signaling. Therefore, we examined the effects of 4th ventricular administration of leptin (1.5, 3, 6 μg) or the melanocortin 3/4R agonist melanotan II (0.1, 0.2 nMol) in POMC-Ptp1b$^{-/-}$ (KO) and control PTP1B$^{fl/fl}$ (WT) mice on food intake, body weight, spontaneous physical activity (SPA), and core temperature (T_C). POMC-Ptp1b$^{-/-}$ mice were hypersensitive to hindbrain leptin- and MTII-induced food intake and body weight suppression, and SPA compared to WT mice. Greater increases in leptin, but not MTII, -induced T_C were also observed in KO vs. WT animals. These studies show hindbrain administration of leptin or MTII alters energy balance in mice, likely via hindbrain POMC neurons. Support: NIHDK 082417&21397, AHA 10POST3910000.

Leptin resistance in vagal afferent neurons drives hyperphagia

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The vagal afferent pathway senses hormones released from the gut in response to nutritional cues and relays these signals to the brain. We have recently demonstrated that vagal afferent neurons (VAN) develop leptin resistance in response to a high fat diet and that this coincides with the onset of hyperphagia. To test the hypothesis that leptin resistance in VAN is responsible for the development of hyperphagia we crossed mice that carry *loxP* sites on either side of exon 1 in the *leptin receptor (lepR)* gene, with *Na$_V$1.8*-Cre mice (promoter specific to sensory neurons) to make mice with VAN deficient in lepR. Real time PCR confirmed *lepR* knockdown in VAN of knockout mice (KO). Furthermore, leptin injection (80 μg/kg, i.p.) increased nuclear phosphoSTAT3 in VAN of wild-type mice (WT), but had no effect in VAN of KO mice, verifying that the KO mice lack functional lepR in VAN. Chow fed KO mice weighed significantly more than WT mice (26.8 ± 0.7 g vs. 24.2 ± 0.8 g at 13 weeks (N = 8); $p < 0.05$) and adiposity was significantly increased in KO mice compared to WT (1.7 ± 0.3 g vs. 0.8 ± 0.1 g at 13 weeks; $P = 0.0013$). Daily food intake was significantly increased in KO mice compared to WT (0.231 ±.0.003 g/day/kg vs. 0.207 ± 0.003 g/day/kg; $p < 0.001$). Interestingly meal patterns differed between sexes. Female KO ate significantly more meals in the dark phase (69.5 ± 11.5 vs. 49.6 ± 9.6; $p < 0.05$), while male KO trended to eat more frequent, longer, and larger meals in both light and dark phase. There was no difference in energy expenditure between groups. This data strongly suggests that leptin resistance in VAN is important in the development of hyperphagia resulting in obesity. Supported by: NIH.

Extracellular dehydration sensitizes sugar intake

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Motivated behaviors (e.g. sodium appetite; sugar intake) may share common reward mechanisms and cross-sensitization. The objective of the present study was to find out if history of dehydration, which sensitizes sodium appetite, also sensitizes sugar intake. Adult male rats with daily access to hypertonic NaCl, water and food were separated in 3 groups, each group receiving a different treatment which was repeated from 2 to 4 times at 1 week interval. Each treatment was followed by a 2-h sodium appetite test for hypertonic NaCl. Treatment 1: Sodium depletion by sc diuretic furosemide combined with the removal of ambient sodium for 24 h. Treatment 2: Combination of furosemide + low dose of captopril (angiotensin converting enzyme blocker). Treatment 3: Water deprivation for 36 + 2 h of partial rehydration with water. The hypertonic NaCl intake was enhanced from the first to last sodium appetite test for every treatment. Control rats that received only vehicle or were not dehydrated showed no enhancement in hypertonic NaCl intake. Rats with history of a treatment tested for sugar intake one week after the last sodium appetite test ingested more 10% sucrose than controls (Treatment 1: 10.0 ± 1.5 vs. 5.8 ± 1.0; Treatment 2: 18.0 ± 3.0 vs. 9.5 ± 2.0; Treatment 3: 9.2 ± 1.5 vs. 5.3 ± 0.6 ml/120 min, n = 7–10/group). The results suggest that history of dehydration, particularly that involving production of angiotensin II, sensitizes sugar consumption. Financial suport: CNPq, FAPESP.

Integrated effects of leptin in the forebrain and hindbrain

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Leptin receptors (ObR) in the forebrain and hindbrain are both known to mediate leptin responses. It is unclear how leptin activity in these areas is integrated. We tested whether ObR activation in both the forebrain and hindbrain is required for a significant change in energy balance. Previous studies used acute high dose leptin injections (1–10 μg); here we used 12 day infusions of low doses of leptin (0.1 μg/24 h in 3rd ventricle, 0.6 μg/24 h in 4th ventricle). Male Sprague Dawley rats (n = 36) were fitted with 3rd and 4th ventricle cannulas and housed in an indirect calorimeter. Saline (S) or leptin (L) was infused from Alzet pumps for 12 days. There were 4 treatment groups SS, SL, LS, LL (3rd–4th ventricle). Leptin in single ventricle infused groups (LS, SL) had no effect when compared to controls (SS). Rats with low dose leptin infusions into both ventricles (LL) showed a 60% reduction in food intake compared to controls that was reversed after day 6 and a 20% weight loss which stabilized at day 6. Body fat of LL rats was decreased by 50% after 12 days despite correction of food intake. LL rats had a lower RER when food intake was low, indicating increased fat oxidation. They displayed normal activity and maintained energy expenditure despite weight loss, indicating inappropriately high thermogenesis. Western blot analysis of leptin signaling proteins revealed increased activation of STAT3 in the brainstem of LL rats, but suppressed activation of ERK 1/2 in the hypothalamus. These data show that chronic activation of ObR in both the hypothalamus and brainstem is required for physiologic doses of leptin to reduce body fat. Supported by: NIDDK 053903.

Fluoxetine dialysis in the nucleus accumbens shell in rats increases blood glucose concentration

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Recently, we showed that deep brain stimulation (DBS) of the nucleus accumbens shell (sNAc) in rats rapidly increases blood glucose and plasma glucagon concentrations in a region- and intensity dependent manner. We hypothesized that the neurobiological mechanism underlying this sNAc-DBS effect on glucose metabolism involves modulation of local neurotransmitter release. To test this hypothesis, we investigated the effect of fluoxetine (a selective serotonin reuptake inhibitor) administration in the sNAc on glucose metabolism. Male Wistar rats received bilateral microdialysis probes in the sNAc in addition to a jugular vein and carotid artery catheter. We subjected the rats to 60 min of reverse microdialysis of either Ringer (vehicle) (n = 8) or fluoxetine (250 mM) (n = 9). Blood samples were drawn prior, during and following cessation of drug administration to measure blood glucose and plasma insulin concentrations. Endogenous glucose production (EGP) was measured by stable isotope dilution. Bi- or unilateral fluoxetine dialysis in the sNAc induced a significant increase in blood glucose compared to vehicle dialysis. This effect was not fully explained by changes in EGP or plasma insulin concentrations. These preliminary data support a role for serotonin in the sNAc-DBS induced effects on glucose metabolism. Supported by: ZonMW.

5-HT1a antagonists reduce food intake and body weight by reducing total meals with no conditioned taste aversion

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Experiments were conducted to determine the effects of 5HT1a antagonists on food intake and taste aversion. Using an automated food intake monitoring system, BioDAQ (Research Diets Inc., New Brunswick, NJ) to determine the microstructure of feeding, male diet-induced obese mice were administered LY439934 (10 or 30 mg/kg, po) or WAY 100635 (3 or 10 mg/kg, sc) for 3 days. Meal pattern analyses revealed that 24 h food intake was reduced through a specific decrease in the total number of meals. Day 1 meal number was decreased ~30–35% compared to vehicle groups in the high dose groups with each compound and was reduced similarly each day. However, average meal size (0.2 g ± 0.0) was not impacted by any of the compounds on any day. In addition, body weight was reduced 2–3% compared by Day 3 to vehicle controls. Subsequently, we sought to determine whether the feeding decrease might be an indicator of visceral illness by using a conditioned taste aversion assay. Using the two bottle preference test, we found none of the compounds produced a conditioned taste aversion. Therefore, the decrease in food intake does not appear to be a response to aversion induced by the compounds. These results indicate that dosing of a 5-HT1a receptor antagonist suppresses feeding, specifically by decreasing number of meals, and induces weight loss without the liability of nausea as an adverse side effect. Supported by: Eli Lilly & Co.

Diet and microbiota interactions in intestinal inflammation, obesity and insulin resistance

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Obesity is associated with inflammatory changes that promote adverse health consequences including insulin resistance, risk of type 2 diabetes, cardiovascular disease and cancers of multiple organs. To date most studies have focused on obesity associated inflammatory changes in adipose tissue, liver or systemic circulation. Our recent studies in wild-type (WT) or NFκB-reporter-Green fluorescent protein reporter mice (NF-κB-GFP mice), demonstrate that high fat western diets (HFD) induce inflammatory changes in multiple cell types within the intestinal wall, including epithelial cells, endothelial cells and immune cells. HFD-induced intestinal inflammation requires the presence of resident microbiota which are also required for high fat diet (HFD)-induced obesity. Furthermore intestinal inflammation precedes and strongly and significantly correlates with HFD-induced increases in fat mass and reduced insulin sensitivity. This presentation will: Explore the evidence that Diet:bacteria induced intestinal inflammation promotes and is an early biomarker of risk of insulin resistance in humans as well as mouse models. Consider mechanisms that may link intestinal inflammation to obesity and insulin resistance including suppressors of cytokine signaling (SOCS), inflammasome activation and a potential role of insulin resistance within the intestinal epithelium itself. Supported by: NIH, NIDDK.

Blockade of cGMP degradation by BAY 73-6691 potentiates and extends amylin's anorectic action

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The satiating effect of peripheral amylin is mediated by area postrema (AP) neurons. Amylin binds strongly to its receptors in the AP where amylin leads to activation of several intracellular signaling cascades. Amylin increases the expression of the second messenger cyclic guanosine monophosphate (cGMP) in the AP approximately 30 min after administration, which parallels the time when amylin's satiating effect reaches its peak. Recent pilot data indicated that a specific subtype of phosphodiesterase (PDE), PDE9, is highly expressed in the AP. PDEs constitute a group of enzymes that degrade cGMP and hence terminate cGMP-mediated actions. We therefore hypothesized that inhibition of PDE9 would potentiate amylin action. Peripheral administration of the specific PDE9 inhibitor BAY 73-6691 (BAY; 0.1 mg/kg) enhanced and extended amylin's (5 μg/kg IP) eating inhibitory action. Amylin reduced eating by about 40% for more than 4 h in BAY-treated rats, but for 2 h only in respective controls. The effects of brain-specific PDE9 inhibition on amylin action will also be explored. Overall, our results provide the first evidence that inhibition of cGMP degradation by a specific PDE9 inhibitor may extend amylin's eating inhibitory effect. Supported by: Swiss National Science Foundation.

Nucleus accumbens GLP-1 receptors contribute to nutrient-induced satiety

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Hindbrain glucagon-like peptide 1 (GLP-1) neurons play a role in the control of food intake, and these cells are activated by meal-related gastrointestinal stimuli. Recent studies have indicated that endogenous GLP-1 receptor (GLP-1R) activation in the nucleus accumbens core (NAcC), limits feeding. Therefore, we hypothesized that the GLP-1 projection to NAcC contributes to meal-induced satiety. Here we asked whether intra-NAcC delivery of the GLP-1R antagonist Exendin 9-39 (Ex9) attenuates satiety induced by an intra-gastric or orally consumed nutrient preload in male Wistar rats. Rats fitted with intra-gastric catheters received an intra-NAcC injection of either saline or 2 mg Ex9, followed by a preload infusion of either saline or 5 ml dilute sweetened condensed milk (SCM) (7.8 kcal, 1 ml/min) 30 min before the onset of the dark cycle. At dark onset, *ad libitum* access to chow was provided and intake was measured. After intra-NAcC saline, SCM preload significantly reduced chow intake (relative to saline load: 68% at 30 min, 58% at 1 h, 37% at 2 h), but the magnitude of the preload effect was significantly reduced by intra-NAcC Ex9 (relative to saline load: 25% at 30 min, 31% at 1 h, 18% at 2 h). In a second experiment, we trained rats to consume the 5 ml SCM preload orally 15 min before dark onset. Similar to the intra-gastric infusion study, we observed that intra-NAcC GLP-1-R blockade prior to the oral preload significantly reduced the magnitude of preload-induced satiety. Based on these data, we conclude that endogenous activation of NAcC GLP-1R plays a role in meal-related satiety.

Nucleus accumbens phasic dopamine signals reward prediction rather than action selection

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Environmental stimuli repeatedly paired with food reward become cues for food delivery as well as initiate approach behavior. These cues evoke brief (<3 s) increases (spikes) in dopamine (DA) concentration in the nucleus accumbens core (NAc). It remains unclear whether DA spikes reflect the associative strength between cues and outcomes, or participate in action selection and generation. Here, we developed a symmetrical Go/NoGo paradigm where one cue (Go) instructs a response on a lever to obtain a sucrose pellet reward. Another cue (NoGo) instructs behavioral inhibition. Successful behavioral inhibition on these trials results in sucrose pellet delivery. Failure to respond on Go and inappropriate responses on NoGo trials resulted in a 'time-out' from the experiment. Cues were therefore predictive of reward but instructed different actions in well-trained rats. Using fast-scan cyclic voltammetry, we measured DA spikes during performance. Both Go and NoGo cues evoked similar spikes in DA concentration (26.5 ± 3.6 vs. 29.4 ± 4.7 nM for Go vs. NoGo, respectively), suggesting that action selection was not encoded in NAc DA signaling. DA modulates the excitability of NAc neurons and we investigated the role of the NAc itself in action selection. NAc inactivations with GABA agonists baclofen/muscimol dose-dependently affected performance on the Go/NoGo paradigm. Taken together, the findings suggest that while DA does not specifically encode action selection, its modulatory role within the NAc may filter NAc inputs whereas NAc output appears critical for cue-evoked approach behavior. Supported by: DA025634, DA027127.

Investigating autonomic regulatory networks controlling energy balance and glucose homeostasis

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The brain plays a critical role in regulating food intake, body weight and blood glucose levels. Dysfunction of this central regulation results in obesity and type II diabetes. Therefore, to understand the causes and to develop treatments for obesity and diabetes, it is first necessary to unravel the brain pathways regulating coordinated energy homeostasis. Metabolic cues and neurotransmitters act on key collection of neurons both within and outside the hypothalamus to regulate food intake and body weight and glucose homeostasis. However, the inherent complexity of these CNS circuits has made it extremely difficult to definitively identify the key neurons that are required to maintain glucose homeostasis and energy balance. Over the past several years the ability to manipulate gene expression in a neuron-specific fashion has become feasible. We will describe some our recent findings using mouse models that allow neuron-specific manipulation of genes regulating energy balance and glucose homeostasis. We will focus on results from studies investigating key autonomic control neurons including autonomic preganglionic neurons including the vagus nerve. It is our hope that these studies have provided insights into the mechanisms through which the nervous system regulates food intake, body weight and blood glucose levels. Supported by: NIH R01DK53301, NIH RL1DK081185, and NIH P01DK088761.

Interaction of dieting status with reward response to palatable food cues. An fMRI study

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Prior neuroimaging research from our lab (Coletta et al., 2009, J. Ab. Psych.)[[Au: The following reference is cited in text but not listed]] has shown that individuals high or low in restrained eating demonstrate brain activation in response to food cues that parallels their food intake in lab studies. We extended these findings by comparing normal weight Nondieters, Historical Dieters (who typically counterregulate), and Current Dieters under the conditions that mimicked past lab studies. Participants were shown pictures of highly and moderately palatable foods while being scanned in an fMRI BOLD paradigm following an 8-h fast and again after a liquid meal. In the Fed state, Historical Dieters showed elevated reward circuitry activation in response to highly palatable food, as compared to Nondieters, Current Dieters and to themselves when fasted. In contrast, Current Dieters showed increased reward activation in the Fasted state in both within- and between-group comparisons, in line with extant behavioral research. The parallels between eating behavior and regional brain activation across groups suggest that a neurophysiological vulnerability to overeat exists in normal weight young women that may increase susceptibility to weight gain in the long term.

Personality as a risk factor for antipsychotic drug induced weight gain and insulin resistance

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Second generation antipsychotics such as Olanzapine (OLZ) are characterized by unwanted side effects: they induce substantial weight gain and insulin resistance. The specific mechanisms through which these antipsychotics induce these effects are still unclear. To study this, we have performed a series of studies, both in humans and experimental animals. In human volunteers, 14 days of oral OLZ treatment led to substantial weight gain, caused by increased food intake and reduced energy expenditure. Body temperature was also reduced and glucose and insulin levels in an oral glucose tolerance test were increased, pointing to insulin resistance. In rats, OLZ treatment leads to similar effects. In both male and female Wistar rats, intragastric administration of OLZ led to insulin resistance, hypothermia, and reduced locomotor activity. Weight gain and increased energy intake was, however, only observed in female rats. Finally, previous studies from our lab revealed that personality may serve as a risk factor for weight gain and insulin resistance. Therefore, we treated both proactive and passive individuals of the Roman Avoidance rat strain with OLZ and found that only the Roman High Avoidance rat is particularly sensitive to the orexogenic and obesogenic effects of OLZ. This finding is relevant since (1) the RHA rat is considered as an animal model for schizophrenia based on its increased compulsive behavior and increased dopaminergic receptor expression; and (2) a 'schizophrenic' personality is particularly at risk for the negative metabolic side effects of antipsychotic drugs.

The circumventricular organs as sensory integrators of critical circulating signals regulating fluid and energy homeostasis

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The area postrema (AP) and subfornical organ (SFO) are classified as "sensory CVOs" in view of their established roles as critical integrative centres where circulating peptides act to regulate the cardiovascular and neuroendocrine systems. An emerging literature now suggests that these sensory CVOs play essential roles in sensing circulating metabolic signals. AP neurons respond to changes in the concentrations of many metabolic signals including amylin, glucose, glucagon like peptide-1 (GLP-1), cholecystokinin (CCK), adrenomedullin, orexin, adiponectin, peptide YY (PYY), and ghrelin. SFO neurons have for some time been recognized as sensors of signals involved in the regulation of fluid balance including angiotensin, calcium, endothelin, osmolarity, and sodium. Additional roles for the SFO in sensing metabolic signals such as calcitonin, amylin, and ghrelin have more recently been demonstrated suggesting additional potentially important roles for this CVO in the regulation of energy balance. Microarray analysis of AP and SFO shows that these CVOs not only contain high densities of receptors for a number of important metabolic signals, but also express high levels of mRNA for neurotransmitters involved in the central regulation of energy balance. These data identify the CVOs as unique CNS sites where integration of metabolic and cardiovascular signals occurs. Such observations also provide some clues as to how pathological changes may in part explain the established comorbidities associated with autonomic dysfunction which link hypertension, obesity, cancer, and stroke. Supported by: CIHR.

Texture and taste influence on food intake for a realistic savoury lunch-time meal

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Many palatable, energy dense and softly textured foods can be ingested quickly and lead to energy overconsumption. Previous studies on liquid and semi-solid foods have shown that increasing a foods texture can lead to lower eating rates and lower ad-libitum food intake. Similarly, studies on taste have shown that higher salt taste intensity can produce lower ad-libitum food intake. The current study aimed to replicate these effects in a realistic solid savoury lunch time meal. Meals consisted of potatoes, carrots, beef steak and gravy varied according to a 2 (texture: mashed vs. whole) $\times$ 2 (taste: standard taste vs. strong taste) design. Using a between groups, single course design, subjects attended a lunch where their ad-libitum food intake was measured. Four groups ($N = 40 \times 4$) were recruited and matched for age, gender, normal BMI and dietary restraint. The four meal conditions did not vary in overall liking between the four groups and covariates (i.e. gender, pre-meal hunger/fullness and overall liking) were also included in the linear-model. Results showed that estimated mean intake in the two mashed conditions were higher than intake for whole meals. The texture effect was much stronger in the savoury tasting meals, with an average of 91g less food consumed in the solid-savoury meal than in the mashed-savoury meal. This effect was not replicated in the standard gravy. Consumers eating-rate in the two mashed conditions was significantly higher than in the whole meal condition (57.0 ± 2.5 g compared to 47.2 ± 2.5 g, $p < 0.05$), with no difference in eating rate between the standard and savoury gravy conditions. Results showed that texture influences eating rate and ad-libitum intake for solid savoury meal components, and suggest that the influence of taste may be linked to individual preferences. The current results demonstrate the impact of sensory properties on ad-libitum energy intake. These findings can be used to design savoury meals with textures that slow the rate of food intake and reduce overall energy intake. Supported by: Nestec – Nestle Research Centre.

Leptin signaling in the nucleus tractus solitarius suppresses motivation to obtain rewarding food

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Leptin signaling in the medial nucleus tractus solitarius (mNTS) suppresses food intake by reducing meal size via amplification of the intake suppressive effects of gastrointestinal-derived satiation signals. Here we examine the hypothesis that mNTS leptin signaling also suppresses feeding by reducing *appetitive* behaviors directed towards obtaining palatable food. Rats were trained in a conditioned place preference (CPP) paradigm to associate one location with rewarding food (diet high in saturated fat and sucrose); a separate location was not associated with food. Leptin (0.5 μg) or vehicle was delivered intra mNTS 3 h before CPP testing. Vehicle-treated rats learned to prefer the food-paired location, whereas this preference was blocked by mNTS leptin delivery. To assess whether mNTS leptin signaling also influences willingness to work for palatable food, rats were trained in an operant paradigm where each lever press yielded access to a sucrose reward. Relative to vehicle treatment, leptin delivered to the mNTS significantly reduced operant responding for sucrose in a progressive ratio reinforcement (PR) test in which the number of lever presses needed to obtain a single sucrose pellet progressively increased. These findings deepen the understanding of the contribution of leptin signaling to feeding control – they reveal that mNTS leptin signaling suppresses feeding not only by amplifying satiation signals, but also by reducing appetitive, motivated behaviors directed towards obtaining rewarding food. NIHDK21397&NIHDK089752.

Ghrelin expression in a rat model of chemotherapy-induced anorexia

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Altered ghrelin expression in the stomach and brain may contribute to chemotherapy associated anorexia, anxiety and depression and were studied during methotrexate (MTX) induced mucositis and anorexia. Sprague-Dawley rats received MTX (2.5 mg/kg, SC for 3 days) and controls received PBS. Forced-swim (FST) and elevated plus maze (EPM) tests and tissues sampling were performed at day 5 after 1st MTX injection during maximal anorexia. We found that preproghrelin mRNA expression levels were lower in the stomach but increased in the hypothalamus of MTX rats. GOAT mRNA was decreased in the stomach and amygdala but not in the hypothalamus of MTX rats. Plasma levels of acyl- and des-acyl ghrelin did not differ significantly from controls but acyl-/des-acyl ghrelin ratios were lower in MTX rats. No significant differences in FST were observed between MTX and control rats, but fewer entries in open and central zone of the EPM were found in MTX rats. These data show that lower rates of acylation of systemic ghrelin in MTX rats may contribute to anorexia and increased anxiety during chemotherapy. Supported by: EU INTERREG IVA 2 Seas Program (7-003-FR_TC2N).

Alterations in brain activity in severely obese women after Roux-en Y gastric bypass surgery

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The Roux-en Y gastric bypass (RYGB) surgery is one of the most successful therapeutic approaches for severe obesity. The aim of this study was the comparison of brain activity pattern between severely obese (OB), normal-weight (NW) and previously severely obese women who had undergone RYGB surgery at least one year before the study. Brain activity was assessed by functional magnetic resonance imaging (fMRI) during the presentation of food and non-food pictures as well as during resting state in 11 OB, 11 NW and 9 RYGB women. As compared to NW and also to RYGB, OB women showed higher cerebellar and lower fusiform gyrus activity during the visual stimulation independent of the stimulus category, a higher hypothalamic activation during the presentation of low vs. high caloric food pictures and a stronger connectivity strength in the Default Mode Network during resting state. There were no differences in brain activity between the NW and RYGB women. RYGB women generally rated lower on hunger while there were no differences in this rating between the OB and NW women. Data indicate profoundly altered brain activity patterns in severely obese women and suggest that RYGB surgery and/or the surgically induced weight loss reverses the obesity-associated alterations.

Effect of gestational caloric restriction on the tendency of the offspring to develop obesity in OLETF rats

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Background & Methods: OLETF rats lack CCK1 receptors and are a model of hyperphagia-induced obesity. Diet restriction (DR) normalizes their phenotype, but this has not been performed in pregnancy. Maternal gestational DR (GDR) has long term consequences on offspring energy regulation. We asked if GDR will affect OLETF offspring's phenotype. Pregnant LETO and OLETF dams were fed ad libitum, or OLETF dams received 75–60% (25–40% caloric restriction) of the amount consumed by LETO controls throughout gestation. From postnatal day (PND) 23–90 offspring received chow. From PND90 they received chow or high fat diet. We measured intake, body mass, fat pads, plasma leptin levels, and performed behavioral tests. *Results*: In the male chow fed groups, GDR produced heavier pups at PND7, 23, and 120, and heavier livers on PND 120. Intake in calories was also, in general higher in GDR compared to controls. In the females, weight-increase was transient, disappearing in adulthood. In HF diet fed males, body weight, fat pads, total white fat, liver weight, percent fat from BW and intake in calories were all significantly increased, compared to HF fed controls. In females, effects were more moderate. In the LETO, the HF diet increased all measures in both sexes. *Conclusions*: GDR did not "normalize" the offspring. In the males, GDR increased body weight and obesity-related measures in infancy and adulthood, as in other, non-obese strains. Female OLETF rats seem to be protected against GDR-induced fat accumulation in adult life.

Intracellular signaling mechanisms of hypothalamic malonyl-CoA in the control of food intake

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Fatty acid metabolism is involved in the hypothalamus-mediated control of food intake. In this regard, malonyl-CoA, an intermediate in fatty acid biosynthesis, has emerged as a key player. In the hypothalamic arcuate nucleus (Arc), malonyl-CoA acts as a downstream mediator in AMP-activated kinase (AMPK) signaling pathways underlying hypothalamic control of feeding. In the Arc, acetyl-CoA carboxylase links AMPK and malonyl-CoA to mediate leptin's hypothalamic effect on feeding. The downstream mediators of malonyl-CoA action on feeding have not been firmly established. In the periphery, malonyl-CoA inhibits the acyltransferase activity of carnitine palmitoyltransferase-1 (CPT-1). However, in the hypothalamus, the CPT-1 acyltransferase activity may not mediate the downstream action of malonyl-CoA's effect on food intake. For example, the classical CPT-1 acyltransferase activity is insignificant in malonyl-CoA signaling pathway in the Arc in leptin's effect on feeding. The brain-specific CPT-1 (CPT-1c) that does not possess the classical acyltransferase activity is a potential downstream player of Arc malonyl-CoA action. Notably, CPT-1c is required in leptin's central effect on food intake, and CPT-1c appears to impact the metabolism of ceramide. Of relevance, ceramide metabolism is implicated in hypothalamic control of food intake, and the actions of leptin and malonyl-CoA both modulate Arc ceramide levels. Thus, malonyl-CoA, CPT-1c and ceramide may constitute a signaling module in mediating aspects of hypothalamic control of food intake.

Acute central neuropeptide Y (NPY) administration increases food intake but does not affect hepatic very low-density lipoprotein (VLDL) production in mice

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Whereas the role of NPY in food intake and glucose metabolism is well-established, its role in lipid metabolism has received less attention. In literature, there is conflicting evidence on a possible role of NPY in hepatic VLDL production in rats and mice. The aim of this study was to establish the effect of central NPY administration on hepatic VLDL production in mice. Male C57Bl/6J mice received a cannula in either the lateral ventricle (LV) or the third ventricle (3V) of the brain. After a week of recovery, after a 4 h-fast, the effect of central NPY administration on hepatic VLDL production was measured in at least 8 mice per group, using the tyloxapol method combined with injection of Tran[35S] label to assess both hepatic VLDL-TG and VLDL-[35S]-apoB production. Both LV and 3V administration of NPY (0.2 mg/kg) significantly increased food intake 1 h after injection (0.34 ± 0.19 vs. 0.90 ± 0.40 g, $p < 0.001$ and 0.21 ± 0.08 vs. 0.98 ± 0.44 g, $p < 0.001$, respectively). However, LV NPY administration did not affect hepatic VLDL-TG production (7.7 ± 0.6 vs. 7.3 ± 1.1 μmol/h, ns) or VLDL-[35S]-apoB production (84 ± 11 vs. 79 ± 21 × 103 dpm/h, ns). 3V injection of NPY also did not affect hepatic VLDL-TG production (6.5 ± 0.6 vs. 6.0 ± 0.9 μmol/h, ns) or hepatic VLDL-[35S]-apoB production (22 ± 3 vs. 22 ± 2 × 103 dpm/h, ns). In mice, acute central administration of NPY increases food intake but does not affect hepatic VLDL production. Supported by: Netherlands Heart Foundation, Netherlands Diabetes Foundation.

Reversing presynaptic central dopamine deficits in obese animals

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Food restriction and exercise are interventions used to combat dietary obesity. It is documented that dietary obesity is associated with a significant presynaptic dopamine deficit in the brain (i.e. Geiger et al., Neuroscience, 2009). It is unclear how these interventions impact on both homeostatic and hedonic central brain circuits that regulate food intake, which could determine their long-term success or failure. In the present study, we used food restriction in *ob/ob* mice and voluntary physical exercise in Long Evans rats to assess effects on presynaptic dopamine plasticity in the mesolimbic system. Body weight of *ob/ob* mice after 6 weeks on a restricted diet decreased by an average of 27.3% producing a significant difference from the *ob/ob* mice fed *ad libitum* chow. Mean evoked dopamine per stimulation in the nucleus accumbens shell was significantly higher in the *ob/ob* food restricted than in the *ob/ob* ad libitum group. The food restricted *ob/ob* mean was $42.7 \times 10^6 \pm 5.0 \times 10^6$ dopamine molecules ($n = 67$ stimulation in 14 slices). The ad libitum *ob/ob* mean was $11.0 \times 10^6 \pm 1.5 \times 10^6$ ($n = 43$ stimulations in 11 slices). In the exercise study, animals were given 24-h voluntary access to running wheels over a period of at least 6 days. Mean number of dopamine molecules released was higher in accumbens slices from runners than from sedentary rats ($p < 0.05$). Acute coronal brain slices from sedentary rats released $7.38 \times 10^7 \pm 1.06 \times 10^7$ molecules as compared to $15.98 \times 10^7 \pm 1.39 \times 10^7$ molecules in slices from runners. We conclude that both chronic food restriction and physical exercise upregulate central dopamine release through presynaptic mechanisms and that this effect is evident across different species. Supported by: DK 065872 (ENP).

Distinct mechanisms mediate glucose-stimulated GLP-1 secretion from small and large intestine

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The glucose-dependent secretion of glucagon-like peptide-1 (GLP-1) from intestinal enteroendocrine L cells is a critical step in the regulation of glucose homeostasis. However, the molecular basis for glucose-stimulated GLP-1 secretion (GSGS) is controversial. We assessed the contribution of two candidate GSGS mediators – the sweet taste receptor (T1R2 + T1R3) and ATP-sensitive K^+ (K_{ATP}) channels – by measuring sugar-stimulated hormone secretion in gene-targeted mice. $T1R3^{-/-}$ mice exhibited impaired glycemic control during an oral glucose challenge and slower kinetics of insulin granule fusion in pancreatic islets imaged by TIRF microscopy. Glucose, fructose and sucralose evoked GLP-1 secretion from $T1R3^{+/+}$, but not $T1R3^{-/-}$, ileum explants in Ussing chambers; this secretion was unaffected by K_{ATP} channel blockers or openers. $T1R2^{-/-}$ mice showed normal glycemic control and partial small intestine GSGS, suggesting that T1R3 can mediate GSGS in the absence of T1R2. Surprisingly, robust GSGS that was K_{ATP} channel-dependent and glucose-specific emerged in the large intestine of $T1R3^{-/-}$ mice. Together, our results demonstrate that the intestine employs two distinct GSGS mechanisms that differ in stimulus selectivity and anatomical localization. Supported by: NIDCD NIDDK Ajinomoto 3ARP.

Transformation of post-ingestive glucose responses in the hindgut after Roux-en-Y gastric bypass in rats

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Roux-en-Y gastric bypass surgery (RYGB) is one of the most effective methods available to produce sustained weight loss and normalize glucose homeostasis. The accelerated delivery of food to the jejunum or/and hindgut is suggested to lead to exaggerated secretion of the insulinotropic hormone glucagon-like peptide 1 (GLP-1). However, the mechanism regulating this GLP-1 release is unknown. We recently found that robust glucose-stimulated GLP-1 secretion (GSGS) from the large intestine emerges in mice with carbohydrate malabsorption. To investigate whether the malabsorptive RYGB results in a similar increase in hindgut GSGS, we measured sugar-dependent GLP-1 secretion from ileum and colon explants from rats receiving sham operation (Sham) or RYGB. Rats were maintained on either normal or high-fat diets (ND, HFD), and were sacrificed 1–3 months post-surgery. Intestinal explants were exposed to sugars, and secreted hormones quantified by ELISA. Glucose and fructose stimulated GLP-1 secretion from ileum in all animals. Glucose, but not fructose, increased GLP-1 secretion in colon from RYGB rats but not Sham rats at all time points and independent of diet, though responses were reduced at 3 months post-surgery. These findings provide an explanation for the increase in GLP-1 levels seen after RYGB and may provide new therapeutic targets for promoting weight loss and improved glycemic control. Supported by: NIDDK (DK080899), NIDCD (DC010110).

Effects of acute treatment with a tryptophan-rich egg white protein on plasma amino acids, emotional and cognitive functioning in older women

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Effective functioning of the neurotransmitter serotonin is important for optimal cognitive performance, maintaining positive mood states and resilience to stressful challenges. We investigated whether two different doses of a tryptophan-rich egg white protein hydrolysate ('lumiVida'™, DSM Nutritional Products, Switzerland) can improve cognitive function, mood state and emotional processing. Sixty healthy women aged 45–65 years were randomised to receive a single drink containing either 2 g or 4 g of lumiVida, or 3.1 g of casein hydrolysate (control), prior to undertaking a battery of cognitive tests (memory, attention, motor control, emotional processing) and completing mental state measures, following a baseline test day without treatment. The expected dose-dependent increase in the ratio of plasma TRP to competing large neutral amino acids was seen. lumiVida (2 g but not 4 g) prevented the decline in wellbeing and increase in fatigue seen over the test session in the other groups, and resulted in a shift in emotional processing towards positive words and reduced bias in assessing negative facial expressions. Cognitively, there was a slight improvement in word memory, and faster reactions in a vigilance task. Thus, a low dose of lumiVida may have modest beneficial effects on emotional and cognitive function. Supported by: DSM Nutritional Products.

Ghrelin antagonizes the stimulatory effect of cocaine on ethanol self-administration

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Recent work has shown that central ghrelin administration stimulates ethanol intake in a free-bottle limited access paradigm. Ghrelin treatment has also been reported to increase ethanol-induced locomotor behavior, operant responding for food and extracellular dopamine release in the ventral striatum. These effects are attenuated by ghrelin receptor antagonism and/or dopamine depletion. The latter finding is consistent with increasing evidence suggesting that ghrelin and dopamine signaling interact in the neural control of motivation and ethanol reward. To further investigate a possible interaction between these two neurochemical systems, in the present study we examined the impact of ghrelin, cocaine and combined injections of ghrelin paired with cocaine, on voluntary ethanol intake. Juvenile male S-D rats were habituated to an 8% ethanol solution until intakes had stabilized. At this point, rats were administered ghrelin (0–10 nmol IP), cocaine (0–10 mg/kg IP) or ghrelin paired with cocaine. Ethanol intakes were examined at 1, 2, 4, 6 and 24 h postinjection. Results indicated that while cocaine reliably increased ethanol intake, systemic administration of the peptide did not alter consumption. Moreover, pretreatment with ghrelin antagonized the effect of cocaine on ethanol intake. In conclusion, while our findings suggest that ghrelin may alter dopaminergic function resulting in a suppression of cocaine-induced intake, our work further indicates that unlike central treatment, which has a stimulatory effect on ingestion, peripheral ghrelin administration fails to reliably potentiate voluntary ethanol intake. Supported by: Reed College Initiative Grant.

Effects of circadian misalignment on sleep, energy expenditure, appetite and related hormones

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Disruption of the circadian system has been associated with the development of obesity. We examined the effects of circadian misalignment on sleep, energy expenditure, substrate oxidation, appetite and related hormones. Thirteen subjects (age 24.3 ± 2.5years, BMI 23.6 ± 1.7 kg/m^2) participated in three light-entrained circadian cycles (3×21 h and 3×27 h) resulting in a phase advance and a phase delay. For each condition, they stayed in a timeblinded respiration chamber. Sleep, energy expenditure, substrate oxidation and appetite were quantified. Blood and saliva samples were taken to determine melatonin, glucose, insulin, ghrelin, leptin, glucagon-like peptide 1 (GLP-1) and cortisol concentrations. Circadian misalignment, either phase advanced or phase delayed, did not result in any changes in appetite or energy expenditure, while meal related blood parameters (glucose, insulin, ghrelin, leptin and GLP-1) followed the new meal patterns. However, phase advanced misalignment caused flattening of the cortisol secretion pattern ($P < 0.001$), increased HOMA-IR ($P = 0.03$) and increased carbohydrate- ($P = 0.03$) and decreased protein-oxidation ($P = 0.001$). Phase delayed misalignment increased REM sleep ($P < 0.001$) and Sleeping Metabolic Rate ($P = 0.02$), increased glucose ($P = 0.02$) and decreased GLP-1 ($P = 0.02$) concentrations and also increased carbohydrate- ($P = 0.01$) and decreased protein-oxidation ($P = 0.003$). The main effect of circadian misalignment, either phase advanced or phase delayed, is a concomitant disturbance of the glucose-insulin metabolism and substrate oxidation, while energy balance and sleep are not largely affected. Chronically eating and sleeping at unusual circadian times, as occurs with shift work and jet lag, may create a health risk through metabolic disturbance. Supported by: Nutrition and Toxicology Research Institute Maastricht (NUTRIM).

Effect of maternal bariatric surgery on metabolic parameters of offspring

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Presently 18.9% of women of childbearing age in the US have a BMI > 35. For women of child-bearing age, obesity presents increased reproductive difficulties. While weight loss improves many complications, it is difficult to achieve. Whereas pharmacologic treatments result in only small reductions in body-weight, several bariatric surgical procedures produce sustained body-weight reduction in obese individuals. The purpose of the current studies was to investigate the impact of maternal vertical sleeve gastrectomy (VSG) on the metabolic health of offspring born following surgery. We hypothesized that maternal weight loss would confer benefit to offspring born following VSG in comparison to controls. Female Long Evans rats were maintained on either a low-fat chow or a palatable high-fat diet (HFD) prior to VSG or sham surgery. At birth, offspring of VSG dams (oVSG) were lighter than offspring born to either dams on Chow or dams on HFD having received sham-operations (oChow and oHFD), respectively. At weaning, circulating plasma triglycerides were significantly elevated in oVSG and oHFD in comparison to oChow. Staining of the liver with Oil Red O revealed ectopic lipid accumulation in both oHFD and oVSG with total liver triglyceride content being greatest in oVSG. In both oHFD and oVSG, hypothalamic AgRP mRNA expression was elevated and POMC was reduced relative to oChow. Collectively these data suggest that maternal loss of adiposity following VSG surgery while continuously maintained on HFD does not confer a metabolic benefit to the offspring during the early postnatal period in the measures we investigated. Supported by: NIH F32 HD 068103.

Maternal high fat/sucrose diet exposure in selectively bred highly active mice causes loss of diet-induced obesity resistance in female offspring

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Maternal high fat/sugar (FS) feeding predisposes offspring to obesity and insulin resistance. While hedonic hyperphagia has been reported to underlie offspring obesity, we focused in CD-1 mice on maternal FS exposure effects on offspring energy expenditure; i.e., daily energy expenditure (dEE), resting metabolic rate (RMR), and energy expenditure attributed to voluntary exercise (NEAT). We also assessed these effects in mice selectively bred for high wheel running behavior (WR). Dams were fed chow (C) or FS during pregnancy and lactation. At weaning half of the mice were given C or FS. Besides energy expenditure, running wheel activity and body composition were assessed. In control mice, either pre- or post-weaning FS diet caused obesity in male and female offspring. While WR males showed similar responses as controls, WR females were obesity resistant when exposed to either pre- or post-weaning FS feeding. The combination, however, did render the WR females (mainly visceral) obese. In control offspring and WR male offspring, none of the diet exposures caused alterations in dEE, RMR or NEAT. WR females had an increased dEE when given post-weaning FS; this response was normalized by a reduced NEAT when pre- and post-weaning FS were combined. Despite the metabolic changes, pre- and post-weaning FS did not change activity levels. We conclude that maternal FS exposure can override offspring obesity resistance by means of metabolic, but not behavioral programming.

Taste-reward conundrum in obesity. Can it be solved by gastric bypass?

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Whereas the reward-deficiency theory of dietary obesity has become broadly accepted, taste changes in obesity and the cause-effect relationship between sensory and hedonic gustatory processing and food reward remain elusive. In a series of behavioral and single-neuron recording studies in the pontine parabrachial taste relays using genetic (OLETF), obesity-prone (OP) and outbred dietary obese (DIO) rats, we investigated the hypothesis that improved food preferences after Roux-en-Y gastric bypass (GBS) are due to improved gustatory and reward functions and the two are causally related. We found that despite baseline differences between strains, GBS increased taste acuity (based on lick and neuronal responses) and preferences for low (0.6 M) concentrations of sucrose in all models. In contrast, GBS either decreased (in OLETF) or increased (in DIO) anticipatory and incentive motivation (operant licks) to alleviate differences characteristic to the model relative to controls (i.e. increased reward in OLETF, decreased in DIO). These findings collectively demonstrate the capacity of GBS to reverse taste and reward deficits in these obese rat models of various etiologies and suggest that taste changes are more likely be related to improved anorexigenic/orexigenic signals, and in turn, reward sensitivity than to the direction of changes in incentive motivation. Future studies are warranted to confirm transferability of findings to human and investigate underlying mechanisms. Supported by: NIH Grant DK080899.

Effects of acute sleep loss on energy intake and expenditure in humans

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Epidemiological studies suggest a relationship between short sleep duration and metabolic dysfunctions like obesity and type 2 diabetes. While the mechanisms behind these observations are largely unknown, experimental investigations point to an orexigenic effect of acute sleep deprivation. In a series of experiments in healthy humans, we investigated the effect of acute sleep loss on hunger, concentrations of the orexigenic hormone ghrelin, and food intake as well as the effect of sleep loss on physical activity (assessed via accelerometry) and energy expenditure (assessed by means of indirect calorimetry). Sleep deprivation dose-dependently increased hunger ratings and ghrelin concentrations, but did not increase food intake. Physical activity was reduced after one night of partial sleep deprivation and total sleep deprivation of one night reduced resting and postprandial energy expenditure. Reductions in energy expenditure due to sleep deprivation might mediate catabolic effects of sleep loss whereas increases in energy uptake – which were observed in comparable studies – could not be observed after sleep deprivation. Our findings support the notion that sleep is critically involved in the maintenance of energy homeostasis, affecting not only energy intake but also energy expenditure.

Portion size perception and anxiety response to food cues in Anorexia Nervosa compared with controls

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Portion size and energy content are cues that affect stress responses to foods in anorexia nervosa [AN] patients. We hypothesized that increased portions of foods will generate greater fear and stress in AN patients [N = 22] than in controls [N = 10]. Participants rated their stress in response to four foods [rice, potatoes, pizza, and M&Ms], each served in five portion sizes [20, 40, 80, 160, 320 kcal]. Maximum tolerable portion size was derived from a 'point of subjective equality' that was generated by asking patients whether or not [yes/no] they could tolerate portions ranging from 20 to 800 kcal. In both groups, stress response increased linearly with log portion size with significantly steeper slopes for potatoes [55 mm/log kcal] and rice [54], than for pizza [35] and M&Ms [26] in patients, but with significantly lower slopes in controls [31, 23, 10, and 9, respectively], and no food by group interaction. Maximum portion sizes were significantly higher for controls [M^{-} = 440 kcal + -45 SE] than for patients [M = 291 kcal + -29 SE] and for M&Ms [M = 463 kcal] and Pizza [M = 512 kcal] than for rice [M = 231 kcal] and potatoes [M = 141 kcal].Consequently, perceived fear and stress induced by increased perceived portion size are significantly higher in AN patients than in controls. Initial nutritional rehabilitation in AN may be more effective with small portions of high density foods. Supported by: Weill Cornell Medical College.

L-cell distribution in the GI-tract of ZDF rats

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Background: In the past different approaches has been used to describe the distribution of the L-cells in the gastrointestinal (GI) tract. Usually the profiles of cells have been evaluated either quantitatively or objectively leading to biased results that are difficult to interpret. Elevated plasma GLP-1 levels in the Zucker diabetic fatty (ZDF) indicate an increased expression of preproglucagon and derived peptides in this model. Because of a general lack of anatomical and morphometric analysis of the gut in type-2-diabetic (T2D) models we performed an unbiased stereological assessment of l-cell distribution throughout the GI tract. *Methods*: The GI-tract from 10 male ZDF rats and 10 lean controls was sampled using stereological sampling principles into 26–30 sections covering the duodenum, jejunum/ileum and colon. The sections were processed for GLP-2 immunohistochemistry and preproglucagon expression by in situ hybridization. *Results*: The total number of L-cells in the GI-tract was more than doubled from 4.8 million cells in lean rats to 10.9 million in the diabetic ZDF rat, supported by an increase in total preproglucagon expression. The L-cells were found evenly distributed throughout the proximal and distal part of the jejunoileum and colon, in correlation to a similar marked hypertrophy of the epithelial mucosa. Gut volumes demonstrated a marked (250%) increase in mucosa volume as well as in total surface area. *Conclusions*: We show a high plasticity of the gut in animal models of T2D. More importantly, and in contrast to the general opinion, GLP-1 releasing L-cells are shown numerous in both the proximal and distal part of the gut. Supported by: gubra.

Liraglutide and linagliptin improves glycemic control but show differential anti-obesity and hypolipidemic efficacy in a novel hamster model of diet-induced obesity and hypercholesterolemia

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Unlike mice and rats, Golden Syrian hamsters fed a high-fat diet with cholesterol supplementation quickly develop hyperlipidemia and hypercholesterolemia, thus showing closer similarity to human lipoprotein metabolism. We developed a novel hamster model of diet-induced obesity (DIO) and evaluated the efficacy of the GLP-1 analog liraglutide and DPP-IV inhibitor linagliptin. Hamsters fed a high fat-high carbohydrate diet with cholesterol supplementation for 12 weeks developed an obese phenotype with impaired oral glucose tolerance and significantly elevated insulin, triglyceride, total cholesterol, LDL and HDL cholesterol levels. Pancreatic and atherogenetic markers are currently being evaluated. Chronic treatment with liraglutide (0.2 mg/kg, b.i.d, s.c., 4 weeks) normalized body weight with a complete reversal of whole-body fat mass gain. Liraglutide also reduced plasma triglyceride and cholesterol levels. Both liraglutide and linagliptin (3.0 mg/kg, q.d., p.o.) normalized glucose tolerance. In conclusion, liraglutide and linagliptin both improved glycemic control. Liraglutide also showed robust anti-obesity and cholesterol-lowering effects in the DIO hamster, supporting the view that chronic GLP-1 receptor agonism may also lower cholesterol-associated cardiovascular risk factors in diabetes and obesity. This DIO hamster model is particularly useful for evaluation of novel anti-obesity, lipid modulating and insulin sensitizing agents.

Role of gut hormone ghrelin in novelty seeking behavior in rodents and men

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Novelty seeking is one of the most reliable predictors of drug use in man. The role of the gut hormone ghrelin in this behavior is unknown. However, recent discoveries regarding (1) ghrelin's importance in drug and alcohol reward and (2) its ability to regulate mesolimbic dopamine activity, suggest a potential role for ghrelin in novelty seeking. This gives rise to our hypothesis that ghrelin might alter novelty seeking. To test this, several complementary rodent models of novelty seeking behavior were applied: the inescapable novelty-induced locomotor activity, novelty-induced place preference and novel object exploration combined with acute ghrelin receptor (GHSR) stimulation or blockade. Furthermore we determined the association in GHSR polymorphisms with novelty seeking behavior in human subjects. Results from the rodent studies indicate an important role for ghrelin in a wide range of novelty seeking behaviors. Blockade of GHSR potently reduced novelty-induced locomotor activity. Ghrelin-injected rats exhibited a higher preference for a novel environment. Conversely, GHSR blockade drastically reduced preference for a novel environment. Ghrelin also increased novel object exploration. Moreover, the GHSR single nucleotide polymorphism (SNP) rs2948694 was significantly associated with the personality trait, novelty seeking, in both men and woman. Collectively this study provides the first evidence for a role of ghrelin in novelty seeking behavior and findings are potentially extended from rodent models to a human population by the discovery of an association of a GHSR SNP with a novelty seeking trait.

Neural mechanisms of self-control in dietary choice

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Self-control is an important component of dietary choices and can be a critical factor in maintaining a healthy body weight. Here we test a two-part hypothesis: (1) the overall value of a food item is represented in ventral medial prefrontal cortex (vmPFC) regardless of self-control level and (2) self-control is implemented by means of interactions between the dorsolateral prefrontal cortex (dlPFC) and vmPFC. We used a combination of fMRI and EEG to compare neural activity during successful self-control (SSC) and non-self-control (NSC) choices. In both cases, the strength of the decision to eat or not eat a food was reflected in the activity of vmPFC and the timing was similar for both SSC and NSC decisions. In other words, the vmPFC represented the overall value the food items. To determine if dlPFC played a role in self-control choices, we compared SSC and NSC decisions. There was greater activity in dlPFC for SSC compared to NSC. Furthermore, we found using fMRI that this region of dlPFC showed increased interactions with vmPFC during choices over unhealthy foods. We also used EEG to confirm that the timing of interactions between dlPFC and vmPFC is consistent with dlPFC signaling to vmPFC at the time of choice. In summary, our data support both aspects of the hypothesis. Supported by: NSF.

Dietary fat, body weight, blood–brain barrier (BBB) integrity, and hippocampal-dependent cognitive functioning

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Rats fed a high energy (HE) diet (i.e., high dextrose and saturated fat) show impaired performance on hippocampal-dependent feature-negative (FN; e.g., tone^{+}; lightàtone^{-}), but not on simple (e.g., clicker^{+}; white noise^{-}) discrimination problems (Kanoski et al., 2010). HE diet also has adverse effects on obesity status and BBB permeability. Here, food-restricted rats fed standard low-fat chow were trained on FN and simple discrimination problems before being fed ad libitum HE diet, ketogenic diet (very high-fat, low carbohydrate), or chow. At 0 (chow baseline), 10, 40, and 90 days after diet initiation, rats were tested on both problems. Body weight, adiposity, and triglyceride and ketone levels were measured after each test. Fasting glucose, insulin, and GLP-1 levels, glucose tolerance and BBB permeability were assessed after the 90-day test. Compared to chow controls, HE-fed obesity-prone but not obesity-resistant rats were impaired on the FN problem at the 10- and 90-day tests and exhibited increased BBB permeability at the level of the hippocampus at the end of testing. These outcomes were not shown by obesity-prone, ketotic rats fed the ketogenic diet, even though they weighed the same, had greater adiposity and were otherwise similar metabolically to the obesity-prone HE-fed rats. However, obesity-resistant, non-ketotic rats fed the ketogenic diet were impaired on the FN problem. This suggests that elevated ketone bodies may afford protection from the adverse effects of high dietary fat on cognitive and BBB function. Supported by: R01HD028792, R01DK078654.

Integrated effects of forebrain and hindbrain leptin on energy balance in non-stimulated conditions

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Activation of leptin receptors (ObR) surrounding the 3rd or 4th ventricle inhibits food intake and weight gain of rats. This study inhibited or stimulated ObR adjacent to the 3rd or 4th ventricle to test how the sites interact to control energy balance. Male Sprague Dawley rats received 12 day 3rd and 4th ventricle infusions of leptin, ObR antagonist (leptin mutein protein) or saline in different combinations. Leptin in only the 3rd (0.1 μg/24 h) or 4th ventricle (0.6 μg/24 h) did not affect weight gain or body fat. When basal tone of ObR was inhibited by mutein in the 3rd ventricle (2 μg/24 h) food intake increased by 10% (P = 0.08) and body fat by 35% (P < 0.05). This was reversed if leptin was simultaneously infused into the 4th ventricle. Mutein blockade of 4th ventricle ObR (2 μg/24 h) had no effect on body fat, but simultaneous infusion of leptin in the 3rd ventricle reduced body fat. These data suggest that in non-stimulated conditions basal levels of leptin in the forebrain suppress food intake to maintain energy balance, but increased activation of ObR in either the 3rd or 4th ventricle when ObR are inhibited at the other site can induce negative energy balance. None of these treatments changed energy expenditure. Surprisingly, infusion of leptin into both the 3rd and 4th ventricle caused a dramatic fall in food intake and body weight, reduced body fat by 75% and lean mass by 20%, but did not change energy expenditure. Thus, simultaneous activation of ObR in both the forebrain and hindbrain produces an integrated catabolic response that inhibits food intake while maintaining energy expenditure. Supported by: NIH DK053903.

Snack frequency. Associations with healthy and unhealthy food choices

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It is important to understand the impact of snack frequency on food choices and healthy lifestyle. Therefore, the study examines the underlying food and lifestyle patterns of high snack consumers to investigate whether various sub-groups of high snack consumers exist. A sample of 6189 adults participating in the Swiss Food-Panel filled in a questionnaire in 2010 (response rate 30%). The sample consisted of men and women with a mean age of 54·4 (sd 13·5) years. The questionnaire included a food frequency questionnaire and questions regarding socio-demographics and lifestyle factors. Data were analysed with analyses of variance, regression analysis, and hierarchical cluster analysis. Gender differences were investigated. There was no correlation between snack frequency and BMI in the regression analysis. Sweets and savouries consumption (β = 0·32) as well as fruit intake (β = 0·14) were positively associated with snack frequency. Age (β = -0·20), higher educational level (β = -0·10), wine and beer consumption (β = -0·10), and having family meals (β = -0·05) were negatively associated with snack frequency. The cluster analysis revealed three groups of high frequency snack consumers: a healthy, an unhealthy, and a moderate dietary pattern cluster. High snack frequency occurred in the context of healthy as well as unhealthy dietary behaviour and lifestyle patterns. Especially women made healthier dietary food choices and were more likely to consume fruits as snack, while men chose unhealthy foods, such as sweets and savouries, more often. Supported by: Swiss Federal Office of Public Health.

Gene expression profiling reveals widespread, weight loss-independent changes in cytoskeletal signaling after RYGB in mice

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Roux-en-Y gastric bypass (RYGB) surgery leads to substantial and durable weight loss (WL) by altering the physiological regulation of energy balance. To assess the system-wide effects of RYGB, we examined gene expression (GE) profiles in RYGB-treated and weight-matched diet-induced obese mice. We randomized mice to RYGB or sham operation with weight matching by food restriction. After 10 weeks, we determined GE of 38,385 transcripts (TS) in 15 tissues, including 8 segments of small intestine (SI); colon; liver; muscle; subcutaneous and epididymal fat; hypothalamus; and brainstem. Fold-change (FC) and *p*-values from one-way ANOVAs were calculated for each TS, with an absolute FC >1.2 and $p < 0.001$ deemed significant. Differentially expressed genes were analyzed for enrichment of canonical pathways. RYGB significantly affected GE of 14,395 TS in the 15 tissues, representing 7600 unique TS and 7086 unique genes. The greatest changes in GE were observed in the surgically altered Roux limb of the SI, with 4517 TS altered. There were also substantial effects of RYGB in the common limb of the SI (906 TS), colon (799) and liver (1633). Among the TS altered by RYGB, the pathways most enriched were cytoskeleton remodeling ($p = 3.4 \times 10^{-11}$) and chemotaxis ($p = 7.5 \times 10^{-8}$). The greatest cytoskeletal changes were seen in the colon, the small intestine, and the liver. RYGB induces profound changes in GE independent of WL, underscoring the widespread physiological effects of this procedure. GE related to cytoskeleton remodeling was profoundly altered by RYGB compared to diet-induced WL. These observations suggest that RYGB induces changes in cellular and tissue remodeling that likely contribute to the powerful therapeutic effects of this operation. Supported by: Merck Research Laboratories and the NIH.

Salt appetite across generations. Aged and middle-aged

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The elderly have an impaired thirst response, and the danger of dehydration is increased. Salt intake decreases with age, as a function of decreased caloric intake, but it is not known how salt appetite may be altered in the elderly. Therefore, using questionnaires and a series of taste tests, we studied salt appetite in a group of elderly 68–85 year olds ($n = 30$), and compared it to a group of middle-aged 54–58 year-olds (30) in a homogenic sample of adults in a small township. In addition, we queried their memories of salt appetite 20 years previously, to evaluate possible changes over time in salt intake, appetite, and cognitive attitudes, in order to construct a "salt appetite biography". In the elderly, we confirm the impaired thirst, and find that they recall greater drinking in the past, and greater liking for water. Similar changes in attitude over time were reported for drinking soft drinks. For salt, we find no impairment in intensity of salt taste, nor in its hedonic profile. Both groups reported greater salt intake, greater salting at table, and more sweetening, 20 years previously. Additional results, including gender differences will be reported. The findings may illuminate means to improve hydrational status and appetite in the elderly. Supported by: The Salt Institute.

Taste receptor expression pattern in brain tissue

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Taste perception is a substantial step mediating motivation and food intake behaviour. The classical model of taste perception involves the stimulation of taste receptors in the gustatory epithelium and transmission of this information to neural circuits that integrate taste perception at the level of the brainstem and hypothalamus. In the past several years, an increasing amount of literature has pointed at the expression of taste receptors in non gustatory organs, like the upper airways, the gastrointestinal tract and the brain. Sweet receptors were reported to be present in the hypothalamus and their involvement in physiological processes has been suggested. In the present study we evaluated the expression pattern of a number of bitter receptors involved in the bitter sensing of glucopyranosides in different organs and areas of the brain. In addition, we investigated the expression of different components of the signalling pathway of these bitter receptors. The results indicate a differential expression of the bitter receptors and their signalling pathways between organs and brain areas, with the highest expression levels, especially of receptor Tas2R116, being found in the hypothalamus and brainstem. These results suggest a direct sensing and influence of bitter tastants on the hypothalamus and brainstem to influence motivation and food intake behaviour. The possible physiological mechanisms are currently been investigated. Supported by: AMC funding.

The dipeptidyl peptidase-IV (DPP-IV) inhibitor, vildagliptin increases energy expenditure and reduces glycaemia, but does not affect energy intake in response to intraduodenal fat infusion in healthy lean males

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There is considerable interest in glucagon-like peptide-1 (GLP-1) as a therapeutic for obesity since studies using long-acting GLP-1 analogues report decreased energy intake and weight loss. DPP-IV inhibitors (e.g. vildagliptin) augment intact GLP-1 and gastric inhibitory peptide concentrations, enhance postprandial lipid mobilisation and fat oxidation, and reduce the glycaemic response to carbohydrate-containing meals. The current study aimed to determine the acute effects of vildagliptin on energy intake and expenditure, as well as the glycaemic response to fat. In a double-blind placebo-controlled cross-over design, 16 healthy lean males (age: 23.7 ± 1.6 years; BMI: 22.6 ± 0.5 kg/m^2) received oral vildagliptin (50 mg) or placebo on separate days, 60 min before an intraduodenal fat infusion (Intralipid® 2 kCal/min for 120 min). Blood glucose levels and energy expenditure were evaluated at intervals during the infusion, and energy intake at a subsequent buffet-meal consumed between 120 and 150 min was quantified. Data are presented as mean values ± SEM. Compared with placebo, vildagliptin reduced glycaemia (AUC 0–120 min: 673 ± 9.7 vs. 645 ± 10.3 mmol/L min^{-1}, $P < 0.05$) and increased energy expenditure (1821 ± 54 vs. 1896 ± 65 kCal/day, $P < 0.05$), but had no effect on the respiratory quotient or energy intake. In conclusion, in healthy males, vildagliptin has the capacity to reduce glycaemia and increase energy expenditure, during intraduodenal fat, without affecting energy intake. The latter may explain why, in contrast to GLP-1 analogues, orally administered DPP-IV inhibitors are usually weight neutral in people with type 2 diabetes. Supported by: Novartis Pharmaceuticals Australia.

Prolonged chewing at lunch decreases later snack intake

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Prolonged chewing has been reported to reduce meal intake. However, whether prolonged chewing influences meal memory and later intake is unknown. We hypothesised that chewing each mouthful for thirty seconds would enhance lunch memory due to an enhancement of meal sensory processing and attention to food. We further hypothesised that enhanced memory would be associated with reduced afternoon snack intake compared with a control condition in which participants chewed normally and a condition in which they chewed normally but took pauses in between mouthfuls to control for effects of prolonged chewing on meal duration. Forty-three participants ate a fixed lunch of sandwiches in the laboratory. Appetite, mood and lunch enjoyment ratings were taken before and after lunch. Intake of candies at a taste test 2 h later that afternoon was measured as well as rated vividness of lunch memory lunch. Participants in the prolonged chewing group ate significantly fewer candies (mean 28 g SEM 9) than participants in the usual chewing group (mean 59 g SEM 8). Intake of candies by the pauses group did not differ from either the prolonged or usual chewing groups (mean 47 g SEM 8). Participants in the prolonged chewing group were less happy, less relaxed and enjoyed their lunch significantly less than participants in other conditions. Appetite ratings did not differ across groups. Rated vividness of lunch memory was negatively correlated with intake but there was no correlation with rated lunch enjoyment. The results suggest that prolonged chewing of a meal can reduce later snack intake and that this may be related to enhanced lunch memory. Supported by: University of Birmingham.

3 Eyes smaller than one's stomach? Repeated consumption of a large volume of liquid and semi-solid foods increases *ad libitum* intake, but doesn't change expected satiation

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Food intake and expected satiation initially rely on sensory properties. People may learn about the food's satiating capacity by exposure. We investigated whether repeated consumption changed the expected satiation and intake of iso-caloric liquid and semi-solid foods in a randomized cross-over study. Participants (n = 53) consumed a dairy food (liquid or semi-solid) in each 5-day test condition. Foods were offered *ad libitum* on day 1 and 5 and in a fixed volume on day 2–4. Appetite sensations were rated up to 180 min after the start of the session. Expected satiation of the semi-solid food was higher than of the liquid food on all days ($p < 0.0001$). *Ad libitum* intake on day 5 was higher than on day 1, and intake of the liquid food was higher than of the semi-solid food (day 1: liquid 391 ± 177 g, semi-solid 277 ± 98 g; day 5: liquid 477 ± 161 g, semi-solid: 375 ± 148 g). On day 2, hunger was rated lower and fullness higher after the semi-solid food. On day 4, no differences were observed. The changes in hunger and fullness suggested that the liquid and solid foods were perceived equally satiating after repeated consumption, but this did not result in the anticipated changes: expected satiation remained lower and *ad libitum* intake higher for the liquid. The effect of texture on expected satiation and *ad libitum* intake appears to be large, also after repeated consumption. Supported by: Top Institute Food and Nutrition, Wageningen, the Netherlands.

Sleep, Obesity and Energy Balance

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Habitually insufficient sleep could contribute towards obesity, metabolic syndrome, etc, via sleepiness-related inactivity and excess energy intake and, more controversially, through direct physiological changes. Epidemiological studies in adult/children point to small clinical risk (albeit statistically significant) only in very short (around 5h in adults), or long sleepers, developing over many years, involving hundreds of hours of 'too little' or 'too much' sleep. Although acute 4h/day sleep restriction leads to glucose intolerance and incipient metabolic syndrome, this is too little sleep and cannot be sustained beyond a few days. Few obese adults/children are short sleepers, and few short sleeping adults/children are obese or suffer obesity-related disorders. For adults, about 7h uninterrupted daily sleep is 'healthy'. Extending short sleep, even with hypnotics, to lose weight, is without basis and might take years, compared with the rapidity of utilising extra sleep time to exercise and evaluate one's diet. The real health risk of inadequate sleep comes from a sleepiness-related accident. However, there may be a subtle key link between REM sleep (REM) and more general aspects of energy balance (different from obesity itself), as mechanisms (e.g. orexins-hypocretins) underlying mammalian REM indicate commonality with feeding and energy balance. REM 'epiphenomena' may facilitate this energy balance, e.g. in providing heat conservation, appetite modulation, and facilitate 'optimal foraging' in wakefulness. A more ecological approach to REM may also help explain the unusual feeding behaviours often found in REM compromised illnesses such as narcolepsy and depression.

The NMDA receptor antagonist MK-801 prevents sensitization of water and sodium intake in the furo/cap model of extracellular dehydration

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When depleted of extracellular fluids, animals seek out and ingest water and sodium. Animals with a history of successive extracellular dehydrations display sensitized water and sodium intake. We hypothesized that NMDA receptor activation is critical for the expression of sensitization. Rats received 3 weekly successive furosemide and low dose captopril treatments to induce thirst and rapid sodium appetite. Rats were pretreated systemically with either the non-competitive NMDA receptor antagonist MK-801 (0.15 mg/kg, IP) or vehicle 20 min prior to each furosemide and captopril treatment. Vehicle treated rats displayed enhanced combined intake of water and hypertonic saline solutions over the course of the treatments. In contrast, MK-801 pretreated rats consumed water and hypertonic saline, but did not elevate combined water and hypertonic saline intake over treatments (mean change in total fluid intake in vehicle pretreated rats: 4.0 ± 1.16 vs. MK-801 pretreated rats: -0.7 ± 1.18). These results support a putative role of central nervous system plasticity in thirst and sodium appetite sensitization.

The role of catechol-*O*-methyl transferase *Val 108/158 Met* polymorphism (RS4680) in the effect of green tea on fat oxidation and energy expenditure

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Green tea (GT) increases energy expenditure (EE) and fat oxidation (FATox) via an inhibition of catechol-*O*-methyl transferase (COMT) by catechins. However, this does not always appear equally clear because of large intra-individual variability. This may be explained by the different SNPs of the functional COMT *Val108/158Met* polymorphism that are associated with COMT enzyme activity; high-activity enzyme, $COMT^H$-allele (*Val/Val* polymorphism), intermediate-activity $COMT^M$-allele (*Val/Met* polymorphism) and low-activity $COMT^L$-allele (*Met/Met* polymorphism). Twenty-one Caucasian subjects (BMI: 22.4 ± 2.4 kg/m^2, age: 21.3 ± 2.2 yrs) were included in a randomized, cross-over study in which EE and substrate oxidation were measured for 3.5 h, after GT and placebo(PL) intervention, with a ventilated hood system. All three COMT-alleles were distributed equally among the subjects. Only for subjects carrying the $COMT^H$-allele, FATox was increased after GT vs. PL (19.1 ± 4.0 g vs. 17.2 ± 3.9 g; $P = 0.05$), corresponding with a decrease in RQ after GT vs. PL (0.79 ± 0.03 vs. 0.83 ± 0.02; $P < 0.05$). Carbohydrate oxidation (17.1 ± 9.5 g vs. 23.9 ± 7.5 g; $P = 0.06$) and EE (60.0 ± 14.2 kJ/3.5 h vs. 33.7 ± 10.3 kJ/3.5 h; $P = 0.49$) seemed to be increased after GT vs. PL for $COMT^H$-allele carriers but did not reach significance. No significant differences were observed for GT and PL between the three SNPs, implying that at group-level, subjects with the $COMT^H$-allele did not respond differently to GT treatment than subjects with a $COMT^M$-allele or $COMT^L$-allele. Although subjects with the $COMT^H$-allele did not respond differently to GT treatment than subjects with a $COMT^M$-allele or $COMT^L$-allele, carriers of the $COMT^H$-allele showed a larger sensitivity for substrate oxidation, especially for FATox after GT. Surprisingly, only subjects with a $COMT^H$-allele increased their FATox and lowered their RQ after GT vs. PL.

Early onset exercise and cessation exacerbates obesity in female DIO rats fed a low fat diet

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Brief exposure to early onset exercise (EOE) provides protection against obesity in male rats selectively bred to develop diet-induced obesity (DIO) when fed a high-energy 31% fat, 25% sucrose diet (HED). To assess the effect of EOE in females, 4 wk old female DIO rats were fed HED or chow (CH), given running wheels for 5 wk and then left sedentary for 4 wk more. Over the first 5 wk, exercising rats ate 43% ($F(1, 9) = 285.37$, $p = 0.001$) and 15% ($F(1, 12) = 45.41$, $p = 0.001$) more CH and HED than respective sedentary controls. Exercising CH rats gained 21% more body weight ($F(1, 11) = 9.33$, $p = 0.01$) while those on HED gained the same amount of weight and had 17% lower feed efficiency (FE) ($F(1, 14) = 19.34$, $p = 0.001$) vs. respective sedentary controls. Also, despite running 41% further ($F(1, 10) = 9.95$, $p = 0.01$) and eating 11% less ($F(1, 11) = 131.69$, $p = 0.001$), exercising HED rats had 32% higher FE ($F(1, 13) = 28.26$, $p = 0.001$) than exercising CH rats. Over the 4 wk after wheel removal, previously exercised CH rats ate 41% more ($F(1, 10) = 158.42$, $p = 0.001$), and had 33% more carcass fat terminally ($F(1, 11) = 8.77$, $p = 0.01$) than 9 wk sedentary controls. However, prior exercise did not affect food intake, body weight gain or carcass adiposity in HED rats over the 4 wk sedentary period. Also, neither exercise nor diet affected menses onset or cycle length. Thus, in contrast to the weight loss during exercise and sustained protection from obesity afforded male DIO rats, EOE offers no obesity protection in female DIO rats, and causes them to overeat and become obese after exercise cessation when fed a low fat diet. Supported by: NIDDK.

Specific amino acids reduce eating and alter gastrointestinal function by distinct mechanism

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The central nervous system senses specific nutrient availability in body and in food to coordinate nutrient intake with homeostasis. Here, we hypothesized that specific amino acids (AA) act as signaling molecules that alter eating behavior and gastrointestinal (GI) function. First, the impact of all 20 individual AA on food intake was tested in rats (6.7 mmol/kg) given by gavage. Arg, Lys and Glu reduced 1 h post-administration food intake by 50%, 47% and 35%, respectively, whereas all other isomolar AA had no effect relative to water control. At the level of the GI tract, Arg and Lys induced strong secretion leading to changes in plasma Cl^- (-8% Arg) and albumin (+9% Arg, +12% Lys) concentrations. Furthermore, delayed gastric phenol red release (25% Lys, 10% Glu) and accelerated phenol red transit into the caecum (428% Arg, 322% Lys) indicated AA induced changes in GI motility. In the brain, cFOS positive cells were observed in the chemosensing area postrema and the nucleus of the solitary tract. Intravenous administered Arg, Lys and Glu (2 mmol/kg) induced a similar anorectic response as oral application, but only Glu led to a similar GI response. Taken together, we have identified Arg, Lys and Glu as specific *in vivo* signaling molecules triggering a decrease in nutrient intake. Additionally Arg and Lys provoked gastric secretion and altered GI transit when given enterally, whereas Glu provoked a slowdown of gastric emptying also when given parentally, suggesting a differential mechanism of action. Supported by: Zurich Center for Integrative Human Physiology.

The contribution of vagal afferents to the effectiveness of the adjustable gastric band. Insights from a rodent model

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Currently, bariatric surgery remains the only effective treatment for morbid obesity. Laparoscopic adjustable gastric banding (LAGB) is a commonly performed bariatric procedure, particularly outside the USA: however, the mechanism(s) underlying its efficacy are unclear. This study aims to elucidate the role of sensory neural pathways in mediating AGB-induced satiety in a rodent model. Adult male Sprague Dawley rats were fitted with an AGB, just below the gastro-oesophageal junction. Our previous data indicate that inflation of the band causes an increase in numbers of Fos-positive neurons in the rostral division of the medial NTS. This could be attributed to a neural, a neural–humoral or a direct humoral link. To test this, capsaicin was used to ablate vagal sensory fibres using CCK- induced anorexia as a biomarker of the extent of the lesion. Capsaicin treatment resulted in a diminution of the acute and chronic effects of AGB on activation of NTS neurons and an amelioration of the AGB – induced reduction in food intake, body weight gain, fat mass and feed efficiency. Approximately 50% of the AGB- induced Fos labeling remained after cap treatment consistent with a residual humoral involvement. These data support the hypothesis that LAGB exerts its effects via the modulation of both, neural and hormonal pathways. Supported by: Allergan, Inc.

Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling

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CNS ghrelin signaling drives higher-order feeding processes related to food reward and food seeking. Here we establish the ventral subregion of the hippocampus (VHPC) as a novel brain substrate of importance in the ghrelinergic control of feeding. Ghrelin delivery to the VHPC (75 and 150 pmol), but not the dorsal hippocampus elevated food intake in rats by increasing both meal size and meal frequency. Results from operant and Pavlovian conditioning paradigms inform about the behavioral mechanisms underlying these effects. Intra VHPC ghrelin delivery increased willingness to work for sucrose reward in an operant lever-pressing test under a progressive ratio reinforcement schedule. VHPC ghrelin delivery also increased spontaneous meal initiation in *ad libitum* fed rats following the presentation of an auditory cue that previously signaled meal access when the rats were food deprived. The food intake enhancing effects of VHPC ghrelin were blocked with co-administration of a phosphoinositide 3-kinase (PI3K) inhibitor (LY294002), indicating that the feeding effects of VHPC ghrelin signaling require intracellular PI3K-Akt signaling. Immunoblot analyses further confirmed that ghrelin activates PI3K-Akt signaling in the VHPC, and revealed that this activation is impaired with high fat diet feeding. Overall these findings illuminate novel neuronal and behavioral mechanisms mediating ghrelin's modulation of cognitive aspects of feeding control. NIHDK21397& NIHDK089752.

Possible role of intestinal fatty acid oxidation (FAO) in the eating-inhibitory effect of the peroxisome proliferator receptor-α (PPARα) agonist Wy-14643

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PPARα plays a key role in metabolism by enhancing FAO. Pharmacological PPARα activation has been shown to reduce food intake and to improve insulin sensitivity and obesity, but the mechanisms of these actions remain unknown. We investigated the effect of acute and repetitive (one dose daily for 6 days) IP administration of the PPARα agonist Wy-14643 (40 mg/kg BW) on energy expenditure, body temperature, food intake and body weight in rats fed a 49% high-fat diet. Acute Wy-14643 administration reduced energy expenditure and body temperature 1 and 2 h after administration (all *P*s < 0.05) and tended to decrease the respiratory quotient (RQ) ($P > 0.05$). Chronic Wy-14643 administration induced a 18.3 and 18.4% reduction in daily and total (6 day) food intake, respectively, and a 55% reduction in body weight gain (all *P*s < 0.05). Given the known stimulatory effect of PPARα on FAO, we measured the protein expression level of carnitine palmitoyltransferase-I (CPT1A), the rate limiting enzyme of mitochondrial FAO, in liver, duodenum and jejunum. Wy-14643 induced a significant increase in the expression of CPT1A in the duodenum and the jejunum but not in the liver, suggesting that Wy-14643 stimulated FAO in the intestine but not in the liver. Our findings are consistent with the view that PPARα activation inhibits eating by stimulating FAO and suggest that the eating-inhibitory effect of Wy-14643 originates in the small intestine rather than the liver.

Role of hindbrain orexin 1 receptors in food reward

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Brain orexin 1 receptors (OX1R) are involved in food-motivated behavior, but most research on this topic has focused on forebrain sites of action. However, hindbrain OX1R activity affects feeding, and we hypothesize that this may involve an effect on the reward value of food. First, we evaluated the effects of 4th-ventricular (4V) injection of orexin-A (OrxA) or the OX1R antagonist SB338467 on rats' operant responding for 45 mg sucrose pellets on a progressive ratio (PR) schedule. In *ad lib*-fed rats ($n = 8$), 4V OrxA significantly increased # of responses (by 81% at 0.1 nmol and 162% at 1 nmol) and breakpoint (50% at 0.1 nmol and 107% at 1 nmol) relative to vehicle. In mildly food-restricted rats (20 g food/day, $n = 9$), 4V administration of SB338467 had no effect on PR responding. However, rats that were food-deprived for 24 h before their PR session showed a small but significant decrease in responding (20%) and breakpoint (21%) after 20 nmol SB338467 delivered 4V ($n = 8$). We then examined the role of hindbrain OX1R in the expression of place preference conditioned by high-fat diet (HFD). *Ad lib*-fed rats were trained to associate one side of a 2-sided chamber with 5 g HFD. Rats were then randomly split into 2 groups for 4V injection, either vehicle or 20 nmol SB338467 ($n = 8$/group) delivered 15 min prior to a preference test. Vehicle-injected rats showed the expected preference for the HFD-paired side, but SB338467-treated rats expressed no side preference. We conclude that hindbrain OX1R activity affects food-motivated operant behavior and may play a role in responding to cues that predict palatable food.

Circadian variations in gastric vagal afferent satiety signals

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Many behaviours including food intake exhibit circadian variation (Physiol Behav. 1987; 40: 437). Disruption of these daily patterns (e.g. shift workers) can increase the risk for developing obesity and metabolic disorders (Int J Obes Relat Metab Disord 1999; 23; 973). Mechanosensitive gastric vagal afferents play an important role in satiety signalling and the control of food intake, but it has not been established whether there is a circadian variation in their sensitivity to mechanical stimuli. Therefore we obtained single fibre recordings of gastric vagal tension and mucosal receptors from mice at 3hr intervals ($N = 5$/time point) starting at 6pm. In mice fed ad libitum, there was 3 times more food in the stomach at 12, 3 & 6am ($p < 0.001$ vs 12pm: 1-way ANOVA). The response of tension receptors to 3 g tension was reduced by up to 70% at 6 & 9pm and 12, 3 & 6am, ($p < 0.05$ vs. 12pm: 1-way ANOVA). Gastric mucosal receptors also displayed circadian rhythm with peak responses to stroking with a 50 mg von Frey hair 3 times greater at 12 & 3pm than the lowest response at 12am ($p < 0.05$ vs. 12am: 1-way ANOVA). Similar findings were obtained in mice fasted for 6hrs or maintained in continuous darkness for 3 days prior to study. Therefore these changes are not mediated by food intake or the light/dark cycle. In conclusion, gastric vagal afferents display circadian variations in mechanosensitivity. Disruption of these oscillations in satiety signalling, caused or exacerbated by shift work, may result in over consumption of food and ultimately obesity. Supported by University of Adelaide.

Obesity induced suppression of gastric satiety signals are not reversed by dietary change

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Diet induced weight loss is seldom maintained suggesting adaptive physiological changes to an increased fat mass. Diet induced obesity (DIO) has been shown to attenuate gastric vagal afferent satiety signalling (J Physiol, 2012: 590; 209). Thus we aimed to determine whether the effect of high fat DIO on vagal afferent mechanosensitivity is reversible by returning DIO mice to a standard laboratory diet (SLD). We placed female C57/BL6 mice (n = 12/group) on a SLD (5% fat) or a high fat diet (HFD) (35% fat) for 24 weeks. A third group was fed HFD for 12 weeks then SLD for a further 12 weeks (RFD). Body weight and food intake were measured weekly. Single fibre recordings of gastric vagal mechanoreceptors were obtained at 24 weeks (J Neurophysiol, 2002: 87; 2095). At 24 weeks the HFD mice had greater weight and adiposity than the SLD and RFD mice ($p < 0.05$), and the RFD mice were heavier and had more body fat than the SLD mice ($p < 0.05$). The RFD mice consumed 45% more food (by weight) than the SLD or HFD mice ($p < 0.05$), but the caloric intake was similar to the HFD mice and 56% more than SLD mice ($p < 0.05$). The mechanosensitivity of gastric tension receptors from both HFD and RFD mice were reduced by 52% compared to the SLD mice ($p < 0.05$). Mechanosensitivity of mucosal receptors was similar in all groups of mice. This indicates that obesity induced effects on gastric satiety signals conveyed through vagal pathways are not reversible by changing the diet to a lower calorie diet, which may explain why weight loss in obese people is hard to maintain.

Hydrogen sulfide as a fluid balance and food intake regulator

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The paraventricular nucleus of the hypothalamus (PVN) is an important relay centre in the brain. Within the PVN there are three distinct cell types, the magnocellular neurons, which project to the posterior pituitary gland and secrete oxytocin (OT) or vasopressin (VP), parvocellular preautonomic neurons (VP, OT corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH)) which project to the brain stem and spinal cord and parvocellular neuroendocrine neurons, which project to the median eminence and secrete CRH and TRH. All three populations of PVN neurons play significant roles in the regulation of fluid balance and metabolism. Recently, hydrogen sulfide (H_2S) has been shown to cause excitatory on neurons in a number of CNS regions, and enzymes responsible for the production of H_2S have been localised to PVN. We sought to investigate the effects of H_2S on PVN neurons using whole-cell current clamp recordings from rat PVN neurons in hypothalamic slice preparations. Bath application of the H_2S donor sodium hydrogen sulfide (NaHS, 10 μM) influenced 100% of cells (n = 13). We observed exclusively depolarizing responses in magnocellular neurons (n = 4, 7.7 ± 1.8 mV), both depolarizing (n = 4, 6.7 ± 1.3 mV) and hyperpolarizing (n = 2, -6.7 ± 0.5 mV), responses in parvocellular neurons and exclusively hyperpolarizing responses in parvocellular neuroendocrine neurons (n = 3, -16.7 ± 5.2 mV). These findings demonstrate important effects of NaHS on all three cell types in the PVN, suggesting a potential role for this gasotransmitter in fluid balance and food intake. Supported by: CHIR.

Chronic intermittent stress associated with highly palatable food results in a binge-like eating with altered corticosterone response to stress challenge

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Male SD rats were divided into four groups; no stress (NS), foot shock on Tuesday, Thursday, Saturday (FS), cookie access for 2 h on Monday, Wednesday, Friday (CNS), cookie on Monday, Wednesday, Friday, and foot shock on Tuesday, Thursday, Saturday (CFS). All rats received chow and water *ad libitum*. The experimental sessions were repeated 4 times, and then rats were subjected to behavioral tests. Foot shock stress acutely suppressed chow intake regardless of cookie access. The increased caloric intake with 2 h of cookie access was greater in CFS than in CNS, suggesting a binge-like eating in CFS, and the difference became more obvious as the experimental sessions were repeated. Activity counts and travel distance during ambulatory test were increased in CFS, but not in CNS, compared to NS. Immobility duration during Porsolt swim test was increased both in CNS and CFS, compared to NS. For the plasma corticosterone assay, tail bloods were collected at 0, 30, 60 and 120 min time points during 2 h of restraint stress. Basal corticosterone levels were elevated and the stress-induced increase was blunted in CFS compared with CNS. Results suggest that chronic intermittent stress leads to the development of binge-like eating when it is associated with highly palatable food, and the HPA axis dysfunction may be implicated in its underlying mechanism. Supported by: Grant from Department of Medical Sciences, The Granduate School, Ajou University.

Effect of subcutaneous injection of butorphanol on exercise-induced suppression of food intake in the rat

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The opioid system plays a major role in the regulation of feeding and exercise, and peripheral injection of butorphanol (BT), which has partial agonist and antagonist activity at mu opioid receptors and agonist activity at kappa opioid receptors, increases food intake more effectively than most other opioid receptor agonists. This study investigated the effects of subcutaneous injection of BT on exercise-induced suppression of food intake. Twenty-four male Sparague-Dawley rats were housed under reverse light conditions and were injected with saline or BT (0.3, 1 and 3 mg/kg) and exposed to treadmill exercise (2500 revolutions/30 min) or non-exercise 10 min post injection. Food intake was measured 1, 2, 3 and 4 h post-exercise. Injections were given in a counterbalanced fashion, with each subject receiving one injection at each dose every other day. The results demonstrated that in the non-exercise condition BT at 1 and 3 mg/kg significantly increased cumulative food intake at 2, 3 and 4 h dose dependently. In the exercise condition BT at 0.3, 1 and 3 mg/kg increased food intake at 1, 2, 3 and 4 h post-exercise, as compared to the non-exercise/saline and exercise/saline groups. These results show that BT increased food intake in the exercise group but to a lesser extent than in the non-exercise group, and indicate that exercise can decrease the orexigenic effect of BT.

Hesperetin stimulates cholecystokinin release in enteroendocrine cells

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Cholecystokinin (CCK) is a neuropeptide secreted from the I cells of the intestine and it has key physiological actions related to satiety control and food intake. Hesperetin exists as both glycoside-hesperidin (hesperetin-7-rhamnoglucoside) and the aglycone-hesperetin and is mainly derived from citrus fruits, especially oranges and lemons. Here, we investigated whether hesperetin and hesperidin can affect CCK release in the STC-1 cells as a model for enteroendocrine I-cells. As a result, hesperetin significantly stimulated CCK release in the cells at the concentration from 0.01 to 1 mM compared to the untreated control. However, hesperidin did not stimulate CCK release at the same concentrations. Furthermore, the effects of antagonist for transient receptor potential (TRP) channel was studied. Ruthenium red (RR, a TRP antagonist) and HC-030031 (a TRPA1 antagonist) inhibited the hesperetin-evoked CCK release. When hesperetin was applied to the cells, a significant increase in the intracellular Ca^{2+} concentration occurred. The Ca^{2+} response evoked by hesperetin was decreased by RR and HC-030031. These results indicate that hesperetin stimulates CCK release and that the activation of TRP channels is involved in hesperetin-stimulated CCK release in STC-1 cells. Supported by: KFRI E0121203.

Adolescence highly palatable food modulates anxiety-related behaviors of rats that experienced neonatal maternal separation

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Functional implication of the nucleus accumbens (NAc) in the psycho-emotional disorders associated with neonatal maternal separation (MS) or long-term exposure to highly palatable food (HPF) has been suggested. Male SD pups were separated from dam for 3 h daily during PND 2-14 (MS) or left undisturbed (NH). Half of NH and MS received free access to chocolate cookie additionally to *ad libitum* chow from PND 28 (NH–HPF, MS–HPF), and the rest half received chow only (NH–CF, MS–CF). Pups were subjected to the behavioral tests during young adulthood. The plasma corticosterone response to stress challenge was analyzed by radioimmunoassay. Tissue contents of FosB and pCREB in the NAc were examined by Western blot analysis. Ambulatory activity tended to be increased in pups received HPF access. Caudal grooming was reduced in MS–CF and NH–HPF, and defecation activity in MS–HPF, but not in NH–HPF, compared to NH–CF. Anxiety-like behaviors during elevated plus maze test and depression-like behaviors during swim test were increased by MS or HPF, and these behaviors were not further affected by HPF in MS pups. Stress-induced corticosterone increase was blunted by MS, and HPF appeared to normalize it. pCREB in the NAc was increased, and FosB decreased, by MS, and HPF normalized them. Results suggest that adolescence cookie access increases anxiety- and depression-like behaviors in NH rats and modulates anxiety-related behaviors in MS rats, likely in relation with neural plasticity in the NAc. Supported by: MOEST (2009K001269&2010-0003642).

Functional knockout of forebrain 14-3-3 blocks conditioned taste aversion learning

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Protein 14-3-3 influences intracellular signaling by sequestering proteins in the cytoplasm, including Transducer of Regulated CREB activity (TORC), a co-factor of CREB. TORC and CREB are activated after visceral neuraxis stimulation, and mediate gene expression in conditioned taste aversion (CTA), a form of single-trial, long-delay associative learning. To determine if 14-3-3 is required for CTA, we tested transgenic (Tg) mice that express a YFP-fused R18 peptide in brain neurons; R18 blocks 14-3-3 binding, resulting in functional 14-3-3 knockouts. Water-restricted Tg mice (n = 16) and C57BL/6 wild-type mice (wt; n = 16) were given 10 min accesss to 0.125% saccharin (CS), followed 10 min later by LiCl or NaCl injection (0.15 M, 20 ml/kg). The next day mice were given 24-h, 2-bottle preference tests of CS vs. water for 6 days. The same mice were tested again with 10 min access to 0.75mM NaCl paired with a higher dose of LiCl or NaCl (0.15 M, 40 ml/kg). As expected, wt mice injected with either LiCl dose showed significantly reduced CS preference (saccharin: 0.57 ± 0.10; NaCl: 0.05 ± 0.01) vs. NaCl-injected wt (saccharin: 0.80 ± 0.10; NaCl 0.46 ± 0.11). However, Tg mice injected with LiCl failed to acquire an aversion at either dose of LiCl (saccharin: 0.90 ± 0.02; NaCl: 0.42 ± 0.12). Preferences in Tg mice injected with LiCl or NaCl were not different from NaCl-injected wt. Thus, 14-3-3 in the brain is critical for CTA learning. The timing, locus, and identity of proteins binding to 14-3-3 and contributing to CTA remain to be investigated. Supported by: NIDCD T32-00044.

Effects of cross-wiring lingual taste nerves on quinine-stimulated fos-labeling in the gustatory cortex in rats

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When cut gustatory nerves reinnervate non-native taste bud fields, restoration of function depends more on the taste field being innervated than on the nerve. We showed previously that cross-wiring the chorda tympani (CT) nerve into the posterior tongue (CT-PT) leads to normal oromotor gaping and partial, if not full, recovery of Fos-immunoreactive (FI) neurons in subcortical taste areas in response to intraoral quinine (Q, 3 mM, 7 ml, 30 min), while cross-wiring the glossopharyngeal (GL) nerve into the anterior tongue (GL–AT) does not, even though the GL normally responds robustly to this bitter substance. We have now begun to count FI-neurons in six 75 μm sections of gustatory cortex (GC, two in the anterior 3rd, two in its middle (MID), two in the posterior 3rd) in the same brain tissues. Many more FI-neurons were observed in Q-stimulated (N = 3) vs. water-stimulated (N = 4) controls, especially in zones approximating dysgranular and dorsal agranular insular subdivisions in MID GC (ranges: 489–759 vs. 81–208). Normal reinnnervation of the PT (GL–PT, N = 3) or cross-reinnervation of PT (CT–PT, N = 3) yielded between 280–652 and 321–570 Q-stimulated FI-neurons, respectively. When just the AT was re-innervated (CT–AT, N = 2; GL–AT, N = 3) or both nerves were cut (N = 2), the numbers ranged only from 47 to 242. These preliminary data imply that regardless of the nerve source, as long as PT is innervated, Q-stimulated neuronal activity in GC is relatively normal, suggesting that GC is capable of adapting to reorganized peripheral nerve input. Supported by: NIH R01-DC01628&NIH R01-DC009821.

Measuring reward value with a Sipometer. Proof of concept

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The Sipometer is a device that measures sipping pressure exerted by a person on a straw and delivers a fixed quantity of liquid reward (beverage), according to a predetermined schedule. Reward value was measured by the time spent sipping as the length of time required after each liquid delivery was increased by 3 sec (progressive ratio schedule = PR). Eight healthy female subjects completed the study in one session of four separate trials, which consisted of two PR trials, and two continuous reinforcement (CR) trials, each with either aspartame sweetened (S) or unsweetened (U) Kool-Aid non-caloric drinks. To prevent post-ingestive influences, subjects sipped and spat out the beverage (modified sham feeding). The subjects were instructed to sip and spit the beverage as long as they wanted on PR, but had 2 min on CR. Sessions of S and U were counterbalanced across subjects. A taste test was conducted before the sham feeding session. Under PR, time spent sipping was significantly (t_7 = 2.56, p = 0.037) longer during S (49 sec ± 12.0 SE) than during U (19 s ± 4.3 SE). Sham intake was significantly (t_7 = 3.71, p = 0.0075) higher for S (36 g ± 4.4 SE) than U (22 g ± 3.0). Differences between S and U in intake and time spent sipping were significantly correlated with corresponding differences in "amount wanted" and "liking" in the taste test. We conclude that the Sipometer testing procedure is a valid measure of reward because it is well known from other studies that for those who prefer S to U, intake is higher on S. Supported by: Brooklyn College Research Foundation, NIH DK26687.

Learned preference for dried-bonito *dashi* (a traditional Japanese fish stock) and its suppression by high fat diet

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The dried-bonito *dashi* is a traditional Japanese fish stock that improves palatability of various dishes, probably via enhancement of umami taste. Here we investigated sensory and physiological mechanisms involved in preferences for dried-bonito *dashi* by using two-bottle choice tests in male C57BL/6 mice. In the ascending concentration series, mice showed only weak preferences for *dashi*: the most preferred concentrations were observed within the 1.2–2% with the maximal preference reaching 65% (just above the water preference levels). In the descending concentration series, however, the maximal preference markedly increased to 95%, with concentration-preference functions shifting to the left at several orders of magnitude. Intriguingly, preference for *dashi* was suppressed by chronic exposure to a high-fat diet. Furthermore, we found that prior exposures to *dashi* for 10 days increased preferences even in ascending concentration tests. Among chemicals present in *dashi*, inosinate (umami taste) produced moderate enhancement while NaCl (salty), lactate (sour) and histidine (bitter) did not. These results reveal the importance of prior experience and dietary fat content for the development of *dashi* preferences. Experience-based enhancement of preference may involve postingestive consequences associated with ingestion.

Heating and eating in genetically obese zucker (fa/fa) rats

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Zucker obese (fa/fa) rats eat less frequently, but average meal size is larger so that total daily food intake is increased. In non-obese Sprague Dawley rats with ad libitum food, eating is episodic, occurring in an irregular ultradian pattern every 1–2 h. Body and brain temperatures increase approximately 15 min before the onset of eating, partially due to thermogenesis in brown adipose tissue (BAT) (1). The present study compares obese Zucker (fa/fa) rats them with lean Zucker rats, determining whether the temporal relationship between meals and episodic increases in temperature is preserved. Continuous recordings of activity, food intake and BAT/body/brain temperature (sampling rate 1 Hz) were made from chronically instrumented animals maintained at 24–26 °C with ad libitum food and water and with a 12 h light/dark cycle. For the dark phase in Zucker obese rats the interval between peaks of the increases in BAT/body/brain temperature was 125 ± 6 min (mean ± SEM, n = 24 rats), significantly longer ($P < 0.01$) than the corresponding interval (95 ± 5 min) in lean controls. Zucker obese rats commenced eating 15 ± 1 min after the onset of the temperature increase, similar to the corresponding time delay in lean control rats (16 ± 2 min, $P > 0.05$). Thus the temporal relationship between increased temperature and food intake is preserved in Zucker obese (fa/fa) rats, but the peaks in temperature occur less frequently. Reference 1. Blessing, W. W., et al., Physiol and Behavior 2012. Supported by: NHMRC.

Association between the serotonin transporter in the hypothalamic infundibular nucleus and BMI. A post-mortem study

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Brain imaging- and genetic studies in humans have shown associations between serotonin transporters (SERT) and BMI. From rodent studies, it is clear that the serotonin system within the arcuate nucleus of the hypothalamus (infundibular nucleus (IFN) in humans) plays an important role in energy metabolism. However, the neuroanatomical resolution of human *in vivo* SERT imaging does not allow for the distinction between separate hypothalamic nuclei. We investigated the relationship between SERT expression and BMI in post-mortem samples of human hypothalamic IFN. We divided hypothalami of n = 22 nondiabetic subjects in two groups: BMI < 25 kg/m^2 (n = 13, median 20.3 kg/m^2 (range 15.2–25)) and BMI > 25 kg/m^2 (n = 9, median 32.0 kg/m^2 (range 26.0–41.0)). Median age was 75 years (range 50–92) and 76 years (range 26–100), respectively. There was no difference in causes of death or co-morbidities. Serial 6 mm coronal sections were cut over the rostro-caudal axis of the hypothalamus and every 100th section with the IFN was used for immunocytochemical staining. Relative SERT protein expression was estimated by computer assisted density measurements of SERT immunoreactivity. In the group with a BMI 2, median SERT density was 16.2 arbitrary units (au) (range 6.5–35.8), while in subjects with a BMI > 25 kg/m^2 median SERT density was 11.3 au (range 3.3–16.9), which was significantly lower (p = 0.046, Mann Whitney U). These findings suggest that hypothalamic SERT is negatively associated with BMI. However, pathophysiological mechanisms remain to be investigated.

Weight gain induced by high-fat diet increases active-period sleep and sleep fragmentation

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Obesity is characterized by excess daytime sleep. It is unknown if body weight (BW) gain by high-fat diet (HFD) alters sleep patterns. We hypothesized that HFD-induced BW gain in rats promotes active period sleep. To test the effect of HFD-induced weight gain on sleep, 3-month old Sprague-Dawley rats (n = 12) were implanted with radiotelemetry transmitters connected to electroencephalogram and electromyogram leads. Following surgical recovery, 24 h baseline recordings were performed. Next, half the rats were fed HFD (45% of fat) for 8 weeks, and the rest were left on control diet. Sleep wake cycles were then monitored for 24 h. Recordings were scored as wakefulness (W), slow-wave (SWS) and paradoxical sleep (PS). Transitions between sleep/wake stages, food intake and BW were also recorded. One-way ANOVA followed by Tukey's multiple comparison test was used to determine the effect of diet on sleep/wake parameters, food intake and body weight. Compared to controls, animals on HFD had significantly increased food intake and BW ($P < 0.05$), SWS time ($P < 0.001$), and number of transitions ($P < 0.001$) between stages, and decreased W time ($P < 0.01$). The sleep/wake changes were primarily observed during the dark (active) phase, as sleep parameters were not different between control and HFD-fed rats during the light phase. In the dark (active) phase, HFD-induced weight gain significantly increased time in SWS ($P < 0.001$) and reduced time in W ($P < 0.001$) and also increased the number of transitions ($P < 0.001$). In conclusion, HFD-induced weight gain is associated with excess active period sleep and fragmented sleep, supporting the hypothesis that excess weight gain alters sleep and wake patterns. Supported by: Department of Veterans Affairs and National Institutes of Health.

Individual differences in children's susceptibility to overeating in obesogenic environments

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Despite the omnipresence of large portions of palatable, energy-dense foods, not all children are equally susceptible to overeating. There is a pressing need to better understand individual differences underlying the controls of appetite and eating in children and their interactions with the food environment. It is possible that obesogenic environments may pose the greatest risk for overeating and excess weight gain in children who find food very reinforcing and whose eating may be guided more by external (environmental) cues than by internal cues of hunger and fullness. The focus of this talk will be to discuss recent data from experimental studies with 5- to 12-year-old children, which aim to identify individual differences in children's susceptibility to overeating and ability to regulate short-term energy intake. Data from a behavioral genetics, crossover design with weight-discordant siblings will be presented which suggest that an impaired ability to adjust for calorie differences in a preload and eating when satiated may represent a behavioral phenotype for obesity in children. The talk will conclude with a discussion of possible strategies which may be used to modify heightened food responsiveness among at-risk children to moderate energy intake and thereby prevent excess weight gain. Supported by: NIH.

Rapid intake of large meals activates glucagon-like peptide-1 (GLP-1) neurons

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Past studies support a role for central GLP-1 in stress-induced hypophagia. GLP-1 neurons in the nucleus of the solitary tract are activated in rats after mechanical gastric distension and interoceptive stress, and central administration of GLP-1 inhibits food intake. However, we previously reported that GLP-1 neurons are *not* activated in rats after voluntary intake of large meals (Am J Physiol 277: R582), arguing against a role for GLP-1 signaling in satiation. The present study re-examined feeding-induced GLP-1 activation using new intake conditions and more sensitive immunohistochemical methods. Adult male rats were food deprived once for 24 h and then given 30-min access to excess or restricted amounts of palatable liquid Ensure or standard chow + water. One hour after food intake, rats were perfused with fixative, residual gastric volumes assessed, and brains sectioned and processed for dual localization of GLP-1 and cFos to identify activated neurons. Significantly more GLP-1 neurons were activated in rats after unrestricted meals of Ensure (~5% BW; ~34% activation) or chow + water (~3.6% BW; ~18% activation) compared to low activation (~3–6%) in rats after restricted meals. GLP-1 activation was positively correlated with intake and post-mortem gastric volume. Thus, and in contrast to our earlier report, GLP-1 neurons are activated in fasted/refed rats that quickly consume a large meal. We hypothesize that rapid intake of large amounts acutely challenges homeostasis, and that GLP-1 signaling in response to this stressor provides an 'emergency brake' that limits further intake. Supported by: NIH grant MH59911.

Fat matters. High fat leads to reduced activity in the orbitofrontal cortex in humans

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Neuroimaging studies evaluating the effect of fat on neural activity in humans are rare and have mainly investigated the immediate effect of fat in the mouth with focus on viscosity, texture, taste and pleasantness. The aim of this study was to investigate the effect of fat on intrinsic whole-brain activity after high versus low fat meal intake. For this purpose, 11 healthy lean male subjects participated on two days after an overnight fast. Intrinsic brain activity was measured using functional magnetic resonance imaging before, 30 and 120 min after intake of 500 g high (8%) and low fat (<0.1%) yoghurt. High fat compared to low fat meal resulted in a pronounced decrease up to 120 min post ingestion in the orbitofrontal cortex (OFC) ($p < 0.05$, corrected for multiple comparison). A reduction of OFC activity can potentially decrease the rewarding value of food terminating food intake. Since subjects reported no difference in subjective feeling of hunger after low versus high fat yoghurt, our results indicate that parts of the OFC responds to the fat content of the meal and not subjective satiety per se as previously reported.

Food texture can induce disgust

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Can texture render a food disgusting? A preliminary survey of 100 subjects identified eight foods often described as having disgusting textural properties. These test stimuli were rice pudding, boiled okra, mayonnaise, graham crackers soaked in milk, large curd cottage cheese, chunky peanut butter, over-ripe banana, and raw tomato. Fifty new subjects were asked how disgusting the test stimuli were. They were then given the opportunity to eat them, to eat a crisp non-absorbent cracker partially covered with them, to clean the cracker and eat the part of the cracker that had been covered with the test stimulus, and to break off and eat part of the cracker that had not contacted the test stimulus. That last task is a test for contamination, the essential defining property of disgust. Five subjects were unwilling to eat the part of a cracker that had not contacted a test stimulus (eight refusals total). This leads to an estimate of 13% as the proportion of the population that would exhibit contamination disgust with at least one of these eight test stimuli. Presumably some of the remaining subjects might have exhibited disgust with some other stimuli. Food texture can give rise to disgust in a considerable number of people.

Vagal afferent signaling contributes to some exendin-4 (Ex-4) effects on ingestive behavior and brain activation patterns

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Glucagon-like peptide-1 receptor (GLP-1R) agonists such as Ex-4 control eating and metabolism and are potential candidates to treat obesity and type-II diabetes. Here we tested whether vagal afferents (VA) mediate the eating-inhibitory and aversive effects of Ex-4. Subdiaphragmatic vagal deafferentation (SDA) blunted the short-term ($p < 0.01$; drug: $p < 0.001$), but not long-term (drug: $p < 0.001$) eating-inhibitory effect of IP infused Ex-4 (0.1 μg/kg) in rats. One μg/kg Ex-4 reduced food intake ($p < 0.001$) in SDA and sham operated rats similarly. These data suggest that intact VA are only necessary for the full expression of the early satiating effect of Ex-4, but not later. To our surprise, SDA rats developed a conditioned taste aversion (CTA) after IP Ex-4 (0.1 μg/kg) (drug × surgery: $p < 0.01$), but not sham animals, suggesting that VA signaling prevented the CTA. Finally, Ex-4 increased the number of c-Fos expressing cells, independent of intact VA, in the dorsal vagal complex ($p < 0.001$), the ventrolateral medulla, the lateral external parabrachial nucleus, the central nucleus of the amygdala, and the nucleus accumbens (all p-values: $p < 0.01$), but not in the dorsal raphe or arcuate nucleus. SDA completely blunted the induction of c-Fos expression by Ex-4 in the paraventricular nucleus (drug × surgery: $p < 0.01$; drug: $p < 0.01$). Together these findings support a role of VA to relay GLP-1R signaling to the brain and to modulate Ex-4's effects on ingestive behavior.

Involvement of nucleus accumbens opioid receptors in a rat model of binge eating

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We hypothesize that opioid receptor activation in the nucleus accumbens (NAc) contributes to binge eating disorder. We developed a rat model of binge eating using intermittent access to a cream, oil and sugar (COS) solution. After 5 weeks of 3 days/week access to COS, rats showed increased rates of initial licking and increased duration of lick bursts, consistent with an increase in palatability of COS. In addition, they showed decreased latency to initiate licking, increased number of lick bursts, and increased number and duration of meals, consistent with an increase in motivation to initiate consumption of COS. To determine the role of opioid receptors in the NAc, we microinjected antagonists of μ (CTAP), d (naltrindole) or k (norBNI) opioid receptors into the NAc core or shell prior to the consumption test. In rats that had been exposed to 5 weeks of intermittent access to COS ($n = 46$), CTAP reduced consumption when injected in NAc core, but, surprisingly, increased consumption when injected in shell. Naltrindole increased consumption when injected in either core or shell, and norBNI increased consumption when injected in core, but had no effect in shell. In striking contrast, none of these antagonists affected COS consumption in control rats ($n = 42$) that were not exposed to the intermittent access (binge) regimen. These results strongly suggest that NAc opioid receptors contribute to binge, but not non-binge consumption, perhaps due to receptor upregulation. The results suggest specific opioid receptor ligands as targets for the development of pharmacotherapies for binge eating disorder. Supported by: Davis Foundation to SL, and Klarman Foundation and R21MH092757 to SMN.

Neutral cues paired with chocolate reward increase food craving in healthy weight non-restrained participants.

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Consistent with incentive salience theories of overeating we have previously shown that neutral cues that have been paired with chocolate reward are able to command attention in an RSVP task (Seage et al., 2011; Appetite). Here we investigate if conditioned stimuli (CS) associated with chocolate reward increase food craving. Paricipants low in dietary restraint ($N = 50$) were trained to associate Chinese characters with either a 0% 10%, 50% or 90% reward contingency. Half of the participants consumed the chocolate reward during the task (the EAT group) and half of the participants earned the reward to consume at the end of the task (the WIN group). Craving was measured using a priming task in which a CS was presented for 2000 ms immediately before participants viewed a food or alcohol picture and rated their 'desire to consume the item right now' on a 0–5 scale where 0 = not at all and 5 = very much. Stimuli consisted of 10 sweet and 10 savory high energy dense foods, and 10 alcoholic drink pictures acted as controls. Each CS was shown as a prime prior to each picture type in a random order. Rated liking of the symbols after training increased from baseline only when paired with reward in the EAT group ($F(3,72) = 5.09$ $P < 0.01$), whereas liking for the CS- decreased in the WIN group ($P < 0.05$). There was a significant effect of conditioned cue type on craving ($F(3,144) = 4.27$ $P < 0.001$) in the WIN group with higher levels of reported desire to eat evident following exposure to the 90% rewarded CS+ ($P < 0.02$ compared to 50%; $P < 0.05$ compared to 10%). Cue induced craving was higher for sweet foods compared to savoury foods ($P < 0.05$) but there was no difference between sweet foods and alcohol. These findings suggest that cues that have acquired incentive salience through classical conditioning are able to increase the desire to eat palatable food items. This provides support for the notion that individual differences in attentional bias for food cues contributes to overeating as cues increase craving and food seeking behaviour. Future work needs to establish whether the presence of cues in the environment are able increase food consumption. Supported by: Swansea University graduate bursary.

Changes in plasma amino acid concentrations in relation to satiety

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In line with Mellinkoff's amino statistic theory protein-induced satiety may be related to the post-prandial increase in amino acid (AA) concentrations. The objective was to compare dynamics of protein-induced satiety ratings on a visual analog scale (VAS) with dynamics of plasma AA concentrations, by using a statistical approach that focuses on within-subject relations of these observations. Subjects (n = 25, age = 22 ± 1y, BMI = 23.9 ± 0.3 kg/m^2) received standardized breakfasts: custards with casein, soy, whey, or whey without GMP as protein type, with 10/55/35 or 25/55/20 En% protein/carbohydrate/fat (randomized cross-over design). VAS satiety scores and AA concentrations were determined for 4 h, at 0, 20, 40, 60, 80, 100, 120, 180, and 240 min. Per subject regression slopes and R^2 values of VAS scores on AA concentrations were calculated. We tested whether the means of the slopes were different from zero. Regardless of the protein type and En% protein, VAS satiety scores and AA concentrations changed synchronously (P < 0.0001, R^2 = 0.2–0.6), implying an explained variation of 20–60%. The AA's phenylalanine, aspartic acid, and arginine contributed most to the development of satiety (P < 0.0001, R^2 = 0.5–0.6). Protein-induced satiety is partly explained by the synchronous post-prandial increase in AA concentrations. Regardless of the protein type and En% protein, the AA's phenylalanine, aspartic acid, and arginine contributed most to the development of post-prandial protein-induced satiety. Supported by: Top Institute Food and Nutrition.

Fibres and proteins combined in a biscuit seemed to have stronger effect than a single enriched one

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Introduction: The objective of this study was to evaluate the single and combined effects of proteins and fibres in biscuits. Methods: In a crossover randomised design, 56 subjects attended the laboratory on 4 days and test one different biscuit each day at breakfast time: control biscuit (C), fibre-enriched biscuit (F), protein-enriched biscuit (P), fibre and protein-enriched biscuit (FP). Energy intake was evaluated by measuring food consumption during *ad libitum* standard lunch, afternoon snack and dinner. They also had to fill VAS at regular time interval in order to evaluate their appetite sensations. Among the 56 volunteers, 16 attended the laboratory on the next day so that different physiological parameters such as gastric emptying, glycemia, insulinemia and gastrointestinal hormones were measured. Results: Although no effect on energy intake, prospective consumption and appetite were lower after FP vs. C, F, and P. Hunger was lower after FP vs. C and F. Desire to consume was lower after FP vs. P. Moreover, F and FP slowed gastric emptying vs. C. Glycemia level is lower after FP vs. C and F and after P vs. C. Peak insulinemia 30 min after breakfast is lower after FP and F vs. P and C. Conclusion: A combination of fibre and protein in a biscuit lower appetite, gastric emptying, insulinemia and glycemia with no effect on energy intake. Supported by: Agence Nationale de la Recherche (ANR) in the framework of BISENS project with AgroParisTech, INRA, Kraft Foods Europe R&D and Polytech Paris-UMPC.

Astrocytes, the unrecognized player in high fat diet intake regulation

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Select metabolic sensing neurons in the brain alter their activity when ambient levels of metabolic substrates such as glucose and fatty acids change. Specific neurons that "sense" nanomolar concentrations long chain fatty acids reside in the ventromedial hypothalamus and many also respond to changes in glucose concentrations. While fatty oxidation accounts for up to 20% of fatty acid sensing in these neurons, up to 50% of such sensing is mediated by the fatty acid translocator/receptor, FAT/CD36. However, astrocytes are critical mediators of the responsiveness of these neurons since astrocytes are the major source of long chain fatty acid oxidation in the brain and they are the only known source of ketone body production. Astrocyte-derived ketone bodies are taken up and utilized by neurons via transport by monocarboxylate transporters. Using in vivo microdialysis, we find that 3 h intake of a high fat diet (60% fat) increases ventromedial hypothalamic fatty acid levels and astrocyte ketone body production while decreasing subsequent intake of either low (5%) or high fat diet over the next 21 h. This interaction between direct neuronal fatty acid sensing and the production of ketone bodies by adjacent neurons represents a novel mechanism for the regulation of feeding on high fat diets. These findings may also suggest that interference with brain fatty acid oxidation alters feeding by affecting astrocyte metabolism of fatty acids as well as possibly neuronal fatty acid sensing. Supported by: NIDDK, VA Research Service.

Wheel running reduces high fat diet preference without altering the expression of reward genes.

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Studies investigating the effects of exercise on food intake and preference in humans have not provided consistent conclusions possibly due to difficulty in controlling multiple variables. Wheel running (WR) in rats provides a good model to address the issue as it has multiple parallels to exercise in human. Male Sprague Dawley rats had 4 weeks free access to chow diet only (naive) or choice between the chow and a high fat (HF) diet (WR and sedentary, Sed). A pair-fed group received the average intake of WR (PF). Initially all rats preferred the HF diet. However, HF diet preference ratio significantly decreased (0.87 ± 0.03 vs. 0.62 ± 0.06) in WR rats in response to 2 weeks free running while it was unchanged in the Sed rats. There were significant effects on percent weight gain i.e. naïve = Sed > PF > WR. To investigate the potential involvement of reward mechanisms in the decreased HF diet preference with running, the expression of reward-related genes e.g. dopamine (DA) and opioid receptors in the ventral tegmental area (VTA), the nucleus accumbens, and the prefrontal cortex was analyzed by RT-QPCR. Gene expression profiles were similar in the Sed and WR rats despite difference in HF diet consumption. On the other hand, although consuming equal amounts of chow and HF diet, the PF group had reduced expression of D1 and D2 receptors in the NAc. The fact that WR rats consumed less HF diet but had similar gene expression profile with Sed rats suggests that running may produce rewarding effects that are additive to palatable food reward.

A classic innate behavior, sodium appetite, is driven by hypothalamic gene-regulatory programs previously linked to addiction and reward

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Sodium appetite is a classic instinct, related to thirst for water, both critically involved in hydromineral homeostasis. Sodium appetite can be evoked by systemic sodium depletion, by stress response mediated via ACTH, and also in reproductive settings. Microarrays with genome-wide coverage of transcripts were employed to investigate hypothalamus and remainder of the brain of mice rendered to crave salt, their equilibrated controls, and of animals that had just gratified their salt appetite. We also analyzed the microarray results with the advanced computational method of gene set enrichment analysis, which led us to identify gene sets previously shown to be associated with addiction. This analysis linked these addiction-associated gene sets with sodium appetite. Administration of dopamine- and metabotropic glutamate-5 receptor antagonists attenuated gratification of sodium appetite evoked by sodium deficiency in mice and rats. Thus, our results (Liedtke et al., PNAS 2011, 108(30): 12509–12514) transformed the more diffuse concept of "addiction hi-jacks brain reward systems" to "addiction hi-jacks hypothalamic genes regulating gratification of sodium appetite, a classic instinct". More recent findings and concepts will be discussed. Supported by: Duke University, Mathers Foundation.

Regulation of adiposity and energy balance by gastric bypass-altered microbiota

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Roux-en Y gastric bypass (RYGB) is among the most effective treatments for obesity. Decreased fat mass accounts for the majority of weight lost after this procedure. This effect is driven by a decrease in food intake and an increase in energy expenditure; however, mechanisms driving these changes are unknown. Previous studies in humans and rats have revealed alteration of gut microbial communities after RYGB, raising the possibility that changes in microbial physiology may contribute to RYGB outcomes, but direct assessments are lacking. In a mouse model, we have observed substantial weight- and diet-independent shifts in microbial communities of fecal samples and along the GI tract after RYGB, as compared to sham-operated (SO) animals. Further, we found that the microbiota can transmit several RYGB-associated metabolic outcomes to recipient, un-operated mice. Colonization of germ free mice with RYGB microbiota resulted in decreased body weight, despite no changes in food intake, and decreased adiposity compared to recipients of SO microbiota. These observations suggest that modulation of the gut microbial community contributes to the beneficial effects of RYGB on energy balance and metabolic function. Further elucidation of the mechanisms by which the RYGB-altered gut microbiota influences host metabolism will extend our understanding of gastrointestinal regulation of metabolism and will likely reveal novel approaches to develop new, more effective, nonsurgical therapies for obesity and related metabolic disorders. Supported by: National Institutes of Health and Ethicon Endosurgery.

Imaging nutrient-induced gut-to-brain signalling pathways in humans. Further analysis reveals increasing complexity

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Using magnetic resonance imaging (phMRI) we have reported that glucose infused into the gut decreases Blood Oxygenation Level-Dependent (BOLD) signal in key areas. Whether peripheral signals or glucose itself mediate this effect is unclear. However, lipid increases BOLD signal, entirely by a CCK_1 receptor mediated pathway. Our initial analysis suggested that CNS responses to glucose were also CCK_1 dependent but ongoing analysis has revealed more complex and discrete response patterns. Data were derived from 12 healthy subjects receiving 1M glucose or 0.9% saline intragastrically on three occasions (blinded, randomised) during phMRI. The glucose arms were conducted ± a CCK_1 antagonist, dexloxiglumide. BOLD signal decreased in the hypothalamus, brainstem and medulla but, unlike the response to lipid, this was not blocked by dexloxiglumide. A partial reversal may be present in the hypothalamus. However, other brain areas that displayed increased BOLD in response to lipid (eg motor cortex and cingulate gyrus) also showed an increase in BOLD following glucose, and all these positive response areas were fully blocked by dexloxiglumide. Moreover, positive BOLD effects were more rapid than negative responses, preceding the rise in plasma glucose. Since intravenously administered glucose also reduces brainstem BOLD signals we speculate that *reduced* BOLD may map to areas within the CNS sensing glucose directly, whilst areas of *increased* BOLD respond only to peripheral (CCK mediated) signals. Supported by *BBSRC/DRINC.*

Effects of the timing of a nutrient load on subsequent energy intake. Relationship with antral area.

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As the time interval between meals increases, the suppressive effects of a nutrient load on subsequent energy intake are reduced (Rolls et al., AJP 260: R756–63, 1991). Previous studies have indicated a strong relationship between antral area immediately before a meal with energy intake (Sturm et al., AJCN 80: 656–67, 2004). Therefore, we hypothesized that the diminishing effects of a nutrient load on energy intake over time would be related to the reduction in antral area as the nutrient load emptied from the stomach. 16 healthy subjects (10 M, 6 F; age: 26 ± 2 y; body mass index: 22.3 ± 0.5 kg/m^2) were studied on 4 occasions in a randomized fashion. Subjects consumed 500 ml of water 180 min (control), or a mixed nutrient drink (Ensure Plus, 750 kcal, 500 ml) 30 (EI30), 90 (EI90) or 180 (EI180) min prior to a cold, buffet-style meal, from which energy intake was quantified. Antral area in response to the drinks was measured using 2D-ultrasound, and appetite perceptions were scored, at regular intervals. Data are presented as mean values ± SEM. The nutrient drinks increased antral area and slowed gastric emptying compared with control ($P < 0.001$, for all). Antral area immediately before the buffet-meal followed the pattern EI30 (12.1 ± 0.9 cm^2) > EI90 (8.8 ± 0.7 cm^2) > EI180 (5.4 ± 0.9 cm^2) > control (3.4 ± 0.3 cm^2). The nutrient drinks all suppressed energy intake when compared with control (EI30: -367 ± 69, EI90: -291 ± 69, EI180: -219 ± 72 kcal, $P < 0.05$, for all). There was a direct relationship between antral area with energy intake ($r = -0.46$, $P < 0.001$), such that the greater the antral area immediately before the buffet-meal, the greater the suppression of energy intake. In conclusion, the diminished suppression of energy intake as the time interval between the drink and the buffet-meal increased can, at least in part, be explained by a reduced intragastric contribution to satiety.

Synphilin-1 alters metabolic homeostasis in a Drosophila obesity model

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Obesity and its related disorder are increasing at an alarming rate worldwide. The pathogenesis of obesity remains incompletely understood. Synphilin-1 is a cytoplasmic protein with unclear function. Using the UAS/GAL4 system to express human synphilin-1 in Drosophila induced metabolic imbalances resulting in obesity-like phenotypes. Overexpression of synphlin-1 in neurons but not peripheral cells increased the body-weight of flies compared to that of non-transgenic controls. Synphilin-1 increased food intake but did not affect locomotor activity. Synphilin-1 increased the levels of triacylglycerol, and the size of fat body cells and lipid droplets, indicating that synphlin-1 increased lipid-fat disposition. Survival studies showed that synphilin-1 transgenic flies were more resistant to food deprivation. Syphilin-1 regulated lipin gene expression that may participate in synphilin-1-induced fat deposition and starvation resistance. These studies demonstrate that synphilin-1 expression affects energy homeostasis in ways that foster positive energy balance and provide a useful obesity model for future pathogenesis and therapeutic studies. Supported by: NIH/NIDDK grant: 083410.

Ginsenoside Rb1 reduces fatty liver in obese rats by activating AMP-activated protein kinase

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Fatty liver is a common metabolic symptom that is strongly associated with obesity and insulin resistance. Recently, we demonstrated that ginsenoside Rb1 (Rb1), a natural and active compound of ginseng, reduces obesity and improves insulin sensitivity in high-fat diet (HFD) induced-obese rats. The current study evaluated the protective effect of Rb1 on fatty liver and investigated the underlying mechanisms. Chronic administration of Rb1 (10 mg/kg, ip) significantly reduced liver weight and hepatic triglyceride content in HFD-induced obese rats. Histological examination of liver sections by H & E and Oil Red O staining confirmed the protective effect of Rb1 on fatty liver. In rat primary-cultured hepatic cells, Rb1 significantly enhanced fatty acid oxidation rates and carnitine palmitoyltransferase 1 (CPT1) activity. Western blots revealed that Rb1 increased the phosphorylation of hepatic AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC). Furthermore, Rb1 significantly stimulated the expression of several lipolytic genes, including peroxisome proliferator-activated receptor-g coactivator-1a (PGC-1a) and proliferator-activated receptor-a (PPAR-a), and it inhibited the expression of lipogenic genes, including sterol regulatory element-binding protein-1 (SREBP-1c) and fatty acid synthase (FAS) in the cultured hepatic cells. These results indicate that Rb1 has the potent ability to reduce hepatic fat accumulation and might be useful as a therapeutic agent for fatty liver disease. Supported by: NIDDK63907 and DK70992.

Lipopolysaccharide inhibits ghrelin-sensitive neurons of the arcuate nucleus via NF-κB dependent nitric oxide signaling

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Lipopolysaccharide (LPS) inhibits food intake via nitric oxide (NO) produced by inducible nitric oxide synthase (iNOS). The iNOS specific inhibitor 1400W leads to a disinhibition of ghrelin-excited ARC neurons after LPS stimulation. We tested by in vitro extracellular recording whether the LPS/NO dependent inhibition of ARC neurons is mediated by NF-κB or STAT, which are transcription factors in iNOS gene expression. We also investigated whether LPS elicits STAT1/STAT3 phosphorylation in vitro and NF-κB signaling after in vivo treatment, respectively. iNOS blockade by 1400W (10^{-4} M) increased the activity of 63% of ARC neurons after 4h in vitro stimulation with LPS (100 ng/ml). The NF-κB inhibitor Bay 11-7085 (10^{-6} M) significantly reduced the number of 1400W sensitive ARC neurons (10%). The percentage of 1400W-excited cells (25%) was non-significantly reduced vs. controls after co-incubation with the STAT inhibitor WP 1066 (10^{-5} M). Incubation of ARC slices with LPS induced a STAT3 phosphorylation but no STAT1 response. While NF-κB immunoreactivity was present in the ARC, no nuclear localization of NF-κB was detectable 4h after LPS treatment (100 μg/kg ip); this indicates that transcriptional activation is likely to occur at an earlier time point. In conclusion, LPS/NO signaling inhibits orexigenic ARC neurons via NF-κB. This pathway might be an interesting pharmacological target for the treatment of disease-related anorexia. Supported by the Swiss National Science Foundation and Krebsliga Zurich.

Nucleus accumbens neuronal responses to reward and aversion are differentially modulated by the basolateral and central nuclei of the amygdala

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The nucleus accumbens (NAc) integrates sensory and affective information to influence behavior. We have shown that the NAc differentially encodes rewarding and aversive stimuli with overall decreases (DEC) or increases (INC) in activity, respectively. It is unclear what contributes to this differential encoding. The amygdala projects both directly, via its basolateral nucleus (BLA), and indirectly, via the central nucleus (CeA), to the NAc. Thus these sub-nuclei may play a role in shaping NAc responses to rewarding and aversive stimuli. To test this, we made electrophysiological recordings of individual neurons in awake and behaving rats (n = 17) while administering intra-oral infusions of sucrose (0.3 M) or quinine (0.001 M). Recordings were made both with and without unilateral BLA or CeA inactivation using baclofen/muscimol. Preliminary results show differential effects of BLA and CeA inactivation on NAc responses. Unilateral BLA inactivation selectively reduced the percentage of DEC responses to sucrose (68 versus 54% DEC phasic responses for control versus inactivation), but had no effect on quinine responses. In contrast, CeA inactivation selectively augmented the percentage of INC responses to quinine (53% versus 73% INC phasic responses for control versus inactivation) without affecting sucrose responses. These data support a contributing role for the amygdala in NAc affective encoding. Furthermore, they suggest the intriguing possibility that this contribution is both region- and value-specific. Supported by: NIDA grant DA025634 (MFR).

Can eating when empty cure Crohn's?

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Hypothesis: Physical hunger pre-meal (Empty Hollow Sensation (EHS)) yields improvements in symptoms and signs of autoimmune disease, specifically Crohn's disease (CD). Background: Cultivating physical hunger before most meals has been associated with improved insulin resistance (IR). IR is associated with degraded immune function as indicated by raised inflammatory markers e.g. C reactive protein (CRP). This suggests a mechanism for clinically observed improvements in autoimmune disorders rheumatoid arthritis, Crohn's and Graves' Disease when subjects have been taught to cultivate EHS pre-meal. Suggested feasibility study: Aim: To test feasibility of subject recruitment and retention and to test the practicality and acceptability of EHS training and repeated data measurements in preparation for a randomised trial in which EHS training is the proposed independent variable and Crohn's Activity Index, CRP and fecal calprotectin the dependent variables. Design: Recorded focus group meetings of CD subjects from secondary care population. Intervention: Prepared questions (approximately 5) to assess receptiveness to training in EHS, likely ongoing participation in training (1 h over three sessions), likely response to training and likely compliance with measures pre, 2 and 12 weeks post intervention. Interpretation of data: Thematic analysis of individuals by group to reveal commonalities and differences in attitude towards the test questions. If upheld, this novel approach to Crohn's disease could help alleviate a serious illness. Supported by: Nil.

Reward sensitivity increases food "wanting" following television "junk food" commercials

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Global obesity rates have been partly attributed to the easy access of cheap, high calorie food. However, many individuals exposed to the same food lie well within the healthy weight range. Reward sensitivity has been proposed as a key trait predisposing some individuals to be highly attracted to cues linked with appetitive food. We tested whether reward-sensitive individuals would experience greater pleasure ("liking") and urge to eat ("wanting") after watching television commercials featuring "junk food", compared with those featuring "healthy food" or no food. 75 men and women watched a 30 min film embedded with "junk food" (n = 27), "healthy food" (n = 26) or no food commercials (n = 22). Participants rated the pleasantness of food images and their urge to eat. As hypothesised, reward sensitivity was associated with an increase in urge to eat in the "junk food" condition (r = 0.31). There was no association in the "healthy food" condition (r = 0.06) and a reduced desire to eat in the "no food" condition (r = -0.24). Reward sensitivity was associated with greater "liking" of "junk food" images, but only for women (r = 0.55). There was no effect of reward sensitivity on "liking" of "healthy food" or non-food images. These findings support the role of greater food "wanting" in high reward sensitive individuals in response to appetitive food cues. Reward sensitivity also plays a role in enhanced "liking" of "junk food" cues, although this was specific to women.

Meal type, gender, and beliefs about body shape

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One way in which a person is judged is by the food that they eat. Individuals, particularly women who eat small meals are typically rated more positively than those who eat larger meals. To further examine the ways in which food intake can influence perception of others, 77 female and 52 male undergraduates were told that other students, who were actually fictitious targets, had participated in a similar study. Participants read a description of a male or female target. Descriptions of the males and females differed only with respect to height and weight. Targets were described as typically eating a light lunch (salad, roll, diet coke and a cookie) or heavy lunch (cheeseburger, fries, coke and piece of cake), and then asked to rate how satisfied they felt the targets were with respect to a total of 31 body parts and physical conditions. For male targets, the only characteristic which varied as a function of lunch condition was appetite. The male target described as eating a light lunch was rated as significantly less satisfied with his appetite than the male eating a heavy lunch. In contrast, the female target described as consuming a light lunch was rated as significantly less satisfied with her weight, waist, hips, neck, nose, thighs, chin, arms, cheeks, figure, condition and appearance than the female described as consuming a heavy lunch. These findings suggest that young women who eat lightly are considered by others to do so, at least in part, as a result of dissatisfaction with their bodies.

Paraventricular hypothalamic (PVN) cFos activation correlates with activation of hindbrain noradrenergic (NA) neurons after systemic cholecystokinin-8 (CCK)

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Systemic CCK dose-dependently inhibits food intake and activates cFos within the nucleus of the solitary tract (NST). High CCK doses also activate the hypothalamic-pituitary-adrenal (HPA) stress axis, but the phenotypic identity of NST neurons recruited by increasing CCK doses has not been reported. The present study used a range of CCK doses (0,1,3,5, and 10 μg/kg, ip) to examine relationships between activation of caudal NST NA and glucagon-like peptide-1 (GLP-1) neurons, and activation of PVN neurons at the apex of the HPA axis. Experimentally naïve adult male rats were anesthetized and perfused with fixative 90 min after ip injection of saline or CCK. Brain sections were processed for dual immunocytochemical localization of cFos plus dopamine-b-hydroxylase (to identify NA neurons), GLP-1, or corticotropin-releasing hormone (CRH). CCK dose-dependently activated neurons within the CRH-rich PVN, and PVN activation was positively correlated with activation of NA NST neurons. Large proportions of GLP-1 neurons were activated across treatment groups, including saline controls, evidence for GLP-1 recruitment by the injection procedure alone. However, the highest dose of CCK further increased GLP-1 activation above control levels. These results support the view that hypothalamic activation after CCK treatment increases along with NST NA activation, and that HPA axis responses to high CCK doses may also result from increased GLP-1 signaling. Supported by: NIH Grant MH59911.

Epigenetic modifications in the hypothalamic Arcuate nucleus by chronic high fat diet in rats

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Proopiomelanocortin (POMC) is a precursor of the anorexigenic neuropeptide α-melanocyte-stimulating hormone (α-MSH) that is critically involved in the regulation of food intake and body weight. Therefore, epigenetic modifications that affect POMC expression can lead to obesity. Expression of POMC in the hypothalamic arcuate nucleus (Arc) is regulated by circulating levels of leptin and insulin. Binding of these hormones to their hypothalamic receptors activates the STAT3 signal transduction pathway which might also result in binding of SP1 transcription factor to the POMC promoter. Here, we studied whether long-term exposure to high fat (HF) diet leads to acquired epigenetic alterations in the promoter region of POMC in the Arc. Male Wistar rats were raised from postnatal day (PND) 22 till PND 90 on either HF diet or standard rodent chow (LF). At PND 90, the HF rats presented increased body weight, significantly higher levels of plasma leptin and SP1 mRNA. In contrast, their POMC mRNA levels did not differ significantly from LF rats. Nevertheless, HF rats presented hypermethylation across the entire length of the Arc POMC promoter region including the SP1 binding site. In addition, HF rats showed significantly lower levels of acetylation on histone H3 lysine 9 (H3K9ac). We suggest that hypermethylation and lower levels of H3K9ac on the POMC gene's promoter region may block the effects induced by high levels of leptin, thus disrupting the activation of the POMC promoter.

Sex differences in HPA axis activity in response to a meal

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Sex may influence the relationship between HPA axis functioning and obesity. This has been suggested to be due to sex-specific differences in body composition, body fat distribution and psychological variables. Age and the use of oral contraceptives may also influence the relationship between HPA axis functioning and obesity. The aim of this study was to systematically investigate the possible contribution of these parameters to differences in HPA axis activity in response to a meal. Subjects were men (n = 19), women in the follicular phase of the menstrual cycle (n = 8), and women using oral contraceptives (n = 11), aged between 18 and 51 years and BMI between 20.3 and 33.2 kg/m^2. HPA axis activity was measured by salivary free cortisol levels before consuming a meal, and at 45, 75 and 125 min postprandial on four repeated test days. No differences between the test days in postprandial cortisol responses appeared. Responses were higher in men compared with women ($p < 0.05$). No correlations were found between cortisol concentrations and sex-specific body composition or body fat distribution. Psychological variables did not contribute to differences in cortisol responses after a meal between men and women. In women, baseline cortisol concentrations correlated inversely with age ($p < 0.05$). Higher HPA axis activity following a meal in men vs. women remained irrespective of sex-specific differences in body composition, body fat distribution, psychological variables, or in age. In women baseline cortisol concentrations were age-dependent.

Flexibility of brain-reward related activation in overweight and normal weight subjects

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Flexibility of brain-reward related activation between food-items and similarly complex non-food-items might differ between overweight and normal weight subjects. Moreover, this flexibility may differ between these subjects in relation to the fasted or satiated condition. We assessed the flexibility in reward related activation in the brain in response to food vs. similar non-food-items in overweight and normal weight subjects in a fasted and satiated state. Twenty normal weight subjects (Age = 22.4 ± 0.4, BMI = 22.7 ± 0.2) and 20 overweight subjects (Age = 24.0 ± 0.7, BMI = 28.1 ± 0.3) completed two fMRI scans: one fasted; one satiated. Subjects arrived fasted and consumed a breakfast comprising 20% of subject-specific DER between scans. Before and after breakfast subjects completed visual analogue scales on their appetite profile. The fasted condition before, and the satiated condition after breakfast were confirmed ($p < 0.0001$). Subjects were scanned in a Siemens Magnetom Allegra magnetic resonance imaging (MRI) system (Siemens AG, Erlangen, Germany), with the standard one-channel head coil. The f-MRI scanning paradigm consisted of blocks of visual stimuli of food and non-food pictures matched on visual complexity. Specific food reward-related activation was determined using a food > non-food contrast. In the fasted state the complete subject group showed a specific food reward-related activation in the prefrontal cortex (PFC) ($p < 0.003$, $t_{(39)} = 3.17$), left ($p < 0.009$, $t_{(39)} = 2.95$) and right insula ($p < 0.005$, $t_{(39)} = 3.12$), cingulate cortex ($p < 0.005$, $t_{(39)} = 3.20$) and thalamus ($p < 0.006$, $t_{(39)} = 2.96$); in the satiated state this activation was limited to the left ($p < 0.005$, $t_{(39)} = 3.21$) and right insula ($p < 0.006$, $t_{(39)} = 3.04$) and cingulate cortex ($p < 0.005$, $t_{(39)} = 3.15$). Food-related activation was significantly decreased from the fasted to the satiated state in the cingulate ($p < 0.005$, $t_{(39)} = 3.15$) and PFC ($p < 0.006$, $t_{(39)} = 3.00$). In the fasted state, specific food reward-related activation in the cingulate cortex was higher in the overweight vs. the normal weight subjects ($p < 0.005$, $F_{(1,38)} = 9.71$). In the satiated condition, specific food reward related activation was lower in the cingulate cortex ($p < 0.006$, $F_{(1,38)} = 9.18$) in the overweight vs. the normal weight subjects. Accordingly, the change in specific food-reward related brain activation from the fasted to the satiated condition decreased more in the overweight than in the normal weight subjects in the cingulate cortex ($p < 0.005$, $F_{(1,38)} = 9.18$) and, additionally in the putamen ($p < 0.006$, $F_{(1,38)} = 9.27$). At the same time, in the satiated state a lower activation in the overweight vs. normal weight subjects was observed in the PFC ($p < 0.006$, $F_{(1,38)} = 8.86$), suggesting a lower control. In conclusion, overweight subjects showed a higher specific food related brain reward activity in the fasted state, and a lower reward and control related brain activity in the satiated state, which, as we speculate, might bring them in a vulnerable position of eating in the absence of hunger. Supported by: TIFN.

Umami and the appetizer effect

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Umami has been described as the fifth basic taste, offering a flavoursome savoury characteristic to foods. However, the hedonic effects imparted by umami's taste profile on sensory experience and meal termination are still unclear. The appetizer effect predicts within-meal consumption and eating rate as modulated by the hedonic properties of a meal, with subjective ratings of hunger increasing early within meals evaluated as more pleasant in flavour. The influence of umami on the appetizer effect was evaluated in 20 non-obese unrestrained individuals (mean BMI: 22.4, mean age: 21) who consumed a standard breakfast returning 3 h later for an *ad libitum* soup lunch over four non-consecutive sessions. The soup was an iso-caloric vegetable soup combined with no MSG, 0.6% w/w MSG or 1% w/w MSG. Ratings of hunger, full-

ness, pleasantness, and thirst were assessed after soup tasting, at 25 g consumption for 250 g intake and at meal termination using the SIPM software. MSG increased meal pleasantness, but intake did not differ significantly between conditions and eating rate tended to decrease with increasing MSG content ($p = 0.087$). There were no significant differences in the pattern of change in rated hunger or pleasantness between MSG conditions during the meal, although there was a trend for an enhanced appetizer effect in the 0.6% MSG condition. These preliminary findings confirmed that MSG enhances palatability but whether this resulted in an increased appetizer effect requires further substantiation.

Effects of the D2-receptor antagonist raclopride on the early-meal microstructure of sucrose licking in rats

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Raclopride (RAC) is reported to decrease real- and sham-intake of sucrose in rats by reducing the size of licking bursts, a measure influenced primarily by orosensation. Here we assess the impact of RAC on early-meal microstructure. During 60-min sessions, male Sprague–Dawley rats (11–12/grp) were given access to water while water-deprived and to an ascending sucrose concentration series (0.1, 0.3, 1.0 M) while food- or nondeprived 20 min after injection of saline (1 ml/kg ip) or RAC (0.2 or 0.4 mg/kg). Licks taken prior to a 5 min pause were organized into bursts (licks separated by pauses of $\geq$1 s). After injection of 0.4 mg/kg, rats licked less water when water-deprived and less sucrose independent of state via a decrease in burst size, despite RAC increasing the number of 1.0 M sucrose bursts taken by food-deprived rats. Water-deprived rats did not lick less water after 0.2 mg/kg, but did take smaller, more frequent bursts. Food-deprived, but not nondeprived, rats licked less 0.1 M sucrose after 0.2 mg/kg, but all rats took smaller bursts, and in food-deprived rats, more frequent ones across concentrations. Importantly, for the 1st minute after the 2nd lick, rats licked less sucrose after RAC independent of state and took fewer licks in the first burst when nondeprived. RAC did not appear to induce motor disturbances, at least as measured by interlick interval. Overall these results suggest that RAC decreased sucrose intake through factors associated with orosensation. We are planning to test the effect of RAC on sucrose taste detection in a 2-response operant task. Supported by: NIH NIDCD F32DC010517 to CMM.

Effects of dried-bonito dashi (a traditional Japanese fish stock) on gastric emptying, gastric myoelectrical activity and hunger-satiety states in humans

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Objective: Dried-bonito (DB) dashi is a traditional Japanese stock that enhances palatability of various cuisines due to its specific flavor. Here we investigated the effects of DB dashi on gastric emptying, gastric electrical activity and hunger-satiety states in Japanese volunteers. *Methods:* We performed a randomized crossover study on 14 healthy young males. Before and just after the ingestion of a liquid test meal (200 kcal) containing ^{13}C-labeled acetate, with 150 ml of DB dashi or energy adjusted water as a control, gastric electrical activity was measured by electrogastrography (EGG) for 30 min. Gastric emptying was investigated by the $^{13}CO_2$ breath test. T_{max} and $T_{1/2}$ were calculated by postprandial 3 h breath collection. Hunger and satiety sensations were rated by visual analogue scales before and after the ingestion at 30 min intervals. *Results:* (1) DB dashi intake significantly decreased both T_{max} and $T_{1/2}$, and increased EGG normogastric power (%) in postprandial state, compared to the control diet. (2) DB dashi produced lower hunger sensations at 60, 90 and 120 min and higher satiety sensations at 90 min after the ingestion. *Conclusion:* DB dashi intake prolongs gastric emptying and improves postprandial gastric motility. In addition, DB dashi reduces hunger and enhances satiety in postprandial state.

Failure of intra-third ventricular infusion of tumor necrosis factor-α (TNF-α) to enhance the anorexigenic effect of leptin in mice

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An overexpression of TNF-α in adipose tissue contributes to the low grade, chronic inflammation and peripheral insulin resistance accompanying obesity. TNF-α also acts as a mediator of the anorexia and cachexia seen in infectious and neoplastic diseases, effects that are presumably due to a central nervous system action of circulating TNF-α, in particular on the hypothalamus. By acting in the hypothalamus, adipocyte-derived circulating TNF-α may enhance rather than attenuate the eating-inhibitory effects of insulin and leptin. We therefore tested the effects of leptin and TNF-α co-infusions into the third ventricle of adult, male C57BL/6 mice fed regular chow. After establishing dose response curves for each compound separately, leptin (0.4 and 0.2 υ;g/mouse) and TNF-α (10 ng/mouse) were infused alone or in combination. Both compounds reduced ($P < 0.05$) food intake alone and in combination, but there was no statistically significant synergistic interaction between them. Leptin in doses of 0.2 and 0.4 υ;g reduced 2 h cumulative food intake by 47% and 57%, 4 h food intake by 36% and 58%, and 20 h food intake by 28% and 27%, respectively. The same doses of leptin in combination with 10 ng of TNF-α reduced the 2 h food intake by 67% and 77%, 4 h food intake by 57% and 78%, and 20 h food intake by 32% and 49%, respectively. These findings do not support the idea of a synergistic central eating-inhibitory effect of TNF-α and leptin.

Subtle changes in the flavour and texture of a drink enhance expectations of satiety

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Previous research indicates that small increases in satiety-relevant oro-sensory properties (thick mouthfeel and creamy flavour) enhance the satiating effects of a high energy yogurt drink. It is possible that oro-sensory cues generate expectations that a food will be filling which enhances our physiological responses to nutrient ingestion. A series of studies investigated the role of thick mouthfeel and creamy flavour in the generation of satiety expectations, and whether these expectations are influenced by individual eating styles. The first study assessed satiety expectations generated by eight versions of the fruit yogurt drink. High and low energy versions of the drinks were presented in four sensory contexts: low sensory, creamy, thick, high sensory (thick and creamy). This design was repeated in study two where participants were grouped based on their tendency to restrict intake (dietary restraint) and tendency to overeat (dietary disinhibition), as measured by the Three-Factor Eating Questionnaire. In both studies thick mouthfeel and not energy content increased expected satiety and filling ratings. Creamy flavour increased filling ratings, but to a lesser extent. In study two participants with a low tendency to both restrict intake and overeat were most sensitive to the sensory manipulations. These data suggests that small manipulations of texture and flavour increase expectations that a fruit yogurt drink will be filling and suppress hunger. Sensitivity to these manipulations may be influenced by an individual's eating style. Supported by: BBSRC-DRINC.

Induction of conditioned taste aversion leads to phasic suppression of dopamine release in nucleus accumbens shell

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Nucleus accumbens (NAc) dopamine is bi-directionally modulated by the palatability of stimuli – it increases to palatable foods (e.g. sucrose) and decreases to unpalatable foods (e.g. quinine). We examined the plasticity of these responses by assessing sucrose-evoked dopamine release in rats that had sucrose rendered unpalatable using a conditioned taste aversion (CTA) paradigm. To induce CTA, one group of rats (paired), received 30 intraoral infusions of sucrose (0.3 M, 200 υ;L/infusion) over a 30 min period and were then injected with LiCl (0.15 M; 20 mL/kg) on Days 1/3. On Days 2/4, these rats received injections of saline in home cage. Another group (unpaired) had the injection order reversed so that they received saline injections immediately after sucrose infusions and LiCl injections in home cage. LiCl caused hypothermia in all rats. On a test day, fast-scan cyclic voltammetry recordings were made in NAc shell of all rats to measure phasic fluctuations in dopamine evoked by infusions of sucrose and water. In paired rats, sucrose infusions suppressed dopamine release relative to water infusions and relative to sucrose infusions in unpaired rats. Sucrose palatability was assessed using taste reactivity which confirmed induction of CTA in all paired rats. In summary, after CTA induction, the affected taste stimulus suppresses dopamine in NAc shell correlating with its dramatic decrease in palatability. This may reflect a form of learning about the stimulus, which is expected to affect animals' future responses towards it. Supported by: NIH Grant DA025634.

Bile acids as TGR5 agonists signaling GLP-1 release in healthy humans

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TGR5 is expressed in GLP-1-secreting cell lines and L cells from mice; gain- and loss-of-function models suggest a physiological role for TGR5 activation on GLP-1 secretion in rodents. TGR5 signaling showed improved postprandial glucose tolerance in obese mice, associated with marked postprandial GLP-1 release and insulin secretion. In contrast, TGR5$^{-/-}$ mice exhibited reduced glucose tolerance. In animals TGR5 may be targeted by natural bile acids (BAs) suggesting a role for postprandial BAs in modulating nutrient-induced GLP-1 secretion. We therefore hypothesized that intraduodenal perfusions of TGR5 agonists stimulate the secretion of GLP-1 in humans. The study was a randomized, double-blind, placebo-controlled, parallel-designed trial. Twelve healthy male volunteers received intraduodenal perfusions (2.0 mL/min for 180 min) of: (i) a bile acid, (ii) a fatty acid or (iii) vehicle. After 1 h subjects performed an oral glucose tolerance test (oGTT). BAs induced a significant secretion of GLP-1 within the first 60 min compared to placebo ($P = 0.016$); however, no differences were observed after the oGTT. Plasma insulin and glucose were not affected by BAs within the first 60 min; a significantly attenuated insulin release compared to placebo was observed after the oGTT ($P = 0.011$). Our results in healthy subjects do not support an important role for BAs in regulating GLP-1 release and postprandial glucose metabolism. A major limitation of the hypothesis is the fact that the evidence is based on animal studies and cell models. Supported by: Grant of the Swiss National Science Foundation (Grant No. 320030_132960/1), the Stiftung zur Förderung der gastroenterologischen Forschung, and an unrestricted Grant of Hoffmann-La Roche.

Effect of A_{2A} adenosine receptor agonists on compulsive binge eating of highly palatable food

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The present study investigated the effect of two A_{2A} adenosine receptor (AR) agonists, CGS 21680 and VT 7, in an experimental model of binge eating (BE) of highly palatable food (HPF) induced in female rats by three 8-day cycles of food restriction/re-feeding followed by acute stress. Two groups of rats were used: NR+NS normally fed and not stressed; R+S exposed to cycles of food restriction/re-feeding and then stressed. R+S exhibited BE of HPF in amounts significantly larger than NR+NS. The two A_{2A}AR agonists were tested at doses of 0.1 and 0.05 mg/kg; VT 7 did not modify locomotor activity at either doses, while CGS 21680 only slightly reduced it at the higher dose tested. Injection of 0.1 mg/kg of both agonists markedly reduced BE in R+S. However, HPF intake was significantly reduced also in NR+NS. The dose of 0.05 mg/kg was inactive. CGS and VT 7, 0.1 mg/kg, reduced also normal food pellets intake in 24 h food-deprived rats; however, they did not reduce water intake, suggesting that their effect on food intake is selective. Again, the dose of 0.05 mg/kg was inactive. Taken together these findings indicate that A_{2A}AR agonists exert a rather general effect on food intake, inhibiting both reward based intake of HPF in sated rats and the intake of food pellets driven by homeostatic needs following food deprivation. A_{2A}AR agonists may represent interesting pharmacological agents to control bingeing-related eating disorders, as well as to reduce food over-consumption associated with obesity.

The food intake- and meal size-suppressive effects of GLP-1 receptor signaling in the VTA are mediated by AMPA/kainate receptors

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Glucagon-like peptide-1 (GLP-1) producing neurons in the nucleus tractus solitarius project directly to the mesolimbic reward system to regulate food intake. Furthermore, endogenous GLP-1 receptor (GLP-1R) signaling in the ventral tegmental area (VTA) is physiologically relevant for control of palatable food intake. Given that increased glutamatergic (Glu) transmission to the VTA decreases rewarding appetitive behaviors, we hypothesized that GLP-1R in the VTA may be expressed on presynaptic Glu terminals and, when activated, stimulate Glu activation of VTA NMDA and/or AMPA/kainate receptors to reduce food intake. Rats maintained on high fat diet received intra-VTA injection of either the AMPA/kainate receptor antagonist CNQX (0.3 μg) or NMDA receptor antagonist MK-801 (0.05 μg), followed by intra-VTA injection of the GLP-1R agonist exendin-4 (Ex-4, 0.05 μg). Consistent with the hypothesis that VTA GLP-1R signaling modulates the within-meal rewarding value of feeding, intra-VTA Ex-4 reduced palatable food intake primarily by decreasing meal size. Additionally, the intake- and meal size-suppressive effects of Ex-4 were attenuated by CNQX at 3 h post-treatment and persisting at various time points throughout a 24 h test. Conversely, intra-VTA MK-801 did not attenuate the intake-suppressive effects of Ex-4. These findings suggest that GLP-1R activation in the VTA increases Glu activation of AMPA/kainate receptors, but not NMDA receptors, to reduce meal size of palatable food. NIH-DK085435 and -DK19525.

Nesfatin-1 influences the excitability of neurons in the nucleus of the solitary tract

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Nesfatin-1 (nes-1), a product of the nucleobindin-2 gene (NUCB2), has been identified as one of the most potent centrally acting anorexigenic peptides. Immunohistochemical studies have revealed the expression of nes-1 throughout the brain and, in particular, in the medullary autonomic gateway known as the nucleus of the solitary tract (NTS). To date, there is minimal data describing the specific functional roles of nes-1, and what information is available has centered only on actions in the hypothalamus. Thus in order to elucidate the mechanisms by which nes-1 exerts its anorexigenic effects, the present study was undertaken to explore the cellular correlates of nes-1 actions in the medial NTS (mNTS). Using current-clamp electrophysiology recordings from rat mNTS neurons in slice preparation, we show that bath applied 10 nM nes-1 directly influences the excitability of the majority of mNTS neurons by eliciting either depolarizing (mean 7.8 ± 0.8 mV, 42%) or hyperpolarizing (mean –8.2 ± 1.0 mV, 21%) responses. These responses were observed in all electrophysiologically defined cell types in the NTS, and were site specific and concentration dependent. *Post-hoc* single cell reverse transcription polymerase chain reaction revealed a depolarizing action of nes-1 on neuropeptide Y and NUCB2 expressing mNTS neurons. Our results suggest important roles for nes-1 in the NTS and highlight this region as a key structure mediating these autonomic actions. Supported by: FQRNT, NSERC, HSFO.

Regulation of protein intake and selection. Branched-chain amino acids and beyond

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Free feeding animals must navigate a complex nutritional space in which food quantity and quality can vary widely. While energy intake is clearly an important component of ingestive behavior, it seems unlikely that food selection is driven solely by energy needs. Protein intake is essential for maximal fitness and ultimately for survival, and multiple studies demonstrate that variations in dietary protein quality and quantity can induce significant changes in food intake and selection. Are these observations consistent with a homeostatic regulation of protein intake, and if so, what are the mechanisms governing protein selection? Recent work indicates that branched chain amino acids (BCAAs), and leucine in particular, can act locally in the brain to alter food intake, likely via regulation of classic cellular nutrient sensors such as mTOR and AMPK. Separate studies suggest that protein quality (amino acid balance) is detected by GCN2-dependent signaling in the anterior piriform cortex. Our recent data indicates that leucine may be unique among amino acids in its capacity to locally signal within the hypothalamus, but that altered dietary BCAA intake was neither necessary nor sufficient for low protein-induced hyperphagia. These data also indicate that protein restriction is associated with unique changes in mitochondrial function and insulin signaling, raising the possibility that protein detection may be mediated in part by metabolic changes in muscle and/or liver. Supported by: NIH 5R01DK081563.

Thermoregulatory leptin action via the dorsomedial hypothalamus and control of energy expenditure

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Leptin is an important regulator of energy homeostasis, which includes thermoregulatory responses via heat produced by brown adipose tissue (BAT). Leptin deficient *ob*/*ob* mice are hypothermic and do not survive cold exposure due to defects in BAT thermogenesis, but the contribution of BAT thermogenesis to body weight regulation has been debated. Leptin induced BAT thermogenesis is well documented, but the central mechanisms of thermoregulatory leptin actions are not clearly understood. Leptin receptors (LepRb) are expressed on dorsomedial hypothalamic (DMH) neurons that are known to regulate sympathetic BAT circuits and are stimulated during cold exposure. Consistently, systemic leptin also stimulates DMH-LepRb neurons and intra-DMH leptin replacement in *ob*/*ob* mice recovered hypothermia and decreased body weight despite unchanged food intake. Furthermore, DMH-LepRb specific expression of DREADDs (designer receptors exclusively activated by designer drugs) allows the acute and specific induction of neuronal excitation in DMH-LepRb neurons. These studies demonstrated that DMH-LepRb neurons are sufficient to generate a powerful increase in energy expenditure and body temperature, while decreasing body weight. Many other LepRb populations innervate the DMH, so that the DMH might be an important relay site for leptin mediated thermoregulatory control and plays a significant role in body weight regulation. *Grant support: MDRTC P&F award, AHA053298N, NIH1 P20 RR02195, NORC P&F award to HM.*

Effect of vertical sleeve gastrectomy in melanocortin receptor 4-deficient rats

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Bariatric surgery is currently the most effective treatment for obesity. Vertical sleeve gastrectomy (VSG) is a bariatric procedure growing in popularity where surgical intervention is limited to the stomach. In humans, partial loss of melanocortin receptor-4 (*Mc4r*) activity is the most common monogenic cause of obesity regardless of lifestyle. At present it is unclear if genetic alteration of Mc4r signaling affects the beneficial effects of VSG. Following VSG, we analyzed body weight, food intake, glucose sensitivity, and macronutrient preference of wild-type, $Mc4r^{+/-}$ and $Mc4r^{-/-}$ rats as compared to sham-operated controls. VSG improved body weight and glucose metabolism, and shifted preference towards carbohydrates and away from fat and this occurred independent of MC4R function. In addition, *MC4R* was resequenced in 46 human subjects who underwent VSG. We observed common genetic variation in *MC4R* in five subjects. However, these variations did not affect the outcome of VSG. We conclude that the beneficial effect of VSG on body weight and glucose metabolism in rats is independent of MC4R function. Combined, our rodent and human data suggest that humans with partial or full loss of MC4R are good candidates for VSG. Supported by: Ethicon-Endo Surgery.

Sensory-specific 'appetition'. IG glucose infusion specifically enhances consumption of the flavor that accompanies it

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Palatable taste stimulates intake, whereas post-oral effects of ingested nutrients are generally inhibitory. But some recent work has identified a rapid, positive 'appetition' effect generated by glucose in the gut. We tested here whether rats accustomed to IG water infusion during daily flavored-saccharin consumption would show an automatic, unlearned acceleration of intake the first time glucose replaced the water infusion. Rats with IG catheters acclimated to routine overnight food/fluid restriction had scheduled 2 h/day access to 0.05% saccharin with a novel flavor added each day. During the first 15 min of each session 5 ml water was infused IG. In a critical test trial, flavored saccharin was accompanied for the first time by 16% glucose infusion. Rats consumed significantly more in this test compared to preceding and subsequent baseline (water infusion) tests, with licking rates elevated over baseline within the first several minutes. This effect was again produced by IG glucose but not fructose or maltodextrin, ruling out thirst due to osmotic effects of the infusion. A final experiment demonstrates this positive feedback is motivationally specific to the flavor that accompanied onset of the post-oral stimulus. Intake stimulation by IG glucose was prevented when the flavor was removed and immediately replaced by a different one, but persisted when the flavor was removed and returned. Thus nutritive effects early in a meal may produce immediate, sensory-specific enhancement in evaluation of a food being consumed.

Depression and anxiety are associated with reduced obesity-related quality of life measures in extremely obese patients attending a specialist weight management service

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Obesity is a worldwide concern yet research on the effectiveness of medical weight management for extreme obesity is limited and there is insufficient information about how co-morbidities affect quality of life and weight loss. Evidence suggests that psychiatric co-morbidities play a role in weight loss. We hypothesised that depression and anxiety would be negatively associated with quality of life measures and weight outcomes in a specialist medical weight management service. 63 men and 181 women (mean age = 43 y and mean BMI = 46 Kg/m^2) were referred to the weight management service where they received advice to promote healthy lifestyle change. At baseline, they completed quality of life, sleep, depression and anxiety questionnaires. Sixty percent of patients had abnormal depression scores and 53% had abnormal anxiety scores. No relationships were found between weight outcome, number of sessions completed and depression and anxiety scores. However, when compared to their non-depressed and non-anxious counterparts, participants with depression and anxiety were significantly more likely to report that their self esteem, physical function, sexual life, level of public distress, career, and overall health was impaired by their weight, even though BMI did not differ according to group status. Depressed and anxious groups also reported poorer sleep. The obese experience appears to be very different for depressed and anxious individuals but for this group of obese patients, depression and anxiety was not associated with weight loss.

Association between impulsiveness and pleasantness ratings for food and drugs

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Those who use drugs and alcohol more frequently eat more food, and a relationship between pleasantness ratings for the effects of drugs and taste of food has been reported. To examine the degree to which impulsiveness plays a role in these relationships, 47 female university students were asked to rate the pleasantness of eating different foods and of drug and alcohol effects on VAS general labeled magnitude scales. They were also asked to complete a survey of drug and alcohol use and the Barratt Impulsiveness Scale (BIS). Results indicated that BIS attentional impulsiveness (but not other BIS subscales) was positively correlated with the pleasantness of eating French fries [$r(44) = 0.366$, $p = 0.012$], pasta [$r(45) = 0.324$, $p = 0.026$] and pizza [$r(43) = 0.490$, $p = 0.001$] as well as the pleasantness of drinking favorite alcoholic beverage [$r(33) = 0.472$, $p = 0.004$] and the intoxicating effects of alcohol [$r(33) = 0.337$, $p = 0.048$] and weekly alcohol consumption [$r(44) = 0.571$, $p = 0.000$], annual alcohol consumption [$r(45) = 0.358$, $p = 0.014$], and binge drinking frequency [$r(45) = 0.322$, $p = 0.027$]. Pleasantness of eating the foods was not consistently correlated with pleasantness of drug effects or frequency of drug use. These results suggest attentional impulsiveness is related to pleasantness of eating and of drug effects and frequency of drug and alcohol use. They also suggest that the relationships among the subjective pleasantness ratings of foods and drugs may be influenced by whether one is rating food taste specifically or the more general experience of eating that food. Supported by: Wagner College.

Effect of various intestinal surgeries on reduction of meal size, prolongation of the intermeal interval and possible weight loss by cholecystokinin-8 and 33

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A combination of intestinal surgery and administration of satiety peptides may form a successful protocol for the treatment of obesity. We tested this possibility by measuring meal size (MS), intermeal interval (IMI) and body weight in response to intraperitoneal (i.p.) injections of cholecystokinin (CCK) -8 or -33 in five groups of rats that underwent the following small intestinal surgeries: (1) Duodenal/Jejunal enterotomy, (2) Jejunal/Ileal enterotomy, (3) Duodenectomy/Jejunectomy, (4) Duodenectomy/Ilectomy and (5) Duodenectomy/Jejunectomy / Ilectomy. We found that CCK-8 and CCK-33 (3 nmol/kg) reduced MS (10% sucrose) in all groups and CCK-33 prolonged the IMI in the sham group. In addition, following the animals for 10 months, the weights of the operated rats in all groups were not different from that of the sham group. As such, the combination of the previous small intestinal surgeries and injections of the short-term satiety peptide CCK failed to reduce body weight. Other gastrointestinal surgeries with injections of different satiety peptides and change of the test diet may still be a valuable option to treat obesity. Supported by: NIH 1SC1DK094972-01A1 and the Birmingham Racing Commission.

Effects of daily timing of saturated fat and liquid sugar intake in obesity development

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Overeating is associated with the development of obesity. However, not only the amount, but also the timing of caloric intake could be an important contributor to obesity. Indeed, mice fed only during the light phase gained more weight compared to mice fed the same amount of calories during the dark phase. We investigated which components of the diet attribute most to this time-of-day dependent increase in weight gain. Twenty-four male Wistar rats were subjected to either chow *ad libitum* or a free-choice diet with access to a dish with saturated fat, a 30% sugar solution, pelleted chow and tap water; either *ad libitum* (fcHFHS), with restricted access to saturated fat only during the light phase (fcHS-dHF), or with restricted access to sugar only during the light phase (fcHF-dHS). Caloric intake and body weight gain were studied during five weeks. Locomotor activity was studied in the fourth week. All animals on a diet showed hyperphagia compared to the control group, with a similar caloric intake among the fcHFHS, fcHS-dHF and fcHF-dHS groups. Animals on a fcHF-dHS diet gained more body weight compared to the other groups despite a similar caloric intake. Thus, consuming both saturated fat and liquid sugar results in hyperphagia, irrespective of when the components are ingested. However, the time when components were ingested was important for body weight gain. Further research will determine which mechanisms are involved in this effect of timing.

Gastric bypass surgery alters gut microbiota profile along the intestine

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Changes in gut microbiota may be responsible for the metabolic changes seen after Roux-en-Y gastric bypass (RYGB) surgery. Altered nutrient flux after surgery might have different effects on the gut microbiota profile along the intestine. Here we assessed the bacterial composition in different anatomically corresponding intestinal segments after RYGB or sham surgery. Fourteen weeks after surgery, intestinal segments and contents were collected. Gut microbiota composition, intestinal preproglucagon, peptide YY (PYY) and cholecystokinin (CKK) expression and intestinal dipeptidyl peptidase-4 (DPP4) activity were measured. Total bacteria, Bifidobacteria, and Bacteroides-Prevotella spp content were significantly increased in the alimentary limb and common channel of RYGB rats compared to sham rats. RYGB rats also had higher Bifidobacteria and Bacteroides-Prevotella spp in the colon, and Bifidobacteria and Lactobacillus in the caecum. Preproglucagon, PYY and CKK expression were increased in the alimentary limb and common channel of RYGB rats, DPP4 activity was unchanged. In conclusion, RYGB surgery leads to changes in the microbiota of the alimentary limb and the common channel which resemble those seen after weight loss by dieting. This may be associated with altered production of gastrointestinal hormones known to regulate energy balance. Our findings suggest that postsurgical microbiota changes in intestinal segments may significantly contribute to the beneficial metabolic effects of RYGB surgery. Supported by: Olga Mayenfisch Stiftung, Grant Nr. 35111013.

Effect of being weighed on responses to eating behavior questions

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A quasi-experimental study was performed to determine whether being weighed prior to completing a questionnaire affects responses to a questionnaire about eating behavior. It was hypothesized that being weighed would serve as a priming stimulus and increase measures of dietary restraint, disinhibition, and hunger. Trained researchers collected a sample of volunteers (n = 355) in 7 cities in the United States on two Saturdays in the summer of 2011. Half of the participants were weighed prior completing the three factor eating questionnaire. Results indicated that being weighed first produced a difference in differences on disinhibition scores between low restraint (95% CI = 4.65–6.02) and high restraint (95% CI = 6.11–7.57) (p = 0.003) compared to being weighed after questionnaire completion. Being weighed is unlikely to be a strong enough prime to significantly change scores on eating behavior questionnaires in everyone, but does allow conditions related to restraint to become evident. Supported by: Cornell University.

Adenovirus-overexpression of liver carnitine palmitoyltransferase 1 (CPT-1a) increases food intake after 24 h fasting

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It has been proposed that hepatic fatty acid oxidation (FAO) contributes to the control of food intake (Scharrer and Langhans, Am J Physiol 1986;250:R1003–6) with an increase (decrease) in FAO inhibiting (stimulating) eating. Therefore we tested the effects of hepatic over-expression of CPT-1 on energy homeostasis. Male adult C57BL/6 mice received either 1×10–9 IU of CPT1a (liver isoform)-mutant adenovirus (n = 6) or beta-galactosidase [n = 6], as control) via the tail vein and placed on metabolic cages to monitor food and water intake as well as activity and energy expenditure parameters. Sixteen days later a 24 h session of fasting was scheduled and animals were behaviorally and metabolically monitored. We confirmed CPT-1 overexpression via western blotting on terminal liver biopsy. No significant difference in the levels of pro-inflammatory cytokines MCP-1 and TNF-α were observed between the groups. Food and water intake were undistinguishable under ad libitum condition, but after 24 h fasting CPT-1 over-expressing animals displayed significantly higher food and water intake than controls, starting 2 h after re-feeding, with no major changes in energy expenditure. All in all, our data are not consistent with the hypothesis that an increase in hepatic fatty acid oxidation inhibits eating.

Physiological alterations upon withdrawal from an obesogenic diet. Implications for dieting

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The high fat high sucrose (HFHS) diet is an established model of diet induced obesity. Wistar rats, exposed to four week HFHS diet show an increase in caloric intake, fat mass and circulating leptin levels coupled with peripheral leptin insensitivity. In the current set of experiments, the dynamics of leptin sensitivity upon withdrawal from a HFHS diet were studied. Our data demonstrate that a 4-week withdrawal from a HFHS diet was insufficient to normalize the obese phenotype encountered upon exposure to it. This was evidenced by persistent leptin insensitivity, pronounced adipose tissue and circulating leptin levels in the backdrop of decreased caloric consumption. Interestingly, the withdrawal phase was accompanied by lower core body- and brown adipose tissue temperature independent of locomotor activity. Levels of mitochondrial uncoupling protein1, an indicator of brown adipose tissue thermogenesis were also attenuated. The current data thus indicate that during food scarcity, modulation of thermoregulatory pathways is one of the essential mechanisms by which animals defend their elevated adiposity. But, the neuronal pathways involved in this modulation still remain unknown. Supported by: The European Community's Seventh Framework Programme FP7/2007-2013(n° 245009).

Subtle goal primes can offset hedonic effects of food on behavior

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Research on the nonconscious processes underlying the common difficulties in weight control has shown that palatable food cues can activate reward expectations and a hedonic eating goal, facilitating overeating. These processes are especially prevalent in people struggling with weight regulation. In this talk, I will briefly review how subtle external goal primes can be used to offset these effects and direct motivated behavior toward pursuit of the dieting goal. Then, I will present a recent field experiment demonstrating the far-reaching motivational effects of diet goal primes and their effectiveness in daily life. Customers of a local supermarket were handed a recipe flyer before grocery shopping. For half of participants, this flyer was furnished with diet-related words to subtly activate the goal of dieting. Afterwards, purchases of healthy and unhealthy items were assessed by means of the receipts. Results show that the subtle diet prime led to healthier grocery shopping among both chronic dieters and overweight participants. Given the impact of grocery shopping for the availability of food in the home and thus for subsequent eating behavior, goal priming in shopping settings constitutes a promising tool for effective diet interventions. These findings enhance our understanding of the mechanisms underlying successful dieting behavior, and they have important practical implications for how successful dieting can be used to reduce the role of reward-activating food cues on our eating behavior. Supported by: NWO Veni awarded to Esther K. Papies, ZonMW Grant awarded to the Netherlands Nutrition Center.

Neuronal activation by ghrelin and angiotensin II

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Although ghrelin is well known for its role in promoting food intake, it also affects fluid intake under a variety of conditions. For instance, ghrelin attenuates fluid intake stimulated by central administration of angiotensin II (AngII). To better understand how ghrelin is participating in the regulation of AngII-induced fluid intake, the current studies used Fos immunohistochemistry to elucidate potential sites at which ghrelin and AngII interact. Rats were given icv injections of vehicle, ghrelin, AngII, or the combination of AngII and ghrelin (AngII+ghrelin). Ninety minutes after the injections, rats were sacrificed by perfusion and their brains were processed for Fos immunoreactivity (Fos-ir). The number of Fos-ir cells was counted in the subfornical organ, the paraventricular hypothalamic nucleus (PVN), and the median preoptic nucleus of the hypothalamus (MnPO), each of which has been implicated in the response to AngII. In the SFO, the number of Fos-ir cells was greater in AngII+ghrelin rats, but no differences were found when rats were given either of the peptides alone. In the PVN, the number of Fos-ir cells was greater in rats given AngII compared to controls, but there were no significant differences between controls and rats in the ghrelin or AngII+ghrelin groups. In the MnPO, the number of Fos-ir cells was similarly increased in the AngII and AngII+ghrelin groups, but not when rats were given ghrelin alone. Although further analysis is needed, these studies highlight the SFO as a potential site for the interaction between ghrelin and AngII and may elucidate other structures relevant to the behavioral effects of these peptides. Supported by: NIHHL-91911.

Glucagon-like peptide-1 in the hindbrain influences unconditioned and conditioned procurement of sucrose in sated rats

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Since discovering that glucagon-like peptide-1 (GLP-1) reduces food intake through the central nervous system, the precise nature of the peptide's role in feeding has become a prominent question with recent attention on the reward system. Thus we investigated where and how GLP-1 may be controlling palatable food intake, first verifying that GLP-1 infused into the 4th ventricle (4V) potently reduces non-homeostatic intake of sucrose (0.3M) in a dose-dependent manner (% reduction from baseline: 2 μg = 52.8 ± 7.3%; 14 μg = 89.3 ± 4.2%), while forebrain infusion using similar doses produced no effect. Animals were then conditioned with sucrose as the operant stimulus on a progressive ratio-10 schedule of reinforcement and the effects of forebrain and hindbrain GLP-1 administration was measured. We found that GLP-1 infused into the 4V, but not the lateral ventricle, lowered the rats' motivation to work for sucrose reward. GLP-1 (6 μg-4V) reduced the number of completed cycles (i.e. break-point) on the empty 'operant spout' (aCSF = 10.6 ± 1.8; GLP-1 = 6.3 ± 0.9; Ex-9 = 10.3 ± 0.8; Ex-9 + GLP-1 = 9.6 ± 1.1), as well as sucrose intake on the 'reward spout', and these effects were blocked by 4V pretreatment with the GLP-1 receptor antagonist, Exendin-9. These findings demonstrate that GLP-1 reduces both anticipatory and consummatory motivation for sucrose in sated rats, and suggest that GLP-1 receptors in the hindbrain rather than the forebrain modulate sucrose reward. Supported by NIH Grants DC011671, DK080899.

Hindbrain expression of dopamine D1 and D2 receptors is differentially affected by free access to a palatable diet in rats

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A strong link between obesity and dopamine (DA) has been established by studies associating body weight status to variants of genes related to DA signaling, including the DA D2 receptor (DRD2) gene. Human and animal studies investigating this relationship have so far focused mainly on the role of DA within the mesolimbic pathway. The aim of this study was to investigate potential DA receptor dysregulation in the hindbrain, where these receptors play a role in taste processing, in rats exposed to a high-fat/high-sugar diet (HFHS). We randomized rats into three groups; ad-libitum access to HFHS (n = 24), restricted HFHS access (n = 10), or controls (chow-fed, n = 10). After 5 weeks, the brainstem was dissected and gene expression was investigated by qRT-PCR. DRD2 mRNA expression was down-regulated in the brainstem of the HFHS ad-lib animals ($P < 0.01$), whereas expression of the DA D1 receptor was upregulated in these rats ($P < 0.01$), compared to chow-fed controls. We also noted an increased POMC expression in the ad-lib rats ($P < 0.01$). The present findings suggest that DA dysregulation after extended access to energy-dense palatable food occurs not only in striatal regions, but also in parts of the hindbrain.

Preload of dietary fibers added to fatty food efficiently reduces energy intake by systemic PYY and vagal CCK signaling in mice

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Most of food actually marketed have large amounts of lipid and have been contributing to the increasing prevalence of obesity. In front of this, adding a satietogenic nutrient such as dietary fiber (DF) to these foods may be an interesting option to fight overfeeding. We aimed to investigate the fate of this satietogenic potency of DF when in a fatty food. We used therefore a model of liquid fatty food (FF, 20% of rapeseed oil in water, 1.2 kcal) in which we have solubilized DF (guar gum and fructo-oligosaccharide, 14% weight per volume of DF). Compared to FF alone, we found that the preload of DF solubilized in fatty food (DF-FF) reduced the daily energy intake by 19% in mice fed with standard diet. Exploring underlying mechanisms exhibited that DF-FF (i) elicited increased Peptide YY (PYY) release at 3 and 6 h after treatment and (ii) upregulated in the hypothalamus both melanocortin type 4 receptor (MC4R) transcripts 4-fold and pro-opiomelanocortin (POMC) protein 2-fold (90 min and 6 h after administration, respectively). Peripheral blockade of cholecystokinin (CCK) -1 receptor abolished the DF-FF effect on food intake while glucagon-like peptide-1 receptor blockade had no influence. Taken together, these data show that DF can maintain its satiating properties even integrated in a fatty food by stimulating anorectic gut-brain signaling which involve systemic PYY, vagal CCK and hypothalamic POMC/MC4R signaling. Supported by: AgroParisTech-INRA.

Physiological responses of food neophobics and food neophilics to food and non-food stimuli

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Individual differences in human food neophobia (the reluctance to try novel foods) and food neophilia (the overt willingness to try novel foods) influence the evaluation of tastes and odors, as well as the sampling of such stimuli. Past research also notes an association of food neophobia to PTC sensitivity, body weight, and cephalic phase salivary response. The present study assessed physiological reactions of food neophobics and neophilics to pictures of food and non-food stimuli. Stimulus pictures were presented in random order on a computer screen for a period of 5 min. No significant differences were found between the groups in relation to non-food stimuli. However, pulse [$F(1,21) = 5.69$, $p = 0.03$], GSR [$F(1,21) = 3.07$, $p = 0.09$] and respirations [$F(1,21) = 4.43$, $p = 0.05$] were significantly increased in food neophobics when presented pictures of food stimuli. Thus, further evidence is provided to support a physiological component at least partially responsible for differences noted between neophobics and neophilics in sensitivity, psychophysical ratings, and willingness to try personality. Such a component may also lead to differences in weight, nutrition, and overall health.

The gut microbiota, gut-brain axis and regulation of food intake

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The gut epithelium is a barrier between the inside of the body and the outside world. Potentially harmful toxins, irritants and bacteria exist in the gut lumen and can disrupt the epithelial barrier, leading to inflammation. The epithelium has to maintain this barrier yet also serve to absorb nutrients, ions and water. Gut enteroendocrine cells (EECs) sense meal components, activate neural and humoral pathways to regulate GI motor, secretory and absorptive functions, and also to regulate food intake and plasma levels of glucose. Recent studies have provided compelling new evidence to suggest that changes in epithelial barrier function and inflammation are associated with and may even lead to altered regulation of food intake and body weight. Our laboratory has obtained evidence to support the hypothesis that changes in the gut microbiota and alteration of gut epithelial function will perturb the homeostatic vagal afferent pathway, leading to altered food intake and control of body weight.

Identifying novel ghrelin receptor neural circuits using a GFP mouse model

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Ghrelin acts on ghrelin receptors (GHSRs) in the brain to regulate a number of diverse functions, including appetite, adiposity, anxiety, stress, learning, memory and addiction. Although the physiological mechanisms controlling these functions are well studied, little is known about the neuroanatomical GHSR populations involved. We used a GHSR GFP mouse model generated by the GENSAT project at Rockefeller University. With immunohistochemistry, we showed GFP expression, as a marker for the GHSR, in hypothalamic nuclei such as the ARC and the VMH. There was a paucity of GHSR expression in the PVN the DMH and the LHA. We also showed significant GFP expression in the hippocampus, anterior cingulate cortex, insular cortex, amygdala, piriform cortex, olfactory bulb, paraventricular thalamic nucleus, reuniens, substantia nigra, ventral tegmental area and edinger-westphal nucleus. In the brainstem, the GHSR was expressed in the periaqueductal grey area, dorsal raphe nucleus, medial and lateral parabrachial nucleus, NTS and area postrema. Coexpression analysis revealed the neurochemical identity of many GHSR populations. In order to determine if brain and brainstem GHSR populations respond to acute peripheral ghrelin, we injected GHSR GFP mice with ghrelin and used Fos as a marker of ghrelin-induced neural activation. Despite the widespread expression of GHSRs throughout the brain and brainstem, we only observed ghrelin-induced Fos activation in a few select hypothalamic and brainstem nuclei. This model improves our understanding of central GHSR circuits controlling appetite, mood, anxiety, learning, memory and addiction.

Comparison of repeated exposure, flavour–flavour learning, and flavour–nutrient learning to increase vegetable intake in weaning infants

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Because vegetable consumption is still below nutritional recommendations, understanding early formation of vegetable acceptance is crucial. The present study compares the efficiency of three learning mechanisms in weaning infants: repeated exposure (RE), flavour–flavour learning (FFL), and flavour–nutrient learning (FNL). A target vegetable (artichoke) prepared according to a basic recipe and a control vegetable (carrot), were proposed to 95 infants (6.4 ± 0.8 months) at two different meal, before and after an exposure period. During the exposure period, infants were randomly assigned to three groups and were exposed 10 times (2 or 3 times a week) to a basic artichoke recipe (RE group; n = 32, 48 kcal/100 g), or a sweet artichoke recipe (FFL group; n = 32, 51 kcal/100 g), or an energy-dense artichoke recipe (FNL group; n = 31, 144 kcal/100 g). Follow-up after 2 weeks, 3 and 6 months were performed. Intake was recorded by parents. During the 10 exposures, artichoke intake increased for both RE and FFL groups, but not for FNL group. The basic artichoke intake increased significantly between the pre and post-exposure in RE (+52 ± 11 g) and FFL (+40 ± 13 g) groups, but not significantly in the FNL group (+9 ± 16 g) and this result was stable at each follow up. To conclude RE and FFL mechanisms equally improved acceptance of the vegetable in weaning infant; but FNL mechanism appeared less efficient than the two other mechanisms. Funding: European Community's Seventh Framework Program FP7-245012-HabEat

Intracarotid infusion of intralipid and glucose towards the brain alters food intake and glucose metabolism

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Different nutrients may specifically alter food intake (FI) and glucose metabolism. When saturated fat and a sugar solution are consumed in addition to chow, rats become hyperphagic and glucose intolerant. This is accompanied by an increase of hypothalamic neuropeptide Y (NPY), an important regulator of both FI and glucose metabolism. These changes were not observed with fat or sugar alone. In addition, an icv infusion of oleic acid decreased FI and hepatic glucose production, accompanied by a decrease in NPY. We hypothesized that fat affects FI and glucose metabolism directly via the brain, and that fat and glucose together alters FI and glucose differently than fat alone. In the current study we used an intracarotid catheter directed towards the brain. Male rats were fasted overnight and infused with IL (20%), G (1%), IL+G or NaCl (5 μl/min) during 2 h prior to feeding. Blood glucose and FI were measured throughout the experiment. The IL+G infusion decreased FI compared to other infusions, most pronounced during the first hour of feeding. All infusions were associated with a decrease in glucose after 2 h except the combination of IL+G that selectively prevented the decrease in glycemia. These data show that a combination of nutrients in the brain alters glucose metabolism and food intake differently than the separate nutrients. Supported by: ZonMW.

Fourth ventricular glucosamine injections stimulate feeding by activating hindbrain catecholamine neurons, but do not elevate blood glucose

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Glucokinase is a pancreatic glucose sensing mechanism that may also contribute to glucose sensing in the brain. Early work showed that lateral (Woods, 1978) or fourth ventricular (4V) (Ritter et al., 1982) injection of alloxan, a beta cell toxin and glucokinase inhibitor, impaired glucoprivic feeding at high doses and stimulated feeding at low doses (Ritter and Strang, 1982). Blood glucose was not affected by either high or low doses of alloxan. Here we examined responses to 4V glucosamine, another glucokinase inhibitor previously shown to enhance glucoprivic feeding and increase NPY/AGRP and orexin gene expression after lateral ventricle injections (Zhou et al., 2011). We found that 4V glucosamine stimulated feeding and increased c-Fos-ir in specific hindbrain catecholamine (CA) cell groups. Both effects were impaired by hypothalamic injection of the retrogradely transported immunotoxin, anti-dbh-saporin, suggesting that glucosamine activates CA neurons required for glucoprivic feeding. Interestingly, glucosamine, like alloxan, did not stimulate a hyperglycemic response. We found similar results using 4V injection of drugs that block or activate AMPK phosphorylation and with phloridzin, the sodium-glucose linked transporter 1 (SGLT1) inhibitor, all of which altered food intake, but not blood glucose (see also Flynn, 1985). Results suggest that different glucosensing mechanisms control feeding and adrenal medullary hyperglycemic responses to glucose deficit. Supported by: NIH DK040498.

The mirror mechanism: Theoretical bases and clinical relevance

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A mere visual perception, without involvement of the motor system would only provide a description of the visible aspects of the movements of the agent, but it would not give precise information about the intrinsic components of the observed action which are critical for understanding "what the action is about, what is its goal, and how to reproduce it". This sentence by Marc Jeannerod beautifully describes the importance of motor system in action perception. In my talk I will elaborate on this theme describing a mechanism – the mirror mechanism – that allows one to understand the others "from the inside". In the first part of my lecture, I will review the basic functional properties of frontal and parietal mirror neurons. I will describe first their motor properties, showing that, as most neurons in the premotor cortex, mirror neurons code the goal of a motor act. I will review then their visual properties showing that mirror neurons represent a mechanism that match the observed motor acts on motor neurons coding them. This includes those motor acts associated with eating and drinking. I will show then that, although there are several mechanisms through which one can understand the behaviour of others, in both humans and monkeys the mirror mechanism is the only mechanism that allows understanding actions from the "inside", giving the observing individual a "first-person" person grasp of others' behavior. In the last part of my talk, I will discuss the clinical relevance of the discovery of the mirror mechanism and in particular its relevance for autism.

Oleoylethanolamide is a gut-derived satiety factor controlling feeding behaviour through the activation of hypothalamic oxytocinergic neurons.

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Oleoylethanolamide (OEA) is a biologically active lipid amide released by small-intestinal enterocytes upon the absorption of dietary fat. OEA decreases food intake in rats and mice. This effect is behaviourally selective and is due to the prolongation of feeding latency and post meal interval. A large body of evidence indicates that the anorectic effect of OEA is mediated by the activation of peripheral PPAR-α receptors, but the central mechanisms downstream to this activation are still unclear. Data obtained from mapping brain c-fos mRNA levels revealed that the systemic administration of OEA evokes a highly localized increase of c-fos transcription in the nucleus of solitary tract, the paraventricular nucleus (PVN) and the supraoptic nucleus (SON). Both PVN and SON release oxytocin (OXY), one of the anorectic hypothalamic neuropeptides and we hypothesized that OXY neurons might play a key role in controling food intake after OEA administration. In agreement with our hypothesis, we found that systemic injections of OEA (10 mg/kg, intraperitoneal) increases OXY mRNA in the PVN and SON after 60 min from the administration, causes an increase of OXY in plasma, enhances OXY peptide expression at both the axonal and the somatodendritic levels of PVN magnocellular neurons and that its anorexiant action can be prevented by the central preadministration of L-368,899 (0.5 nmol) a selective OXY receptor antagonist. Our data strongly suggest that central release of OXY mediates OEA-induced anorexia.

Hindbrain GLP-1 receptor-mediated suppression of food intake requires PI3K/AKT signaling

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The CNS glucagon-like peptide-1 (GLP-1) system is physiologically required for normal control of energy balance. While GLP-1 receptors (GLP-1R) are expressed throughout the brain, nucleus tractus solitarius (NTS) GLP-1R are required for food intake control and, when pharmacologically activated, intake is potently suppressed for over 24 h. Recently, NTS GLP-1R-mediated control of feeding was shown to occur via PKA-induced suppression of AMPK and activation of MAPK signaling. While these pathways are identified as essential *rapidly-induced* intracellular responses mediating GLP-1R-suppression of intake, unknown are the intracellular signaling pathways that account for the *long-term* food intake suppressive effects of GLP-1R activation. As PI3K/AKT signaling can be modulated by PKA activity and regulate gene transcription, we hypothesize that PI3K/AKT signaling is required to mediate the anorectic effects of hindbrain GLP-1R activation by agonist exendin-4 (Ex-4). Inhibition of hindbrain PI3K or AKT activity by 4th icv administration of the inhibitors LY294002 (3.07 μg) or triciribine (2 and 10 μg), respectively, attenuated the food intake-suppressive effects of 4th icv Ex-4 (0.3 μg) over 24 h. *Ex vivo* tissue analyses revealed that 4th icv Ex-4 increased phosphorylation of AKT in the NTS. Preliminary *in vitro* data show that GLP-1R-mediated AKT signaling responses occur in GLP-1R-expressing neurons (GT1-7). Thus, long-term suppression of food intake by hindbrain GLP-1R activation requires PI3K-dependent activation of AKT signaling. NIH-DK085435 and -DK19525.

Working memory and attention to food

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Attentional biases towards food cues may be linked to the development obesity. The present study investigated the mechanisms underlying attentional biases to food cues by assessing the role of top down influences, such as working memory (WM). We assessed whether attention in normal-weight, sated participants was drawn to food items specifically when that food item was held in WM. Lean and overweight participants took part in a laboratory based study assessing reaction times to food and non-food stimuli. Participants were presented with an initial cue stimulus to either hold in WM or to merely attend to, and then searched for the target (a circle) in a two-item display. On valid trials the target was flanked by a picture matching the cue, on neutral trials the display did not contain a picture matching the cue, and on invalid trials the distractor (a square) was flanked by a picture matching the cue. Cues were food, cars or stationery items. We observed that, relative to the effects with non-food stimuli, food items in WM strongly affected attention when the memorized cue re-appeared in the search display. In particular there was an enhanced response on valid trials, when the re-appearance of the memorized cue coincided with the search target. There were no effects of cue category on attentional guidance when the cues were merely attended but not held in WM. These data point towards food having a strong effect on top–down guidance of search from working memory, and suggest a mechanism whereby individuals who are preoccupied with thoughts of food, for example obese individuals, show facilitated detection of food cues in the environment. Supported by: Marie Curie Intra European Fellowship.

Comparative effects of intraduodenal lipid and protein on gut hormones, glycemic control and energy intake in healthy males

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Intraduodenal (ID) lipid suppresses energy intake, at least in part through its stimulatory effects on gastrointestinal (GI) hormone release, particularly CCK, and pyloric contractions. Protein is regarded as the most satiating macronutrient, and it also stimulates gut hormone as well as insulin release, although, when compared with lipid, its effects on GI function are less well established. We have recently reported (Appetite 2011;57(Suppl 1):S38) that in healthy lean humans 90-min ID infusions of pure lipid (L3) and protein (P3) at a load of 3 kcal/min, but not isocaloric combinations of 2:1 (L2P1) or 1:2 (L1P2) lipid:protein, suppressed energy intake compared with saline control (C) (L3: 973 ± 42 kcal; P3: 1031 ± 34 kcal; L2P1: 1185 ± 34 kcal; L1P2: 1137 ± 35 kcal; C: 1240 ± 33 kcal; n = 20), although L3 stimulated GLP-1 release and isolated pyloric pressure waves much more potently than P3. Here, we report the effects of L3, P3, L2P1, L1P2 and C on plasma CCK, serum insulin and blood glucose. 20 healthy males (age 27 ± 3 yr, BMI 22.4 ± 0.3 kg/m^2) were studied on five separate occasions in double-blind, randomized fashion. Plasma CCK, serum insulin and blood glucose were measured in response to each 90-min infusion at 15-min intervals. Plasma CCK (AUC, pmol/L.min; L3: 501 ± 49; L2P1: 395 ± 42; L1P2: 354 ± 35; P3: 295 ± 33; C: 147 ± 15) was stimulated in a lipid-load dependent manner ($P < 0.001$), while serum insulin (AUC, pmol/L.min; L3: 470 ± 22; L2P1: 946 ± 103; L1P2: 1474 ± 151; P3: 1702 ± 182; C: 231 ± 22) was stimulated in a protein-load dependent manner ($P < 0.001$). Blood glucose concentrations did not differ between infusions. Collectively, our data suggest that in healthy males (1) the effect of ID lipid on energy intake is mediated by GI mechanisms, (2) protein has comparable effects to lipid on the suppression of EI, but this appears to be mediated by mechanisms other than GI function, and (3) protein stimulates insulin, while maintaining normoglycemia.

A cautionary tale. Drinking and feeding behavior as a consequence of altered autonomic function

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Numerous peptides act in CNS to alter drinking and feeding and although both behaviors occur simultaneously under most conditions, the two can be separated experimentally. We have reported the discovery of a novel peptide, neuronostatin (NST), encoded in the somatostatin preprohormone. When administered i.c.v. NST inhibited food intake and water drinking in rats, stimulated pressor hormone release, and activated sympathetic outflow. Since NST is co-produced with somatostatin we could not address the issue of physiologic relevance by gene ablation or RNA compromise. Instead we hypothesized that passive immunoneutralization of endogenous, brain-derived NST would result in exaggerated food and water intakes. On the contrary, it resulted in significant inhibition of both. Thus both the peptide and compromise of its function yielded the same result, an apparent paradox. We then examined the cardiovascular status of the antibody treated rats and discovered severely unstable arterial pressures, orthostasis, and altered baroreflex sensitivity, which may explain the antidipsogenic and anorexigenic effects. Was this specific or a spurious side effect of antiserum administration? We detail here our discovery of the identity of the NST receptor and the in vitro and in vivo evidence of its selectivity and physiological relevance. These studies serve not only to establish the importance of NST in appetite, thirst and cardiovascular function, but also as a cautionary tale of off-target effects altering thirst and appetite that should be considered in all experimental models of feeding and drinking. Supported by: NIH HL066023.

Influence of color and viscosity on milk pleasantness and intensity ratings in disordered eaters

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While previous research on viscosity and taste perception tends to be in agreement, the research on color and taste perception exhibits a tremendous amount of inconsistency. Possible reasons for this inconstancy are the multi-modal nature of gustation and possible confounds, such as level of disordered eating, which are rarely accounted for. The purpose of the present study was to evaluate the effect of both color and viscosity on the pleasantness and intensity ratings of milk, while taking level of disordered eating into account. Thirty-eight participants (22 male, 16 female) completed the study. The stimuli were distinct milk samples which varied in color (red, blue, brown, white) and viscosity (skim, 2%, whole), which led to 12 possible combinations. Food-neophobics (those individuals who are unwilling to try new foods) rated milk as less pleasant and more intense, as well as consumed less of it, when compared to food-neophilics (those individuals who are particularly willing to try new foods). However, the significant pleasantness and intensity ratings were found to disappear when Food Neophobia Score was used as a covariate, suggesting that significance within the data relied upon whether an individual was classified as food-neophilic or food-neophobic. While this does not provide an all-inclusive explanation for the past ambiguity, it does open up a new direction for future research on the multi-modal influences of taste perception where level of disordered eating should remain a covariate.

Diacylglycerol acyltransferase-1 (DGAT-1) inhibition decreases meal size and postprandial respiratory quotient (RQ) in high fat diet (HFD)-fed rats

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DGAT-1 catalyzes the final committed step in triacylglycerol (TAG) synthesis; it is highly expressed in the small intestine. Here we investigated the effects of a small molecule DGAT-1 inhibitor (DGAT-1i) on eating, fat metabolism, and energy expenditure in rats fed a HFD or chow and adapted to an 8 h feeding/16 h deprivation schedule. Intragastric (IG) DGAT-1i (10 mg/kg body weight (BW)) infusions reduced ($P < 0.05$) the size (44%) and duration (33%) of the second meal and increased the inter-meal interval (IMI) and the first meal satiety ratio compared to vehicle. First meal size and duration was unaffected. In HFD-fed, but not in chow-fed rats, IG DGAT-1i (9 mg/kg BW) infusions prior to a 3 g HFD or isocaloric chow meal offered at the onset of the 8 h feeding period blunted the increases in circulating TAG and increased β-hydroxybutyrate levels compared to vehicle, suggesting a metabolic shift from TAG synthesis to fatty acid oxidation (FAO). Likewise, the DGAT-1i (10 mg/kg BW) lowered RQ, indicating increased FAO, but did not affect whole-body energy expenditure. Furthermore, DGAT-1i enhanced FAO in CaCo2 ($\log EC_{50} = -0.4566$ ($EC_{50} = 0.3494$)) and HuTu80 ($\log EC_{50} = -2.1223$ ($EC_{50} = 0.00762$)) cells, as assessed by the uptake of ^{14}C-oleate or ^{14}C-palmitate and release of $^{14}C–CO_2$ and ^{14}C-acid soluble products. These data support the hypothesis that DGAT-1 inhibition reduces food intake by stimulating enterocyte FAO. Supported by: AstraZeneca Pharmaceuticals, UK.

Roux-en Y gastric bypass surgery decreases bitter and umami taste perception thresholds in severely obese subjects

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Roux-en Y gastric bypass (RYGB) surgery has been shown to affect eating behaviour and dietary habits in severely obese subjects but underlying mechanisms are incompletely understood. Here we tested the hypothesis that RYGB surgery affects taste perception thresholds. 32 severely obese subjects (9 men, BMI 43.6 ± 0.9 kg/m^2) were tested before and 3 months after RYGB surgery. Perception thresholds for sweet, salty, sour, bitter, and umami taste were assessed by a standard taste detection task. Further, subjects rated on a series of high caloric sweet (hcs) and non-sweet (hcns) as well as low caloric (lc) food pictures on a 'liking' and 'wanting' scale. Trait measures of eating behaviour were assessed by the Three Factor Eating Questionnaire (TFEQ) and by the Power of Food Scale (PFS), which captures the hedonic drive to consume palatable foods. After surgery BMI was reduced to 35.5 ± 0.7kg/m^2. Subjects showed higher scores on the cognitive restrained but lower scores on the disinhibition and hunger scale (TFEQ; all $P < 0.001$) and also reduced hedonic hunger (PFS; $P < 0.001$). 'Liking' as well as 'wanting' of hcs and hcns (both $P < 0.05$) but not of lc foods were reduced. Perception thresholds for bitter and umami taste were decreased (both $P < 0.05$), while those for sweet, salty, and sour were unaltered. Data clearly show profound changes in eating behaviour and food preferences in severely obese subjects after RYGB. Further, they provide some evidence that alterations in taste perception could contribute to postoperative changes in eating behaviour.

Estradiol stimulates apolipoprotein A-IV gene expression in the nucleus of the solitary tract via estrogen receptor-α

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Compelling evidence suggests that estrogen reduces food intake indirectly by enhancing the potency of other satiation factors. Recently, we demonstrated that 17b-estradiol (E2) stimulates *apo A-IV* gene expression in the nucleus of the solitary tract (NTS), and the increased local apo A-IV levels might partially mediate E2-induced reduction of food intake. To determine which estrogen receptor (ERa or ERb) mediates E2's action on apo A-IV, using immunohistochemistry, we found that ERa was co-localized with apo A-IV in the NTS of female rats. Treatment with 10 nM E2 or PPT (an ERa agonist), but not DPN (an ERb agonist), significantly increased *apoA-IV* gene expression in cultured primary neuronal cells of rat embryonic brainstems, compared with those in vehicle-treated cells. Interestingly, this effect of E2 was abolished when the cells were incubated with E2-BSA (E2 linked to bovine serum albumin to prevent E2 from entering cells), implying that a nongenomic mechanism is not involved in E2's effect on *apoA-IV* gene expression. Cyclic injection of E2 (2 υ;g, s.c.) or PPT (150 υ;g, an effective dose at reducing food intake) for 8 cycles (4 days/cycle) in adult OVX female rats significantly reduced food intake and body weight, and significantly increased *apo A-IV* gene expression in the NTS, compared with vehicle-controls. These data collectively suggest that the ERa is the primary mediator of E2's action on apo A-IV in the NTS. Supported by: NIDDK 63907 and DK 70992.

Peripheral modulation of taste responses by angiotensin II

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Modulation of gustatory function critically influences food preference, food intake and metabolic homeostasis. Even so, the mechanisms for modulating taste sensitivity are poorly understood. Here, we report that angiotensin II (AngII), which plays important roles in the maintenance of sodium and water homeostasis, modulates salt and sweet taste sensitivities in mice. The chorda tympani nerve recording demonstrated that AngII suppresses amiloride – sensitive taste responses to NaCl. Surprisingly, AngII enhances sweet taste responses, without affecting responses to KCl, sour, bitter and umami tastants. These effects of AngII on gustatory nerve responses are blocked by an AngII type1 receptor (AT1) antagonist. Behavioral tests showed that lick rates (per 10 s) for NaCl and sweeteners are significantly reduced by the AT1 antagonist in water deprived mice. Expression analyses revealed that AT1 are co-expressed with αENaC (amiloride – sensitive epithelial sodium channel α-subunit. A salt taste receptor) or T1r3 (a sweet taste receptor component) in a subset of taste cells. These results suggest that taste organ is a new peripheral target of AngII, and the specific modulation of amiloride – sensitive salt and sweet taste sensitivities may play important roles in regulating sodium and glucose homeostasis. Supported by: Grant-in-Aids 18077004 (Y.N.), 18109013 (Y.N.) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Human dorsal striatal response to milkshake is associated with body weight, dopamine signaling, and impulsivity

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Prior fMRI work has identified a negative relation between BMI and the blood oxygen level dependent (BOLD) response to milkshake in the dorsal striatum (Stice et al., 2008). This effect appears to be a consequence, rather than a cause of obesity, since weight gain, but not risk for obesity (by virtue of parental obesity), is associated with reduced DS response to palatable food (Stice et al., 2010, 2011). Here we sought to (1) determine the cognitive and perceptual correlates of this effect by assessing BOLD and perceptual responses to milkshake, self-report measures of eating style, impulsivity, sensitivity to reward and willingness to work for food and (2) test whether the effect is associated with dopamine (DA) D2/3 receptor binding potential using [^{11}C]PHNO positron emission tomography. Replicating prior work BMI was negatively related to BOLD dorsal striatum response to milkshake (n = 40). Extending prior knowledge we show a strong negative association between impulsivity and BOLD dorsal striatum response in overweight and obese but not lean individuals. No associations were observed with milkshake perception, willingness to work, eating style or sensitivity to reward. In contrast to BOLD, DA D2/3 binding potential was positively related to BMI the dorsal striatum (n = 7). Taken together, these findings indicate that the adaptation in the dorsal striatum previously identified with fMRI is associated with impulsivity, which is characteristic of habitual rather than goal directed behavior, and changes in DA signaling. Supported by: NIH.

The Sensory circumventricular organs. Alternative targets for circulating leptin

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Adipose tissue secretes leptin which acts centrally to suppress food intake, effects which have been attributed to direct actions in the arcuate nucleus. The subfornical organ (SFO) and area postrema (AP) are sensory circumventricular organs (CVOs) which lack the normal blood brain barrier (BBB) and contain the leptin receptor. These characteristics confer to these regions the unique ability to detect circulating leptin that would otherwise not have access to CNS tissue behind the BBB, and convey this information to autonomic centers of the brain to control feeding. The present study was undertaken to examine the effects of leptin receptor activation on AP and SFO neurons using whole cell current clamp techniques in dissociated rat AP and SFO neurons. Leptin (10^{-8} M) influenced 64% (46/72) of SFO neurons tested and was found to depolarize the majority of responsive neurons with a mean change in membrane potential of 7.3 ± 0.6 mV (n = 28). The remaining cells that responded to leptin hyperpolarized (–6.9 ± 0.7 mV, n = 18). Leptin also influenced 48% (27/55) of AP neurons tested and was found to depolarize the majority of responsive neurons (5.7 ± 1.0 mV, n = 15) while the remaining responsive cells hyperpolarized (–6.4 ± 1.1 mV, n = 12). Leptin was also found to influence the same population of SFO and AP neurons influenced by amylin as 3 of 4 cells tested for the effects of both amylin and leptin depolarized to both peptides. These observations identify the SFO and AP as CNS locations at which leptin may act to influence central control of energy balance. Supported by: Canadian Institutes of Health Research.

Regional taste loss disinhibits intact oral sensations and may induce weight gain

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Clinical and laboratory data identify health conditions predicting regional oral sensory loss: severe childhood ear infections (otitis media, OM) damage the chorda tympani (CT) and block anterior taste cues, while tonsillectomy damages the glossopharyngeal nerve (IX) and blocks posterior taste/tactile cues. Our data link these conditions to long-term obesity risk (e.g., body mass, high-fat food avidity) induced by shifting interactions among flavor elements. Regional taste loss disinhibits intact oral loci; for example, CT block elevates glossopharyngeal, trigeminal, and whole-mouth sensation, particularly in supertasters of 6-*n*-propylthiouracil. Recent findings extend this model to oral sensory insults carrying obesity risk: subjects with medical histories and local hypogeusia indicating either CT damage (i.e., OM) or IX damage (i.e., tonsillectomy, head/neck radiation) show elevated whole-mouth taste, oral burn and viscosity, and retronasal olfaction (RO). However, those with damage to both nerves show reduced whole-mouth taste and RO, failing to counteract extensive loss. These data affirm reports linking RO to oral sensation, underscoring the importance of whole-mouth input in flavor integration. Differential obesity risk with whole-mouth gain vs. loss remains to be seen, but relative intensity changes among flavor components appear sufficiently robust to influence high-fat intake. Overall, oral disinhibition sustains whole-mouth taste and flavor by moderating the impact of limited spatial loss, but the strength of this effect may shape long-term food choice and dietary health. Supported by: NIH (DC 00283).

Effects of sleep restriction on body weight and food intake in healthy adults

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Short sleep duration disrupts the release of hormones that play a key role in appetite control and thus may be a novel risk factor for obesity and type 2 diabetes. This study examined the effect of chronic sleep restriction (SR) on weight gain and food intake under ad libitum feeding conditions (3 meals/day, access to snacks and an optional late night meal during SR). Healthy subjects (31.42 ± 7.59 y; 103 males) participated in a laboratory-controlled protocol. N = 166 subjects underwent 2 baseline nights (BL;10 h time in bed [TIB]/night) followed by 5 SR nights (4 h TIB/night) and 1 recovery night (REC;12 h TIB/night). N = 22 served as a 10 h TIB/night control group. Height and weight were measured at study entry and end. Though control and SR groups did not differ in weight at entry, SR subjects gained more weight than controls (–0.05 vs. 1.14 kg; p = 0.001). Among SR subjects, males gained more weight than females (p = 0.003) and African Americans gained more than Caucasians (p = 0.001). In a subset of SR subjects (n = 27), food intake was recorded daily and analyzed using The Food Processor SQL program. Compared with BL, more food was consumed during SR1 (p < 0.001) and less food was consumed during REC (p = 0.046). Though total food intake did not differ between BL and SR2-5, a greater percentage of total daily calories was consumed after 1600 h during SR2-5 (51.4%) compared with BL (36.4%; p < 0.001). These results suggest that sleep restriction disrupts energy balance: it increases food intake acutely and then delays the majority of caloric intake to evening and nighttime hours. NIH NR004281; CTRC UL1RR024134; NASA NCC 9-58; ONR N00014-11-1-0361.

Psychophysical analysis of the contribution of the T1R family of receptors in saccharide taste

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In taste receptor cells, the T1R2 and T1R3 proteins form a heterodimer that binds with sweeteners. Since their seminal discovery, questions have been raised as to whether these proteins are necessary and sufficient for normal taste perception of sweeteners. We have been taking a behavioral approach to this problem using knock-out (KO) mice that are lacking T1R2 or T1R3 or both subunits (T1R2+3). We have found that in brief access taste tests, T1R2 KO and T1R3 KO mice are initially unresponsive to saccharin, sucrose, glucose, maltose, and maltotriose relative to wild-type (WT) mice, but with repeated testing with carbohydrate stimuli, can express concentration-dependent licking responses to the sugar stimuli, but not saccharin. This is thought to be due to some form of post-oral conditioning. T1R2+3 KO mice do not display such change in responsiveness. Interestingly, Polycose responsiveness is relatively normal in all KO mice upon initial testing, but with some slight decrease in lick rate in the T1R2+3 KOs. We recently used an operant two-response taste signal detection procedure and found that T1R2 KO and T1R3 KO mice had severely impaired or absent ability to detect sucrose, glucose, and maltose, but had relatively normal psychometric functions for Polycose, albeit with minor impairment in performance at higher concentrations. These findings functionally confirm the primacy of the T1R2+3 heterodimer in sweet taste perception, and are also consistent with the postulated existence of a T1R2+3-independent mechanism that contributes to the oral sensing of polysaccharides. Supported by: NIH-R01-DC004574.

Cholecystokinin mediates hypoxia. Induced inhibition of gastric emptying

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Hypoxia-induced anorexia is one of several symptoms of acute mountain sickness (AMS). We hypothesized that hypoxia also affects gastric emptying by modifying gastrointestinal hormones. In a field study, gastric emptying and ad libitum eating were analyzed in volunteers at baseline in Zurich (490 m above sea level) and on day 2 and 4 at high altitude (4560 m; approx. 12% O_2). Gastric emptying was measured by breath test after a 400 kcal muffin test meal, labeled with 13C octanoic acid. Ad libitum eating was recorded after a 400 kcal preload. In parallel experiments gastric emptying after a 3 g meal and the effect of the CCK receptor antagonist devazepide (1 mg/kg) was studied in rats kept at hypoxia (10% O_2). Volunteers ate less on day2 at high altitude but the 13C exhalation test suggested accelerated gastric emptying; however, metabolic parameters indicated that the effect was probably based on an artifact due to accelerated octanoic acid metabolism under hypoxia. In fact, hypoxia significantly slowed the rate of gastric emptying (20 min) in rats by 23% ($p < 0.01$). This effect was partly attenuated by pretreatment with devazepide ($p = 0.016$). The data suggest that the 13C exhalation method that is routinely used to study gastric emptying in humans may yield erroneous results if metabolic test conditions (e.g. citric acid cycle activity) differ between test conditions. Further, reduced eating at high altitude may be partly associated with decreased gastric emptying. The latter effect may be CCK mediated. Supported by: ZIHP, Zurich, Switzerland.

Hepatic portal vein (HPV) peptide tyrosine–tyrosine (PYY) and eating in rats

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PYY inhibits eating, but the site(s) and mechanism(s) of its action are still unclear. We previously showed that HPV PYY concentration increases after a normal meal in rats and that experimentally induced increases in HPV PYY acutely inhibit eating. Whether this effect is physiologically relevant remained uncertain. Here we implanted HPV and jugular vein (JV) catheters in adult male rats to measure (a) the increase in JV plasma PYY in response to a dose of PYY that inhibited eating when infused into the HPV and (b) simultaneous prandial changes in HPV and JV PYY levels, and to test (c) whether doses of PYY that inhibited eating after HPV infusion caused a flavor aversion. We found that a chow meal (12.5 kcal) given at dark onset after 3 h of food deprivation increased ($p < 0.05$) plasma PYY concentrations within 15 min after meal onset in HPV and JV similarly. HPV PYY infusion increased JV PYY levels ($p < 0.05$) substantially more than a meal. Both PYY doses that inhibited eating did not cause a flavor aversion ($p > 0.05$). These data show that HPV infusions of PYY (1 and 3 nmol/kg) inhibit eating in rats without causing aversion, but as plasma PYY levels after infusion were much higher than postprandial levels, the physiological relevance of the observed inhibition of eating remains questionable. The parallel meal-induced increase of PYY in the HPV and JV suggests a systemic, endocrine action for endogenous PYY. Further studies should examine whether PYY administrations that mimic the meal-induced increase are sufficient to inhibit eating.

Chronic postnatal hyperghrelinemia disrupts hypothalamic development and induces long term metabolic dysfunctions

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Ghrelin is an important hormonal signal that can act directly within the arcuate nucleus of the hypothalamus (ARH) to block axon growth. During neonatal life, ghrelin levels increase gradually starting at postnatal day (P) 10 to reach a plateau by P14. Although levels of ghrelin are low during the first two weeks of life, ARH neurons appear sensitive to ghrelin as early as birth. Here we investigated whether premature increases in ghrelin levels, induced by daily injections of ghrelin from P4 to P12, have an impact on metabolic regulation and hypothalamic development. Chronic administration of ghrelin during postnatal life causes higher body weight, hyperleptinemia, hyperglycemia and hyperinsulinemia as compared to control animals. These metabolic impairments are associated with a disruption in the maturation of ARH axonal projections. Neonates injected with ghrelin display a reduction in the density of ARH projections to the paraventricular nucleus that persisted throughout life. In vitro experiments using ARH explants further show that ghrelin is capable of blunting the documented neurotrophic effect of leptin on ARH neurons. Consistent with these observations, chronic administration of ghrelin attenuates leptin-induced pSTAT3 in ARH neurons. Collectively, these data suggest that a correct timing of ghrelin action during neonatal life is required for normal hypothalamic development and optimal metabolic regulation throughout life. Supported by: National Institute of Health (Grant DK84142, to SGB), the "Fondation pour la Recherche Médicale" (to SGB), the EU FP7 integrated project (grant agreement n° 266408, "Full4Health", to SGB), and the "Ag.

Optogenetic & pharmacogenetic deconstruction of hypothalamic feeding circuits

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Hunger is a complex behavioral state that elicits intense food seeking and consumption. These behaviors are recapitulated in well-fed mice by activation of starvation-sensitive AGRP neurons, but the relative contribution of different AGRP neuron projection targets to acute feeding behavior are poorly understood. To investigate the neural circuits underlying hunger, we mapped synaptic interactions of AGRP neurons with multiple cell types and probed the contribution of these distinct circuits to feeding behavior using optogenetic and pharmacogenetic techniques. These experiments establish functional contributions of specific neural circuits that regulate hunger and pathways associated with overeating disorders. Supported by: Howard Hughes Medical Institute.

Blockade of melanocortin 3/4 receptors in Fisher 344 x Brown Norway rats does not prevent the reduction of high-fat diet intake during voluntary wheel running

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The act of voluntary wheel running (WR) in rats results in a decrease in *ad libitum* high-fat diet (HF) intake. Alternatively, food deprivation can increase WR. Brain melanocortin pathways are implicated in both food intake and activity behaviors. We hypothesized that blockade of MC3/4R with the antagonist SHU9119 (SHU) would prevent WR from attenuating HF intake. To address the role of SHU on WR dependent HF intake, we used 6 mo. old male Fisher 344 x Brown Norway rats. Treatments were delivered with 28 day osmotic-pumps linked by catheter to a cannula targeted to the left lateral ventricle; control rats were infused with artificial cerebrospinal fluid (aCSF) while treated rats received 0.2 nmol/rat/day SHU. Rats were further split into sedentary (Sed) or WR groups (n = 6–8). Since SHU is known to increase HF intake, we included SHU rats pair-fed (PF) to aCSF controls. SHU increased daily HF caloric consumption irrespective of WR (Two-Way ANOVA, $p < 0.0001$). Fifteen days of WR reduced average daily HF consumption from 78.6 ± 2.9 to 61.3 ± 5.4 (aCSF-Sed v aCSF-WR), and with SHU from 140.0 ± 5.7 to 123.1 ± 5.6 kcal ± SEM (SHU-Sed v SHU-WR). Changes to body weight were significant with both SHU ($p < 0.0001$) and WR ($p = 0.0116$). SHU significantly reduced WR, and SHU/PF rats showed a trend toward increased WR ($p = 0.07$) compared to SHU. Our results indicate that attenuation of HF diet intake following voluntary wheel running is not dependent on the action of the MC3/4 receptor. Supported by: National Institute on Aging Grant T32 AG000196.

Choice influences the effect of nutrient intake on brain response

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Weight-loss programs often promote the use of fixed-portion meals to limit energy intake. In this case meal termination is dictated by portion size rather than a conscious choice to stop eating. This is an important distinction because reward circuits implicated in feeding are influenced by top-down cognitive control and thus may be sensitive to whether meal termination is voluntary vs. externally imposed. We used fMRI to investigate the influence of voluntary meal termination on brain response to a dessert (milkshake) following a fixed-portion or *ad libitum* meal in 13 healthy non-dieting subjects. We predicted greater responses in the amygdala, a region strongly implicated in non-homeostatic feeding via its hypothalamic projections, following the fixed vs. *ad libitum* meal. Meals consisted of sandwiches and apples, and a counter-balanced repeated measures design was employed. The fixed meal size was selected to be satiating and was adjusted for gender. Critically, macronutrient intake did not differ between the fixed and *ad libitum* meals, with subjects ingesting similar amounts of calories in both conditions. As predicted, greater amygdala response to milkshake minus a control solution was observed following the fixed compared to the *ad libitum* meal. In contrast, lower responses were observed in hypothalamus. These differential brain responses were not correlated with meal intake or subjective ratings of hunger or fullness. These results demonstrate that voluntary meal termination influences the effect of nutrient intake on brain responses to food. Supported by: R01 DK085579.

Circadian rhythm powerfully regulates expression of intestinal sweet taste receptors in mice

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Circadian rhythms strongly influence digestive processes, with disruption (e.g. shift work) linked to increased risk of obesity and diabetes in humans[1]. It is unknown how circadian rhythms influence nutrient uptake and satiation signalling via effects on nutrient sensing G-protein coupled receptors (GPCR) within the proximal intestine[2]. Accordingly, we assessed the expression of intestinal nutrient sensing GPCR at different stages of the light/dark cycle in mice. Proximal intestine mucosa was collected from six C57BL mice at 3 h intervals over a 12 h light, 12 h dark cycle (dark phase onset at 6 pm). Relative expression of GPCR for sweet taste (T1R2), protein hydrolysate (GPR93), amino acid (calcium sensing receptor, CaSR) and long chain fatty acid (GPR40) sensing were assessed by quantitative RT-PCR; gastric content was also assessed. GPR93, CaSR and GPR40 expression was unaltered over 24 h. In contrast, intestinal T1R2 expression displayed marked circadian variation, with peak levels evident at 6 pm, increased 23-fold over nadir (12 am, p 1Nutr Res Rev 2010;23:155 [2]J Clin Invest 2007;117:13.

Genome-wide assessment of differential DNA methylation in offspring of rat dams fed high-fat diet

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Maternal high fat diet (HF) during gestation and lactation results in rat offspring that are obese, and have impaired glucose tolerance, and attenuated pSTAT3 in response to leptin by postnatal day 21 (PND21). In adulthood, both male and female pups from HF dams are more susceptible to diet-induced obesity. Epigenetic processes have been suggested to contribute to the long-term consequences of early nutrition and may contribute to increased metabolic vulnerability in offspring of HF-fed dams. We used a genome-wide, array-based method (rCHARM, NimbleGen) to assess one such epigenetic modification, DNA methylation (DNAm) in the rat. Genomic DNA from micropunches of the arcuate (ARC) were analyzed by rCHARM to identify differential DNA methylation (DNAm) between CHOW and HF male pups on PND21. Among the top FDR significant hits ($P < 0.05$) in the ARC were centrosomal protein 290 (*CEP290* or *BBS14*) and intraflagellar transport protein *IFT52*, both of which were hypermethylated. BBS and IFT proteins have been attributed to the obese phenotype in patients with Bardet-Biedl Syndrome and are implicated in leptin receptor (LepRb) trafficking in the hypothalamus. Leptin receptor-overlapping transcript (*LEPROT* or *OB-RGRP*) was also hypermethylated and is a negative regulator of LepRb. Thus, differential DNAm of genes that encode proteins involved in LepRb trafficking and function in the ARC could be responsible for leptin resistance observed in maternal HF diet offspring. Support: HD055030.

Dissociating intra-gastric vs. oral routes of feeding in high-fat induced obesity

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Most often, the hedonic orosensory properties of fats are considered to be the main drivers of excessive caloric intake. However, digestive functions adapt to the chronic consumption of dietary fats raising the possibility that post-oral signaling is impacted by chronic high-fat exposure. We tested the hypothesis that prolonged exposure to high-fat diets impairs post-oral reinforcement, i.e. attenuates the motivation to eat in the absence of orosensory stimulation. We show that (1) during I.G. feeding, when no oral detection of lipids is involved, high- fat (HF)-fed mice consumed markedly lower levels of fat compared to low-fat (LF)-fed mice; (2) Caloric intake differed between oral and I.G. routes in HF-fed, but not in LF-fed, mice; (3) HF-fed mice display stronger attraction towards high-calorie emulsions than LF-fed mice during both short- and long-term oral tests; (4) HF-fed mice lack calorie-dependent dopamine release in striatum following gut Infusions of fat emulsions, showing that HF-induced dopamine deficiency is recapitulated by gastrointestinal stimulation; (5) The behavioral abnormalities associated with HF-feeding were not recapitulated in morbidly obese ob/ob mice. In summary, our data provide support for our contentions of post-oral impairment (underfeeding and homeostatic dysregulation) in HF-fed mice; for dysregulated dopamine transmission associated with intra-gastric feeding; and for a gastrointestinal rather than general metabolic root for the impairment in post-oral reinforcement. Supported by: R01 DC009997 (NIDCD).

Effects of *E. coli* on α-MSH-reactive immunoglobulins, food intake and anxiety

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Anorexigenic effect of α-melanocyte-stimulating hormone (α-MSH) can be enhanced by low affinity α-MSH-reactive immunoglobulins via peptide transport. Subjects with anorexia nervosa have elevated plasma α-MSH IgM. Here we studied if commensal *E. coli K12* may stimulate α-MSH IgM relevant to food intake and anxiety. Wistar rats received by gavage 4 ml of culture medium with 10^8 *E.coli* for 21 days; controls received the medium. Then, half of rats from both groups had five days of food restriction (1 h/day) and 15 min before return to *ad libitum* food all rats received α-MSH (100 mkg/kg, I.P.). Five days later, rats were tested in O-maze. Blood samples for ELISA assay of α-MSH IgM were taken the day before the gavage and after the O-maze. We found that *E.coli* rats had transitory (2 days) decrease in food and water intake which normalized thereafter and did not significantly differ from controls during either *ad libitum* or restricted schedules. However, after α-MSH injection, the following 24 h food intake was lower in *E.coli* rats which were food restricted. *E.coli* treated rats also showed a tendency of increased anxiety in the O-maze test. Plasma levels of α-MSH IgM autoAbs were higher in *E.coli* than in controls rats. These data show that commensal *E.coli* stimulate α-MSH-reactive IgM which may enhance α-MSH anorexigenic and anxiogenic effects and thus suggest a microbial risk factor for anorexia nervosa. Supported by: EU INTERREG IVA 2 Seas Program (7-003-FR_TC2N).

GABAa receptors play a role in the long term increases in food hoarding in Siberian hamsters (*Phodopus sungorus*)

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The number of overweight/obese individuals is increasing, a situation that has multiple dire secondary health consequences. The physical and financial gravity of obesity has led to a substantial amount of research on reducing food intake (FI) to modest effect. Two often overlooked facets of ingestive behavior are food foraging and food hoarding (FH), behaviors exhibited by humans that may play a role in the promotion/maintenance of obesity. After food deprivation (FD) Siberian hamsters, like humans, increase FH, but not FI. FD triggers release of a stomach-derived hormone, ghrelin, in humans and Siberian hamsters. FD and exogenous ghrelin increase the expression of hypothalamic orexigenic peptides [neuropeptide Y (NPY) and agouti-related peptide (AgRP)] subsequently increasing foraging, FH (lasting ∼7 d), and FI (lasting ∼1 d) by Siberian hamsters. We have found that NPY is involved in the 0–2 d increases in FI and FH, but the mechanism of the 3–7 d FH increases remains elusive. Some AgRP neurons contain GABA and project to the lateral parabrachial nucleus (LPBN), which has GABAa receptors. GABAa-R agonism prevents AgRP neuron ablation-induced anorexia and increases FI in mice. Using a semi-natural foraging/hoarding housing apparatus we asked: does GABAa-R signaling in the LPBN lead to FD/exogenous ghrelin-like increases in FH? Nanoinjections of muscimol (0.44, 0.88, or 1.76 nmol) caused increased FH at 4–7 d post-injection. These results suggest that LPBN GABAa signaling is involved in the extended FH increases in Siberian hamsters.

The effect of growth hormone supplementation on feed intake and plasma leptin in crossbred dairy goat during early lactating period

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Long-term Somatotropin supplementation in dairy cattle fed in the tropics increased milk yield together with feed intake. In addition, the increased feed take was coincided with the lower plasma leptin. We hypothesized further that the decreased plasma leptin may precede the increased feed intake effect of somatotropin. The experiment used dairy goat during early lactation supplemented with either recombinant somatotropin (rbST, 250 mg/doe) or sesame oil (once at day 7th post-parturition). Feed intake from the individual animal was measured throughout the experiment. Blood was collected once per day started from day 6th to 21st post-parturition. Milk yield from rbST treatment group was not significantly different from control group. Unexpectedly, rbST apparently decreased feed intake. Recombinant bST increased plasma IGF-1, insulin and glucose within 24 h. In addition, plasma leptin was increased prior the feed intake effect. In conclusion, short term rbST supplementation during early lactation decreased rather than increased feed intake in dairy goat. The coupling of plasma leptin and feed intake effect suggested that rbST may activate leptin secretion and subsequently decrease feed intake. Supported by: Faculty of Veterinary Science, Chulalongkorn University.

The effect of meta-chlorophenylpiperazine (mCPP) on appetite ratings and food intake in healthy volunteers.

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The 5-HT_{2C} receptor agonist meta-chlorophenylpiperazine (mCPP) significantly reduces food intake in healthy volunteers but the underlying mechanism is unclear. In the present study, we used the Sussex Ingestion Pattern Monitor (SIPM) to investigate the effects of mCPP on eating microstructure and changes in appetite and mood ratings in a between-subjects double blind placebo controlled design. Twenty-four male and 24 female participants were randomly assigned to either placebo, 15 mg or 30 mg mCPP treatment groups (8 males and 8 females per group). Participants completed appetite and mood ratings, and ate an ad libitum lunch of pasta. There was no significant effect of drug on amount eaten, number of forkfuls consumed, or eating ratings. However, 30 mg mCPP significantly reduced appetite ratings and increased physical symptoms, including nausea, in both males and females. Calculation of meal satiety quotients (SQs: change in hunger ratings, divided by caloric intake) showed that, compared to placebo, females exhibited significantly higher SQs during the first two quartiles of the meal after 30 mg mCPP, and a higher SQ during the second quartile after 15 mg mCPP. These data suggest that mCPP reduces appetite, and in females, enhances satiation with an earlier onset at the higher drug dose. Supported by: P1vital.

Renal basis for glucocorticoid potentiation of salt appetite in rats.

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Experiments tested if renal responses to glucocorticoids, such as dexamethasome (DEX), may explain exaggerated sodium ingestion by rats treated with glucocorticoids, and if these renal responses change with age. Male rats aged 4, 12 and 30 mo received DEX (0.3 mg/kg bw, sc) or sham injections under basal conditions (no load) or followed by loads of water or isotonic saline (3% of bw, ip). Urine was collected at 2, 4 and 6 h. DEX more than doubled urine volumes across all load conditions and ages compared to results after sham injections. DEX had little effect on urinary sodium concentration, yet nearly tripled cumulative sodium excretion across all load conditions and ages due to increased urine volumes. DEX generally reduced urinary potassium concentration while more than doubling cumulative potassium excretion across all loads via increased urine volume. However, DEX caused smaller increases in potassium excretion with age. Urinary sodium and potassium concentrations declined dramatically with age, especially under basal conditions and saline loading. In conclusion, increased turnover of water and electrolytes due to glucocorticoid-enhanced renal excretion of these elements likely explains exaggerated ingestion of sodium and water by rats treated with glucocorticoids in models of salt appetite. Age-related reductions in renal sodium and potassium concentrating ability may help explain reduced sodium ingestion by old animals in models of salt appetite. This work was supported by AG-025465 to RLT and HL-14388 to AKJ.

Macronutrient selection of mice is influenced by *Itpr3*, the inositol 1,4,5-triphosphate receptor type 3 gene, or a nearby gene

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There has been scant work to investigate the mechanisms underlying macronutrient choice by mice. We used a two-cup choice method developed by Smith Richards and colleagues to measure the consumption of carbohydrate- and fat-containing diets by several mouse strains. We found that mice of the NZW/LacJ (NZW) strain voluntarily eat more carbohydrate and less fat than do mice of the BTBR T^+ tf/J (BTBR) strain. Mice with a BTBR background and BTBR/NZW congenic region on chromosome 17 between 25.7 and 27.5 Mb (N_{10} generation) or 26.7 and 27.5 Mb (N_{12} generation) also eat more carbohydrate and less fat than do BTBR/BTBR littermate controls. The congenic interval appears to capture the previously described quantitative trait locus (QTL) Mnic1 [macronutrient intake (carbohydrate) 1]. Of the 21 known and predicted genes between 26.7 and 27.5 Mb, we raise as a likely responsible candidate Itpr3, the inositol triphosphate receptor type 3 gene. The protein product of this gene is a component of the GPCR-mediated taste transduction cascade. Consistent with this, the congenic mice differ from littermate controls in preferences for several representative sweet, umami, bitter and calcium taste compounds. We have recently discovered a small deletion in exon 23 of the BTBR form of Itpr3. Perhaps this mutation renders the BTBR strain unable to detect some taste components of the macronutrient-containing diets, which consequently influences its diet selection. [Supported by NIH DK-46791 and DC-10393].

Intrasolitary pathways. Connecting the rostral and caudal NST

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The capacity of decerebrate rats to adjust intake of sapid substances based on visceral signals implies brainstem integration. Previous studies suggest that the 2nd order taste/visceral relay, the parabrachial nucleus, is a major site for this process. Evidence that orosensory and vagal signals interact in the nucleus of the solitary tract (NST) has been less compelling. Recent findings, however, suggest that cross-talk between the rostral, gustatory and the caudal, visceral NST (rNST, cNST) has been underestimated. Injections of anterograde tracers in rNST at orosensory-responsive sites produces terminal labeling extending past obex. Similarly, cNST injections result in terminal labeling reaching the rostral pole. Interestingly, the caudal to rostral projection is strongest at the medial tip of rNST, which contains preganglionic parasympathetic neurons. Nevertheless, cNST projections also extend more laterally, to the dorsal zone, which receives primary afferent taste input and ventrally, to a region involved in oromotor reflexes. Preliminary studies combining retrograde tracer injections in rNST with cholesytokinin-induced Fos and immunostaining for DBH reveal retrogradely labeled cNST neurons dually stained for DBH and/or Fos. In vitro neurophysiological experiments in a horizontal slice preparation further demonstrate that stimulation of vagal afferents elicits polysynaptic responses in rNST. Collectively, these results suggest a bi-directional functional pathway between cNST and rNST and further imply that a component of the caudal to rostral pathway is catecholaminergic and involved in signaling satiety or visceral malaise. Supported by: DC00416&DE14320.

High fat maternal diet significantly reduces the appetitive component of behavior but not concentration-dependent licking responses to sucrose in a brief-access taste test

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Maternal high-fat diet appears to disrupt several energy balance mechanisms. Offspring from dams fed high fat (HF) did not significantly differ in body weight from offspring of chow-fed (CHOW) dams when weaned onto chow. Yet when these animals were presented both chow and high fat, high fat intake was significantly higher in HF compared to CHOW rats. To assess taste-based hedonic responsiveness, offspring (12 weeks old) were tested in 30-min sessions (10-s trials) to a sucrose concentration series. This brief-access taste test allows for some segregation of the appetitive and consummatory components of ingestive behavior. The HF rats initiated significantly fewer trials than the CHOW group, but did not significantly differ in concentration-dependent licking. Thus, rather than lick response (consummatory), the effect of maternal diet appears to primarily be on spout approach (appetitive) which may be attributed to motivation-related mechanisms. Consistent with this possibility, naltrexone, an opioid receptor antagonist, further reduced trial initiation, but not licking in both groups. The group difference was not apparent with naltrexone administration suggesting differential endogenous opioid activity. Furthermore, when trial initiation was not required in one-bottle intake tests, two-way ANOVAs revealed no main effect of maternal diet when intake of a range of sucrose and corn oil concentrations were compared across the two groups. Support: NIH DK019302, HD055030.

Hypothalamic glycogen-synthase-kinase 3β has a central role in energy- and glucose metabolism

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Glycogen-synthase-kinase3b (GSK3b) is a ubiquitous kinase that plays a key role in multiple intracellular signaling pathways, and increased GSK3b activity is implicated in disorders ranging from cancer to Alzheimer's disease. Here, we provide the first evidence of increased hypothalamic signaling via GSK3b in leptin-deficient $Lep^{ob/ob}$ mice and that intracerebroventricular injection of a GSK3b inhibitor acutely improves glucose tolerance in these mice. The beneficial effect of the GSK3b inhibitor is dependent on hypothalamic signaling via phosphatidylinositol 3-OH kinase (PI3K), a key intracellular mediator of both leptin and insulin action. Conversely, neuron-specific over-expression of GSK3b in the mediobasal hypothalamus increased food intake and body weight, impaired glucose tolerance, without reducing energy expenditure in mice fed a high-fat diet, while having little effect in controls fed standard chow. These results demonstrate that increased hypothalamic GSK3b signaling contributes to deleterious effects of leptin deficiency and exacerbates high-fat diet-induced weight gain and glucose intolerance. Supported by: Ministry of Research and Education (BMBF).

Ipsilateral lateral hypothalamic NMDA receptor antagonism suppresses accumbens shell-mediated eating in a behaviorally specific manner

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The lateral hypothalamus (LH) and the nucleus accumbens shell (AcbSh) interact to regulate food intake. Feeding induced by AcbSh AMPA receptor blockade, using DNQX, is suppressed by bilateral LH NMDA receptor blockade using D-AP5. However, this eating suppression might be an artifact of sedation or hypolocomotion. To investigate the behavioral specificity of the intake suppression, we implanted adult male Sprague-Dawley rats with guide cannulas terminating unilaterally in the AcbSh and bilaterally in the LH. We elicited eating via AcbSh injection of DNQX and attempted to suppress this eating with concurrent injection of D-AP5 either (1) bilaterally into the LH, or (2) into the LH ipsilateral or contralateral to the AcbSh injection site. Behaviors were monitored and rated minute-by-minute 10 min prior to and 60 min post injection. AcbSh DNQX elicited eating in both experiments. Concurrent bilateral LH D-AP5 infusion halted the elicited intake, suppressed grooming, and hastened the onset of sleep. In contrast, ipsilateral LH D-AP5 suppressed AcbSh DNQX-induced feeding with no significant effects on any other behaviors. These results suggest that bilateral LH NMDA receptor inactivation may suppress AcbSh-mediated feeding through non-specific means such as decreased arousal. However, ipsilateral LH NMDA receptor blockade appears to suppress AcbSh feeding in a behaviorally specific manner, consistent with the linked roles these brain areas have in controlling feeding behavior.

Central leptin sensitivity in rats on a free choice high-fat high-sugar diet

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Rats on a choice diet of saturated fat and sugar solution in addition to chow (high-fat high-sugar, HFHS) have been shown to be resistant to peripherally administered leptin and to have disregulated NPY and POMC signaling. The aim of this study was to investigate whether these important hypothalamic targets within the leptin pathway are still responsive to centrally administered leptin. Rats were subjected to either a CHOW control or HFHS diet for 2 weeks. After 1 week, food intake was measured 2, 5 and 24 h after intracerebroventricular (ICV) administration of leptin (15 μg) or vehicle. In the second week, rats were killed 2 h after ICV of leptin or PBS and SOCS3 and NPY mRNA expressions were measured by *in situ* hybridization in different hypothalamic regions. ICV leptin decreased 24 h total caloric intake in rats both on CHOW and on HFHS diet. In the HFHS diet, leptin significantly inhibited fat intake, but not chow or sugar solution. Leptin decreased NPY mRNA expression in the arcuate nucleus of rats on CHOW, but not in rats on HFHS diet. SOCS3 mRNA expression was not affected by either leptin or diet in the arcuate nucleus or DMH. Moreover, SOCS3 mRNA in the premammillary nucleus significantly increased upon leptin in rats on CHOW diet, but not HFHS diet, supporting leptin insensitivity in various hypothalamic nuclei in rats on HFHS diet. In conclusion, as the arcuate nucleus appears to be insensitive to leptin, extra hypothalamic areas may account for the leptin-induced feeding suppression in rats on HFHS diet.

Behavioral traits of deregulated eating behavior and energy balance; evolutionary considerations from man to mouse

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Anorexia Nervosa (AN), which is associated with leanness and often with hyperactivity, and Binge Eating Disorder (BED), which is associated with obesity, are derangements in humans with devastating consequences on sustainable health. It is not clear which of the behavioral and physiological concomitants are traits or state-dependent factors associated with these syndromes, but it is likely a mix between the two. These traits may represent extreme examples of behavioral strategies (i.e., activity-based anorexia and obesity/thrift) that have evolved to survive unanticipated famine. These different traits can be studied in genetic mouse reference populations such as the A/J and C57Bl/6J strain, to unravel the underlying mechanisms and relations. C57Bl/6J mice are more prone to activity-based anorexia (i.e., an animal model for AN) as well as to diet-induced obesity than A/J mice. Recent evidence suggests that the genetic basis for these phenomena is rather divergent. Thus, while substitution of some distinct C57Bl/6J-chromosomes by A/J chromosomes affects the expression of the diet-induced obesity as well as the expression of ABA, the majority of substituted genes affect one or the other trait. We interpret these data to indicate both traits may be rooted deep in the genetic code of mice and potentially other species as well, and represent two default strategies beyond the homeostatic regulation of energy balance.

Cats prioritize taste preference over macronutrient content of food in ingestion choices with nutritionally balanced complete foods.

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These studies used complete and balanced foods for adult cats to evaluate taste preference and subsequent ingestion choices with foods of controlled macronutrient concentration. Taste preferences were measured by offering two foods in two different bowls for two days in 50 cat panels with cats trained to choose between the offered foods. A subsequent study with 12 cats measured food choices in a four bowl setting where foods were offered with controlled macronutrient variation and known preference from the two bowl test. In the first series of experiments a food was prepared with high palatability that was high (50% of the calories from that macronutrient) in protein, moderate palatability and high in carbohydrate and moderate palatability and high in fat. The fourth bowl was a high carbohydrate low palatability choice. As previously shown, cats both preferred ($P < 0.05$) the high protein food in the two pan test and also in the four bowl test when offered with the foods with varying macronutrient concentration. In the second series of tests a food was prepared with high palatability and high carbohydrate content and compared to foods with high protein, fat and carbohydrate but lower palatability. In this experiment, when offered foods with varying macronutrient content cats chose ($P < 0.05$) the high palatability high carbohydrate food. These studies show that cats regulate intake reflecting palatability preference and choose the more palatable food without regard to carbohydrate or protein concentration. Supported by: Hill's Pet Nutrition.

Role of the anteroventral third ventricle region in angiotensin II-induced behavioral desensitization.

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Angiotensin II (AngII) acts centrally to cause increases in water and salt intake. Repeated intracerebroventricular (icv) injections of AngII, however, result in a reduction in the dipsogenic response to a subsequent AngII injection. This AngII-induced behavioral desensitization requires angiotensin type I receptors, is not the result of some broader behavioral deficit, and likely reflects changes in AngII-responsiveness at the level of the receptor, but a neuroanatomical locus for this phenomenon is unknown. The anteroventral third ventricle (AV3V) region has been shown to be important in mediating the dipsogenic effects of icv AngII and, as such, is likely involved in the effects of repeated AngII administration. Consistent with previous studies, we found that injection of AngII directly into the AV3V region stimulated water intake, using doses that do not stimulate water intake when injected icv (1 ng). To investigate the role of this brain area in AngII-induced behavioral desensitization, we made repeated injections of AngII into the AV3V region, using a dose of AngII that did not produce desensitization when delivered icv. We found that when all injections were made into the AV3V region, rats given repeated injections of AngII (100 ng) drank less water after a test injection of AngII (100 ng) compared to rats given repeated injections of vehicle (TBS) before the same AngII test injection. The results provide additional support for the AV3V region in mediating the central actions of AngII and suggest a role for this region in AngII-induced desensitization. Supported by: NIH award HL-91911.

Arcuate nucleus controlled locomotor activity and glucose metabolism is depending on melanocortin signalling

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POMC- and AgRP-expressing neurons in the ARC of the hypothalamus play a critical role in the regulation of energy and glucose homeostasis. They are targets of leptin and insulin in the control of food intake and energy expenditure. We have previously demonstrated that overactivation of transcription factor Stat3 specifically in AgRP neurons controls locomotor activity and glucose metabolism, thus assigning AgRP neurons in the ARC an unexpected role in these responses. AgRP neurons are not only characterized by their ability to modulate melanocortin signalling via inverse agonism of the MC4 receptor but they also express other neurotransmitters such as GABA. To directly address the contribution of melanocortin dependent signalling in mediating the effect of Stat3 signalling in AgRP neurons in the control of locomotor activity and glucose metabolism, we have crossed mice expressing the constitutively active version of Stat3 in AgRP neurons with those overexpressing the mutant agouti (Ay mice). In contrast to Stat3-C^{AgRP} mice on a BL6 background, locomotor activity and body weight regulation are indistinguishable between Ay/Ay mice and Stat3-C^{AgRP} mice on an Ay background. In contrast, while Stat3-C^{AgRP} mice are more glucose tolerant, these animals on an Ay background appear glucose intolerant. Collectively, these data indicate that regulation of locomotor activity and glucose metabolism via activation of Stat3 signaling in AgRP neurons depends on functional MC4 receptor signalling.

Effects of adolescent dietary exposure on adult feeding and metabolism

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Adolescence is a critical period of development. These experiments were designed to determine how adolescent dietary experiences affect adult feeding and metabolism. For our initial experiments, body weight and feeding behavior were measured in adolescent rats. Four groups of male Sprague Dawley rats (PND 32) were exposed to the following dietary conditions: High Fat Diet (60% fat; $n = 8$), High Fat Diet with optional 8% sucrose solution access ($n = 8$), Control Diet (10% fat; $n = 8$), Control Diet with optional 8% sucrose solution access ($n = 8$) for 18 days. During this period, a significant effect was demonstrated for total caloric intake between groups ($P < 0.05$), with those groups receiving optional sucrose access having higher caloric intakes. This effect was demonstrated regardless of diet. In adulthood (PND 73), a saccharin preference test (0.011–1%) was performed to determine if there were sensory changes based on previous dietary exposure. There was a diet effect ($P < 0.05$) with adolescents exposed to a high fat diet preferring lower concentrations of saccharin. In order to assess metabolic impairments, an oral glucose tolerance test (2.0 g/kg/bw) was administered. There was an overall diet effect ($P < 0.05$), with those exposed to a high fat diet having an elevated blood glucose response to oral glucose. The findings from these preliminary experiments will have important implications for understanding how obesogenic dietary conditions during adolescence influence the neural and behavioral controls of feeding in adulthood. Supported by: NIFA-USDA NJ06156.

Cafeteria diet programmes behaviour in rats

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The timing of exposure to obesity is particularly of interest, as sensitive developmental periods have been identified where dietary extremes play a critical role in determining adult risk of physiological dysfunction. Despite evidence for such a programming effect on several physiological systems, the behavioural consequences of hyperenergetic maternal diets are yet to be elucidated in detail. To this end, we fed female rats either on chow or cafeteria diet (CD) and analysed emotional and feeding behaviour and brain serotonin (5-HT) metabolism in their offspring. Maternal obesity reduced locomotor activity in both male and female offspring. Lactational CD had an anxiolytic effect in male offspring as shown in the Elevated Plus Maze (increased entries into and more time on the aversive open arms) and the Open Field (shorter latency to enter the unprotected centre). Although Behavioural Satiety Sequence analysis revealed no lactational CD effect on food consumption itself, female offspring showed greater frequency of eating bouts, paralleled by an increased latency to rest and reduced duration of resting. In males resting was only delayed. Hypothalamic 5-HT metabolism was reduced. The present study demonstrates that high caloric maternal diet, in particular during lactation impacts on the offspring's emotional behaviour with the strongest anxiolytic effect observed in males. In both sexes lactational CD impacted on the BSS and hypothalamic 5-HT metabolism. Together these findings indicate a significant nutritional programming effect induced by a hyperenergetic maternal diet. Supported by: RSF5103, University of Nottingham.

Influence of the circadian and estrous cycles on calcium and sodium intake in the rat

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The circadian and estrous cycles both influence water and mineral metabolism. However, the influence of these cycles on calcium intake has not been studied, and there are scant data to elucidate how the estrous and circadian intake cycles interact. To investigate this, 22 adult female Long Evans rats housed in a TSE Systems Phenomaster apparatus were given a series of two-bottle tests with a choice between water and 10 mM $CaCl_2$, 10 mM NaCl or 316 mM NaCl. In each case, intakes were recorded every 60 s for two consecutive 4-day estrous cycles. Total fluid intakes fluctuated over the estrous cycle: they decreased during the last 5 h of proestrus and first 5 h of estrus, and increased during the beginning of the light period of estrus (relative to the same periods during other estrous stages). There were fluctuations in 10 mM $CaCl_2$ intake but these were likely secondary to changes in total fluid intake. Fluctuations over the estrous cycle in 10 and 316 mM NaCl intake could be partially but not completely accounted for by changes in total fluid intake. Specifically, during the first 5 h of the dark period of estrus, water and 10 mM NaCl intakes decreased but 316 mM NaCl intake did not. During the last 5 h of the dark period of estrus, 316 mM NaCl intake increased but water and 10 mM NaCl did not. The results illustrate that patterns of mineral solution intakes over the estrous cycle are the product of the interaction between a demand for fluids and a relatively subtle appetite for minerals. Supported by: NIH NIDCD DC-10149.

Multiple pieces of food are more rewarding than an equicaloric single piece of food in both animals and humans

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Previous work by E.J. Capaldi and colleagues (1989) investigated preference by showing that rats trained in a T-maze preferred four pellets of 75 mg each (totaling 300 mg) to a single, 300 mg pellet of food. Our study measured both preference and running speed in a T-maze where 39 male, Sprague-Dawley rats were trained to associate one arm with a single 300 mg pellet and another with 30 pieces of 10 mg pellets. Thirty pellets were used to be comparable to snack foods consumed by humans in multiple, bite-sized pieces. Results showed rats preferred and ran faster for the arm associated with multiple pellets than that associated with the single pellet. Experiment 2 investigated the satiating effects of a single, uncut preload (bagel) and a cut, equicaloric preload (bagel cut in quarters) on subsequent test meal intake by 93 college students. The results showed that test meal intake was lower in subjects who got the cut preload than those who got the single, uncut preload. Thus in both rats and humans, food in pieces was more rewarding than a single equicaloric piece. Perhaps food in pieces appears bigger and is therefore more rewarding and satiating to both animals and humans.

Pectin with bulking, viscous or gelling properties have different effects on appetite

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Different fibre properties may differently affect appetite related signals in the oral cavity and gastro-intestinal tract. The objective of this study was to determine the effects of bulking, viscous and gelling pectin on appetite and ad libitum energy intake, and to identify underlying mechanisms. The study was a randomized crossover study with 29 healthy subjects (21 ± 2 y, BMI 21.9 ± 1.8 $kg{\cdot}m^{-2}$). Test foods were dairy based liquids and contained either: no added pectin (control), bulking pectin (10 g), viscous pectin (10 g), or gelling pectin (10 g). Appetite, glucose, insulin and gastric emptying were measured at baseline and 15, 30, 45, 60, 90, 120, 150 and 180 min after ingestion of the test foods. For gastric emptying measurements test foods were enriched with ^{13}C. An ad libitum lunch was given after 3 h to measure energy intake. After gelling pectin, AUC for appetite ratings was 14% lower than after control ($p = 0.007$). Bulking and viscous pectin did not change appetite. Incremental AUC for insulin was 25% lower for viscous pectin ($p = 0.003$) and 29% lower for gelling pectin ($p < 0.001$) than after control. The time to maximum excretion of ^{13}C increased with 17% after gelling pectin compared to control ($p = 0.01$). Glucose response and energy intake did not differ between the test foods. We conclude that pectin with gelling properties decreased appetite ratings, decreased insulin response, and slowed down gastric emptying. Viscous pectin reduced insulin response. Pectin with different properties affect appetite and its possible underlying mechanisms differently. Supported by: Dutch Ministry of Economic Affairs, Agriculture and Innovation (project KB-05-009-003).

Isotonic sodium chloride prevents the negative energy balance associated with angiotensin converting enzyme inhibition

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The renin-angiotensin system (RAS) appears to play an important role in metabolic processes. Angiotensin converting enzyme (ACE) inhibitiors, drugs that prevent formation of ANG II, have been shown to reduce body weight in rodents. The weight loss appears to be magnified when animals are fed synthetic diets (low in NaCl) compared with chow diets. The possibility that dietary NaCl intake can interfere with ACE inhibition-induced weight loss was investigated. After a 7-day baseline period (free access to a high fat diet (w/w 21% fat) with water to drink, male C57Bl/6J mice ($n = 48$) were fed a high fat diet with free access to water (H_2O), 0.9% NaCl solution (NaCl), water with captopril (0.05 mg/ml) added (CAP-H_2O), or 0.9% NaCl solution with captopril added (CAP-NaCl) for 28 days. Food and fluid intakes and body composition (DEXA) were determined. The results demonstrated that food intakes were not altered by the captopril treatment. Water, but not NaCl intake was increased by the captopril treatment. By the end of treatment, CAP-NaCl animals weighed less than and had less body fat than NaCl animals, CAP-H_2O animals weighed less than and had less body fat than animals in any of the other groups. In conclusion, the results suggest that high NaCl intake may interfere with but does not completely eliminate the weight and fat loss caused by captopril treatment. Supported by: Australian Research Council.

Sleep, energy balance and endocrinology

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Epidemiologically, BMI is inversely related to sleep duration. Inter-individual relationships between energy balance (EB = Energy expenditure – Energy intake MJ/d) and quality sleep (QS = percentage (slow-wave-sleep(SWS) + Rapid-eye-movement(REM)-sleep of total sleeping time) were investigated, as well as intra-individual effects of sleep-fragmentation on QS, EB and endocrinology. Sixteen men (age 23 ± 4 y, BMI 23.9 ± 1.9 kg/m^2) stayed in the respiration chamber twice for 46 h, fed in EB, + 2 h for the last supper offered ad lib. Polysomnography was used to monitor sleep (2330-0730 h). Ad lib energy intake was determined. Hunger and fullness were scored by VAS; blood-samples were taken hourly; total energy expenditure (TEE) and its components sleeping metabolic rate (SMR), diet-induced thermogenesis (DIT), activity-induced thermogenesis (AEE) and substrate-oxidation were measured continuously. Intra-individually, sleep-fragmentation reduced QS ($P < 0.05$). Then postprandial insulin secretions increased ($F = 4.0$, $P < 0.05$) without affecting glucose concentrations. GLP-1 concentrations, and fullness scores were reduced, while desire to eat was increased ($P < 0.05$). TEE was not affected (9.96 ± 0.17 MJ/d vs. 9.83 ± 0.13 MJ/d; NS), nor were SMR or DIT, yet AEE was higher after fragmented sleep:1.63 ± 0.15 MJ/d vs. 1.42 ± 0.13 MJ/d, $P < 0.05$). Then also physical activity(PA), exhaustion, sleepiness, respiratory quotient (RQ), and carbohydrate (CHO) oxidation were elevated: PA counts: 2371 ± 118 vs. 2204 ± 124 counts/d, $P < 0.02$; exhaustion: 40.1 ± 3.8 vs. 21.8 ± 2.4 mmVAS, $P < 0.001$; sleepiness: 47.4 ± 4.2 vs. 33.9 ± 4.6 mmVAS, $P < 0.001$; RQ: 0.95 ± 0.01 vs. 0.93 ± 0.01, $P < 0.01$; and CHO oxidation: 525.6 ± 18.4 vs. 486.1 ± 15.6 g/d, $P < 0.01$; while fat oxidation was reduced: 37.5 ± 4.5 vs. 44.1 ± 5.1 g/d, $P < 0.05$. Inter-individually QS was positively related to preserving EB by avoiding overeating and relatively higher TEE and AEE. A positive energy balance was mainly due to overeating from the ad lib supper, related to relatively higher ghrelin concentrations, higher hunger scores. Fragmented sleep reduced quality sleep, insulin sensitivity, satiety, fat-oxidation, and increased AEE, physical activity, exhaustion, sleepiness, RQ and carbohydrate oxidation. Inter-individually reduced QS was related to a positive energy balance underscored by overeating and reduced AEE. Reduced QS and may create a vulnerable condition in the etiology of overweight. Supported by: NUTRIM, Maastricht University.

Effects of oral exposure duration and gastric energy content on energy intake and appetite ratings

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Both oral and gastric signals contribute to satiety. In a previous study we showed that 8 min of oral exposure to cake through modified sham feeding (MSF) had a stronger effect on subsequent satiety than a 800 ml/100 kcal gastric load (GL). The small effect of the 800 ml/100 kcal GL on satiety may have been due to its low energy content. In this study we investigated the effect of independent manipulations of oral exposure duration and energy density of GL on satiety and energy intake (EI) of an *ad libitum* test meal 30 min later. It was hypothesized that both GL and MSF would equally contribute to satiety. Thirty-seven lean men were studied in a randomized crossover trial with 5 treatments. Men were orally exposed to food via 1 or 8 min MSF and simultaneously received a 500 ml GL via a naso-gastric tube (100 or 700 kcal). As control only a naso-gastric tube was inserted. Compared to the control, 700 kcal GL with 1 or 8 min MSF decreased EI ($p < 0.0001$), while 100kcal GL treatments did not ($p = 0.52$). Seven hundred kilocalories GL treatments increased satiety ratings more than 100 kcal GL treatments ($p < 0.0001$). Eight minutes MSF increased satiety ratings more than 1 min exposure ($p < 0.004$). In conclusion, appetite feelings were both affected by long oral exposure duration and high energy

density of the gastric load. However, subsequent energy intake was mainly affected by energy density of the gastric load. For energy intake 30 min after treatment the effect of 700 kcal gastric energy content is of more importance than the effect of 8 min oral exposure. Supported by: Nestlé Research Centre.

Sugar can mask the bitter taste of vegetables

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The unpleasant bitter taste found in many nutritious vegetables may deter people from consuming a healthy diet. We explored ways to mask the taste of bitterness and increase liking for broccoli and cauliflower through added seasonings. Two hundred and twenty undergraduate volunteers tasted and rated one piece of each vegetable plain, completed a distracter task, then tasted and rated one piece of each vegetable that had been seasoned with salt, sugar, lemon, or butter. Analyzed with a repeated-measures ANOVA, we found that sugar decreased bitterness and increased liking, lemon did the opposite, and salt and butter had no effect. Supertasters liked the texture of the vegetables more and tasted more sweetness in the sugared vegetables than did moderate tasters and nontasters. Adding sugar to vegetables appears to be an effective way to mask their bitter taste and increase their pleasantness. Previous work in our lab suggests that through flavor–flavor and flavor-calorie learning, consuming a vegetable with sugar will increase subsequent preference for that vegetable plain. Bitterness masking to improve initial palatability may therefore be the first step towards permanently increasing acceptance and consumption of healthy vegetables.

Weight loss in response to a controlled diet intervention is linked to ad libitum eating behavior at a buffet

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Eating behavior was examined in overweight women ($n = 48$) in response to a diet-controlled weight loss intervention. A 3-week baseline for weight maintenance was followed by a 12-week intervention with energy needs restricted by 500 kcal/day. An *ad libitum* dinner was administered to study food intake, intermeal interval (IMI) and meal duration (MD) at the beginning and end of intervention. On test days, participants consumed a standard breakfast and lunch that provided 25% and 30% of their prescribed energy intake, respectively. Dinner was provided upon request and consisted of a wide variety of foods. Mean body weight loss was 5.9 ± 2.8 kg ($p < 0.001$), and ranged from 0.5 to 12.5 kg. Energy intake and MD at the dinner did not change in response to the intervention but IMI was lower following the intervention (291 ± 36 vs. 276 ± 41 min, $p = 0.02$). Participants who lost less weight (lower tertile), consumed more energy, fat, and carbohydrate than those who lost more weight (upper tertile): 1579 ± 483 vs. 1223 ± 372 kcal ($p = 0.01$), 74.8 ± 25.3 vs. 56.3 ± 19.9 g ($p = 0.01$) and 158.7 ± 56.9 vs. 124.0 ± 45.3 g ($p = 0.03$), respectively, at dinner. IMI between lunch and dinner was also shorter: 260 ± 39 vs. 300 ± 33 min ($p = 0.01$). These results indicate that IMI decreased with energy restriction and energy and macronutrient intake at a buffet were relatively stable across the intervention but associated with magnitude of weight loss. Supported by: Dairy Management Incorporated.

Peptides, food intake and body weight. Problems of interpretation

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Many compounds, including many metabolically important peptides, have been demonstrated to influence food intake. In a typical experiment, subjects are administered the compound (e.g., CCK, insulin and many others) and the impact on subsequent food intake and body weight assessed; and whereas the results oftentimes align with prior results or hypotheses, there are many instances when they do not; i.e., the actual effect of a peptide on food intake appears to be probabilistic rather than certain, implying the existence of robust yet unknown influences. An important implication is that except in rare, usually experimentally-introduced circumstances, food intake should not be considered a homeostatic response that reflexively responds to signals of energy deficits. Rather, both the onset and termination of meals can be quite flexible, allowing for myriad influences to come into play. Over long intervals, of perhaps several days or more in humans, homeostatic considerations can be seen to have an effect, but in the short term, the impact of the same homeostatic signals can be variable. In this light, food intake should be viewed as a feedforward behavior whose properties have been shaped by past experience. Supported by: DK 017844 and DK 067550.

Effects of sex, body size and physical task on personal attribute

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Body image remains a serious topic of modern society with numerous media outlets placing emphasis on body size, body composition, and weight status. The present study asked 96 participants to rate pictures based on several dimensions, such as attractiveness, intelligence, healthiness, successfulness, trustworthiness, sexiness, promiscuity, willingness to kiss, willing to date, and willingness to have intercourse with. The pictures varied in sex (male and female), body size (small, medium or large), and activity being engaged in (control, treadmill, watching television, eating from a bowl, and drinking a beer). As body size increased, ratings decreased. Males rated the treadmill task pictures as healthiest, while females rated the treadmill task pictures as most successful, healthy and intelligent. In terms of sex of the pictures and task, the female treadmill picture was rated the highest while the male beer picture was rated the lowest. Overall, researchers found four main areas that were consistent throughout the experiment; (1) Females were more critical of ratings than males, (2) Females were rated higher by both sexes, (3) As body size increased attribute ratings decreased, and (4) Physical activity improves perceived personal ratings, while watching television, drinking a beer, or eating diminishes such ratings. These results are particularly salient in relation to how a person is judged based on the physical task in which they are engaged.

Deciphering a neuronal circuit that mediates appetite

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Hypothalamic neurons that co-express AgRP, NPY and GABA are known to promote feeding and weight gain by integration of various peripheral and neuronal signals. Ablation of these neurons leads to starvation that is accompanied by *Fos* activation in most post-synaptic regions. Previous experiments indicate that the ensuing starvation is due to aberrant activation of the parabrachial nucleus (PBN) as a consequence of deleting GABAergic input from AgRP neurons. However, the source of the excitatory inputs to the PBN was unknown. Here we show that glutamatergic neurons in the nucleus tractus solitarius (NTS) and caudal serotonergic neurons control the excitability of PBN neurons and inhibit feeding. Blockade of 5-HT_3 receptor signaling in the rostral NTS by either chronic administration of ondansetron or genetic inactivation of *Tph2* in caudal serotonergic neurons that project to the NTS protects against starvation when AgRP neurons are ablated. Moreover, genetic inactivation of glutamatergic signaling by the NTS onto NMDA-type glutamate receptors in the PBN prevents starvation. We also demonstrate that suppressing glutamatergic output of the PBN reinstates normal appetite after AgRP neuron ablation, whereas it promotes weight gain without AgRP neuron ablation. Hence, we identify the PBN as an important hub that integrates signals from several brain regions to bidirectionally modulate feeding and body weight.

Ablation of Sim1 Neurons causes obesity through hyperphagia and reduced energy expenditure

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Sim1 is a transcription factor necessary for development of the paraventricular nucleus of the hypothalamus (PVH). This nucleus is a critical regulator of appetite, energy expenditure and body weight. Previously we showed that *Sim1*$^{+/-}$ mice and conditional postnatal *Sim1*$^{-/-}$ mice exhibit hyperphagia, obesity, increased linear growth and susceptibility to diet-induced obesity, but no decrease in energy expenditure. Bilateral ablation of the PVH causes obesity due to hyperphagia and reduced energy expenditure. It remains unknown whether Sim1 neurons regulate energy expenditure. In this study, Sim1creiDTR mice were generated and Sim1 neuron ablation was performed by ICV injection of diphtheria toxin. Compared to controls, mice with Sim1 neuron ablation became obese on a chow diet due to increased food intake and reduced energy expenditure. We observed a strong inverse correlation between the degree of obesity and hypothalamic *Sim1* expression. The reduction in baseline energy expenditure observed in these mice was accompanied by a reduction in activity, decreased resting energy expenditure, decreased body temperature, and decreased brown adipose tissue temperature suggesting an impairment of thermogenesis. Hypothalamic gene expression of *Sim1*, *OXT* and *TRH* was reduced by 50%. These results demonstrate Sim1 neurons in adult mice regulate both food intake and energy expenditure. Supported by: The Children's Hospital of Philadelphia.

Evidence for noradrenergic (NA)/glutamatergic co-transmission within the dorsal vagal complex (DVC), hypothalamus, and limbic forebrain in rats

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NA neurons within the caudal DVC (A2 cell group) participate in diverse autonomic, endocrine, and behavioral adjustments that contribute to body energy homeostasis. Stornetta and colleagues reported that ~80% of A2 neurons express mRNA for vesicular glutamate transporter 2 (VGLUT2; J. Comp. Neurol. 444: 191–206, 1999), suggesting that these NA neurons co-release glutamate from their axon terminals within the brain. To test this hypothesis, we combined anterograde tracing from the A2 region of the caudal DVC with dual immunofluorescence and confocal microscopy in order to visualize tracer-labeled and NA inputs to the paraventricular nucleus of the hypothalamus (PVN) and bed nucleus of the stria terminalis (BNST), as well as NA terminals within the caudal DVC. A subset of tracer-labeled inputs to the PVN and BNST were VGLUT2-positive, as were many NA terminals in both forebrain regions and within the caudal DVC. Conversely, glucagon-like peptide-1-positive terminals within the PVN, BNST, and DVC were not VGLUT2-positive, nor were cortical or hippocampal NA terminals that arise from A6 neurons in the locus coeruleus. These results provide anatomical support for the proposal that A2 NA neurons use glutamate as a co-transmitter within the DVC, PVN, and BNST, and presumably within other brain regions that receive A2 neuronal input. Future studies should investigate potential interactions between NA and glutamatergic signaling in brain regions that control food intake and energy balance. Supported by: NIH grant MH59911.

Anorexigenic effects of brain-derived neurotrophic factor is regulated by estradiol

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Estradiol (E2) suppresses food intake by enhancing other anorexigenic signals. BDNF administration decreases caloric intake. We previously found that female rats at estrous stage responded to a lower dose of BDNF to suppress feeding than females at other stages of estrous phase, indicating that the anorexigenic effect of BDNF may be modulated by E2. To test this hypothesis, adult female rats received intra-third-cerebroventricular (i3vt) cannulation and ovariectomy (OVX). One group OVX rats received E2 cyclic replacement, with one subcutaneous injection of 2 υ;g E2 every four days (OVX-E2). The other group OVX rats received vehicle injections (OVX-oil). OVX-E2 and OVX-oil rats were i3vt injected with BDNF or its vehicle saline one day after E2 or oil subcutaneous injection. 0.1 υ;g BDNF-injected OVX-E2 rats had significantly lower food intake than saline-treated OVX-E2 rats. In contrast, OVX-oil rats had similar food intake after 0.1 υ;g BDNF or saline injection. Although a higher dose 0.3 υ;g BDNF-injected OVX-oil rats decreased food intake compared with saline-injected OVX-oil rats, the anorectic effect was less than that of 0.3 υ;g-injected OVX-E2 rats. These findings suggest that the minimally effective anorectic dose of BDNF is lower in E2-replaced OVX rats, and physiological dose of E2 facilitates BDNF-induced feeding reduction. We determined whether BDNF gene expression was regulated by E2. OVX-oil rats had a lower VMH BDNF mRNA level compared with that of sham-operated females. This alteration was almost restored by cyclic replacement with E2. We conclude that E2 regulates BDNF gene expression and enhances the anabolic effects of BDNF. Supported by: R15DK090823.