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Abstracts

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Guest Editors: Wolfgang Langhans*, Christine Zuberbuehler

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Note: Authors marked with # were recipients of New Investigation Awards.

The effect of injection of a D2 dopamine (DA) receptor agonist into hypothalamic ventromedial nucleus (VMN) on food and water intake in adult male rats

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Dopamine (DA) as a member of catecholamine has a crucial role in the regulation of feeding behaviors. The peripheral injection of DA agonists such as bromocriptin (BR) as a D2 receptor agonist causes anorexia in rat. The aim of this study is to show the effect of injection of BR into ventromedial nucleus of hypothalamus (VMN) on food and water intake. For this purpose, 42 adult male rats (280–320 g) were used. Guide cannulae were implanted bilaterally into VMN. After one week recovery period, rats were divided into sex groups ($n = 7$) as follows: sham-operated, BR 12.5, BR 25, BR 50 (injected 12.5, 25 and 50 μg BR, respectively), SBR (Sulpiride, a potent D2 receptor antagonist, +BR), SCBR (SCH23390, a D1 receptor antagonist, +BR). The injections were made bilaterally with 24 h intervals for 7 days period. Food and water intake were measured 30 min before and both 30 min and 24 h post-injection. Data analysis (Paired *t*-test, One Way ANOVA and Tukey–Kramer Test) indicated that BR caused a significant decrease in food and water intake ($P \leq 0.05$). Pretreatment with Sulpiride but not SCH23390 attenuated the effect of BR. It can be concluded that the VMN D2 receptors have a crucial role in decreasing food and water intake in rat.

Insensitivity of body weight settling-points to prior dietary history in C57BL6 mice

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The widespread inability to sustain weight loss has been attributed to the recruitment of homeostatic mechanisms favoring restoration of body weight (BW) to its prior 'settling-point'. The present study was conducted to determine the extent to which such homeostatic mechanisms occur in mice. We show that mice maintained for 20 weeks on a high-fat diet (HFD) have 25% higher BW compared to mice maintained on a low-fat diet (LFD) despite similar caloric intake and energy expenditure (EE). When half of the obese mice were subsequently food restricted and maintained at a BW level equivalent to that of LFD mice, their resting EE was reduced by 24–33%. LFD mice and post-obese mice were then switched to ad lib HFD feeding and BW was monitored weekly. Post-obese mice subsequently gained weight at a faster rate, reaching (but not exceeding) the BW of the obese group more rapidly than did the previously LFD-fed mice. All animals were then maintained on the HFD ad lib feeding regimen for an additional 20 weeks. During this period, no significant differences in body weight were observed among the three groups. These results indicate that physiological mechanisms through which dietary history influences weight regain are distinct from those that influence settling points. Body weight settling points appear to be more strongly determined by age, diet and genetic endowment than by prior dietary history.

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Sucrose is a more potent post-ingestive reward than ethanol in conditioning flavor preferences

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Previous oral conditioning data suggested that isocaloric ethanol and sucrose have equivalent post-ingestive reward effects. To compare post-ingestive reward effects directly, without the influence of ethanol and sucrose flavors, we trained rats to drink flavored solutions paired with IG infusions of isocaloric sucrose and ethanol solutions. Male Sprague-Dawley rats were trained in 22-h one-bottle sessions with flavored saccharin solutions: a CS + E flavor paired with 5% ethanol infusions, a CS + S paired with 7.18% sucrose, and a CS-paired with water infusion. In two-bottle tests in Experiment 1, the rats preferred both the CS + S (97%) and CS + E (75%) flavors over the CS − , and strongly preferred the CS + S over the CS + E (96%). The rats drank substantially more of the sucrose-paired flavor in training, which may have contributed to the stronger CS + S preference. Therefore, Experiment 2 examined the effect of limiting the training intakes of all three CS flavors to 30 g/day. Although preferences were somewhat attenuated, the same pattern of results was found, except that the rats did not prefer the CS + E to the CS − (55%). In Experiment 3, rats drank matched amounts of 5% ethanol and 7.18% sucrose solutions containing CS + E and CS + S flavors. In flavor-only choice tests, the rats preferred CS + S to CS + E (67%). Thus, under these training conditions, sucrose is a more potent reward than ethanol in flavor preference learning. [Supported by: NIAAA AA11549]

Long term effect of physical activity induced weight-loss on GLP-1 release in overweight subjects

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Glucagon-like peptide 1 (GLP-1), as an incretin, potentiates glucose-related insulin secretion at the level of pancreatic b-cells. Moreover, exercise has been shown to improve insulin sensitivity in humans with impaired glucose tolerance. To assess the effect of moderate exercise on GLP-1 (7–36 amide) secretion, we compared 10 healthy normal weight subjects performing exercise with 20 subjects who rested (14 men, 16 women; BMI 22.9 ± 1.8; age 35.9 ± 13 yr; body fat% 22.9 ± 8.3). AUC of GLP-1 release in lean subjects was significantly increased during one hour of exercise compared to the resting condition ($P < 0.05$). To assess whether the acute beneficial effect of exercise on GLP-1 release in healthy, normal weight subjects is reproducible in overweight subjects after weight loss, and whether GLP-1 release is improved by weight-loss, 30 overweight (BMI 29.7 ± 2.7) subjects in a resting and active condition (60 min ergometer based on 25% of maximal power output W_{max}) before (W_{max} : 205 ± 55.4) as well as after a 3 month weight loss (3.41 ± 4.1 kg, W_{max} : 220 ± 47.7) period were investigated. In the obese subjects GLP-1 levels were increased after weight-loss ($P < 0.05$), whereas short term exercise did not have any effect on GLP-1 release before nor after weight loss. From these results we conclude that modest weight-loss was effective for improving GLP-1 sensitivity in the overweight, but did not improve acute, exercise induced GLP-1 release.

The role of social influences on food neophobia in tufted capuchin monkeys (*Cebus apella*)

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Many animal species exhibit food neophobia and the social context is one of the factors that increases the acceptance of novel foods. We carried out four experiments, based on an observer-demonstrator(s) paradigm, to investigate the extent to which social influences on feeding behavior foster a safe diet. In Experiment 1, observers ate significantly more of a novel food when group members were eating a different food than when alone. In Experiment 2, there were two conditions: (a) Same color condition: group members eating a food of the same color as the observer's novel food; (b) Different color condition: group members eating a food of a color different from the observer's novel food. Observers spent more time eating the food matching the color of the demonstrators' food but did not ingest more of it. In Experiment 3, the subject had a choice between two novel foods, whose color matched, or did not match, the food eaten by group members. Observers did not eat the matching food more than the non-matching food. A similar result was obtained in Experiment 4, in which the demonstrator was presented with two foods of the same two colors as the novel foods presented to the observer, and the demonstrator was eating only one of these two foods. Overall, we did not find evidence that social influences were directed to a specific food target. In conclusion, although in capuchins social influences on food neophobia occurred, the hypothesis that social influences foster a safe diet was not supported.

Intranasal administration of the calcium channel blocker diltiazem decreases food intake in hyperphagic rats

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Food intake is known to be influenced by a multitude of complex endogenous central and peripheral neurochemical systems, in addition to numerous external environmental stimuli. Of these external stimuli, olfaction is believed to play a very important role as evidenced by dysmosic patients' disordered ingestive behaviors. Additionally, taste is thought to be strongly influenced by olfactory function. Since the normal function of many olfactory neurons appear to be influenced by Ca^{+2}-mediated processes via Ca^{+2} channels, a novel approach at influencing the ingestive behaviors of animals might therefore involve interference with olfaction via Ca^{+2} channel blockade. Thus, we tested the ability of a Ca^{+2} channel blocker, diltiazem (D), to alter food intake in hyperphagic rats. Using a reversed-lighting environment, male SD rats, which had been food-deprived for 4 h at the beginning at the onset of the dark cycle, were administered intranasally doses of D (0.08, 0.8, or 8 mg/animal) or vehicle and the amounts of food consumed were measured. Intranasal administration involved direct application of the drug solution in a volume of 20 μl/nare. Food intake at 1, 2 and 4 h post-D administration was significantly ($P < 0.05$) decreased in a dose-dependent manner. As intranasally administered drugs can act locally on the nasal mucosa, peripherally after systemic absorption, and/or centrally via a preferred entry into the brain via the olfactory neuron, one or more of these mechanisms may have accounted for the observed anorectic activity of D. Further studies are needed to determine the mechanism/site of action of intranasally administered D.

Localized glucoprivation of hindbrain but not hypothalamic sites stimulates corticosterone and glucagon secretion

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Decerebration, aqueduct occlusion and cannula mapping studies concur in showing that glucose-sensing cells capable of eliciting feeding and adrenal medullary responses to glucoprivation are present in and restricted to the hindbrain. In this experiment, we implanted cannulas throughout the hindbrain and hypothalamus to localize glucoreceptive areas controlling two additional glucoregulatory responses, corticosterone and glucagon secretion. Chronic intraatrial catheters were also inserted. After recovery extensive habituation, rats were placed without food in remote blood sampling chambers. One hour later, the antiglycolytic agent, 5-thioglucose (5TG, 24 μg in 100 or 200 nl) or control solution was injected through the cannula. Blood was collected at 0, 15, 30, 60, 90, 120, 180 and 240 min after the injection (volume and hematocrit were maintained). Corticosterone, glucagon and glucose were measured in each sample. Localized glucoprivation of particular hindbrain sites elicited significant corticosterone and glucagon responses, both peaking at 30 min after 5TG. Averaged values at 0 and 30 min were 50 and 300 ng/ml, respectively, for corticosterone and 57 and 95 pg/ml, respectively, for glucagon. Glucose concentration and, in separate tests, food intake, doubled basal values in response to 5TG. There were no hypothalamic sites where corticosterone, glucagon, glucose or food intake was elevated by 5TG injection. Control injections did not significantly increase any of these responses at either hindbrain or hypothalamic sites. Thus, corticosterone secretion and a neural control of glucagon secretion can be added to the growing list of glucoregulatory responses controlled by hindbrain glucose-sensing cells.

Cerebral ischemic injury induced hyperphagia—involvement of PPAR beta and consequence of previous chronic infection

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Various aspects of energy balance were studied after middle cerebral artery occlusion (MCAO) in mice. In this model of stroke in humans, there is an initial decrease (first day) and a subsequent (days 2 and 3) regain of appetite. These changes are followed by a hyperphagic phase of about a week, after which food intake returns to pre-ischemia values. Body weight parallels food intake changes. The usual hyperphagic phase after MCAO did not occur in PPAR beta knockout (KO) mice (obtained from Prof. Wahli University of Lausanne), indicating that PPAR beta is involved in the hyperphagic response following MCAO. As PPAR beta is expressed mainly in microglia, the data suggest that microglia can influence appetite following MCAO. Moreover, neuropeptide Y may be involved in the hyperphagia after MCAO since it was reduced by 30% in the PPARKO compared to the WT mice in the hyperphagic phase. Finally, mice chronically infected with *Toxoplasma gondii* or *Spirometra mansonoides* also displayed hyper-

phagia following MCAO. In fact food intake in toxoplasma-infected mice exceeded the pre-ischemic food intake level by nearly 125%. This suggests that even the apparently stable chronic anorexia of *Toxoplasma gondii* infection in mice can be overcome by the hyperphagic effect of MCAO.

Differential effect of estradiol (E) treatment on c-Fos expression induced by intraduodenal (ID) infusions of intralipid (IL) and L-phenylalanine (PHE) in the nucleus tractus solitarius (NTS) of ovariectomized rats

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E selectively increases the inhibition of sham feeding produced by ID infusions of IL (whose satiating potency is CCK-dependent), but not that of infusions of PHE (not CCK-dependent) in ovariectomized rats (Geary, Peptides 22 (2001) 1251). We sought to determine whether these infusions differentially affect the expression of c-Fos protein, a surrogate for neuronal activity, in the NTS and hypothalamus. Ovariectomized rats with ID infusion catheters were s.c. injected with 10 μg E benzoate or oil vehicle alone on Tues and Wed and on Fri received 10 min, 0.44 ml/min, 300 mOsm ID infusions of saline, IL or PHE. Brains were processed for c-Fos immunocytochemistry 90 min later. In the NTS subregion just caudal to the AP, both IL- and PHE-induced c-Fos expression were increased by E, with the IL response increased more. Neither IL nor PHE increased c-Fos in oil-treated rats or in NTS regions subjacent to the AP or rostral to the AP in E or oil rats. Infusion type did not affect c-Fos expression in the magnocellular region of the PVN, but there was more c-Fos in E-treated rats. Neither ID infusion type nor E affected c-Fos in the arcuate nucleus. These data suggest that E affects processing of CCK-dependent vagal afferent signals in specific populations of NTS neurons caudal to the AP. The roles of these neurons in the control of eating remain to be established. [Supported by: NIH DK 54523]

Different effects of angiotensin II and III microinjections into the zona incerta in the regulation of drinking behaviour of rat

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Different doses of angiotensin II (AII) and III (AIII) microinjections into the zona incerta have been studied on drinking of rats in separate experiments during the consequent 60-min-daily-drinking period. Also, the dipsogen power of only the effective dose of AII and AIII were compared to vehicle treated rats. After, angiotensin receptor (AT1, AT2) antagonists on AII or AIII induced drinking were tested. In the first and second experiments only the 100 ng AII and the 200 ng AIII increased water intake significantly. In the third experiment the AII started its dipsogen effect earlier, at the 5 min measuring time, compared to the AIII. Both effects kept on lasting parallel from the 10 min on. Considering the antagonist pre-treatments in the fourth experiment, animals were injected by 90 ng losartan, an AT1 antagonist, 180 ng PD 123319 or 200 ng CGP 42112, both AT2 antagonists, respectively. Both AII and AIII increased water consumption. The effect of AII could be blocked by losartan, but not by PD 123319 or CGP 42112. On the other hand, the effect of AIII could not be blocked by losartan, but by both the PD 123319 and CGP 42112. The effects of AII, AIII, losartan, PD 123319 and CGP 42112 have not been tested in the zona incerta. The finding that water intake increased after AII or AIII injections and it could be blocked only by either of the antagonists suggests that AT1 and AT2 receptors play partially different roles in the regulation of water intake.

Meal replacement with an ethyl oleate emulsion reduces food intake and body weight in rats

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Satiety is triggered in part by nutrient absorption in the GI tract, which produces signals that act via the CNS to terminate ingestion. Previous work has demonstrated that intestinal infusion of linoleic acid suppresses food intake to a greater degree than predicted by its caloric value and therefore results in weight loss. However, because linoleic acid would be difficult to use in human contexts, we attempted to reproduce its effects using ethyl oleate (EO). To this end, we examined the effect of EO consumption on food intake and body weight in rats using a meal-feeding paradigm similar to human meal patterns. Animals received an emulsion containing 11.3% EO or soybean oil followed by meals of standard rodent diet. Relative to the control emulsion, EO reduced total caloric intake and body weight gain. This finding was confirmed in two replications with different lengths of chow access. Additionally, EO consumption did not significantly alter plasma glucose or insulin levels. Finally, when subjected to 75% chow restriction, rats receiving EO lost more weight and recovered less weight

upon return to ad lib chow access. These findings suggest an important interaction between EO absorption and short-term satiety mechanisms that results in reduced body weight. Further examination of these mechanisms may reveal nutrient supplement strategies that could serve as aids to weight loss regimens.

Distraction during meals induces increased intake in adult women: comparison of two distractors (television versus auditory stimulus)

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Background. Distraction during meal eating facilitates increased intake, especially in persons with chronic dietary restraint. Television viewing is often associated with increased body adiposity, but the mechanism of this association is not elucidated. *Objective*. To measure meal size in distracted versus non-distracted conditions, and compare the effects of two distractors: television viewing and listening to an auditory stimulus. *Design*. Healthy women ($N = 58$; age: 30 ± 1.3; BMI: 22 ± 2) participated in four once-weekly standardized laboratory lunches. Subjects ate alone and ad libitum. The first and last lunches were presented without any distractor; in the other two tests (random order) subjects ate while either watching television or listening to a recorded radio program. The distractors contained no food-related material. Subjects filled the Three Factor Eating Questionnaire and the Dutch Eating Behavior Questionnaire. *Results*. Meal size was significantly higher in both distraction conditions than in both undistracted lunches ($+11\%$; $P < 0.001$). No difference in energy intake was observed between distraction conditions, or between undistracted conditions. This demonstrates that the appeal of the presented food had not changed during the test series, and that television did not exert a more potent stimulation than the other distractor. In contrast to earlier reports, the stimulating effect of distraction was not related to personal characteristics, such as chronic dietary restraint. Conclusions: Distraction during meal eating is associated with increased intake. Television viewing might influence energy intake and body weight by, among other mechanisms, inducing mealtime distraction. The potential modulating effect of program content should be investigated.

The influence on memory of breakfasts differing in the content of rapidly and slowly available glucose

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There are a growing number of studies that report that eating rather than missing breakfast influences aspects of cognition. There are also a large number of reports, based on both animals and humans, that the raising of blood glucose is associated with better memory. The hypothesis was considered that breakfast products that over time differ in their ability to provide blood glucose, may differentially influence memory. Subjects were randomly allocated to groups that either fasted, consumed breakfast biscuits high in rapidly available glucose (RAG) or thirdly consumed breakfast biscuits high in slowly available glucose (SAG). The two types of breakfast biscuits provided identical amounts of carbohydrate (34 g). The consumption of breakfast, as opposed to fasting, was associated with better mood and hunger. However, both hunger and mood benefited equally from the two breakfasts. The most interesting findings occurred with memory. Although after 40 and 105 min there were no significant differences, 160 and 215 min after a breakfast high in SAG, recall of a word list was better than after a meal high in RAG. When the ability to recall the more easily recalled concrete words was considered SAG influenced their recall only later in the morning. In contrast, SAG improved the recall of the more difficult to recall abstract words throughout the morning, a finding consistent with the supply of glucose influencing in particular more demanding tasks.

Effective treatment of eating disorders

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Fewer than 50% of patients with anorexia (AN) or bulimia nervosa (BN) recover within 10 years, about 25% develop into chronic cases and both disorders have a sadly high mortality. Also, most patients treated to remission relapse shortly after treatment. This is because there are no realistic models of AN/BN. Most of these hypothesize that a mental disorder, most often lacking clear neural reference, elicits disordered eating but fail to offer a mechanism to explain how this is mediated. Neurobiology has also failed to give a clue to how to treat eating disorders, probably because of its overly simplistic brain-elicits-behavior approach. A new framework for treatment is therefore needed. As a start, we suggest that AN/BN develop because the neural mechanisms or reward is activated by dieting and enhanced physical activity, the main risk factors for eating disorders, and that they are maintained because the mechanism of attention is also activated, thus conditioning disordered eating behavior to the stimuli providing reward. On this framework patients are taught how to eat and perceive satiety using computer support, they are provided with warmth, their physical hyperactivity is reduced and they are

removed from the site where they developed the disorder. This method was evaluated in a randomized controlled trial and has an estimated rate of remission of 75% and a rate of relapse of about 10% during 5 years of follow-up.

How do environment and mind override the homeostatic regulatory system controlling food intake and body weight?

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Under conditions of a restrictive food environment the homeostatic control system regulates body weight and adiposity with remarkable precision. However, this regulation appears to brake down in many individuals with a genetic predisposition under the conditions prevailing in the modern era, characterized by a sedentary life style and easy availability of large portions of palatable and calorically dense food. The nervous system is the main interface by which food-related environmental factors influence the regulatory process. Thus, focusing on the neural systems mainly located in the telencephalon dealing with environmental factors, and on their connections with the homeostatic regulatory system, distributed mainly in the hypothalamus and brainstem, should result in new drug targets and behavioral strategies for prevention and therapy. The structures providing this interface with the environment are involved in the execution of mainly the initiation, procurement, and appetitive phases of ingestive behavior and associative learning before during and after the consummatory phase. It is thought that learned or unlearned representations of foods and food cues in the orbitofrontal and other cortical areas are filtered for affective/emotional value in the amygdala and for motivational salience in the nucleus accumbens/ventral striatum to initiate goal-directed motor programs. Internal state signals generated by the metabolic sensing mechanisms in the hypothalamus, interact with each of these cortico-limbic structures through reciprocal connections. While many projections from the hypothalamus contain the various 'feeding peptides', the neurochemistry of projections to the hypothalamus has not been well characterized. [Supported by: NIH DK47348]

OLETF rats lacking CCK-A receptors exhibit altered responses to acute food deprivation

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Otsuka Long-Evans Tokushima Fatty (OLETF) rats lacking cholecystokinin (CCK)-A receptors are hyperphagic and obese, and exhibit deficits in meal size control and in neuropeptide Y (NPY) gene expression in the dorsomedial hypothalamus (DMH). The present study was intended to determine whether these deficits would affect OLETF rat's response to an acute 24 h period of food deprivation. OLETF rats lost more body weight in response to deprivation but recovered their weight more quickly during refeeding than did lean Long Evans Tokushima Otsuka (LETO) rats. Food deprivation decreased plasma glucose and leptin levels to a similar degree in both strains. Both groups increased intake during refeeding but the magnitude of increase was significantly greater in OLETF rats. Deprivation resulted in a significant elevation in arcuate NPY gene expression (~47%) in LETO rats, but only produced a small non-significant increase in the already decreased level of expression in OLETF rats (~24%, $P > 0.05$). DMH NPY gene expression was not changed by deprivation in either OLETF or LETO rats. Although paraventricular corticotropin-releasing factor (CRF) expression was decreased by deprivation in LETO rats, CRF expression was not affected in OLETF rats. Together, these data suggested that OLETF rats lacking CCK-A receptors are not only capable of increasing their food intake in response to food deprivation, but also exhibit differential sensitivity to the effects of deprivation during both the food deprivation and refeeding periods. [Supported by: DK57609. The OLETF and LETO rats were a generous gift of Otsuka Pharmaceutical, Tokushima, Japan]

Leptin and cortisol in the night eating syndrome

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The night eating syndrome (NES), first described in 1955 by Stunkard et al. has recently experienced increased study and is a diagnostic candidate for a new eating disorder. The NES pattern comprises skipping breakfast, consuming most food at night, difficulty with sleeping, and awakenings from sleep to eat. In a study performed in Norway, 12 night eaters and 21 control women stayed in the Clinical Research Center for 24 h. Bloods were drawn every 2 h during the wakeful and sleep periods. Despite repeated episodes of nocturnal eating, leptin did not increase as much during the night ($P < 0.001$) in the night eaters compared to the controls. Additionally, circadian cortisol was higher in the night eaters. In a further study, five women with NES and five controls underwent a 2 h CRH stimulating test. Blood samples were drawn for plasma ACTH and cortisol. In night eaters compared with controls, the CRH-induced ACTH and cortisol response was significantly decreased by

47 and 71%, respectively, consistent with an overactive HPA axis. These neuroendocrine findings, especially the lower leptin and the greater cortisol, both of which can increase food intake, may be contributing to NES.

Post-preload feeding and brain c-fos immunoreactivity in Long-Evans and OLETF preweanling rats

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Feeding suppression by 5% BW gastric preloads of corn oil and mineral oil was assessed in 18–20 days old Otsuka Long-Evans Tokushima Fatty (OLETF) rats [lacking cholecystokinin-A (CCKA) receptors] and LETO controls, and in Long-Evans pups on postnatal 9–11 days. c-fos Immunoreactivity was examined in area postrema (AP), the nucleus of the solitary tract (NTS), and in hypothalamic areas implicated in feeding regulation. 18–20-days old LETO pups ingested significantly less after corn oil compared to mineral oil or sham preloads, while 9–11 days-old Long-Evans pups did not display lesser intake after corn oil compared to mineral oil preloads, replicating previous findings in Sprague-Dawley rats. c-fos Immunoreactivity was low after sham-preload, in NTS and AP, in OLETF, LETO, and Long-Evans rats. Preliminary analyses in corn-oil treated pups ($N = 3–5$ rats/treatment/group) showed lower levels of c-fos immunoreactivity in OLETF vs. LETO rats, in caudal, medial and intermediate NTS, but not in AP or commissural NTS. These findings, together with additional c-fos results, still in progress, provide support for the role of CCKA receptors in mediating intake-reduction early in ontogeny. [Supported by: the US-Israel Binational Science Foundation and the Rich Center, Bar-Ilan University]

Measuring food preference may influence energy metabolism. Changed characteristics in food intake, glucose challenge, and fasting in rats on carbohydrate- or fat-enriched chow

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Background. When having access to multiple food types, individual preference is often used to characterize subjects. However, subsequently observed differences in other parameters may not be related to individual characteristics, but instead be the result of predominantly eating one diet. Therefore the metabolic profiles were investigated of rats fed one of three different diets. *Methods*. Rats were allocated to normal (N), carbohydrate-enriched (CE), or fat-enriched chow (FE). After three weeks, i.v. glucose tolerance was tested. After that experiment, continuous measurement of O_2, CO_2, activity, and cage temperature was started. After an overnight baseline period, the animals were fasted for 48 h. Blood samples were regularly taken for glucose and insulin. *Results*. Body weight gain was highest for N and lowest for CE, while food intake was similar between N and CE but increased in FE. Baseline glucose and insulin were lower in FE, while glucose tolerance was unchanged. Respiratory quotient (RQ) was highest in CE and lowest in FE. Oxygen consumption, temperature, and activity peaked at the end of the night in N and CE, and at the beginning of the night in FE. During fasting, oxygen consumption, RQ, glucose, and insulin decreased. Glucose and insulin decreased the least in N, while the insulin decrease in FE started remarkably earlier, before RQ or glucose changed. *Conclusion*. Enriched chow alters metabolism within a few weeks. Care should therefore be taken in studies with continuous access to multiple food types, as predominantly eating one type can influence experiment interpretation.

Neither dopamine D2 receptors nor norepinephrine are required for amphetamine anorexia

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Amphetamine causes the release of biogenic amine neurotransmitters and prevents normal re-uptake, flooding the synaptic cleft with excess neurotransmitter. Both systemic and centrally administered amphetamine results in decreased food intake. The anorectic effect of amphetamine is greatest when injected locally into the anterolateral hypothalamus (LH), particularly on the lateral surface of the fornix. The anorexia produced by peripheral administration of amphetamine can be attenuated by microinjection of either β-adrenergic or dopamine D2 receptor antagonists into the LH. Based upon these and other studies, it is widely accepted that amphetamine acts via β-adrenergic and dopamine D2 receptors in the LH to inhibit feeding. We sought to test this hypothesis using mice lacking either D2 receptors ($D2r -/-$) or the ability to make norepinephrine ($Dbh -/-$). Because an antagonist at either receptor counteracts amphetamine-induced hypophagia, we predicted that both $D2r -/-$ and $Dbh -/-$ mice would have reduced anorexia following a modest dose of amphetamine (2 mg/kg). However, both $D2r -/-$ and $Dbh -/-$ mice responded the same as controls. We have obtained similar results using $D1r -/-$ and dopamine-deficient mice. Thus

neither dopamine nor norepinephrine is required for amphetamine anorexia.

Dopamine D2 receptors are necessary for weight gain during chronic treatment with the antipsychotic sulpiride

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Recent reports implicate the dopamine type 2 (D2) receptor in human obesity, and drugs that interact with the D2 receptor have effects on body weight. The antipsychotic sulpiride is an antagonist at all D2-like receptors (D2, D3 and D4), but does not bind to D1-like receptors (D1 and D5). Sulpiride causes body weight gain in humans and in young female rats. In the present work, we replicated this effect in young female mice. However, mice lacking D2 receptors ($D2r-/-$) do not gain weight when given sulpiride, indicating that the weight gain requires intact D2 receptor signaling. Chronic sulpiride treatment did not diminish the hypophagic effect of amphetamine in either $D2r+/+$ or $D2r-/-$ mice, but did sensitize the $D2r-/-$ mice to the locomotor effects of amphetamine. Because dopamine and neuropeptide Y (NPY) interact in the control of feeding, in a subsequent study we tested the effect of sulpiride on body weight gain in $Npy-/-$ mice. We observed that mice lacking NPY respond identically to controls, thus NPY is not required. We conclude that dopamine D2 receptors are necessary for weight gain during chronic treatment with the antipsychotic sulpiride.

Reward in dopamine deficient (DD) mice

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Dopamine (DA) plays an important role in reward. Nucleus accumbens (NAcc) DA, in particular, has received substantial attention because release of DA in the NAcc correlates with events considered to be rewarding, such as food intake, sexual interaction, and drug-taking. We study a line of transgenic mice that lack the ability to make dopamine (DA). The loss of DA in these mice has a devastating effect on motor and feeding behaviors. They are far less active than wildtype littermates, and at or before the time when normal littermates are weaned and begin to eat solid food DA-deficient (DD) mice do not eat enough to survive. Without our intervention, they would starve to death. Dopamine is obviously critical for sufficient, life-sustaining feeding behavior. Is dopamine also necessary for reward? We have used licking behavior as an operant response in a series of experiments designed to assess the response of DD and WT mice to rewards such as sucrose, saccharin, and saccharin paired with intraperitoneal glucose injection. By measuring responses with a lickometer system, we are able to detect preference even when the volume intake of DD mice is low. From these studies we have concluded that dopamine is not required for reward; DD mice express marked preference despite diminished ingestive behavior.

High fat diets promote increased hepatic 11-beta HSD-1 mRNA

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Adrenal steroids play a critical role in obesity. Curiously, elevated circulating glucocorticoids only rarely have been reported in the obese. Recently, Flier and colleagues genetically engineered a mouse whose adipocytes over-express 11 beta hydroxysteroid dehydrogenase-1, (11β HSD-1) an enzyme that is thought to convert the inert steroid metabolite 11-dehydrocorticosterone into the active hormone corticosterone. Mice bearing the trait become obese. Elevated 11β HSD-1 mRNA and enzyme activity have been reported in the omental adipose of obese humans and genetically obese rodents. The purpose of this study was to determine if rats that become obese by eating a high fat diet have increased 11β HSD-1. Quantitative PCR was performed using liver samples from rats fed either a standard diet or high fat diet for 10 weeks. Livers taken from high fat fed rats had 88% more 11β HSD-1 mRNA than controls. In a second experiment, a Northern blot analysis of liver RNA was performed, using radiolabeled riboprobes. After 24 h film exposure the blot was then stripped, and labeled with L32 probe hybridized for 24 h and again washed, and exposed to film for 24 h. Densitometry was then performed, and blot OD volume was calculated for both hybridizations. Livers from rats fed the high fat diet had 2.04 times more 11β HSD-1 mRNA than did their controls. These data confirm our hypothesis: access to a high fat diet CAN induce an increase in 11β HSD-1 message.

Dose-dependent effects of alcohol on appetite and food intake

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Evidence suggests that energy derived from alcohol is not only additive to the habitual diet, but may stimulate appetite leading to over consumption. To investigate a dose response effect of alcohol on appetite and food intake, 12 males attended the laboratory on three occasions. On each occasion they were given a standard breakfast and returned to the laboratory for an ad libitum lunch and dinner. Ss received 330 ml of no-alcohol lager (62.62 kcal: no-alcohol condition), the same amount of lager spiked with 1 unit (8 g ethyl alcohol, 118.62 kcal) or 4 units of alcohol (32 g ethyl alcohol, 286.62 kcal) 30 min before lunch. Ss rated appetite and mood at intervals before and after the preload, lunch and dinner. Ad libitum intake at lunch (excluding energy from the pre-load) was significantly higher following 4 units of alcohol (1377.65 + /− 236 kcal) compared to 1 unit (1173.31 + /− 296 kcal). Intake at dinner did not differ significantly in any of the conditions. Total energy intake at lunch and dinner (including energy from the pre-load) was significantly higher following 4 units of alcohol (2859.82 + /− 366 kcal) in comparison to 1 unit (2523.86 + /− 513 kcal). Hunger was rated higher following 4 units of alcohol across the day in comparison to no alcohol and one unit. The stimulatory effect of alcohol on food intake appears to occur only at doses above one unit, and this is not compensated later in the day.

Food intake in school children: energy compensation or adjustment?

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Previous research has shown that pre-school children regulate energy precisely in the short term. In contrast, the accuracy of energy compensation in older children and adults varies, raising the possibility that accurate energy compensation may diminish with age. To further investigate this hypothesis, ad libitum food intake from a test-meal was measured in 43 school children aged 6–9 years, 90 min following ingestion of either a no energy (NE), low energy (LE) or high energy (HE) preload. Preloads were water (250 ml), LE (187 kcal): muffin (56 g) + orange juice (250 ml), and HE (389 kcal): muffin (56 g) + orange juice (250 ml). All conditions were conducted in school, separated by a week. Data analyses revealed a significant effect of preload on energy intake from the test-meal ($P < 0.001$), with a dose-related response, i.e. most consumed after the NE preload, least consumed after the HE preload. Analysis of the compensation index (COMP-X) indicated no effect of preload condition on ability to compensate at the test-meal, and no main effects of sex or weight category (lean, overweight, obese). Notably, mean COMP-X values were low in all conditions (NE/LE: 43.0 ± 9.5; NE/HE: 35.7 ± 5.2; LE/HE: 29.0 ± 9.4), despite individual variation in accuracy. These data demonstrate that whilst older children can 'adjust' food intake according to differences in preload energy density, the accuracy of this as reflected by COMP-X is poor, supporting the idea that accurate energy compensation may be attenuated as children grow, which may in turn lead to intake in excess of requirements.

A role for GLP-1 in stress-induced hypophagia and body weight reduction

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Previous work from our lab has demonstrated a role for glucagon-like peptide-1 (7–36) amide (GLP-1) in the response to various stressors. Centrally administered GLP-1 has been shown to reduce food intake and induce c-fos immunoreactivity in the paraventricular nucleus of the hypothalamus (PVN). Central GLP-1 elevates stress-related hormones corticosterone and ACTH and also activates corticotropin-releasing hormone (CRH) neurons in the PVN. Repeated restraint stress results in a temporary inhibition of feeding activity but a long-term reduction of body weight. The purpose of this study was to test the hypothesis that activation of central GLP-1 receptors plays a critical role in the reduction of body weight and food intake following repeated restraint stress. Male Long-Evans rats were equipped with cannulas directed at the third ventricle for administration of either des-His1, Glu9-exendin-4 (exendin, 50 μg in 2 μl), a potent GLP-1 receptor antagonist, or saline. Following recovery from surgery, rats were matched for body weight and assigned to one of four groups: saline/-nonstressed, saline/stressed, antagonist/nonstressed, antagonist/stressed. The GLP-1r antagonist or an equivalent volume of saline was administered into the third ventricle. Following injection, stressed rats were immobilized for 1 h and non-stressed rats were placed back in their home cage without access to food or water for 1 h. This protocol was repeated over 3 days. Rats treated with the GLP-1r antagonist showed less body weight loss following repeated restraint than saline treated controls consistent with a role for the GLP-1r in stress-induced weight loss.

Alterations of short-term intake in mice with genetic or vagal surgical manipulations

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?twb=.4w>Mice with genetic mutations provide a useful alternative to traditional methods in examining gastrointestinal controls of feeding. To compare the effects of mutations with surgical manipulations, automated feeders were used to examine the microstructural feeding behavior of mice. In an initial experiment, C57BL/6 mice were given ventral, dorsal, bilateral, or sham truncal vagotomies. On a pelleted diet, unilaterally vagotomized mice had significant changes in meal size (↓18–31%), meal duration (↓21–34%), and meal number (↑18–22%), with the bilateral mice having larger changes (↓35%, ↓46%, ↑26%, respectively). In another experiment, W/Wv mutant mice (with a significant reduction of gastric intramuscular arrays, or IMAs) had significant changes in meal size (↓22%), meal duration (↓24%), and meal number (↑24%) compared to controls, possibly due to an earlier satiety and a decreased gastric accommodation reflex caused by the reduction of IMAs. A preliminary study with neurotrophin-4 knock-in mice showed changes in meal number (↑45%), intermeal interval (↓55%), and rate of eating (↓ 38%) compared to controls. These data indicate that genetic manipulations are able to provide information previously unattainable with surgical manipulations, such as the study of animals with alterations limited to individual mechanoreceptor types. The experiments also show that meal pattern/microstructural analysis reveals differences in short-term mechanisms of feeding previously undetected in cumulative intake studies. [Supported by: NIH DK27627]

Low dose furosemide modulates taste responses in the nucleus of the solitary tract (NST) in the rat

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With only one exception, treatments that induce sodium appetite reduce the magnitude of gustatory responses to sapid NaCl. The exception occurred when 10 mg of the diuretic furosemide (Furo), which produces a robust Na-appetite, resulted in increased responses to NaCl in NST neurons. This high dose of Furo not only induces Na-appetite, but also supports a conditioned taste aversion (CTA). A lower, 2 mg dose of Furo induces an equivalent Na-appetite, but not a CTA. To determine whether the anomalous electrophysiological results reflected the 10 mg of Furo, we replicated the original experiment but used only 2 mg of the diuretic. Chronically prepared, but lightly anesthetized rats were injected with either 2 mg Furo or saline alternately once a week over 8 weeks. A total of 49 NST neurons were tested with 4 standard taste stimuli (0.1 M NaCl, 0.3 M sucrose, 0.01 M citric acid, and 0.01 M quinine HCl) and a concentration series of NaCl and sucrose. A trend toward increased responsiveness to 0.1 M NaCl in cells during the treated condition failed to reach significance [$F(1,47) = 0.92$, $P = 0.342$]. In the NaCl concentration series, however, the two higher concentrations did produce larger responses during 2 mg Furo with treatment than during saline [$F(4,188) = 2.916$, $P < 0.05$]. No such effect occurred in the sucrose series. Therefore, it appears that, unlike other methods for raising a Na-appetite, Furo increases NST neural responsiveness to salt. [Supported by: NIH DC05435]

Food intake was suppressed in the rats treated with 5-hydroxy-L-tryptophan, in spite of the increased NPY expression in the arcuate nucleus

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We previously found that the intraperitoneal administration of 5-hydroxy-L-tryptophan (5-HTP), precursor of 5-hydroxytryptamine (5-HT), increases 5-HT level in the hypothalamus and the brainstem, decreases food intake and body weight gain in rats. It has been suggested that the brain 5-HT may be negatively correlated with neuropeptide Y (NPY), positively with pro-opiomelanocortin (POMC), in the hypothalamic control of food intake. To investigate if the anorectic effect of 5-HTP administration correlates with the expression level of NPY and/or POMC, we performed in situ hybridization with cDNA probes of NPY or POMC in the hypothalamic tissue sections with 5-HTP administration. Male SD rats (260–300 g) received 5-HTP (100 mg/kg, i.p.; an acute or three daily injections), perfused with 4% PFA in 0.1 M PB at different post-injection time points. The pair-fed and the saline injected groups were also processed as the control groups. In the results, POMC mRNA level in the arcuate nucleus did not differ in all groups. However, NPY mRNA significantly increased in the chronic (three daily injections), but not in the acute, 5-HTP group as high as in the pair-fed group, compared to the saline group. These results demonstrate that food intake was suppressed, in spite of the increased NPY expression in the 5-HTP group, and suggest that an increase in the brain 5-HT level might inhibit the stimulatory effect of NPY in the control of food intake. Additionally, it seems that the brain 5-HT level may not be directly related with POMC expression.

Chronic treatment with fenfluramine increases hypothalamic neuropeptide Y but not corticotropin releasing factor expression in the rat

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Fenfluramine (FEN) reduces food intake by increasing serotonin (5HT) neurotransmission. Chronic administration of FEN reduces feeding for 2–3 days, but then, food intake returns to control values despite continued FEN treatment. 5HT release is known to inhibit the action of potent appetite-stimulating neuropeptide Y (NPY) neurons and stimulates the anorectic corticotropin releasing factor (CRF) neurons in the hypothalamus. Thus, we examined hypothalamic NPY and CRF mRNA to assess if these peptides were modified during the initial suppression or subsequent recovery of food intake during chronic FEN treatment. Male rats received once daily 10 mg/kg d,l-FEN (ip) for 15 days. On treatment days 2, 6 and 15, brains from drug or vehicle treated rats were analyzed for NPY and CRF mRNA expression by in situ hybridization. FEN reduced body weight during the entire treatment period, but food intake was suppressed only until day 4 of treatment (compared to controls). Surprisingly, posterior arcuate NPY mRNA expression was elevated in FEN treated rats at both 2 and 6 days of treatment, and by day 15 had returned to control values. CRF mRNA expression measured in the hypothalamic paraventricular nuclei was unaffected by FEN treatment. Thus, chronic FEN treatment did not inhibit NPY nor stimulate CRF neurons, suggesting that these peptides may not be involved in FEN-induced suppression of feeding. It is possible the early rise in NPY mRNA expression is a reflex response, i.e. an indirect response to the FEN-induced reduction in feeding and body weight that is intended to promote food intake.

Environmental temperature between meals and xylose absorption

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Intestinal absorption might positively correlate to energy expenditure that depends on environmental temperature. Oral tests with D(+)xylose might show negative correlation between absorption and temperature after demonstration of no urine excretion difference. Eight voluntary lean adults received 40 g cake for breakfast and remained in a room at 18 °C ambient temperature for 6 h. After 4 h, they received 0.4 g/kg body weight of D(+)xylose in 10% solution by mouth. Urine was collected just before and 2 h after xylose intake. The volume was measured and the concentration analyzed by the method of Roe and Rice. The eight subjects repeated the experiment at 28 and 33 °C ambient temperature in the same room and with the same clothing at intervals of 48 h. Two further experiments at 18 and 28 °C were performed by i.v. administration of 0.1 g/kg body weight of D(+)xylose in 100 min. D(+)xylose emission after i.v. administration was 49.3 ± 22.6% (SD) at 18 °C, and 54.7 ± 35.1% of administration at 28 °C (NS). D(+)xylose emission after oral administration were: at 18 °C 14.4 ± 7.4%, at 28 °C 10.5 ± 6.2% and at 33 °C 7.7 ± 4.2% of administration. The absorption decrease from 18 to 28 °C amounted to 27% of the value at 18 °C and was significant at $P = 0.03$, and between 28 and 33 °C amounted again to 27% of the absorption at 28 °C, at $P = 0.01$. *Speculation*. Assuming that D(+)xylose absorption test is representative of food absorption, meal intake has to follow the changes in absorption and expenditure during the intermeal interval, and inversely those in environmental temperature to prevent nutrient accumulation in intestine.

Estimates of blood glucose are accurate after training by pairing of self-sensing with glycemia measurement

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Human subjects were trained to estimate blood glucose levels by self-measurement of glucose level when they felt hunger. One hundred and forty-nine adults, who had functional complaints but no disease or glucose intolerance, were randomized in training (75) and control (74) groups. Subjects delayed intake until they felt hunger, measured glycemia and ate to raise mild hunger feelings in the hour before next mealtime and to prevent intense hunger for two further hours. Instructions on food content and expenditure factors, and high fruit and vegetable consumption helped adjustments in trials and errors. After 7 weeks, 64 trained and 72 control subjects attended the laboratory before breakfast. All subjects estimated (i.e. gave their best guess of) their current level of glycemia without knowing the measured level, using glycemia scale anchors from 110 mg/dl after a meal to 60 mg/dl during intense hunger. Their actual levels were measured by autoanalyzer. A correlation between estimations and measurements was significant for the trained ($r = 0.82$, $P = 10^{-6}$) but not for the untrained group ($r = 0.14$). Among those who said they were hungry, 18 trained subjects had 80.1 ± 6.3(SD) mg/dl glycemia, and estimated their glycemia with an average absolute difference from measurement (accuracy) of 3.2 ± 2.4% of the measurement, whereas the values were 89.8 ± 12.1 mg/dl ($P = 10^{-4}$) and 17.3 ± 11.6% in 42 untrained controls ($P = 10^{-9}$). Among those who denied that they felt hungry, 46 trained subjects had glycemia at 90.0 ± 6.6 mg/dl with 5.3 ± 3.6% estimation accuracy, while 30 untrained subjects had 90.8 ± 11.3 mg/dl with 18.0 ± 12.9% accuracy ($P = 10^{-5}$). The difference in accuracy between the trained group (4.7 ± 3.5%) and the untrained group (17.5 ± 12.1%) was highly significant ($P = 10^{-13}$).

A comparison of NPY, AgRP, MCH, and Orexin-A on multiple ingestive measures

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Several orexigenic neuropeptides are upregulated in the hypothalamus after food deprivation and stimulate food intake when administered centrally. We hypothesized these orexigenic neuropeptides would have disparate effects on a number of behavioral measures. We compared equally orexigenic doses of NPY, AgRP, MCH and Orexin-A on several measures: (1) food intake, (2) water intake, (3) macronutrient selection, (4) interaction with opioid receptors and (5) taste aversion. MCH, Orexin-A, and NPY increased food intake in both phases of the light/dark cycle, whereas AgRP more potently stimulated food intake in the dark phase. NPY, AgRP and Orexin-A selectively increased intake of a high fat diet, whereas MCH increased consumption of both high and low-fat diets. To assess the involvement of opioidergic signaling, we assessed the ability of naloxone (a non-specific opioid antagonist) to attenuate the effects of each peptide. NPY, AgRP and Orexin-A induced hyperphagia were attenuated by a sub-threshold dose of naloxone; whereas MCH induced hyperphagia was not affected. Water intake, independent of food intake, was only increased in animals that received MCH. Finally, we compared the ability of each peptide to elicit a taste aversion. Only NPY caused a significant taste aversion. These studies suggest that while all 4 peptides stimulate food intake, there are more similarities of action between NPY, AgRP and Orexin-A with less commonality with MCH.

Sex hormones determine body fat distribution and sensitivity to adiposity signals

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We hypothesized that there are differences in fat distribution in males and females that are directly determined by gonadal hormones. Specifically, we found that females have more subcutaneous fat than males, and conversely, males have more visceral fat than females. In the present studies, we assessed the relationship between gonadal hormones and fat distribution. In females, ovariectomy increased the visceral fat depot. In males, castration increased subcutaneous fat depot. Further, the addition of estradiol (2.5 mg/pellet) to males also increased subcutaneous fat. We also hypothesized that sensitivity to the adiposity hormones leptin and insulin would be influenced by fat distribution. Results demonstrate that male and female rats are differentially sensitive to central administration of these signals as measured by food intake and body weight. Specifically, males, despite having higher amounts of visceral fat, are more sensitive to centrally administered insulin than females. Conversely, females with higher amounts of body fat, and specifically subcutaneous fat, are more sensitive to centrally administered leptin. Results suggest that lack of estrogen in females decreases sensitivity to leptin administered centrally, yet has no effect on sensitivity to insulin. Additionally, castrated males became less sensitive ($P < 0.05$) to insulin and more sensitive ($P < 0.05$) to leptin. The addition of estradiol to intact males increased their sensitivity to leptin ($P < 0.05$). Our data suggest that body fat distribution is influenced by sex hormones, and that fat distribution influences sensitivity to adiposity signals.

The consequences of massive gustatory deafferentation on feeding and drinking patterns in the rat

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The contribution of orosensory signals, more specifically, the role of taste, in feeding and drinking patterns in rats was examined with cages that monitor ingestion. Gustatory deafferentation was produced by bilateral transection of the chorda tympani, glossopharyngeal, and greater superficial petrosal nerves. The deafferented rats showed reduced responsiveness to oil-chow mash, diluted sweetened condensed milk, and water. Body weight and food, milk, and water intake remained below control levels for at least 30 days post-surgically. The decreased body weight in deafferented rats was accompanied by decreases in overall intake, number of bouts, and rate of ingestion during bouts, but not bout duration. Thus, experimental rats did not initiate as many meals as sham-transected rats, but when they did, the meals lasted as long, but were consumed less efficiently. Apparently significant loss of gustatory input reduces the frequency with which rats approach food and fluid sources and affects how vigorously they consume them during ingestive episodes. However, it is equally clear that nongustatory signals can maintain ingestion, but not to the degree seen in intact animals. One way to interpret these findings is that in the absence of taste input, body weight was more stable compared with the more progressive course of weight gain observed in controls. The low sample size [GUSTx, $n = 3/4$; SHAM, $n = 4$] precluded comprehensive statistical analysis, but a replication is planned. We are

processing the tissue for histological verification of the absence of nerve regeneration. [Supported in part by: NIDCD R01-DC01628]

Nitric oxide synthase inhibitors reduce hyperphagia induced by raphe injections of the 5-HT1A agonist 8-OH-DPAT

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8-OH-DPAT elicits hyperphagia via the activation of 5-HT1A somatodendritic autoreceptors in the midbrain raphe nuclei with the increase in eating resulting from an apparent reduction in 5-HT synthesis and release. Several recent studies indicate that systemic administration of the non-selective nitric oxide synthase (NOS) inhibitor L-NAME and the neuronal NOS inhibitor 7-NI suppress eating elicited by subcutaneous injection of 8-OH-DPAT. Previous reports also indicate that these same NOS inhibitors decrease eating elicited by chlordiazepoxide, morphine and 2-deoxy-D-glucose. The present study was designed to examine the central interaction of NOS inhibition and 8-OH-DPAT. In male rats ($n = 8–10$/group), systemic or dorsal raphe injections of L-NAME and 7-NI inhibited eating resulting from dosal raphe 8-OH-DPAT administration. All drugs were administered during the mid-dark cycle and food intake was measured 2 h post-injection. In female rats ($n = 8–10$/group), 8-OH-DPAT did not reliably increase eating behavior. However, the 5-HT1A agonist did increase food intake in ovariectomized females. Both L-NAME and 7-NI inhibited this response, similar to that observed in male rats. Finally, we have previously reported that 8-OH-DPAT reverses the anorectic effect of fluoxetine (FLU) in male rats but not in intact female rats. In the current study, pretreatment with L-NAME and 7-NI blocked the action of 8-OH-DPAT on FLU anorexia. Similar findings were observed in ovariectomized female rats. These data suggest that central nitric oxide may modulate the action of 8-OH-PAT on food intake.

Sex differences in behavioral and chorda tympani nerve responses to NaCl

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A number of researchers have reported sex differences in the ingestion of concentrated NaCl solutions. The present studies investigated the possibility that differences in taste responses to NaCl underlie sex differences in the ingestion of NaCl. Male and female rats differed in their behavioral taste responses to NaCl solutions (0.028–0.5 M) in short term (10-s) tests. Compared to male rats ($n = 8$), female rats ($n = 8$) licked more of both 0.28 M NaCl (61.6 ± 2.2 licks/10 s vs. 39.4 ± 6.8 licks/10 s) and 0.5 M NaCl (49.5 ± 4.3 licks/10 s vs. 27.0 ± 4.4 licks/10 s). These sex differences in behavioral taste responses to NaCl may be attributable, in part, to differences in the sensory neural input related to NaCl taste. Specifically, electrophysiological responses of the chorda tympani nerve to lingual stimulation with NaCl (0.075–0.6 M) showed the expected concentration-dependent relationship in male rats ($n = 4$). In contrast, the NaCl concentration-neural response function plateaued earlier in female rats ($n = 4$) beginning at 0.3–0.45 M NaCl. Sex differences in chorda tympani nerve responses do not appear to be the result of differences in taste receptors, as the number of fungiform papillae on the anterior tongue of male rats ($n = 5$; 43.2 ± 2.3 papillae/side) was not different from that on the tongue of female rats ($n = 6$; 40.7 ± 3.8 papillae/side). These studies suggest that sex differences in taste responses to NaCl may contribute to differences in the ingestion of NaCl. In addition, sex differences in behavioral taste responses appear to have a peripheral neural component that does not involve differences in the number of taste papillae.

C-fos identification of brain areas involved in high protein diet-induced satiety

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Transition from a normal (P/E 14%) to a high protein diet (P/E 55%) induces a depression in food intake the first day and a progressive return to the initial intake during the following days. Although numerous lesions studies have been carried out the mechanisms and the brains areas involved in these effects are still unknown. In the present study, C-fos activation was determined after a meal in different brain area in three groups of rat: (i) 21 days adapted to a P14 diet (control), (ii) two days after transition from a P14 to a P55 diet (non-adapted), or (iii) 21 days adapted to the P55 diet (adapted). C-fos expression has been measured in brain areas involved in the control food intake (solitary tract nucleus, anterior piriform cortex, lateral hypothalamus, arcuate nucleus, posterior para ventricular nucleus, medio

ventral hypothalamus, dorso medial hypothalamus, amygdala and accumbens nucleus). During the transition phase, structures such as the dorso-medial hypothalamus, amygdala and the accumbens nucleus were activated. Furthermore, after adaptation to the high protein diet only the nucleus of the solitary tract remained activated. The results agree with the idea that in the non-adapted situation an urgency signal probably associated to the peripheral postprandial increase in plasma amino acids led to hypothalamic area activation. In contrast after adaptation these effects were no more observed, and the regulation of nutrient handling in visceral tissues (gut, liver) only produced a vagus-mediated activation of the nucleus of the solitary tract.

Differences in preference to sensory attributes of food is associated with PROP status

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Sensitivity to *n*-propylthiouracil (PROP) may identify an individual's preference for particular foods (Pasquet et al., 2002). It has been suggested that PROP sensitivity correlates with fat preference (Yackinous & Guinard, 2001) however food preference is determined by other attributes of food. This study aimed to identify whether attributes of a simple food were associated with PROP status. Volunteers were tested for perceived intensity of PROP using Green's scale of magnitude estimation (Tepper et al., 2001) and also sensory appraised three yoghurts with differing fat contents. Attribute scores for different yoghurts were analysed for associations with PROP status and between individuals classified as PROP tasters or non-tasters. PROP tasters preference for full fat yoghurts was significantly lower ($P < 0.05$) for flavour and creaminess ($P < 0.01$) than non-tasters. Low fat yoghurts were rated lower for overall mouth feel ($P < 0.05$). For the full fat yoghurts only PROP intensity score was inversely correlated with flavour ($r = -0.34$, $P < 0.05$) and creaminess ($r = -0.29$, $P < 0.05$). PROP status may provide a useful tool in predicting preference for foods with a significant fat content. This may have a benefit in identifying individuals where compliance to low fat dietary strategies is promoted.

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A role for food viscosity in caloric intake and body weight regulation

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Recent studies with humans indicate that calories contained in high viscosity foods (i.e. foods that resist flow) are more effective at reducing subsequent caloric intake and hunger ratings than are calories contained in low viscosity foods (i.e. foods that flow easily). These reports suggest that the ability of humans to adjust their intake to compensate for calories consumed depends, at least in part, on the viscosity of the food they eat. Using a rat model, we studied the role of food viscosity on intake and body weight regulation. In one series of experiments, fasted rats ate 10 g pre-meals (Chocolate Royal Ensure® brand liquid dietary supplement) that were matched in terms of nutritive content and caloric density but varied in viscosity (55,800 cps (pudding-like) or <100 cps (similar to chocolate milk). During subsequent 4-h feeding tests, rats given the high viscosity pre-meal consumed significantly less lab chow than did rats that received the low viscosity pre-meal. In another series of studies, nondeprived rats given overnight exposure to 15 g of the high viscosity, high calorie (1.3 kcal/ml) Ensure® dietary supplement gained less weight over a 30-day period that rats given the same amount of a low viscosity, high calorie diet, or a lower calorie (0.6 kcal/ml) Ultra Slim-fast® diet of either high or low viscosity. The results provide preliminary support for the hypothesis that caloric intake and body weight regulation is influenced by food viscosity. [Supported by: NIH grant R01 HD28792-09 (to TLD)]

Learning mechanisms involved with energy homeostasis

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Learning about events and relationships among events enables animals to anticipate the occurrence of important environmental and behavioral consequences. It seems very likely that the tendencies to eat and refrain from eating are also based, at least in part, on what animals know about the consequences (appetitive, aversive, or neutral) of ingestion. Accordingly, one way to investigate the control of food intake is to study the learning

mechanisms and processes that enable animals to anticipate these consequences. This presentation explores the possibility that changes in the performance of appetitive and consummatory behavior is based, in part, on learning about changes in interoceptive stimulation that accompany departures from energy homeostasis. The case for this possibility is based on converging evidence that (a) interoceptive cues produced by different levels of food deprivation can 'set the occasion' for the occurrence of appetitive reinforcers; (b) parallel variations in the intensity of conventional occasion setting stimuli (e.g. punctate auditory, visual, and diffuse contextual) and in degree of food deprivation have parallel effects on appetitive behavior; (c) The ability of conventional occasion setters and of changes in level of food deprivation, respectively, to modulate the performance of appetitive responses depends on the hippocampus—a structure long considered to be a substrate for learning and memory processes. The data are consistent with the possibility that food intake and body weight regulation in response to metabolic and other challenges may depend, in part, on hippocampal-dependent learning and memory processes. [Supported by: NIH grant R01 HD28792-09]

Agouti-related peptide increases food hoarding more than food intake in Siberian hamsters

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Agouti-related peptide (AgRP) is a receptor antagonist of the melanocortin system and appears to play an important role in the control of food intake and energy balance because exogenous administration in rats and overexpression in mice result in hyperphagia and body mass gain. Because AgRP mRNA increases during a fast in Siberian hamsters (*Phodopus sungorus*), but food intake is not increased, we hypothesized that AgRP may be involved in the fasting-induced increases in food hoarding seen in this species. Hamsters were trained in a hoarding/foraging apparatus where they had to run a programmed number of wheel revolutions to earn food pellets. Four doses of AgRP (83–132) or vehicle were injected intracerebroventricularly into the third ventricle at the beginning of the dark phase. Food hoarding, intake and foraging were measured at various time points. Food hoarding was increased more than food intake and hoarding was increased the greatest at the lowest dose (0.1 nM), whereas food intake was increased the greatest at the second lowest dose (1.0 nM). Foraging was increased at all doses. These results suggest that AgRP triggers the search for food in this species and once they find it, they hoard rather than eat it.

The behavioral genetics of restrained eating

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Dietary restraint affects food intake and body weight, but, little is known about its origins. The influence of heredity, familial environment, and individual environment on dietary restraint and their relationship to body size and food intake was investigated by obtaining scores on the Three Factor Eating Questionnaire and the Restraint Scale, as well as the height, weight, body mass index, and 7-day diary reported nutrient intakes from 39 identical, 60 fraternal same-sex, and 50 fraternal opposite-sex adult twin pairs who were living independently. Significant influences were found of the genes and individual environment, but not familial environment, on cognitive restraint, perceived hunger, and Restraint Scale scores. In contrast, the familial environment was found significantly influence disinhibition. The heritability of cognitive restraint and perceived hunger did not result secondarily from the heritability of body weight, height, or body mass index, while the heritability of Restraint Scale scores was found to be related to body size. Cognitive restraint was negatively correlated with nutrient intake. Finally, the differences in cognitive restraint between individuals in identical twin pairs were found to be related to differences in the body sizes. Hence, dietary restraint appears to be part of a set of genetically determined physiological, sociocultural, and psychological processes that control energy balance.

Immunoreactivity for immediate-early gene proteins and orexin A in the brains of mice after food restriction and refeeding

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Restricted daily food access entrains a circadian oscillator that triggers increased activity in anticipation of feeding time. The identity of this endogenous oscillator and the nature of its afferent signals are not known. Neurons in the lateral hypothalamus that contain orexin (hypocretin) project to a variety of brain regions involved in the regulation of arousal, feeding and circadian rhythms. Orexin expression increases in food deprived animals, and central injections of orexin have been shown to induce feeding. Cellular activation in response to the release of orexin could function in the signaling pathway that establishes or maintains food anticipation. We compared immunoreactiv-

ity (IR) for immediate-early gene (IEG) proteins and orexin A in brains of mice maintained on a restricted feeding schedule. Animals were fed for 4 h daily and were killed immediately before, or 1 h into the scheduled feeding. Results were compared to those of mice fed ad lib, acutely food deprived for 20 h, or refed after 20 h of deprivation. More orexin containing neurons showed c-Fos IR in animals acutely fasted than in those fed ad lib. The same was true for animals on a restricted feeding schedule once they had been refed, but not if they were still anticipating the meal. Furthermore, IEGs were differentially regulated among the five feeding conditions in other brain regions involved in feeding and arousal. These regions may be involved in mediating the effects of food availability on the system regulating anticipatory arousal and activity. [Supported by: NSERC and CIHR of Canada]

Differential effects of long-term central melanocortin or leptin administration on metabolism and body weight

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The central melanocortin (MC) system is implicated in the signaling cascade of leptin in the CNS. It consists of two receptors (MC-3 and MC-4), found in brain regions involved in the regulation of food intake, and both an endogenous agonist (a-Melanocyte Stimulating Hormone) and antagonist (Agouti-Related Peptide, AGRP). While the function of the MC-3 receptor still remains unclear, studies have shown that central MC-4 receptor stimulation results in decreased food intake and bodyweight. Oppositely, blockade of the central MC system with AGRP or SHU-9119 (synthetic antagonist) causes hyperphagia and obesity. Since both in humans and rodents mutations in this system are associated with obesity and diabetes, an important research topic is the development of potent MC agonists for therapeutic use. We compared the effects of long-term central infusion of either specific MC receptor agonists (MC4-a, MC3-a) or leptin on nutrient homeostasis in rats. Whereas chronic infusion of leptin resulted in permanently reduced food intake and bodyweight, MC4-a, but not MC3-a treated animals normalized food intake while maintaining a lower bodyweight. Together with elevated locomotor activity and body temperature this implicates that increased MC-4, but not MC-3 activation results in increased energy expenditure. In addition, plasma adiponectin levels, implicated in enhancing insulin action, are increased. These results emphasize the possible therapeutic role of MC-agonists in fighting obesity and diabetes. Research was funded by the Dutch Foundation for Scientific Research (NWO) and sponsored by the Groningen School of Behavioral and Cognitive Neurosciences (BCN).

Insulin-induced hypoglycaemia causes a tendency to increase fat consumption in humans

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Diabetics often suffer from prolonged periods of hypoglycaemia, which is believed to cause an increase in food intake. Little data is currently available on the magnitude of this effect, or on subjects ability to make appropriate food choices to correct hypoglycaemia. Sixteen healthy volunteers (mean age 29.8 years) enrolled for a double-blind cross over study: either insulin (0.05 units/kg) or saline was given as a bolus intravenously. Blood glucose was measured at 5 min intervals for 20 min after intravenous administration and then every 20 min till 120 min. Participants were presented with a selection of foods (ad libitum) at 20 min following injections. Blood glucose remained constant following saline (4.3 ± 0.4 to 4.4 ± 0.3 mmol/l) (mean $\pm$ SD). A transient fall in blood glucose after insulin was observed, with a zenith at 20 min (4.31 ± 0.34 to 2.41 ± 0.45 mmol/l), returning to baseline at 40 min. Participants attempted to recover from hypoglycaemia by consuming more high fat foods (muffins) ($P = 0.009$) after the insulin dose. A non-significant rise in high carbohydrate food (toast and toasted potato cakes) consumption was also observed in the insulin administration ($P = 0.07$ and 0.09, respectively). However, foods with the highest carbohydrate values (malt loaf and pancakes) were not chosen ($P = 0.91$ and 0.95, respectively). Overall, this resulted in a 17% increase in energy consumption ($P = 0.026$) after the insulin condition. High fat foods have a low glycaemic index, which might prolong hypoglycaemia, leading to passive over consumption and weight gain in hypoglycaemic individuals.

Rats that have recovered from activity-based anorexia can regulate their body weight when re-exposed to a restricted-feeding schedule

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Activity-based anorexia (ABA), an animal model of anorexia nervosa, is induced by placing rats on a restricted-feeding schedule while providing free access to running wheels (RWs). Because relapse is high in recovered

anorexia nervosa patients, we examined how previous exposure to the ABA paradigm affects body weight regulation in female rats ($n = 12$) re-exposed to the paradigm. Following baseline measurements of food intake and activity, food was restricted to 2 h/day until individual rats lost 25% of their baseline body weight. Rats were then allowed free access to food until they regained the weight lost and displayed regular estrous cycles. Following recovery, rats were re-exposed to the restricted-feeding schedule. During the first restricted-feeding phase, rats displayed a 61% suppression in food intake and an 84% increase in activity, compared to baseline. Food-restricted rats reached the 25% body weight loss criterion within 3–8 days. When food was freely available, rats displayed hyperphagia and hypoactivity. During the second restricted-feeding phase, rats displayed a 47% suppression in food intake and a 116% increase in activity, compared to baseline. The increase in food intake, compared to the first restricted-feeding phase, enabled rats to maintain a body weight loss of ~20% by the third day of restricted feeding. This adaptive increase in food intake following re-exposure to the ABA paradigm suggests that it is not a good animal model of the relapse observed in anorexic women. [Supported by: NIH Joint Neuroscience Predoctoral Training Grant (NIH, NIDCR, NIGMS, NIMH, NINDS, NINR) and MH 63787]

Daily central injections of NPY prepare rats for meal consumption

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Because eating relatively large meals may be a necessity of life in many environments, an important adaptation for minimizing the impact of the postprandial elevations of plasma fuels is to anticipate the meal by making responses that lessen postprandial hyperglycemia. When neuropeptide Y (NPY) is administered centrally, many meal-anticipatory responses are elicited, including increasing plasma insulin and corticosterone, and increasing locomotor activity. If NPY is a signal that heralds an imminent large meal, then timed daily injections of NPY should condition meal-anticipatory physiological and behavioral responses that facilitate ingesting a large meal. To test this, rats were given 4-h access to food (9 a.m.–1 p.m.). Rats received timed (4 p.m.), daily 3rd-ventricular (i3vt) injections of NPY (9.5 μg) or saline for one week. On test day (Day 8), animals were injected with the conditioning drug (NPY or saline) or with the opposite drug. Food was made available immediately after injection on test day, and intake was measured. Rats conditioned with NPY and then given saline ate significantly more than rats conditioned with saline and then given saline at 1, 2 and 4 h. In fact, rats anticipating receiving NPY and given saline ate the same amount as rats given NPY on that day. Collectively, these data suggest that NPY plays a role in mediating conditionable food-anticipatory responses that help to cope with the effects of large meals.

A low-fat yoghurt eaten when hungry in the afternoon has a more efficient short-term satiety power than a chocolate bar or a fromage frais

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The aim was to compare the satiety effect of three iso-energetic preloads varying in nutrient content: low-fat yoghurt, chocolate bar, fromage frais. Sixteen young normal-weight males were submitted to four test-sessions in counter-balanced order. Subjects were time-blinded and instructed to request food when hungry. In three sessions subjects were given 70% of their usual lunch energy content at 12.30. At their first request they received the test snack (30% of their usual lunch energy content). At their second request, they were offered an ad libitum dinner. In the control session, subjects had 100% of their usual lunch and dinner was freely requested. Hunger and fullness sensations, desire to eat were rated throughout the day. Inter-meals intervals and intake at dinner were measured. Compared to control, the interval of time between lunch and dinner request was longest in the yoghurt session only ($P < 0.05$). The interval between snack and dinner tended to be higher in the yoghurt than in the fromage frais session ($P = 0.07$). In the 2 h following the snack consumption, hunger feelings were lower in the yoghurt session than in the two others ($P < 0.001$). However, in the 2 h preceding the dinner request, this effect had disappeared. Energy intake at dinner was lower in the yoghurt session than in the others ($P < 0.05$). Compared to a fromage frais or a chocolate bar, a low fat yoghurt consumed when hungry has a more efficient satiety power. These results could be explained by a combination of volume and nutrient effects.

Reversal of ghrelin hyperphagia by NPY Y1R antagonist delivery to the fourth ventricle

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The orexigenic effect of central ghrelin administration is thought to be mediated primarily by NPY neurons in the

arcuate nucleus of the hypothalamus. It is also clear, however, that GHS-R is present in the brainstem and low-dose ghrelin administration to the brainstem parenchyma induces robust hyperphagic responses. We explore the hypothesis that NPY Y1 receptors also mediate brainstem ghrelin action by delivering the Y1 antagonist, 1229U91 (5 nmol/1 μl) 2 min prior to ghrelin (150 pmol/1 μl) administration to the 4th ventricle. Results were compared with those obtained from rats receiving the same treatments via 3rd-i.c.v. cannula. As previously, we found comparable hyperphagic responses after 3rd- and 4th-i.c.v ghrelin delivery, 1.5 and 3 h after treatment. The Y1 antagonist by itself was without effect but in combination with ghrelin it completely reversed the hyperphagic effect at 1.5 h with a significant but partial reversal 3 h after treatment. The same profiles were obtained with both i.c.v. placements; there were no significant placement *x* treatment interactions. The results are consistent with the hypothesis as stated and suggest a functional parallelism between the hypothalamus and brainstem with respect to ghrelin and NPY interactions. [Supported by: DK21397 and DK42284]

Serotonin reuptake inhibitors do not prevent 5,7-dihydroxytryptamine-induced serotonin depletion in rat brain

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Although the selective toxicity of 5,7-dihydroxytryptamine (5,7-DHT) is thought to depend on the drug's transport into serotonin (5HT) neurons via the 5HT transporter, few data have verified this postulation. We evaluated if 5,7-DHT-induced reductions in 5HT concentrations and synthesis in rat brain are blocked by 5HT-selective reuptake inhibitors. Rats given desipramine (to prevent catecholamine depletion) received intracerebroventricular 5,7-DHT 30 min after fluoxetine (20 mg/kg ip), and 48 h later, *m*-hydroxybenzylhydrazine (100 mg/kg ip) 30 min before sacrifice. 5HT and 5-hydroxytryptophan (5HTP, an index of 5HT synthesis) were measured in hypothalamus, cortex and brainstem. 5,7-DHT produced marked reductions in 5HT and 5HTP in all regions, effects that were not blocked by fluoxetine. Two other 5HT reuptake blockers (chlorimipramine, alaproclate) also did not block the 5HT and 5HTP depletions caused by 5,7-DHT. Desipramine did block 5,7-DHT-induced norepinephrine depletion. Pretreatment with the 5HT receptor antagonist metergoline, or the 5HT1A agonist 8-hydroxy-(di-n-propylamino)tetralin (which slows 5HT neuronal firing rate) also did not antagonize the 5HT depleting action of 5,7-DHT. Together, the data suggest that 5,7-DHT-induced depletion of brain 5HT does not involve 5HT transporter or 5HT autoreceptor interaction, and does not depend on 5HT neuronal firing rate.

Preweanling rats are sensitive to nutrient-conditioned flavor preferences

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Adult rats learn to prefer arbitrary flavors paired with sweet taste or with the postingestive effects of nutrients. As these learning abilities are not well understood, studying their ontogeny may yield insight into their organization. Prior work (Myers & Sclafani, 2001) has shown that preweanlings can learn to associate a flavor with glucose, but those experiments did not clearly dissociate the reinforcing properties of sweet taste and of nutrition. The current experiment sought specifically to determine whether preweanling rats are sensitive to postingestive reinforcing properties of nutrients in flavor preference conditioning. On postnatal days (P) 16–19, infant rats were removed from the dam and trained in a Pavlovian conditioning paradigm. Training consisted of intraoral infusions of a flavor mixed with 0.05% saccharin paired with simultaneous intragastric (IG) infusions of a nutritionally complete milk solution, and a different flavor mixed with 0.05% saccharin paired with no IG infusion. Each infusate was administered five times within a 96 h period (2 ml/6 h). Following weaning, on P24 preferences were measured in a 4-h two-bottle intake test with each flavor cue mixed with 0.05% saccharin. On P30, a retention test was conducted with the same method, with intakes measured at 4 and 24 h. Although no preference was evident on P24, in the 4 h test on P30 rats significantly preferred the flavor previously paired with IG nutrients over the flavor that was not. Studying the ontogeny of flavor preference conditioning should yield insight into this complex, dynamic system.

Autoimmune component in anorexia and bulimia nervosa. A new hypothesis for eating disorders

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We identified in the plasma of anorexia nervosa (AN) and bulimia nervosa (BN) patients autoantibodies against one or several neuropeptides such as α-melanocyte stimulating hormone (α-MSH), adrenocorticotropic hor-

mone (ACTH) and/or luteinizing hormone releasing hormone (LHRH). Additionally, several unidentified autoantibodies which bound to distinct neuronal populations in the hypothalamus and other brain areas as well as to the tanycytes and or to other glial cells were found in AN/BN plasma. Although some autoantibodies such as those against α-MSH also appear to be present in control subjects, their more frequent occurrence in AN/BN patients raise the possibility that these autoantibodies play a role in the development of eating disorders in a subset of patients. We propose a hypothesis that suppression of appetite in AN and/or binge eating behavior in BN are associated with autoantibodies penetrating into the brain, where they can target appetite-controlling neuronal circuitries. Since more than half of AN/BN patients displayed binding of their sera to α-MSH-synthesizing melanotrophes, and since the α-MSH-ergic neuronal circuitry in the brain is among the most relevant systems in appetite regulation, it is possible that dysfunction of the central melanocortin system compromised by autoantibodies in AN/BN patients contributes to for their disturbed appetite. This hypothesis must be tested in *in vivo* models, to establish under what conditions circulating antibodies against neuropeptides may cross the blood–brain-barrier; and if so, what is their effect on signalling between neuropeptide-producing and -receptive cells and on appetite and food intake.

Insulin, leptin, and food reward

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In addition to well-documented actions of the adiposity signals insulin and leptin to regulate food intake and energy balance at the medial hypothalamus, new evidence suggests that these metabolic hormones may modulate brain reward pathways as well. Our laboratory has focused on modulation of the rewarding aspects of food, and these studies will be reviewed. Food reward assessed by the conditioned place preference task is enhanced by chronic food restriction. This can be reversed by peripheral leptin administration in food-restricted rats, and blunted by intraventricular insulin and leptin in free-feeding rats. This task is dopamine-dependent and we have recently identified both insulin receptors and leptin receptors on midbrain dopamine neurons, suggesting that these neurons can serve as a direct target for insulin or leptin. Short-term ingestion of sucrose, and its stimulation by opiates, can be decreased by intraventricular insulin administration. These studies—together with findings from other labs that both insulin and leptin can modulate brain self-stimulation activity, as well as reports of enhanced self-administration or relapse to psychostimulant administration (i.e. drugs which directly stimulate midbrain dopamine pathways) in association with food restriction—support the conclusion that insulin and leptin can have direct CNS effects to blunt reward pathway activity, including, but perhaps not limited to, the rewarding aspects of food. [Supported by: NIH DK40963; Dr Lattemann is a VA Merit Review Program awardee]

The effect of orexigenic neuropeptides on intraoral intake of sucrose

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The ingestion of food can be divided into two distinct phases: appetitive and consummatory. While most food intake paradigms include both phases, the intraoral intake test emphasizes the stereotyped consummatory phase by infusing a liquid food directly into the oral cavity. Several hypothalamic peptides have been shown to increase intake of chow in standard food intake paradigms and the current experiments sought to test whether these peptides would increase food intake in the intraoral intake paradigm. NPY, melanin-concentrating hormone (MCH) and Orexin-A were infused into the third ventricle (i3vt) at equally orexigenic doses in a counter-balanced latin-square design just prior to rats getting 0.1 M sucrose solution infused via indwelling intraoral catheters and compared it to intake on bottle tests with access to the same sucrose solution. In the first experiment, naïve rats received eight daily sessions of intra-oral sucrose delivery. On the first day, each peptide increased intra-oral intake relative to saline in the between-subjects comparison (a finding at odds with previous work with NPY). Moreover, intake of sucrose following i3vt saline increased as a function of training. By day 8, rats receiving saline consumed as much sucrose as rats receiving NPY, MCH, or Orexin-A. This finding was conceptually replicated in the second experiment in which rats drank sucrose freely from a bottle on the home cage. These results are consistent with two conclusions: (1) NPY, MCH and Orexin-A increase appetitive and consummatory-phase ingestive behaviors; (2) repeated training interacts with the effects of these orexigenic peptides.

Peripheral baroreceptor signals stimulate intraoral water and NaCl intake in intact rats but not in chronic decerebrate rats

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The experiment tested the hypothesis that afferent signals from cardiac baroreceptors are integrated by hindbrain systems to control behavioral responses that correct deficits in plasma volume in rats. In adult male rats, a supracollicular transection was made above the medulla, which should not interfere with the neural signal of volume depletion. Decerebrate ($n = 5$) and control rats ($n = 7$) were given subcutaneous injections of isotonic saline or 30% polyethylene glycol (PEG). Four hours later, intraoral intake of water or 0.1 M NaCl was measured until rejected. As expected, control rats ingested more NaCl than water after PEG treatment (11.3 ± 2.3, 6.5 ± 1.3 ml, respectively; $P < 0.05$), and they ingested more of either fluid after PEG treatment than after saline treatment ($P_s < 0.01$). In contrast, decerebrate rats ingested comparable amounts of water (1.0 ± 0.3 ml) and NaCl (1.2 ± 0.2 ml) after PEG treatment, and those intakes were not significantly different from those that followed saline treatment. Similar changes in heart rate and blood pressure were observed in control and decerebrate rats following intravenous injections of phenylephrine and nitroprusside. These results indicate that hindbrain systems mediate the cardiovascular reflexes in response to deficits in plasma volume, but midbrain or forebrain systems mediate the behavioral responses associated with hypovolemic thirst.

Palatability shifts in flavour preference conditioning

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Although palatability shifts in taste aversion conditioning have been widely studied, relatively little work has been conducted to determine whether palatability shifts occur as a function of flavour preference conditioning. In the present study, hungry rats drank mixtures of kool-aid flavour cues mixed with sweet-tasting, calorific reinforcers in a long-exposure conditioning paradigm. In test, conditioned preferences using the two-bottle choice test were employed as well as the taste reactivity test to assess palatability shifts (Grill & Norgren, 1978). When tested hungry, rats preferred CS + whether they had acquired flavour-calorie or flavour-taste associations. However, CS + became more palatable only for rats that acquired flavour-calorie associations. These results suggest that acquisition of flavour preferences, as measured by 2-bottle tests, may not always be accompanied by enhanced palatability.

Orexins play important roles in palatability induced hyperphagia in rats

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We examined the effects of intraventricular injection of orexin-A on the intake of taste solutions in rats. Orexin-A (3 nmol) increased the intake of all solutions used, but the enhancement effect was most robust for sweet solutions such as sucrose and saccharin. Moreover prepro-orexin mRNA expression was elevated by saccharin ingestion. These results suggest that orexins plays an important role in palatability-induced hyperphagia. We also examined the effects of orexin-A on gastric motility. The centrally injected of orexin-A facilitated gastric contractility in the distal stomach and induced relaxation in the proximal stomach. These gastric motor responses may promote digestive functions of the stomach. In fact centrally injected orexin-A increased food output from the stomach. These findings suggest that orexin-A induces hyperphagia of palatable diets at least by facilitating digestive functions.

Effect of IP CCK infusion on insulin-induced hyperphagia and hypothalamic neuropeptide expression in male rats

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High doses of insulin induce hypoglycemia and hyperphagia, but the central neural mechanisms of the hyperphagia are largely unknown. Also, it is unclear whether peripheral satiety peptides such as cholecystokinin (CCK) antagonize insulin-induced hyperphagia. We used male rats to address these questions. In the early light phase and 1 h after IP injection of 50 IU/kg insulin, 32 μg/kg CCK-8 or PBS

(control) were infused for 60 min through previously inserted intraperitoneal catheters. IP insulin increased ($P < 0.05$) meal size and cumulative food intake compared to baseline. Compared to PBS control infusion, CCK attenuated this effect by reducing meal size and cumulative food intake ($P < 0.05$). In a terminal experiment in which rats had no access to food, animals were decapitated at the end of infusion and 2 h after IP insulin injection, and brains were snap-frozen for analysis of orexigenic neuropeptide mRNAs. Insulin increased hypothalamic orexin (ORX) mRNA expression by 30% and melanin concentrating hormone (MCH) mRNA expression by 52% ($P < 0.05$). Insulin tended to increase neuropeptide Y (NPY) mRNA expression ($P = 0.07$; +20.5%). CCK infusion blunted the insulin-induced increase of ORX and of MCH ($P < 0.05$ for both), but not of NPY mRNA expression ($P = 0.28$). These results are consistent with a role of ORX, MCH and NPY neurons in the mediation of insulin-induced hyperphagia. In addition, they show that signals triggered by peripheral CCK converge on neural circuits containing ORX and MCH.

Intracerebroventricular (ICV) leptin administration stimulates peripheral lipolysis in rats at a feeding-inhibitory dose

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Centrally administered norepinephrine (NE) stimulates peripheral lipolysis through activation of the sympathetic nervous system and results in an increase of plasma free fatty acids (FFA). Previously, stimulation of CNS leptin receptors has been shown to decrease food intake and body weight through effects on feeding behavior, energy expenditure, and lipid metabolism. The present study examines the ability of centrally administered leptin to stimulate peripheral lipolysis. In this study, separate groups of male Sprague-Dawley rats received ICV infusions of artificial cerebrospinal fluid (5 μl), NE (100 nmol/5 μl), or leptin (6.5 μg/5 μl). This dose of leptin significantly suppressed ad libitum feeding at 4, 8, 12 and 24 h post-injection in a separate group of rats ($P < 0.01$). Three hours prior to microinjection, rats were deprived of food. Blood was drawn from the tail of each rat immediately prior to injection and at 30, 60 and 90 min post-injection. To quantify peripheral lipolysis, we measured non-esterified fatty acids in plasma obtained at each time point. As expected, an increase of plasma FFA was detected at 60 and 90 min following NE administration ($n = 5$, $P < 0.01$). No increase was observed in vehicle-injected controls ($n = 9$). A significant increase of plasma FFA was detected 60 min after ICV leptin administration ($n = 6$, $P < 0.01$) and FFA continued to rise through 90 min post-injection (P for trend < 0.01). As opposed to an abrupt increase following NE, plasma FFA increased gradually in response to leptin. This study is the first demonstration that centrally administered leptin triggers peripheral lipolytic activity resulting in an increase of plasma FFA.

Endocrine signals in the control of eating

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Endocrine signals contribute importantly to the control of eating. These include (1) phasic endocrine feedback signals arising from the release of hormones from the gastrointestinal tract and pancreas during and between meals, (2) tonic endocrine feedback signals arising from hormones whose release is related to more delayed consequences of eating on metabolism and energy balance, and (3) non-feedback signals related to other endocrine systems, such as the HPA axis. Analysis of endocrine controls of eating has been facilitated by the relative accessibility of humoral mechanisms as well as by the well-developed strategies and methods of classical endocrinology. Indeed, endocrine signals number among the best understood controls of eating. Here three hypothesized endocrine feedback signals, cholecystokinin (CCK), ghrelin (GHR), and leptin, are reviewed against empirical criteria based on the last 30 y of progress in behavioral endocrinology. According to these criteria, CCK can be considered to be a proven physiological control of meal size in rats and humans. The crucial evidence includes reports that administration of doses of CCK that mimic prandial CCK levels or administration of CCK secretogogues selectively reduce meal size, that administration of CCK-1 receptor antagonists blocks the effects of CCK or CCK secretogogues, that administration of CCK-1R antagonists alone increases meal size, and that these effects can be obtained by local infusion of CCK or CCK-1R antagonists to the critical receptor sites in the proximal duodenum. In contrast, comparable evidence for leptin as a control of meal size and for GHR as a control of meal initiation is suggestive, but not yet complete. [Supported by: DK54523]

Ghrelin and binge eating disorder

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A subset of obese individuals, 30% of those seeking treatment, has binge eating disorder (BED) without the associated vomiting as in bulimia nervosa. Ghrelin, a

recently described orexigenic hormone, released primarily by the stomach, is elevated prior to a meal and declines during and after a meal, unlike most other peripheral appetitive hormones which rise after meals. Ghrelin injected into humans also increases food intake. Somewhat surprisingly, fasting ghrelin is lower in obese than lean individuals. We hypothesized that fasting and postprandial ghrelin would differ between BED and non-BED obese individuals. Three groups of obese women participated: BED ($n = 11$), subthreshold BED ($n = 12$) and non-BED ($n = 13$). Characteristics including age, BMl, and % body fat from air displacement did not differ among groups. Following a 12 h overnight fast, ghrelin and other appetitive hormones, as well as glucose were measured before and after a fixed liquid meal. Blood was drawn and fullness rated during a 2 h period. Fullness ratings and glucose, insulin, glucagon, leptin, and CCK did not differ among groups before or after the meal. However, ghrelin declined more after the meal from a higher baseline in the non-BED group than in both the subthreshold BED and BED groups ($P = 0.009$). The ghrelin AUC was also greater for the non-BED group than the other groups ($P = 0.001$). As appears for obesity, ghrelin may be down regulated in BED, and by not falling as much after a meal may contribute to overeating. A ghrelin antagonist has potential for treating obesity and BED, although it may not be successful, if as it seems, ghrelin level is secondary to obesity and BED. [Supported by: NIH Grant DK 54318]

Effects of high fiber oatmeal breakfast on appetite, body weight, and cholesterol

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We conducted a short and longer term study to examine the satiety impact of oatmeal breakfast cereal (1463 J) compared to equienergetic frosted corn flakes cereal and non-caloric water control. In the short-term study, 36 subjects (18 m, 18 f; 50% overwt, 50% normal wt) received each of the three breakfasts at least two days apart. Fullness was rated just before blood draws at − 15, 0 15, 30, 60, 90, 120, 150, 180 min. Test meal intake at 180 min was lowest following the oatmeal ($P < 0.0005$) and still lower for the overweight ($P = 0.004$) subjects. Fullness area under the curve (AUC) was greatest ($P = 0.00001$) following oatmeal. After cornflakes, glucose fell the most at 180 min ($P = 0.0001$), and insulin peaked the most ($P < 0.00001$). Acetaminophen tracer peaked later ($P = 0.00006$) following oatmeal, reflecting slower emptying. In the longer-term study, 36 overwt subjects (18 m, 18 f) were randomized to receive one of the three breakfasts daily for 4 weeks. Body composition, resting metabolic rate (RMR), and fasting bloods were measured at the start at end. The oatmeal group did not lose weight but had a 5.5% reduction ($P = 0.04$) in total cholesterol. The control group lost 1.2 kg ± SD ($P = 0.04$) but had a 7.9% elevation in cholesterol ($P = 0.02$). Weekly fullness ratings at 30 min declined only after oatmeal ($P = 0.04$). Glucose, insulin, and RMR changes did not differ among groups. Thus, oatmeal breakfast induced more satiety, possibly by slowing gastric emptying, and after 4 weeks, although not resulting in weight loss, lowered total cholesterol. [Supported in part by: a grant from Quaker Oats]

Attenuated dopamine efflux in the rat nucleus accumbens during consummatory negative contrast

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Animals trained to lick for a sucrose solution of a given incentive value that subsequently encounter an incentive downshift, display decreased licking relative to unshifted controls (i.e. successive or consummatory negative contrast or SNC). A contrast effect supports the assertion that the reward value associated with a stimulus is not a static, intrinsic property of that stimulus. Rather, animals can assign different appetitive values to a stimulus as a function of their internal states (a) at the time the stimulus is encountered and (b) as a function of their experience with the stimulus. In vivo microdialysis with HPLC-ED was used to monitor changes in dopamine (DA) efflux in the nucleus accumbens of food-restricted rats which had experienced either an incentive downshift ('shifted': 32–4%) or no change in incentive value ('unshifted': 4–4%) after 10 days of training. In the first 5 min of access to the 4% solution, shifted animals initiated significantly fewer bouts than unshifted animals despite no significant differences in the frequency of licks or the bout duration of licking between the two shift groups. The effect of the downshift on bout frequency disappeared in the second 5-min of access. DA efflux in the nucleus accumbens (NAC) reflected this initial difference in bout frequency, suggesting that DA activity is influenced by changes in the sensory incentive properties of sucrose solutions. Further, in the post-ingestive period, DA efflux in shifted animals remained significantly lower than in the unshifted animals suggesting a role for DA in the mediation of behavioural responses to incentive contrast.

Learnt protein appetite in human beings: involvement of cortisol in postingestive reinforcement by protein intake

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Human beings, as well as rats and other species, can rapidly learn an appetite for flavours associated with protein meals, which is expressed only when lacking in protein. This is learnt protein appetite. A possible neurohormonal pathway underlying this learnt protein appetite is activation of the hypothalamic pituitary adrenal axis and release of cortisol. For instance, a rise in cortisol secretion induced by a midday meal is known to be protein-dependent. A possible link with protein appetite and cortisol secretion was studied by measuring changes in salivary cortisol after a protein-rich meal, in low- and high-protein diet groups. The protein-rich lunch elicited a sharp rise in Positive Affect 2–3 h after the meal, but only in the group previously low in dietary protein. This implies a postingestive, not orosensory, reaction that is protein-state dependent. This increase in Positive Affect correlated positively with the meal-induced rise in salivary cortisol ($r = 0.77$, $P < 0.01$). The enhanced Positive Affect, and its associated rise in cortisol, may reflect the known reinforcing effect of a protein-rich meal in acutely protein-deprived people. Over all the subjects, the cortisol response to the protein-rich meal was inversely related to body mass index ($r = -0.54$, $P < 0.01$). The results are considered in the context of other recent findings concerning nutritional influences on cortisol release.

Biochemical correlates of the early (15–30 min) anorectic response to amino acid (AA) deficient diets in the AA sensor, anterior piriform cortex (APC)

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Provision of essential AAs, obligate precursors for protein synthesis, provides a challenge to dietary selection for most animals. Omnivores robustly reject a diet that leads to AA deficiency as early as 15–20 min after initial exposure to an essential AA deficient diet (Koehnle et al., SSIB, 2002), leading to questions of very early mechanisms. Therefore, we studied factors involved in initiation of existing mRNA translation. The concentration of the limiting AA is decreased in APC before 30 min. The first step in recognizing this depletion is likely to be the lack of charged tRNA for the limiting AA. To evaluate this possibility we injected threoninol, an inhibitor of tRNA synthetase, into the APC and measured basal diet (BAS) intake. After 2 nM of threoninol, animals decreased their BAS intake (<0.05). Uncharged tRNA may serve as a kinase to phosphorylate the alpha subunit of eukaryotic initiation factor 2 (eIF2a), part of the initiation complex. We measured phosphorylated eIF2a in the APC, and in perirhinal cortex (PRC), a control brain area, after feeding the rats a BAS, or the same diet devoid of the essential AA threonine (BDEV). The results (in pixels/mm^2) showed a significant increase in peIF2a only in APC of the animals fed BDEV: 2.18 ± 0.37 vs BAS: 1.52 ± 0.34; PRC: BDEV 0.17 ± 0.05 vs BAS: 0.15 ± 0.05. These data suggest roles for uncharged tRNA and initiation of translation in the earliest mechanisms of AA recognition in mammals. [Supported by: NIH NS33347 and USDA 2000-01049]

Obese women with binge eating disorder (BED) report more stress and pain, related to increased hunger and desire to binge eat following a cold pressor test (CPT)

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Stress is linked to both overeating and undereating, but little is known about the directionality. We examined responses to a cold stress test in thirty-five overweight women (BMI = 36.7 ± 6.5 [SD], age = 29.8 ± 8.1) classified by binge eating category (11 normal, 13 subthreshold [BE], 11 BED). During the CPT, participants immersed their hand in ice water for 2-min. Visual analogue scale ratings included stress, pain, hunger, and desire to binge eat. Blood was drawn and assayed for cortisol. The BE and BED groups had a greater increase in hunger ($P = 0.008$) following the CPT. Compared to the normals, the BED group had a greater area under the curve (AUC) for desire to binge eat ($P = 0.04$), a trend towards greater AUC for hunger ($P = 0.08$), greater increase in pain ($P = 0.02$), and AUC for pain ($P = 0.05$). Basal cortisol and AUC for cortisol did not differ between groups. Positive relationships were observed only in the BED group between AUC's for desire to binge eat with stress ($P = 0.004$), pain ($P = 0.008$) and hunger ($P = 0.001$). Our findings show a positive relationship between pain and stress on the one hand, and hunger and desire to binge eat on the other, only in women with BED. This suggests that in BED perceived pain and stress, which may be enhanced, contribute to desires to binge eat. [Supported by: NIH grants DK 54318 (AG), DK 07559 (MG), and GCRC grant MO1 RROO64529]

Arcuate-neuropeptide Y (ARH-NPY) neuronal projections develop prenatally in the third trimester in the nonhuman primate (NHP)

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ARH-NPY neurons are key components of the circuitry that controls food intake. Specifically, these neurons

transmit peripheral metabolic signals to important downstream target sites, such as the paraventricular nucleus (PVH). In the rodent, ARH-NPY projections to the PVH do not fully develop until the 3rd postnatal week. In this study, we determined the ontogeny of the development of ARH projections in the NHP. The unique colocalization of agouti related protein (AGRP) was used to identify and map ARH-NPY projections. On gestational ages G100, G130, G170 (full term is 175 days), a C-section was performed and the fetus was immediately perfused with saline, the brain quickly removed and placed in fixative. Animals were also killed at various postnatal ages. Double label immunofluorescence was used to visualize NPY-ir and AGRP-ir. At G100, most ARH-NPY-ir neurons coexpressed AGRP-ir, and in the PVH, there was an abundance of NPY-ir fibers, but only a small number contained AGRP-ir. By G130, the PVH contained an abundance of both single label NPY-ir and double label NPY/AGRP-ir fibers. The density of ARH-NPY/AGRP projections to the PVH continued to increase from G130 through the postnatal period. These data indicate that ARH projections start to develop between G100 and G130 in the NHP. This is in stark contrast to the rodent. The physiological significance of these findings is that maternal diet and health are likely key factors that could influence the development of ARH-NPY projections in the primate, whereas the postnatal environment would be more important in the rodent.

Relation between sleep and peripheral energy metabolism?

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The observation that sleep strongly varies in relation to the energetic status and that anabolism in the periphery is strongly dependant on sleep led us to propose that one function of sleep is to monitor the body energetic status and to tune accordingly feeding behaviour and anabolic/catabolic balance in peripheral tissues. The aim of this study was to test if correlations exist between sleep stages and peripheral accretion of lean or fat mass. *Materials and methods*. The specific accretion of lean or fat mass (quantified post hoc by carcass analysis) was induced by manipulations of the amount and quality of the food in rats bearing permanently implanted cortical electrodes enabling to quantify the time spent in wakefulness, slow wave sleep (SWS) and paradoxical sleep (PS). *Results*. This experiment confirmed the strong dependence of sleep on energy availability by showing that food deprivation reduced sleep by 50% within 4 days. On the other hand, it showed that sleep recovery during refeeding was accompanied by an relative increase in PS (PS/SWS = 30%) when refeeding favoured the accretion of lean body mass, and by a relative increase in SWS when refeeding favoured the accretion of fat mass (PS/SWS = 10%). *Conclusions*. These results are consistent with our hypothesis of a quantitative and qualitative link between sleep and energy metabolism and deserve the research of causal relationships. The involvement of various brain nuclei in both sleep and feeding behaviour suggests that sleep could modulate feeding behaviour and energy metabolism by affecting the activity of various brain nuclei.

Meal size and development of fullness depends on eating rate in patients with Bulimia Nervosa and healthy controls

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One characteristic of binge eating among patients with Bulimia Nervosa (BN) is that their rate of food consumption is elevated. It is possible that this increased eating rate is associated with a decrease in the rate of development of fullness, leading to increased food consumption. This hypothesis was tested by comparing rates of eating (g/min) with rates of fullness development among patients with BN and healthy controls, across a previously collected sample of subjects. Fullness development was quantified with a three-parameter, nonlinear model that fit fullness, which subjects rated (on 150 mm Visual Analog Scales-VAS) at 75-g increments throughout a test meal (energy density = 1.04 kcal/g), to increasing intake. We found that meal size correlated significantly and positively with eating rate, among both patients and controls ($r^2 = 0.65$; $n = 47$; $P < 0.0001$, for patients and controls combined). Furthermore, rate of eating correlated inversely with rate of fullness development ($r^2 = 0.13$; $n = 44$; $P = 0.003$; for both groups combined). These findings suggest that the faster subjects eat the slower their rate of fullness development and the larger their meals, regardless of eating pathology. Future studies will test whether slowing the rate of eating among patients with BN reduces the amount consumed during a binge episode. [Supported by: NIMH Predoctoral Training Grant MH12901, MH42206 and NY ORC]

Dietary obesity depresses the mesoaccumbens dopamine system: is food intake compensating?

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We examined the effects of chronic exposure to a cafeteria-type diet on the midbrain dopamine systems. Following a 30% weight gain in female rats kept on cafeteria-type diet, we observed a 70% reduction in basal extracellular accumbens dopamine. Amphetamine (1.5 mg/kg i.p.) or a laboratory chow meal increased accumbens dopamine levels significantly less in dietary obese than in normal weight animals. Only consumption of a cafeteria-diet meal readily elevated dopamine levels to those of controls. The low basal dopamine levels and the subnormal response to amphetamine and regular chow suggest a significant reduction in dopamine stored in synaptic vesicles in the accumbens terminals of obese rats. Apparently, only a cafeteria diet meal overcomes the dopamine deficit. Is this reduced dopamine quantal size present in early age in animals likely to develop dietary obesity? To consider this issue, we cultured midbrain dopamine neurons from inbred obesity-prone and obesity-resistant rat pups (age P0–P3) and measured differences in the density of the neuronal monoamine vesicular transporter VMAT2. VMAT2 expression directly correlates with changes in dopamine quantal size (Pothos et al., J. Neurosci. 20, 7297–7306). We have now obtained evidence that VMAT2 density is 50% less in cultures from obesity-prone than in cultures from obesity-resistant rats. We conclude that a reduction in accumbens dopamine quantal size is characterizing dietary obesity and may drive a compensatory increase in food intake. The same effect is present in offspring of inbred obesity-prone animals before any actual exposure to high-energy diets. [Acknowledgements: The Chestnut Hill Charitable Foundation]

Sham-feeding of sucrose increases dopamine and decreases norepinephrine in the nucleus accumbens of the rat

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Central norepinephrine long has been known to influence feeding behavior and other rewards including opiate abuse. Some projections of the ventral noradrenergic bundle reach the caudal shell of the nucleus accumbens (NAcc), a structure implicated in both reward and feeding behavior. Despite this relationship, the effect of food reward on norepinephrine in the NAcc remains uninvestigated. In the course of assessing dopamine overflow in the caudomedial NAcc during sucrose ingestion (Hajnal et al., 2001, 2003), we also collected data on norepinephrine flux from 14 ad libitum fed, male rats. Sham-feeding with 0.03, 0.1, 0.3 M sucrose resulted in an increase in NAcc DA (+20–47%), but an overall decrease in NE overflow (−20%; $F(7, 368) = 11.58$; $P < 0.001$). While the dopamine effect was a positive linear function of the sucrose concentration, there was no correlation between norepinephrine levels and sucrose concentration ($r = 0.11$, NS). Nevertheless, dopamine levels returned to the baseline within one, 20 min sampling period after sucrose licking, but the depression in norepinephrine levels during ingestion was followed by an elevated baseline (+10%) that persisted for at least 1-h after licking. These results demonstrate a dynamic relationship between two neurotransmitters within the same structure during orosensory stimulation with a normally preferred taste. [Supported by: NIH grants DC 04751 and DC 00240]

Cooperation of 5HT-3 and CCK-A receptors in CCK-induced satiation

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Recent evidence supports a role for the 5HT-3 receptors in the modulation of CCK-induced satiation. In this study, we used selective antagonists to investigate the roles of CCK-A and 5HT-3 receptors in CCK-induced satiation. Specifically, we examined the effects of ondansetron, a highly selective 5HT-3 antagonist on CCK-induced suppression of liquid (15% sucrose) and solid food (rat chow) intake in animals that were either deprived or non-deprived of food. Ondansetron (1 mg/kg, ip) significantly attenuated CCK (2 μg/kg, ip)-induced reduction of liquid food intake but was ineffective in attenuating CCK-induced suppression of solid food intake. Ondansetron significantly attenuated CCK-induced suppression of sucrose intake in nondeprived as well as in deprived animals. To determine whether ondansetron interacts with CCK to augment intake of palatable foods, in a separate experiment, we examined the effects of ondansetron on CCK-induced suppression of cookies in satiated animals. Ondansetron failed to increase intake of CCK-induced suppression of cookies. Finally, to determine if a relationship exists between CCK-A and 5HT-3 receptors in the regulation of CCK-induced satiety, both antagonists were administered together and tested for their effectiveness to reverse the satiating effects produced by CCK. Pretreatment with lorglumide (1.0 mg/kg) reversed CCK-induced inhibition of sucrose intake. Co-administration of lorglumide and ondansetron as well as simultaneous administration of the two antagonists with CCK produced a significant increase in sucrose intake compared to intakes after administration of saline, CCK, or either

antagonist alone. These studies show that CCK-A and 5HT-3 receptors participate and cooperate in CCK-induced satiation.

Abdominal vagal mediation of the satiety effects of CCK in rats

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Cholecystokinin (CCK) is a peptide found throughout the brain and in neurons and endocrine cells of the gastrointestinal tract. CCK secreted from the upper intestine in response to duodenal delivery of nutrients is postulated to act through a common afferent pathway—paracrine or neurocrine stimulation of type A CCK receptors (CCKAR) on vagal sensory neurons—to inhibit gastric emptying, stimulate pancreatic enzyme secretion, and produce satiety. In this study CCKAR antagonists differing in blood–brain barrier permeability were used to test the hypothesis that satiety is mediated in part by CCK action at CCKARs on vagal sensory nerves. Devazepide is a CCKAR antagonist that penetrates the blood–brain barrier; A-70104, the dicyclohexylammonium salt of Na-3-quinolinoyl-D-Glu-*N*,*N*-dipentylamide, does not. At dark onset, non-fasted control rats and rats with bilateral subdiaphragmatic vagotomy received a bolus injection of devazepide (2.5 mmol/kg iv) or a 3-h infusion of A-70104 (3000 nmol/kg/h iv) either alone or co-administered with a 2-h intragastric infusion of peptone (0.75–1 g/h). Food intake was determined from continuous computer recordings of changes in food bowl weight. In control rats both CCKAR antagonists stimulated food intake and attenuated the anorexic response to intragastric infusion of peptone. In contrast, only devazepide was effective in vagotomized rats. These results support the hypothesis that endogenous CCK acts both at CCKARs beyond the blood–brain barrier and at CCKARs on vagal sensory nerves to inhibit food intake. [Supported by: the Department of Veterans Affairs and NIH grants DK52447 and DK55830]

Effects of baclofen on appetitive and consummatory behavior examined in the runway

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The motivational mechanisms underlying the effects of systemic administration of the GABA-B agonist baclofen on feeding behavior were examined using a runway paradigm. Food-deprived male Hooded Lister rats were trained to traverse a straight runway for sucrose pellets over a series of 10 successive trials (5 blocks of 2 trials). Baclofen (1 mg/kg i.p.) increased sucrose consumption on the final two blocks of testing only. No significant effects of this dose were observed on measures of appetitive motivation (starting and runway speed), although the usual positive relationship between running/starting speed and intake was preserved. The 2 mg/kg dose of baclofen significantly increased running speed without altering intake. At the highest dose tested (4 mg/kg), no significant effects were observed on either consummatory or appetitive behaviors. However, runway speed was lower than that in the vehicle condition at the beginning of testing. In addition, the usual decline over the course of testing was absent and instead running speed remained relatively constant over blocks. This resulted in a non-significant correlation between running speed and intake, which may be indicative of non-specific motor effects. These data suggest that low doses of baclofen may enhance the consummatory phase of ingestion by attenuating the natural signals associated with onset of satiation. However, baclofen also has a biphasic effect on appetitive behavior that may interfere with changes in consumption.

Labyrinthectomy blocks acquisition of conditioned taste aversions induced by high strength magnetic fields

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We have previously shown that following exposure to a high strength static magnetic field (comparable to those of research MRI machines), rats will walk in circles, acquire a conditioned taste aversion (CTA), and express c-Fos in vestibular and visceral relays of the brainstem. These results suggest that the magnetic field may interact with the vestibular system producing effects similar to rotation or motion sickness. To determine if the vestibular apparatus of the inner ear is required for magnet-induced CTA, labyrinthectomized rats were tested. Rats ($n = 24$) received intratympanic injections of sodium arsanilate (50–100 μl/ear) which destroys the hair cells of the semicircular canals; sham-lesioned rats ($n = 24$) received saline vehicle injections. Rats were placed on a water deprivation schedule, then given 1 or 3 pairings of 10-min access to 0.125% saccharin with 30-min restraint within a 14 T magnetic field or restraint with sham exposure ($n = 6$ per group). CTA extinction was measured with 24-h, two-bottle preference tests. Sham-exposed lesioned and sham-lesioned rats showed high saccharin preferences and thus did not acquire taste aversions. Sham-lesioned rats exposed to the 14 T magnetic field expressed aversions to saccharin after 1 or 3 pairings that lasted 5 and 14 days, respectively.

Labyrinthectomized rats that received either 1 or 3 pairings of saccharin with the 14 T magnetic field did not acquire taste aversions and were behaviorally indistinguishable from sham-exposed rats. We conclude that the vestibular apparatus of the inner ear is a critical site for the behavioral effects of high magnetic fields. [Supported by: NIDCD04607]

Selective immunotoxin lesion of hindbrain catecholamine neurons with projections to the medial hypothalamus impairs the consummatory feeding response to glucoprivation, but not to lipoprivation or food deprivation

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Previously we injected the retrogradely transported immunotoxin, anti-dopamine-β-hydroxylase (dβh) conjugated to the ribosomal toxin, saporin (DSAP) bilaterally into the paraventricular nucleus of the hypothalamus (PVH) to selectively lesion norepinephrine (NE) and epinephrine (E) neurons projecting to the PVH. These lesions abolished glucoprivic but not lipoprivic or deprivation-induced feeding in conventional feeding tests. Here we injected the PVH with DSAP or unconjugated saporin (SAP) control solution to examine the importance of hindbrain catecholamine neurons for consummatory responses to these same challenges. Insulin (1.5 U/kg), 2-deoxy-D-glucose (2DG, 200 mg/kg), mercaptoacetate (MA, 68 mg/kg), 0.9% saline and 18 h food deprivation were tested. During tests, food (40% lactose-free milk) was infused at a constant rate through a chronic intraoral fistula until rejected. SAPs consumed significantly more milk after both insulin and 2DG than after saline, but DSAPs did not increase their intake above baseline to either challenge. MA and food deprivation stimulated feeding significantly in both SAPs and DSAPs and responses did not differ between groups, indicating that DSAP did not destroy the circuitry for consummatory responding, but only the mechanism for activating this circuitry specifically by glucoprivation. Thus, NE/E neurons that innervate the PVH are required for both the appetitive and the consummatory responses to glucoprivation. The fact, that consummatory responding to glucoprivation persists after decerebration (Flynn & Grill, 1983) indicates that hindbrain collaterals of PVH-projecting neurons, not their PVH terminals, stimulate this response. Hindbrain catecholamine neurons, therefore, appear to be involved in multilevel processing of behavioral responses to glucoprivic stimulation.

Immunotoxic destruction of distinct catecholaminergic neuron populations disrupts the reproductive response to glucoprivation in female rats

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Chronic glucoprivation suppresses estrus in hamsters (Schneider et al., 1997) and rats (I'Anson et al., 2003). This suppression can be viewed as an adaptive glucoregulatory response since by delaying pregnancy, it conserves metabolic fuels for maternal survival. Our previous work shows that adrenal medullary, corticosterone and feeding responses to glucoprivation are mediated by hindbrain glucose sensing cells and require activation of ascending or descending catecholamine neurons. Glucose sensors responsible for the delay of estrus also appear to be located in hindbrain, since fourth ventricular infusion of low 2-deoxy-D-glucose (2DG) doses suppresses pulsatile LH secretion in rats (Nagatani et al., 1996). Here we tested the involvement of catecholamine neurons in suppressing estrus during chronic glucoprivation. We microinjected the retrogradely transported immunotoxin, anti-dâh-conjugated to saporin (DSAP), bilaterally into the paraventricular nucleus of the hypothalamus (PVH) of female rats to selectively destroy dbh-containing catecholamine neurons projecting to this area. Neither DSAP nor unconjugated saporin (SAP) control injections altered basal estrus cycle length. To assess effects of chronic 2DG, rats were injected with 2DG (200 mg/kg every 6 h for 72 h) beginning 24 h after detection of estrus following two normal 4–5 day cycles. Chronic glucoprivation increased cycle length significantly in 7/8 SAP controls but in only 1/8 DSAP rats. Thus, hindbrain catecholamine neurons with projections to the PVH are not required for estrus cyclicity when metabolic fuels are abundant, but are required for inhibition of reproductive function during chronic glucose deficit.

Effect of hepatic portal vein (HPV) caprylic acid (CA) infusion on saccharin preference and gastric emptying in male rats

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Recently, we demonstrated that HPV CA infusion reduces food intake in male rats, but the mechanism of this feeding suppression is still unknown. In the present study, we examined the effect of HPV CA infusion on saccharin preference and gastric emptying. In Experiment 1, 16 h food

and water deprived rats ($n = 16$) had once access to a 0.125% saccharin solution for 120 min just prior to dark onset. At dark onset the rats received food and water, and CA (total dose: 207 mg; 0.35 mol/l), equimolar saline and equimolar lithium chloride (LiCl) was infused (40 μl/min) into the HPV for 90 min. Three and 4 days later, a 2 bottle choice test (water vs. saccharin solution) was performed. Percentage intake of saccharin solution differed between groups (saline, $n = 5$: 80.7 ± 9.1%; LiCl, $n = 5$: 9.5 ± 2.1%; CA, $n = 6$: 36.4 ± 10.5%; $P = 0.002$). In Experiment 2, 18 h food deprived rats received 4 g rat chow for 30 min at dark onset. Thereafter, CA (total dose: 207 mg; 0.35 mol/l) or equimolar saline was infused (40 μl/min) into the HPV for 90 min. At the end of infusion rats were sacrificed and gastric content was removed, dried and weighed. HPV CA infusion tended to inhibit gastric emptying (saline, $n = 7$: 62.9 ± 4.8%; CA, $n = 8$: 48.8 ± 5.1%; $P = 0.068$). Thus, CA reduced the preference for saccharine solution and possibly also gastric emptying. Whether these effects are responsible for the feeding suppressive effect of the HPV CA infusion still has to be clarified.

The effect of intracerebroventricular (ICV) injection of oxytocin (OT) on feeding behaviors of meat-type chickens

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Many investigations have documented the presence of oxytocin (OT) in different regions of the brain, including limbic system, hypothalamus and brain stem. According to this fact and other evidences, it has been proven that OT not only plays a role as a hormone, but also it act as a neurotransmitter in the central nervous system. Different behavioral functions have been attributed to OT, including its anorectic effects in rat. In birds, OT has been replaced with mesotocin, which is different with the former only in one amino acid. According to the authors' knowledge, there are no reported investigations concerning the role of this neurotransmitter in behaviors of birds till now. Therefore, in this study the effects of intracerebroventricular (ICV) injection of OT on feeding behaviors of Ross broiler cockerel were investigated. For this purpose, one week after implantation of a canulla into the right lateral ventricle, 0–10 μg OT was injected i.c.v. and all the behaviors following injections were video recorded to be evaluated with every detail later on. Data analysis (ANOVA and Duncan's Multiple Range Test) showed that OT caused a significant decrease in feed intake, feeding time, feed pecking frequency and feeding and pecking rhythms on 15 and 30 min post-injection ($p \leq 0.05$). These results indicated that central OT decrease feed intake and change feeding pattern in birds, via acting on mesotocin or vasotocin receptors.

Diacylglycerol affects fat oxidation and appetite in humans

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The aim of the study was to assess effects of partial replacement of triacylglycerol (TG) by diacylglycerol (DG) on substrate oxidation, energy expenditure (EE), blood parameters and appetite measures. Twelve healthy, dietary unrestrained females participated in a single blind, placebo-controlled cross-over design. For 3 days prior to and throughout a 36 h stay in the respiration chamber, subjects ate an energy balance diet of 55/15/30 energy % as carbohydrate/protein/fat. In the respiration chamber, 40% of fat was consumed as DG or TG oil with similar fatty acid profile. Fat oxidation was higher and RQ lower with DG vs. TG. Appetite profile on day 1 did not differ between DG and TG, but feelings of hunger, prospective food intake, appetite and desire to eat were all lower with DG on day 2. Plasma β-hydroxy-butyrate tended to be higher with DG, and was significant at 11.30 on day 2. DG did not affect plasma lipid levels. There were no differences in EE between DG and TG. We conclude that partial replacement of TG by DG does not alter total energy expenditure, but produces metabolic effects, particularly increases in fat oxidation, which may be associated with improved appetite control and energy balance.

The role of linoleic acid taste perception in the etiology of obesity

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The aim of the study was to determine taste perception of a 10 μM linoleic acid solution and its relationship with body mass index (BMI), body composition, resting metabolic rate, respiratory quotient, age, macronutrient selection and dietary restraint. Two hundred twenty-one subjects conducted a linoleic acid perception test. Subjects had to taste 10 pairs of 2 samples with one sample containing 10 μM LA and the other containing the placebo

solution. All samples were tasted in a standard manner. Subjects had to answer the question: 'Which sample contains the fatty acid?'. Subjects were classified as linoleic acid tasters with ≥ 9 correct answers; otherwise they were classified as linoleic acid non-tasters. BMI, body composition, RMR, RQ, and dietary restraint were measured. Furthermore, a subgroup of 35 subjects conducted a macronutrient specific food choice test. Of the 221 subjects, 46% were classified as linoleic acid tasters. Linoleic acid tasters were younger ($P < 0.0001$) and less dietary restraint ($P < 0.01$) than linoleic acid non-tasters. They did not differ in body composition, resting metabolic rate, respiratory quotient or macronutrient selection. Within the non-obese subgroup (BMI < 30 kg/m^2), 10 μM linoleic acid tasters had a significantly lower BMI ($P < 0.05$) and were less dietary restraint ($P < 0.05$) compared to linoleic acid non-tasters, whereas in the obese subjects (BMI ≥ 30 kg/m^2) these effects were not observed. We conclude that linoleic acid sensitivity might play a role in the etiology of obesity, in subjects in the range of normal weight to overweight.

Complex—'endogenous' and 'exogenous'—chemosensitivity is a general characteristic of glucose-monitoring neurons in the rat forebrain

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The mediodorsal and ventrolateral prefrontal (orbitofrontal) cortex (mdPFC and vlPFC, respectively), the nucleus accumbens (NAcc) and the ventromedial hypothalamic nucleus (VMH), constituents of the forebrain limbic circuitry, play important roles in various homeostatic regulatory functions. Classic and more recent studies provided evidence for the existence of special, 'glucose-monitoring' (GM) neurons in these structures, nevertheless, feeding-associated 'endogenous' and 'exogenous' chemosensitivities of these units are yet to be defined. In order to elucidate the above, complex chemical attributes of the cells, extracellular single neuron activity of the mdPFC, vlPFC, NAcc and VMH of Wistar rats was recorded by means of tungsten wire multibarreled glass microelectrodes during (1) microelectrophoretic administration of D-glucose and other chemicals, and (2) gustatory stimulations. Approx. 8–12% of the units tested in these forebrain regions changed in activity in response to micro-osmo-electrophoretically applied glucose. These chemosensitive cells were found to respond to a broad array of microiontophoretically administered neuromodulators as well. In addition to their endogenous chemical responsiveness, a substantial majority of the GM neurons of these structures were also modulated by intraoral gustatory stimulations. In parallel behavioral studies, GM neuron destroying bilateral streptozotocin (STZ) microinjections into either of the above forebrain regions, led to the development of characteristic taste perception deficits. The present and previous findings show an overlapping of the endogenous and exogenous chemosensory systems in these forebrain structures, further substantiating the significant adaptive role of GM neurons in integrative processes of the central regulation of feeding. [Supported by: Richter G. Ltd, HAS, Hungarian National Research Fund]

Food deprivation increases pERK, decreases pCREB in the rat PVN

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We previously found that food deprivation decreases pCREB (cAMP-response element binding protein), a member of MAP kinase signaling pathway, immunoreactivity in the rat PVN, and that RU486 blocked this decrease. This suggested that the increased plasma glucocorticoid during fasting might suppress CREB phosphorylation. In this study, we examined phosphorylated ERK-1/2 (extracellular signal-regulated kinase-1/2) level in the PVN of free fed or 48 h food deprived rats. Another group of rats received RU486 twice daily during the fasting period. Rats were transcardially perfused with 4% PFA in 0.1 M PB 48 h after food deprivation, the PVN tissue sections processed for immunohistochemistry with an antibody specific for pERK-1/2. A marked increase in pERK-1/2 immunoreactivity was detected in the PVN of deprived rats, and RU 486 failed to inhibit this increase. These results suggest that the fasting-induced up-regulation of pERK-1/2 in the rat PVN may not be mediated by plasma glucocorticoids. From in vitro studies reported, it can be suggested that glucocorticoid has an inhibitory effect in CREB phosphorylation, likely via the suppression of ERK-1/2 phosphorylation, and this suppression may occur by glucocorticoid-induced increase in the expression of the MAP kinase phosphatase-1. However, our results show that pCREB in the PVN was down-regulated during food deprivation, in spite of the up-regulation in pERK-1/2, upstream molecule of CREB in MAP kinase pathway. It is concluded that food deprivation may produce a multiple effects on MAP kinase pathway in the PVN.

Lithium-induced expression of TH and ICER in the locus ceruleus and the adrenal gland of rat

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Lithium has been widely used as a therapeutic agent for mood disorders, as a toxic for conditioned taste aversion (CTA) learning. Activity of the hypothalamic–pituitary–adrenal gland (HPA) axis is implicated in both the cases. We previously found that intraperitoneal lithium at a dose enough to induce CTA activates HPA axis, likely through its central action. Variety of stressful stimuli activates not only the HPA axis, but also the sympatho-adrenal (S/A) system. In this study, we examined gene expression of tyrosine hydroxylase (TH), rate limiting enzyme for norepinephrine (NE) biosynthesis, in the locus ceruleus (LC) and adrenal medulla (ADM) of rat after an intraperitoneal lithium injection (0.15 M LiCl, 12 ml/kg). The expression of inducible cAMP early repressor (ICER), which reported to be involved in TH expression and lithium-induced HPA activation, is also examined. Rats were perfused with 4% PFA in 0.1 M PB at each time point (0, 1, 6, and 24 h) after LiCl, the brains and adrenal glands sectioned, processed for in situ hybridization using cDNA probes of TH or ICER. TH expression level both in LC and ADM was increased until 6 h, returned to the base line by 24 h after the injection, interestingly, the adrenal expression appeared to be stronger than in LC. ICER expression showed the peak at 1 h in ADM was not detected in LC. These results suggest that lithium may stimulate NE synthesis, likely by a different mechanism in LC and ADM. [Supported by KISTEP grant (JWJ)]

Cholecystokinin (CCK) and bulimia nervosa (BN)

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One possible contributor to the increased meal size during binge eating in patients with BN is reduced release of CCK, compared with controls. Because CCK reduces food intake in humans and CCK antagonists increase it, CCK is an important modulator of food intake, which may operate by increasing the afferent vagal nerve activity that participates in food intake inhibition. Patients with BN have both decreased gastric emptying and decreased CCK release after a standardized food load. The difference in CCK release between patients and controls diminished and was no longer significant, when the food load was infused intraduodenally. Therefore, the disturbance in CCK release may be secondary to a disturbance in gastric emptying, which reduces the rate of delivery of CCK secretagogues to the small intestine, thereby reducing the rate of CCK release. Consequently, pharmacological agents that accelerate gastric emptying have potential in the management of bulimia nervosa by facilitating one of the inhibitory signals controlling food intake. Besides, reduced gastric emptying, patients with BN also exhibit greater tolerance to gastric distension than do control subjects. One possibility for CCK's role in excessive eating specifically during binge meals is that diminished CCK release reduces sensitivity to gastric stretch, thereby enabling the greater tolerance to gastric distention. Patients with BN may lose control of eating because one, or possibly more, inhibitory signals for eating are blunted. [Supported by: MH-42206 and DK-26687]

Measuring the effectiveness of glucose-sensing in controlling human intake of food

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We will show how data collected by others (Ciampolini, Teff, Westerterp) can be used in a paradigm that relates glucose metabolism or concentration to controls of normal food intake. In this paradigm an intake-related response must vary with the level of a potentially sensed glucose stimulus, with the usual influences on eating present, but not co-varying with the glucose signal. Two experimental measurements are needed: (1) stimulation of the sensors by glucose in the presence of all the other influences on intake (i.e. the stimulus); (2) the effect of that glucose level on the current tendency to ingest food (the response). The first clear evidence of an effect of glucose-sensing on food intake and sating of hunger in human volunteers is a stimulus of high and low levels of signals from diet-derived glucose and three responses: (a) decreasing pleasantness of eating, summed over staple foods, (b) amount wished to eat of specific usual foods, and (c) energy content of a test meal of ordinary foods. We then suggest ways of estimating the stimulus from slowly changing blood glucose, from the glucose dip and from short-term infusion of glucose. Finally we compare responses open to all influences on hunger, such as meal requests, cessation of an ad libitum meal, or rating 'how much of that food I'd like to eat right now', with ingestive microbehavioral parameters or wordings of ratings that may be more sensitive to glucose-sensing than to gastric fill, sensed food composition or memory of previous eating.

Transgenic over-expression of carnitine palmitoyltransferase 1α (CPT-1α) increases fatty acid oxidation

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CPT-1α catalyses the rate limiting step in mitochondrial β-oxidation of long-chain fatty acids. The aim of the present study was to examine the effect of transgenic CPT-1α over-expression on fatty acid oxidation. CPT-1α cDNA was amplified by PCR and placed under the control of a *tet* promoter. Transcription was controlled by a doxycycline-inducible transcription activator (rtTA) expressed from a second plasmid. Human 293T-line kidney cells were transiently transfected either with both plasmids or with only rtTA. Transfected cells were incubated for 24 h with a medium containing doxycycline and the fatty acid C17:1 bound to albumin. Cells were then harvested and broken up with ultrasonication and methanol, and fatty acid methyl esters were analyzed by gas chromatography. The concentration of C15:1, which is a unique product of C17:1 oxidation, was about five times higher in the double-transfected than in the single-transfected cells or untransfected cells. These data indicate (1) that the double-transfection protocol led to overexpression of CPT-1α in cultured cells and (2) that overexpression of CPT-1α in turn increased mitochondrial β-oxidation of fatty acids in these cells. The generation of rats with this inducible CPT-1α transgene in hepatocytes would permit a novel test of the putative contribution of hepatic fatty acid oxidation to the control of eating behavior.

Effects of green tea on weight maintenance after body weight loss

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This study was conducted to investigate whether green tea may improve weight maintenance by preventing or limiting weight regain after weight loss of 5–10% in overweight and moderately obese subjects. The study had a randomized, parallel, placebo-controlled design. 104 overweight and moderately obese male and female subjects (age, 18–60 y; BMI, 25–35 kg/m^2) participated in a very low energy diet intervention (VLED, 2.1 MJ/d) of 4 weeks followed by a weight maintenance period of 13 weeks in which they received green tea (caffeine, 104 mg/d; catechins, 573 mg/d: epigallocatechin gallate, 323 mg/d) or placebo. Subjects lost (mean ± SD) 6.4 ± 1.9 kg or 7.5 ± 2.2% of their original body weight during VLED ($P < 0.001$). Body weight regain was not significantly different between the green tea and the placebo group (30.5 ± 61.8 and 19.7 ± 56.9%, respectively). However, these results may have been affected by an increase in cognitive restraint during the weight maintenance period in the placebo group. In the green tea treatment, habitual high caffeine consumers showed a higher weight regain compared to habitual low caffeine consumers (39 ± 17 and 16 ± 11%, respectively; $P < 0.05$). We conclude that weight maintenance after 7.5% body weight loss was not affected by green tea treatment and that habitual caffeine consumption affected weight maintenance in the green tea treatment.

Analysis of AP-1 gene expression during conditioned taste aversion using laser capture microdissection of amygdala

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Conditioned taste aversion (CTA) occurs after pairing of a novel taste with a toxin (e.g. sucrose with LiCl). Expression of multiple transcription factors (e.g. Fos- and Jun-family members) after contingent taste and toxin pairing may be required for learning. In order to screen a large number of genes from small tissue samples, we analyzed the expression of the AP-1 transcription factors by PCR after laser capture microdissection of the amygdala. After implantation of intraoral catheters, rats were infused with 5% sucrose (6 ml over 6 min) or injected with LiCl (12 ml/kg, 0.15 M, i.p.) or given sucrose paired with LiCl, or not treated ($n = 3$ per group); 1 h later their brains were dissected. The lateral, basolateral, and central subnuclei of the amgydala of single 5 μm sections from individual rats were dissected using the Arcturus PixCell II system. RT-PCR for all AP-1 genes was performed. c-Fos expression was highly increased in all three amygdalar regions after LiCl and sucrose/LiCl treatment. Sucrose infusion alone did not increase c-Fos expression. unD expression was decreased in the lateral and basolateral amygdala after LiCl and sucrose/LiCl paired treatment. There was a tendency for Fra-2 to increase in basolateral amygdala after sucrose infusion. There were no differences between groups in c-Jun, JunB, FosB, Fra-1, or ICER expression. In summary, the high sensitivity of RT-PCR revealed AP-1 expression in the amygdala not previously reported during

CTA acquisition. However, no AP-1 family gene was uniquely expressed after the pairing of sucrose and LiCl. [Supported by: NIDCD03198]

Corticosterone promotes adiposity and food intake but does not determine lard ingestion (in diabetes)

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Insulin and corticosterone serve opposite roles in maintaining energy balance. Insulin inhibits feeding and stimulates overall energy storage, whereas corticosterone stimulates feeding and inhibits energy storage. In untreated diabetes, hyperphagia only occurs when corticosterone concentrations are at least ~4 μg/dl. On the contrary, in many models of obesity, adrenalectomy reverses many metabolic deficits. Untreated diabetic rats overeat on a high carbohydrate diet, show high NPY peptide and mRNA levels, and low CRFmRNA and peptide levels. Feeding untreated diabetic rats a high fat diet normalizes these changes. Since corticosterone is essential for the hyperphagia when fed a high carbohydrate diet, we tested whether adrenalectomy with replacement of corticosterone (B) in different doses would influence the fat-induced inhibition in food intake in diabetic rats. We adrenalectomized rats and provided them with 100% B, 35% B or no pellet. Rats were made diabetic with a s.c. injection of streptozotocin. Corticosterone was positively correlated with food intake in adrenalectomized diabetic rats replaced with different doses of corticosterone. Eating lard however decreased food intake significantly in diabetic rats independent of corticosterone. Interestingly, in non-diabetic control rats seven days of lard consumption increased total caloric intake, abdominal fat depots, insulin and leptin concentrations significantly, whereas body weight gain did not change. Adrenalectomy with 35% corticosterone pellets (with ~5 μg/dl levels) totally prevented these obesity features. These data suggest that corticosterone is not involved in the lard-induced decrease in food intake in diabetes, however it does play an important role in lard-induced obesity in non-diabetic rats. [Supported by: 'Dutch Diabetes Research Foundation' and DK28172]

Effects of feeding-related peptides in bombesin receptor subtype-3 deficient mice

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Bombesin receptor subtype 3 (BRS-3) is one of several bombesin (BN) receptor subtypes present in mammals. Unlike other BN receptor subtypes, the function of BRS-3 has been difficult to evaluate due to the lack of an identifiable endogenous ligand. However, BRS-3 deficient mice (KO) develop hyperphagia and obesity beginning at ~14 weeks of age suggesting that BRS-3 may participate in feeding and body weight regulation. It was demonstrated that hyperphagia in KO mice is accounted for by increased meal size, with no increase in meal number. This pattern suggests that BRS-3 may play a role in meal-related food intake and that increased feeding in KO mice could result from deficits in the response to short-term satiety signals. To examine this possibility, we compared the ability of cholecystokinin (CCK), BN and gastrin-releasing peptide (GRP) to suppress food intake in KO and wild-type (WT) mice. Mice were injected i.p. with either 0.9% saline or CCK (0.32, 1, 3.2, 10 nmol/kg), BN (1, 3.2, 10, 32 nmol/kg) and gastrin-releasing peptide (10, 32, 100 and 320 nmol/kg) and given 30 min access to a 0.5 kcal/ml glucose solution. We found no differences between WT and KO mice in either sensitivity to, or the magnitude of suppression by, CCK, BN or GRP. These data suggest that (1) alterations in the responsiveness to short-term satiety signals are not likely to contribute to hyperphagia in KO mice and (2) functional BRS-3 is not required for feeding suppression by CCK, BN and GRP. [Supported by: NIH grant DK46448]

Tonic regulation of satiety by 5-HT1B receptors in the mouse: converging evidence from antagonist studies and CP-94,253-induced c-fos immunoreactivity

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The preferential 5-HT1B receptor agonist CP-94,253 selectively decreases food intake in both rats and mice, supporting an important role for 5-HT1B receptors in the serotonergic control of satiety. Consistent with this, mice lacking 5-HT1B receptors are hyperphagic and weigh more compared to age-matched wild-type controls. Here we challenged the hypophagic action of CP-94,253 with the selective 5-HT1B antagonist SB224289 in mice given access to a test meal of palatable mash for 40 min. Wild-type sv129 mice were pre-treated with SB224289 (0, 2.5, 5.0 mg/kg i.p.) 30 min prior to treatment with CP-94,253 (0, 20 mg/kg i.p.) and 60 min before testing. CP-94,253 reduced mash intake ($P < 0.001$) by 65% compared to vehicle-treatment, an effect that was completely reversed by SB224289 at 2.5 and 5.0 mg/kg (pre-treatment × treatment interaction $P < 0.01$). SB224289 treatment alone produced

a significant and dose-dependant increase in food consumption ($P < 0.01$). Furthermore, analysis of time-sampled behavioural observations revealed that antagonist pre-treatment delayed the onset of satiety. In order to elucidate the brain loci involved we examined the pattern of c-fos expression in mouse brain following an anorectic dose of CP-94,253. Intense expression of c-fos was evident in hypothalamic and hindbrain regions (PVN, VMH, DMH, ARC, lPBN, NTS) and was also apparent in other structures including the nucleus accumbens and amygdala. These data indicate that acute stimulation of 5-HT1B receptors located in key feeding-related nuclei, causes hypophagia, complementing studies carried out in 5-HT1B receptor knockout mice. [Supported by: BBSRC-LINK award LKD/12007]

Proteome profiles of the rat brain regions after an intraperitoneal LiCl

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Lithium chloride induces c-Fos expression in the brain regions involved in conditioned taste aversion (CTA) learning. Induction of c-Fos expression implicates that numerous genes are going to be expressed in the c-Fos expressing neurons. New protein synthesis is required in CTA learning. In order to identify the protein(s) implicated in the lithium-induced CTA acquisition, we examined the proteome patterns of tissue lysates prepared from the hypothalamus, the amygdala, the hippocampus and the NTS regions by using proteomic analysis technique. Male SD rats (300–350 g) received an intraperitoneal LiCl (0.15 M LiCl, 12 ml/kg) were decapitated 1 h after the injection, the brain tissues were rapidly dissected on ice, frozen in LN_2, then stored at $-80\,^{\circ}C$ until used. Tissues were homogenized in Reagent R/S buffer and centrifuged. Supernatants were isoelectrofocused using Bio-Rad PROTEAN IEF CELL with IPG strip (pH 5–8), electrophorased on 12% of SDS gel. Gels were stained with coomassie blue. Five protein spots were newly detected in the lithium-injected brain samples. There were 23 spots showing stronger staining density, 32 weaker, detected in the gel prepared with the hypothalamic tissue lysate of lithium treated rat, compared to each relevant spot in the control. Fifty-five spots were determined to be up-regulated, 25 down-regulated in the amygdala, and 24 spots up-regulated, 34 down-regulated in the hippocampus, and 45 spots up-regulated, 23 down-regulated in the NTS samples by lithium, respectively. These protein spots were analyzed by MALDI-TOF peptide analysis system. [Supported by: KISTEP grant (JWJ)]

Additional protein intake limits weight regain after weight loss in humans

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This study was conducted to investigate whether addition of protein to the diet may limit weight regain after weight loss of 5–10% in overweight subjects. The study had a randomized parallel design. One hundred and twenty overweight subjects followed a very low energy diet (VLED) for 4 weeks after which a 6-month weight maintenance period followed. During weight maintenance subjects were randomized over a protein and a control group. The protein group received 48.2 g/d protein in addition to their diet, partly replacing protein intake at lunch. Body mass, body composition, waist circumference, eating behavior, substrate oxidation and relevant blood parameters were measured at baseline, after VLED and during and after weight maintenance. During VLED no differences between the groups were seen. During weight maintenance the protein group showed, compared to the control group, a significantly higher protein intake (18 vs. 15%, $P < 0.05$), a significantly lower weight regain (0.8 vs. 3.0 kg, $P < 0.05$), a significantly decreased waist circumference (-1.2 ± 0.7 vs. 0.5 ± 0.5 cm, $P < 0.05$), and a significantly smaller increase in RQ (0.03 ± 0.01 vs. 0.07 ± 0.01, $P < 0.05$). Weight regain in the protein group consisted of only fat free mass, while the control group gained fat mass as well. Satiety in the fasted state before breakfast increased significantly more in the protein group compared to the control group.

Effect of capsaicin on substrate oxidation and weight maintenance after modest body weight loss in humans

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This study was conducted to investigate whether capsaicin may improve weight maintenance (WM) by limiting weight regain after weight loss of 5–10%. In this randomized double blind placebo controlled study 91 moderately overweight subjects were randomly assigned to a RP and a placebo group. Four-week very low energy diet (VLED) was followed by a 3 months WM period. Measurements were done before and after VLED and after 3 months WM. The mean ± sd BM loss during the VLED (6.6 ± 2.0 kg $= 7.8 \pm 1.9\%$ of initial BM) was not different between the subsequent treatment and

placebo group. During weight maintenance mean% regain in the treatment was not significantly different compared to placebo (33.3 ± 35.5 vs 19.2 ± 41.8). Fat oxidation was greater in the capsaicin group compared to placebo (4.2 ± 1.1 vs 3.5 ± 0.9). A trend for a higher resting energy expenditure as a function of fat free mass was seen in the capsaicin group. While attitude towards eating, appetite profile, relevant blood parameters and physical activity did not differ significantly between both groups. These results indicate that capsaicin treatment has no limiting effect on 3 months weight regain after modest weight loss. However, capsaicin treatment showed a greater fat oxidation during weight maintenance compared to placebo.

Effect of the β3-adrenergic-receptor agonist CGP-12177A and the α1-adrenergic receptor antagonist Prazosin on food intake and hepatic fatty acid oxidation in male rats

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β3-Adrenergic-receptor agonists reduce food intake only when β3-adrenergic-receptors are present on white adipose tissue. The mechanism of this effect is unknown. A stimulation of lipolysis and/or an increase in hepatic fatty acid oxidation might be involved. α1-Adrenergic activation due to an increase in portal venous fatty acid levels might also contribute to the anorectic effect of β3-adrenergic stimulation. We therefore examined whether the α1-adrenergic receptor antagonist Prazosin modulates the effect of the β3-adrenergic-receptor agonist CGP-12177A on food intake and hepatic fatty acid oxidation. In a within-subject design, 12 h food deprived male rats ($n = 24$) were injected i.p. with saline, CGP-12177A (1 mg/kg BW), Prazosin (0.5 mg/kg BW) and a combination of both substances just prior to lights off, when food cups were reopened and food intake was measured. Only the combination of CGP-12177A and Prazosin reduced 30 min food intake (control: 5.2 ± 0.3 g; CGP-12177A: 4.5 ± 0.3 g; Prazosin: 4.4 ± 0.3 g; CGP-12177A/Prazosin: 2.7 ± 0.3 g; $P < 0.001$). In blood samples taken 30 min after injection from rats without access to food, plasma concentrations of free fatty acids (FFA) and β-hydroxybutyrate (BHB) of the two groups that received CGP-12177A were significant higher than those of the control and Prazosin groups. Plasma FFA and BHB concentrations did not differ between the CGP-12177A and the CGP-12177A/Prazosin groups. These findings do not support the idea that an increase in hepatic fatty acid oxidation or α1-adrenergic activation is involved in the feeding suppressive effect of β3-adrenergic stimulation.

The Freshman 15: a model for the study of techniques to curb the 'epidemic' of obesity

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In the first of two studies, 60 freshman were weighed during the first week of the semester, then 12 weeks later. The mean weight gain was 1.9 ± 2.4 kg (range −5.9 to +8.6 kg) and was significantly different from 0 ($P < 0.01$). Analysis of questionnaires completed at the end of the semester indicated that the variance in the weight gain could be attributed to (a) eating in an 'all-you-can-eat' dining halls [20%], (b) eating evening snacks [10%], (c) consumption of high fat foods [12%], (d) consumption of 'junk' food [8%] and other variables. Only 41% of the variance of the weight gain could not be accounted for by variables measured in the questionnaire. In a subsequent study, freshman gained 3.1 ± 2.0 kg during the first semester. In this study, the weight gain was totally prevented by giving bathroom scales to one group of students and having them email their weights daily during the semester. This group gained only 0.1 ± 3.1 kg, a value significantly less than the controls. The study demonstrates the importance of environmental cues to weight gain and the possibility of preventing the weight gain by self-monitoring. It also suggests the value of using the freshman weight gain as a model for studying potentially effective techniques to inhibit gain in weight.

Total sub-diaphragmatic vagotomy does not suppress the depression in food intake produced by a high protein diet in the rat

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Transition from a control (14%) to a high (50%) protein diet induces a rapid depression in food intake the first day and a progressive but incomplete return to the initial intake during the following days. This study was undertaken in order to evaluate whether the sub-diaphragmatic vagus nerve represents a main route involved in the depression in food intake produced by ingestion of a high protein diet in the rat. The results showed that a total sub diaphragmatic vagotomy did not (i) modify the daily food intake of the control diet, (ii) suppress the dramatic depression in food intake produced by an acute transition to a high protein diet and (iii) but in contrast slightly reduced the daily intake of a

high protein diet in acute condition or even after adaptation. (iv) Analysis of meal parameters and behavioral satiety sequence after adaptation indicated no presence of major metabolic distress. In conclusion, this study suggested that the sub diaphragmatic vagus nerve should not be an obligatory pathway to transfer information to the brain leading to the depression in a high protein diet intake. In contrast, the vagus could play a role in the peripheral recording of the presence of nutrients in the intestine in order to adapt the delivery of nutrients to the small intestine through a retro-control of gastric kinetic. Defect in this visceral regulating system could reinforce the metabolic-associated food intake depression signal and limits the ability of the animal to regulate protein and energy intake on the short-term.

Satiety and palatabiity factors involved in the depression of food intake produced by a high protein diet in rat

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The respective roles of conditioned food aversion, satiety and palatability in the depression in food intake produced by a high protein diet remained unclear. The influence of (i) the amount of proteins (50 or 70%), (ii) the type carbohydrates or protein sources, (iii) the amount of ingested fluid, was evaluated using different paradigms including determination of weight gain, food intake, meal pattern and behavioral satiety sequence. The results showed that behavioral and food intake parameters were disturbed during the first day of the transition phase from a normal to a high protein diet and that most of the parameters returned to baseline values after three days of high protein diet. Rats adapted to a high protein diet (P50 or P70) did not acquire a conditioned taste aversion but exhibited satiety associated to a normal behavioral satiety sequence. These experiments did not indicate a role for pre-absorptive factors in the depression of food intake induced by a high protein diet. Neither the low palatability of high protein diets nor physical factors associated to their ingestion (e.g. stomach volume) seemed to explain the depression. The initial reduction in high protein diet intake appeared to result from the lower palatability of the food combined with the satiety effect of the high protein diet and the delay required for metabolic adaptation to the higher protein level. After adaptation, the lower intake observed with the high protein intake is mainly related to the protein-induced satiety.

2-Deoxy-D-glucose (2DG) increases NPY mRNA expression in hindbrain neurons

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Previous results suggest that the orexigenic peptide, neuropeptide Y (NPY), may participate in glucoprivic feeding. NPY mRNA is increased by glucoprivation in the arcuate nucleus (Sergeyev et al., 2000; Fraley & Ritter, 2003) and NPY immunoneutralization in the paraventricular nucleus of the hypothalamus (PVH) impairs glucoprivic feeding (He & Edwards, 1998). Hypothalamic NPY innervation is derived substantially from hindbrain NPY neurons, most of which co-express norepinephrine or epinephrine and are located in catecholamine cell groups A1 and C1. Selective immunotoxin lesions have demonstrated that these hindbrain catecholamine neurons are required for glucoprivic feeding, as well as for glucoprivic stimulation of corticosterone secretion and suppression of estrus. However, the specific contribution of hindbrain NPY to these glucoregulatory responses has not been examined. Therefore, we examined the effect of glucoprivation on NPY mRNA expression in the A1 and C1 areas using in situ hybridization. One hour after 2-deoxy-D-glucose (2DG, 250 mg/kg), NPY mRNA expression in A1 and C1 neurons was increased. In A1 caudal to the area postrema, NPY mRNA level in each neuron was increased, but the number of NPY-positive neurons did not change. In the area of A1/C1 overlap, both NPY mRNA level and number of NPY-positive neurons were increased by glucoprivation. PVH microinjection of the retrogradely transported catecholamine immunotoxin, saporin conjugated to anti-dopamine-β-hydroxylase, destroyed A1 and A1/C1 neurons and abolished NPY expression in these areas. Thus, NPY mRNA is inducible in hindbrain neurons by glucoprivation, suggesting that NPY may contribute to glucoprivic feeding and other glucoregulatory responses.

Furosemide supports acquisition of a learned taste aversion

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The diuretic and natriuretic action of furosemide (Furo) is well known, but only modest attention has been given to its apparent anorectic action. We investigated the avoidance of Na^+-free chow (ICN) following treatment with 10 mg of Furo given in a single or divided dose in rats that had pre-treatment access to either the ICN or the

standard Na^+-replete chow (Teklad). After Furo, all animals reduced their intake of the ICN food. Nevertheless, dosing schedule and immediate dietary history influenced the magnitude of suppression. In general, the magnitude of suppression was divided dose > single dose and pre-Furo access to ICN > Teklad food. The effect of dosing schedule suggests that the post-treatment suppression of ICN intake reflects an ongoing malaise produced as a direct consequence of the Furo administration. On the other hand, the effect of pre-exposure to the ICN diet, independent of dosing schedule, suggested that some aspect of the post-furo anorexia was learned. To test this, additional groups of animals were used to determine whether furosemide (2 and 10 mg) could serve as an unconditioned stimulus in a conditioned taste aversion paradigm using 0.2 M sucrose as the conditioned stimulus. A saline injection group served as control. The results show clearly that animals developed an aversion to sucrose when paired with 10 mg Furo, but not with 2 mg Furo or saline. Thus, the suppressive effects of high dose Furo on food intake might be due in part to a conditioned response. [Supported by: NIH DC 00240, DC 05156, and DC 05435]

Leukotrien and purinergic receptors are involved in the hyperpolarizing effect of glucagon on the hepatic membrane potential

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Previous studies have shown that pancreatic glucagon (G) infusion reduces, and glucagon antibody (GAb) infusion increases food intake in rats. In agreement with Russek's potentiostatic hypothesis, which postulates a link between the hepatic membrane potential, the discharge rate of vagal afferents and the control of food intake, we recently found that G dose-dependently hyperpolarized the liver cell membrane whereas infusion of GAb depolarized the liver cell membrane. While G's effect appears to depend on opening of K-channels, it was the aim of the present study to further analyze the mechanisms underlying G's hyperpolarizing effect on the liver cell membrane (approx. 5 mV) under in vitro conditions. Leukotrien (LT) signaling appears to be involved in G-induced hyperpolarization because blockade of this system reduced G's effect on the liver cell membrane potential [(1) Ca-free superfusion conditions; (2) blockade of phospholipase A2, which is Ca-dependent; (3) blockade of lipoxygenase; (4) specific LT-B4 antagonist]. Further, signaling via purinergic receptors may also be involved in G's effect through G-induced release of purinergic agonists like ATP or UTP. G's effect was blocked by an antagonist of purinergic receptor subtypes (P2Y1, P2Y4, P2Y6) and by inhibition of phospholipase C which is involved in the signaling cascade of purinergic receptors. In addition, UTP or an ecto-ATPase stable ATP analogon hyperpolarized liver cells similar to G. G-induced hyperpolarization of liver cells seems to involve leukotrien and purinergic receptor-mediated opening of K-channels.

Amylin's anorectic effect depends on an intact lateral parabrachial nucleus (lPBN)

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Amylin is a satiating hormone acting via area postrema (AP) neurons. Peripheral amylin's anorectic effect is ablated in AP-lesioned (AP-X) rats, and amylin specifically activates AP neurons. Amylin also leads to a strong expression of c-Fos protein in the nucleus of the solitary tract (NTS), lateral parabrachial nucleus (lPBN) and central nucleus of the amygdala (AMG). The effects in the NTS, lPBN and AMG are blocked in AP-X rats. We now wanted to test whether the amylin-induced activation of lPBN neurons is a necessary component in the signalling cascade of amylin's anorectic effect. We produced rats with an electrolytic lesion in the lPBN (lPBN-X) and compared the effect of amylin on food intake in these rats to sham-operated controls (SHAM). Amylin (i.p. injection in 24 h food deprived rats; 5 μg/kg) potently reduced food intake in SHAM but not in lPBN-X rats (e.g. 1 h food intake, SHAM control 7.4 ± 0.8 g vs. amylin 4.0 ± 0.5 g [$P < 0.01$]; lPBN-X 8.9 ± 1.0 vs. 8.0 ± 0.6 g [n.s.]). After completion of the feeding studies, rats were used for immunohistochemical detection of c-Fos protein following amylin injection. While amylin produced strong c-Fos induction in the AMG in SHAM rats, no induction was seen in lPBN-X rats. Amylin, however, produced a similar c-Fos response in the AP in either SHAM or lPBN-X rats. We conclude that amylin's anorectic effect depends on an intact lPBN and that the AMG is the subsequent relay station in the central signalling cascade mediating amylin's anorectic action.

Alterations in food intake by opioid and dopamine signaling pathways between the Ventral Tegmental Area and the shell of the Nucleus Accumbens

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Reward is an important factor motivating food intake in satiated animals. Two sites involved in the reward response are the Ventral Tegmental Area (VTA) and the Nucleus Accumbens shell region (sNAcc), between which communication is partially regulated by opioids and dopamine (DA). Previous studies have shown that the m-opioid agonist Tyr-D-Ala-Gly-MePhe-Gly(ol)-enkephalin (DAMGO) dose-dependently enhances food intake in satiated animals when injected into either the VTA or the sNAcc. The enhanced intake elicited by DAMGO injected into the sNAcc was dose-dependently blocked by injection of naltrexone (NTX) bilaterally into the VTA, indicating an opioid-dependent signaling pathway from the sNAcc to the VTA in mediation of food intake. In the present study, we cannulated animals bilaterally in both the VTA and the sNAcc to further study the nature of opioid- and DA-dependent communication between the sites. Food intake elicited by DAMGO (2 or 5 nmol) injected unilaterally into the VTA was dose-dependently diminished by bilateral injection of NTX (2.5, 5, and 25 mg per side) or the D1 antagonist SCH 23390 (3, 1, 0.3, 0.15, 0.05, and 0.015 nmol per side) into the sNAcc. When DAMGO (5 nmol) was injected into the sNAcc, the resulting food intake was decreased by doses of SCH 23390 ranging from 0.05 to 100 nmol per side injected bilaterally into the VTA, but not by equimolar doses of Raclopride, a D2 antagonist. These results, combined with previous findings, suggest a signaling pathway between the VTA and the sNAcc in which opioids and DA facilitate feeding in an interdependent manner.

Mercaptoacetate stimulates eating in untreated ovariectomized rats, but not in estradiol treated ovariectomized rats

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Mercaptoacetate (MA) blocks hepatic mitochondrial β-oxidation of long-chain fatty acids, an important energy metabolism pathway in rats or humans adapted to diets with medium (MF) or high (HF) fat content. MA also stimulates feeding in male MF- or HF-fed rats, indicating that hepatic fatty acid oxidation is a physiological controller of feeding. Here we examined the effect of MA in female rats for the first time. Long-Evans female rats fed a MF diet (19% fat by weight) were ovariectomized treated with estradiol (2 μg once each 4th d, SC) or the sesame oil vehicle (100 μl) alone. MA (2 ml/kg of 300 μmol/kg MA) or equiosmotic saline was injected i.p. in the middle of the light phase, and food intake was measured 2–12 h later. MA significantly increased feeding for at least 4 h in oil-treated ovariectomized rats, which is similar to its effect in males. In contrast, MA tended to decrease feeding in estradiol-treated ovariectomized rats, so that the difference in MA's effect between oil- and estradiol-treated rats differed significantly. These data demonstrates that estradiol can profoundly modulate the feeding effect of MA. It is not clear whether this estradiol-dependent feeding response results from altered hepatic metabolism, altered vagal signaling, or altered central information processing. [Supported by: DK 54523]

MTII, AGRP(83-132) and SHU9119 do not influence ethanol intake in Marchesian-Sardinian alcohol-preferring rats

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The Marchigian-Sardinian alcohol-preferring (msP) rat exhibits high spontaneous alcohol intake. To investigate the central mediation of this, we tested the effects of lateral ventricle (LV) injections of a melanocortin 3/4-receptor (MC3/4-R) agonist and antagonists on 10% ethanol intake in male msP rats. First, we tested chronic (0.01 nmol/rat-d for 9 d) and acute (0.1 nmol/rat) LV injections of the MC3/4-R agonist MTII. Injections were at dark onset, and access to 10% ethanol was limited to the first 2 h of the dark. Chow and water were available ad libitum. Next we assessed the acute effects of dark-onset LV injections of two non-selective MC3/4-R antagonists, AgRP(83-132) (0.01, 0.1 and 1 nmol/rat) and SHU9119 (0.1, 0.5 nmol/rat) in rats that had unlimited access to ethanol, chow and water. None of the treatments affected ethanol intake. In contrast, MTII reduced food intake, and AgRP(83-132) and SHU9119 each increased food intake. Therefore, MC3/4-R reached by LV injections of these three drugs do not appear to play an important role in the control of ethanol intake in male msP rats. This contrasts with one report that LV MTII decreased ethanol intake in another strain of alcohol-preferring rats (Ploj et al., 2002). [Supported by: NIH AA 12880]

Ploj, K. et al. (2002). *Brain Research Bulletin* 59(2), 97–104.

Palatability and energy density: an industrial perspective

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The food industry promotes intake of specific foods as well as providing options and solutions for problems of

weight control. How can these roles be balanced, and where can research on palatability and energy density be translated into product development? While palatability may be an important stimulant to intake, offering less acceptable products is clearly not a viable alternative. Energy content, portion size, and marketing are the main arenas in which industry can make changes promote weight control within mainstream foods. Energy density by itself is a difficult concept to apply commercially, partly because it is only meaningful in the context of food type/category, actual usage patterns, and portion size. For many products, there is little incentive to make modest changes change in compositions, while large changes are technically demanding and often result in significantly reduced product performance or consumer acceptance. Commercial food products must meet regulatory and technical quality standards, and generate a significant return on investment. The challenges are: (1) to foster positive economic incentives for low- and reduced-energy foods (consumer education, marketing, food labelling and economic policies), (2) continued technical quality improvement in lower-energy versions of popular commercial foods, and (3) ensuring best uptake and long-term acceptance of such products. The latter issue must take account of possible acquired changes in palatability, e.g. linked to the physiological effects of foods. Lastly, functional agents may increasingly be used to bridge the gap, offering improved weight control without other changes to product quality.

Exercise attenuates the hyperphagia associated with a palatable diet in male, but not female, rats

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Maintaining rats on a palatable diet promotes hyperphagia and body weight gain. It is not known, however, whether these effects are attenuated by giving rats the opportunity to exercise. To investigate this hypothesis, male and female rats ($n = 8$/group) were housed in cages connected to running wheels (RWs) and food intake, activity, and body weight were monitored under two test conditions. During an 8-day baseline phase, rats were given free access to chow, with and without access to RWs. During a 16-day hyperphagia phase, rats were given free access to a palatable diet (sweetened condensed milk diluted 1:2 with tap water) and chow, with and without access to RWs. Introduction of the milk diet increased daily food intake in males (baseline: 37.7 ± 1.0 kcal/100 g BW vs. hyperphagia: 41.9 ± 0.9 kcal/100 g BW, $P < 0.05$) and in females (baseline: 34.0 ± 0.9 kcal/100 g BW vs. hyperphagia: 45.9 ± 0.5 kcal/100 g BW), when access to RWs was denied. This expression of hyperphagia was greater in females than in males ($P < 0.001$) and resulted from females consuming more of the milk diet than males (females: 37 ± 1.1 kcal/100 g BW vs. males: 29.9 ± 1.1 kcal/100 g BW, $P < 0.001$). When access to RWs was permitted, males decreased food intake to baseline levels, while females remained hyperphagic. These data suggest that females are more vulnerable than males to body weight gain when maintained on a palatable diet that promotes hyperphagia. [Supported by: MH 63787]

CCK and GRP receptor deficient mice demonstrate alterations in meal patterns consistent with disruptions in satiety

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Cholecystokinin (CCK) and gastrin releasing peptide (GRP) inhibit food intake following their exogenous administration. Roles for endogenous CCK and GRP in mediating satiety have been suggested by demonstrations of the ability of specific receptor antagonists to increase feeding in rats. Targeted gene deletion provides the opportunity to more completely assess the role of a specific peptide system in ingestive behavior. Although a spontaneous mutation resulting in a 6 kb deletion in the gene for CCK-A receptors resulted in clear hyperphagia and obesity in OLETF rats, targeted deletion of the CCK-A receptor gene in a mouse did not appear to affect food intake in that these mice had normal 24 hour intakes and normal body weight. Examination of meal patterns in the OLETF rat had revealed that an 80% increase in meal size and an incomplete compensatory decrease in meal number accounted for the hyperphagia. Examination of patterns of spontaneous food intake in CCK-A receptor knockout mice revealed a similar, although smaller, alteration in meal patterns. CCK-A receptor knockout mice had significantly elevated nocturnal meal size. A similar pattern of results was found in GRP receptor knockout mice. An evaluation of meal patterns showed that GRP-R knockout mice ate significantly more at each meal than wild type mice, although total 24 h food consumption was equivalent. These data demonstrate that CCK-A and GRP receptors participate in the termination of meals in mice and show the importance of examining meal patterns in revealing how targeted genetic alterations can affect ingestive behavior. [Supported by: DK46448 and DK57609]

PYY(3-36) inhibits food intake and gastric emptying in rhesus monkeys

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PYY(3-36) has been recently demonstrated to inhibit food intake following peripheral administration. To characterize the mechanisms through which PYY(3-36) acts to affect food intake, we monitored daily 6 h patterns of food intake in male rhesus monkeys following IM administration of 0.3, 1 and 3 nmol/kg PYY(3-36) administered 10 min prior to food access. PYY(3-36) significantly inhibited 6 h food intake at doses of 1 and 3 nmol/kg. Analysis of meal patterns through the 6 h feeding period indicated that 1 and 3 nmol/kg doses of PYY(3-36) resulted in increased latencies to the first meal, and a reduction in average meal size with no change in meal number. The major effect of PYY(3-36) occurred during the first half of the 6 h feeding period. Rates of intake during the second 3 h were comparable. We also assessed the effects of the two larger doses of PYY(3-36) on gastric emptying in monkeys with chronic indwelling gastric cannulas. PYY(3-36) significantly inhibited the 10 min emptying of 0.9% NaCl. 1 nmol/kg suppressed emptying by 75% and emptying was completely prevented at the 3 nmol/kg dose. These data demonstrate the effectiveness of PYY(3-36) in inhibiting food intake in rhesus monkeys and suggest that it may do so through mechanisms that enhance satiety. [Supported by: DK19302]

Dysregulated insulin release in rats following blockade of the melanocortin system during acute, but not chronic exposure to a high-fat diet

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In previous work, we observed that blockade of the brain melanocortin system in rats by 14-day i.c.v. infusion of SHU9119 caused obesity, which was more pronounced under chronic high-fat (HF: 60% of energy) feeding relative to high-carbohydrate (HC: 60% of energy) feeding. Despite exaggerated hyperleptinemia in SHU9119-treated HF rats relative to HC rats, plasma insulin and adiponectin levels were comparable among diet groups. The present study investigated whether these secretion profiles of adipose and pancreatic hormones are influenced by the duration of adaptation to HF feeding before SHU9119 treatment. Therefore, rats were either adapted to HF feeding starting 2 months prior to the onset of SHU9119-infusion (LT), or were switched from HC to the HF diet at the onset of SHU9119 infusion (ST). Following 14-day SHU9119 treatment, early light phase plasma leptin levels were not different among groups (44.4 $\pm$ 7.7 ng/ml in LT and 36.5 $\pm$ 5.3 ng/ml in ST rats). Baseline plasma adiponectin levels were significantly higher in LT (7.9 $\pm$ 0.9 mg/ml) than in ST rats (5.0 $\pm$ 0.4). Interestingly, plasma insulin levels were markedly higher in ST (33.0 $\pm$ 7.4 ng/ml) than in LT (8.3 $\pm$ 1.1 ng/ml) rats. Thus, despite comparable increases in food intake, plasma adiponectin was 36% lower, whereas plasma insulin was 400% higher in ST relative to LT rats. This dramatic increase in plasma insulin concentration in ST rats might indicate severe insulin resistance as a consequence of acute HF exposure and low brain melanocortin activity. [Supported by: QLK4-CT-2001-51977 to CM]

Dissociation of conditioned taste aversion and conditioned immune response resulting from lesions of the central gustatory system

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The present study examined the effect of bilateral ibotenic acid lesions of the gustatory nucleus of the thalamus (THLx), and the medial and lateral parabrachial nuclei (MPBNx, LPBNx) on acquisition of conditioned taste aversion (CTA) and conditioned immune response (CIR). First, we tested the ability of lesion and control rats to acquire a CTA when either 0.1 M NaCl or 0.2 M sucrose was paired with the immunosuppressant drug, cyclophosphamide (CY, 15 mg/kg BW). Half of the rats in each group received NaCl and the other half sucrose. Two weeks later, the rats were tested for acquisition of a LiCl-induced CTA (0.1 M, 1.33 ml/100 g BW) using the same sapid stimuli in counterbalanced order. The results showed that the control and THLx groups learned to avoid consuming the taste associated with CY and LiCl treatment, while both PBNx groups did not. One month later, lymphocytes were collected from tail blood prior to (baseline) and 7 days following re-exposure to the sapid stimulus previously paired with CY. Re-exposure to the conditioned stimulus increased concanavalin A-induced T lymphocyte proliferation above baseline levels in the control and pontine lesion animals. The results suggest dissociation between the effects of the thalamic and pontine lesions on conditioned responses. Specifically, the THLx lesion altered CIR, but not CTA, compared with controls, while the MPBNx and LPBNx had the opposite effect. [Supported by: NIH DC 00240 and DC 05156]

Conditioned taste aversion after repeated high dose CCK

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Systemic administration of cholecystokinin octapeptide (CCK) activates gastric vagal afferents carrying viscerosensory signals to central brain regions. We have previously established that CCK activates noradrenergic (A2) inputs to the amygdala, where such mechanisms have been shown to exert profound effects on learning and memory. Pharmacological doses of CCK (10–100 μg/kg) are anorexigenic, and produce central neural and endocrine response profiles indistinguishable from those produced by LiCl, a classic model of toxemia and conditioned taste aversion (CTA). Previous studies have suggested that CCK treatment does not reliably support the formation of CTA. These findings may be attributed to the short-lived effects of systemic CCK (~10–15 min) rather than its inability to recruit central neural substrates important in aversive learning. In the present study, we prolonged the effect of CCK by giving adult male Sprague-Dawley rats either one or two i.p. injections (spaced 15 min apart) of CCK (10 μg/kg) or saline. A sensitive indicator of CTA using a flavor preference test was subsequently performed. The double-CCK paradigm produced a clear CTA (66%:34% preference ratio for saline-paired flavor vs. CCK-paired flavor in the flavor preference test; $P < 0.0001$), whereas the single-injection paradigm did not (46%:54%; $P = 0.3$). These findings are consistent with the view that noradrenergic inputs to the hypothalamus and amygdala are recruited to mediate the aversive properties of high-dose CCK. Thus, CCK is an adequate model for investigating the underlying neural substrates of viscerosensation and emotional learning.

Effects of milk availability during nursing on subsequent fat appetite and conditioned responses to fat

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The intake-stimulating effects of fat taste do not emerge until relatively late in preweaning development. It is uncertain whether fat appetite is influenced by early experience. We have recently demonstrated that milk exposure during nursing has lasting effects on fat appetite after weaning. Rats reared in small litters (increased milk availability) treat fat as less acceptable and less preferred after weaning than rats reared in large litters (decreased milk availability). The motivational basis for this difference is uncertain. The present experiment assessed the 'oral reward' value of fat in these rats by repeatedly pairing an arbitrary flavor with fat, and then measuring conditioned preference for that flavor. Juvenile rats from large litters ('LL', 12/pups litter) and small litters ('SL', 6 pups/litter) were compared. Rats were given a fat solution (10% corn oil + 0.2% saccharin) with a particular flavor (CS +) added, and on alternate days a different flavored solution (CS −) without the fat (0.2% saccharin only). Solutions were provided 30 min/day during food restriction, for 10 days. Then rats were tested for a preference between the CS + and CS − flavors, each presented in saccharin only, in 30-min/day 2-bottle tests. The LL rats and SL rats significantly preferred the CS + flavor over the CS − flavor. The strength of this conditioned preference did not differ between the LL and SL rats. Therefore, although SL rats treat fat as less acceptable and less preferred than LL rats, this may not be due to a change in the oral reward value of fat.

Chronic psychosocial stress effects on body composition, behavioral, and neuroendocrine profiles of mixed-gender VBS rat colonies

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The Visual Burrow System (VBS) model serves as a potent inducer of chronic psychosocial stress in mixed-gender rat colonies (four males, two females per colony) by allowing the animals to create a dominant (DOM)/subordinate (SUB) social hierarchy. Depending upon their social status, differences in neuroendocrine stress measures, behavior, body weight, and body composition were observed among these animals when housed in mixed-gender colonies. Because these differences were apparent only within mixed-gender colonies, we wanted to assess their occurrence after varying the colonies' composition (4 males, 2 females vs. 4 females, 2 males). Social status was determined by standard criteria. In both types of colonies, SUB had higher corticosterone and lower testosterone levels than DOM and control (CON) rats. Carcass analysis revealed that SUB had a higher percentage of water than CON or DOM, and a lower percentage of fat and lean body mass than CON and DOM. Consistent with this, plasma leptin levels were higher in SUB relative to DOM. A striking finding was that the SUB in the 4 female, 2 male colonies had a significantly higher percentage of body fat than the SUB in the 4 male, 2 female colonies. These data suggest that

changes associated with chronic psychosocial stress include body composition as well as body weight, and that specific changes vary with the composition of the colony. [Supported by: NARSAD, Guggenheim Foundation, and NSF]

Recognition of food stimuli including amino acids in the brain

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It has been suggested that the forebrain such as the amygdala (AM) and hypothalamus is a higher center to modulate activity of a basic neural system for ingestive behavior in the brainstem by descending projections to the brainstem. In the present study, neuronal activity was recorded in the AM and parabrachial gustatory nucleus in the brainstem of rats during discrimination of conditioned sensory stimuli associated with rewards and ingestion of taste solutions including amino acids. In the parabrachial nucleus, interneuronal correlation coefficients and factor analysis indicated that both the sodium cation and glutamic anion contributed to the neural activity elicited by MSG. Guanosine potentiated the responses to MSG, but only in neurons that also responded to sucrose. These results suggest that the gustatory contribution to the flavor of umami may be mediated by neurons that also responded to chemicals described by humans as sweet. In the amygdala, patterns of the correlation coefficients between the taste stimuli and the multidimensional scaling derived from the neuronal responses suggest that taste is encoded in the AM based on the palatability of the taste chemicals, and the AM plays a role in the association of taste stimuli with other sensory stimuli. Along with the resent studies indicating a role of the forebrain in modulating taste responses in the lower brainstem taste center, the results suggest a complementary role of the forebrain and brainstem in ingestive behaviors.

Preference for sweet foods and higher BMI in patients on methadone maintenance

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Past research indicates that patients on methadone maintenance treatment frequently have poor nutritional status and consume sweet foods in excess (Best et al., 1998; Zador, et al., 1996). This study attempted to confirm these findings and to measure any food cravings methadone users might experience. Fourteen participants on methadone maintenance and 14 controls were administered a questionnaire containing questions regarding food preferences and eating behaviors. Participants were asked to consider a number of eating behaviors and rate how often they engaged in them. They were also asked to rate their desire at the moment of testing for specific foods and how much of that food they would like to consume. Statistical analyses (ANOVA) revealed that the participants on methadone had significantly higher cravings for desserts, chocolate, candy and pizza and wished to eat them in higher quantities than controls. Significant differences were also found for their reported eating behaviors: participants reported higher intake of sweet foods than controls. The methadone patients had a higher mean body mass index (BMI) than the members of the control group. While the findings of this study allow only for limited conclusions to be made, the results support the hypothesis that patients on methadone maintenance report food cravings for and higher consumption of sweets. Higher consumption of sugars and fat may be responsible for the patients' higher BMI.

Self-esteem and BMI predict dietary restraint in women

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Body dissatisfaction increases the risk for bulimia (Stice & Shaw, 2002) probably via restriction of food intake (van den Berg et al., 2002). Negative body image, low self-esteem, and degree of disturbed eating are correlated and it has been suggested that both self esteem and body image predict eating disorders (Mintz & Betz, 1988). The Three-Factor Eating Questionnaire, Rosenberg Self Esteem Scale, and figure rating scale (current − desired = figure rating difference, FRD) were administered to 71 women (mean age = 20.79 years) with mean body mass index (BMI) = 22.75 (SEM = 0.29). Significant correlations were found between restraint and self esteem ($r = -0.311$, $P = 0.010$), FRD ($r = 0.382$, $P = 0.001$), and BMI ($r = 0.394$, $P = 0.001$). Restraint was not correlated with disinhibition which was correlated with FRD ($r = 0.333$, $P = 0.006$) and self-esteem ($r = -0.443$, $P = 0.000$). Multiple regression revealed that 23% of the variance in restraint was predicted by BMI and self esteem. 23.3% of the variance in disinhibition was predicted by self-esteem and FRD. Independent group t-tests revealed no differences in self-esteem, restraint or disinhibition between overweight women ($n = 15$) and normal weight ($n = 56$) although there was a difference in FRD, $t(69) = -2.928$, $P = 0.005$. Women in the bottom half of the self-esteem range had higher disinhibition ($t(66) = -2.430$,

$P = 0.018$), higher restraint ($t(66) = -2.377$, $P = 0.020$), and higher FRD ($t(69) = -2.081$, $P = 0.041$). These data confirm that self-esteem is an important factor in the prediction of restraint and disinhibition and that body dissatisfaction is related to low self-esteem. BMI was a better predictor of restraint than self-esteem.

Behavioral and physiologic responses to reduced caloric availability in mice

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Controlled reductions in food availability produce marked adaptive responses in mouse behavior and physiology. The symposium presentation will summarize recent experiments examining the behavioral (ingestive behavior patterns and locomotor activity) and physiologic (oxygen consumption and heart rate) responses to fasting and caloric restriction in mice. The crucial role of ambient temperature (T_a) in the study of mouse behavior and physiology will be emphasized. If T_a is increased from standard conditions (T_a = 23°C) to thermoneutrality (T_a = 30°C), mouse food intake is rapidly reduced in proportion to energetic requirements. The behavioral and physiologic responses to negative energy balance are fundamentally altered by the mild cold stress associated with standard housing conditions (T_a = 23°C). Torpor and light-phase hyperactivity are evident in mice subjected to caloric restriction when housed at T_a = 23°C, but not evident during caloric restriction at T_a = 30°C. Recent studies with A^y mice will be summarized to examine the role of melanocortin signaling in these responses. The influence of ambient temperature on behavior and physiology has important implications for the use of mouse models in obesity, cardiovascular and aging research.

Melanocortin agonist treatment of the melanocortin type 4 receptor increases phosphorylated MAPK in vitro

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Central administration of melanocortin agonists or antagonists is well known to alter food intake. MTII, a synthetic melanocortin agonist, produces long-term decreases in food intake while SHU9119, a synthetic melanocortin antagonist, produces long-term increases in food intake. The long-lasting actions of melanocortins suggest that changes in gene expression are involved; however, a mechanistic connection between gene expression and melanocortin receptors has not been demonstrated. Using COS-1 cells transfected with melanocortin receptor type 4 (MC4-R) DNA and treated with MTII, we showed that in addition to the previously demonstrated increases in cAMP formation through MC4-R, activation of this receptor by MTII increased phosphorylated (activated) MAPK. The activation of MAPK through MC4-R was dose-dependent with concentrations ranging from 1×10^{-10} to 1×10^{-6} M increasing phosphorylated MAPK. A time course analysis showed that phosphorylated MAPK levels increased significantly 5, 10, 30, and 60 min after MTII treatment with a maximum increase at 10 min. We are currently investigating the effect of antagonist treatment on the phosphorylation of MAPK. These data provide a novel signaling mechanism for MC4-R and suggest that activation of MAPK may be one way that MC4-R regulates gene transcription and possibly modulates food intake. [Supported by: NIH awards DK64012 (D.D.), HL58792 (D.K.Y.), and DK052018 (S.J.F.)]

Multiple conductances modified by CCK and leptin induce activation of vagal afferent neurons

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Vagal afferent neurons carry gastrointestinal sensory information, including satiation signals, to the central nervous system, and thereby participate in the control of food intake. Recently we demonstrated that both CCK and leptin induce electrical activation as well as increases in cytosolic calcium in cultured vagal afferent neurons. Furthermore, CCK and leptin appear to act synergistically to reduce food intake and activate of vagal afferents. In the present study, we used simultaneous electrophysiological and calcium imaging measurements on cultured vagal afferent neurons, to demonstrate that oscillatory increases in cytosolic calcium induced by CCK are dependent upon bursts of action potential firing. To investigate the electrical basis for this response we examined the effects of CCK and leptin on background currents in cultured nodose neurons using patch-clamp electrophysiology. We found that only CCK increased a depolarizing cationic current, while both CCK and leptin inhibited a hyperpolarizing K^+ current. The fact that both CCK and leptin inhibited the K^+ current suggests that modulation of this conductance might be a mechanism through which these two substances cooperate to activate vagal sensory neurons and enhance each other's effects on food intake. Additional studies will be needed to determine whether CCK and leptin affect other ionic

conductances. [Supported by: NS20561 and a grant from the Autzen Endowment]

Some considerations for the use of hepatic portal vein cannulation in the study of food intake

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The liver plays a major role in energy balance, and hepatic portal vein cannulation often has been used to assess the contribution of the liver to the control of food intake. Typically, nutrients, such as glucose, that are infused into the portal vein suppress feeding more than when they are infused into the jugular vein; however, we have observed significant variability in the inhibitory effects of intraportal nutrient infusions. The current study was conducted to determine whether catheter tip placement and liver perfusion were critical factors in this variability. Catheters were inserted into the portal vein of rats to rest close to the liver (LC, $n = 8$) or far (3 cm upstream) from the liver (LF, $n = 8$). Four weeks later, rats were anesthetized and methylene blue was infused into the portal vein. The five liver lobes were homogenized and the amount of dye in each lobe was determined by spectrophotometry. Dye was concentrated primarily in the caudal and right lobes in LC animals, whereas dye was evenly distributed across lobes in LF animals. The placement of the catheter tip close to the liver also resulted in significant thickening/necrosis of the portal vein. These results show that placing the cannula tip close to the liver can produce inadequate hepatic lobe perfusion and may account for some of the variability observed in the feeding responses of animals following intraportal nutrient infusion. [Supported by: NIH grants DK 36339 and DK 02894. M. Piñon is a fellow of DEDICT-COFAA, IPN; Mexico]

Individual differences in high-fat diet hyperphagia

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Rats fed a liquid high-fat (HF) diet have greater average caloric intake than weight-matched rats fed isocaloric (kcal/g) high-carbohydrate (HC) diet. HF is avidly sham-fed, indicative of its high palatability, but this attribute is not necessary for the hyperphagic response to HF since overeating also occurs during self-regulated intragastric feeding. However, diet palatability presumably contributes since manipulating fat palatability in other paradigms influences intake. Among rats fed HF there is considerable variability in the magnitude of hyperphagia; of interest is whether certain behaviors/responses discriminate between rats that have relatively high intake when offered HF ('High eaters'), and rats that have lower intake ('Low eaters'). High eaters ate more meals per day than Low eaters, but meal size did not differ. Current studies investigate whether individual differences in sensitivity to the orosensory and/or postingestive effects of HF predict the eating response to HF. Sham-feeding intake of HF assesses taste sensitivity, while the degree of suppression of test meal intake following a HF preload indicates sensitivity to the satiating effect of fat. Following these tests, HF is consumed ad lib for >10 days. Preliminary results indicate that taste responsivity predicts intake: rats with high sham-feeding intake of HF were subsequent High eaters, while lesser sham-intake was characteristic of Low eaters. [Supported by: NIDDK 55367]

Fenfluramine-induced hypophagia, but not hypoactivity, is sexually dimorphic

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Increased activity within the serotonergic system has been implicated in anorexia nervosa, an eating disorder that is more prevalent in women than men. It is possible, therefore, that females may be more sensitive to the hypophagic effects of drugs that enhance serotonergic neurotransmission. To test this hypothesis we examined food intake in rats treated with the serotonin agonist fenfluramine. Male and female rats ($n = 8$/group) were housed in cages connected to running wheels (RWs). After establishing baseline levels of food intake and activity, female rats were i.p. injected with 0, 0.25, and 1.0 mg/kg fenfluramine, 30 min prior to dark onset, on diestrus 2 (D2) and estrus (E) of three consecutive 4-day estrous cycles. Male rats received the same schedule of injections at 2-day intervals. While fenfluramine dose-dependently decreased food intake in all rats, the effect was greater in females, compared to males, at 2, 6, and 24 h following fenfluramine. At each of these time points, fenfluramine-induced hypophagia was greater during E, compared to D2 in females, but it did not differ between the two injections in males (e.g. reduction in food intake 2 h post-injection of 1.0 mg/kg fenfluramine: E-female: 83%; D2-female: 48%; male-injection 1or 2: 25% $P < 0.001$). Fenfluramine also dose-dependently decreased activity in all rats, but this effect did not differ between D2-females, E-females, and males. These data demonstrate that female rats are selectively more sensitive to the hypophagic effects of fenfluramine than male rats. [Supported by: NIH Joint Neuroscience Predoctoral Training Grant and MH 63787]

Brain mechanisms that analyse glutamate taste and their relation to the control of feeding

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Single neuron recordings in macaques show that there is a representation of umami taste, which can be activated by the taste of monosodium glutamate, glutamic acid, or inosine 5′-monophosphate in the mouth, in the primate primary and secondary taste cortex. The findings also show that the pleasantness of umami taste is represented in the primate secondary taste cortex, in that the neuronal responses here to the taste of food are decreased by feeding to satiety (see Rolls, 1999). There is neurophysiological convergence of glutamate and corresponding olfactory stimuli onto some neurons in the orbitofrontal cortex. This suggests that umami flavour could be produced in a strong form by a combination of glutamate taste and corresponding olfactory stimuli, and this has been confirmed in a human psychophysical study in which the savory odor was methylfuryl disulphide. Neuroimaging studies using fMRI are showing that an insular (primary) and orbitofrontal (secondary) taste area can also be identified in humans, and that these areas can be activated by umami taste. Moreover, there is an anterior part of the orbitofrontal cortex which shows a synergistic (supralinear) activation to a combination of monosodium glutamate and inosine 5′-monophosphate (De Araujo et al., 2003).

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Glucosensing neurons

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Neurons within the hypothalamus change their firing rate in response to changes in extracellular glucose levels. These neurons exist in nuclei such as the arcuate (ARC) and ventromedial hypothalamic nucleus (VMN) which are important for the regulation of food intake and energy balance and/or body fat content. However, early studies of these neurons used very non-physiologic levels of extracellular glucose. This raises concern about the physiologic relevance of these neurons. We have found that neurons within the ARC and VMN do respond to physiologic changes in extracellular glucose. Moreover, VMN glucosensing involves a complex convergence of pre- and postsynaptic mechanisms. That is, there are two subtypes of VMN glucosensing neurons which intrinsically sense glucose using mechanisms similar to that of the pancreatic β-cell. These neurons, as well as non-intrinsically glucosensing VMN neurons, receive presynaptic input from other glucosensing neurons. The exact origin of these presynaptic influences remains unknown, however they must originate within the brain slice itself. Thus, it is likely that glucosensing neurons from adjacent hypothalamic nuclei (e.g. ARC) regulate VMN glucosensing neurons. In addition, these glucosensing neurons may also serve as 'metabosensors' by responding to other indices of metabolic status such as leptin. Finally, both pre- and postsynaptic glucosensing mechanisms in the VMN are dysfunctional in a rodent model of diet-induced obesity (DIO) and type II diabetes in which central glucosensing is abnormal. This suggests that these mechanisms are important for the central regulation of glucose homeostasis.

Carbohydrates' quality affects learning performance in the rat

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Glucose is the main energy nutrient of the brain and is needed for mental functioning. A large variety of food items are glucose suppliers but the delivery rate of glucose is highly variable, depending on the physicochemical properties of the various carbohydrates contained in the diet. This variability is reflected by the glycemic index (GI) of the food. We assessed the effect of several cereal-based foods of different GI (ranging from 44 to 84) on the learning performances of Wistar male rats by mean of an operant conditioning test. A meal, consisting of 20% of the daily energy intake, was given to the rats at the beginning of the dark phase, followed by 2.5 h deprivation before the animals were submitted to the learning test. The latter was an operant conditioning task based on the rats' aversion to brightness: the rats had to learn which one among two levers permitted lights extinction for 30 s. In general, rats fed with a medium or low GI diet ($GI < 70$) succeeded to significantly discriminate between the active lever (generating light extinction) and the inactive lever (no effect). On the opposite, animals fed with a high GI diet were not efficient to discriminate between the two levers. The relationship between the nutritional characteristics of the diet and learning performance are discussed with special attention to carbohydrates. In particular, our data provide some evidence that the quality of dietary carbohydrates affect learning performance measured 3 h later.

Influence of the suprachiasmatic nucleus on daily lipid profiles

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Plasma glucose concentrations in the rat display a daily rhythm independent of the timing of food intake. This rhythm is controlled by the hypothalamic biological clock, located in the suprachiasmatic nucleus (SCN). It is not known, however, how the SCN causes the glucose rhythm. Besides hormones that affect glucose homeostasis, such as insulin and glucagon, also other factors interact with glucose metabolism. For instance, it is well known that free fatty acids (FFA) compete with glucose for oxidation. Therefore, daily fluctuations in the availability of plasma FFA could affect plasma glucose levels. Our aim was to determine whether plasma lipids display a daily rhythm, and whether this is modulated by the SCN. Intact rats were either fed ad libitum or entrained to a feeding schedule with six equal meals a day. Rats with SCN-lesions were fed ad libitum or fasted. Via jugular vein catheters, hourly blood samples were taken and triacylglycerides (TAG), glycerol, FFA and glucose were measured. A daily rhythm in TAG concentrations was found, similar for ad libitum fed and scheduled-fed rats. Thus, the daily TAG profile seems not to be affected by the feeding pattern, but to be controlled by an endogenous factor. FFA and glycerol profiles differed between and scheduled-fed rats, indicating more influence of food intake. FFA and glucose profiles did not seem to correlate directly. Data obtained from the SCN-lesioned rats will give insight in the role of the SCN in modulating daily lipid profiles. [Supported by: the Dutch Diabetes Foundation]

CCK-33 mediates the satiating effect of duodenal preloads of intralipid or oleic acid in rats with abdominal vagal innervation restricted to the liver

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When surgery restricts the abdominal vagal innervation to the liver (H), hepatic vagal afferent fibers are sufficient to mediate the normal satiating potency of CCK-33 (i.p.), but the potency of CCK-8 (i.p.) is markedly reduced (Eisen et al., 1998). Thus, H rats can serve as a bioassay for the release of endogenous CCK-33. Because fats and fatty acids are potent stimuli for the release of endogenous CCK, we administered duodenal infusions (10 ml, 0.44 ml/min) of Intralipid (10, 15 and 20%), oleic acid (0.065, 0.130 and 0.195%), or saline (0.15 M) to H and sham vagotomized, male Sprague Dawley rats that had been deprived of food for 4 h. The infusions began 10 min before access to 10% sucrose for 30 min. All preloads decreased intake of 10% sucrose significantly and equivalently in H and sham vagotomized rats compared to intakes after isovolumteric preloads of saline. Pretreatment with a CCK-1 antagonist markedly reduced or abolished the inhibitory effects of the preloads in H and sham vagotomized rats, demonstrating that the decreased intake of 10% sucrose in H and sham rats was mediated by endogenous CCK. Since CCK-8 has very little inhibitory effect in H rats, we suggest that endogenous CCK-33 mediated most, if not all, of the inhibitory effects of the preloads. This is the first evidence for the satiating effect of endogenous CCK-33 in the rat. The relative importance of endogenous CCK-33 and CCK-8 for satiation in vagally intact rats remains to be determined. [Supported by: MH 40010]

Third ventricular (3V) alloxan temporarily impairs glucoprivic feeding and hyperglycemic responses

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Glucokinase (GK) is expressed in glucosensing neurons located in the PVN, ARC, VMN and DMN and wall of the 3V. GK is a potential gatekeeper for neuronal glucosensing. However, the physiological role of GK in glucose homeostasis, hypoglycemic counterregulation and the regulation of food intake and body weight is poorly understood. Alloxan (ALL) is an inhibitor of GK. At low doses, central injection of ALL stimulates food intake (Ritter & Strang, 1982), while high, toxic doses abolish the feeding (Woods & McKay, 1978; Ritter et al., 1982), but spare the sympathoadrenal response to glucoprivation. We injected ALL (200 μg/5 μl) or Saline (SAL) into the 3V to target GK containing cells located in nearby hypothalamic sites. After 4 d, compared to SAL, ALL rats had significant deficits in both 2DG-induced feeding (SAL 5.8 ± 0.6 g vs. ALL 2.9 ± 0.5 g; $P < 0.01$) and hyperglycemic responses (SAL 169 ± 12 mg/dl vs. ALL 104 ± 6 mg/dl, $t = 60$; $P < 0.01$), yet ate significantly more in 3 h after an overnight fast (SAL 8.2 ± 1 g vs. ALL 12.8 ± 0.9 g, $P = 0.01$). However, 13 d after ALL, 2DG-induced feeding and hyperglycemic responses and 3 h refeeding after an overnight fast were similar to SAL rats. Results indicate that the neural substrates targeted by 3V ALL are important for both feeding and sympathoadrenal responses to glucoprivation. The finding that these deficits reversed indicate that the lesion induced by ALL is temporary, possibly due to GK compensatory mechanisms.

Serotonin type-3 receptor participation in nutrient-induced satiation

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Serotonin (5HT) released in response to intraintestinal stimuli during a meal is known to reduce food intake.

Recent evidence implicates serotonin type-3 receptors (5HT3R) in mediating satiation signals generated by duodenal preloads (Ensure). In a series of studies, we examined participation of 5HT3R in suppression of 15% sucrose intake in response to fat (intralipid) and glucose polymer (polycose). Rats received intraduodenal loads (8 ml/20 min) of either intralipid (0.125, 0.25, 0.5 kcal/ml) or polycose (0.18, 0.25, 0.5 kcal/ml) under food deprived and non-food deprived conditions. Pretreatment with ondansetron (1 mg/kg, ip), a selective 5HT3R antagonist, significantly attenuated 60-min suppression of sucrose intake produced by 0.25 kcal/ml loads of intralipid in fed but not fasted rats. At the highest caloric load tested ondansetron enhanced intralipid-induced suppression of intake following an overnight fast. Treatment with ondansetron had no effect on polycose-induced suppression of intake in either fed or fasted rats. Ondansetron alone did not affect food intake. To determine if 5HT3R blockade was necessary prior to the intestinal stimulation by nutrients, in a separate experiment, we administered ondansetron either before or after duodenal infusion. Ondansetron significantly attenuated intralipid (0.25 kcal)-induced suppression of intake when given before the infusion and reversed the suppression when administered after the duodenal load. These results support participation of 5HT3R in suppression of food intake in response to the presence of fat within the intestine. Since fats and not carbohydrates release CCK, these results suggest that 5HT3R may mediate nutrient induced satiation through a CCKergic mechanism.

Oral and post-oral food rewards

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It is well known that the flavor (taste, odor, texture) of food can have positive (stimulatory) effects on food choice and intake during a meal as well as over longer time periods. In contrast, the post-oral consequences of food are usually considered to be inhibitory (satiating) in nature. However, recent findings demonstrate that nutrients can have positive postingestive actions that increase meal size and total daily consumption via a flavor conditioning process. Nutrients differ in their ability to reinforce flavor preferences, and flavor quality (e.g. sweet vs. non-sweet) can affect preference conditioning. Little is known about the viscerosensory systems that mediate post-oral food reward although they operate in food deprived and sated animals. The central neural processing of post-oral food reward is also poorly understood. Recent data suggest that post-oral nutrient feedback modifies the evaluation of flavor stimuli in multiple ways. Thus, the rewarding properties of food that stimulate intake and influence food choice may result from the combined actions of oral and visceral stimuli. [Supported by: NIDDK 31135]

Flavor preference conditioning in C57BL/6J mice by gastric nutrient infusions

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This study determined the feasibility of conditioning flavor preferences in mice by self-administered intragastric (IG) nutrient infusions. Male C57BL/6J mice were surgically fitted with an IG catheter that was attached by a tether system to an infusion pump. The mice were given ad libitum access to chow and a flavored solution 23 h/day. Drinking was monitored with lickometers and a computer which controlled the infusion pumps. During 6 one-bottle training days, drinking one flavored solution (CS +, e.g. grape-saccharin) was paired with matched infusions of 8% maltodextrin, and drinking another solution (CS −, e.g. cherry-saccharin) was paired with water infusions. The mice drank more CS + than CS − during training which was due to an increase in bout size but not bout frequency. In subsequent two-bottle choice tests, the mice strongly preferred (91%) the CS + to the CS −. These data indicate that mice, like rats, acquire an increased acceptance and preference for flavors paired with the postingestive actions of nutrients. Our understanding of flavor-nutrient learning can be advanced by studying this process in selected mouse strains and genetically modified animals. [Supported by: NIH grants DK 31135, DK59630, and DC007475]

Dietary glucose reward sensing

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Glucose derived from sugar and starch is a major source of nutrition. Glucose-containing saccharides (glucose, gucrose, maltose, polycose) are sensed in the oral cavity by sweet taste and perhaps 'polycose' taste receptors, providing a significant source of food reward. Animal studies indicate that the post-oral actions of glucose also have a potent reward effect. This is indicated by the robust flavor preference and acceptance conditioned by intragastric glucose infusions. The site and mechanism of action of this post-oral reward effect remain uncertain. A generic energy-related signal may be involved, but the more potent conditioning effect of glucose, relative to other nutrients, suggests the participation of glucose-specific sensors. Such sensors may be located in the intestines and/or liver based on results obtained with duodenal and hepatic-portal glucose infusions. How post-oral glucose reward signals reach the brain is not known. Transections of vagal afferents and splanchnic fibers do not block flavor conditioning by intestinal glucose infusions which implicates a humoral pathway in post-oral reward signaling. The sensory physiology of dietary glucose reward

and its role in carbohydrate appetite require further investigation. [Supported by: NIDDK 31135]

Adipose tissue mRNA levels of leptin, TNFα, LPL, and GLUT-4 in dairy cows in relation to parturition and lactation

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The high energy demand related to the onset of milk production after parturition in dairy cows is often not fully covered by an increase in food intake. The resulting energy deficit causes lipolysis and ketogenesis, which can ultimately lead to clinical ketosis. We measured adipose tissue mRNA levels of leptin, tumor necrosis factor-α (TNFα), lipoprotein lipase (LPL), and glucose transporter-4 (GLUT-4), which have all important regulatory functions in metabolism and may contribute to ketosis susceptibility and development. During the dry period (3rd week before the calculated calving), in early lactation (4th week after calving) and in the 25th week of lactation subcutaneous adipose tissue samples were taken from dairy cows and mRNA for leptin, TNFα, LPL, and GLUT-4 was measured in adipocytes using the Taqman RT-PCR technique. Adipocyte TNFα mRNA levels were 139 arbitrary units before calving and 199 and 99 arbitrary units in the 4th and 25th week of lactation, respectively. Leptin and GLUT-4 mRNA were highest before calving (17.3 and 0.2533 arbitrary units) and consistently low thereafter (4th week: 2.9 and 0.0008, 25th week: 3.2 and 0.0009 arbitrary units). LPL showed lowest mRNA levels in the 4th week of lactation (0.1421, 0.0915, and 0.1867 arbitrary units prepartum, 4th, and 25th week of lactation, respectively). These results are consistent with a regulatory function of adipocyte TNFα, leptin, LPL, and GLUT-4 in dairy cow metabolism around parturition and early lactation. Whether the observed differences in expression contribute to the metabolic changes and/or have predictive value for the development of ketosis remains to be determined.

Further evidence for a role for serotonergic 5-HT1B receptors in feeding in rabbits: a suggestion about mechanism

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Activating serotonergic 5-HT1B receptors via systemic, hypothalamic paraventricular or parabrachial administration of agonist drugs reduces food intake in rats. We tested male Dutch belted rabbits ($n = 6$) that were adapted to eat their regular pelleted chow after 3-h deprivation during the light cycle, with water freely available. The 5-HT1B/1D agonist, GR46611 (0.5 μmol/kg, s.c., 60 min pretreatment) reduced 30-min intake by 59% in six rabbits. Pretreatment with the 5-HT1B antagonist, SB216641 (0.005–0.5 μmol/kg) antagonized the hypophagic action of GR46611 with complete blockade at 0.05 μmol/kg. Bilateral infusion of GR46611 (10 nmol/0.5 μl) into the lateral parabrachial nucleus (LPBN) reduced intake from 10.5 + 1.7 g/30 min to 5.9 + 0.5 g; the 5-HT1B antagonist SB216641 (10 nmol) blocked this action completely without itself altering baseline. The 5-HT1D antagonist, BRL15572, did not reverse GR46611. Administration of 10 nmol of GR46611 into the nucleus accumbens (shell) did not alter eating. Systemic administration of SB216641 (1, 2 and 4 μmol/kg) increased food intake at the middle dose only and only during the 90-min interval following an initial 8 g ration (30-min exposure). Systemic injection of the agonist, GR46611 (0.5 μmol/kg) did not change the percentage of 30 trials on which rabbits moved their jaws in response to intraoral infusion of sucrose (baseline, 80%; GR46611, 82%). The data suggest that 5-HT1B receptors normally mediate inhibition of feeding in rabbits. The LPBN appears to be one central site subserving this receptor role. Finally, oral stimuli are insufficient to enable this function. [Supported by: DK58669 to KJS]

Short-chain acyl-CoA dehydrogenase (SCAD) deficiency in mice suppresses self-selected dietary fat intake

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In a survey of 13 mouse inbred strains, fat intake ranged from 26 to 83% of energy (Smith et al., 2000). BALB/cByJ mice self-selected a lower percentage of fat intake (36%) than all other strains except for the CAST/Ei. BALB/cByJ mice have a naturally occurring deficiency in mitochondrial beta-oxidation of fatty acids due to a mutation in Acads (Wood et al., 1989). We hypothesized that this deficiency would alter the animals' response to dietary fat. SCAD normal mice (BALB/cBy) and SCAD-deficient mice (BALB/cByJ) were provided for 10 d the choice between two diets containing fat/protein or carbohydrate/protein. On days 2–4, SCAD-deficient mice decreased their fat intake in a stepwise manner suggesting a conditioning effect, and maintained a >2 fold lowering of fat intake compared to controls [genotype × day: $F(9, 29) = 6.72$, $P < 0.0001$]. Next, in 2-bottle, 48-h tests, chow-fed SCAD-deficient mice displayed a significantly lower preference for 50% corn oil vs. vehicle compared to controls. Finally, naïve mice were trained to drink water in a computer-controlled lickometer. On the test day, mildly deprived (6 h) mice were given 15-s access to ascending

concentrations of corn oil (0.5, 1.5, 5, 15 and 50%). There was no effect of concentration on lick responses of SCAD-deficient mice and they licked significantly less than controls at 50%. Their indifference to corn oil in brief-access tests is consistent with the absence of a postingestive signal from fat oxidation. Alternatively, the Acads mutation may affect taste responses. [Supported by: NIH DK-53113]

Food preferences mediate relationships between otitis media and body mass index

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Otitis media (OM) is a common childhood illness with effects that may persist into adulthood. Since the chorda tympani (VII) taste nerve passes through the middle ear, OM alters taste perception. VII inhibits other oral inputs (V, IX) centrally; so OM damage may also alter oral touch (i.e. fat perception). Oral sensation guides food preference/intake, thereby affecting body mass index (BMI). Recent survey data ($N = 3876$) assessing age, sex, OM history, food preferences, and PROP (6-*n*-propylthiouracil) intensity indicate that OM may lead to health risk by contributing to increased BMI. Men aged 30 + with severe OM history have significantly higher BMIs than those without; within this group, PROP supertasters (ST) have higher BMIs than nontasters. We show here that these men also have the highest preference for a group of high-fat foods (including sausage, whole milk, and whipped cream). Increased preferences for some bitter foods (e.g. beer, grapefruit) suggest taste loss. Overall, men aged 30 + with OM history (especially STs) may be at high obesity risk due to their increased preference for high-fat foods. We believe that severe OM history reduces taste input by damaging VII, thereby releasing inhibition on the trigeminal nerve and increasing sensations from fat. This may produce increased fat preferences in men, leading to increased fat intake and higher BMI. STs may be at special risk, as they experience the most intense oral sensations and may incur greater release of inhibition. [Supported by: DC 00283]

Enteroendocrine changes following ileal transposition in the rat

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Gastric bypass is currently the most effective treatment for morbid obesity. With this procedure the mid- or terminal-jejunum is connected to the stomach, bypassing the upper intestine, and delivering partially digested nutrients into the distal gut. The mechanism by which gastric bypass causes weight loss is incompletely understood but one possibility is that augmented secretion of hormones from the distal intestine enhances satiety. To study the effects of nutrients delivered directly into the ileum on the release of regulatory peptides we performed ileal transposition surgery in rats. A 10 cm segment of distal ileum was transposed to the duodeno-jejunal junction resulting in the delivery of gastric contents into the ileum while maintaining an intestinal tract of normal length. Consistent with the hypothesis that activation of ileal activity contributes to the effect of gastric bypass, rats subjected to ileal transposition ate less food and lost more weight than rats receiving a sham surgery. Five minutes following an oral glucose gavage rats with ileal transposition exhibited a three-fold greater GLP-1 response than sham-surgery controls. Consistent with the elevated levels of GLP-1, rats with ileal transposition had higher peak insulin levels following the gavaged glucose load compared to the controls. These findings support a role for an ileal endocrine response in mediating the effects of gastric bypass to cause weight loss.

The response of syndecan-3 deficient mice to melanocortin receptor agonists

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Syndecan-3 is a member of the heparan sulfate proteoglycan family and has been implicated in mediating the orexigenic effects of agouti-related peptide (AgRP), the endogenous melanocortin receptor antagonist. Hypothalamic syndecan-3 expression is increased by food deprivation and decreases rapidly following ingestion. Syndecan-3 is bound to cell membranes and acts as a co-receptor to facilitate AgRP antagonizing melanocortin-4 receptors. Consistent with the hypothesis that syndecan-3 is important for the orexigenic effects of AgRP, syndecan-3 null mice exhibit attenuated compensatory hyperphagia following food deprivation. We hypothesized that genetic deletion of syndecan-3 would alter the ability of melanocortin receptor ligands to influence food intake. To test this hypothesis, we assessed food intake following peripheral administration of the synthetic melanocortin receptor agonist melanotan-II (MT-II) and leptin, an upstream activator of MC signaling, in mice with a targeted deletion of the syndecan-3 gene. Syndecan-3 −/− mice were more sensitive than wild-types to the anorexic effects of both MTII and leptin. These data are consistent with the hypothesis that targeted deletion of syndecan-3 attenuates the ability of AgRP to antagonize activity at MC4 receptors. Finally, we hypothesized that attenuated AgRP activity would prevent the development of diet-induced obesity. Syndecan-3 −/− and +/+ mice were fed high-fat and low-fat diets and body weights were tracked for 16 weeks. Contrary to our prediction, syndecan-3 −/−

and wild-type mice similarly developed diet-induced obesity. Thus, while syndecan 3 appears to play an important role in the actions of melanocortin antagonists, it may not be essential to the development of obesity.

Increased water intake by rats maintained on high NaCl diet: analysis of ingestive behavior

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To determine the temporal relation between the ingestion of dry food containing 8% NaCl and the increased daily consumption of water that occurs when rats eat this diet, rats were placed in specially designed cages linked to microprocessors that allowed the continuous monitoring of food and water ingestion. The increase in water intake was found to result from increases both in the number and the size of individual drinking bouts. More specifically, the number of drinking bouts doubled while bout size was 1.5-times control values, which together computes to the observed threefold increase in daily water intake. Approximately 75% of the water intakes were consumed in drinking bouts that occurred within 5 min after feeding bouts. Indeed, rats rarely consumed 8% NaCl diet without also drinking water in the same ingestive episode, and the volume of water they drank was proportional to the food intake in that episode. This increase in food-associated drinking began on the first day that rats were given a diet containing 8% NaCl, and there was no evidence that rats learned to drink more water in anticipation of dehydration resulting from the ingested NaCl load. These and other observations suggest that ingestion of the high salt diet stimulated thirst rapidly. As such, they are consistent with previous reports that visceral osmoreceptors (or Na^+-receptors) detect osmolytes passing through the gastrointestinal tract and provide an early stimulus of thirst in rats that precedes large increases in systemic plasma osmolality.

Rapid inhibition of vasopressin secretion when thirsty rats drink water

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Water deprivation elicits two adaptive and well-studied responses in rats, thirst and neurohypophyseal secretion of vasopressin. Both responses have long been believed to be mediated by osmoreceptors located in the basal forebrain. However, recent evidence for the existence of visceral osmoreceptors (or Na^+-receptors) in rats allows the possibility that these peripheral receptors participate in mediating the two prominent regulatory responses to dehydration. The present experiments examined this possibility. Rats were maintained either on standard laboratory diet containing 1% NaCl or a high salt diet containing 8% NaCl, before being deprived of drinking water (but not food) overnight. One group of rats was placed in special cages that allowed continuous monitoring of food and water ingestion. As expected, the rats reduced their intake of dry food when water was withheld (i.e. 'dehydration anorexia'), and they did so by reducing both the size and the number of feeding bouts. Other experiments determined how quickly the ingested water emptied from the stomach into the intestines, and how quickly plasma vasopressin levels decreased after water ingestion. The results indicated that the water ingested by dehydrated rats passed quickly into the intestines and inhibited vasopressin secretion well before the drinking bout ended, and well before substantial dilution of plasma osmolality occurred and could have influenced cerebral osmoreceptors. These and other findings suggest that visceral osmoreceptors can provide potent signals for inhibiting vasopressin secretion and thirst during rehydration in rats.

Does television viewing influence food intake?

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Television viewing is an ambient factor that may influence food intake. It is the third most time-consuming activity in the United States, only ranking behind work and sleep. The energy expenditure while watching television is low and television watching seems to be associated with less physical activity, as well as with a general increase in food intake. However, the underlying mechanisms for an increase in food intake while watching television are less clear and more insight regarding this factor is needed. A diet-diary was used for data collection. College students ($n = 73$) were instructed to maintain a detailed diary about their food intake including their daily activities and television viewing for seven consecutive days. Meals eaten with the television on were not significantly different from other meals but television watching was significantly associated with an increase in meal frequency. About one additional meal was added to the usual food intake on days when television was viewed compared to days when no television was watched. The participants also reported less hunger when eating while viewing television. In addition, less physical activity over the day was reported when television was watched. The results suggest that television viewing decreases physical activity and influences food intake by increasing meal frequency and thereby the overall daily caloric intake.

TRH mRNA expression in neurons of the caudal raphé nuclei is modulated by nutritional state, diurnal cycle, and orexin: possible role in thermogenesis and gastrointestinal functions related to energy homeostasis

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The caudal raphé nuclei contain premotor neurons for autonomic outflow and have been implicated in thermo-, cardiovascular- and gastrointestinal regulation. Caudal raphé neurons expressing thyrotropin releasing hormone (TRH) project to the dorsal vagal complex, where they stimulate gastric functions, and to brown adipose tissue, where they stimulate thermogenesis. To investigate the role of nutritional state and diurnal cycle, we measured pro-TRH mRNA expression in the various groups of medullary TRH neurons by non-radioactive in situ hybridization. Male Sprague-Dawley rats maintained on a 12/12 h light/dark cycle (lights off 1800 h), were either fed ad libitum and killed at various times or food deprived for 24 h. In the raphé pallidus, pro-TRH mRNA expression in ad lib fed rats was low at 0900 h (11.5 ± 2.8 cells/3 sections), and significantly ($P < 0.01$) higher at 1700 h (35.7 ± 3.6) and at 2200 h (35.5 ± 4.0), as well as in food deprived rats at 0900 h (37.5 ± 4.9). Combination in situ hybridization/fluorescence immunohistochemistry revealed a dense plexus of orexin-A, CART and fewer alpha-MSH, and MCH-immunoreactive axon terminals near TRH neurons, suggesting a role for these hypothalamic feeding-related peptides in the modulation of TRH neuronal activity throughout the dark/light cycle and by metabolic state. We conclude that TRH neurons in the caudal raphé nuclei are responsive to nutritional state and thus may mediate physiological changes in gastric function and energy expenditure that are important in maintaining energy homeostasis. [Supported by: DK 47348, Community Foundation of Southeastern Michigan]

Intraperitoneal CCK administration increases phosphorylation of MAPK in rat solitary nucleus neurons

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CCK released by protein and fat from small intestinal endocrine cells is thought to help limit a meal via activation of vagal afferent fibers terminating in the solitary nucleus (NTS). How activation of primary vagal afferents, and in turn of NTS neurons, leads to inhibition of ingestive behavior is an open question. We hypothesized that visceral afferent inputs mediated predominantly by vagal afferent fibers, and other inputs to NTS neurons including those mediated by hypothalamic peptidergic projections, are integrated into signals that are then propagated to other brainstem neurons involved in oromotor control, and that this integration is accomplished by rapid phosphorylation and de-phosphorylation of intracellular signaling proteins. To start analyzing the critical signaling events, we monitored phosphorylated MAPK (MAPK, Thr203/Tyr204), calmodulin kinase (CaMK, Thr286), cAMP-regulated binding protein (CREB, Ser133), as well as various phosphorylated GABA and glutamate receptors, following CCK administration (100 mg/kg, ip) by Western blotting of NTS micropunches and by immunohistochemistry using phospho-specific antibodies. Relative abundance of pMAPK was increased 8 and 15 min after CCK injection as compared to saline. The number of NTS neurons specifically and darkly stained with pMAPK-immunoreactivity was also increased 10 min following CCK as compared to saline. These findings demonstrate the feasibility of phospho-specific antibodies to monitor protein–protein interactions in specific neuron populations, and suggest that the MAPK pathway is involved in CCK's satiety effects. [Supported by: DK47348]

Chronic social stress and recovery: changes in body composition

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Rat colonies (4 males and 2 females each) form dominance hierarchies when housed in a visible burrow system (VBS), producing 1 dominant (DOM) and 3 subordinate males (SUB). When housed for extended periods of time in the VBS, animals display characteristics consistent with chronic social stress. In this experiment, rats were housed in the VBS for 14 days before being allowed to recover in individual cages for 21 days. SUB lost significantly more weight compared to DOM or control (CON) over 14 days in the VBS. During recovery, all animals re-gained body weight, but neither DOM nor SUB reached CON levels. Plasma leptin was significantly lower in DOM and SUB than CON after stress. However, after the recovery period, SUB had higher leptin than DOM. Consistent with this, body composition analysis indicated that after 14 days, weight loss in DOM is attributable to loss of adipose tissue while weight loss in SUB is attributable to loss of lean and adipose tissue. Plasma testosterone was significantly reduced in SUB compared to DOM and CON,

and was similar to CON level after recovery. Together these data indicate that chronic social stress in the VBS suppresses testosterone and results in decreased fat and lean body mass in SUB. In addition, when the stress is removed, SUB re-gain body weight primarily as fat during the recovery period. [Supported by: DK-59803 (KLKT), NARSAD, The Guggenheim Foundation, NSF (RRS)]

Central administration of Ghrelin has prolonged effects on feeding and locomotor activity—predominantly mediated via the melanocortin system

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Ghrelin, an endogenous ligand of the growth hormone secretagogue receptor (GHS-R), is expressed and secreted by gastric A-like-cells prior to meal onset. Consistent with this secretion pattern, Ghrelin stimulates food intake and promotes adiposity when administered peripherally or centrally. It has previously been shown that peripheral and central administration of Ghrelin and Ghrelin analogues activate neurons in the medial portion of the arcuate nucleus and that these neurons stain positively for NPY/AGRP and the GHS-R. Since the activation of these neurons stimulates both a short-term oxrexigenic peptide (NPY) and a peptide with longer lasting effects on feeding (AGRP), we studied the effect of a single dose of Ghrelin on both short and long term feeding. In addition we examined Ghrelin's effect on locomotor activity as this parameter could contribute to the positive energy balance following chronic Ghrelin treatment. Central administration of increasing doses of Ghrelin (0.2, 1.0, and 5.0 μg, 4 h into the light phase) dose-dependently increased food intake during the 24-h observation period, but interestingly reduced spontaneous locomotor activity in same period. In a set of separate experiments, we observed increased cumulated food intake and decreased locomotor activity for more than 72 h without any sign of compensation, following a single i.c.v. Ghrelin injection (5.0 μg) in rats. In a fourth set of experiments we demonstrated that the acute orexigenic effect of ghrelin is identical with NPY induced feeding patterns, whereas its long-term component as well as its effect on locomotor activity appear to be mediated by the hypothalamic melanocortin receptor system. In summary, Ghrelin not only stimulates caloric intake and reduces fat oxidation but also plays a previously unknown role as an independent suppressor of spontaneous physical activity. NPY and AGRP co-mediate acute and chronic orexigenic effects of ghrelin, respectively, while only AGRP is a candidate for triggering decreased motor activity. It appears teleologically reasonable that an afferent hormone signalling lack of caloric intake to the brain triggers both, increased hunger and reduced spontaneous locomotion. Ghrelin receptor antagonists might therefore not only reduce appetite but also increase spontaneous physical activity and as such be as attractive compounds for the treatment of obesity.]

Quantitative "behavioral informatics" approaches to genetic influences on ingestive behavior

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The regulation of spontaneous patterns of behavior exhibited by rodents in their home cages reflects the integrated functional output of many neural circuits, each of which is influenced by large numbers of genes. We are pursuing a technology development initiative to determine the extent to which detailed quantitative home cage behavioral assessment strategies may provide insights into mechanisms through which genetic factors influence patterns of locomotor activity and ingestive behavior. Quantitative assessments of behavioral patterns were performed in lines of hyperphagic mice bearing mutations of single genes implicated in energy balance regulation. Perturbations of activity and ingestive behavior patterns were readily observed, and found to differ among the hyperphagic lines. These findings reveal heterogeneity in neural mechanisms through which mutations may lead to hyperphagia. In addition, the impact of strain on behavioral patterns is under examination in conjunction with the Jackson Laboratory Mouse Phenome Project. Results to date indicate that behavioral patterns are also highly sensitive to strain and that "behavioral informatics" approaches may be applied to extract features of behavioral patterns characteristic of particular strains. The potential utility of "behavioral fingerprint analysis" to provide insights into neural mechanisms through which genes influence ingestive behavior will be discussed.

Effect of sibutramine on macronutrient selection in male and female rats

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Sibutramine is a serotonin-noradrenalin reuptake inhibitor which has been shown to be a safe and effective weight-loss drug. The purpose of the present study was to examine whether sibutramine has an effect on macronutrient selection and total food intake in rats of both sexes. Wistar rats were divided into three groups and each group was offered a different set of three sensorily contrasting macronutrient-

specific diets, each set including a carbohydrate-rich diet, a protein-rich diet and a fat-rich diet. The rats were given ten days of adaptation to the diets and environment, which included 7 days adaptation to a 4-h food deprivation period. On the experiment days, at the end of the 4-h food deprivation period, the rats received by gavage either saline or one of three different doses of sibutramine (2.5, 5 and 10 mg/kg; Meridia®, Ross Laboratories Canada), dissolved in water, at the beginning of the dark phase. Sibutramine (10 mg/kg) was shown to decrease carbohydrate and fat intake at all data points regardless of gender and diet, which constituted a nutritional effect. The effect of sibutramine on protein intake was diet- and gender-specific, suggesting that this effect was sensory. All doses of sibutramine decreased total food intake regardless of gender and diet group beginning at 6-h post-administration. In conclusion, sibutramine affects macronutrient selection and emphasis on dietary recommendations, as well as appropriate dosage according to gender should be considered during therapy. [Supported by: a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC)]

Circadian rhythmicity of macronutrient intake by reversed feeding rhythms induces low nocturnal secretion of serum melatonin and pineal NAT in rats

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The effect of a single chow diet or a dietary choice between a protein-rich and a carbohydrate-rich diet ingested ad libitum or during daytime (between 0800 and 1600 h) on the rhythms of food ingestion and nocturnal peaks of serum melatonin and pineal *N*-acetyltransferase (NAT) activity was studied in Wistar rats. Animals fed ad libitum the single or the choice diets displayed a standard circadian rhythmicity of total food intake. Rats fed the dietary choice paradigm also displayed a preference for carbohydrate at the beginning of the dark phase and a preference for protein toward the end of that phase. In contrast, on the daytime feeding access schedule, both dietary conditions resulted in only one peak of total intake at the beginning of the access period, followed by a gradual decrease in food consumption. Nocturnal peaks of serum melatonin and pineal NAT activity were lower with daytime access to dietary choice compared to ad libitum access while these parameters did not differ significantly with single chow feeding. It is suggested that with dietary selection of macronutrients, feeding cycle can directly act on the rhythms expressed within the pineal gland. [Supported by: a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC)]

Centrally administered orexin-A does not increase motivation for sweet pellets under a PR5 schedule

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Centrally administered orexin-A elicits a feeding response in rats, as well as an increase in arousal and locomotion; suggesting that feeding may be secondary to a general increase in activity. To test the hypothesis that orexin-A does not specifically enhance motivation to eat, male Sprague-Dawley rats implanted with lateral hypothalamus (LH) cannulae were trained to press a lever for sweet pellets on a progressive ratio of 5 (PR5) schedule. Under PR5, the 1st pellet is delivered after 5 presses; thereafter, each successive pellet requires an additional 5 presses. The study ended when the animal did not press a lever for 5 min. The number of presses required to reach this stage is termed breakpoint and is thought to correlate to a change in motivation to eat. Orexin-A (0, 250, 500 or 1000 pmol) was injected in a counterbalanced design with 72 h between sessions. As a positive control we also tested the effect of neuropeptide Y (NPY, 0, 58, 117 and 185 pmol) on breakpoint. Although both the high doses of orexin-A and NPY stimulated feeding of sweet pellets in the home cages (mean above baseline 1 h post-injection $= 4.4 \pm 1.5$, $P < 0.05$, NPY; 3.85 ± 2.2, $P < 0.05$, orexin-A), only NPY (117, 185 pmol) significantly altered the breakpoint on a PR5 schedule ($P < 0.05$). These data indicate that although LH orexin-A enhances feeding, the motivation to press a lever for sweet pellets was not manifest under a PR5 schedule, suggesting that orexin A does not enhance motivation to eat.

Forebrain inputs to the parabrachial nucleus under conditioned taste aversion in the rat

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We recorded taste activity of neurons in the parabrachial nucleus (PBN) to investigate the neural mechanisms of conditioned taste aversion (CTA). In Experiment 1,

sham-conditioned control rats and experimental rats exhibiting CTA to NaCl were used. Rats were anesthetized with urethane and decerebration was made at the beginning of the recording session. The taste stimuli were Sucrose, NaCl, HCl, quinine, KCl and Amiloride mixed with NaCl. We have recorded 42 units for each group. No statistical differences were observed among the taste responses of the two groups. In Experiment 2, we examined the effects of electrical stimulation of the gustatory cortex (GC) and the central nucleus of the amygdala (CeA) on PBN taste activity. The experimental procedures except for decerebration were the same as those of Experiment 1. We have so far recorded 29 units in control and 31 units in experimental rats. Among them, only the amiloride-sensitive NaCl-best units in experimental rats showed larger responses to NaCl than in control rats. CeA stimulation enhanced responses in 12.9% of units in the experimental group where no enhancing effects were observed in the control rats. These results suggest that PBN taste responses are modulated by centrifugal influences under CTA.

Influence of dietary calcium on the body weight of obese rats and mice

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Epidemiological findings and an experiment with agouti mice has led to the hypothesis that low dietary calcium intakes contribute to obesity. Here, we evaluated whether calcium influenced the body weight of rodents made obese by feeding a high-energy diet. In Experiment 1, female C57BL6/J mice were fed diets with either normal (3.3 kcal/g) or high (4.7 kcal/g) energy density. Some of the mice also had access to 30 mM $CaCl_2$ solution to drink, either alone or mixed with saccharin to stimulate intake. Eating high-energy diet induced obesity and drinking calcium had no effect on this. The mice were then reassigned to new groups that were fed diets with the same energy content as they had previously received but differing in calcium content (50, 150, or 450 mmol Ca^{2+}/kg). Dietary calcium had no effect on body weight at any time during the 6-wk test. In Experiment 2, female Sprague Dawley rats were fed the normal or high-energy diet for 12 wk to allow obesity to develop. They then received diets of the same energy content but three levels of calcium (50, 150, or 450 mmol Ca^{2+}/kg). Once again, there were no significant effects of dietary calcium on body weight (after 32 days). These data challenge the hypothesis that low dietary calcium contributes to the etiology or maintenance of obesity.

Vagus nerve response to amino acid as chemical sense and control of preference

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Gustatory and anticipatory cephalic stimuli that are detected during a meal yield nutritional information and aid in the efficient digestion of food. It is possible that animals including humans can detect the amount of dietary protein and its quality via cephalic relay to initiate proper digestion in the alimentary tract. The vagus nerve conveys primary afferent nutritional information from the digestive system to the brain. Intestinal luminal or hepato-portal stimulations of vagal afferent fibers by amino acids (AAs) modulate food intake and digestive functions such as gastric emptying and pancreatic secretion. In the present study, we recorded electrophysiological responses to 20 different AAs in celiac and hepato-portal branches of the vagus under urethane anesthesia. All AAs had regulatory effects on the afferent nerve activity. Threonine, methionine, isoleucine and glycine evoked inhibitory and others excitatory signals on the afferent nerve fibers in the both branches. Moreover, the AA-sensing ability of hepato-portal branch showed large plasticity during an essential AA-deficiency such as lysine. In this case, compared to the normally fed controls, the sensitivity for lysine was increased an approximately 100-fold in the lysine-deficient rats. This plasticity is quite comparable to quantitative ingestion of lysine solution in choice paradigm under lysine deficiency. In addition, our recent results about the signal transduction mechanisms of AAs in vagus nerve activation in the alimentary tract indicated to involve the type 5HT3 receptor at the vague nerve end. The integration of each AA information via vagus nerve is significantly important to maintain the AA homeostasis metabolically.

Food aversion learning based on intragastric macronutrient cues

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Can rats identify different macronutrients based solely on stimuli arising in the gut? We assessed the ability of rats to differentiate fat and carbohydrate in the gut in the absence of differential orosensory cues or caloric density. Animals consumed two differently flavored, non-caloric solutions and received an equicaloric gastric infusion containing either corn oil or Polycose as they drank, each associated

with a particular flavor. Following this flavor®nutrient training, animals were given a separate infusion of each nutrient while consuming a novel flavored solution. Half the animals were made ill via a LiCl injection following the Polycose infusion, while an injection of isotonic saline followed the corn oil infusion. The remaining half of the animals received the reverse illness®nutrient pairing. If illness was associated with specific properties of the infused nutrient, rather than just the novel flavor or the infusion itself, animals should then avoid the flavor previously trained with the poisoned infusate. The results of post-aversion tests under various conditions indicate that animals are capable of differentiating gastric infusates composed of two separate pure macronutrients in the absence of differential orosensory information. Furthermore, the data suggest that for animals exposed to the pairing of illness with a compound stimulus containing both an orosensory and a postingestive (e.g. nutritive) component, conditioning occurs to both of these components and there may be greater learning about the occurrence of a specific postingestive stimulus than the taste. [Supported by: NIH R01 HD28792-09 (TLD), DK27627 (TLP) and an NSF Graduate Research Fellowship (ALT)]

Modulation of ingestive function by medullary NOS containing neurons

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Neurons containing nitric oxide synthase (NOS), the synthetic enzyme for nitric oxide (NO), are found in numerous brainstem regions, including the nucleus of the solitary tract and the lateral division of the medullary reticular formation (RF), an area critical in the control of oromotor behavior. That NO neurons in the medullary RF are involved in oromotor behavior is suggested by our previous study demonstrating that the number of neurons double-labeled for FOS and NADPH increases following gustatory stimulation, and current experiments indicating that some NOS-labeled cells project to the hypoglossal nucleus. To obtain direct evidence for the hypothesis that NO is involved in gustatory-modulated oromotor function, we infused the NOS inhibitor L-NAME (80 mM/100 nl) bilaterally into the lateral medullary RF and observed effects on oromotor responses evoked by intra-oral infusion of sucrose, quinine, and water, as well as appetitive licking of sucrose. A preliminary analysis revealed multiple effects of the infusions on different components of ingestion and rejection. Immediately after the infusion, gapes to QHCl were replaced by licks and the number of swallows was reduced. Later in the test session (approximately 45 min), appetitive licking of sucrose was disrupted, taking on an intermittent rather than continuous pattern. These data suggest an important role for medullary NO in taste-motor processing. However because NO is involved in a number of autonomic processes including vagally-mediated GI control, further experiments in nearby autonomic structures are necessary to specify the relative contributions of different brainstem structures to ingestive behavior. [Supported by: DC00417 and DC00416]

Complete subdiaphragmatic vagotomy does not abolish increased food intake following NMDA ion channel blockade

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Previously, we reported that subdiaphragmatic (SD) vagotomy abolishes increased feeding following MK-801, suggesting that SD vagal fibers were necessary for increased food intake. However, recent reexamination indicates that our earlier interpretation was in error. In the current study, rats were adapted to eat 15% sucrose after a 16 h fast. Rats then received either total SD vagotomy ($n = 12$) or a sham procedure ($n = 12$). After recovery, both groups were retested. As previously reported, vagotomized rats exhibited a significant increase in meal size, compared to controls, following vehicle injection. Control rats significantly increased their intake of sucrose following MK-801 injection (80 μg/kg) (9.6 ± 0.7 ml, vehicle; 12.7 ± 0.9 ml MK-801), while the average intake of vagotomized rats following MK-801 did not differ from intake following saline injection. However, when vagotomized rats were examined individually, we found that rats eating ≥15 ml ($n = 5$) following vehicle injection did not consume more after MK-801 (19.2 ± 0.7 ml, vehicle; 15.9 ± 1.9 ml MK-801). Vagotomized rats that consumed ≤15 ml after vehicle ($n = 6$), substantially and significantly increased their sucrose intake after MK-801 (12.6 ± 0.5 ml, vehicle; 18.8 ± 1.3 ml MK-801). Response to i.p. CCK-8 (2 μg/kg), and failure of vagal transport of i.p. fluorogold confirmed completeness of vagotomies. These data indicate that intact SD vagal fibers are not necessary for increased food intake following MK-801. The data further suggest that attenuation of the response to MK-801 may be due to a combination of vagotomy-induced increase in meal size and MK-801-induced motor impairment in vagotomized rats. [Supported by: NIDDK grant DK-52849]

Central and peripheral cholecystokin in the control of feeding behavior and meal induced thermogenesis

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This study addressed the involvement of central and peripheral CCKA receptors in feeding and meal induced thermogenesis (MIT) using neonatal capsaicin (CAP) treated rats as a tool for vagal deafferentation and vehicle (VEH) as a control. Intraperitoneal (i.p.) CCK (3 and 6 μg/kg) did not have an effect in CAP, but induced a dose dependent reduction in sucrose intake in VEH, hereby confirming earlier data. CCK did not have an effect on MIT in CAP, but blunted MIT in a dose dependent way in VEH. This indicates the involvement of primary C-afferents in the effects of CCK on temperature. The role of endogeneous CCK was studied by using i.c.v./i.p. injection of devazepide (CCKA receptor antagonist). Peripheral injection of devazepide (2 or 200 μg/kg) did not show significant effects on sucrose intake in CAP or VEH Indicating that peripheral endogenous CCK is not involved in satiety signalling. However, central injection of the lowest dose (10 ng) of devazepide increased sucrose intake significantly in VEH; the highest dose (1 μg) had no effect. Surprisingly, ICV administration of devazepide in CAP did not affect sucrose intake, suggesting that brain regions involved in CCK signaling might be destroyed in CAP. Devazepide (i.p./i.c.v.) had no significant effect on MIT in CAP or VEH. In summary, these findings suggest that only central endogenous CCK is involved in the control of feeding. The physiological role of endogenous CCK in MIT is unclear, since i.c.v./i.p. administration of devazepide did not show any effects on MIT.

Interactions between social stress and chronic high-fat feeding as reflected in stress responsivity and metabolic functioning in male rats

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Several studies suggest that stress and dietary fat impose a risk for development of the metabolic syndrome. The present studies were designed to assess the consequences of a change in dietary macronutrient composition on the acute and long-term response to stress. Two months prior to experiments, rats were either provided with a high-fat (HF) diet (19% carb, 20% protein and 61% fat) or remained on standard high-carbohydrate (HC) laboratory chow (63% carb, 23% protein and 14% fat). In addition, rats were implanted with telemetry transmitters (for recording of body temperature and locomotor activity) and jugular vein catheters to allow stress-free blood sampling. Following social defeat (with the 'resident–intruder' paradigm) or lipopolysaccharide injection, HF rats showed a faster recovery of body temperature and locomotor activity relative to HC rats. The loss in body weight following these stressors was also less severe in HF rats. Furthermore, HF rats showed a reduced 5-HT1a receptor desensitization following social stress as compared to HC rats implicating a role for the serotonin system in these effects. HF fed rats also showed a reduced ACTH response to conditioned social exposure to the resident male. However, intravenous glucose tolerance testing before and 3 weeks after defeat indicated that social stress induced a doubling of the insulin response in HF rats, but not in HC rats. Thus, HF feeding decreases certain aspects of the stress response but that stress in combination with a HF diet makes animals more susceptible to develop insulin resistance.

A rat model of antipsychotic drug-induced weight gain

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Atypical antipsychotic drugs minimize extrapyramidal side effects associated with treatment, but induce significant weight gain in patients. We developed a rat model of olanzapine (OLZ)-induced weight gain to investigate this effect. Previous studies utilizing a palatable high-fat diet (HF) fed to female Osborne-Mendel (OM) rats with OLZ administered by daily subcutaneous (s.c.) injection, demonstrated significant body weight (BW) gain, but tolerance to the drug's effect. To lower the total OLZ dosage received during treatment, and thus minimize the development of drug tolerance, we administered OLZ in silastic capsules implanted s.c. Semi-permeable medical-grade silastic tubing plugged at each end and containing OLZ suspended in sesame oil was used. Four groups of $n = 5$ female OM rats fed HF were implanted with one to four silastic capsules, resulting in the following total doses of OLZ per animal: 0, 0.6, 1.2 and 2.0 mg. After 20 days of treatment, the original capsules were replaced at the same doses. After 40 days of treatment, the 1.2 mg group exhibited significantly greater body weight gain than the other groups ($P < 0.05$). An inverted U-shaped OLZ dose-response effect on BW gain was seen. However, the development of tolerance was not observed in the 1.2 mg group, indicating that silastic capsule administration of OLZ may be superior to other forms of drug delivery. An inconsistent trend towards greater daily caloric intake was seen in the 1.2 mg group, suggesting that OLZ may be stimulating BW gain via additional energy-altering mechanisms. [Supported by: Pfizer Central Research]

Grape seed extract affects energy intake in humans

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Substances that reduce energy intake (EI) without a strong reduction in satiety are potentially useful in the context of obesity treatment. Therefore we assessed the efficacy of grape seed extract with respect to EI and satiety, since grape seed extract has been shown to stimulate lipolysis in vitro. In a randomized, placebo-controlled, double-blind, cross-over study 51 subjects (age 18–65 y, BMI 22–30 kg/m^2) ate for 3 days an ad libitum lunch and dinner in the University Restaurant. Standard breakfasts and snacks were provided. Supplements were taken 30–60 min prior to each meal. We found no difference in EI between grape seed extract and placebo in the total study population. However, in the subgroup of subjects ($n = 23$) who ate 8.9 ± 1.3 MJ/day on average (during placebo), EI was reduced by 4% (DEI 352.1 kJ/24 h, $P = 0.05$) after grape seed extract compared to placebo. Meanwhile there were no significant differences in macro-nutrient composition, appetite/satiety profile over the day, mood or tolerance. So the conclusion is that grape seed affected 24 h EI with 4% reduction in subjects with an intake of 8.9 MJ/day on average without further effects on appetite, mood or tolerance. These findings suggest that grape seed could be effective in reducing EI in normal to overweight, dietary unrestrained men and women. We suggest that a sustained reduction of 4% EI could play a role in body weight management.

Effect of serotonergic agents and CCK on food intake in transgenic rats with low brain angiotensinogen

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The octapeptide angiotensin II is a potent regulator of blood pressure. The more recent finding of interactions between angiotensin and fat cell metabolism could support a role of angiotensin in obesity related symptoms. Recently, a transgenic rat model, TGR(ASrAOGEN)680 expressing an antisense RNA against angiotensinogen mRNA specifically in the brain, has been established. In these animals, brain angiotensinogen protein concentration is reduced by more than 90%. This transgenic rat model provides an opportunity to study the role of brain angiotensin in feeding behavior. Since angiotensin interacts with serotonin (5-HT), a mediator of satiety, the efficacy of serotonergic drugs on food intake has been tested first in these rats. TGR(ASrAOGEN)680 responded more strongly to the hyperphagic action of the 5-HT1A-agonist 8-OH-DPAT. On the contrary, the satiating effect of fenfluramine was also augmented in the transgenics. To characterize the underlying mechanisms, hypothalamic 5-HT was measured. In TGR(ASrAOGEN)680, 5-HIAA/5-HT ratio was significantly reduced, whereas no changes in 5-HT itself were found when compared with the parent Sprague-Dawley strain. Cholecystokinin (CCK) is another important satiety factor and interacts with 5-HT to control feeding. CCK was also more effective to reduce food intake in the transgenic rats as compared to the wildtype. Taken together, these findings suggest a higher sensitivity to serotonergic and CCKergic signals controlling food intake in rats with low brain angiotensin. This indicates a possible action of angiotensinogen or angiotensin on brain serotonergic mechanisms involved in the control of feeding.

CD14 may affect energy balance and lipid metabolism independent of its immune function

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The cell surface molecule CD14 is an important component in mediating the mammalian immune responses to bacterial products such as lipopolysaccharide (LPS). We used male mice with null mutations of CD14 (CD14-KO) and their wild-type littermates (CD14-WT) to investigate the role of CD14 in the anorexia produced by LPS. Mice were injected intraperitoneally at dark onset with LPS (40 mg/mouse) or saline, and food intake was measured every 24 h for the following 5 days. LPS produced significantly more anorexia in CD14-WT mice than CD14-KO mice each day (all $P < 0.05$). Saline-injected CD14-KO mice ate constantly more than saline-injected WT mice (5-d totals 20.8 vs. 16.4 g, respectively). Despite their increased food intake, however, CD14-KO mice had a lower body weight than the CD14-WT mice (31 vs. 36 g, $P < 0.05$). This paradoxical dissociation between food intake and body weight suggests that CD14 affects metabolism, physical activity, or some other function influencing energy balance. As CD14 has been implicated in diseases associated with altered lipid metabolism, we measured plasma triacylglycerol (TAG) and free fatty acid (FFA) concentrations in CD14-KO and CD14-WT mice. FFA and TAG concentrations were both increased in CD14-KO mice. Thus, in addition to its role in mediating the

anorectic effect of LPS, CD14 also appears to play roles in the control of lipid metabolism and energy balance.

The effects of three different Stroop tasks on perceived anxiety and chocolate intake

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This study was designed to test the effects of the modified Stroop colour-naming task on stress and chocolate intake. An ego-threatening Stroop task could provide a standardised method to induce stress in eating behaviour research. In order to establish if any induced stress is due to experiencing the stress word condition or simply to the cognitive demands of the Stroop task, three conditions were employed: stress, matched neutral and incongruent colour words. The stress-word condition incorporates three types of ego threat (from others, self-directed and sociotropy threat) and allows examination of attentional bias towards these different forms of threat. The tasks were presented using SuperLab software to ensure accurate measurement of reaction time and errors and to control the response-stimulus interval (32 ms has previously been found to provide sufficient time pressure to produce anxiety). Mood and appetite variables were assessed before and after completing the task. An ad libitum amount of chocolate buttons was offered after the post-task ratings. A preliminary analysis of 19 participants found a significant difference between stress ratings such that the greatest stress was produced by the stress-word condition (mean = 38.89), followed by the incongruent condition (mean = 35.89) and the lowest stress was reported in the neutral condition (mean = 22.06) [$F(2, 34) = 4.51$; $P = 0.018$]. Chocolate intake showed a similar, but non-significant trend in the same direction. Results suggest that the traditional Stroop task and the ego-threatening Stroop task induce similar amounts of stress and increase food intake to the same extent.

Assortment structure's influence on perceived variety and consumption quantities

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Increasing the actual variety of an assortment has been shown previously to increase the quantity consumed. We show, however, that consumption quantities are also influenced by the perceived variety of an assortment. In combination, six lab and field studies show that the structure of an assortment (e.g. organization and entropy) moderates the effect of actual variety on perceived variety. We further show that—and it is perceived variety, which, in turn, influences consumption quantities through anticipated consumption utility. Making salient other 'consumption rules', such as size of the assortment, moderates this effect. These findings are of immediate relevance to interdisciplinary researchers and to consumers and health practitioners who wish to better control food consumption.

Bottoms up! The influence of elongation on pouring and consumption volume

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Although the effects of shapes on area perceptions have been widely investigated, we examine the extent to which the shapes of containers influence how much people pour and consume in a series of controlled field experiments. Two experiments in cafeterias show both children and adults pour and consume more juice when given a short, wide glass compared to those given a tall, narrow glass, but they perceive the opposite to be true. We conclude the elongation of glasses negatively influences consumption volume in a single-serving context. A third potentially policy relevant field experiment conducted with Philadelphia bartenders and liquor shows that the effect of elongation is moderated—but not eliminated—with pouring experience.

Perinatal dietary omega-3 fatty acid manipulation affects neural control of sodium homeostasis in adulthood

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Polyunsaturated omega-3 fatty acids are the most abundant fatty acids found in the central nervous system and are essential, as mammals are unable to synthesize them de novo and therefore must rely on dietary supplementation to acquire them. We determined whether dietary manipulation of omega-3 fatty acid levels during the perinatal period would lead to alterations in the central control of sodium homeostasis. Pregnant rat dams were fed either an omega-3 sufficient (CON) or deficient (DEF) diet. Pups were continued on the same diet as their mothers (CON-CON, DEF-DEF) or switched to the opposite diet at weaning (CON-DEF, DEF-CON). Salt appetite was induced by furosemide (20 mg/kg, i.p.) and low-dose captopril (20 μg/kg, i.p.). Animals had continuous access to 0.5 M NaCl except during the 2 h prior to injection. No significant differences in baseline sodium intake were found between groups. Sodium

intake was increased ($p's < 0.001$) in all groups during the 24 h period following treatment with furosemide and low dose captopril. Sodium intake returned to baseline during the next 24 h in animals that had access to the CON diet prior to weaning (CON-CON and CON-DEF). The increased salt intake persisted for 5 days in animals that had access to the DEF diet prior to weaning (DEF-DEF and DEF-CON). These results suggest that a deficiency of essential omega-3 fatty acids during the perinatal period can lead to dysregulation of central sodium homeostatic mechanisms. These findings are consistent with the reported effects of perinatal omega-3 fatty acid supply on blood pressure.

Differential impact of cocaine on meal patterns in female and male rats

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Female rats exhibit greater behavioral activation to cocaine and other psychostimulants. The present study extends that literature through an examination of the impact of sex and the estrous cycle on the hypophagic properties of cocaine. Meal patterns were recorded in automated food hoppers during the first 3 h of the dark phase in adult female and in male rats after administration of ascending cocaine doses (0, 7.5, and 15 mg/kg cocaine, i.p.) on successive trials. Estrous phase was determined using daily measures of vaginal resistance and of food intake. The hypophagic properties of cocaine were similar in male and female rats during the first hour after cocaine treatment. In contrast, cocaine produced a greater suppression of feeding, as well as reduction in meal number, over a 3-h test period in female rats during estrus, relative to that noted during diestrus. No significant differences were noted between estrus and diestrus with regard to changes in meal size induced by cocaine over a 3-h test period. During the 3-h test period, male rats showed minimal hypophagic responses to 7.5 or 15 mg/kg cocaine. These results extend the range of behavioral perturbations induced by cocaine that are modulated by sex and by the estrous cycle and are consistent with the notion that estradiol may modulate the neurochemical actions of cocaine.

Blood glucose dynamics and feeding patterns

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Synchronization between transient (i.e. starting from stable baseline as a relatively small dip in blood glucose) or dynamic (i.e. after ingestion starting from the peak and going through baseline) blood glucose declines and spontaneous meal initiation in time blinded young lean men lends support to satiating efficiency being higher after high-fat than after simple CHO preloads. Varied blood glucose responses after aspartame were related to sweetness perception, which was negatively related to hunger suppression. This explains the controversy over the aspartame effects. The synchronization between blood glucose dynamics and meal initiations may be explained by glycogen dynamics. In cases of depleted glycogen buffers after exercise, post-absorptive meal initiation was not synchronized with blood glucose declines; post-prandially the synchronization with dynamic declines but not with transient declines reestablished. In a negative energy balance synchronization was also absent, except between dynamic declines and meal initiation. These observations lend support to the 'glycogen dynamics hypothesis'. The significance of the synchronization has been shown in energy intake regulation. In lean young men energy intake, and the accuracy of energy intake regulation was a function of meal frequency, related to the frequency of blood glucose declines, related to macronutrient intake. With a higher relative CHO intake frequency of blood glucose declines and of meals, respectively, increased, while energy intake decreased and tuning of energy intake to energy expenditure was more accurate. In conclusion, in young lean men the relationship between blood glucose dynamics and feeding pattern plays an important role in energy intake regulation.

Managing energy density effects on the long term

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On the short term, i.e. during a meal, energy density (ED) determines energy intake (EI) of food to a great extent. This occurs irrespective of ED being determined by the macronutrients or water, or fibre. On the longer term ED only determines EI when ED is determined by the macronutrients (and water, fibre) but not if it is only determined by water. However, culturally determined portion sizes of well-known foods represent a 'learned satiety' in that portion size is anticipated and inversely related to energy density. Moreover, on the long term dietary restraint compensates for increases in ED, while unrestraint compensates for decreases in ED. Taken together: with daily food intake learned satiety translated into culturally determined portion sizes, and dietary restraint prevent running away with energy density determining energy intake as it does during a meal.

Habitual caffeine intake affects weight-loss and affects the effect of green tea on weight-maintenance thereafter

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Since thermogenetic effects of green tea have been shown, we investigated the effect of green tea on weight-maintenance after weight-loss in relation to habitual caffeine intake. In a randomized placebo-controlled double blind parallel trial in 76 subjects, (BMI = 27.5 ± 2.7 kg/m^2; 40 ± 10 yrs) matched for habitual caffeine intake, a 4-week very low energy diet (2.1 MJ/d) was followed by 3 months green tea (epigallocatechin gallate + caffeine), or placebo. In the women leptin concentration was inversely and in both genders satiety was positively related to habitual caffeine intake ($r^2 = 0.7$; $r^2 = 0.6$; $P < 0.01$). Reduction of weight (6.7 ± 0.7 vs 5.5 ± 0.6 kg), fat-mass (4.4 ± 0.4 vs 3.1 ± 0.3 kg), waist-circumference (6.7 ± 0.6 vs 5.1 ± 0.5 cm), respiratory quotient (RQ) (0.06 ± 0 vs 0.04 ± 0) and of decrease in resting energy expenditure (REE) (0.5 ± 0.06 vs 0.8 ± 0.09 MJ/d) was larger in the high- than in the low-caffeine consumers ($P < 0.01$). During weight-maintenance green tea reduced body-weight (−0.6 ± 0.1 vs +3 ± 0.4 kg), waist (−2.0 vs +0.1 cm), RQ (−0.01 ± 0 vs +0.2 ± 0) and body-fat (−1.6 vs +0.3 kg) further, and increased REE more (+1.0 vs 0.5 MJ/d) in habitual low-caffeine consumers, compared to placebo or high-caffeine consumers ($P < 0.01$). We conclude that thermogenesis and fat-oxidation increased body-weight loss in high caffeine consumers, and weight-maintenance in low caffeine consumers receiving green tea.

High protein intake supports weight maintenance after body weight loss in humans

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Since high-protein diets have been shown to be relatively more satiating, thermogenetic and having a low energy efficiency during overfeeding, we investigated the effect of addition of protein on weight-maintenance after weight loss of 5–10%. In a randomized parallel trial in 148 subjects (age 44.2 ± 10.1 yrs; BMI 29.5 ± 2.5 kg/m^2), with stratified characteristics a 4-week very low energy diet (2.1 MJ/d) was followed by 3 months' consumption of 25 g/d additional protein or not, while all subjects got dietary counseling. Protein intake was determined from 24 h urinary nitrogen. Both groups lost 6.4 ± 1.8 kg body mass ($P < 0.001$). Thereafter the additional-protein group compared to the non-additional protein group showed 18 vs 15 en% protein intake, a 50% lower body weight regain (1.0 ± 0.2 vs 2.0 ± 0.3 kg) only consisting of fat free mass (+2 ± 0.2 kg FFM −1 ± 0.1 kg FM vs +0.8 ± 0.1 kg FFM +2.2 ± 0.2 kg FM), a 50% decreased energy efficiency; increased satiety (8.4 ± 0.8 vs 0.5 ± 0.1 mm VAS), and a lower increase in triacylglycerol (+160 ± 74 vs +300 ± 138 μM/l) and in leptin (+6.4 ± 4.6 vs + 9.5 ± 6.1 μg/l) ($P < 0.01$). Changes in RMR, RQ, TEE, glucose, insulin and free fatty acids did not differ. We conclude that an 18 vs 15 en% protein intake during weight-maintenance showed 50% less weight-regain, due to effects on body-composition, satiety and energy efficiency.

Acute 3rd ventricular administrations of leptin decrease food intake and modify food choice of rats

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Peripheric administration of leptin reduces food intake and body weight gain and modifies food choice. The aim of this study was to examine the effect of acute cerebral injections of leptin on food selection in rats. Male rats were first adapted to the food choice paradigm (protein, carbohydrate, fat) since the age of 3 weeks. When body weight reached 250–270 g, rats were implanted with a cannula in the third ventricle. The placement of cannula was tested by angiotensine injection. Leptin injections (5 μg in 5 μl saline) were performed either at the beginning or at the end of the night at 72 h interval. Food intake was recorded 3 days before, during and after injections. Rats were sacrificed 72 h after the second injection. After both injections body weight gain and food intake were reduced. The reduction in food intake was however different according to the time of injection. When injection was done at the beginning of night, after 2 h latency, the reduction was 50 and 27% during the first 24 h and the following 24 h, respectively. For the 2d injection, the same effects were observed immediately (respectively, 32 and 30%). Leptinemia (5.4 ± 2.1 μg/ml), insulinemia (34.4 ± 2.1 μg/ml) and glycemia (1.12 mg/ml) at time of sacrifice, 6 h after food deprivation were identical to those obtained in control rats. ICV leptin reduces food intake differently according to their food preferences, it seems that protein and fat intakes are more decreased. Food intake records are under investigations.

Hydroxycitrate (HCA) reduces the insulin response to an intragastric glucose load

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(-)-Hydroxycitric acid (HCA), the active ingredient in the herbal compound Garcinia Cambogia, may be used as a dietary supplement for influencing food intake and metabolism/energy balance. In our initial studies we showed that HCA reduces short term food intake. The effects on glucose and fat metabolism are still unknown. In the present study we focused on the possible effects of HCA on glucose homeostasis. Intragastrical administration of HCA (Regulator, 310 mg/kg) dramatically reduced the insulin response to an intragastric (ig) infusion of 9 ml Nutrison (a highly nutritive solution). This reduction in plasma insulin was also found when HCA was intragastrically administered 2 h prior to an infusion of glucose (12% glucose in 9 ml in 5 min) into both (1) the stomach and (2) the duodenum. HCA had no effect on blood glucose and plasma insulin profiles during an intravenous glucose tolerance test (an infusion of 200 mg glucose – 10 mg/min during 20 min). Taken together, these data reveal that HCA reduces the insulin response to a glucose load. The insulin lowering effect of HCA seems secondary to an effect of HCA on glucose entrance from the gut into the blood. A direct action of HCA on pancreatic insulin secretion is less likely.

Vagotomy dissociates consumption- and deprivation-related controls of endogenous ghrelin

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Plasma ghrelin levels are responsive to short- and long-term nutrient fluctuation, rapidly decreasing with food consumption and rising with food deprivation. The mechanism(s) underlying these two responses is not clear, but it seems reasonable to hypothesize a vagal contribution to both. Nutrient-related ghrelin suppression may be mediated by load-related vagal afferent activity, or depend upon vagal efferent input to the gastrointestinal tract. Similarly, the deprivation-related ghrelin rise could involve state-related vagal afferent or efferent signals. Here, we examine the role of the vagus in the regulation of plasma ghrelin by sampling blood from rats with subdiaphragmatic vagotomy and sham-operated controls over 48 h of food deprivation, and before and after gavage of liquid diet. Vagotomized rats showed load-related ghrelin suppression, but failed to increase plasma ghrelin with food deprivation, providing a clear dissociation in the mechanisms underlying these responses. These results are reminiscent of a previous study in which we demonstrated that chronic decerebrate rats show an intact loading response, but no deprivation-related increase in plasa ghrelin level. It is clear, then, that neuroanatomically separable mechanisms mediate the short- and long-term ghrelin responses. The nutrient-related ghrelin reduction requires neither the vagus nerve nor communication between forebrain and hindbrain. The deprivation-induced rise in ghrelin, by contrast, requires both an intact vagus and connections between the forebrain and the caudal brainstem.

A2 lesions block LiCl and CCK anorexia

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LiCl and CCK (large doses) promote anorexia, increase plasma oxytocin (OT), and induce conditioned taste aversion (CTA). The CTA response to LiCl is absent in rats with lesions of the area postrema and adjacent portions of the nucleus of the solitary tract (NST); however, such lesions do not block anorexia or OT secretion after LiCl (Brain Research, 663, 30). Such lesions largely spare noradrenergic (A2) NST neurons, which are robustly activated by LiCl and CCK. In the present study, we examined the role of A2 neurons in LiCl and CCK anorexia by specifically lesioning these neurons while sparing the AP. Female rats (~140 g) received bilateral microinjections of vehicle ($n = 4$) or DβH-saporin toxin ($n = 3$) into the A2 region. Intake experiments were performed a few weeks later, after rats recovered from lesion effects on feeding and BW, and after acclimation to daily 4-h food access. In separate trials, rats were injected i.p. with LiCl (0.15 M, 2% BW), CCK (10 μg/kg), or saline before food access. Intake was recorded every 30 min for 4 h. In the final experiment, rats were injected with saline or CCK and perfused 60–90 min later for lesion verification and cFos analysis. *Results*. LiCl and CCK did not inhibit food intake in rats with bilateral A2 lesions, and CCK-induced cFos expression was reduced in relevant brainstem and forebrain regions (including hypothalamic OT neurons) in lesioned rats. We conclude that A2 neurons are a necessary component of the central neural circuits mediating LiCl- and CCK-induced anorexia. [Supported by: NIMH 59911]

Exogenous amylin inhibits ethanol intake in Sardinian alcohol-preferring rats

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Sardinian alcohol-preferring (sP) rats are a Wistar substrain selectively bred for high spontaneous alcohol

intake (ad libitum 10% ethanol:water intake ratio >3; Alcohol Alcoholism, 32 (1997) 443). Because amylin is a peptide that selectively decreases meal size in rats (i.e. Peptides 24 (2003) 91), we investigated its effect on 10% ethanol intake in sP rats. Rats had free access to food, water, and alcohol except for two weekly 60-min tests that followed 22 h alcohol deprivation. Intraperitoneal injections of 1, 2 and 4 μg/kg amylin just prior to ethanol access produced a significant dose-related inhibition of 30-min alcohol intake (21–45%). Across these doses, amylin had no reliable effect on 1% sucrose intake in sP rats or in normal Wistar rats. We do not know whether this difference is due to a selective effect of amylin on alcohol intake or can be accounted for by some other difference in the test situation, such as the differing nutrient compositions of the fluids. This is the first evidence that exogenous amylin inhibits alcohol intake. [Supported by: AA12880]

Increased fullness and appetite suppression by exercise in postmenopausal women

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Experimental evidence for appetite suppression by exercise is limited. We designed a study where postmenopausal women expended approximately 800 kcal divided between AM and PM walking bouts on 2 days, one day they exercised 1 h before eating (ExBE), and the other one hr after eating (ExAE), to discover how walking in a fasted state or after a meal affects ratings of hunger, fullness, desire to eat and estimation of capacity to eat on a visual analog scale (VAS). *Results*. *Hunger*: ExBE, increased hunger after the two meals by 86%. *Fullness*: ExBE, increased the sense of fullness before the AM meal by 58%. ExAE increased the sense of fullness following both meals by 22 and 13%, respectively, as well as before the PM meal, 15%. *Desire to eat*: ExBE, decreased the desire to eat by 32% 1 h before the AM meal, and by 18% at the PM meal. ExAE, had an 82.5% decrease in the desire to eat, following the meals. *How much they wolud like to eat*: ExBE, reduced the amount subjects wanted to eat before the meal by 41%. ExAE, showed a 50% decrease following the PM meal. *Conclusion*: Exercise will augment feelings of fullness and attenuate the desire to eat and the amount of food they would like to eat. Exercising fasted, will increase hunger after the meal. Further work is needed to determine the role of hormonal influence.

Effects of manipulated palatability and energy density on appetite

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Energy density (ED) is strongly correlated with rated food palatability. This is even true with sub-classes of food where variance in ED is minimal, for example fruit and vegetables (Gibson et al., 2003, Appetite, in press). Two different explanations for this relationship are explored. Firstly that there is some innate preference for high-ED foods. It is known that humans have an innate preference for sweet tastes, and sweetness, palatability and ED are inter-correlated. The recent finding of an oral fat receptor leads to the hypothesis (untested) that the same may be possible for fats. Alternatively, palatability may reflect the acquired liking for foods based on conditioned associations between flavour and consequence. Thus energy dense foods may be liked because there flavours predict delivery of energy. There is a substantial animal literature supporting this idea, but the human literature in this area is weak. In contrast, the concept of learned satiety suggests that flavours come to control meal-size by predicting post-ingestive effects. These two concepts therefore make different predictions about the ED/palatability relationship which were explored through recent studies where palatability and energy density were independently manipulated. Where flavour differences existed which predicted ED, flavour but not palatability predicted meal-size, but when foods matched in ED varied in flavour, palatability predicted intake. These data suggest that the palatability/ED relationship is therefore a complex one, and the consequences of these findings for understanding short-term over-eating are discussed.

Responsivity to manipulated palatability as a function of scores for restraint and disinhibition from the Three Factor Eating Questionnaire

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Manipulating palatability affects short-term intake, and variation in sensitivity to palatability could explain individual susceptibility to weight gain. One possibility is that palatable food cues lead to a breakdown in dietary restraint. However, since sufferers from binge-eating disorder score low in restraint but still binge-eat, some variability in responsivity to palatability may operate independent of restraint. To test these ideas, 40 normal weight women, all of whom had

completed the Three Factor Eating Questionnaire, ate a simple pasta lunch on two occasions, with intake and changes in appetite recorded using the Sussex Meal Pattern Monitor (see Yeomans, Neurosci. Biobehav. Rev. 24 (2000) 249–259). On one occasion, the pasta was served in a bland sauce, and the other an isoenergetic palatable sauce. Overall, women ate more of the palatable than bland version ($F(1,36) = 12.86$, $P < 0.001$), but there were significant food*restraint ($F(1,36) = 4.91, P < 0.05$) and disinhibition*restraint interactions ($F(1,36) = 6.93$, $P < 0.05$). High restraint was associated with less response to palatability, whereas high disinhibition was associated with a large response to palatability. Pleasantness did not differ with restraint or disinhibition, and the palatable food was consistently rated more pleasant than the bland version ($F(1,36) = 10.44$, $P < 0.005$). Appetite ratings revealed a larger appetiser effect of the palatable food in women scoring high in disinhibition, and a tendency for women scoring low in disinhibition but high in restraint to end the meal with higher hunger ratings. Together these data imply a complex relationship between palatability and individual attitudes to food.

Orexin-A administration to dorsal vagal complex augments food intake by counteracting visceral satiety signals

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Orexin neurons located in the hypothalamus project profusely throughout most of the brain, including brainstem structures involved in the execution of ingestive behavior and in autonomic control mechanisms serving energy homeostasis. Orexin-A-immunoreactive axon terminals and orexin receptors are numerous in the NTS and dorsal motor nucleus of the vagus, and local microinjection of orexin-A potently stimulates gastric motility and acid secretion (Krowicki et al., Am. J. Physiol. (2002) 283). As the inhibitory effect of i.p. administered CCK was negated by ICV orexin (Asakawa et al., Diab. Obes. Metab. (2002) 4), we hypothesized that orexin's effects on food intake might be partly mediated by actions in the caudal medulla. Orexin-A (2×0.2 nmol) injected bilaterally into the NTS significantly stimulated by 40% ($P < 0.01$) high-fat chow intake (known to strongly release endogenous CCK) but not intake of 15% sucrose solution (a weak stimulator of CCK). Preliminary results from medulla slice preparations in neonatal rats show that orexin-A has direct postsynaptic effects by depolarizing 2 of 5 neurons and hyperpolarizing 1 neuron. Together, the results demonstrate that orexin-A can change activity of NTS neurons and thereby influence food intake under certain conditions. Thus, part of orexin's modulation of ingestive behavior achieved by intraventricular injections might be mediated by an interaction with CCK-induced afferent signaling from the gut at the level of the dorsal vagal complex. [Supported by: DK47348]

Modulation of brainstem feeding and autonomic mechanisms by hypothalamic peptides

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The hypothalamus senses the internal nutritional state and initiates control mechanisms serving homeostatic regulation, and the modern environment influences energy balance mainly via motivational, cognitive, rewarding, and emotional processes residing in the forebrain. Critical information pertaining to these hypothalamic and forebrain processes reaches the brainstem, which organizes oropharyngeal motor behavior and autonomic concomitants of eating, via rich descending projections. We have started to analyze the organization of projections descending from hypothalamic peptidergic neurons to the dorsal vagal complex and other brainstem areas. Analysis at the anatomical and neurochemical level shows significant innervation of the solitary nucleus, vagal motor nuclei, and the surrounding reticular formation by orexin, MCH, alpha-MSH, CART, GRP, and oxytocin neurons located in specific hypothalamic areas. The simple, straight geometry of varicose axon terminals found in solitary nucleus suggest chemical rather than anatomical addressing, likely achieved by volume release of peptides. Whole cell recording from solitary nucleus neurons in medulla slices and bath application of the various peptides revealed both presynaptic modulation via vagal afferent terminals and postsynaptic modulation of glutamate currents. The findings support the idea that part of the effects of these hypothalamic peptides on feeding is mediated by their actions on medullary reflexes of meal ingestion and autonomic control. [Supported by: NIH grant DK47348]