1

The Central Melanocortin System: 20 Years On

R CONE

Vanderbilt University School of Medicine Nashville, TN, USA

The central melanocortin receptors were cloned twenty years ago, and soon thereafter discovered to play a critical role in feeding behavior. What have we learned since then about the central melanocortin system and its role in ingestive behavior? The system is at the heart of the adipostat, but also required for acute signaling of hunger and satiety. Activation of melanocortin signaling mimics cachexia. Even a partial decrease in MC4R activity causes hyperphagia and morbid obesity, thus the MC4R acts as a rheostat on food intake. Indeed, the elegant studies of Palmiter and Sternson argue that AgRP neurons are an essential accelerator of food intake, via regulation of MC4R target neurons. Curiously, this accelerator does not seem to increase the reward value of food. Indeed, MC4R deletion actually decreases the consumption of palatable foods in a two choice model. Current studies in the laboratory are focused on understanding the rheostat-like nature of the MC4R, atypical of a GPCR, and on capitalizing on this aspect of MC4R signaling to develop a new class of drugs for treating melanocortin obesity syndrome. Since partial loss of MC4R signaling causes morbid obesity, we reasoned that bringing MC4R receptor activity back to normal levels should treat melanocortin obesity syndrome. Small molecule allosteric modulators of the MC4R are described that can increase the efficacy of a-MSH at the MC4R. We also describe a novel signaling pathway of the MC4R, identified in neurons of the PVN, that may provide a molecular basis for the rheostat-like function of the receptor.

2

Application of Behavioral Economics to Childhood Obesity Prevention

KL KELLER

The Pennsylvania State University, Dept of Nutr Sciences and Food Science University Park, PA, USA

The long-term goal of this research is to use theories from behavioral economics to improve eating behaviors and prevent obesity in children. Previous research has largely relied on changing *individual* behaviors to treat childhood obesity. In our model, we are testing whether using behavioral economics to change the packaging and presentation of foods in the home can impact children's intake of these foods. We conducted a 7-week randomized-control trial with 4-5 year-olds at risk for obesity to determine the efficacy of using promotional characters on packages of fresh fruits and vegetables (F&V) for improving dietary habits. Children enrolled in the treatment group increased their F&V consumption by ~ 50% from baseline; children in the control group showed no change. In addition, children in the treatment group decreased BMI z-scores from baseline, while the control group increased BMI z-scores ($p<0.05$). To improve upon these effects, we are currently testing the efficacy of combining the use of promotional characters with a presentation manipulation that teaches parents to serve the vegetable as the optimal default, or primary snack or meal option. "Optimal defaults" implies the positioning of choices in an optimal manner to achieve a positive outcome. The concept has been successful at improving behaviors in the public health arena, but no previous studies have tested this in the home. Preliminary analyses of these data suggest improvements in eating behavior and overall diet quality. While more rigorous testing is needed, food packaging and presentation are easily implemented by parents and may enhance current practices for obesity prevention.

3

The impact of thirst on stress responsiveness and mood.

E.G. KRAUSE

Department of Pharmacodynamics, University of Florida Gainesville, FL, USA

Challenges to body fluid homeostasis are met with behavioral and humoral responses aimed at restoring hydromineral balance. Decreased blood volume increases circulating angiotensin-II (ANGII), which maintains perfusion pressure and stimulates water intake, thereby alleviating hypovolemia. Hypernatremia, another threat to body fluid homeostasis, elicits thirst and the secretion of oxytocin (OT) and vasopressin into the systemic circulation, which decrease the plasma sodium concentration by promoting water intake, sodium excretion, and water retention. Using rodents, my studies have determined that although hypovolemia and hypernatremia similarly stimulate water intake, these challenges have differential effects on stress-responsiveness and mood. Pharmacologically-induced hypovolemia or exposure to psychological stress elevates circulating levels of ANGII, which activates the hypothalamic-pituitary-adrenal (HPA) axis and promotes anxiety-like behavior. These effects are elicited by neurons expressing angiotensin type-1 receptors in the subfornical organ that have an excitatory influence on parvocellular neurons in the paraventricular nucleus of the hypothalamus (PVN). In contrast, acute hypernatremia decreases stress-induced activation of the HPA axis and is anxiolytic, especially in social situations. These effects are mediated by centrally-released OT, which alters activation of neurons in the PVN and other brain nuclei implicated in the control of anxiety-like behavior. Collectively, the results suggest that hydration state profoundly influences neural circuits controlling stress responsiveness and mood.

4

Modeling emetic behavior in the animal: how do we study nausea?

BC DE JONGHE
University of Pennsylvania Philadelphia, PA, USA

New discoveries and improved methods for controlling nausea in diverse populations via dietary, behavioral, and/or pharmacological means are desperately needed. Clinically available anti-emetic drugs have over the last two decades greatly reduced the severity and appearance of vomiting, and thus increased the tolerance of treatments associated with emetic side effects. However, many patients still exhibit severe treatment-induced nausea which continues to unnecessarily obviate successful treatment adherence. Traditional animal species models have been focused on modeling emesis without the clarity of delineation between sensations of sickness (i.e., nausea) and vomiting behaviors. While rodents lack the physiology needed to vomit, rats and mice may still represent a viable model for the study of nausea. Important considerations in choice of species also include fundamental metabolic and behavioral similarities to humans with respect to energy balance in the neuroendocrine control of sickness behavior. Here, the focus will be centered on recent advances in the field of nausea research in widely used animal models of energy balance research which have heightened our understanding of the underlying mechanisms thought to control nausea and emetic behaviors. Support: NIH DK19525 & DK093874, Penn McCabe Fund, Penn Biobehavioral Research Center.

5

Cognitive Correlates of Food Intake

PS HOGENKAMP
Dept. of Neuroscience, Uppsala University Uppsala, Sweden

Food intake is a process controlled by a complex interplay of psychological, physiological, environmental, and lifestyle factors. A variety of these factors encourage excessive consumption in the absence of energy needs. In order to test whether food texture is one of the factors that can be altered to regulate intake, I investigated the role of food texture in learned satiation in a series of conditioning studies. In one of these studies, participants repeatedly consumed low (LE) and high energy (HE) dense foods, which were either low (n=24) or high (n=22) in viscosity. In another study, LE and HE foods were consumed with a straw (liquid yogurt, n=34) or a spoon (liquid yogurt, n=36; semi-solid yogurt, n=35). Our dependent variable was *ad libitum* intake. Interestingly, changes in meal size through learning were limited, and the amount consumed depended on texture rather than energy density. After finding that elements of the food items that were unrelated to total calories could significantly influence intake, we anticipated that cognitions regarding the food may be similarly potent. To test this we examined the effects of both calorie anticipation and actual food intake on intake in a follow up meal. As cognitive cues that modulate food intake may also influence the release of gut hormones, we also measured ghrelin levels. During four breakfast sessions, participants (n=12) consumed either a LE or a HE preload and were provided with either consistent or inconsistent calorie information (i.e., stating the caloric content of the preload was low or high). Ghrelin levels did not respond in an anticipatory manner; however, calorie information affected the amount of food consumed following low-caloric food consumption, suggesting that cognitive cues significantly impact food intake. In sum, my studies show that cognitive processes, qualities of the food and metabolic responses interact to determine food intake.

6

Altered intestinal morphology may account for differences in nutrient sensing

MJ DAILEY, EC GELFAND, TH MORAN
Johns Hopkins University Department of Psychiatry and Behavioral Sciences Baltimore, MD, USA

Every day we are eating a variety of foods and our body needs to be able to assess what we are eating. Our body senses the quantity and quality of our food in order to get us to stop eating and to be able to direct the nutrients to the appropriate target tissues and metabolic pathways. Differences in how we sense nutrients may profoundly affect our ingestive behaviors. I previously found that obese and lean rats respond differently to the same nutrients whether they are delivered directly into the intestine or eaten orally, as measured by changes in food intake, body weight and satiety peptide levels. The purpose of this study was to examine whether the functional differences in response to different nutrients in lean/chow fed and obese/high fat fed rats could be accounted for by changes in the number or signalling of different cell types within the intestinal epitheluim. Male Sprague-Dawley rats were fed either chow or a high fat diet. When there was a significant difference in body weight, food intake was measured. Rats were then sacrificed, intestinal tissue collected and processed. We found that there were no differences in intestinal length or jejunal villi length between the two groups. In contrast, the obese/high fat fed rats had a significantly greater number of enteroendocrine, enterocyte, paneth and goblet cells in the jejunum and ileum of the intestine compared with lean/chow fed animals. These morphological differences in the intestine between lean and obese animals may account for differences in nutrient-driven changes in ingestive behaviors.

7

Forebrain-hindbrain nutrient sensing neurocircuits in the regulation of energy balance.

C BLOUET
Albert Einstein College of Medicine New York, NY, USA

Detection of nutrient availability and coordination of effectors that determine energy intake and utilization is a critical process in the maintenance of energy homeostasis, and is impaired in metabolic disorders such as obesity and type 2 diabetes. In these pathologies, cellular exposure to excess nutrients is emerging as a common putative cause for multiple deleterious cellular consequences across diverse cell types, underscoring the need to better identify and characterize nutrient sensors and their downstream targets. The central nervous system (CNS) has emerged as an important contributor to the nutrient-sensing mechanisms underlying the regulation of energy homeostasis. Distributed systems of specialized metabolic sensing neurons are responsive to both nutrients and fuel related signals (insulin, leptin and gut-secreted peptides), integrate all class of signals and consequently engage a complex set of neurochemical and neurophysiological responses to produce a coordinated negative feedback response regulating multiple effectors of energy balance. We will discuss the recently characterized molecular and neurochemical mechanisms underlying central amino acid detection and the forebrain-hindbrain circuits engaged by amino acid to regulate food intake. We will also present data supporting the integrative capacities of caudomedial nucleus of the solitary tract in the acute regulation of meal size. Last, we will present data supporting the plasticity of CNS amino acid sensing mechanisms.

8

Discovery of an Endogenous Inhibitor of Angiotensin II Signaling

GLC YOSTEN[1], *J LIU*[2], *K SANDBERG*[2], *WK SAMSON*[1]
[1]*Saint Louis University St. Louis, MO, USA,* [2]*Georgetown University Washington, DC, USA*

Two transcripts (E1,2,3 and E1,3) are synthesized from the rat angiotensin type 1a receptor ($rAT_{1a}R$) gene. Both transcripts encode the entire $rAT_{1a}R$ protein in exon 3. Within the 5' leader sequence, exon 2 contains a short open reading frame that encodes a seven amino acid peptide (PEP7) that we find *in vitro* selectively inhibits angiotensin II (Ang II) activation of Erk1/2 but not PKC. We hypothesized that PEP7 would inhibit saline, but not water, drinking after Ang II infusion since Ang II-induced saline drinking depends upon the Erk1/2 pathway while water drinking does not. Administration of 1.0 nmol PEP7 i.c.v. 10 min prior to a second injection of 25 pmol Ang II, failed to alter water drinking but significantly abrogated saline (1.5% NaCl) drinking in male rats. This inhibitory effect lasted the entire 2 hr observation period. Although Ang II can induce saline ingestion through the Erk1/2 pathway, it can also inhibit saline drinking through the central release of the inhibitory peptide oxytocin (OT). We hypothesized that when PEP7 blocks Ang II-stimulated Erk1/2 activation, animals no longer ingest saline to balance the continued water intake, due to the release of OT and its subsequent inhibitory effects on saline drinking. The action of PEP7 to inhibit Ang II-induced saline ingestion was rapidly reversed by a subsequent i.c.v. injection of the OT antagonist, OVT (8.7 nmol given 30 min after Ang II). Thus we have identified an endogenous peptide encoded in Exon 2 of the $rAT_{1a}R$ that both *in vitro* and *in vivo* selectively inhibits Erk1/2 activation resulting in physiological consequences for sodium ingestion.

9

Activation of ERα decreases overnight and stimulated water intake by female rats.

J SANTOLLO[1], *A MARSHALL*[1], *KS CURTIS*[2], *D DANIELS*[1]
[1]*University at Buffalo Buffalo, NY, USA,* [2]*Oklahoma State University Tulsa, OK, USA*

Estradiol decreases both overnight and stimulated water intake by female rats. Which estrogen receptors (ER), either classical ERα and/or ERβ, or the more recently identified GPR30, mediate this effect remains an open question. In the present studies, we examined overnight and angiotensin II (AngII)-stimulated water intake and angiotensin type 1 receptor (AT1R) expression in ovariectomized female rats treated with either vehicle, the ERα agonist PPT, the ERβ agonist DPN, a combination of PPT and DPN, or the GPR30 agonist G1. Only treatment with PPT decreased overnight water intake, and the reduction occurred only during the first 6 h after drug treatment. Analysis of drinking patterns revealed that the decrease in intake was a function of fewer bursts and smaller burst size. The change in burst number, however, was more transient, whereas the effect of PPT on burst size was longer lasting. Likewise, AngII-induced water intake was decreased by PPT, but not by the other agonists. Analysis of drinking patterns revealed a PPT-mediated decrease in burst number with no change in burst size. Finally, preliminary RT-PCR studies found that treatment with PPT decreased AT1R expression in the subfornical organ. Together, these findings demonstrate that activation of ERα is sufficient to decrease water intake by female rats and suggests minimal, if any, involvement from other ER subtypes. Furthermore, the lick pattern analysis suggests that estrogens decrease intake through changes in both satiety and appetitive behavior.

10

Endogenous glucagon-like peptide-1 influences water intake in rats

NJ MCKAY, D DANIELS
Department of Psychology, University at Buffalo Buffalo, NY, USA

Glucagon-like peptide-1 (GLP-1) plays an important role in energy homeostasis. Injections of GLP-1 receptor (GLP-1R) agonists suppress food intake, and endogenous GLP-1 production and release are stimulated by the presence of nutrients in the gut. There is also growing evidence that the GLP-1 system is involved in the regulation of body fluid homeostasis. Exogenous GLP-1R agonists suppress water and saline intake, but, to the best of our knowledge, there have been no studies testing whether endogenous GLP-1 regulates fluid intake. To address this open question, we designed the present experiment to test if endogenous GLP-1 plays a role in water intake. Approximately 30 min before the start of the dark phase, food was removed, and rats were given a lateral ventricle injection of either the GLP-1R antagonist exendin (9-39) (Ex-9; 20 µg) or vehicle (1 µl 0.9% saline) and overnight water intake was measured with a contact lickometer. Rats given Ex-9 licked more ($p<0.05$) for water during the first 3 h post injection than controls, suggesting that under normal circumstances central GLP-1 restricts fluid intake. To test if this effect was a function of appetitive or post-ingestive feedback, we analyzed the licking patterns during the first 3 h of intake. This analysis found that the number of licking bursts were greater ($p<0.05$) in rats that received Ex-9, however, there was no difference in the burst size (p=ns). These data indicate that GLP-1 decreases fluid intake through post-ingestive feedback mechanisms. More broadly, these data add to the growing evidence that the effect of GLP-1 is not specific to food intake.

11

MOXONIDINE INTO THE LATERAL PARABRACHIAL NUCLEUS MODIFIES BOTH OROSENDORY AND POSTINGESTIVE SIGNALS RELATED TO SODIUM INTAKE.

S GASPARINI[1], J.V. MENANI[1], D. DANIELS[2]
[1]São Paulo State University,UNESP Araraquara, Brazil, [2]University at Buffalo.The State University of New York Buffalo, NY, USA

Activation of α_2-adrenoceptors by bilateral LPBN injections of moxonidine strongly enhances 0.3 M NaCl intake induced by subcutaneous treatment with the diuretic furosemide (FURO) combined with captopril (CAP). Moreover, FURO + CAP treated rats that received bilateral injections of moxonidine into the LPBN presented low number of aversive and the high number of ingestive responses to an intra-oral infusion of 0.3 M NaCl in the next hour after strong ingestion of water and hypertonic NaCl. In the present study, we used analysis of licking behavior to test if LPBN injections of moxonidine affects saline intake by changing orosensory or postingestive feedback. Bilateral injections of moxonidine (0.5 nmol/0.2 µl) into the LPBN of male Sprague Dawley rats increased FURO + CAP-induced 1.8% NaCl intake (29.7 ± 5, vs. sal: 4 ± 1 ml) and the number of licks/bin from 15 to 60 min (737.67 ± 266.98, vs. sal: 0.0 ± 0.0 at 60 min). These increases were caused by the increase in the number of bursts/bin (36.67 ± 7.43, vs. sal: 0.0 ± 0.0) for 1.8% NaCl and also by the increase in number of licks/burst for 1.8% at 60 minutes of the test (26.58 ± 8.16, vs. sal: 0 ±0). These results suggest that moxonidine into LPBN affects both orosensory and postingestive signals related to sodium intake.

12

Age-related fluid intake and cardiovascular responses to aldosterone-dexamethasone combination in rats.

RL THUNHORST[1,2], B XUE[1,2], TG BELTZ[1], AK JOHNSON[1,2]
[1]Department of Psychology, University of Iowa Iowa City, IA, USA, [2]The Cardiovascular Center, University of Iowa Iowa City, IA, USA

We tested effects of aldosterone (ALDO, 60 ug/day by minipump) given alone, or in combination with dexamethasone (DEX, 2.5 or 20 ug/day) on water and 0.3 M NaCl intakes, mean arterial pressure (MAP) and heart rate (HR) of young (3-4 mo), middle-aged (12 mo) and old (29-30 mo) rats. Compared to baseline values, ALDO significantly increased both daily water and saline intakes. ALDO plus DEX (20 ug/day) further doubled the saline intakes compared to ALDO alone, without affecting water intakes. There were no effects of age or the lower dose of DEX (i.e., 2.5 ug/day) on the behavioral responses to hormone treatment. MAP increased for all ages of rats in response to ALDO and increased significantly more with the addition of DEX (20 ug/day). Increases in MAP were also age-related, increasing in young > middle-aged > old rats. HR decreased equivalently in response to ALDO alone or with DEX (20 ug/day) for young and middle-aged rats but HR decreased in old rats only with the addition of either dose of DEX. Thus, age- and treatment-related changes in MAP were not accompanied by compensatory age- and treatment-related changes in HR. The increases in sodium ingestion and MAP associated with the combination of ALDO plus DEX (20 ug/day) were accompanied by significant weight loss consistent with glucocorticoid-induced excess urinary excretion and, possibly, catabolism.

13

Acute Blood Pressure Responses Differ Following a Sodium and Water Bolus

MA MURPHY[1], PAS BRESLIN[1,2]
[1]Department of Nutritional Sciences, Rutgers University New Brunswick, NJ, USA, [2]Monell Chemical Senses Center Philadelphia, PA, USA

High Blood Pressure (BP) is a leading cause of death in the US and prevention may save millions of dollars in health care costs. Early identification of hypertension-related disease risk may require investigation of short term BP regulation. We have previously shown that BP changes in response to the mere taste of NaCl. To investigate further the acute effects of sodium on BP, we determined whether blood pressure would change in individuals ingesting a salt solution. On 6 different days, 15 healthy normotensive subjects (age 18-35) sat at a desk while we measured their BP with a mercury brachial sphygmomanometer every 10 minutes for 2 hours post ingestion of 475 mL of either 30 degree C 157 mM NaCl (1,714 mg sodium) or DI water. Thirteen of fifteen subjects BP response pattern differed following NaCl ingestion relative to DI water ingestion. Of these thirteen, nine subjects' BP decreased following a NaCl bolus but not following a DI water bolus, suggesting this may be a typical acute response to oral sodium. In all of these subjects, the stimulus –dependent response occurred 30 minutes post ingestion, potentially marking a key point in time for these regulatory mechanisms. These data confirm that there is a BP reflex acutely following sodium intake. Our previous data suggest that salt taste alone participates in these reflexes. And together these studies suggest that regulatory controls of BP anticipate plasma volume increases and respond acutely to salt ingestion with decreases in vascular tone.

14

Can exposure to diet-congruent food reduce restrained eaters' and dieters' intake of tempting foods?

N.J. BUCKLAND, G. FINLAYSON, M.M HETHERINGTON
University of Leeds Leeds, United Kingdom

Exposure to tempting food elicits overconsumption by dieters. Three studies tested whether exposure to diet-congruent cues (odours and images) activate diet cognitions and reduce female dieters' intake of tempting food. A repeated measures design (study 1) exposed restrained dieters (n = 13) and unrestrained non-dieters (n = 21) to a diet-congruent (orange) and tempting (chocolate) odour and measured subsequent energy intake of food. A mixed factorial ANOVA showed restrained dieters reduced energy intake after exposure to a diet-congruent compared to a tempting odour. Study 2 exposed dieters (n = 26) and non-dieters (n = 41) to diet-congruent or control non-food images. Diet cognitions were measured with a lexical decision task, followed by measures of food intake. A mixed factorial ANOVA showed no effect of diet-congruent cue exposure on diet cognitions in dieters. A univariate ANOVA showed dieters and high restrained/high disinhibited eaters reduced food intake after diet-congruent cue images compared to non-food images. A final repeated measures study exposed dieters (n = 25) and non-dieters (n = 19) to a diet-congruent (orange), tempting (chocolate) and non-food odour (soap). A lexical decision task was administered and subsequent intake of snacks was recorded. Mixed factorial ANOVAs showed no effect of diet-congruent cue exposure on diet cognitions or energy intake in dieters. Dieters consumed less overall than non-dieters. Diet-congruent cues may be used as an effective strategy to resist tempting food cues, in dieters most susceptible to succumb to temptation.

15

The ability to delay gratification in both men and women predicts BMI

MJ PRICE[1], S HIGGS[2], MD LEE[1]
[1]Dept Psychology, Swansea University Swansea, United Kingdom, [2]School of Psychology, University of Birmingham Birmingham, United Kingdom

Individual differences in impulsivity may explain why some people are able to stick to long-term health goals whilst others are susceptible to momentary temptation. In the modern food environment the ability to exert inhibitory control is critical for successful control of eating and bodyweight. Here we explore the relationship between the ability to delay gratification, eating characteristics and BMI using a monetary delay-discounting task. Previous research has suggested that discounting behaviour is related to overweight and obesity, but this has only been shown in women. Male (N=25) and female (N=54) participants, aged 18-51 years (M=28.3; SD=8.85) with a BMI range of 18.3-43.6 (M=25.4; SD=5.18) completed a computerized delayed discounting task. All participants also completed a number of self-report measures of eating behaviour, impulsivity and rated current hunger levels. Rate of discounting was significantly steeper in the overweight/obese participants (N=38, *k* est 0.002) compared to the healthy weight (*k* est 0.004) participants, $F(1,77)=8.016$); $p=0.006$; $R^2=9.3\%$), when controlling for gender and age. Hunger levels at time of testing accounted for a further 7.6% of the unexplained variance in discounting. No differences were found between the weight groups for any self-report measures of eating behaviour, and no significant interactions between discounting behaviour and eating style were detected. These findings indicate the ability to delay gratification is an important and independent predictor of BMI in males and females, across age groups.

16

Food intake attenuates brain responses to unpleasant images in humans

SN ALI[1], E HENNESSY[1], C ORBAN[2], G DURIGHEL[1], J MCGONIGLE[2], AR LINGFORD-HUGHES[2], DJ NUTT[2], JD BELL[1], AP GOLDSTONE[1]

[1]Metabolic and Molecular Imaging Group, MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital London, United Kingdom, [2]Centre for Neuropsychopharmacology Imperial College London, Hammersmith Hospital London, United Kingdom

Emotional eating is a maladaptive behavior predisposing to weight gain. We hypothesised that brain responses to negative emotional stimuli would be reduced by food intake, and may be influenced by psychological traits e.g. disgust sensitivity and emotional eating. Healthy, non-obese adults (n=18, mean ± SEM age 23.6 ± 0.7y, BMI 23.0 ± 0.6 kg/m^2) had functional MRI while viewing unpleasant vs. neutral pictures (IAPS). Subjects were scanned after fasting overnight (~16h) and when fed (110 min after ingestion of a 1200 kCal liquid meal) in a randomized, cross-over design. Correlations of ROI activation were made with trait measures of disgust sensitivity (DS-R) and emotional eating (DEBQ). BOLD activation to unpleasant images was significantly lower when Fed than Fasted in the orbitofrontal cortex (OFC), caudate and putamen (P<0.01), but not insula, nucleus accumbens or amygdala (P=0.25-0.44). Positive correlations were seen between DS-R (but not DEBQ-emotional eating) and BOLD activation to unpleasant pictures when Fasted (r=+0.57-0.72, P<0.01), and the decrease in activation from Fasted to Fed state (r=+0.45-0.59, P=0.01-0.06), in the amygdala, insula, OFC and hippocampus. In conclusion, brain responses to negative emotional stimuli are attenuated after food intake, which may reflect the stress alleviating effects of food consumption underlying emotional-induced eating, perhaps via changes in gut hormones such as ghrelin.

17

Is trait binge eating a hedonic subtype of obesity? 48hr free-living and laboratory-based examination of reward, food selection and energy intake.

M DALTON, J BLUNDELL, G FINLAYSON
University of Leeds Leeds, United Kingdom

Trait binge eating has been proposed as a hedonic subtype of obesity characterised by enhanced food reward, increased food intake and a preference for sweet-fat foods under laboratory conditions. This study assessed whether these observations are evident in free-living behaviour. In a matched pairs design, 24 overweight/obese females (BMI: 30.30±2.60kg/m^2; Age: 25.42±3.65yrs) were recruited into Obese Binge (O-B) and Obese Non-binge (O-NB) groups using the Binge Eating Scale. Energy intake was measured using combined total day laboratory test meals and 24-hr dietary recall over a 48-hour period. Food reward was assessed using the Leeds Food Preference Questionnaire. O-B individuals consumed more energy in the laboratory compared to O-NB with no group differences in free-living energy intake. O-B individuals reported greater wanting and craving for sweet-fat foods and this was supported by proportionally greater intake of these foods in the laboratory and free-living energy intake measures. In the laboratory, O-B consumed significantly more than their estimated daily energy needs suggesting that they over-consumed compared to O-NB. Trait binge eating in obese women was associated with enhanced wanting, preference and consumption of sweet-fat foods under laboratory and free-living conditions, and overconsumption in the laboratory. These findings support trait binge eating as a hedonic subtype of obesity and highlight the relevance of this subtype for habitual intake behaviour.

18

Selective indulgence: Delay discounting, appetitive responsivity and the prediction of hedonic overeating

AV ELY, JB HOWARD, MR LOWE
Drexel University Philadelphia, PA, USA

Impulsive decision-making and high appetitive responsivity (as measured by the Power of Food Scale, PFS) have been identified in obese individuals as predictive of hedonic overeating in behavioral lab studies (Appelhans et al, 2011). We extended these findings by investigating whether these characteristics are related to non-homeostatic snack food intake in normal weight participants, which would suggest a vulnerability to dysregulated eating behavior. Seventy-eight participants ate a standard meal, completed self-report measures and a delay discounting task, and participated in a sham taste test of palatable foods. PFS and delay discounting were both significantly correlated with BMI, but neither variable nor their interaction was predictive of hedonic intake in regression analyses when controlling for BMI, preload intake and dietary restraint. When examining each type of snack food individually, the same model was predictive only of pretzel intake, and further exploration showed that those who had a history of dieting ate significantly more pretzels than nondieters. The phenomenon seen in earlier studies may emerge only after an individual gains weight, while those normal weight participants most prone to overeating may not do so under the circumstances of a laboratory-based eating behavior study. However, it is hypothesized that weight-gain prone individuals may judge certain foods as 'safer' to indulge in than others.

19

Bingeing rats show gene expression changes in the VTA and PFC that may contribute to binge-type intake.

RL CORWIN[1], RK BABBS[2], LE MCGRATH[3], DR OLIVOS[3], DJ ZIMMER[3], EG MIETLICKI-BAASE[3], MR HAYES[3]

[1]Penn State Nutrition University Park, PA, USA, [2]Yale/JB Pierce Lab New Haven, CT, USA, [3]U Penn Philadelphia, PA, USA

Circuitry involving midbrain dopamine (DA) neurons and their terminal areas may be critical to binge-type consumption of fatty food. To explore this hypothesis, we assessed gene expression in the ventral tegmental area (VTA), nucleus accumbens (NA) shell and core, and prefrontal cortex (PFC) of bingeing rats and controls. Male rats had 1h shortening (fat) access either daily (controls; C) or intermittently (INT; i.e. Mon, Wed, and Fri). Rats had chow when fat was not available. After 8 wk, INT 1h fat intake significantly exceeded that of C, i.e. INT rats binged. Rats were then sacrificed either pre-or post-/during fat presentation. Gene expression was analyzed in brain tissue punches. In the VTA, tyrosine hydroxylase (TH), dopamine transporter, and D2 receptor expression were enhanced in INT relative to C pre-fat presentation. However, post-fat presentation, the elevated mRNA levels for these genes returned to C levels. In contrast, D1 and GABA-A receptor gene expression in the VTA, and TH, GABA-B, AMPA, and mu opioid receptor expression in the PFC were significantly reduced pre- and post-fat presentation in INT rats. There were no significant effects in the NA. These data indicate that the VTA and PFC, but not NA, may be critical to binge-type eating and may contribute to loss of control during a binge. Also, the act of bingeing may "normalize" some of the changes, at least temporarily. Funding: MH67943 (RLC), DK096139, DK085435 (MRH).

20

Public Health Approaches to Changing Eating Patterns of African Americans: Can They Work?

S KUMANYIKA

University of Pennsylvania Philadelphia, PA, USA

Recognition of the population-wide nature of the obesity epidemic has led to an expansion of approaches used to foster changes in eating patterns and physical activity. In addition to individually-oriented educational or counseling approaches, there is now an increasing focus on interventions that take a classical public health approach by attempting to change policies and environmental factors that shape contexts for individual eating and physical activity behaviors. Individuals are engaged in community activities to support and increase acceptance of these changes. Environmental and policy approaches to changing eating patterns are particularly important for addressing obesity in black Americans. Obesity prevalence is higher in blacks than whites among both children and adults, even when adjusting for socioeconomic status differences. Available evidence points to many factors in the physical, economic, policy, and sociocultural aspects of food environments of black Americans that contribute to this excess risk. These factors may be very difficult to change. Quantitative assessments of neighborhood characteristics and media promotions document that, compared to whites or general populations, black Americans experience relatively lower availability and promotion of healthful foods and higher availability and promotion of unhealthful foods. Qualitative studies with black consumers, retailers who do business in black communities, and policy makers who represent black communities reveal the complexity of their perceptions of and responses to these food marketing inequities. Thus, from a community perspective, there may be mixed support for changes thought to be essential from a public health perspective. Given that changes in eating patterns will be essential for addressing obesity and other diet-related chronic disease disparities that affect black Americans, a process of rethinking potential approaches with a lens that is specific to the environmental contexts of black Americans is needed to identify promising approaches going forward.

21

Genetic predisposition to obesity: what does it mean for you?

JR FERNANDEZ

University of Alabama at Birmingham Birmingham, AL, USA

The prevalence of obesity has been increasing on a global scale over the past few decades. Obesity has been associated with many chronic diseases that directly affect health outcomes and reduce overall quality of life. The specific factors influencing the accumulation of adipose tissue are not completely understood. Genetics have been identified as a major contributor to obesity, particularly body composition and fat accumulation. Candidate genes and genome-wide association studies (GWAs) have identified genetic markers across the human genome that contribute to obesity-related traits. It is generally understood that these genes do not act independently, but rather synergistically, and recent approaches have been developed to incorporate information of multiple genes into a quantitative measure that encompasses multi-gene information. Taking into account that obesity is considerably influenced by environmental factors, focusing research into the evaluation of gene by environment interactions is paramount toward the understanding to the nature versus nurture etiology of obesity. Scientific research is beginning to explore approaches where the multifactorial nature of obesity is considered in experimental designs, such as the inclusion of individual's genetic ancestral background,

socioeconomic status, cultural factors and behavioral practices. These approaches are believed to provide better insights into the etiology of obesity and provide hope for the development of policy and intervention strategies to effectively address the obesity epidemic.

22

Prevalent, but often overlooked, risk factors for the development of obesity among adolescents and young adults.

AE FIELD
Harvard School of Public Health Boston, MA, USA

Background: Approximately 1/3 of adolescents are overweight or obese. Although it is widely accepted that dietary intake is related to weight gain and the development of overweight and obesity, few dietary factors or patterns have consistently predicted excessive weight gain. Methods: Using prospective data from 14,549 girls and 10,439 boys in the Growing Up Today Study (GUTS) and the Growing Up Today Study II (GUTS II), two ongoing studies of adolescents and young adults across the United States, we have studied the prospective association of eating behaviors and eating disorders with the development of overweight and obesity. Methods: Information on weight, height, dietary intake, and eating disorder behaviors has been collected via online and paper questionnaires every 12-36 months since 1996 (GUTS)/2004 (GUTS II). To account for siblings, generalized estimating equations were used for the analysis. Results: Although soda is the most commonly consumed sugar-sweetened beverage, sports drinks and energy drinks are gaining in popularity, particularly among the adolescent and young adult males. Among both males and females we find that independent of age, earlier BMI, time spent watching TV, physical activity and intake of regular and diet soda, intake of sports drinks is predictive of greater changes in BMI ($p<0.001$). Although the association is observed in both genders, the public health burden is greater among males since they are more frequent consumers of sports drinks. In contrast, eating disorders are more common among females than males, but a non-trivial number of youth report frequent overeating episodes. Most females who report frequent overeating endorsed a loss of control over the eating during the episode and thus were labeled as binge eaters. Fewer males reported losing control. Among both males and females, independent of age, earlier BMI, dieting, time spent watching TV, and geographic region, youth who engaged in at least weekly binges were 90% more likely (odds ratio=1.94, 95% confidence interval CI 1.18-3.18) to become overweight or obese. The association was weaker with overeating episodes during which no loss of control was reported. We observe that 5-6% of females develop eating disorders involving at least weekly binge eating, thus the public health burden of binge eating is non-trivial. Conclusions: Although sports drinks are marketed to appear healthier than regular soda, we find that they are at least as predictive of weight gain, thus the public must be made more aware. Given their popularity among males, they represent an important area to target to prevent weight gain among adolescents, particularly adolescent males. Our findings also suggest that disordered eating, particularly binge eating, should be targeted in obesity prevention programs. Rather than obesity and eating disorders being addressed separately, our results suggest that preventing binge eating might make a meaningful difference in preventing overweight and obesity.

23

The Calculus of Calories: Understanding Obesity through Mathematics

K HALL
NIDDK

Health practitioners and policymakers need to make decisions about whether a proposed intervention will cost-effectively contribute to reversing the obesity epidemic. Quantifying the likely impact of an intervention is facilitated by translating between changes in the calories eaten or expended per day and body weight. Unfortunately, the usual methods for making this translation were recently revealed to be highly inaccurate and have led to misleading conclusions about the energy gap underlying the obesity epidemic as well as incorrect predictions about the intervention magnitude required to prevent and reverse obesity. Mathematical models of human metabolism and body weight change have now been developed that correct the deficiencies of the previously standard calculations. These new models provide accurate predictions of body weight dynamics resulting from interventions in both individuals and populations. In this presentation, I will describe some insights obtained from these models such as how obesity prevalence would likely be affected by policy changes such as a soda tax. I will also describe how mathematical models can be used interpret the relationship between the changing food environment, food waste, and the genesis of the obesity epidemic.

24

Parabrachial nucleus GLP-1 receptor signaling contributes to food intake control

AL ALHADEFF, MR HAYES, HJ GRILL
Depts. of Psychology and Psychiatry, Univerisity of Pennsylvania Philadelphia, PA, USA

Glucagon-like peptide-1 (GLP-1) signaling in the periphery and CNS is widely investigated for the treatment of type-2 diabetes mellitus and obesity. While CNS GLP-1-synthesizing neurons are located almost exclusively in the nucleus tractus solitarius (NTS) of the caudal hindbrain, the GLP-1 receptor (GLP-1R) is distributed throughout the brain. The parabrachial nucleus

(PBN) in the rostral hindbrain expresses GLP-1R and is generally understudied for its role in energy balance despite being a major integrative relay site for sensory and visceral information. We hypothesize that central GLP-1 treatment may reduce food intake through signaling in the PBN, perhaps via monosynaptic NTS GLP-1 projections. To test this hypothesis, we (1) unilaterally injected the GLP-1R agonist exendin-4 into the PBN and measured food intake and (2) performed double immunohistochemistry (IHC) on NTS brain sections to visualize GLP-1 producing neurons and the monosynaptic retrograde tracer Fluorogold (2%, 300nl) that was unilaterally injected into the PBN. A ventrical subthreshold dose of exendin-4 (0.025µg/100nl) in the PBN significantly reduced cumulative chow intake and high fat diet intake at 6-24h post-injection. Preliminary IHC data suggest that 30-40% of NTS GLP-1-producing neurons project directly to the PBN. Together, these data suggest that PBN GLP-1 receptor signaling may reduce food intake in part via monosynaptic projections from NTS GLP-1 neurons.

25

NMDA receptor participation in reduction of food intake and NTS synapsin 1 phosphorylation following hindbrain MC4 receptor activation

CA CAMPOS, H SHIINA, RC RITTER
Programs in Neuroscience, Washington State University Pullman, WA, USA

Others have reported that hindbrain injection of the melanocortin 3/4 receptor (MC4R) agonist, MTII, reduces food intake primarily by reducing meal size. Our own work indicates that NMDA-type glutamate receptors (NMDAR) on vagal afferent endings in the nucleus of the solitary tract (NTS) participate in control of meal size and reduction of food intake by CCK. This effect appears to be mediated by increased phosphorylation of synapsin 1 (serines 62/67) in vagal afferent endings. Vagal afferent terminals express MC4R, and our recent results indicate that central vagal afferent terminals contribute to reduction of food intake by MTII. Therefore, we postulated that NMDAR activation participates in reduction of food intake by hindbrain injection of MTII. After an overnight fast, hindbrain injection of MTII (50 pmoles) reduced food intake at 0.5, 1, 2, 4 and 24h post injection. Co-injection of an NMDAR antagonist with MTII attenuated reduction of intake for at least 4h post injection. MTII injection resulted in no increase in synapsin 1 phosphorylation at serines 62/67 (pERK1/2 sites), but triggered significant phosphorylation at serine 9 (PKA/CaMK I site). Increased synapsin phosphorylation was sustained for 6h, and was prevented by NMDAR antagonism. Our data indicate that NMDAR contribute to reduction of food intake by hindbrain MC4R activation, and suggest that increased synapsin phosphorylation, which results in increased synaptic strength, may be a mechanism by which NMDAR participates in MTII's reduction of food intake.

26

Effects of Roux-en-Y gastric bypass (RYGB) and estradiol (E2) on hindbrain c-Fos following intrajejunal (IJ) nutrient infusion in ovariectomized (OVX) rats

L. ASARIAN[1,2], M BUETER[3], K ABEGG[1], TA LUTZ[1,2]
[1]Inst. of Vet. Physiol. Zurich, Switzerland, [2]Zurich Center for Integrative Human Physiol., Univ. of Zurich Zurich, Switzerland, [3]Dept. of Surgery, Div. of Visceral and Transplantation Surgery, Univ. Hosp.l Zurich Zurich, Switzerland

E2 amplifies the effects of RYGB on eating and body weight in OVX rats. To determine if this is associated an E2-induced increase in the central neural processing of meal-related stimuli during a meal, we compared the effects of IJ infusions of Ensure on c-Fos and estrogen receptor-a (ERa) expression in RYGB and sham-operated (SHAM) OVX rats. RYGB, OVX and catheterization were done in one surgery. Chronic IJ catheters ended ~2 cm distal to the gastro-jejunal anastomosis in RYGB rats and at an equivalent jejunal location in SHAM rats. Rats received either cyclic E2 (2 ug E2/4d, SC) or oil treatment. After 6 wk, all rats received an IJ infusion of 2.5 ml Ensure and were sacrificed 90 min later. Brains were processed for c-Fos and ERa expression. E2 did not increase the number of c-Fos(+) cells. RYGB both (1) increased cNTS, but not spNTS, c-Fos(+)/ERa(+) cells in E2- and oil-treated rats and (2) decreased cNTS and spNTS c-Fos(+)/ERa(-) cells. The selective activation of cNTS ERa(+) cells independent of E2 suggests that these cells are distinguished from ERa(-) in an additional way, perhaps due to distinct afferent projections. The decrease in activation of NTS ERa(-) cells suggests that these cells inhibit rather than stimulate satiation. Further characterization of these cells may also provide additional clues as to the efficacy of RYGB. Supported by NIDDK.

27

TRPV1 regulates leptin expressing neurons in the dorsal motor nucleus of the vagus

Y JIANG[1,2], IJ ANWAR[1], KR ZADEH[3], H MUENZBERG-GRUENING[3], A ZSOMBOK[1,2]
[1]Neuroscience Program, Tulane University New Orleans, LA, USA, [2]Department of Physiology, Tulane University New Orleans, LA, USA, [3]Central Leptin Signaling, Pennington Biomedical Research Center Baton Rouge, LA, USA

Activity of the preautonomic neurons in the dorsal motor nucleus of the vagus (DMV) determines the vagal tone, although the underlying synaptic mechanisms are poorly understood. Recent studies including our work demonstrated that TRPV1 (transient receptor potential

vanilloid type 1) plays a pivotal role in synaptic regulation. Here, we tested the hypothesis that TRPV1 regulates the activity of leptin expressing ($LepRb^{GFP}$) DMV neurons. First, patch-clamp recordings were performed from leptin-GFP neurons to verify the responsiveness to leptin. Leptin decreased mEPSC frequency and produced a baseline shift. Then, we determined the effect of TRPV1 activation on leptin neurons. Administration of capsaicin, a TRPV1 agonist increased the frequency of mEPSCs in 50% of the leptin-positive cells (1.9±0.6 vs. 5.1±1.6, n=7, $p<0.05$), indicating that TRPV1 regulates excitatory synaptic inputs to leptin neurons. Interestingly, leptin expressing neurons responded with an inward shift of baseline to TRPV1 activation (n=14, $p<0.05$), suggesting postsynaptic effect of capsaicin. Furthermore, application of leptin in the presence of capsaicin failed to reduce mEPSC frequency or cause a baseline shift, indicating that activation of TRPV1 camouflaged the effect of leptin. In conclusion, our data demonstrate that TRPV1 is involved in the regulation of leptin expressing DMV neurons via pre- and postsynaptic mechanisms. This data suggest potential interaction between TRPV1 and leptin signaling in DMV neurons.

28

Hindbrain norepinephrine (NE) neurons may be involved in the satiating effect of GLP-1

SJ LEE, JP KRIEGER, M ARNOLD, W LANGHANS
Swiss Federal Institute of Technology Zurich, Switzerland

Peripheral administration of glucagon-like peptide 1 (GLP-1) inhibits eating and activates brain regions involved in eating control. Yet, the exact CNS mechanisms that mediate the satiating effect of peripheral GLP-1 are still unclear. Elimination of vagal afferent signaling by subdiaphragmatic vagal deafferentation (SDA) attenuated the initial satiating effect of IP administered GLP-1R agonist exendin-4 (Ex-4) and reduced the Ex-4-induced increase in c-Fos expression in the paraventricular hypothalamus (PVH), suggesting the vagal-hindbrain-hypothalamic connection is critical for the satiating effect of peripheral GLP-1R activation. Here we attempted to identify the CNS pathways involved in processing the GLP-1R activation-induced satiation signal. IP injection of Ex-4 (0.1μg/kg) in male SD rats induced a robust c-Fos expression mainly in the medial nucleus tractus solitarii (NTS). Only in the NE A2 region, 22% of total c-Fos expressing neurons were co-localized with dopamine-β-hydroxylase (DBH). After injection of fluorogold (FG) into the PVH, 50% of FG labeled cell bodies in the A2 region also co-expressed c-Fos and DBH, and this co-localization was reduced in SDA rats after IP Ex-4 stimulation. In addition, the c-Fos/DBH cells were in close proximity to the axonal swellings of vagal afferent neurons labeled by AAV-GFP, suggesting that the DBH neuronal activation was vagally mediated. The findings raise the possibility that IP Ex-4 induces satiation by recruiting NE projections from the NTS to the PVH, possibly via GLP-1R-activated vagal afferent signaling.

29

4th Ventricular Infusion of the Dopamine D2-Receptor Antagonist Raclopride Decreases Intake and Psychophysically Assessed Detectability of Sucrose by Rats.

CM MATHES, GD BLONDE, AC SPECTOR
Florida State University Tallahassee, FL, USA

Peripherally injected raclopride (RAC) decreases sucrose intake in rats in a manner suggestive of effects on motivational and hedonic factors. Recently we demonstrated that RAC also decreases sucrose taste detectability. Here we sought to assess if these hedonic and sensory effects are mediated by D2 receptors in taste-related hindbrain regions. After implanting 4th ventricular cannulae in male Sprague-Dawley rats (n=6), we measured 30- and 60-min intake of 0.3M sucrose solution immediately following infusion of 3 μl of saline or RAC (4,12,22,40,70 μg). At 22 & 40 μg RAC decreased 30-min sucrose intake (corrected $p\leq0.05$). We then assessed the impact of RAC on the ability of cannulated rats (n=10) to discriminate water and sucrose in an operant 2-response taste signal detection test. Three sucrose concentrations (0.02,0.08,0.3 M) were chosen to represent the dynamic portion of the previously established mean psychometric function, and performance at these concentrations versus water was measured after saline and 22 μg RAC infusion. RAC significantly decreased percent correct responding for all sucrose concentrations tested, suggesting that RAC in the hindbrain disrupts the discrimination between the taste of sucrose and water. Importantly, responding under RAC remained concentration-dependent, suggesting that the action of RAC was sensory-related. Collectively these results suggest that the effects of D2-receptor antagonism on sucrose intake and taste sensitivity are mediated, at least in part, by the hindbrain.

30

The combination of peripheral CCK and apo AIV in the NTS inhibits food intake via both peripheral and NTS CCK-1R

CC LO, SC WOODS, SK HIBBARD, P TSO
Univesity of Cincinnati Cincinnati, OH, USA

CCK and apolipoprotein IV (apo AIV) combine to reduce food intake. When both are administered ip, CCK-1R on vagal afferents are required. However, both Apo AIV message and CCK-1R are present in the NTS. Methods. We tested the hypothesis that apo AIV inhibits food intake by itself within the NTS, or alternatively that it requires peripheral CCK activity. We assessed food intake and neuronal activation in the NTS following

combinations of ip CCK and/or 4th-ventrcular (i4vt) administration of apo AIV. Results. I4vt administration of rat apo AIV at 4 μg and above suppressed food intake at 30, 60 and 120 min. Blockade of peripheral or NTS CCK-1R by the CCK-1R antagonist, lorglumide, attenuated the satiating effect of i4vt apo AIV. While combinations of subthreshold doses of ip CCK and i4vt apo AIV did not inhibit food intake, the combination of an effective dose of i4vt apo AIV and either a subthreshold dose or an effective dose of ip CCK caused a greater reduction of food intake than the apo AIV alone, and this satiating effect was abolished by i4vt lorglumide, suggesting that ip CCK and i4vt apo AIV interact to suppress food intake via a pathway requiring NTS CCK-1R activity.

31

Hindbrain noradrenergic input to PVN mediates the activation of oxytocinergic neurons induced by the satiety factor oleoylethanolamide

A ROMANO[1], CS POTES[2], B TEMPESTA[1], V CUOMO[1], T LUTZ[3], S GAETANI[1]
[1]Dept. of Physiology and Pharmacology "V. Erspamer", Sapienza Univ. of Rome Rome, Italy, [2]Dept. of Experimental Biology Univ. of Porto Porto, Portugal, [3]Inst. of Veterinary Physiology, Univ. of Zurich Zurich, Switzerland

Oleoylethanolamide (OEA) is a gut-derived lipid that stimulates vagal fibres to induce satiety. We previously showed that peripheral OEA activates c-fos in the nucleus of solitary tract (NST) and in the paraventricular nucleus (PVN), where it enhances oxytocin (OXY) transmission. OEA anorexiant action can be prevented by the i.c.v. administration of a selective OXY receptor antagonist, suggesting a crucial role of OXY system in the pro-satiety effects of OEA. The NST is the source of noradrenergic input to hypothalamic OXY neurons. We hypothesized that the activation of this pathway might mediate OEA effects on PVN neurons. Therefore we subjected rats to intra-PVN administration of the toxin saporin (DSAP) to destroy hindbrain noradrenergic neurons, and evaluated the effects of OEA (10 mg/kg i.p.) on feeding behavior, on c-fos expression in the PVN and OXY immunoreactivity in the PVN and neurohypophysis. DSAP lesion prevented OEA effects on food intake, c-fos activation and OXY expression, while sham operated rats responded normally to OEA, showing a 60% decrease of food intake, a 3-fold increase of c-fos and OXY levels in the PVN and a 47% increase of pituitary OXY. These findings support the hypothesis that noradrenergic NST-PVN projections are involved in the central release of OXY, which mediates OEA pro-satiety action, and shed new light on the role of OEA in satiety.

32

Appetition in rats: rapid post-oral stimulation of intake by glucose

K ACKROFF, A SCLAFANI
Brooklyn College of CUNY Brooklyn, NY, USA

Control of intake by post-oral nutrient actions has been viewed as largely inhibitory, yet the acquisition of flavor preferences suggests positive feedback that stimulates appetite. When rats are trained in short alternating sessions to associate a CS+ flavor with intragastric (IG) glucose infusion and a CS- flavor with IG water, their intake of the CS+ does not increase selectively although a CS+ preference is displayed. Using a different procedure, we observed a rapid appetition effect in mice first given several successive sessions with a CS- paired with IG water: intake increased within minutes in the first test session with a CS+ paired with IG glucose. The present study extends this method to rats. Food-restricted rats were trained to consume a CS- flavor (e.g., grape saccharin) paired with IG water in 5 daily 1-h tests. In the next 3 tests, they drank a CS+ (e.g., cherry saccharin) paired with IG glucose. Rats trained with 8% glucose increased intake significantly on CS+ test 1, but those trained with 16% glucose showed only a small increase in intake, which may reflect a counteracting satiating effect. Both groups further increased CS+ intakes in tests 2 and 3, and preferred (81%) the CS+ to the CS- in a two-bottle test without infusions. These data show that rats, like mice, rapidly detect the positive post-oral effects of glucose that can stimulate intake within the first hour of exposure. The test protocol described here can be used to investigate the peripheral and central processes involved in stimulation of intake by post-oral nutrients.

33

Dose-dependency and time-course of effects of a soluble dietary fibre (pectin) on appetite, gut size, adiposity and gut satiety hormone secretion in rats

CL ADAM, PA FINDLAY, LM THOMSON, AW ROSS
UNIVERSITY OF ABERDEEN, ROWETT INSTITUTE OF NUTRITION AND HEALTH ABERDEEN, United Kingdom

Macronutrient-induced satiety offers a natural means of bodyweight regulation. It has been shown that soluble dietary fibre increases satiety and decreases adiposity in rats and this experiment examined whether these responses are dose-sensitive, quantified changes in gut size and compared responses in the short- and long-term. Isocaloric diets, based on the control standard AIN-93 and containing 3.3%, 6.7% or 10% w/w soluble fibre as apple pectin (P), were offered *ad libitum* for either 1 week or 4 weeks to individually-housed adult male rats (12 wks old, 444±11g) (n=8/group). Daily voluntary food intake was measured, initial and final MRI scans performed, final dissected gut weights obtained and final (trunk) blood

samples analyzed (by RIA kits) for GLP-1 and PYY concentrations. At 1 and 4 weeks (respectively) dietary P inclusion rate correlated negatively with cumulative food intake (n=32; r=-0.89 and r=-0.73) and final % body fat (r=-0.51 and r=-0.66) and positively with small intestine weight (r=0.95 and r=0.89), caecum weight (r=0.84 and r=0.90), colon weight (r=0.61 and r=0.35), plasma GLP-1 (r=0.91 and r=0.66) and plasma PYY (r=0.84 and r=0.72). Therefore, increasing soluble fibre in the diet decreased appetite and body fat proportionately in both the short- and long-term. The data are consistent with GLP-1 and PYY mediating fibre-induced satiety and demonstrate how the gut's morphological and endocrine adaptations are dose-sensitive, occur within a week and are sustained.

34

Peripheral blockade of cannabinoid receptors decreases food intake, body weight and body composition while improving glucose handling.

J ALEXANDER-CHACKO, DK SINDELAR
Eli Lilly and Company Indianapolis, IN, USA

Centrally penetrant cannabinoid (CB1) receptor antagonists while showing remarkable efficacy to reduce food intake, have had psychiatric liabilities associated with their use. Recently, significant efforts have been undertaken to synthesize and characterize peripherally restricted molecules. One such molecule is JD5037 which in a recent publication has been shown to produce similar effects to a centrally penetrant CB1 molecule (Tam et al, Cell Metab, 2012). We tested the hypothesis that JD5037 will produce significant reductions in food intake, body weight and fat mass along with improved glucose metabolism following an OGTT. Male DIO C57Bl/6 mice were sc injected with JD5037 (1,3,10 mg/kg) and its inactive enantiomer (10 mg/kg) for 14 days. In these mice, 14 day cumulative food intake showed a 20% decrease at the highest dose relative to vehicle and weight loss of 5g relative to Vehicle. Fat mass at the 10mg/kg dose was reduced by 4g. When challenged with an oral glucose load, both glucose and insulin AUCs were significantly suppressed, indicating increased insulin sensitivity. In a subset of animals, liver triglyceride levels were determined and showed a dramatic decline (from 401 mg/g for the Vehicle group vs 105 at the 10 mg/kg dose). The inactive isomer of JD5037 did not produce any measurable changes relative to Vehicle. These results suggest that antagonism of peripheral CB1 receptors provide an effective path forward for treatment of obesity along with an improved diabetic profile.

35

Roux-en-Y gastric bypass effects on vascular reactivity and liver metabolism

C CORTEVILLE[1], E OSTO[2], K SPLIETHOFF[1], T LUESCHER[3], M BUETER[4], T LUTZ[1]
[1]Inst Vet Physiol UZH Zürich, Switzerland, [2]Cardiovasc Res Inst Physiol Zürich, Switzerland, [3]Clinic Cardiol Zürich, Switzerland, [4]Dep Surg USZ Zürich, Switzerland

Roux-en-Y gastric bypass (RYGB) reduces body weight and improves obesity-related co-morbidities such as insulin resistance, fatty liver disease and endothelial dysfunction. Some effects appear before significant weight loss, but the underlying mechanisms are still unclear. We therefore investigated the immediate weight-independent effects of RYGB on vascular reactivity and liver metabolism. Male Wistar rats were fed 7 weeks with high-fat high-cholesterol diet and underwent RYGB or sham surgery. Sham rats were fed ad lib (n=12) or weight-matched (n=12) to RYGB rats (n=24). Rats were sacrificed 8 days after surgery. Before surgery and at termination, they were tested for systemic insulin sensitivity. Vascular reactivity and markers for insulin signaling (pAkt/Akt, pIRS/IRS) were investigated in aorta and liver. Systemic GLP-1 and bile acid concentrations were measured. The vasorelaxant effects of insulin and GLP-1 were significantly improved 8 days after RYGB. This was consistent with a higher pAkt/Akt ratio and GLP-1R expression in the aorta. Systemic insulin sensitivity and hepatic pAkt/Akt ratio were unchanged. Plasma fasting levels of GLP-1 and bile acids were increased after RYGB compared to AL and BWM (GLP-1: 10±2.8 vs 0.8±0.1 vs 2.0±0.9 pg/ml; bile acids: 20.7±11.5 vs 7.8±4.8 vs 9.3±3.2 pg/ml). Our study suggests that vascular reactivity is improved after RYGB and that GLP-1 and bile acids may be potential mediators of immediate, weight-independent effects after RYGB.

36

Involvement of TGR5 and TAS2R38 in the effect of compound K on GLP-1 release from enteroendocrine cells

HY KIM, K KIM, M PARK, YM LEE
Korea Food Research Institute Sungnam-si, South Korea

Orally ingested saponins of ginseng (*Panax ginseng* C.A. Meyer, Araliaceae) are metabolized to compound K (CK; 20-O-beta-D-glucopyranosyl-20(S)-protopanaxadiol) in the intestine, and CK is absorbed into the body. Our recent studies demonstrated that CK stimulated glucagon-like peptide (GLP)-1 release from the enteroendocrine NCI-H716 cells. GLP-1 plays a key role in maintaining glucose and energy homeostasis. G-protein-coupled receptors (GPCRs), such as the bile acid receptor TGR5 and taste receptors, have been reported to be involved in GLP-1 secretion in enteroendocrine cells. To reveal the molecular targets of CK-induced GLP-1 release, the involvement of TGR5 and TAS2R38 (a bitter taste receptor) in the GLP-1 release was studied using gene

silencing. The transfection with small interference RNA (siRNA)(100 nM) targeted for human TGR5 and TAS2R38 for 48 hours significantly inhibited the GLP-1 secretion and intracellular Ca^{2+} increase mediated by CK at 1, 10 and 100 micromolar in NCI-H716 cells. In conclusion, TGR5 and TAS2R38 exhibited the roles in the GLP-1 release induced by CK. The findings suggest that CK might displays regulatory effects for maintaining glucose and energy homeostasis at least in part via activating TGR5 and TAS2R38. Acknowledged for KFRI E0131203 and NRF 2010-0024475.

37

The role of the vagus nerve in glutamate appetite: a study of mouse strains selectively bred for high and low glutamate intake

A KITAMURA[1], M INOUE[2], TM NELSON[1], ML THEODORIDES[1], AA BACHMANOV[1]
[1]Monell Chemical Senses Center Philadelphia, PA, USA, [2]Lab of Cellular Neurobiology, Dept of Life Science, Tokyo University of Pharmacy and Life Science Tokyo, Japan

L-glutamate (Glu) is abundant in dietary proteins, is present in a free form in many foods, and is widely used as a flavor supplement (typically as monosodium Glu, MSG). Glu elicits umami taste sensation, and ingested Glu evokes multiple physiological responses. Therefore, ingestive behavior towards Glu can be influenced by both its sensory and postingestive effects. The purpose of this study was to understand the mechanism of Glu appetite. In our survey of 28 inbred mouse strains, we found that mice from the C57BL/6ByJ (B6) and 129P3/J (129) strains had large differences in voluntary MSG consumption. To develop a better model for genetic and physiological studies of Glu appetite, we had intercrossed the B6 and 129 strains and then used selective breeding to produce mouse strains with high and low MSG intake (MSG-H and MSG-L, respectively). After 13 generations of selective breeding, MSG-H mice drink 6 times more 300 mM MSG than MSG-L mice. We used MSG-H and MSG-L mice to examine 1) neural taste responses to amino acids and other tastants, 2) behavioral responses to MSG in brief-access tests of naïve and MSG-exposed mice, 3) blood glucose after MSG gavage, and 4) effect of vagotomy on MSG consumption in preference tests. We found that strain differences in MSG intake most likely depend on postingestive effects mediated by the vagus nerve. These data support the role of the vagus afferent nerve pathway in Glu appetite.

38

Glucose-Stimulated Insulin Secretion is Inhibited *in vivo* and *in vitro* by 2-Mercaptoacetate (MA).

A-J LI, Q WANG, TT DINH, S RITTER
Programs in Neuroscience, Washington State University Pullman, WA, USA

We recently found that MA, known for its ability to inhibit fatty acid oxidation and stimulate feeding, blocks the effect of long and medium chain fatty acids (FA) and GW9508 on calcium influx in cultured nodose neurons. GW9508 is an agonist at the G-protein coupled FA receptor, GPR40. Fatty acids have been shown previously to potentiate glucose-induced insulin secretion via beta cell GPR40. Therefore, we examined MA's effect on this response. We hypothesized that MA would antagonize glucose-induced insulin secretion if MA blocks the GPR40 receptor. Rats maintained on 50% fat diet were injected with saline or MA (68 mg/kg, i.p.) 15 min before a glucose injection (1g/kg, i.p.). Blood glucose and insulin levels were determined from remotely-collected venous catheter blood. Blood glucose reached similar peak levels in both groups by 15 min after glucose injection and returned to baseline 45 min after glucose injection in the saline treated group. However, glucose levels were still significantly elevated at 105 min in the MA treated group. Surprisingly, glucose-stimulated insulin secretion was completely suppressed in the MA-pretreated group during the entire measurement period, though robustly stimulated in the saline group. Similar effects of MA on insulin secretion were obtained from cultured insulinoma cells (INS-1) cells. Results reveal a novel effect of MA on glucose-stimulated insulin secretion and also suggest that non-metabolic actions of MA on membrane receptors may contribute to its effects on food intake.

39

The effect of L-cysteine on gastrointestinal hormone release and food intake.

AK MCGAVIGAN[1], HC GREENWOOD[1], C WONG[1], A LEHMANN[2], MA GHATEI[1], SR BLOOM[1], KG MURPHY[1]
[1]Section of Investigative Medicine Imperial College London, United Kingdom, [2]AstraZeneca R&D Mölndal, Sweden

Protein is the macronutrient that induces the strongest feeling of satiety. Protein induced satiety may be due to the specific amino acids generated by protein digestion. We have identified L-cysteine (L-cys) as an amino acid with anorexigenic properties. Oral gavage (OG) of 2 and 4mmol/kg L-cys significantly reduced 60minute food intake (FI) compared to water treated controls (water: 6.8±0.6g vs. 2mmol/kg L-cys: 4.3±0.6g, $p<0.05$; vs. 4mmol/kg L-cys: 2.7±0.3g, $p<0.001$, n=7-8) in overnight fasted male Wistar rats. OG of L-cys did not induce aversive behaviour in a conditioned taste aversion protocol, suggesting the reduction in food intake was not secondary to unpleasant post-ingestive consequences. OG

of L-cys significantly increased plasma insulin levels (water: 68.0±7.7pmol/l vs. L-cys: 125.7±20.7pmol/l, $p<0.05$, n=7-8) and significantly reduced plasma acyl-ghrelin levels compared to control (water: 8.8±1.6pmol/l vs. L-cys: 3.6±0.9pmol/l, $p<0.05$, n=7-8) 30mins post administration. L-cysteine had no effect on plasma glucagon-like peptide-1 or peptide YY levels. Repeated administration of L-cys over 5 days significantly reduced cumulative FI compared to glycine (negative control) and water controls (day 5, water: 144.7±3.5g, glycine: 145.7±3.0g, vs. L-cys: 130.1±2.5g, $p<0.001$, n=6-9). L-cysteine may therefore contribute towards protein induced satiety through suppressing the release of the orexigenic hormone ghrelin. Specific amino acids may be useful in modulating appetite to treat obesity.

40

Intraintestinal dairy macronutrient constituents augment the food intake and glycemic suppressive effects of DPP-IV inhibition

DR OLIVOS, O MONTAUBIN, CA TURNER, SL PRESTOWITZ, LE MCGRATH, BA JEFFREY, EG MIETLICKI-BAASE, MR HAYES
University of Pennsylvania Philadelphia, PA, USA

Glucagon-like peptide-1 (GLP-1) is an incretin hormone released from intestinal L-cells in response to food entering into the GI tract. GLP-1-based pharmaceuticals improve blood glucose regulation (BGR) and may hold promise for obesity treatment, as GLP-1 drugs reduce food intake (FI) and body weight in humans and animals. In an effort to improve GLP-1 pharmacotherapies, we focused our attention on specific macronutrients that, when ingested, may trigger robust GLP-1 secretion and improve BGR and FI suppression when combined with systemic administration of sitagliptin (SIT), a pharmacological inhibitor of DPP-IV (enzyme responsible for GLP-1 degradation). *In vitro* data suggest that specific macronutrient constituents found in dairy foods may act as potent secretagogues for GLP-1 and possible adjunct behavioral therapy in combination with SIT. To explore this hypothesis, rats received IP injections of SIT (6mg/kg) or saline (VEH) followed by 20min intraduodenal infusions of milk protein concentrate (MPC; 80/20% casein/whey; 0.5kcal/ml), soy protein (non-dairy control infusate; 0.5kcal/ml) or 0.9% NaCl. FI was assessed 30min post-infusion and in separate studies, BGR was examined via a 2hr oral glucose tolerance test (25% glucose; 2g/kg). MPC significantly enhanced both the FI and glycemic suppressive effects of SIT, but not soy protein, suggesting that MPC may augment endogenous GLP-1 signaling and the BGR and FI suppressive effects of DPP-IV inhibition.

41

Intraintestinal dairy macronutrient constituents enhance the food intake suppressive effects of exendin-4, a GLP-1 receptor agonist

DR OLIVOS, O MONTAUBIN, CA TURNER, SL PRESTOWITZ, EG MIETLICKI-BAASE, MR HAYES
University of Pennsylvania Philadelphia, PA, USA

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted from intestinal L-cells following the ingestion of nutrients. In addition to regulating blood glucose, long-acting GLP-1 receptor (GLP-1R) agonists such as exendin-4 (EX4) reduce food intake and body weight in humans and animal models. As a result, GLP-1-based pharmaceuticals are potential treatments for obesity. Combined with exogenous stimulation of the GLP-1 system, foods that trigger robust GLP-1 secretions may result in a greater suppression of food intake. Specific macronutrient constituents found in dairy foods may act as potent secretagogues for GLP-1 and thus possible adjunct behavioral therapy in combination with EX4. To explore this hypothesis, adult rats received IP injections of EX4 (3µg/kg) or saline (VEH) followed by 20min intraduodenal infusions of milk protein concentrate (MPC; 80/20% casein/whey; 0.5kcal/ml), soy protein (non-dairy control protein; 0.5kcal/ml), oleic acid (0.5kcal/ml), intralipid (non-dairy control fat; 0.5kcal/ml), 0.9% NaCl (control for proteins) or phosphate buffered saline in bovine serum albumin (control for fats). FI was assessed 30min and 60min post-infusion. MPC and oleic acid significantly enhanced the FI suppressive effects of EX4, whereas soy protein and intralipid did not. These data suggest that intraintestinal dairy macronutrient constituents can enhance the food intake suppressive effects of GLP-1R agonists.

42

2-Mercaptoacetate (MA) Blocks Fatty Acid (FA) Induced Calcium Influx in Cultured STC-1 Cells and Reduces GLP-1 Secretion *in vivo*.

S RITTER, A-J LI, S SIMASKO, Q WANG, TT DINH
Washington State University Pullman, WA, USA

We recently found that MA, known for its ability to inhibit fatty acid oxidation and stimulate feeding, blocks calcium influx into cultured nodose neurons exposed to long and medium chain fatty acids (FA). MA also blocked Ca++ influx in response to GW9508 (GW), an agonist at the G-protein coupled receptor 40 (GPR40), a receptor for long and medium chain FAs. These data suggest that MA is a GPR antagonist. FAs stimulate GLP-1 secretion from enteroendocrine L cells by binding to GPR120 membrane receptors. We postulated MA might inhibit GPR-mediated GLP-1 secretion. In an in vivo test of this hypothesis, we found MA attenuated GLP-1 secretion triggered by olive oil gavage in rats. In vitro we measured effects of MA on Ca++ influx in cultured STC-1 cells. STC-1 cells are known to express GPR40 and GPR120

and to secrete GLP-1 via GPR120. Linoleic acid (LA, 0.5 mM) and GW (5 uM) increased Ca^{++} influx in STC-1 cells and MA (0.2 mM) blocked this effect. The GPR120 agonist, grifolic acid (0.1, 0.2, 0.5 and 1uM), also increased Ca++ influx in STC-1 cells and MA (0.2mM) blocked the effect. MA did not block effects of high K^+ (55 mM), suggesting a selective action of MA, as we observed previously in nodose neurons. GLP-1 is insulinogenic and has both central and peripheral effects that promote satiety. GLP-1 antagonists have been shown to increase food intake. Therefore, inhibition of GLP-1 secretion by MA, an effect examined here, could potentially contribute to MA-evoked food intake.

43

Hindbrain parabrachial nucleus lesions attenuate anorexic responses to a glucagon-like peptide 1 receptor (GLP-1R) agonist

JP BAIRD, J SWICK, P ANGARITA, H ROH
Amherst College Amherst, MA, USA

To better understand the brain structures that contribute to GLP-1 effects on food intake, we evaluated the effect of bilateral ibotenic acid lesions of the parabrachial nucleus (PBNX) on the consumption of chow, 0.3M sucrose, and 4mM sodium saccharin solutions after systemic injections of the GLP-1R agonist, exendin-4 (EX4; 1 ug/kg), or vehicle. EX4 significantly reduced cumulative chow intake in the SHAM lesion (n=18) and INTACT (n=12) control groups at 1,2,4, and 8 h measurement intervals relative to vehicle conditions (ps<0.05). Intake at 24h was reduced in the SHAM (p<0.03) but not the INTACT control group, and in both groups the 24h differences were more modest, suggesting delayed compensatory intake after EX4 anorexia. PBNX rats showed no significant intake reduction after EX4 injection at any time measurement. Analysis of 90-min sucrose consumption revealed a modest 25% reduction in meal size that was significantly smaller in the SHAM (p<0.01) but not the intact control group (p=0.11). PBNX rats consumed significantly more sucrose under vehicle conditions than either control group, but EX4 injection had no significant effect in this group, reducing sucrose intake by <8%. Non-caloric saccharin intake tended increase after EX4 injection in all 3 groups, but the differences were variable and not statistically significant relative to vehicle conditions. Overall, the results suggest that EX4 intake effects depend on the caloric and gustatory properties of the food stimuli. The results also suggest that the PBN is necessary for the expression of EX4 anorexia exhibited for caloric foodstuffs.

44

Insulin effects on inhibitory transmission in the vagal complex is regulated by cAMP in control, but not diabetic mice

CB BLAKE, BN SMITH
Department of Physiology, College of Medicine, University of Kentucky Lexington, KY, USA

Brainstem nuclei, specifically the dorsal motor nucleus of the vagus (DMV) and nucleus tractus solitarius (NTS), vitally regulate visceral function. Insulin is transported into the brainstem and affects whole-body metabolism through central mechanisms. The NTS receives viscerosensory vagal input, and projects heavily to the DMV, which supplies parasympathetic vagal motor output to the abdominal viscera. Pathologies in which insulin is dysregulated, including diabetes, can disrupt this circuit, leading to gastric and other autonomic dysfunction. Previously, we found that insulin decreases excitatory activity of DMV neurons, with no effect on inhibition, in both control and streptozotocin-induced, hyperglycemic mice. We hypothesized that the lack of effect on GABAergic transmission may be due to low resting cAMP levels in GABAergic terminals. We used whole-cell patch-clamp recordings in brainstem slices from mice to identify effects of insulin on inhibitory neurotransmission in the DMV in the presence of elevated cAMP levels. Preliminary data suggest that with pre-incubation with the adenylate cyclase activator, forskolin, insulin reduces the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs, 6 of 7 cells) and miniature IPSCs (3 of 5 cells) from control, but not in hyperglycemic mice. This suggests that hyperglycemic mice may have a dysregulated cAMP system in the brainstem and/or may be resistant to forskolin. More studies are currently being conducted to determine the mechanism of this difference.

45

The role of the AP in the anorectic effects of amylin and sCT—behavioral and neuronal phenotyping

F BRAEGGER, CN BOYLE, TA LUTZ
Institute of Veterinary Physiology, University of Zurich Zurich, Switzerland

It is well established that amylin reduces meal size via a direct action on the area postrema (AP). Salmon calcitonin (sCT) is an amylin receptor agonist that, like amylin, potently reduces food intake, but reportedly exerts additional effects, such as improved blood glucose. It is unknown if sCT recruits additional populations of hindbrain neurons to bring about these actions. Here, we tested whether sCT, which in addition to the amylin receptor also binds to the calcitonin receptor, activates similar hindbrain pathways to amylin, and if its anorectic properties also depend on the AP. The role of the AP in mediating sCT's anorectic action was examined in feeding

experiments testing the dose-response effects of sCT in AP-lesioned (APX) versus sham-lesioned male rats. Further, the effect of sCT to induce c-Fos expression was compared in the two surgery groups, and in relation to amylin-induced c-Fos. The phenotype of c-Fos-expressing neurons in the hindbrain was examined by testing for the co-expression of tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH), or tryptophan hydroxylase (TPH). Our results showed that similar to amylin, an intact AP is necessary for sCT to reduce eating. Further, the pattern of co-expression between c-Fos activation and TH or DBH after amylin or sCT did not differ markedly. There was only little c-Fos/TPH colocalization under any condition. Our study suggests that the hindbrain mechanisms activated by amylin and its agonist sCT share many similarities and that the effects induced by sCT seem to be mainly mediated by AP neurons.

46

Reduction of food intake by hindbrain MC4R activation depends on central vagal afferent endings.

CA CAMPOS, H SHIINA, RC RITTER
Washington State University Pullman, WA, USA

Injection of MTII, a melanocortin 3/4 receptor (MC4R) agonist into the nucleus of the solitary tract (NTS) reduces food intake (Grill et al. 1998). MC4R transcript is present in vagal afferent neurons, and patch clamp experiments indicate that most NTS responses to MTII are mediated by presynaptic vagal afferent MC4R activation (Wan et al. 2008). Consistent with these reports, we recently observed that 4th ventricle MTII produces long lasting increase in synapsin 1 phosphorylation (serine 9) in NTS vagal afferent endings, an effect known to enhance neurotransmitter release in other neural systems. Based on these results, we postulated that reduction of food intake by hindbrain MC4R agonists depends on activation of central vagal afferent endings. To test this hypothesis we subjected rats to unilateral nodose removal, resulting in degeneration of vagal afferent endings in the ipsilateral, but not contralateral, NTS. Nodosectomized rats were implanted with cannulas for MTII injection either ipsilateral or contralateral to the nodose removal. We found that unilateral nodosectomy attenuated reduction of food intake when MTII was injected in the NTS ipsilateral to nodose removal, but did not affect reduction of food intake when MTII was injected in the contralateral NTS. We conclude that reduction of food intake by hindbrain action of MC4R agonist depends on the presence of intact central vagal afferent endings. MC4R modulation of transmitter release from central vagal afferent endings could adjust the strength of vagal afferent synapses involved in satiation.

47

Hindbrain NMDA-receptors participate in reduction of food intake by hypothalamic leptin

CA CAMPOS, H SHIINA, RC R
Programs in Neuroscience, Washington State University Pullman, WA, USA

A growing number of observations suggest that reduction of food intake by the adipokine, leptin, acting in the hypothalamus, is mediated in part by descending neural projections that modulate satiation signals in the nucleus of the solitary tract (NTS). Our own results indicate that N-Methyl-D-aspartate receptors (NMDAR) activation in the NTS play an essential role in mediating reduction of food intake by peripheral CCK and hindbrain melanocortin agonist injection. Therefore, we postulated that hindbrain NMDAR might also contribute to reduction of food intake following activation of hypothalamic leptin receptors. To test this hypothesis we used rats implanted with multiple intracranial injection cannulas aimed for the hypothalamic arcuate nucleus (ARC) and the NTS. We found that ARC injection of leptin (300 ng) significantly reduced food intake at 1, 2, 24 and 48h post injection. Over a 24h period, most of leptin's effect on food intake was due to reduction of meal size. DCPPene, a competitive NMDAR antagonist, injected into the NTS just prior to ARC leptin injection, significantly attenuated reduction of food intake by leptin at all of the above time points. This effect of hindbrain NMDAR antagonism was due entirely to reversal of leptin's effect on meal size, with no effect on meal frequency. Additional experiments in progress are aimed at establishing the cellular and synaptic mechanisms by which hindbrain NMDAR contribute to reduction of food intake by leptin.

48

Lipopolysaccharide induces inflammatory STAT and NF-kB signaling in the area postrema

L LOI[1], T BORNER[1,2], C CORDANI[1], TA LUTZ[1,2], T RIEDIGER[1,2]
[1]Institute of Veterinary Physiology Vetsuisse Faculty, University of Zurich Zurich, Switzerland, [2]Zurich Center for Integrative Human Physiology (ZIHP) Zurich, Switzerland

Lipopolysaccharide (LPS) treatment is a commonly used model for inflammation. Among other disease symptoms, LPS induces hypophagia and induces conditioned taste aversion (CTA). The area postrema / nucleus of the solitary tract (AP/NTS) region controls food intake but also mediates CTA responses. Moreover, pharmacological (bupivacaine) inactivation of this brain area blocks LPS-dependent social withdrawal and neuronal activation of forebrain areas, and induction of LPS tolerance by repeated LPS administration attenuates LPS-induced CTA. STAT and NF-kB are intracellular mediators of inflammatory stimuli. We investigated whether LPS induces NF-kB or STAT in the AP/NTS

region and whether inflammatory signaling in this brain area is attenuated after induction of LPS tolerance induced by repeated LPS treatment (3 x 100µg/kg ip). Single injection of LPS significantly induced STAT3 phosphorylation in the AP and NTS 4h but not 2h after injection. This response was completely blunted in LPS tolerant rats. Compared to STAT3, the NF-kB pathway was activated already at an earlier time point (1h) as measured by nuclear translocation of Nf-kB immunoreactivity. NF-kB activation appeared to be restricted to vascular cells. In conclusion, inflammatory STAT and NF-kB might function as intracellular transducers of AP/NTS dependent behavioral changes occurring under inflammatory disease conditions. Supported by the Swiss National Science Foundation and Krebsliga Zurich.

49

Systemic leptin is insufficient to confer sensitivity of glucagon-like peptide-1 (GLP-1) neurons to cholecystokinin-8 (CCK) in fasted rats

JW MANISCALCO, L RINAMAN
University of Pittsburgh Pittsburgh, PA, USA

We recently reported that i.p. saline or CCK (3 ug/kg) increases cFos expression within hindbrain GLP-1 neurons by ~30% compared to non-injected controls when rats are fed *ad libitum*, whereas GLP-1 neuronal sensitivity to i.p. treatment is nearly abolished in rats after overnight food deprivation (DEP). Leptin enhances CCK's ability to depolarize vagal sensory neurons and to activate neurons in the nucleus of the solitary tract (NTS). Since plasma leptin falls during overnight fasting, we hypothesized that systemically administered leptin in DEP rats would rescue the ability of i.p. saline and CCK to activate GLP-1 neurons. To test this, adult male rats were food deprived overnight for 16-18 hr, then perfused with fixative 90 min after i.p. injection of leptin (0, 100, 200, or 400 ug/kg) alone or in combination with CCK (3 ug/kg). Brain sections were processed for immunolocalization of cFos and GLP-1, or pSTAT3. Compared to low pSTAT3 labeling in i.p. saline controls, all leptin doses robustly and similarly increased pSTAT3 within the arcuate nucleus. Additionally, leptin produced a dose-dependent increase in pSTAT3 within the caudal NTS and dorsomedial division of the ventromedial hypothalamic nucleus. Despite this central pSTAT3 induction, leptin alone or combined with CCK did not activate GLP-1 neurons. Thus, leptin signaling is insufficient to restore GLP-1 responsiveness to i.p. saline or CCK in food-deprived rats. We are exploring whether systemic leptin can rescue the blunted sensitivity of A2 noradrenergic neurons to systemic CCK following fasting.

50

Prolactin-releasing peptide (PrRP) neurons in the nucleus tractus solitarius (NTS) are differentially activated by stressors that reduce meal size

LE RUPPRECHT, JW MANISCALCO, L RINAMAN
University of Pittsburgh Pittsburgh, PA, USA

The majority of noradrenergic (NA) neurons in the caudal NTS co-express PrRP, and central administration of PrRP reduces meal size. PrRP neurons express cFos after a wide array of stressful stimuli that reduce food intake. Interestingly, however, PrRP neurons reportedly are not activated in rats after LiCl, which increases latency to feed without reducing meal size. Thus, PrRP neurons may be selectively recruited by stimuli that reduce meal size. To test this, we assessed whether PrRP neurons are activated in rats after restraint or water deprivation, both of which reduce meal size. Adult male rats were anesthetized and perfused with fixative after 24 hr water deprivation, or 90 min after the onset of 30-min restraint. Controls were non-manipulated. Brain sections were processed for triple immunolocalization of cFos, dopamine-b-hydroxylase (DbH), and PrRP, and the percentage of DbH+/PrRP+ and DbH+/PrRP- neurons expressing cFos was quantified. In control rats, 35% of DbH+/PrRP+ and 5% of DbH+/PrRP- neurons expressed cFos. Water deprivation reduced food intake by 44.4%, but did not significantly alter recruitment of either DbH+/PrRP+ or DbH+/PrRP- neurons, as compared to controls. Conversely, restraint significantly increased cFos activation of DbH+/PrRP+ and DbH+/PrRP- neurons to 81% and 21%, respectively. Thus, PrRP neurons are recruited by some, but not all, stimuli or treatments reported to decrease food intake by decreasing meal size.

51

Regional arterial infusions indicate that the gastrointestinal tract contains site(s) of action regulating meal size and intermeal interval length by gastrin-releasing peptide.

AI SAYEGH[1], AH AGLAN[2], MC WASHINGTON[1], JR REEVE[3]
[1]College of Veterinary Medicine, Tuskegee University Tuskegee, AL, USA, [2]School of Medicine, Wayne State University Detroit, MI, USA, [3]David Geffen UCLA School of Medicine Los Angeles, CA, USA

Mammalian gastrin-releasing peptide (GRP), homologous to amphibian peptide bombesin, reduces meal size (MS) and prolongs intermeal interval length (IMI). The site(s) regulating these actions are unknown. Here, we measured these feeding responses by regional gastrointestinal arterial infusions of GRP-10 (found in all mammals), GRP-27 (found in most mammals) and GRP-29 (found only in rat) (doses 0, 0.1, 0.2, and 0.5 nmol/kg) in the celiac artery (CA, supplying stomach and upper duodenum, n=6), cranial mesenteric artery (CMA, small

and large intestine, n=6) and femoral artery (FA, the systemic control, n=4) in freely-fed rats, food-deprived 50 min prior to dark onset. We found that no GRP form had any action on MS or IMI after FA infusion. For reduction of MS, the order of potency was GRP-29>GRP-27>GRP-10 GRP-29 displayed its greatest potency in the CA (75% decrease at maximally effective dose; $p<0.001$). In the CMA, all forms of GRP reduced MS similarly. For prolongation of IMI, GRP-10 was ineffective at all sites; GRP-27 produced moderate responses only in CA (33% increase, $p<0.04$); GRP-29 produced major, consistent responses only in CA (three-fold increase, $p<0.002$). Likewise, calculations of satiety ratios (IMI in min/MS in g) revealed that GRP-29 in CA clearly produced the most pronounced responses (eight-fold increase, $p<0.001$). These results indicate that the vascular bed of the CMA contains site(s) of action for MS reduction and strongly and uniformly suggest that the vascular bed of the CA contains site(s) of action for IMI prolongation by exogenous rat GRP-29.

52

CCK-58, but not CCK-8, prolongs the intermeal interval.

AI SAYEGH [2], MC WASHINGTON[2], AH AGLAN[1], JR REEVES JR[3]
[1]1School of Medicine, Wayne State University Detroit, MI, USA, [2]College of Vetinary Medicine Tuskegee Universty Tuskegee, AL, USA, [3]3David Geffen UCLA School of Medicine Los Angeles, CA, USA

Cholecystokinin-58 (CCK-58) is the only endocrine form of CCK in the rat (Reeve et al, 2003), but CCK-8 is the common form employed to study the actions of this peptide. We compared the effects of exogenous CCK-58 and CCK-8 (0, 0.1, 0.5, 0.75, 1.0, 3.0, and 5.0 nmol/kg intraperitoneally) on first meal size (MS), postprandial intermeal interval (IMI) length and second MS in overnight food-deprived rats before access to 10% sucrose. Both peptides reduced first MS in almost identical patterns (35% decreases at threshold doses, both $p<0.001$; 65% decreases at maximally effective doses; $p<0.001$). CCK-58 produced major prolongations of the IMI (three-fold increase at the maximally effective dose; $p<0.001$). No dose of CCK-8 had any effect on IMI length. Calculation of the satiety ratio (SR; IMI length in min/MS in mL) demonstrated a similar major action of CCK-58, with an inverted U-shaped dose-response function and a peak effect at a dose of 0.75 nmol/kg (ten-fold increase; $p<0.001$). No dose of CCK-8 had any effect on SR. No dose of either CCK-58 or CCK-8 had any effect on second MS. We conclude: (1) exogenous CCK-58 decreases MS in a pattern almost identical to that of exogenous CCK-8; (2) exogenous CCK-58, but not CCK-8, produces a major prolongation of IMI length; and (3) these actions of CCK-58 do not result in a compensatory increase in second MS. Whether the two major actions of exogenous CCK-58 on meal pattern reflect physiological functions of endogenous CCK-58 remain to be demonstrated.

53

Evidence for co-expression of TRPV3 with TRPV1 in primary vagal afferent neurons.

S WU[1], J PETERS[2]
[1]Washington State University Seattle, WA, USA,
[2]Washington State University Seattle, WA, USA

Post-ingestion activation of vagal afferent neurons innervating the gastrointestinal tract relays satiety information to the brain. Centrally vagal afferents form strong excitatory synapses with neurons in the nucleus of the solitary tract (NTS). Calcium permeable TRPV1 channels residing in these central terminals specifically control spontaneous and asynchronous neurotransmitter release pathways which help shape the information transfer across this synapse. Our recent preliminary data suggest that TRPV3, in conjunction with TRPV1, also contributes to neurotransmitter release. To investigate the potential role of TRPV3 in vagal afferent neurons we used a combination of approaches including; ratiometric calcium imaging, patch-clamp electrophysiology, RT-PCR, and western blot analysis. We found that TRPV3 agonist ethyl vanillin (EVA) activated a subpopulation of neurons, all of which were sensitive to TRPV1 specific-agonist capsaicin (CAP). But, not all CAP reactive neurons responded to EVA. Repeated application of EVA caused channel sensitization; a characteristic unique to TRPV3. Analysis of calcium influx in the presence of EVA and channel selective antagonists across wild-type and transgenic mice indicate that TRPV3 may form hetero-tetramers with TRPV1. Whole-cell patch clamp experiments also suggest this interaction. These preliminary findings suggest that TRPV3, together with TRPV1, provide important calcium influx pathways in vagal afferent neurons which may participate in the control of neurotransmitter release at the central synapses onto NTS neurons.

54

Anatomical evidence for central glucagon-like peptide-1 (GLP-1) and glutamatergic co-transmission in rats

H ZHENG, L RINAMAN
University of Pittsburgh Pittsburgh, PA, USA

Hindbrain GLP-1 neurons target brainstem and forebrain regions that shape food intake and energy balance in rats and mice. The present study examined whether GLP-1 neurons express vesicular glutamate transporter 2 (VGLUT2), similar to noradrenergic neurons within the caudal nucleus of the solitary tract (NST) (Stornetta et al., 2002). VGLUTs ensure vesicular uptake of glutamate and are unambiguous markers for glutamatergic transmission (Mestikawy et al., 2011). Tissue sections from adult male Sprague-Dawley rats were processed for dual

immunofluorescence localization of GLP-1 and VGLUT2, and then examined using confocal microscopy and a 100x oil-immersion objective. The large majority of GLP-1+ varicosities within the hypothalamus (paraventricular, dorsomedial, arcuate nuclei), paraventricular thalamus, bed nucleus of the stria terminalis, and NST also were VGLUT2+. Fluorescence *in situ* hybridization confirmed that many GLP-1+ neurons within the caudal NST expressed VGLUT2 mRNA in adult male rats. We conclude that at least a subset of hindbrain GLP-1 neurons are equipped to use glutamate as a co-transmitter within brain regions that receive GLP-1 axonal input and are implicated in controlling food intake and energy balance. This new evidence invites further research to investigate potential functional interactions between glutamate and GLP-1 signaling within these regions.

55

Neurobiological underpinnings of psychic secretion

RK BABBS[1,2], I DE ARAUJO[1,2], DM SMALL[1,2]
[1]John B. Pierce Laboratory New Haven, CT, USA, [2]Yale University, Dept. of Psychiatry New Haven, CT, USA

In 1927 Pavlov described anticipatory salivation to cues that predict food availability and termed this phenomenon "psychic secretion". Since this time the neural circuits for what is now termed classical conditioning have been well established. In the current study we extended this research to ask whether the acquisition of "psychic secretion" predicted brain response to conditioned cues. We measured salivation in response to milkshake aromas before and after the aroma was associated with immediate delivery of milkshake in 25 people. The difference in salivation (ΔSAL) was taken as a physiological marker of conditioning. Following conditioning, brain response to the predictive milkshake aroma was assessed with fMRI. A regression analysis revealed a significant positive association between ΔSAL and response to the predictive milkshake aroma in the caudate nucleus, a region known to respond to palatable food cues, especially in obese individuals (Berns et al., 2012; Rothemund et al., 2007). This result indicates that caudate response to food cues reflects the acquisition of cephalic phase pre-ingestive responses or psychic secretions.

56

Ghrelin regulates phasic mesolimbic signaling evoked by food stimuli

JJ CONE[1], JD ROITMAN[2], MF ROITMAN[1,2]
[1]Neuroscience Chicago, IL, USA, [2]Psychology Chicago, IL, USA

The peptide ghrelin signals energy need and promotes food intake via action in multiple brain regions. Recent work demonstrates that one such site is the ventral tegmental area (VTA) whose dopamine neurons project to the nucleus accumbens (NAc). Brief (phasic) fluctuations in NAc dopamine and in the firing of NAc neurons signal aspects of food reward including the predictive nature of cues and goal-directed behavior for food. We previously showed that lateral ventricular administration of ghrelin increased phasic dopamine signals evoked by food reward in ad libitum fed rats. Here, in vivo electrochemistry was used to determine a site of action for ghrelin to influence phasic dopamine signaling. Phasic spikes in NAc dopamine evoked by food reward were recorded before and after infusion of either saline or ghrelin into the VTA or lateral hypothalamus (LH) – another locus of ghrelin-stimulated feeding. Infusion of ghrelin (0.4μg) into the LH (131.6+/-6% of baseline post-ghrelin versus 101.9+/-2% post-vehicle), but not the VTA (95.5+/-2% of baseline post-ghrelin versus 99.4+/-7% post-vehicle), increased phasic dopamine evoked by food. Interestingly, both sites supported ghrelin-enhanced food consumption after the recording session. Parallel studies are being conducted in which the electrophysiological responses of NAc neurons are recorded. Our data demonstrate that: 1) central ghrelin potently modulates phasic mesolimbic signaling evoked by food reward and 2) the LH is an important locus for ghrelin action on the neural circuitry underlying motivated behavior. DA025634, TL1TR000049.

57

Nucleus accumbens GLP-1 receptors influence meal size and palatability.

AM DOSSAT, RD DIAZ, L GALLO, A PANAGOS, KE KAY, DL WILLIAMS
Psychology Department & Program in Neuroscience, Florida State University Tallahassee, FL, USA

Recent evidence suggests that the glucagon-like peptide 1 (GLP-1) neuronal projection to the nucleus accumbens core (NAcC) influences food intake. To investigate the role of endogenous stimulation of GLP-1 receptors (GLP-1R) in NAcC, we examined the effects of the antagonist Exendin (9-39) (Ex9) on meal pattern and microstructure of licking behavior in rats. Intra-NAcC Ex9 treatment selectively increased meal size relative to vehicle in rats consuming 0.25M sucrose by 37-94%, $p < 0.05$) or sweetened condensed milk (by 18%, $p < 0.05$). Microstructural analysis showed that NAcC Ex9 increased initial lick rate and the size and duration of licking bursts in rats ingesting 0.1M or 0.25M sucrose ($p < 0.05$), suggesting that blockade of NAcC GLP-1R increases palatability. NAcC Ex9 did not affect licking for 0.1% saccharin, so we suggest that the presence of nutrients in the gut is required for endogenous stimulation of NAcC GLP-1R. Consistent with this, we also found that the meal size-suppressive effects of intra-gastric sucrose infusion (2.4 kcal) were attenuated by NAcC delivery of Ex9 at a dose that had no effect when delivered alone ($p < 0.05$). Analysis of licking patterns revealed that NAcC Ex9 blunted the effect of intra-gastric nutrients on meal size primarily by increasing the size and

duration of licking bursts ($p < 0.05$). Our results suggest that NAcC Ex9 influences taste evaluation. We conclude that GLP-1 released in NAcC in response to gastrointestinal nutrients influences the hedonic value of food.

58

Ventral tegmental area orexin-A stimulates feeding.

KE KAY[1], JF DAVIS[2], DL WILLIAMS[1]
[1]Psychology Department & Program in Neuroscience, Florida State University Tallahassee, FL, USA,
[2]Department of Psychiatry, Metabolic Diseases Institute, University of Cincinnati Cincinnati, OH, USA

Central orexin-A treatment increases food intake, and brain orexin 1 receptors (OX1R) are thought to be involved in food-motivated behavior. Orexin neurons project to many brain regions, including the ventral tegmental area (VTA), which is of particular interest because of its involvement in food reward. Here, we investigated the effects of intra-VTA orexin-A injection on palatable food intake in two experiments. First, we asked whether VTA orexin-A treatment could increase intake of standard rat chow or high-fat (60%) diet (HFD) when rats were satiated. Rats ($n = 9$) with cannulas targeting the VTA were food-deprived for 48 h, then given ad lib access to standard chow for 1.5 h. At that point, rats received intra-VTA injection of vehicle or 0.5 nmol orexin-A. Chow remained available, and HFD was introduced 30 min post-injection. Intake of chow and HFD were measured 60 min later. We observed that VTA orexin-A injection more than doubled chow intake relative to vehicle during the 30 min post-injection period ($p < 0.05$), and significantly increased HFD intake by 32% relative to vehicle during the 60 min of HFD access ($p < 0.05$). In a second study, we examined the orexin-A effect on non-deprived rats' ($n = 8$) intake of 0.1M sucrose during daily 30-min test sessions. Intra-VTA injection of 0.5 nmol orexin-A 30 min before the test session increased sucrose intake by 175% relative to vehicle ($p < 0.05$). Based on these data, we conclude that VTA orexin receptor stimulation can robustly drive food intake.

59

Encoding of reward prediction by dopamine in nucleus accumbens core and shell

JE MCCUTCHEON, MF ROITMAN
Psychology, University of Illinois Chicago Chicago, IL, USA

Nucleus accumbens (NAc) dopamine correlates with rewards and reward-predictive cues. However, the nature of reward delivery (e.g. food pellets or sipper tube) often confounds three processes: reward receipt, reward prediction, and motor generation necessary for reward collection. We used intraoral reward delivery in an attempt to resolve this confound. Rats were trained to associate distinct cues with subsequent delivery of either a sucrose pellet or an infusion of an identical amount of sucrose solution. Fast scan cyclic voltammetry was then used to measure phasic dopamine in NAc core and shell while rats experienced both cued and uncued rewards. Behavioral discrimination between pellet-paired and infusion-paired cues was seen during training sessions and the test session. As such, rats made more head entries into the food receptacle during presentation of the pellet-paired cue relative to the infusion-paired cue. Despite this difference, both cues evoked dopamine release of similar magnitude in both subregions. In contrast, responses to the rewards themselves differed - pellets evoked dopamine release, whether cued or not, in core and shell, whereas infusions evoked dopamine in shell but not core. Apart from the response to uncued infusions, the primary difference between NAc subregions was that decay of dopamine events was broader in shell than core, which has implications for tuning of temporal relationships between rewards and cues. In conclusion this work supports a role for NAc dopamine in encoding the reward-predictive nature of cues rather than either reward receipt or motor generation.

60

Amylin interacts with leptin in the ventral tegmental area to control food intake

EG MIETLICKI-BAASE, BA JEFFREY, DR OLIVOS, MR HAYES
U Penn Philadelphia, PA, USA

Amylin is a neuropeptide produced by pancreatic β-cells that reduces food intake (FI) following CNS activation. Systemic coadministration of amylin and the adipose-tissue derived hormone leptin suppresses FI beyond that produced by either peptide alone. CNS nuclei mediating this interaction have only been partially ascribed to processing by the hypothalamus and area postrema; however, other CNS structures are clearly required to mediate the interaction between leptin and amylin. The ventral tegmental area (VTA) is a likely site of action as both leptin and amylin receptor signaling in the VTA are physiologically relevant for the control of FI. To test whether VTA amylin and leptin signaling interact to control FI, we administered into the VTA moderately suprathreshold intraparenchymal doses of amylin (0.4μg) and leptin (0.3μg) alone or in combination. Both individually reduced FI, while the combination further suppressed FI compared to either drug alone. The FI suppression by leptin was primarily due to a decrease in meal size, whereas amylin induced early reductions in meal number and size and later suppression of just meal size. Further evidence for an intra-VTA interaction of amylin and leptin signaling was provided by the finding that the FI and body weight suppression produced by intra-VTA leptin (0.6μg) were attenuated by intra-VTA pretreatment with the amylin receptor antagonist AC187

(0.1µg). These findings highlight the VTA as an important site mediating the cooperative effects of leptin and amylin.

61

A Role for Vitamin D3 in Diet-Induced Obesity and Dopamine-Related Behaviors

JR TRINKO, BB LAND, RJ WICKHAM, WB SOLECKI, LA TELLEZ, J MALDONADO-AVILES, IE DE ARAUJO, NA ADDY, RJ DILEONE
Dept. of Psychiatry, Yale University School of Medicine New Haven, CT, USA

Obesity rates have increased over the past two decades. Vitamin D3 deficiency rates have increased within a similar timeframe, and evidence suggests an inverse relationship between circulating vitamin D3 levels and obesity. Western diets typically involve high-fat items that are low in vitamin D3, yet a causative role in the development of obesity has not been explored. We show that mice consuming a modified high-fat diet with reduced vitamin D3 displayed enhanced diet-induced obesity and food intake, while naïve mice treated with exogenous vitamin D3 had reduced high-fat intake and weight gain. Dopamine (DA) neural circuits are involved with the overconsumption of palatable foods and drugs of abuse. We show that the receptor for vitamin D3 (VDR) is expressed throughout these circuits. Additionally, exogenous vitamin D3 enhanced the effects of amphetamine (AMPH) on DA release and locomotor activity, while dietary deficiency reduced the responses to AMPH. As VDR can function as a transcription factor, canonical genes involved in DA transmission were assessed by qPCR. Finally, naïve mice trained to orally consume AMPH decreased their intake after exogenous vitamin D3, while mice in the vitamin D3 deficient state displayed increased AMPH consumption. Our data provide causative evidence for dietary levels of D3 in the development of obesity, and potentially drug intake.

62

Distribution and Characteristics of Lateral Hypothalamic Neurons that Innervate the Dorsomedial Accumbens Shell

KR URSTADT[1], NG BOUTROS[2], KC MOSCHKIN[2], BG STANLEY[1,2]
[1]Psychology Dept., UC Riverside Riverside, CA, USA, [2]Cell Bio & Neurosci Dept., UC Riverside Riverside, CA, USA

The nucleus accumbens shell (AcbSh) and the lateral hypothalamus (LH) interact to control feeding and reward, and are reciprocally connected. However, few studies identify the specific LH subregion locations and chemical phenotypes of AcbSh-projecting neurons. To explore this issue, four male Sprague Dawley rats received iontophoretic injections of Fluorogold (FG; 2%) into different rostrocaudal regions of the dorsomedial AcbSh. After a 7-day survival period, their brains were extracted, fixed, and sliced into 40 um coronal sections. Sections containing the LH were stained with antibodies against Orexin-A, melanin concentrating hormone (MCH), cocaine and amphetamine regulated transcript (CART), leptin receptors (ObR), or GAD67 (a marker for GABA), followed by an AlexaFluor 647-conjugated secondary antibody. FG was excited directly with a UV laser. In each subject, the highest density of FG-labeled cells was seen throughout the rostrocaudal extent of the peduncular LH, with moderate FG labeling in the lateral perifornical area and tuberal LH and fewer labeled cells in other hypothalamic areas. At least half of FG-labeled cells were GAD67-positive, and a substantial minority of cells in these areas were FG and CART or FG and ObR positive. Rarely were FG-labeled cells within these regions orexin or MCH positive. This evidence suggests that the projections from LH regions to the dorsomedial AcbSh are likely to be GABAergic, leptin-receptive, and/or CART-secreting but rarely utilize orexin or MCH.

63

Inhibitory Control of Eating as Assessed by a Novel Go/No-Go Sipping Task

LA BERNER, H AYAZ, M IZZETOGLU, S WINTER, A MATTEUCCI, M LOWE
Drexel University Philadelphia, PA, USA

Individuals with bulimia nervosa (BN) demonstrate impaired self-regulation and less activation in lateral prefrontal cortex when engaging self-control in cognitive tasks. Potential direct relations between these impairments and the sense of "loss of control" characteristic of binge eating remain unexplored, as no food-adapted self-regulation task has included participant-controlled eating responses. To directly assess self-regulatory control over eating, we developed a go/no-go (GNG) task in which individuals sip and swallow a palatable yogurt shake (0.74kcal/g) in response to all letters except X (208 trials; ISI=2-4s; 15.4% no-go). Video analysis software codes responses. Five healthy women completed, after 3-h fast, the sipping task and a matched-parameter standard GNG task, while functional near-infrared spectroscopy measured prefrontal cortical activity. Task performance was comparable (*M* total sipping task commission errors=17.5%; *M* standard task commission errors=21.3 %). In both tasks, activations associated with inhibition were observed in right middle and inferior frontal gyri, with additional activation in left inferior frontal gyrus in the sipping task. These initial results support the feasibility of a GNG sipping task to assess eating-specific inhibitory control. Data collection to compare the performance of women with BN to controls is underway. This first integration of a cognitive task and a lab-based test meal could elucidate binge eating mechanisms and permit distinction of deficits

related specifically to eating inhibition from those related to general response inhibition.

64

Food exposure, cravings, and physiological reactivity in overweight subjects and healthy-weight

C CAMACHO[1,2], R GEVIRTZ[1], V RISBROUGH[2], K BOUTELLE[2]
[1]Alliant University San Diego, CA, USA, [2]UCSD San Diego, CA, USA

Altered food cue reactivity is implicated in the path to weight gain and obesity. The autonomic nervous system (ANS) has been identified as one marker of cue reactivity in overweight individuals (Matsumoto et al., 2001). The aim of this study was to investigate the ANS responses to palatable food-cues in overweight and healthy-weight adults. Heart rate variability (HRV) and skin conductance (GSR) were measured during a food cue exposure task. Participants included 50 adults (25 overweight; 25 healthy weight) between the ages of 18-26 (Female=74%; Caucasian=36%). Participants were guided through a baseline period, food exposure task, and recovery period and reported subjective craving every 30 sec on a 1-100 scale. Repeated measures ANOVAs found an effect for time, with increased cravings during exposure and habituation to baseline levels during recovery ($F=25$, $p=.000$); an increase in low frequency HRV ($F=7.5$, $p<.001$) and GSR ($F=22.8$, $p<.001$) after exposure; and a decrease in high frequency HRV ($F=7.7$, $p<.001$) after exposure. No differences were found between overweight and healthy weight individuals in the ANOVA analyses. However, one-way ANOVAs, using power or area under the cuve (AUC), found greater AUC low frequency HRV in the overweight group ($F=5.2$, $p=.027$) compared to the normal weight group. Linear regressions found a positive association between food cravings and GSR ($\beta=.25$, $p=.03$). Results indicate increasing sympathetic activity, decreasing parasympathetic activity following food exposure, and evidence for sympathetic dysregulation in overweight individuals. Additionally, GSR should be evaluated further as a marker of food cravings. Sympathetically-mediated cephalic phase responses may be subjectively experienced as food cravings, providing support for the cue-reactivity model.

65

Food reinforcement value after food deprivation

FJ DIAZ[1], K FRANCO[1]
[1]UDG JALISCO, Mexico, [2]UDG JALISCO, Mexico

Food is a positive reinforcer that can increase its consumption even the food access interval is short. In this experiment frequency access to food and food deprivation interval were varied according to a factorial design intra-subject. In successive conditions nine rats were exposed to 720, 180 and 45 minutes of food deprivation interval. These values were combined with 40, 10 and 2.5 minutes of access to food. To probe the value of food an operant response was established during the access to food. Every lever press was reinforced with a 25 mg standard pellet. Before starting experimental conditions all subjects had ad-libitum access to food. Body weight described an U-inverted function during 180 and 45 minutes of food deprivation. Response rate increased systematically as access to food decreased. Latencies for food consumption decreased while access to food decreased. Food consumption was higher during night than day. In this period food intake increased as access to food decreased. In contrast, at night food intake decreased as access to food interval increased. This data suggest that food reinforcement value depends on restriction but access duration interval also.

66

Interaction deprivation food consumption

EC FLORES, SI BRAVO, FJ DIAZ
UDG JALISCO, Mexico

Two variables that modify the value of food reinforcement are food deprivation and the duration of access to food. In the present experiment de effect of maintaining constant the deprivation period and varying the access to food periods were studied. Nine rats were exposed to conditions of food deprivation periods of 300, 100 and 30 minutes, for each group the food access period was 100, 30 and 10 minutes in intervals of 15 days each. All rats had ad libitum access to food and water before they were exposed to the periods of deprivation. The results for the groups with 300/100 minutes and 300/30 minutes showed a stable pattern in food consumption in relation with the baseline data. None the less the group with 300/10 minutes had stable consumption in each one of the food access intervals. For all groups the food and water consumption was greater during the dark cycle as well as greater water consumption for the first food access interval. Behavior prior to a food access interval consisted of approaching towards and sniffing the food dispenser, as a result we can state that a discrimination contingency was made between time and the access to food period. In conclusion results showed that varying the food access and food deprivation periods modulates food consumption as well eating behaviors.

67

Small Weight Fluctuations Impact Impression Formation

JF HAYES[1,2], EG ROTHSTEIN[1], KE D'ANCI[1], RB KANAREK[1]
[1]Tufts University Medford, MA, USA, [2]Natick Soldier Research, Development and Engineering Center Natick, MA, USA

Overweight and obese individuals are judged more

negatively than there lean counterparts in a wide variety of domains. Less is known about impression formation of individuals losing and gaining small amounts of weight that may be more common in daily life. This study examines perceptions of a normal-weight female target (BMI= 23.2) who has lost or gained fifteen pounds over a period of two months, or who has maintained a steady weight. Female undergraduate participants (N=105) read a questionnaire describing a fictitious female target and rated her on a variety of physical and personality characteristics. Results demonstrated a negative bias towards the weight-gain target, where she was believed to be less satisfied with weight-related (i.e. hips) and fitness-related (i.e. stamina) physical characteristics and was perceived more negatively on personality characteristics related to health (i.e. discipline) than was the weight-loss or weight stable target. There were no observed differences between the weight-loss and the weight-stable target. Findings suggest that gaining a relatively small amount of weight is perceived to negatively impact body satisfaction and may reflect poorly on health-related personality characteristics, but that losing a relatively small amount of weight does nothing to influence perceptions if an individual. This study contributes to existing weight stigmatization literature and demonstrates that relatively small weight fluctuations may influence impression formation.

68

Food reinforcement is associated with food intake, BMI, and reward sensitivity in preschool children

BY ROLLINS[1,2], E LOKEN[2], JS SAVAGE[1], LL BIRCH[1,2]
[1]Center for Childhood Obesity Research, Penn State University University Park, PA, USA, [2]Health and Human Development, Penn State University University Park, PA, USA

Progressive ratio (PR) schedules of reinforcement have been used to measure the relative reinforcing value (RRV) of palatable, energy-dense foods in humans as young as 8 years old; however, developmentally-appropriate measures are needed to measure RRV of food in younger children. Study objectives were to demonstrate the validity of an RRV of food task (Epstein et al., 2004) adapted for use among preschool children (3 to 5y), and examine individual differences in RRV performance (number of responses and response rates). Thirty-three children completed the RRV task in which they worked to access graham crackers. Based on a pilot study, modifications were made to the RRV task to make it developmentally-appropriate and a PR schedule of '4, 8, 16, 32, 64…' was selected. Children's intake of the graham crackers was measured in a standard snack session; heights/weights were measured. Parents reported on their child's reward sensitivity using the BAS. Overall, children were willing to work for palatable snack foods. Boys and older children made more responses in the task, while children with higher BMI percentiles and reward sensitivity responded at a faster rate. Children who worked harder in terms of total responses and response rates consumed more calories in the snack session. This study demonstrates that the RRV of food task is a valid and developmentally appropriate measure for assessing individual differences in food reinforcement among very young children.

69

State hunger relates positively to power of food and Extraversion, and negatively to eating competence.

U VAINIK[1], E LäTT[2], J MäESTU[2], J JüRIMäE[2]
[1]Institute of Psychology, University of Tartu Tartu, Estonia, [2]Faculty of Exercise and Sport Sciences, University of Tartu Tartu, Estonia

Several personality traits have been suggested to regulate eating behaviours. However, these traits' relation to hunger is not known. Behaviourally, measures capturing food motivation and self-control have been shown to be influenced by state hunger. Here we tested how similar traits measured by questionnaires would relate to self-reported hunger or hunger hormones. 130 adolescent boys (13-15 years) were asked to restrain from breakfast before coming to test site at approximately 8:30am. After arrival, their blood was sampled and perceived hunger (100mm visual analogue scale) was measured. Then, the participants snacked ad libitum and completed measures capturing Five-Factor Model (FFM) personality traits, power of food, food impulsivity, and eating competence skills. Preliminary analysis suggests that higher hunger level relates positively to food motivation (Power of Food Scale (r=.21, p=.03)) and also general sensation seeking (Extraversion (r=.25, p=.01)), and negatively to eating-related self-control (ecSatter eating competence (-.22, p=.034)). The hormonal levels are currently being analysed and the interrelationships between hormonal levels, reported hunger and personality traits will be reported at the meeting. While the direction of the relationship is yet unknown, current results hint that subjective and objective hunger measures should be considered when collecting self-report data on power of food, Extraversion and eating related self-control.

70

Previous Exposure to Food Does Not Influence Subsequent Food Choice In Dogs

JC VONDORAN[1], E CHAMBERS IV[2], DA BALOGA[1], DE JEWELL[1]
[1]Hill's Pet Nutrition Topeka, KS, USA, [2]Kansas State University Manhattan, KS, USA

Previous exposure to food was investigated to determine the influence on subsequent food choice. This study was performed using 25 pet dogs of varying age, breed, and size in the home setting. This study complied with the Hill's Global Animal Welfare Policy and dogs were

maintained in their homes throughout the duration of the evaluations. Eleven palatability tests were performed. An automated feeding station was employed to provide collection of data pertaining to intake of food over a specified period. This device utilizes a two bowl feeding system with each bowl holding a specific amount of food for the subject conducting the test. Each food amount was calculated for the test subject using the feeding guidelines of the specific products being tested in combination with the age of the pet. Of the eleven tests, seven found a significant difference ($p<0.05$) in intake ratio between the tested foods. Intake ratios were calculated by dividing the amount of test food consumed by the total amount of food. Ten percent of the dogs had previous exposure to one of the tested foods. Statistical significance was calculated by using either previous exposure or lack of exposure as the independent variable and the SAS PROC MIXED model to evaluate differences. By comparing the intake ratios of dogs that were previously fed one of the tested foods (0.4853) to those not previously fed one of the foods (0.4815), it was found that previous exposure to a food did not have a significant effect on subsequent preference for that food.

71

Mechanisms underlying metabolic risk of passively coping prenatally stressed rats.

GJ BOERSMA, ZA CORDNER, AA MOGHADAM, TH MORAN, KL TAMASHIRO
Psychiatry& Behavioral Sciences, Johns Hopkins Univ, Baltimore, MD, USA

Prenatal stress (PNS) and a passive stress-coping style are risk factors for metabolic disease. Pregnant Sprague-Dawley rats were subjected to variable stress during the 3rd wk of gestation while control (CON) dams were undisturbed. Male offspring were tested in a defensive burying test to determine their stress-coping style (passive v. proactive). On a high fat diet (HFD), passive PNS offspring were heavier, hyperleptinemic, and had impaired glucose tolerance compared to CON and proactive PNS offspring, while there were no differences among the groups on CHOW diet. We used RT-PCR to measure mRNA expression of *Npy* and *Pomc* in the arcuate nucleus (ARC) of CON and PNS offspring. PNS reduced both *Npy* and *Pomc* expression ($p<0.05$), independent of stress-coping style. Passive PNS rats had greater HPA-axis reactivity compared to proactive PNS rats. There was no difference in glucocorticoid receptor (*Nr3c1*) expression, but *Fkbp5*, coding for a protein that lowers glucocorticoid sensitivity, was increased ($p<0.05$) in passive PNS rats in brain areas involved in stress reactivity and emotionality, including amygdala (AMG), prefrontal cortex (PFC) and paraventricular nucleus. *Bdnf*, which is involved in brain development, glucocorticoid signaling, and body weight regulation, was lower in passive PNS rats in AMG and PFC ($p<0.05$). The data suggest that alterations in expression of genes involved in HPA-axis regulation and emotionality may contribute to the increased susceptibility of passive PNS rats to metabolic dysregulation on HFD compared to CON and proactive PNS offspring.

72

Optogenetic enhancement of food 'liking' versus 'wanting' in the ventral pallidum hotspot and lateral hypothalamus.

DC CASTRO, KC BERRIDGE
University of Michigan Ann Arbor, MI, USA

Lateral hypothalamus (LH) has long been known to play a role in eating behavior and reward. More recently, ventral pallidum (VP) has also become a major site of interest in motivational systems. Previously, we have shown that a cubic-millimeter hedonic hotspot is located within the caudal portion of VP, where mu opioid receptor stimulation can enhance the hedonic impact of a sweet solution. Similarly, orexin receptor stimulation in this area can also enhance the sensory pleasure of food. Because orexin neurons are only produced within a small region of hypothalamus, it is likely that a functional connection exists between orexin neurons and the VP hotspot. Here, we show that optogenetic stimulation of either LH or LH projections to the VP hotspot can both enhance food 'wanting' for palatable chocolate candies. However, while LH stimulation alone does not affect hedonic reactions, stimulation of LH-VP hotspot projections can double the number of hedonic 'liking' reactions. Similarly, direct optogenetic stimulation of the VP hotspot both enhances 'liking' and 'wanting' for palatable food. These results suggest that while LH itself is not a pleasure-amplifying hotspot, it does send hedonically relevant projections to the VP hotspot, which may then act to recruit local hedonic systems.

73

The role of central angiotensin type-1a receptors in energy balance and blood pressure regulation

AD DE KLOET[1], L WANG[2], H HILLER[2], SC WOODS[3], C SUMNERS[1], EG KRAUSE[1]
[1]Dept of Physiology Gainesville, FL, USA, [2]Dept of Pharmacodynamics, Univ of Florida Gainesville, FL, USA, [3]Dept of Psychiatry, Univ of Cincinnati Cincinnati, OH, USA

Obesity increases levels of angiotensin-II (Ang-II), which activate angiotensin type 1a receptors (AT1a) to influence cardiovascular function and energy homeostasis. To test the hypothesis that AT1a within the paraventricular nucleus of the hypothalamus (PVN) mediate diet-induced weight gain and hypertension, we used the Cre/lox system to delete AT1a from the PVN of mice. When maintained on high-fat diet, mice lacking PVN AT1a had increased food intake and decreased energy expenditure that augmented body mass and adiposity relative to controls.

Despite this increased adiposity, PVN AT1a deletion reduced systolic blood pressure, suggesting that this receptor population mediates the positive correlation between adiposity and blood pressure. Gene expression studies revealed that PVN AT1a deletion decreased hypothalamic expression of corticotrophin-releasing hormone and oxytocin, neuropeptides known to control food intake and blood pressure. Central inflammation is associated with metabolic and cardiovascular disorders and PVN AT1a deletion reduced indices of hypothalamic inflammation during diet-induced obesity. Collectively, these studies demonstrate that PVN AT1a regulate energy balance and blood pressure during environmental challenges that promote metabolic and cardiovascular pathologies. The implication is that during high-fat diet feeding, Ang-II serves as a negative feedback signal that activates PVN neurons to reduce weight gain.

74

Satiating meals differentially activate prolactin-releasing peptide (PrRP)-positive noradrenergic (NA) neurons in the caudal nucleus of the solitary tract (NST) in rats

AD KREISLER, EA DAVIS, L RINAMAN
University of Pittsburgh Pittsburgh, PA, USA

PrRP is expressed by a large subset of NA neurons within the caudal NST (caudal A2 cell group). Central PrRP signaling in rats and mice reduces food intake by reducing meal size. Since satiety signals also reduce meal size, natural feeding-induced satiety might be mediated via central PrRP signaling. If so, then PrRP-positive neurons should be differentially recruited by satiating vs. non-satiating meals. To test this hypothesis, adult male rats were food deprived once for 24 hr and then were either not re-fed, or were given 30-min access to an unrestricted or restricted volume of palatable liquid Ensure. One hour later, rats were perfused with fixative, residual gastric volumes assessed, and brains sectioned and processed for triple localization of dopamine-beta hydroxylase (DBH), PrRP, and cFos to identify activated A2 neurons. In food deprived, non-fed rats, less than 1% of PrRP-positive A2 neurons and ~3% of PrRP-negative A2 neurons were activated. Intake of an unrestricted meal (2-8% BW) activated ~66% of PrRP-positive A2 neurons, but only ~26% of PrRP-negative A2 neurons. Restricted meals (1-3% BW) activated only ~3% of PrRP-positive A2 neurons, and ~6% of PrRP-negative A2 neurons. Thus, feeding to satiety after overnight deprivation differentially recruits the PrRP-positive subpopulation of A2 neurons, evidence that these neurons are particularly sensitive to sensory feedback generated by intake of a large, satiating meal.

75

Sex-dependent effects of prenatal immune challenge on energy balance across aging

MA LABOUESSE[1], J RICHETTO[2], MA RIVA[2], W LANGHANS[1], U MEYER[1]
[1]Physiology and Behavior Laboratory, ETH Zurich Zurich, Switzerland, [2]Department of Pharmacological and Biomolecular Sciences, University of Milan Milan, Italy

We showed previously that an acute prenatal immune challenge in late pregnancy results in hippocampal-dependent cognitive abnormalities in aged mice. Such deficiencies may arise from infection-induced neuroinflammation which disrupts the normal development of neuronal networks. We here investigated whether the same prenatal immune challenge also affects the regulation of energy balance and metabolism across aging. Pregnant mouse dams were IV injected with vehicle (CON) or polyI:C (POL), a synthetic substance known to induce a cytokine-acute phase response. Aged POL female (15 months), but not male, offspring, were much heavier (51.7±1.4 vs. 41.9±2.5g; $p<0.01$) and more obese ($p<0.05$) than CON mice, but showed no change in energy intake or expenditure. Adult POL female (3 months) were still normal weight and not obese, but showed an increase in energy intake ($p<0.05$) and a mild hypothalamic inflammation. Interestingly, adult POL female showed early decreases in accumbal dopamine receptor 1 (D1R) ($p<0.01$) and D2R ($p<0.01$) gene expression levels, which, in CON animals, were only manifest in aging, suggesting accelerated alterations in the reward system. Thus, a single adverse event in prenatal life can lead to abnormalities in energy balance circuits across aging in a sex-dependent fashion. Future studies should address the possible role of sex-specific hormones in these effects and whether they are related to a modulation of gene expression in hypothalamic or accumbal nuclei.

76

Gastrointestinal Satiation Signals in Healthy Obese Persons

AC MEYER-GERSPACH, B WÖLNERHANSSEN, RE STEINERT, C BEGLINGER
Department of Biomedicine, University Hospital Basel, Switzerland

Introduction: The gastrointestinal tract plays a key role in the control of satiation; discrepancies exist, however, for the role of gastric and intestinal parameters in the control of satiation in relation to body mass. To achieve a better understanding for the reciprocal control between gastric functions and intestinal parameters in the development of satiation we compared satiation parameters, gastric emptying and plasma glucagon-like peptide-1 (GLP-1), peptide tyrosine tyrosine (PYY) and

ghrelin levels between normal weight and obese healthy volunteers. **Methods:** Fifty-one normal weight and forty-three obese subjects participated in the study. We measured 1) Time needed to reach maximal satiation and total calorie intake by a standardized nutrient drink test (Ensure Plus®); 2) Gastric emptying of solids and liquids by a ^{13}C-octanoic acid breath test; and 3) Plasma GLP-1, PYY and ghrelin levels after Ensure Plus®. **Results:** 1) Obese subjects reached maximal satiation faster (P = 0.006), and total intake of calories was higher (P = 0.013); 2) Gastric emptying rates were delayed in obese (P <0.001); 3) The increase in postprandial plasma GLP-1 and PYY were reduced in obese (P <0.001, respectively) and postprandial suppression of ghrelin was smaller compared to normal weight subjects (P = 0.001). **Conclusions:** We suggest that the delay in gastric emptying leads to impaired interaction of nutrients with the intestine, which results in decreased GLP-1 and PYY secretion. As a consequence, obese subjects need more calories to reach maximal satiation and to stop eating.

77

Amylin receptor signaling in the VTA controls food intake and motivation to obtain palatable food

EG MIETLICKI-BAASE[1], JJ CONE[2], LE RUPPRECHT[1], DR OLIVOS[1], MD ALTER[1], RC PIERCE[1], HD SCHMIDT[1], MF ROITMAN[2], MR HAYES[1]
[1]Psychiatry, U Penn Philadelphia, PA, USA,
[2]Neuroscience, UIC Chicago, IL, USA

The CNS nuclei mediating the anorectic effects of the pancreatic β-cell-derived peptide amylin have been primarily ascribed to the area postrema (AP). However, as amylin binds throughout the brain, further investigation of extra-AP sites mediating these effects is warranted. Given the critical role of the mesolimbic system in the control of feeding, and that amylin receptors are expressed in the ventral tegmental area (VTA), we tested the effects of intra-VTA administration of the amylin receptor agonist salmon calcitonin (sCT) on food intake and body weight. Unilateral VTA sCT (0.004, 0.04, 0.4µg/100nl) dose-dependently reduced chow intake in *ad lib*-fed rats 1h-24h post-injection. Intra-VTA sCT (0.04µg) decreased sucrose self-administration on a progressive ratio in *ad lib*-fed and food restricted rats, suggesting that VTA amylin signaling reduces motivation for a palatable food. Intra-VTA administration of the amylin receptor antagonist AC187 (0.17, 0.3µg/100nl) increased 24h chow intake, suggesting that endogenous amylin acts in the VTA to control feeding. Analyses of pica and open field locomotor activity showed that these effects are not due to nausea/malaise or persistent drug-induced changes in locomotion. Intra-VTA sCT (0.04µg) reduced the magnitude of the dopamine spike evoked during sucrose reward in the nucleus accumbens (73±5% of baseline post-sCT versus 100±5% post-vehicle), providing a potential mechanism by which VTA amylin receptor activation suppresses food intake.

78

Amygdala response to food cues in the absence of hunger predicts weight change.

X SUN[1], MG VELDHUIZEN[1,2], AE WRAY[2], IE DE ARAUJO[1,2], DM SMALL[1,2]
[1]The J.B. Pierce Laboratory New Haven, CT, USA, [2]Yale University New Haven, CT, USA

Animal work has shown that food-predictive cues can elicit eating in the absence of hunger (Weingarten 1983). This behavior is dependent upon the integrity of amygdalar and prefrontal pathways to the lateral hypothalamus (Petrovich & Gallagher 2007). The omnipresence of food cues in our modern environment may therefore contribute to the obesity epidemic by stimulating cue-induced responses in the amygdala to override hypothalamic homeostatic circuits and promote eating in the absence of hunger. To test this hypothesis we used fMRI to assess whether amygdala response to food cues when individuals are sated, but not when they are hungry, predicts weight gain over 1 year. 14 healthy non-dieting subjects (6 male, BMI M=24.6 SD=4.9) underwent fMRI scanning while tasting and smelling a palatable milkshake when hungry (4h fast) and when full (after consuming a fixed-portion lunch). Scans took place in randomized order on different days. Separate runs measured brain response to flavors (milkshake and tasteless) and aromas (food and floral). Subjects returned after 1 year to assess weight change. As anticipated, amygdala response to food cues (milkshake-tasteless flavors and food-floral aromas) positively predicted weight change only in the absence of hunger. Interestingly, a strong negative correlation between weight change and response to milkshake while hungry was observed in ventromedial prefrontal cortex. These data support the possibility that amygdalar and prefrontal responses to food cues serve as biomarkers for weight gain susceptibility.

79

Deciphering a Neural Circuit that Mediates Anorexia

R PALMITER
University of Washington Seattle, WA, USA

Ablation of neurons that express agouti-related protein (AgRP) in adult mice results in starvation within 6 days. During this time, the mice initiate feeding bouts less often and consume very little nutrient delivered by intraoral cannula. The anorexia was linked to hyperactivity (induction of Fos) of neurons in the external lateral region of the parabrachial nucleus (PBNelo). Because expression of the*Calca* gene, which encodes calcitonin gene-related protein (CGRP), is prominent in the PBNelo, we targeted Cre recombinase to this locus to allow expression of virally delivered effector genes selectively in CGRP neurons in the PBNelo. Selective expression of mCherry

in CGRP neurons revealed that Fos is induced in these neurons in response to satiety signals (cholecystokinin and amylin), nausea (LiCl, i.p.), inflammation (lipopolysaccharide, i.p.) and ablation of AgRP neurons. Photoactivation of channel rhodopsin (ChR2) in CGRP neurons inhibited food intake at thebeginning of dark cycle or even after a 24-h fast. When photoactivation ceased the mice resumed eating. Chronic activation of HM3q DREADD in CGRP neurons resulted in sustained anorexia and severe weight loss. The main projection of these CGRP neurons is to the lateral capsule of the central nucleus of the amygdala (CeNlc). Photoactivation of the ChR2 in CeNlc of mice after viral injection into the PBNelo also inhibited feeding. These experiments delineate a circuit from the vagus > NTS > PBNelo > CeNlc that when activated inhibits the appetite of hungry mice.

82

Behavior economics of food and beverage reinforcement in adults and children

JL TEMPLE
Universuty at Buffalo Buffalo, NY, USA

A basic principal of behavioral economics is that as the cost of or effort to obtain a product increases, purchasing or consumption of that product will decrease. In order to understand the impact of behavioral economics on ingestive behavior, our laboratory utilizes an operant behavior paradigm to measure how much work an individual will engage in to get access to a portion of a food or a beverage. This task provides an objective measure of the reinforcing value of food and beverages. We are especially interested in determining ways to shift the behavioral economic curve in order to find ways to decrease the reinforcing value of unhealthy food increase the reinforcing value of healthy food. We have shown that consumption of the same unhealthy food every day for two weeks reduces its reinforcing value in lean individuals, but increases its reinforcing value in obese individuals. This increase in the reinforcing value of food predicts future weight gain. Similarly, we have shown that repeated intake of caffeinated soda increases its reinforcing value in boys, but not in girls. This increase does not correlate with usual caffeine consumption, but may relate to positive, subjective effects of caffeine that are reported in boys, but not in girls. Because food and beverage reinforcement relates to real-world consumption, it is important to determine factors that increase or decrease the reinforcing value and determine the consequences of these responses.

83

PUTTING THE ENVIRONMENT BACK INTO THE NEUROBIOLOGY OF FEEDING

N.E. ROWLAND
University of Florida Gainesville, FL, USA

For the past few years, we have been investigating the effects in mice of environmental costs of food on the amount and pattern of food consumed. In general, when cost in the form of responses per food pellet (the unit price) is imposed, then daily intake declines as price increases. This is a classic demand function and can be described mathematically, with the curvature equivalent to elasticity. Consistent with externality theories of eating in humans, we have found that elasticity is greater in obese than lean mice. Elasticity is greater in males than females: this resilience of females to price increase appears to be mediated by estrogen receptors α. This relative sensitivity of females to reduced energy reserves might be linked to reproductive significance. In other studies, we have found that increased energy expenditure in the form of voluntary exercise, and decreased net energy gain in the form of caloric dilution, is associated with decreased elasticity or greater work output. This indicates that changes in energy stores and/or flux are readily translated into changes in decision-making about working for food. Some aspects of these demand functions can be recapitulated by imposing a minimum delay between earing successive pellets (i.e. slowing eating rate) rather than response effort per se. Further, when access to food is restricted to a few discrete meals or opportunities per day and moderate food costs, mice eat far less at each meal than they could earn in the time allotted and so lose weight rapidly – apparently an elective anorexia.

84

Behavioral Economic Approaches to Grocery Store Health Interventions

CR PAYNE
New Mexico State University Las Cruces, NM, USA

While packaged food companies have become very good at providing purchasing rules-of-thumb regarding their products' nutritional (e.g., "low-fat") and non-nutritional benefits (e.g., "new"), rules-of-thumb for purchasing fruits and vegetables are almost non-existent. Considering current health issues facing the nation (e.g., obesity) and over 60% of US food expenditures occur in the grocery store environment, it is imperative to create purchasing rules-of-thumb that benefit the health of the consumer *and* are economically sustainable for the grocery store. We provide evidence of three novel purchasing rules-of-thumb that could increase fresh fruit and vegetable purchases without significantly increasing consumer budgets, yet are economically sustainable for the grocery store. Furthermore, we provide evidence that WIC

(Women, Infant, Children) program participants may be particularly sensitive to these types of rules-of-thumb. Finally, we discuss ways in which collaboration between three seemingly disparate entities (i.e., retailers, health foundations, and universities) can take the lead in helping promote systemic change in consumer health behaviors.

85

Pharmacological manipulations of neural responses to food stimuli in humans

PC FLETCHER
University of Cambridge, United Kingdom

Given the profound problems posed by over-eating and obesity, and with a growing recognition that vulnerability to such problems may emerge from patterns of reward-related behaviors rather than primary metabolic disturbances, there has been a major research drive towards understanding the higher brain basis for appetite and eating. This has encouraged a large body of functional neuroimaging work which, though it has provided strong evidence for activation of predicted reward circuitry in response to food-related stimuli, has produced variable and inconsistent results with respect to identification of altered patterns of response in association with over-consumption and obesity. I will suggest that, used alone, functional neuroimaging provides only a very limited tool for understanding appetite and its alterations and that more powerful, and complementary, insights may be obtained through its combination with behavioural studies and precise pharmacological manipulations. I will present evidence to suggest that appetite-suppressing pharmacological manipulations involving serotenergic and opioid transmitter systems produce distinct and dissociable effects and that these dissociations are reflected both in cognition and behaviour and at the underlying neural level. This linking of different levels of observation may further our understanding of how the brain responds to an environment heavily loaded with palatable and easily-obtained foods.

86

Role of VMH CD36-Mediated Fatty Acid Sensing in Energy and Glucose Homeostasis Regulation in DIO and DR Rats.

C LE FOLL[1], AA DUNN-MEYNELL[2], S MOUSATOV[3], BE LEVIN[1,2]
[1]Dept Neurology and Neurosciences, NJ Med School Newark, NJ, USA, [2]VA Medical Center East Orange, NJ, USA, [3]Weill Cornell Medical College New York, NY, USA

The ventromedial hypothalamus (VMH) contains "metabolic sensing neurons" which play a key role in the regulation of glucose and energy homeostasis and the development of obesity and diabetes. Many of these neurons utilize both glucose and long chain fatty acids to alter their activity as a means of monitoring the metabolic status of the body. We previously found that 50% of these neurons responded to oleic acid, by utilizing the fatty acid translocator/receptor, CD36. Here, P21 DIO and DR rats were injected in the VMH with AAV expressing CD36 shRNA (CD36 rats) to assess how VMH fatty acid sensing regulates their energy and glucose homeostasis. After 10 wk on a 45% fat diet, DIO CD36 rats ate 10% more ($P<0.05$), and weighed 7% more ($P<0.05$), while DR CD36 rats weighed 6% less than comparable controls ($P<0.05$). Leptin levels were 212% and 115% higher in DIO and DR CD36 rats in association with 74% and 59% heavier subcutaneous, but not visceral fat pads compared to respective controls ($P<0.05$ all comparisons) Importantly, DIO and DR CD36 rats were 7% and 8% shorter than their respective controls ($P<0.05$). Finally, DIO CD36 rats also had a markedly abnormal oral glucose tolerance with 142% higher glucose ($P<0.05$) and 244% higher insulin ($P<0.05$) AUC than controls. These results demonstrate that VMH CD36-mediated fatty acid sensing is a critical factor in the regulation of energy and glucose homeostasis, as well as linear growth, in DIO and DR rats.

87

Restoring gastrointestinal lipid sensing recovers dopamine signaling in high-fat fed mice

LA TELLEZ[1,2], S MEDINA[1], JG FERREIRA[1,2], X REN[1,2], W HAN[1,2], GJ SCHWARTZ[3], IE DE ARAUJO[1,2]
[1]The John B Pierce Laboratory New Haven, CT, USA, [2]Psychiatry, Yale Univ. Sch. of Med New Haven, CT, USA, [3]Albert Einstein Col. of Medicine, Yeshiva Univ New York, NY,

Excessive intake of dietary fats leads to diminished brain dopaminergic function. While it has been proposed that diminished dopamine function leads to compensatory overfeeding, the physiological link between prolonged high-fat intake and dopamine deficiency remains unknown. We show that administering oleoylethanolamine, a gastrointestinal lipid messenger whose synthesis is suppressed after prolonged high-fat exposure, restores gut-stimulated striatal dopamine release in high-fat fed mice. The treatment also eliminated motivation deficits during flavorless intra-gastric feeding in high-fat fed mice, while increasing oral intake of low-fat emulsions. Our recent findings specifically show that 1. These restorative effects were eliminated in both vagotomized and Ppar-alpha knockout mice; 2. Elimination of vagal afferents mimicked some of the behavioral choice deficits observed in high-fat fed mice; 3. Dopamine receptor antagonism in dorsal straiatum abolishes the suppressive effects of oleoylethanolamine on fat intake. Our findings suggest that high fat-induced gastrointestinal dysfunctions play a critical role in obesity-associated dopaminergic and behavioral deficiencies.

88

Dissection of the neural mechanism underlying the sensing of sugars in *Drosophila*

M. DUS, G. SUH
Molecular Neurobiology Program, Skirball Institute of Biomolecular Medicine, NYU School of Medicine, NY, USA

Feeding behavior is orchestrated by the interplay between nutritional needs and food palatability. Robust signaling mechanisms exist to ensure energy homeostasis: thus, variations in internal energy are rapidly detected and coupled with an increase in food consumption. However, how changes in metabolic needs function in the selection of food is poorly understood. We previously described that food-deprived fruit flies prefer calorie-rich sugars over zero-calorie sweeteners even in the absence of taste, suggesting the existence of a taste-independent metabolic sensor that functions in food selection. Manipulating the levels of circulating glucose in the blood had a direct effect on food choice, suggesting that the cells directing the preference for metabolizable sugars sense and respond to the levels of glucose. To elucidate the mechanisms animals use to sense sugars and calories, we tested flies carrying mutations in glucose transporters genes and identified *cupcake*, a putative fly homologue of mammalian Sodium-Solute Transporters (SLC). *cupcake* mutant flies select food only based on palatability and not metabolic needs, suggesting that this gene is required for the choice of metabolizable sugars during starvation. *cupcake* is expressed in a small population of neurons in the fly brain (~20 cells) and it is upregulated in food-deprived flies. Silencing of Cupcake-expressing neurons by expression of the inwardly rectifier K+ channel *Kir2.1* interferes with metabolic feeding, indicating that this circuit is required for responding the nutritional value of sugar. Ongoing experiments are aimed at understanding how these neurons sense the presence of metabolized glucose in the hemolymph and how they trigger a value-dependent behavioral shift to ensure that the animal's energetic needs are met.

89

How does monosodium glutamate interact with macronutrients to influence appetite and subsequent intake?

U MASIC, M.R. YEOMANS
University of Sussex Brighton, United Kingdom

Monosodium glutamate (MSG) enhances savoury food flavour and has been shown to reduce satiation but enhance satiety over time. This ‘MSG paradox’ may be due to specific MSG-macronutrient interactions and was explored in a fixed portion of a soup preload differing in MSG (MSG added or no MSG), energy and macronutrient (low energy control, high energy carbohydrate or high energy protein) content. Appetite before, after tasting and after meals and subsequent *ad-libitum* intake of a test meal (pasta main course and ice cream dessert) delivered 45 minutes post-preload ingestion were assessed in 35 low-restraint male participants (mean BMI: 22; mean age:21) over six sessions. Data were subjected to repeated measures ANOVA and measures of energy compensation (COMPX) and satiety quotient (SQ) analyses for the whole meal and course-by-course. Protein preloads decreased intake at the *ad-libitum* course ($F(1.8,59.6) = 4.5$, $p = .02$) and improved COMPX ($F(1,34) = 4.19$, $p = .05$). Appetite did not differ across MSG, macronutrient or energy conditions over preload ingestion or 45 minute post-intake. MSG protein preloads displayed the strongest SQ scores after the savoury course ($F(1.8,60.9) = 5.72$, $p = .007$) indicating that appetitive satisfaction was high despite consuming the fewest calories in this course. These findings suggest that protein satiety may be further maximised with the addition of MSG, decreasing intake in a savoury course whilst maintaining satisfaction.

90

Behavioral Assessment of the Detection of Glutamate Stimuli in Mice Lacking T1R2 and T1R3.

KR SMITH, AC SPECTOR
Dept of Psychology, Florida State University Tallahassee, FL, USA

The T1R2+T1R3 heterodimer is a critical receptor in the mediation of sweet taste in mammals. Monosodium glutamate (MSG), although shown to be transduced by the T1R1+T1R3 receptor, has a complex taste quality including “sweet-like” as assessed by CTA procedures in rodents. We thus assessed the detection thresholds to MSG stimuli in T1R2+T1R3 KO and WT mice in a two-response operant procedure. Thirsty mice were trained in a specialized gustometer to respond to a left or right reinforcement ball depending upon the sample stimulus (taste or water) delivered to obtain a water reinforcer. KO and WT mice did not differ in their ability to detect NaCl (N=8/group) and MSG (N=7/group). However, when mice (N=8/group) were presented with a mixture (M+A+I) of MSG with the sodium channel blocker amiloride and inosine 5’ monophosphate (IMP), KO performance significantly differed from WT such that only 4 KO mice were able to perform the discrimination task, but only at the high concentrations, while WT mice performed at asymptotic levels across concentrations. When presented with IMP alone, KO mice were unable to detect the stimulus, whereas WT mice responded with high accuracy thus explaining why their performance never declined with lower MSG concentrations in the mixture. These results match those from a similar study we recently conducted with T1R3 KO mice suggesting that 1) T1R2 was not contributing to performance in the T1R3 KO mice of the prior experiment, 2) M+A+I is a weak signal in mice lacking T1R3, and 3) this weak signal is likely based on a T1R1 homodimer or a T1R1+3-independent receptor.

91

Effects of intraduodenal L-tryptophan (L-Trp) infusions on energy intake, antropyloroduodenal (APD) motility, glycemia, insulinemia and plasma glucagon-like peptide-1 (GLP-1) concentrations in healthy men

RE STEINERT, ND LUSCOMBE-MARSH, TJ LITTLE, T FATEHI, S STANDFIELD, M HOROWITZ, C FEINLE-BISSET

University of Adelaide Discipline of Medicine and Centre of Research Excellence (CRE) in Translating Nutritional Science to Good Health Adelaide, Australia

We hypothesized that amino acids mediate the anorectic and glucoregulatory effects of dietary protein via changes in gut motor and hormone functions. We investigated the effects of L-Trp on energy intake, APD motility, blood glucose, plasma insulin and GLP-1. Eight healthy, lean men were studied on 3 separate days in randomized, double-blind fashion. APD motility, blood glucose and hormones were measured during 90-min intraduodenal infusions of L-Trp at 0.075 ("Trp-0.075"; total 1.6 g, 6.75 kcal) or 0.15 ("Trp-0.15"; total 3.3 g, 13.5 kcal) kcal/min, or saline control ("C"; rate: 4.5 ml/min). Energy intake at a buffet meal following the infusions was determined. L-Trp dose-dependently reduced energy intake ($r=-0.71$, $P<0.01$), stimulated tonic and phasic pyloric ($r=0.70$, $P<0.01$ for both), and suppressed antral ($r=-0.69$, $P<0.01$), pressures, and modestly stimulated GLP-1 ($r=0.61$, $P<0.01$) and insulin ($r=0.54$, $P<0.05$) without affecting blood glucose or inducing nausea. The mean reduction in energy intake by Trp-0.15 *vs.* C was 265 kcal ($P<0.05$). In conclusion, acute intraduodenal administration of L-Trp reduces energy intake markedly, in excess of its own caloric content, and in the absence of nausea. This effect is associated with marked effects on pyloric pressures, and small effects on GLP-1 and insulin.

92

Load-dependent effects of oral protein on gastric emptying, glycemia, appetite and energy intake in healthy men

AT RYAN[1], D GENTILCORE[2], M HOROWITZ[1], C FEINLE-BISSET[1], ND LUSCOMBE-MARSH[1]

[1]University of Adelaide, Discipline of Medicine Adelaide, Australia, [2]University of South Australia Adelaide, Australia

The ingestion of nutrients modulates gastrointestinal (GI) function, including the slowing of gastric emptying (GE), associated with the postprandial glycemic response and suppression of energy intake (EI). We recently reported that whey protein, infused intraduodenally (ID) to bypass orosensory and gastric influences, at rates mimicking the normal range of GE (i.e. 0.5 - 3 kcal/min), stimulated pyloric motility and insulin release, while maintaining normoglycemia, and suppressed EI, in a load-dependent manner. Whether the load-dependent effects of protein are upheld when protein is consumed orally, allowing 'gastric' mechanisms to come into play, is unclear. We hypothesized that oral protein would load-dependently slow GE, modulate postprandial glycemia and suppress EI, and that both glycemia and EI suppression would be related to the slowing of GE. 18 lean males (age 24.7±3 yr, BMI 22±1 kg/m^2) received, on 3 separate occasions, in randomized, double-blind order, iso-osmolar, lime-flavoured, equally palatable drinks (450 mL), containing either 30 (L) or 70 (H) g whey protein, or saline as control (C). Immediately after the drink (i.e. t=0), GE (3D ultrasound), plasma insulin, blood glucose and hunger (visual analog scales) were measured at 15-min intervals for 180 min. EI was measured at a buffet-style lunch (t=180-210 min). Whey load-dependently slowed GE ($r=0.9$, $P<0.05$) (half-emptying time T_{50}, min; C: 21±2, L: 69±9, H: 116±12), increased insulin ($r=0.9$, $P<0.01$) (AUC, pmol/L.min, C: 503±63, L: 1463±175 H: 2275±300) but did not change blood glucose, and decreased hunger ($r=-0.38$, $P<0.05$; AUC, mm.min; C: 8703±1164, L: 7206±971, H: 7187±1005). L and H suppressed EI compared with C ($P<0.05$) (kcal, C: 1174±91, L: 1027±81, H: 997±71). L, but not H, suppressed EI in excess of its caloric load (L: 128±48%, H: 65±19%). The suppression of EI was related inversely to T_{50} ($r=-0.49$) and insulin ($r=-0.53$) and directly to hunger AUC ($r=0.45$) (all $P<0.05$). In conclusion, oral protein slows GE, and, like ID protein, stimulates insulin release without changing blood glucose, and suppresses hunger, load-dependently. Given these effects, the lack of difference in EI suppression between L and H suggests a threshold load for suppression of EI by orally-consumed protein may exist.

93

Elucidating the neural circuitry linking gastrointestinal fat sensing to action selection and reward

I E DE ARAUJO[1,2], J G FERREIRA[1,2], L A TELLEZ[1,2], S J SHAMMAH-LAGNADO[3]

[1]The J.B. Pierce Laboratory New Haven, CT, USA, [2]Department of Psychiatry Yale University New Haven, CT, USA, [3]Department of Physiology São Paulo, Brazil

Gut nutrient sensing is a powerful regulator of food reward, as attested for instance by the remarkable behavioral modifications that follow bariatric surgeries. However, the neural circuitry linking gastrointestinal sensing to action selection and attribution of reward value to foods remains to be elucidated. Because these behavioral functions are entirely dependent on dopaminergic signaling, we aimed at characterizing the neural circuitry that allows gut signals to be transmitted to midbrain dopaminergic cells. Our studies reveal a circuitry where the pontine parabrachial nucleus is posited as the visceral afferent nucleus of the midbrain

dopaminergic system. Data from a series of studies confirm that the midbrain dopaminergic system is robustly targeted by vagal-*nucleus tractus solitarii* gastrointestinal signals via this pontine relay. Anterograde/retrograde tracing, behavioral, and optogenetic experiments established the necessity and sufficiency of the parabrachial relay nucleus for dopamine visceral sensing. Specifically, pontine parabrachial lesions disrupt gut fedding and gut-stimulated dopamine release in both ventral and dorsal aspects of striatum; conversely, optogenetic stimulation of pontine parabrachial cell bodies is sufficient to evoke gut-like dopamine responses in these regions. We conclude that the parabrachial nucleus is a critical mediator of the link between gut fat sensing and the regulation of motivated appetitive behaviors.

94

Regulation of feeding in *Drosophila melanogaster*

K SCOTT
1 Berkeley, CA, USA

For an animal to survive in a constantly changing environment, its behavior must be shaped by the complex milieu of sensory stimuli it detects, its previous experience and its internal state. Although taste behaviors in the fly are relatively simple, with sugars eliciting acceptance behavior and bitter compounds avoidance, these behaviors are also plastic and modified by intrinsic and extrinsic cues such as hunger and sensory stimuli. Using a combination of molecular genetic, calcim imaging and behavioral approaches, we are identifying and characterizing modulatory neruons that influence feeding decisions. These studies have identified neurons that promote or inhibit feeding and provide a foundation to examine neural mechanisms of feeding modulation in the fly.

95

Synphilin-1 alters metabolic homeostasis in a novel Drosophila obesity model

WW SMITH[1], J LIU[1], R MA[1], D YANG[1], T LI[1], TH MORAN[2]
[1]*Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy Baltimore, MD, USA,*
[2]*Johns Hopkins University School of Medicine Baltimore, MD, USA*

Drosophila have conserved neuroendocrine and digestion systems with human and are thus an excellent model system for studying energy homeostasis and novel protein functions. Synphilin-1, a cytoplasmic protein, with enriched expression in neurons has been shown to be involved in regulating energy balance in mice. We have generated a novel obesity Drosophila model, in which expression of human protein, synphilin-1 (SP1), in neurons fosters positive energy balance. Overexpression of SP1 in neurons, but not peripheral cells, increased the body weight of flies compared with that of non-transgenic controls. SP1 also increased food intake but did not affect locomotor activity. SP1 increased the levels of triacylglycerol, and the size of fat body cells and lipid droplets, indicating that SP1 increased lipid-fat disposition. Survival studies showed that SP1 transgenic flies were more resistant to food deprivation. SP1 regulated lipin gene expression that may participate in SP1-induced fat deposition and starvation resistance. These studies demonstrate that SP1 expression affects energy homeostasis in ways that enhance positive energy balance and provide a useful obesity model for future pathogenesis and therapeutic studies.

96

INTERNAL NUTRIENT SENSING BY A DROSOPHILA FRUCTOSE RECEPTOR

T MIYAMOTO, D MISHRA, A YAVUZ, H AMREIN
Department of Molecular and Cellular Medicine, Texas A&M Health Science Center College Station, TX, USA

Internal nutrient sensors play important roles in feeding behavior, yet their molecular structure and mechanism of action are poorly understood. Recently, we showed that the gustatory receptor 43a (Gr43a) acts as an internal nutrient sensor in the fly brain. Using Ca^{2+} imaging and behavioral assays, we showed Gr43a is specifically activated by fructose in a small group of neurons in the posterior superior lateral protecerebrum. Interestingly, hemolymph fructose levels are tightly linked to feeding status: after nutritious carbohydrate consumption, fructose levels rise several fold and reach a concentration sufficient to activate Gr43a in the brain. By using different feeding paradigms and artificial activation of *Gr43a*-expressing brain neurons, we show that Gr43a is both necessary and sufficient to promote feeding in hungry flies, but suppress feeding in satiated flies. Thus, our studies indicate that the *Gr43a*-expressing brain neurons function as a nutrient sensor for hemolymph fructose and assign opposing valence to feeding experiences in a satiation-dependent manner. *Gr43a* is also expressed in taste and many brain neurons of *Drosophila* larvae. Here, it functions both as the main external sugar receptor as well as an internal fructose sensor. Using a two choice residence/feeding assay, we show that *Gr43a* mutant larvae fail to sense not only fructose, but also non-fructose sugars, including glucose, trehalose and the sugar alcohol sorbitol. Gal80 mediated suppression and cell inactivation experiments allowed us to map the critical neurons for sensing fructose containing sugars to the taste neurons and non-fructose sugars to the brain. Thus, we postulate a two-phase taste mechanism: An immediate taste response is established within the first two minutes and mediated by the larval taste neurons, while a late preference (established only after ~ 8 to 16 minutes) for non-fructose sugars requires the function of

the brain neurons, which sense fructose derived from conversion of dietary sugars.

97

Taste-independent Nutrient Selection is mediated by a Brain-specific Na+/solute cotransporter-like in *Drosophila*

M DUS, M AI, GSB SUH
Skirball Institute/ NYU New York, NY, USA

During the past 20 years, there has been a significant increase in obesity around the world. This growing epidemic is due in part to poor eating behavior, which is controlled by multiple factors including food palatability and nutritional needs. External chemosensory sugar receptors primarily detect palatable food, but animals without sugar receptors can still develop a preference for sugars based on their nutritional value. This suggests the existence of a taste-independent sugar sensing pathway. Here, we describe a mutation in a Na+/solute cotransporter-like protein in *Drosophila melanogaster*, designated *dSLC5A11*, that is completely insensitive to the nutritional value of sugar, but responds only to the concentration (i.e. sweetness). dSLC5A11 is structurally similar to mammalian Na+/glucose cotransporters (SGLTs) that transport sugar in bulk across the intestinal and renal lumen. However, dSLC5A11 has a prominent expression in 10-13 pairs of R4 neurons of the ellipsoid body (EB) in the brain. Its activity appears to differ from that of its mammalian counterparts, in that it functions in R4 neurons for selecting appropriate foods without the influence of taste. Given their restricted expression pattern and function, we propose that *dSLC5A11* and EB R4 neurons carry out a critical signaling function in sensing glycemic levels of the internal milieu, which, in turn, may dictate the desire for nutritive sugar. Our finding may reveal information that can serve as a valuable framework for studying the mechanism(s) by which nutritional need influences eating behavior in normal and obese individuals.

98

Selective Defects in Arcuate Nucleus (ARC) Leptin Signaling in P4 and P7 DIO Rats

MD JOHNSON[1], *BE LEVIN*[1,2]
[1]*Grad Sch Biomed Sci, Dept Neurol & Neurosci, NJ Med Sch, UMDNJ Newark, NJ, USA,* [2]*VA Med Ctr E. Orange, NJ, USA*

We have shown that offspring of rats selectively bred to develop diet-induced obesity (DIO) when fed a 31% fat, 25% sucrose diet have decreased leptin-induced phosphorylation of STAT3 (pSTAT3; a marker of leptin signaling) in the ARC and blunted ARC-paraventricular nucleus (PVN) axonal outgrowth compared to diet resistant (DR) rats as early as P10. To determine how early this decrease in leptin signaling occurs postnatally, we assessed ARC leptin-induced pSTAT3 expression in P4 DIO and DR neonates. DIO neonates had 33% more ($F(1,14)=8.40$, $p=0.01$) pSTAT3-positive neurons in the rostral and 22% more in the caudal ARC $F(1,15)=7.31$, $p=0.02$). But, overall, there were no differences from DR rats throughout the entire ARC. Although P4 DR and DIO rats had the same total number of POMC neurons and POMC neurons expressing pSTAT3, DIO rats had 23% fewer POMC neurons that expressed pSTAT3 selectively in the rostral ARC ($F(1,11)=13.16$, $p=0.004$) . However, by P7, DIO neonates had 19% fewer leptin-induced pSTAT3 expressing neurons through the entire ARC ($F(1,13)=6.18$, $p=0.03$), with the largest decrease (32%) in the caudal ARC. Therefore, while DIO neonates have similar overall leptin-induced ARC pSTAT3 at P4, they have a selective reduction of leptin signaling in a subpopulation of POMC neurons and an overall reduction in ARC pSTAT3 expression by P7. These data suggest that DIO rats have an early, selective postnatal defect in leptin receptor signaling that continues throughout life and is likely to predispose them to become obese on a high fat diet.

99

Rat pups of dams maintained on a high-fat diet display early independent feeding

S KOJIMA, L RINAMAN
University of Pittsburgh Pittsburgh, PA, USA

Maternal high-fat diet (HFD) promotes long-lasting increases in offspring body weight (BW) and adiposity in rats. Before weaning on postnatal day (P)21, laboratory rat pups derive most of their energy via suckling. However, independent chow intake in the home cage emerges by P18 (Thiels et al., 1990). Here, we examined whether pups raised by dams on HFD display earlier independent feeding that might contribute to their increased BW. Pregnant female rats were fed with either HFD (60% kcal fat; Research Diets D12492) or standard chow (Purina 5001) throughout gestation and lactation. Dams and pups were video-monitored for 3 hr during each light and dark cycle from birth (P0) until P21, when pups were weaned to chow. Caloric intakes of HFD- and chow-fed rats were comparable during gestation (dams) and lactation (dams+pups). Nevertheless, HFD pups weighed significantly more than chow pups by P7, and this BW difference increased through P21, as previously reported (Sun et al., 2012). Both HFD and chow pups sampled solid food from the home cage floor after P14, but the incidence of feeding from the hopper was significantly more frequent in HFD pups by P16. Nipple shifting (an index of milk letdown) was comparable between HFD and chow pups from P14-16, suggesting that increased independent feeding by HFD pups may contribute more significantly than increased suckling to their greater BW gain during the week before weaning. These new findings indicate that maternal diet effects on offspring physiology and behavior should be carefully

dissociated from effects of the pups' own independent feeding.

100

MATERNAL CONSUMPTION OF DIETARY FAT STIMULATES CENTRAL CHOLINERGIC ACTIVITY AND NICOTINE REWARD IN OFFSPRING

I MORGANSTERN, O LUKATSKAYA, S-H MOON, W-R GUO, O KARATAYEV, SF LEIBOWITZ
Laboratory of Behavioral Neurobiology, Rockefeller University New York, NY, USA

The current study was designed to determine whether exposure to a high-fat diet (HFD) early in development increases propensity towards the use of nicotine and if this phenomenon is accompanied by disturbances in central nicotinic cholinergic signaling. Rat offspring exposed to a perinatal HFD or a Chow diet were characterized based on their nicotine self-administration behavior in a series of operant response experiments and their activity of acetylcholinesterase (AChE) as well as their density of nicotinic ACh receptors (nAChRs). Perinatal HFD compared to Chow exposure increased acquisition of nicotine-taking behavior, produced a vertical shift in dose-response curves and an increase in breakpoint using progressive ratio testing; however, it led to a reduction in nicotine-seeking in response to nicotine prime-induced reinstatement. Neurochemical analyses revealed a reduction in activity of AChE in the midbrain, hypothalamus and striatum which was accompanied by an increased density of β2 –nAChRs in the ventral tegmental area and substantia nigra, and of α7-nAChRs in the lateral and ventromedial hypothalamus of HFD compared to Chow offspring. Perinatal exposure to HFD increases the vulnerability of the offspring to excessive nicotine use by enhancing its reward potential resulting from stimulation of nicotinic cholinergic signaling in mesostriatal and hypothalamic brain areas important for reinforcement and consummatory behavior.

101

Maternal hyperleptinemia alters anorexigenic innervation of hypothalamic paraventricular nucleus in male offspring

RL LESHAN[1], G CECCARINI[1,2], DW PFAFF[1]
[1]The Rockefeller University New York, NY, USA,
[2]University Hospital of Pisa Pisa, Italy

The perinatal period represents a time of rapid neural development that is sensitive to maternal physiology. Absence of leptin signaling prevents normal development of projections from the hypothalamic arcuate nucleus (ARC) to paraventricular nucleus (PVN), a deficiency recovered by neonatal, but not adult, leptin administration. Acute leptin delivered to pregnant dams or neonates, or high maternal leptin due to obesity produce variable metabolic effects in offspring. To determine the effect of chronic maternal hyperleptinemia (without obesity) on offspring ARC-PVN circuitry, we examined the progeny of transgenic female mice overexpressing leptin (LTNC). At three weeks old, male offspring of LTNC dams weighed significantly less than offspring of control dams fed normal chow (CNC) and offspring of diet-induced obese dams (controls fed high fat diet; CHF). The decreased body weight persisted through adulthood (12 weeks old), and was not observed in females. Consistent with the decreased body weight, we observed an increase in anorexigenic alpha-melanocyte-stimulating hormone- (aMSH-) immunoreactive (IR) fibers in the PVN of three week old male LTNC offspring. No differences were detected in female aMSH-IR, or in agouti-related peptide (AgRP) fibers in either sex. Our results demonstrate that perinatal exposure to maternal hyperleptinemia sex-specifically affects male offspring body weight and hypothalamic organization, reinforcing the importance of leptin in development. Future studies will further characterize this altered circuit.

102

Prenatal exposure to dietary fat induces changes in the transcription factors, TEF and YAP, which may stimulate differentiation of hypothalamic peptide neurons

K POON, S MANDAVA, K CHEN, SF LEIBOWITZ
The Rockefeller University New York, NY, USA

Prenatal exposure to high-fat diet (HFD) stimulates differentiation of hypothalamic peptide neurons in offspring. To examine mechanisms that mediate this phenomenon, this study examined the transcription factors, transcription enhancer factor-1 (TEF) and Yes-associated protein (YAP), which when inhibited activate neuronal differentiation. In rat embryos and postnatal offspring prenatally exposed to HFD, hypothalamic TEF and YAP mRNA and protein were measured and related to the peptide, enkephalin (ENK). HFD offspring at postnatal day 15 showed in the paraventricular nucleus reduced YAP mRNA and protein, and increased total and inactive TEF protein with no change in mRNA. Similarly, HFD-exposed embryos at embryonic day 19 showed in hypothalamus reduced YAP mRNA and protein and TEF mRNA, and increased inactive TEF protein. This HFD-induced suppression of TEF and YAP was accompanied by increased density and fluorescence intensity of ENK neurons. A relationship between TEF and ENK was suggested by the finding that TEF co-localizes with ENK and that HFD reduced the density of TEF/ENK co-labeled neurons, even while the number and intensity of single-labeled TEF neurons were increased. YAP function was similarly suppressed by HFD as indicated by a decrease in the number and intensity of YAP neurons, although in contrast to TEF the density of YAP/ENK co-labeled neurons was unaltered. These findings suggest that prenatal HFD exposure suppresses TEF and YAP

function and contributes to the HFD-induced increase in differentiation of ENK neurons.

103

A one-session attention modification training to decrease overeating in obese children

K BOUTELLE[1], J KUCKERTZ[1,2], J CARLSON[1], N AMIR[2]
[1]UCSD San Diego, CA, USA, [2]SDSU San Diego, CA, USA

Attentional bias to food cues can index individual differences in saliency and reward.The purpose of this study was to evaluate the impact of attention modification training on overeating.Twenty–four obese children who eat in the absence of hunger were recruited for two visits and were assigned to an attention modification program (AMP) or attentional control condition (ACC).The AMP program trained attention away from food words (cake) to neutral words (pencil) 100% of the time.The ACC program trained attention 50% of the time to neutral and 50% of the time to food.In visit 1, children completed an eating in the absence of hunger free access session, and measures of craving, taste and salivation. At visit 2, they were randomized to a one-session AMP/ACC training and completed the same measures as in visit 1. Results revealed that children in the AMP condition showed a significant decrease over time in the number of calories consumed in the free access session compared to those in ACC (between-group difference t=-77.3 kcal; p=.038) as well as the percent of daily caloric needs consumed in free access (between-group difference t=-3.28%; p=.028). There was also a marginally significant difference in cravings (between-group difference t=-1.15; p=.052). Changes in attention bias, taste and saliva were not significantly different between groups (ps=.178 to .527). This is the first study to demonstrate the efficacy of AMP in decreasing overeating in obese children. AMP programs may have an immediate effect on eating and cravings in this population and should be explored further.

104

Effects of restriction on children's intake differ by child regulatory and appetitive tendencies

BY ROLLINS[1,2], E LOKEN[2], JS SAVAGE[1], LL BIRCH[1,2]
[1]Center for Childhood Obesity Research, Penn State University University Park, PA, USA, [2]Health and Human Development, Penn State University University Park, PA, USA

Parents' use of restrictive feeding practices is counterproductive, increasing children's intake of restricted foods and risk for excessive weight gain. Evidence is needed to identify characteristics of children who show greater susceptibility to the negative effects of restriction, who could benefit most from more responsive feeding practices. An experiment was conducted to 1) replicate Fisher and Birch's (1999) original findings that a short-term restriction increases children's selection, intake, and behavioral response to a restricted food and 2) extend these findings by evaluating individual differences in the effects of restriction among preschool children (3 to 5 y). The experiment used a within-subjects design; thirty-seven children completed a food reinforcement task and heights/weights were taken. Parents reported on child inhibitory control and approach. The experiment replicated and extended Fisher and Birch (1999), revealing that the effects of a short-term restriction to graham crackers (GC) differed by children's regulatory and appetitive tendencies. Greater increases in GC intake in response to the restriction were observed among children lower in inhibitory control, higher in approach, and who found GC highly reinforcing. The results confirm that parents' use of restriction may not be an effective approach to managing children's consumption of snack foods, particularly among children with lower regulatory or higher appetitive tendencies.

105

Effect of child's gender on mother's food choices: a virtual reality-based buffet

S BOUHLAL[1], C MCBRIDE[1], D WARD[2], S PERSKY[1]
[1]Social and Behavioral Research Branch, NIH/NHGRI Bethesda, MD, USA, [2]Department of Nutrition, University of North Carolina Chapel Hill, NC, USA

National guidelines recommend a higher energy intake for boys than girls beginning as young as 4-5 years. Few studies have investigated how mothers apply these recommendations while making food choices for their child, especially when the latter is at risk of becoming overweight. As part of an experimental study of mothers' food choices in a virtual buffet restaurant, we collected data on the composition of a lunch plate that each mother prepared for her 4-5 year-old. Of the 221 overweight mothers recruited, 55% identified a daughter as the child for whom they chose the food. Mothers donned a head-mounted display to be immersed in the virtual buffet. Mothers reported that the food selected in the virtual buffet was the same kind of food they typically fed their child (5.6±1.3 SD; on a 7-point scale), and regarded the virtual buffet as highly realistic (5.5±1.1 SD; on a 7-point scale). Mothers selected larger servings for boys than for girls (p=.008). The caloric content of boys' plates was 40 calories higher than that of girls' (p=.02); this difference was mainly due to an additional 1.76±0.78 g of fat in the food selected for boys (p=.02). Differences in dietary choices that mothers make for girls and boys encourage lifelong gender differences in eating patterns, and likely contribute to the higher prevalence of obesity observed among boys. Additional analyses evaluating the influence of parental BMI and mother's attitudes will be presented to further investigate these gender differences.

106

CaCo-2 cells have a higher fatty acid oxidation (FAO) capacity than HuTu-80 or HepG2 cells

R CLARA, W LANGHANS, A MANSOURI
Physiology and Behavior Laboratory, ETH Zurich Schwerzenbach,, Switzerland

The small intestine is the major site for fat digestion and absorption. Recently, we found that a stimulation of intestinal, in particular jejunal, FAO and ketogenesis was associated with an inhibition of eating in rats (Schober et al., J. Lipid Res. 2013). Because little is known about the capacity of enterocytes to oxidize fat, we investigated the metabolic behavior of immortalized cell lines from different intestinal segments in comparison to a hepatic cell line. We used the Seahorse Extracellular Flux Analyzer XF24 to measure oxygen consumption rate (OCR) and media acidification rate in HuTu-80 (a human duodenal adenocarcinoma cell line), CaCo-2 (a colon adenocarcinoma cell line) and HepG2 (a hepatoma cell line) cells incubated with octanoic acid (C8:0), oleic acid (C18:1) or glucose. Also, we performed a mitochondrial stress test, determined the maximum mitochondrial respiratory capacity (MMRC), and the response to the FAO inhibitor etomoxir (Eto). We found that CaCo-2 cells were more sensitive to Eto (min. Eto concentration for 50% OCR inhibition: CaCo-2 50, HuTu-80 and HepG2 200µM) and had a higher MMRC than HepG2 and HuTu-80 cells (min. FCCP concentration needed to reach the MMRC: CaCo-2 1.50, HuTu-80 1.0 and HepG2 0.5 µM, respectively), indicating that CaCo-2 cells have a higher oxidative phosphorylation capacity than the other two cell lines. Overall, these results suggest that the distal part of the gut oxidizes more fatty acids than the duodenum. To draw strong conclusions, the data must be confirmed in primary isolated enterocytes.

107

Effects of sugar content on neural responsivity during ingestion of palatable foods.

SN DURANT, KS BURGER, E STICE
Oregon Research Institute Eugene, OR, USA

Advances in neuroimaging have provided considerable insight into the neural correlates of taste, however few data are available regarding the impact of increasing sugar content of beverages. In 106 healthy-weight adolescents (aged 14-16y; BMI = 21.32 +/- 2.4), we used fMRI to test the neural responsivity to ingestion of a high-sugar (HS: 23.7g sug/100mL), versus a low-sugar (LS: 8.7g sug/100mL) chocolate milkshake and a calorie-free tasteless solution. After correcting for multiple comparisons, the HS milkshake vs. tasteless solution elicited robust activity in the basal ganglia, insula, and postcentral gyrus, and the LS milkshake vs. tasteless elicited activity in the thalamus, central operculum, and caudate. Using a direct contrast to test the effects of sugar ((HS > tasteless) vs. (LS > tasteless)), we observed significant activity in the bilateral insula and right postcentral gyrus (Z's = 4.4 - 5.3; k's = 57-85). Though significant activity was not observed in typical reward regions using this contrast, results dovetail the theory that high sugar food elicits greater activity in regions previously associated with gustatory, and somatosensory processing, which are essential in feeding. This hyper-responsivity to excess sugar is supported by theories that individuals have an innate predisposition for sweet taste. Further data are needed to assess the effects of fat and energy density.

108

DEMAND FUNCTIONS OF MICE FOR A CELLULOSE-DILUTED DIET: IMPLICATIONS FOR OPTIMAL FORAGING.

A.M. GIDDINGS, N.E. ROWLAND, K.L. ROBERTSON, V. MINERVINI, J.L. HARP, Z. KHAN
University of Florida Gainesville, FL, USA

Optimal foraging theory predicts that the demand for a calorically dilute food should decline more rapidly as price increases than for a dense food. Male CD1 (Harlan, ~ 3 mo of age) mice were tested in standard operant behavior chambers fitted with a nose poke response device and trough into which 20 mg pellets were delivered. Except for a servicing and weighing period each day, mice lived in their chamber and earned all of their food (closed economy). Completion of a specified number of nose pokes (the fixed unit price, FUP) delivered one 20-mg pellet; each FUP was in force for 4 days and was then increased (sequence: 5, 10, 25, 50, 100). Pellets were either standard grain (~3.3 kcal/g) or grain with 50% w/w cellulose (~1.65 kcal/g). Control mice fed the standard pellets showed expected elasticity of demand as price increased. At low costs, mice fed the diluted diet consumed approximately twice as many pellets as the controls, so realizing similar digestible energy intake. As FUP increased, the dilute group maintained energy intake similarly to controls, but had to emit twice as many responses (and in twice the time). Thus, contrary to a prediction of optimal foraging, halving the energy yield of a food item did not produce the expected decline in effort (or time) spent to obtain the commodity. In preference tests, regular pellets were highly preferred over cellulose-diluted pellets indicating that mice are willing to work hard for the diluted food even when it is unpalatable. Translational implications will be discussed.

109

Effect of macronutrient composition of breakfast on total daily intake of calories among adults with type 2 diabetes

S JARVANDI, S RACETTE, M SCHOOTMAN
Washington University School of Medicine St. Louis, MO, USA

Little is known about the effect of breakfast composition, independent of energy content, on the daily intake of calories in persons with type 2 diabetes. Our objective was to test the relationship between macronutrient content of breakfast and daily energy intake among people with type 2 diabetes. We used data from adults with self-reported history of diabetes who completed the 1999-2004 National Health and Nutrition Examination Survey (NHANES). Dietary data were collected by a 24-hour recall method. We calculated energy and macronutrient composition of breakfast, as percent of breakfast energy. Multiple regression models were used to estimate the association between protein and fat content of breakfast (independent variables) and total daily calories (dependent variable), controlling for age, sex, race/ethnicity, body mass index, smoking, alcohol intake and physical activity. A total of 1026 persons were included in this analysis. On average, breakfast provided (mean ± standard error) 415 ± 8 calories, 15% ± 0.20% protein (% of breakfast calories), 31% ± 0.52% fat, and 56% ± 0.65% carbohydrate. Consuming a high-protein breakfast (> 20% vs. <15% of breakfast calories) was associated with 188 ± 72 fewer daily calories (P = 0.01). Also, consuming a relatively low-fat breakfast (<30% vs. ≥ 30% of breakfast calories) was associated with 241 ± 62 fewer daily calories (P <0.01). In sum, eating a low-fat, high-protein breakfast may be a practical strategy to reduce daily intake of calories, which is a central part of the dietary management of type 2 diabetes.

110

Sensory properties and novelty as factors influencing the efficacy of leptin and ghrelin in the control of high-fat diet intake

E LEIDMAA, AV PATCHEV, A PISSIOTI, M HARB, OFX ALMEIDA
Max Planck Institue of Psychiatry Munich, Germany

The regulation of feeding behaviour depends on a fine balance between peripheral signals and central sensory, motivational and affective signals. We hypothesized that the efficacy of peripheral homeostatic signals governing food intake will be altered by exposure of mice to highly palatable food rich in carbohydrates and fat. Consistent with the literature, we observed that adult male mice show a strong preference for high-fat diet (HFD) compared to standard chow (SC). We found that this preference persists even after administration of the anorexigenic hormone leptin (3 or 5 mg/kg). When comparing the effect of leptin on HFD and SC intake, we observed that leptin was less efficacious at suppressing HFD as compared to SC intake in fasted animals. This effect was strongest when HFD was introduced for the first time. In addition, administration of the orexigenic peptide ghrelin (0.3 mg/kg) in satiated animals increased ingestion of HFD but not of SC. These results suggest that sensory properties of HFD as well as its "novelty" may contribute to the overriding of the peripheral satiety signals and potentiating the effect of peripheral hunger signals.

111

RATE OF ADAPTATION OF MICE TO UNIT PRICE CHANGE IN FOOD ACROSS REPEATED CYCLES

V MINERVINI, E TSISKAKIS, K.L. ROBERTSON, A. GIDDINGS, Z. KHAN, N.E. ROWLAND
University of Florida Gainesville, FL, USA

Studies of closed food economies in several species have shown changes in overall intake and meal patterns as a function of price manipulation. Neither the latency for change in eating behavior following a change in price nor the potential role of learning have been studied systematically. In the present experiment, using 23-h/day sessions, 8 male C57BL/6 mice could obtain 20-mg food pellets upon completion of a fixed unit price or ratio of nose poke responses. Each ratio was in effect for 1 day and was then incremented to the next in the series 5, 15, 45, 90, 180, 360, 500. At the end of the incrementing series, the whole cycle was restarted for a total of 5 consecutive cycles. The 23-h intake data for each mouse in each cycle were used to compute demand curve parameters; we hypothesized that if learning occurred then demand elasticity would decrease as cycle number increased. This hypothesis was supported in 4 of the 8 subjects. To analyze the latency for change in behavior, the number of pellets consumed were plotted in 6-h bins. An interaction was identified between unit price ratio and cycle number. At low fixed ratios, consumption tended to shift across cycles from early in a session toward a more constant rate of intake. In contrast, at high fixed ratios, consumption tended to shift across cycles from low early in a session to higher. These results suggest that, after repeated exposure to price changes, mice show more rapid changes in behavior, appropriate to optimal strategy in an inconsistent environment.

112

Antagonism of CRF type 1 receptors does not alter appetite following exposure to an acute stressor

CJ MOORE[1], D TOUFEXIS[2,1], ME WILSON[1]
[1]Yerkes Natl. Primate Res. Center, Emory Univ. Atlanta, GA, USA, [2]Univ. of Vermont Burlington, VT, USA

Exposure to acute and chronic stressors significantly alters feeding behavior. However, effects can be differential depending on stressor type/duration and diet

availability. This study was undertaken to test whether acute stress superimposed on a background of chronic stress increases appetite when both a highly palatable diet and a standard chow diet are available and to assess whether the administration of a CRF type 1 receptor antagonist normalizes this response. Study subjects were adult, female rhesus monkeys housed in nine small social groups. Because social subordination is a known chronic stressor, we focused on the most dominant (n=9) and most subordinate females (n=9) in each group. The acute stressor was a 30-minute social separation task. Using a counterbalanced design, females were treated with saline or antalarmin (1 mg/kg), and caloric intake was quantified during the 6-hours following the acute stressor. Repeated measures ANOVA revealed no significant effect of antalarmin on caloric intake compared to the placebo condition (442 ± 97 vs. 389 ± 54, $p = 0.61$). There was no effect of social status ($p > 0.05$). A significant diet effect emerged as animals consumed more calories from palatable food (295 ± 53) compared to chow (120 ± 33, $p = 0.02$) in both experimental conditions. Findings suggest pathways other than the HPA-axis may influence acute stress-induced changes in eating and that access to palatable food may override commonly observed anhedonic effects of acute stressors.

113

***Drosophila* prandial behavior—a new paradigm for invertebrate feeding**

KR MURPHY[2], JL WEISSBACH[1,2], CF TRIVIGNO[1], A AMADOR[1], BE HUNTER[3], M MADRIGAL[3], KD DAWSON-SCULLY[2], WW JA[1]
[1]The Scripps Research Institute Jupiter, FL, USA, [2]Florida Atlantic University Jupiter, FL, USA, [3]California Institute of Technology Pasadena, CA, USA

The fruit fly, *Drosophila melanogaster*, has been an exceptional model for dissecting the relationship of genes with behavior and disease. Feeding is a fundamental behavior that is understudied due to the lack of sensitive assays for measuring fly food consumption. The study of prandial behavior—including measurements of meal size and frequency—is also neglected despite its central role in the pathogenesis of metabolic disorders. We previously characterized the capillary feeder (CAFE) assay to measure adult food intake. Here, we take advantage of real-time CAFE measurements to resolve prandial behavior of individual flies. We have identified single gene mutations that alter meal timing, size, and/or frequency—but not total food intake. Given the increasingly recognized importance of meal timing and prandial habits—as opposed to total caloric consumption—on human health, our studies will further the development of *Drosophila* models of feeding behavior and metabolic disease and improve our understanding of the central mechanisms that underlie appetite and satiety.

114

Does muscle communicate with the CNS via a mechanism similar to adipose – leptin signaling?

R.J. R.J. MARTIN, JR[1], B.D WHITE[2], M.J. AZAIN[3], G.L. EDWARDS[3], C.D. MORRISON[4]
[1]1Western Human Nutrition Research Center Davis, CA, USA, [2]Auburn University Auburn, AL, USA, [3]3University of Georgia Athens, GA, USA, [4]3University of Georgia Athenes, GA, USA, [5]4Pennington Biomedical Research Center aton Rouge, LA, USA

When given a choice of 2 diets, high protein and low protein, rodents will balance dietary choice to regulate protein intake within a range consistent with normal growth. Dietary protein decreases food intake when fed in excess of protein requirements for growth and increases food intake when fed below protein requirement. Furthermore, when dietary protein requirements are increased by growth hormone (GH) enhanced muscle growth, rodents select a higher protein intake when given a choice of a low and a high protein diet. If the muscle tissue communicates with the brain in a manner similar to adipose tissue and leptin, the following criteria for putative signaling molecules could be applied: muscle cells release the candidate molecule when optimal growth is achieved, and decrease the release of the molecule when growth is suboptimal receptors for the molecule are found in brain areas associated with feeding activation of these receptors decreases selection for dietary protein inactivation of the receptor should increase food selection for protein One candidate molecule is a member of the transforming growth factor superfamily that is predominantly secreted by skeletal muscle. It is predicted that GH enhanced muscle growth and dietary protein selection will be regulated by interactions of muscle derived factors and brain systems involved in feeding behavior.

115

Developing and Testing a Smartphone Based Attentive Eating Intervention

E ROBINSON, S HIGGS, AJ DALEY, K JOLLY, D LYCETT, A LEWIS, P AVEYARD
University of Birmingham Birmingham, United Kingdom

Attentive eating means eating devoid of distraction and increasing awareness and memory for food being consumed. Encouraging individuals to eat more attentively could help reduce calorie intake, as evidence suggests that memory and awareness of food being consumed influence energy intake. Here we report the development and initial feasibility testing of a smartphone based attentive eating intervention. Informed by models of behavioral change, we developed a smartphone application and invited twelve overweight and obese volunteers to use the application during a four week trial. Participants used the application regularly, reported it was easy to use and lost weight during the trial. Qualitative

analyses indicated that participants felt that the application raised their awareness of what they were eating. They outlined barriers to using a smartphone application to change dietary behavior. An attentive eating based intervention using smartphone technology is feasible and testing of its effectiveness for dietary change and weight loss is warranted.

116

The GLP-1 receptor antagonist exendin9-39 fails to increase food intake in healthy men

RE STEINERT[1], J SCHIRRA[2], AC MEYER-GERSPACH[1], C BEGLINGER[1]
[1]University Hospital Basel, Switzerland, [2]Ludwig Maximilian University Munich, Germany

Exogenous GLP-1 inhibits food intake in healthy subjects and diabetic patients. We used the specific GLP-1 receptor antagonist exendin9-39 as a tool to further explore the physiology of GLP-1 as an endogenous satiation signal. Two double-blind, 4 period cross-over studies were performed, each with 10 healthy men. In part 1, subjects received either an intravenous (iv) infusion of exendin9-39 or saline (control) plus an oral glucose preload and an intraduodenal (ID) infusion of glucose or saline for 60 min; in part 2, iv infusions were identical, but an oral ensure preload and a 60 min ID infusion of oleic acid or saline were administered. An *ad libitum* test meal was served 30 min after the preloads/start of the ID infusions and subjects were invited to eat as much as they wished. The amount of food eaten and fluid drunk, and the time to complete the meal was quantified. Appetite sensations, plasma GLP-1, insulin, glucose and glucagon were measured. In both studies, the increase in GLP-1 and glucagon was significantly higher with iv exendin9-39 than with iv saline ($P \leq 0.001$). Insulin was lower with iv exendin9-39 in response to ID glucose ($P \leq 0.05$). Appetite sensations, energy intake, fluid intake, or eating duration were not affected by iv exendin9-39 in either study. In conclusion, we confirm the usefulness of exendin9-39 as a probe of the physiological activity of endogenous GLP-1 revealing (i) a central role of endogenous GLP-1 in the control of insulin and glucagon secretion, but (ii) a limited role in the control of appetite and food intake.

117

Commensal *E. coli* increase affinity of alpha-MSH-reactive immunoglobulins in rats

N TENNOUNE, W OUELAA, J BRETON, R LEGRAND, N LUCAS, P DECHELOTTE, SO FETISSOV
Nutrition, Gut & Brain Laboratory, Inserm U1073, Rouen University Rouen, France

Autoantibodies (autoAbs) against α-melanocyte-stimulating hormone (α-MSH) correlate with behavioral traits in eating disorders but the underlying mechanisms are unknown. We have shown sequence homology between α-MSH and proteins from *Escherichia coli* (*E.coli*), suggesting that they may influence production of α-MSH autoAbs. To test this possibility, female Wistar rats received daily by gavage 4 ml of medium with 10^8 of *E.coli K12* for 21 days; control rats received the medium only. Food intake, body weight and plasma levels of α-MSH IgG autoAbs did not significantly differ between two groups. Affinity kinetics between plasma-extracted IgG and α-MSH was analyzed using surface plasmon resonance. *E.coli* group showed significant increase (by 1.6 times) in IgG affinity vs. controls (6.0×10^{-7} M vs. 1.0×10^{-6} M) which was mainly due to lower dissociation rates. α-MSH-induced cAMP release by HEK293 cells overexpressing human melanocortin receptor MC4R was detected at α-MSH smallest dose of 750 nM. Adding rat IgG (0.5 mg/ml) to α-MSH resulted in significant cAMP release detected at α-MSH dose of 250 nM with IgG from controls but at 500 nM with IgG from *E.coli* group; adding rabbit anti-α-MSH antiserum inhibited cAMP. These data show that α-MSH reactive autoAbs in rats enhance α-MSH signaling on MC4R and that *E.coli* can make it less efficient by increasing affinity of these autoAbs. It suggests that bacteria may interfere with peptidergic signaling relevant to eating disorders.

118

DO BREAKFAST SKIPPERS EXHIBIT AN ALTERED PATTERN OF CIRCULATING CORTISOL THAT PROMOTES OVEREATING AT A SNACK BUFFET?

MG WITBRACHT[1], AM WIDAMAN[1], SM FORESTER[1], WF HORN[2], KD LAUGERO[1,2], NL KEIM[1,2]
[1]Department of Nutrition, University of California Davis Davis, CA, USA, [2]USDA, ARS, Western Human Nutrition Research Center Davis, CA, USA

Breakfast skipping is associated with increased BMI and poor dietary habits, although the reasons for these relationships remain unclear. Evidence suggests that breakfast skipping may modulate cortisol concentrations in such a way that promotes overeating. We hypothesized that breakfast skippers will display a pattern of circulating cortisol that is marked by sustained elevations after the typical morning peak and an exaggerated response to a psychological stressor. Additionally, skippers will consume more total calories and more calories from fat at a snack buffet. Female breakfast "eaters" (n=23) and "skippers" (n=11) participated in two 5-h protocols that included a standard lunch, an afternoon snack buffet, and either a stress test or a relaxation task. Before testing, participants followed their normal breakfast routine. Saliva was collected at multiple times on both days for the measurement of free cortisol. Data was analyzed using repeated measures ANOVA. Cortisol concentrations were higher in the skipper group ($p=0.03$). Although the stress test generated a cortisol response, it was not different between groups. Furthermore, food intake was not different. Our results demonstrate that while breakfast

skipping was associated with sustained elevations in circulating basal cortisol, this endocrine disturbance was not associated with changes in stimulated cortisol or eating behavior. Supported by NIFA and ARS.

119

The Effects of Leptin on the Neuronal Circuitry of Rats in an Activity-Based Anorexia Paradigm

J ALBERTZ, G BOERSMA, K TAMASHIRO, TH MORAN
1 Baltimore, MD, USA

Anorexia nervosa (AN) is a psychiatric illness, involving decreases in food intake and participation in excess activity, leading to unhealthy weight loss. There are few successful treatments and the underlying neural mechanisms remain unclear. Leptin, an adipose-tissue derived hormone, typically low in AN patients and negatively correlated with motor restlessness, has been suggested to decrease patients' excessive activity. In the current study, we examined the effects of leptin administration on multiple aspects of activity-based anorexia (ABA) in rats. ABA is characterized by decreases in food intake and body weight due to increased running wheel activity (RWA) during conditions of restricted food access. We compared the effect of multi-day infusion of peripheral leptin on the RWA, food intake, body weight, and neural peptide mRNA expression in ABA (RW, 1.5hr food access/day) and Food Restricted (FR) (1.5hr food access/day, no RW) adolescent female Sprague Dawley rats. Leptin treatment decreased RWA in ABA rats compared with saline treated ABA rats without affecting either food intake or body weight. The dose of leptin did not result in significant differences in body weight or food intake in the FR group. Understanding the underlying mechanisms responsible for the leptin mediated decreases in activity may help in developing a novel pharmacological tool for AN.

120

Leptin Alterations in Eating Disorders: Associations with Food Intake and Clinical Features in Bulimia Nervosa and Purging Disorder

LP BODELL[1], LA ECKEL[1], LM MCCORMICK[2], DC JIMERSON[3, 4], PK KEEL[1]
[1]Florida State University Tallahassee, FL, USA, [2]University of Iowa Iowa City, IA, USA, [3]Beth Israel Deaconess Medical Center Boston, MA, USA, [4]Harvard Medical School Boston, MA, USA

Bulimia nervosa (BN) is characterized by large binge episodes and compensatory behaviors such as self-induced vomiting. In contrast, purging disorder (PD) is characterized by purging in the *absence* of binge episodes. Research supports decreased leptin levels in BN and PD compared to controls, suggesting a role for leptin in the pathophysiology of these illnesses. Few studies have examined clinical correlates of leptin levels, and none have examined whether leptin levels predict food intake during an *ad lib* test meal in eating disorder participants, despite leptin's role in regulating food intake. Women with BN, PD, and healthy controls underwent a fasting blood draw and a single-item *ad lib* test meal as part of a larger study on the pathophysiology of eating disorders. Plasma leptin was determined by radioimmunoassay. Analyses controlled for body mass index, which was a significant correlate of leptin levels. Preliminary analyses show significant correlations between lower leptin levels and higher weight suppression (the difference between highest prior and current weight) and greater food intake. In BN, lower leptin levels were associated with increased food intake ($t=-1.86$, $p<.08$). In PD, higher leptin levels were associated with greater purging frequency ($t=3.49$, $p<.05$), but not food intake. Results provide preliminary evidence that leptin abnormalities may contribute to binge eating in BN and may be associated with purging in PD.

121

Brief intermittent access to banana-flavored candy did not induce binge-like behavior in marmoset monkeys (*Callithrix penicillata*)

AC BORGES, RBM DUARTE, JR COSTA, AGL JESUS, M BARROS
Universidade de Brasília Brasília, Brazil

Intermittent access to an alternative palatable food source induces binge-like behavior in non-food deprived rodents. However, this has not yet been tested in non-human primates. Therefore, this study evaluated the intermittent access model in marmoset monkeys *(Callithrix penicillata)*. Fifteen free-feeding pair-housed animals were individually tested, once a day, during a 15-min interval (13:00-16:00 h), over a 4-week period. They were divided into three matching groups (sex, body weight and consumption of test-food): (1) High Restriction (HR) group received banana-flavored sugar candy three times a week; (2) Low Restriction (LR) group received the same food item daily; and (3) Control (CG) group was not given access to this food item. All three groups had daily access to their regular diet (fresh fruits/vegetables). The mean amount of candy ingested weekly on binge days did not differ significant between- or within-groups. However, CG-marmosets consumed significantly more of the regular diet than the other two groups, with a significant increase in the amount ingested over the 4-week period. Body weight did not differ between groups during the study period. Therefore, the intermittent access model presently used did not contribute to the development of a binge-like behavior in the marmosets. Methodological issues related to the high palatability of the test-food (banana-flavored sugar candy), its access interval (15-min), the time of testing (afternoon) and the use of both male and female marmosets may have contributed to the result presently observed.

122

Peripheral Glucagon-Like Peptide 1 Levels in Purging Disorder and Bulimia Nervosa

AM DOSSAT[1, 2], DL WILLIAMS[1,2], LA ECKEL[1,2], PK KEEL[1]
[1]Department of Psychology Tallahassee, FL, USA,
[2]Program in Neuroscience Tallahassee, FL, USA

A previous study found that individuals with bulimia nervosa (BN) report lower satiation, assessed via visual analog scale (VAS) ratings, before and after consumption of a standardized test meal (900 kcal Ensure Plus), compared to controls and women with purging disorder (PD). In addition, women with PD endorsed higher post-prandial fullness compared to women with BN and controls. These patterns could not be fully attributed to post-prandial cholecystokinin (CCK) response because CCK levels were significantly lower in individuals with BN relative to controls and PD, and there were no differences between PD and control participants. Rodent and human studies demonstrate that glucagon-like peptide 1 (GLP-1) is released within the periphery and central nervous system in response to food intake, and GLP-1 receptor activation in both areas limits energy consumption. Due to the ability of GLP-1 to promote satiation, examination of post-prandial GLP-1 levels in individuals with PD and BN may provide useful information about post-prandial fullness and satiation patterns. Prior to, 15 and 30 min after ingestion of a standardized test meal (900 kcal Ensure Plus) blood samples were collected for assay. Preliminary analyses suggest lower GLP-1 levels before and after the test meal in individuals with BN (n=8) compared to controls (n=12) and PD participants (n=8) (p=.08), similar to patterns observed for group differences in subjective reports of satiation. Future research should examine these patterns in larger samples to determine whether reliable differences emerge.

123

Nutrient Deficiencies Exacerbate Addiction-Like Behaviors in Rat

CB JENNEY[1], DN ALEXANDER[1], EL UNGER[2], BC JONES[3], PS GRIGSON[1]
[1]Neural and Behavioral Sciences, College of Medicine, The Pennsylvania State University Hershey, PA, USA,
[2]Nutritional Sciences, The Pennsylvania State University State College, PA, USA, [3]Biobehavioral Sciences, The Pennsylvania State University State College, PA, USA

Acute iron deficiency (ID) and chronic omega-3 fatty acid (n-3) deficiency (OD) both alter dopamine signaling. Thus, we hypothesized these deficiencies would exacerbate drug-taking. Iron deplete (postnatal days 4-21), iron replete (IR), OD (10 weeks before and during testing), or n-3 replete (OR) rats self-administered cocaine on a fixed ratio (FR20) schedule, with periods of signaled non-availability (SNA), and on a progressive ratio (PR) schedule. Addiction-like behavior (ALB) scores were calculated such that rats earned 1 point when in the top 33% for active responding during SNA period or for infusions during PR. Thus, rats earned a 0, 1, or 2. ID rats self-administered as much cocaine as IR rats and exhibited greater ALB during SNA and PR (% with a Score of 2; IR: 14%, ID: 25%). This behavior, however, was not goal-directed, as ID rats also exhibited far more responding on the inactive spout (IR: 91.4±34.3; ID: 1375.9±915.0). ID, then, may interfere with learning the task as much or more than motivation. The ALB score for OD rats was the same as for OR rats, but OD rats made more active (OR: 1891.5±851.5; OD: 2030.0±793.6) vs. inactive spout (OR: 625.2±524.7; OD: 143.0±30.9) licks, and during SNA persisted more on the active (OR: 770.0±296.1; OD: 771.5±214.3) vs. inactive spout (OR: 154.1±88.3; OD: 148.9±50.1). Thus, unlike ID rats, OD rats were more goal-directed.

124

The impact of weight cycling on caloric intake and bingeing.

S.B. OGDEN, B. JAMESON, L.A. ECKEL
Program in Neuroscience, Florida State University Tallahassee, FL, USA

Overconsumption of palatable food is a leading cause of obesity. Once obese, individuals struggle to lose excess body weight since attempts to lose weight are often accompanied by repeated bouts of weight loss and regain, a phenomenon known as weight cycling. The degree to which weight cycling may promote feeding patterns that have adverse consequences on energy homeostasis remains unclear. To investigate the impact of weight cycling on subsequent binge eating behavior, female rats were assigned to a control (chow-fed) group or a weight cycle group that received two weeks access to a milk diet (Borden's sweetened condensed milk, diluted 1:2 with water) in addition to chow, followed by two weeks of restricted (80%) access to chow only. After two weight cycles, all animals were given brief (1-h) access to vegetable shortening (Crisco) on an intermittent schedule known to induce bingeing behavior. As expected, the weight cycle group consumed more calories and gained more body weight than controls during both weight cycling phases (ps <0.01). However, this history of weight cycling did not appear to impact shortening intake in the binge protocol as shortening binges were similar in both groups. Interestingly, a preliminary analysis involving the weight cycle group (n = 7) revealed a positive correlation between weight suppression (highest body weight minus current body weight at the beginning of the binge protocol) and shortening intake ($r = 0.57$, $p = 0.09$). The current data suggest that weight suppression induced by a brief history of weight cycling increases the propensity to binge on palatable food. (Supported by DK73936)

125

Binge-like sugar overconsumption is mediated by ghrelin signaling in the ventral midbrain in mice

Y YASOSHIMA, T SHIMURA
Division of Behavioral Physiology, Department of Behavioral Sciences, Graduate School of Human Sciences, Osaka University Suita, Japan

Binge-like sugar overconsumption is suggested to be regulated by the brain reward system including the ventral tegmental area (VTA). However, it still remains to be solved what kind of molecular mechanism of the VTA is involved in expression of the binge-like behavior. To explore the issue, we developed a mouse behavioral model to establish binge-like overconsumption of a 0.5 M sucrose solution by 10-day training with intermittent, limited access to the sugar solution and chow only for 4 h under food deprivation. On training day 7-8, the sugar consumption for the first hour reached 4-fold increase to that at pre-training basal level. Next, we investigated a possible function of ghrelin-related signaling in the VTA for the binge-like sugar-taking behavior. An intraperitoneal injection of a ghrelin receptor antagonist, D-Lys3-GHRP-6 (DG-6), remarkably reduced binge-like sugar overconsumption in trained mice. Intra-VTA infusions of DG-6, but not vehicle (saline), also suppressed the binge-like sugar consumption. Finally, the amount of food intake elicited by intra-VTA ghrelin infusion in the training group was significantly larger than that in the non-trained control group, suggesting that the limited access training enhances the reactivity to a given ghrelin in the VTA. These results suggest that the enhanced ghrelin signaling in the VTA induced by the limited access training plays a crucial role in the hedonic motivation to express binge-like sugar-taking behavior.

126

Parabrachial Nucleus Contributions to Benzodiazepine Hyperphagia

JP BAIRD[1], J SWICK[1], S DIXIT[1], J SCOTT[1], DL PITTMAN[2]
[1]Amherst College Amherst, MA, USA, [2]Wofford College Spartanburg, SC, USA

Benzodiazepines are well known to increase the consumption of palatable foodstuffs, in part by increasing ingestive taste reactivity. In a recent analysis of licking for a variety of taste stimuli we determined that systemic benzodiazepine injections produce manifold effects on ingestive behavior (Pittman et al., 2012). Systemic chlordiazepoxide (CDP) injections increased behavioral measures associated with taste reactivity (increased burst size and initial lick rate) and appetitive sampling behaviors (reduced pause duration). CDP had no effect on measures associated with gut feedback (meal duration and number of bursts). We hypothesized that different brain structures contributed to the dissociable behavioral effects of CDP. Prior work showed that parabrachial nucleus (PBN) benzodiazepine injections increase ingestive taste reactivity (Soderpalm & Berridge, 2000). We therefore evaluated whether PBN lesions selectively affected licking behaviors associated with taste reactivity, but not appetitive sampling, after CDP injection. Rats received bilateral ibotenic acid (PBNX) or sham (SHAM) lesions of the PBN prior to analysis of 90-min licking for a 0.3M sucrose solution 15 min after 10mg/kg CDP or vehicle injection. As observed in prior studies, CDP increased meal size in the SHAM group (n=7) by more than 50% of vehicle conditions ($p<0.05$). We were surprised to observe that CDP increased meal size more so, almost 3-fold, in the PBNX group (n=11; $p<0.05$). As hypothesized, PBN lesions abolished CDP-induced increases in initial lick rate and burst size, which were reliably observed in the SHAM group (interaction $p<0.007$). By contrast, CDP profoundly increased the meal duration and number of bursts in PBNX rats by more than 300%, whereas SHAM rats showed no increases for these measures after CDP (interaction $ps<0.01$). We conclude that the PBN is necessary for CDP-induced increases in behavioral measures associated with hedonic evaluation. We speculate that PBN lesions also attenuated gut-related feedback inhibition of CDP-induced increases in appetitive behaviors. The results are consistent with the hypothesis that separate brain structures independently contribute to systemic benzodiazepine-induced hyperphagia.

127

ANTERIOR THALAMIC PARAVENTRICULAR NUCLEUS IS INVOLVED IN INTERMITTENT ACCESS ETHANOL DRINKING: ROLE OF THE OREXIN 2 RECEPTOR

JR BARSON, SF LEIBOWITZ
The Rockefeller University New York, NY, USA

The paraventricular nucleus of the thalamus (PVT), which participates in motivated arousal, is activated by experimenter-administered ethanol. This nucleus receives input from neurons containing orexin (OX), which can act at the OX 1 (OX1R) or OX 2 receptor (OX2R). The present study investigated whether the PVT is also involved in voluntary ethanol drinking, perhaps in a regionally-selective manner, and whether OX contributes to this process. Long-Evans rats maintained on chow were trained to drink ethanol using the 20% intermittent-access paradigm or were given only water (n=8/group). Ethanol intake averaged 3.5±0.8 g/kg/24 h, resulting in blood ethanol concentrations averaging 53±14 mg%. Ethanol drinking increased the number of c-Fos-positive neurons in the PVT, an effect which occurred specifically in the anterior PVT (aPVT) but not posterior PVT (pPVT), shown using immunohistochemistry. Drinking also increased the percentage of c-Fos-positive neurons double-labeled with OX2R, in the aPVT but not pPVT, but had no effect on double-labeling with OX1R. In cannulated ethanol-drinking rats (n=6-8/group), OX-A

and OX-B (1, 0.5 nmol in 0.3 ul) both stimulated ethanol drinking when injected in the aPVT but not pPVT, while aPVT injection of an OX2R (TCS OX2 29) but not OX1R antagonist (SB 334867; 10 nmol) suppressed ethanol intake. These results support the idea that ethanol drinking activates neurons specifically in the anterior PVT and that this occurs, in part, via activation of the OX2R which in turn promotes further ethanol drinking.

128

The antipsychotic Olanzapine reduces insulin sensitivity and increases hepatic polyol pathway related proteins.

SS EVERS[1], KA PAAL[2], N GOVORUKHINA[2], PL HORVATOVICH[2], G VAN DIJK[1], RPH BISCHOFF[2], AJW SCHEURINK[1]
[1]Dept. of Neuroendocrinology, University of Groningen Groningen, Netherlands, [2]Dept. of Analytical Biochemistry, University of Groningen Groningen, Netherlands

The antipsychotic drug Olanzapine (OLZ) is notorious for its unwanted side-effects: weight gain and increased risk for developing type 2 diabetes. Our studies with female rats showed that OLZ increases body weight and food intake and reduces insulin sensitivity. In similar studies in male rats, OLZ treatment led to a reduction in food intake and body weight with somewhat reduced insulin levels when compared with ad lib fed controls. However, OLZ treated male rats were still markedly insulin resistant when compared to a weight controlled pair fed control group. Post mortem blood samples showed OLZ treated animals have increased triglyceride levels. To further analyze the metabolic state of the OLZ treated animals we measured the protein profile of the liver using phosphoproteomics. Results showed that OLZ significantly increases aldose-reductase-related-protein, glucose-6-phosphate 1-dehydrogenase, and 3α-hydroxysteroid dehydrogenase. The increase of these compounds suggests that OLZ promotes a different metabolic pathway of glucose utilization, polyol pathway i.o. glycolysis. Increases of these compounds are related to a diabetic state and explain why OLZ increases circulating triglyceride levels. The exact mechanism via which OLZ promotes this pathway is still unclear, nor if it is a direct effect of OLZ or a consequence of long-term treatment.

129

Estradiol administration regulates hypothalamic expression of QRFP and QRFP-induced food intake

KP ARCENEAUX[2], A MOUTON[1], MJ BARNES[2], SD PRIMEAUX[1,2]
[1]LSU Health Sciences Center New Orleans, LA, USA, [2]Pennington Biomedical Research Center Baton Rouge, LA, USA

Central administration of the hypothalamic neuropeptide, QRFP, selectively increases high fat diet intake in male and random-cycling female rats. In female rats fed a chow diet, hypothalamic prepro-QRFP mRNA levels fluctuate across the estrous cycle. The current experiments were conducted to determine the role of estradiol on the feeding effects of QRFP and the hypothalamic expression of QRFP in female rats. In Experiment 1, female rats were habituated to either a high fat (60%) or a low fat (10%) diet, ovariectomized and implanted with an indwelling cannula aimed at the lateral ventricles. Estradiol benzoate (EB, 4ug/100ul) was administered once weekly to all rats. For testing, QRFP (1nM) was administered via cannula either two days prior to EB administration or 1 day following EB administration and food intake was assessed at 1h, 2h, and 4h. QRFP administration increased high fat diet intake, which was attenuated by EB administration. In Experiment 2, rats were habituated to high fat or low fat diet prior to ovariectomy, EB was administered every 4 days to mimic a rats' estrous cycle. Following 9 cycles, brains were removed and hypothalamic prepro-QRFP mRNA and QRFP protein levels were measured. In the ventromedial hypothalamus/arcuate nucleus, EB administration significantly decreased prepro-QRFP mRNA levels. Immunohistochemical analysis of QRFP protein expression is underway. These data suggest that estradiol regulates the hypothalamic expression and feeding effects of QRFP in female rats.

130

Roux-en-Y gastric bypass (RYGB), but not estradiol (E2), increases the density of PYY cells in the common channel of ovariectomized (OVX) rats

T BACHLER[1], M BUETER[3], TA LUTZ[1,2], L ASARIAN[1,2]
[1]Inst. of Vet. Physiol. Zurich, Switzerland, [2]Zurich Center for Integrative Human Physiol., Univ. of Zurich Zurich, Switzerland, [3]Dept. of Surgery, Div. of Visceral and Transplantation Surgery, Univ. Hosp. Zurich Zurich, Switzerland

RYGB surgery in rats leads to hypertrophy of the Roux limb and an increase in the number, but not the density, of enteroendocrine cells, such as those producing CCK and GLP-1. We showed that E2 potentiates the effects of RYGB on eating and body weight in OVX rats. Here we examined whether (1) RYGB or (2) E2 increases the number of GLP-1 or PYY-producing L-cells in the common channel of RYGB or SHAM-operated rats. OVX and RYGB were performed in one surgery; 12 d later rats in each surgery group began chronic, cyclic E2 (2 mg/rat, SC, every 4 d) or OIL treatment. After 6 cycles samples of ileum 5 cm proximal to the cecum were processed for GLP-1 and PYY immunoreactivity. RYGB significantly increased the number of PYY-positive cells/vilus in RYGB OIL vs. SHAM OIL rats. Unexpectedly, there were less PYY cells/villus in RYGB E2 vs. RYGB OIL rats. In the SHAM rats E2 had no effect. These results suggest that the potentiating effects of E2 on eating and

body weight in RYGB rats do not depend on the density of PYY cells in the common channel. There was no difference in GLP-1 cells between any groups. Whether this was due to a technical issue, such as antibody affinity, or a differential effect of RYGB or E2 on GLP-1 versus PYY synthesis in L-cells, of some other reason requires further investigation. Supported by NIDDK.

131

Sex differences in exercise induced novel palatable diet avoidance in Sprague Dawley rats

N-C. LIANG, L. MOODY, J. LIANG, T.H. MORAN
Department of Psychiatry and Behavioral Sciences, The Johns Hopkins University Baltimore, MD, USA

Recent work has demonstrated that access to a running wheel and the resulting exercise reduces preference to a previously preferred high fat diet in male rats. In the present study, we tested the hypothesis that exercise may also promote avoidance of novel highly palatable diet independent of fat content and assessed whether there were sex differences in this response. Rats were initially maintained sedentary (Sed) with access to a standard chow diet. Once the experimental procedure began, rats were divided into sets of Sed and wheel running (WR). The Sed rats were given access to a highly palatable high fat (HF) or high sugar (HS) diet in addition to the chow diet. For the WR rats, the access to the palatable diet was given simultaneously with free access to running. The results revealed that male WR rats completely avoided either palatable diets during 6-day access to the running wheel while the Sed rats showed hyperphagia and high preference for both HF and HS diet. The avoidance to the HF or HS diet in male WR rats persisted even when the rats were no longer running. In female rats, similar procedures resulted in a complete HF diet avoidance in WR rats for only 3 days. Intakes of the palatable diet recovered gradually with running and some WR females even preferred the HF diet after 15 days of running. Overall, the study demonstrates that while exercise results in avoidance to a novel palatable diet in both sexes, the effect declines in female rats and can result in a reversal of preference with continued running wheel access.

132

Estrogen receptors and HSD2-containing neurons in the rat ventromedial hypothalamus

HD MUCKELRATH, ML ASKEW, KS CURTIS
Oklahoma State University Center for Health Sciences Tulsa, OK, USA

Estrogen decreases food intake and body weight in rats during basal conditions. One central site that may be important in these effects is the ventromedial hypothalamus (VMH). A dense population of estrogen receptor α (ERα) is located in the VMH, and silencing these receptors increases food intake and body weight. Moreover, 11-β-hydroxysteroid dehydrogenase type 2-containing (HSD2) neurons are located in the VMH; however, their function is unknown. We sought to determine whether ERα in the VMH are associated with estrogen effects to suppress feeding during basal conditions, and whether HSD2 neurons contribute to the estrogen suppression of feeding. We used immunohistochemical methods to assess ERα in the VMH of male rats and ovariectomized (OVX) rats with or without estrogen, and to examine the distribution of HSD2 neurons in the VMH. We found no difference in numbers of ERα among the groups.
Moreover, HSD2 neurons are located more laterally in the VMH and likely do not co-localize with ERα. However, HSD2 neurons occur in greater numbers in estrogen-treated OVX rats, and are located in close proximity to a plexus of fibers labeled for catecholamines. These results suggest that estrogen suppression of feeding during basal conditions does not involve numbers of ERα in the VMH. It remains unclear whether there is a role for HSD2 neurons in the VMH in the control of feeding. If there is, it may involve interactions between HSD2 and catecholamines, but is unlikely to involve direct actions of estrogen on ERα in HSD2 neurons.

133

Inhibitory Peripheral Signals, Feeding and Body Weight in Estrogen- vs Oil-treated Ovariectomized Rats

RJ NAUKAM, KS CURTIS
Oklahoma State University Center for Health Sciences Tulsa, OK, USA

Treatment with 17-β-estradiol-3-benzoate (EB) decreases *ad libitum* food intake and body weight in ovariectomized (OVX) rats. Our recent work shows that food intake and body weight are also reduced in EB-treated rats on a restricted feeding schedule compared to vehicle-treated controls (OIL). Specifically, EB-treated rats ate less than did OIL-treated rats during the first week that feeding was restricted to two hours/day, but ate as much as did OIL-treated rats during the subsequent week. We hypothesize that this transient effect of EB on food intake is due to changes in inhibitory peripheral signals. One possibility is gastric distention signals, carried by vagal afferents to the hindbrain nucleus of the solitary tract (NTS). Alternatively, CCK, a gut hormone that inhibits food ingestion, may play a role. Accordingly, we examined c-fos labeling, a marker of neural activation, in the NTS, and measured plasma CCK levels in OIL- and EB-treated rats during food restriction. Both OIL- and EB-treated rats that were on the restricted feeding schedule and allowed to eat had greater c-fos immunolabeling in the NTS than did rats that were not allowed to eat. However, despite the fact that EB-treated rats ate less during the first week on the restricted feeding schedule, we found no differences in c-fos labeling in the NTS between EB- and OIL-treated groups, nor did plasma CCK levels differ between the

groups. Thus, there appears to be a transient increase in sensitivity to peripheral inhibitory signals in EB-treated rats on a restricted feeding schedule.

134

FEMALE MICE HAVE INELASTIC FOOD DEMAND RELATIVE TO MALES: ROLE FOR ESTROGEN RECEPTOR-ALPHA

N.E. ROWLAND, K.L. ROBERTSON, V. MINERVINI, T.C. FOSTER, A.M. GIDDINGS, J.L. HARP, Z. KHAN
University of Florida Gainesville, FL, USA

In study A we examine whether males and females differ in their food demand curves. C57BL/6 mice (Harlan ~3 mo of age; both sexes) were tested individually in standard operant behavior chambers fitted with a nose poke response device and trough into which 20 mg grain-based pellets were delivered. Except for a servicing and weighing period each day, mice lived in their chamber and earned all of their food (closed economy). Completion of a specified number of nose pokes (the fixed unit price, FUP) delivered one pellet; each FUP was in force for 4 days and was then increased (sequence: 5, 10, 25, 50, 100). At low costs, male mice consumed more than females, strictly in accordance with their higher body weight (2/3 exponent). Both male and female mice decreased food intake as FUP increased but the decline was more marked in males who lost more body weight. To determine whether estrogen acting via ERα or ERβ may underlie this relative resilience in food intake of females, genomic knockout (KO) and wild type (WT) females (C57BL/6 and 129/SvJ background: 6-14 mo of age) were used in Study B with the same procedure as Study A. WT and ERβKO mice showed similar elasticity of demand to the females in Study A, while ERαKO mice showed higher elasticity of food demand, and comparable to the males in Study A. This work has uncovered a previously unreported resilience in food intake and body weight maintenance of female mice in the face of higher food costs, and suggests a role for estrogen acting via ERα in this phenomenon.

135

Fetal growth restriction programs an increased hedonic response to sucrose: possible contribution to catch-up weight gain and early obesity

R DALLE MOLLE[1], FS CUNHA[1], CS BENETTI[1], C NOSCHANG[1], AK PORTELLA[1], M DESAI[2], MG ROSS[2], PP SILVEIRA[1]
[1]Hospital de Clínicas de Porto Alegre - FAMED, UFRGS Porto Alegre, Brazil, [2]Dept of Ob/Gyn, Harbor-UCLA Med Ctr Torrance, CA, USA

Clinical evidence suggests that intrauterine growth restriction (IUGR) can persistently program the subject's preference for palatable foods. Considering that the taste reactivity to different solutions is a behavior homologous in humans and animals, we aimed at investigating the hedonic response to sucrose in the first day of life in an animal model of IUGR, resulting from maternal food restriction (FR; 50% food restricted diet from pregnancy day 10 to term). Within 24 hours after birth, pups (n=90) derived from FR or Control (fed *ad libitum*) dams were weighed and randomized to receive a droplet (10µl) of sucrose solution (0.3M) or distilled water. Hedonic facial responses exhibited within 60 seconds (sec) were analyzed by a blinded observer. Analyzing the period between 8 and 20 sec, Control pups exposed either to water or sucrose resolved their hedonic responses after 16 and 18 sec, respectively, while FR responses persisted over 20 sec. The sum duration of the hedonic responses from 8 to 20 sec was higher in FR pups exposed to sucrose (3.9±2.7 sec) as compared to water (1.4±1.8 sec), but no differences were seen between the Control groups. Thus, as compared to Controls, IUGR newborns demonstrate an increased hedonic response to sucrose versus water, and more prolonged hedonic responses. Upregulation of hedonic responses may contribute to catch-up weight gain and the development of obesity in IUGR offspring.

136

A maternal junk food diet alters the response of the mesolimbic reward system to naloxone in offspring post-weaning.

JR GUGUSHEFF[1], ZY ONG[1,2], BS MUHLHAUSLER[1,2]
[1]University of Adelaide Adelaide, Australia, [2]University of South Australia Adelaide, Australia

A maternal 'junk food' diet alters the response of the mesolimbic reward system to naloxone in offspring post-weaning. JR Gugusheff[1], ZY Ong[1, 2] and BS Muhlhausler[1, 2] [1]Foodplus Research Centre, School of Agriculture, Food and Wine, The University of Adelaide, South Australia, Australia.[2] Sansom Institute for Health Research, School of Pharmacy and Medical Science, University of South Australia, South Australia, Australia. The present study aimed to determine whether the response to the opioid antagonist naloxone in rat offspring was influenced by exposure to a junk food diet in the perinatal period. To explore this, 17 Albino Wistar female rats were provided with either a junk food (JF, n=9), or control chow diet (C, n=8), during pregnancy and lactation. At weaning, pups received daily i.p. injections of either naloxone (5mg/kg) or an equivalent volume of saline for 10 days. qRT-PCR was used to determine the expression of mu-opioid receptor (MOR) and the opioid enkephalin in the nucleus accumbens (NAc) and ventral tegmental area (VTA) in the brains of offspring. In male offspring, there was a significant interaction ($P<0.05$) between maternal diet and saline/naloxone treatment on MOR expression in the VTA, such that MOR expression was decreased by naloxone treatment in the JF group, but not controls. In the NAc, MOR expression in males (C 0.003±0.0007, JF 0.006±0.0008, $P<0.05$) and enkephalin expression in

females (C 1.29±0.04, JF 1.43±0.04, $P<0.05$) was increased in JF offspring independent of naloxone treatment. These results suggest that perinatal exposure to a junk food diet alters the response of the mesolimbic reward pathway to opioid receptor blockade and may indicate functional consequences on the regulation of opioid signaling in junk food exposed offspring.

137

Early life stress, anxiety and feeding behavior are related to changes in HPA axis

TD MACHADO, R DALLE MOLLE, DP LAUREANO, AK PORTELLA, CS BENETTI, IC WERLANG, PP SILVEIRA
Hospital de Clínicas de Porto Alegre – FAMED, UFRGS Porto Alegre, Brazil

Chronic stress increases anxiety and prones to use palatable foods as "comfort foods", which seems to be mediated by altered function of the hypothalamic–pituitary–adrenal (HPA) axis. We aimed at verifying if an early life stress affects anxiety, feeding behavior, stress responses in adult female rats. By the day 2 of life litters of Wistar rats were subjected to reduced nesting material (Early–Life Stress; ELS) or standard care (Controls). Anxiety was evaluated using the novelty supressed feeding test (NSFT), and stress reaction was measured by corticosterone (CORT) at 0 and 20, 40, 60 and 90 min. of restrain stress. Feeding preference for comfort food (CF-diet) rich in fat (34%) and sugar (20%), was measured in a computerized system (BioDaq, Research Diets®) in rats receiving only standard chow (std-diet) or exposed to both diets for 30 days. ELS increased adulthood anxiety in the NSFT (incresead latency to eat in a new environment p=0.005, decreased chow consumption at the homecage p=0.045), as well as increased CORT levels in response to restraint stress (p=0.02). On the preference test, while the control group chronically receiving the CF-diet showed a diminished preference for the CF-diet compared to the control group exposed only to std-diet, the ELS rats did not demonstrate this reduction in preference after the chronic exposure (p<0.001).The anxiety and altered feeding behavior seen in ELS to be related to changes in the HPA axis response to acute stress. The consumption of CF-diet possibly is used by ELS to inhibit the anxiety symptoms.

138

Effect of exposure to cafeteria diet during gestation and after weaning on metabolism and body weight of adult male offspring of rats

AB MUCELLINI[1,4], ACA DA CUNHA[1], RC CACERES[1], C NOSCHANG[2], CS BENETTI[2], JF GOULARTE[1], PP SILVEIRA[2,3], GL SANVITTO[1]
[1]Physiolgy Graduation Program, UFRGS Porto Alegre, Brazil, [2]Department of Pediatrics, UFRGS Porto Alegre, Brazil, [3]Neuroscience Graduation Program, UFRGS Porto Alegre, Brazil, [4]Medical Sciences: Psychiatry Graduation Program, UFRGS Porto Alegre, Brazil

We investigated if maternal cafeteria diet affects the offspring metabolism and whether there is a cumulative effect with the exposure to cafeteria diet during the offspring life course. Female rats were fed a control (CON) or cafeteria (CAF) diet from weaning until their offspring's weaning. After 21 days of life, their male offspring were divided into 4 groups, considering diet during gestation/post-weaning diet (CON/CON, CON/CAF, CAF/CON, CAF/CAF). Half of the animals were decapitated at 30 days of age and the other half at 120days. Using 2-way ANOVA, caloric intake of CAF rats was higher than CON rats, regardless of maternal diet. Litters showed equal body weight at weaning and 30 days, but, at 120, CON/CAF rats were heavier. At 30 and 120 days, CAF rats had heavier adipose tissue than CON rats, regardless of maternal diet. At 30 and 120 days, triglycerides and cholesterol were similar between the groups, as well as blood glucose levels at 30 days. However, at 120 days, puppies that ingested CAF showed hyperglycemia, as hiperleptinemia and hyperinsulinemia, irrespective of maternal diet. These data suggest that maternal diet modulates body weight of offspring and its effects on metabolism are influenced by postnatal diet, but more studies are needed to understand the origins of metabolic changes.

139

Food Preference and Metabolic Parameters are Affected by Prenatal Stress in Wistar Rats

A WELLER[1,3], M SCHROEDER[1,3], S YARKONI[2,3], N KRONFELD-SCHOR[4]
[1]Psychol Dept, Bar-Ilan University Ramat-Gan, Israel, [2]Life Sciences Faculty, Bar-Ilan University Ramat-Gan, Israel, [3]3Gonda Brain Res Ctr, Bar-Ilan University Ramat-Gan, Israel, [4]Zoology Dept, Tel-Aviv University Tel-Aviv, Israel

The risk for depression and anxiety in offspring may be increased by prenatal stress (PNS). We asked whether PNS (maternal restraint stress randomly scheduled over gestation) may also affect offspring's eating behavior and metabolism, in Wistar rats. In Experiment 1, we examined food preference and body composition, from weaning to pre-puberty (day 39), focusing on sex differences. Animals had free access to standard chow and high fat diet (HFD) (40%) throughout the study. Also, a novelty test assessed anxiety-like behavior at weaning. In Experiment 2, we examined long term metabolic outcomes and food preference focusing on PNS Wistar female offspring, from weaning till adulthood (day 90), with and without access to HFD (60%). In Experiment 1, PNS reduced the initial (expected) preference for HFD and reduced body weight and fat, in males and females. PNS affected bone morphology, decreased core temperature and increased anxiety at weaning, specifically in female offspring. In Experiment 2, PNS

females showed lower total HFD preference and total caloric intake from weaning to adulthood, lower percent fat, and a tendency towards lower bone density at day 90. Chronic HF diet availability reduced insulin sensitivity, an effect also seen in PNS females reared on a low-fat (chow)-only diet. Thus, PNS appeared to induce a "depressive-like" behavioral (anhedonia) and physiological/metabolic profile.

140

Impact of sweet, savoury and fat on appetite and reward in individuals at risk of overeating.

G FINLAYSON
University of Leeds United Kingdom

Introduction: Taste and fat are involved in food preference and choice, and thought to modulate appetite and reward. Recent studies have investigated the effect of savoury or sweet taste and high or low fat containing foods on reward (liking & wanting), satiety and food intake; according to individual differences in psychobiological markers underlying susceptibility to overeating. **Methods:** Behavioural procedures have been developed to evaluate the strength of liking and wanting using a range of visual food stimuli varying in sweet/savoury and fat. Susceptibility to overeating was assessed by the eating behaviour traits of disinhibition and binge eating, along with measures of body composition and fasting leptin concentrations. **Results:** When high and low fat meals were matched for energy and taste, post-meal satiety was weaker and the recovery of hunger more rapid after high fat. When meals were matched for fat, the impact of savoury or sweet taste on hunger was similar. Susceptibility to overeating was associated with enhanced wanting for sweet/fat foods when fasted and weaker satiety after fixed energy preloads. When given a test buffet containing a variety of foods, those with high scores specifically selected foods in the sweet/fat combination. Independent of adiposity, fasting leptin concentrations were positively associated with disinhibition, binge eating and wanting for high fat foods. **Conclusion:** Some individuals (susceptible to overeating) show a tendency to prefer and consume, high sweet/fat foods, indicating an impact on reward and satiation. The role of leptin as a potential marker for susceptibility to overeating is consistent with the hypothesis that leptin resistance may weaken satiety and enhance reward for sweet/fat food. **Funding:** EU FP7/KBBE under grant agreements 266408 & 289800.

141

Effects of dietary habits on the neural response to food stimuli: Evidence from neuroimaging studies.

KS BURGER
Oregon Research Institute Eugene, OR, USA

Roughly one-third of Americans are able to maintain a healthy weight despite the omnipresence of high-fat and high-sugar foods, whereas the majority experience unhealthy weight gain. This has prompted multiple theories regarding individual difference factors that may increase risk for overeating, many of which are founded in the idea that aberrant neural responsivity to food stimuli increases intake. Data from neuroimaging studies indicate that obese versus lean individuals show an increased neural response in brain regions associated with visual, gustatory, and reward processing when exposed to food cues, yet show decreased activity in reward-related regions during intake of food. However, few data are available that provide insight to the initial emergence of these neural patterns and the degree that ingestive behavior (relative to excess adipose tissue) impacts neural responsivity to food stimuli. We performed a series of studies aimed to evaluate the acute effects of food-based reward learning, and the impact of habitual eating patterns and weight change on neural responsivity to food stimuli. While preliminary, studies investigating habitual eating behavior echo cross-sectional studies comparing obese vs. lean individuals and prospective weight change studies. Collectively, data point toward possible food reward neuroplasticity resulting from overeating that may perpetuate further intake. Gaining a better understanding of how reward from food varies as ingestive behaviors are repeated and become dietary habits can directly inform obesity prevention and treatment efforts.

142

The Hypothalamus and Beyond: Neural and Hormonal Pathways Governing Human Appetite

LM HOLSEN[1,2,3]
[1]*Division of Women's Health, Department of Medicine, Brigham and Women's Hospital Boston, MA, USA,* [2]*Department of Psychiatry, Brigham and Women's Hospital Boston, MA, USA,* [3]*Harvard Medical School Boston, MA, USA*

The use of neuroimaging techniques in the study of human appetite has grown rapidly over the last decade, providing rich data on the neural mechanisms underlying various basic and complex facets guiding normal and abnormal food intake. In addition to more fully characterizing the well-established role of the hypothalamus, studies employing fMRI, PET, and other modalities have illuminated the unique functions of mesolimbic reward and cortical inhibitory regions in the processing of internal and external cues related to appetite and food intake, both in healthy individuals and in

populations with disordered eating. However, the goal of translating results from human studies into targeted treatment for conditions from anorexia nervosa to extreme obesity will require a multisystem approach. For example, given the evidence from in vivo animal studies of acute and chronic effects of appetite-regulatory peptides on functioning in these same brain circuits, the incorporation of hormone assessment in relation to brain activation patterns in humans would create a synergistic effect on increasing our understanding of both of these systems, and their interaction. Additional lines of research utilizing a combination of these types of techniques, in the context of clinical trials for hormone treatment, brain stimulation, and surgical intervention, will help unravel the complexities of the role of the brain in human appetite by focusing on the critical systems involved in predicting therapeutic response, allowing for more targeted and effective treatment.

143

Role of central GLP-1: implications for food and alcohol reward

KP SKIBICKA
Sahlgrenska Academy Göteborg, Sweden

Glucagon-like-peptide-1 (GLP-1R) and its long acting analogues, a novel class of type 2 diabetes (T2D) treatment, reduce food intake, a great benefit considering the comorbidity of T2D and obesity. It is often expressed in the literature that GLP-1 regulates feeding via its action on the classic central nervous system areas involved in energy balance regulation like the hypothalamus and the hindbrain. In contrast to this common view, recent evidence points to the mesolimbic reward circuitry as a potential target for the anorexic action of GLP-1. Emerging evidence supports the idea that hunger-driven feeding, the hedonic value of food and food-motivation are impacted by GLP-1 action in the mesolimbic system. GLP-1R mediated inhibition of food reward can be driven from two key mesolimbic structures, the ventral tegmental area (VTA) and nucleus accumbens. Furthermore our recent results suggest that the range of action of GLP-1 on reward behavior is not limited to food-derived reward but extends to alcohol reward. Peripherally applied and intra-VTA GLP-1 reduces alcohol intake and reward. Importantly, a contribution of the endogenously released GLP-1 to alcohol intake is highlighted by the observation that blockade of GLP-1 receptors alone results in increased alcohol intake. These findings implicate GLP-1R signaling as a novel modulator of alcohol intake and reward. The new discoveries concerning GLP-1 action on the mesolimbic reward system significantly extend the potential therapeutic range of this drug target. Support: Swedish Research Council 2011-3054, European Council FP7-KBBE-2010-4-266408, Full4Health.

144

The clock gene Bmal1 regulates ghrelin levels and induces inflammation during restricted feeding

J LAERMANS, C BROERS, T THIJS, J TACK, I DEPOORTERE
Gut Peptide Research Lab, TARGID, University of Leuven Leuven, Belgium

Introduction Food-entrainable circadian oscillators (FEOs), used to predict food availability, can be entrained by restricted feeding (RF). Ghrelin-releasing cells from the stomach may act as FEOs. We previously showed that RF increases octanoyl ghrelin levels and induces stomach inflammation, leading to contractility changes. We aimed to study the role of the clock gene Bmal1 in these effects. **Methods** Wild-type (WT) and Bmal1 knockout (Bmal1-KO) mice were fed *ad libitum* or put on RF (food access: 12-4PM) for 1 week with a canola oil-enriched diet. Plasma ghrelin levels (radioimmunoassay) and fundic myeloperoxidase (MPO) activity (O-dianisidine assay) were determined. *In vitro* contractility changes were measured isometrically in fundic smooth muscle strips. **Results** The high-fat diet abolished the RF-induced increase in plasma octanoyl ghrelin in WT mice and was also without effect in Bmal1-KO mice. In Bmal1-KO, but not in WT mice, RF increased ($P<0.005$) plasma total ghrelin from 1776±128 to 2367±78 pg/ml. This was accompanied by a decrease in stomach ghrelin mRNA expression. In WT mice, RF increased MPO activity (2.2 fold) and IL-1α/IL-1β mRNA expression (1.9 fold) in the stomach. These inflammatory changes were not observed in Bmal1-KO mice. As a result, contractility changes towards acetylcholine were normalized in fundic muscle strips of Bmal1-KO mice. **Conclusion** Bmal1 is involved in the regulation of ghrelin expression and secretion during RF and in RF-induced inflammation, since loss of Bmal1 dampens cytokine levels and recruitment of neutrophils to the stomach.

145

Obesity disrupts circadian variations in gastric vagal afferent satiety signals

S KENTISH[1], T O'DONNELL[2], D KENNAWAY[1], G WITTERT[1], A PAGE[1,2]
[1]University of Adelaide Adelaide, Australia, [2]Royal Adelaide Hospital Adelaide, Australia

Mechanosensitive gastric vagal afferents are part of a co-ordinated set of peripheral mechanisms involved in the regulation of food intake. Previously we have shown that (i) gastric vagal afferent response to mechanical stimulation exhibits circadian variation, and (ii) obesity reduces vagal afferent mechanosensitivity and instead of potentiating mucosal receptors leptin inhibits tension receptors. However, the effect of obesity on circadian variation of gastric vagal afferent activity is unknown. Therefore we fed 8wk old female C57BL/6 mice either a standard laboratory diet (SLD; 7% energy from fat,

N=12) or high fat diet (HFD; 60% energy from fat, N=12) for 12wks then sacrificed mice at 6hr intervals starting at 18:00. Stomach contents were measured and single fibre recordings from gastric vagal tension and mucosal receptors were obtained at each time point, in the absence and presence of leptin (1nM). In SLD mice, at 00:00 as compared to 12:00, stomach contents were 168% greater, response of tension receptors to tension (3g) was reduced by 59% and that of mucosal receptors to stroking (50mg) was reduced by 62%. Leptin potentiation of mucosal receptor responses to stroking (50mg), were 70% less at 00:00 than 12:00. In HFD mice circadian variation in stomach contents, vagal afferent mechanosensitivity, and leptin inhibition of tension receptors, was minimal. In conclusion, HFD induced obesity suppresses circadian variations in gastric vagal afferent mechanosensitivity, responsiveness of tension receptors to leptin and alters food intake patterns.

146

Ability of GLP-1 to Decrease Food Intake is Dependent on Nutritional Status

CC RONVEAUX[1,2], G DE LARTIGUE[1], HE RAYBOULD[1]
[1]Dept. of Anatomy, Physiology and Cell Biology, UC Davis School of Veterinary Medicine Davis, CA, USA,
[2]AgroParisTech, UMR914 Nutrition Physiology and Ingestive Behavior Paris, France

Glucagon like peptide-1 (GLP-1) is released from the gut in response to a meal and acts on GLP-1 receptors (GLP-1R) at central and peripheral sites. There are discrepancies in the literature about the ability of GLP-1 to inhibit food intake and moreover, the pathway of action is unclear. GLP-1 has a very short half-life in the circulation; the terminals of vagal afferent neurons (VAN) in the gut are a likely site of action. Expression of receptors by VAN varies with nutritional status; we hypothesize that GLP-1R expression on VAN increases in the fed state and mediates inhibition of food intake in response to a meal. **METHODS:** To test whether GLP-1 effects on FI are nutrient-dependent, FI was recorded following administration of GLP-1 (30nmol/kg) or saline (IP) in Wistar rats fasted for 18h or fasted and re-fed 3g chow for 40 min. GLP-1R expression on VAN was detected by RT-PCR and western blots in animals fasted for 18h or fasted and refed *ad libitum* for 2h. **RESULTS:** In response to a meal, GLP-1 significantly reduced FI by 64% after 40 mins and 60% after 60mins compared to saline ($p<0.05$); however, GLP-1 had no effect on FI in fasted rats. Rats refed for 2h after an overnight fast had similar GLP-1R mRNA and protein levels on VAN compared to fasted rats. **CONCLUSION:** Feeding rapidly changes the biological activity of GLP-1. The mechanism is unclear but this effect is independent of changes in receptor expression by VAN.

147

"Gut feelings": Vagal afferent signaling modulates innate anxiety-like behaviors in rats

M KLARER, M ARNOLD, W LANGHANS, U MEYER
Physiology and Behavior Laboratory, ETH Zurich Schwerzenbach, Switzerland

The central nervous system and viscera are engaged in constant bidirectional communication via diverse pathways. This allows for information exchange pertaining to the regulation of body homeostasis and control of ingestive behavior. It has long been speculated that afferent signals from the viscera may also modulate other behaviors than eating. This putative link has often been conceptualized as "gut feelings" and may involve vagal afferent signaling, but direct proof of such a connection is still missing. We started to address this issue by assessing the effects of subdiaphragmatic vagal deafferentation (SDA) on emotional and cognitive behavioral functions in rats. SDA was verified by the abolition of cholecystokinin satiation. We employed tests assessing innate anxiety-like behavior (elevated plus maze, open field, and food neophobia test), social interaction (two-compartment social approach test), and short-term memory (spontaneous alternation in the Y-maze). We found that compared to Sham-operated control rats SDA rats consistently displayed reduced anxiety-like behavior in the elevated plus maze, open field, and food neophobia tests. On the other hand, social approach behavior and short-term memory performance were not affected by SDA. Our findings indicate that abdominal vagal afferent signaling can modulate behavioral functions beyond eating. The observed selectivity of the behavioral effects suggests that afferent vagal pathways are particularly important for innate anxiety-like behaviors.

148

EFFECT OF COVERTLY REDUCING PORTION SIZE OF A MEAL ON PYY, GLP-1, INSULIN, PERCEIVED APPETITE AND SUBSEQUENT ENERGY INTAKE

HB LEWIS, I SOLIS-TRAPALA, SA JEBB
MRC Human Nutrition Research Cambridge, United Kingdom

Larger portion sizes (PS) have been associated with greater energy intake (EI). There is little evidence on the effects of PS reduction or the mechanisms controlling EI. This study investigated the effect of covertly reducing breakfast PS on postprandial profiles of PYY, GLP-1, insulin, perceived appetite and subsequent EI. A randomised crossover study was conducted in 20 overweight and obese men and women (mean BMI 29kg/m^2, mean age 43y). Condition A provided a standard breakfast based on 25% of gender-specific average estimated energy needs. PS was reduced by 20%(B) and

40%(C). Blood samples were taken at fasting, 30, 60, 120, 180 and 240 mins after breakfast for analysis of gastrointestinal hormones. Perceived appetite was measured using visual analogue scales. EI was measured at an *ad libitum* lunch at 240 mins, and over the rest of the day by weighed diet diary. Mixed models were used to determine the effect of PS condition on profiles of gastrointestinal hormones, perceived appetite and EI. Condition C postprandial profiles of PYY, GLP-1, insulin and fullness were lower and hunger was greater ($p<0.05$) than in condition A. Profiles in conditions A and B were similar. EI at lunch (A: 2930±203; B: 2853±198; C: 2911±179kJ) or later in the day (A: 3865±332; B: 4011±369; C: 3798±357kJ) did not differ by condition. Covert PS reduction led to attenuated profiles for postprandial gastrointestinal hormones and perceived fullness, and higher perceived hunger. Despite this, EI at subsequent eating occasions did not differ between conditions. MRC funded U105960389.

149

Combinations of long acting GLP-1R and CCK-1R agonists produce additive reductions of food intake in nonhuman primates.

TH MORAN[1], E OFELDT[1], C SUN[2], JD ROTH[2]
[1]Psychiatry and Behavioral Sciences, Johns Hopkins Medcine Baltimore, MD, USA, [2]Amylin Pharmaceuticals San Diego, CA, USA

The gut peptides CCK and GLP-1 reduce food intake following their exogenous administration and similar actions of the endogenous peptides have been demonstrated. In the current experiments we assessed the interaction of CCK1 and GLP-1 receptor stimulation in male rhesus monkeys maintained with daily 6 hr access to 1 gm banana flavored chow pellets. Monkeys received individual or combination IM injections of a selective CCK1-R agonist ([Hpa-Nle-Gly-Trp-Lys(2-tolylaminocarbonyl)-Asp-(N-methyl)Phe-NH_2]) and/or a GLP-1R agonist ([Leu(14)]exendin-4) immediately prior to the daily 6 hr feeding period. The CCK1-R agonist alone (0.32-10 µg/kg) dose dependently suppressed food intake by up to 72% at the highest dose tested. All doses exerted the greatest suppression during the first hour with the dose effect being more clearly demonstrated at later times. Likewise, the GLP-1R agonist alone (0.1–1 µg/kg) suppressed intake in a dose related fashion throughout the feeding period with a maximal 6 hr suppression of 81% at the highest dose. Combinations of the lower doses produced additive suppressions of food intake. In general, the rate of eating followed that produced by the GLP-1 analog with a greater suppression during the first hour so that the cumulative intake curves were parallel. These data demonstrate that combined CCK and GLP-1 receptor stimulation can result in increased feeding suppression across the period of daily food intake with the effects of each individual compound clearly evident.

150

Insights into underlying mechanism and improved efficacy of the adjustable gastric band

A STEFANIDIS[1], J KAMPE[2], K COHEN[1], N FORREST[1], WA BROWN[3], BJ OLDFIELD[1]
[1]Department of Physiology, Monash University Melbourne, Australia, [2]Institute for Diabetes and Obesity, Helmholtz Zentrum München Munich, Germany, [3]Department of Surgery, Monash University Melbourne, Australia

Currently, bariatric surgery remains the only effective treatment for morbid obesity. Laparoscopic adjustable gastric banding (LAGB) is one of the most commonly performed bariatric procedures; however, the mechanism(s) underlying its efficacy are unclear. This study aims to elucidate the role of sensory neural pathways in mediating AGB-induced satiety in a rodent model and assess the effectiveness of adjuvant therapies on AGB-induced weight loss. Adult male Sprague Dawley rats (n=8/group) were fitted with an AGB, just below the gastro-oesophageal junction. Our previous data indicate that inflation of the band causes an increase in numbers of Fos-positive neurons in the rostral division of the medial NTS in the brainstem. This could be attributed to a neural, a neural - humoral or a direct humoral link. To test this, capsaicin was used to ablate vagal sensory fibres using CCK- induced anorexia as a biomarker of the extent of the lesion. Capsaicin treatment resulted in a complete elimination of the elevated Fos labelling in the NTS but only a partial attenuation of AGB induced weight loss. Furthermore, AGB induced reductions in body weight and fat mass in obese rats are associated with reductions in energy expenditure that can be effectively ameliorated by co-treatment with factors such as thyroid hormone, which increase energy expenditure in brown adipose tissue ($p<.05$). These data support the hypothesis that LAGB exerts its effects via the modulation of both, neural and hormonal pathways. Adjuvant therapies that increase energy expenditure can enhance the effectiveness of the AGB.

151

Correlations between food selection preferences and the novel peptide hormone adropin in human females

M-P ST-ONGE[1], S GAO[2], A SHECHTER[1], JD SHLISKY[1], C TAM[3], E RAVUSSIN[3], AA BUTLER[2]
[1]New York Obesity Nutrition Research Center New York, FL, USA, [2]The Scripps Research Institute Jupiter, FL, USA, [3]Penningtin Biomedical Research Center Baton Rouge, LA, USA

Adropin is a peptide hormone linked to metabolic and cardiovascular disorders. Mouse studies indicate regulation by dietary macronutrients, however, whether diet regulates adropin levels in humans is not known. The objective of this investigation was to assess whether plasma adropin levels measured the morning prior to food

self-selection (Pre-FS) and the following day (Post-FS) would correlate with food choices. Our hypothesis was that Post-FS plasma adropin levels would correlate with fat intakes. The study used fresh plasma samples from a study examining energy homeostasis in sleep restricted and normal sleep conditions in 15 men and 15 women of healthy body weight (BMI 22-26 kg/m2) aged 30-45y. Participants were provided with a controlled diet (30% energy from fat, 55% carbohyrates and 15% protein) designed to maintain energy balance for 4 days. Serum samples were collected at 07:30 the next day (day 5/Pre-FS) after an overnight fast. Participants were then allowed to self-select food intake from foods available at the research center and self-purchased foods from a local grocery store. Another fasting blood sample was drawn at 07:30 on day 6 (Post-FS). Sleep state did not affect serum adropin. Controlling for sex, race, and weight, the saturated fat content of the foods selected by females, but not males, correlated with *both* Pre-FS (r=0.927, $P<0.01$) and Post-FS adropin values (r=0.842, $P<0.05$). Pre- and Post-FS adropin also correlated positively with percent energy intakes from fat (r=0.867 and 0.797, respectively; $P<0.05$) and negatively with carbohydrate [r=-0.894 (Pre-FS) / -0.929 (Post-FS); both $P<0.01$]. The increased fat content of the diet selected by individuals with high adropin was associated with increased total energy intake, suggesting potential orexigenic activities. Adropin treatment increased consumption of a high fat diet in female mice with normal weight gain suggesting compensatory mechanisms. These are the first data linking adropin with the control of ingestive behavior in humans, and suggest that adropin may have orexigenic actions. High plasma adropin levels correlate with self selection of foods high in saturated fat content in females.

152

From evolution to ecology to economics: Some curious conjectures connecting social dominance, energetics, perceptions, adiposity, and lifespan.

DB ALLISON
University of Alabama at Birmingham Birmingham, AL, USA

The evolution and ontogeny of lifespan at the species and individual level, the energetics of the organism in its environment, the storage of metabolizable energy as body fat, and socioeconomic disparities within populations all seem intricately related and yet the nature of these interrelations is poorly understood. Indeed questions as fundamental as why people age remain open and subsidiary questions such as why caloric restriction leads to increased lifespan and why lower socioeconomic status is related to obesity in developed countries also remain unanswered. I will discuss a hypothsized unified model informed by evolutionary thinking about life strategies which connects these phenomena. In this model, aging or more precisely senescence is not something that passively happens to organisms as the result of environmental insults or from metabolizing fuel. That is, mortality rate or the rate of aging is seen as (partially) internally regulable phenomenon much like the control of body temperature in homeotherms in which the regulated rate is responsive to perceptions about the energetic state of the environment. From this perspective, it is perceptions of the energetic security of the environment that are a key factor in linking these phenomena. Ongoing tests of this theory using model organisms will be described and connections to human data which suggest some avant-garde approaches to reducing obesity based on the principles of *Liberté, Égalité, Fraternité*.

153

Endocannabinoid signaling and the brain-gut-brain control of dietary fat preference

NV DIPATRIZIO[1], D PIOMELLI[1,2]
[1]Department of Anatomy and Neurobiology, University of California Irvine School of Medicine Irvine, CA, USA, [2]Istituto Italiano di Tecnologia Genoa, Italy

Fatty foods are highly palatable to mammals and recent studies reveal that this attraction is primarily driven by gustatory signals from the oral cavity. We recently reported that oral exposure to solutions containing dietary fats (i.e., corn oil)–but not carbohydrate or protein alone–causes an accumulation of the endocannabinoids (eCBs), via the vagus nerve, in the rat jejunum, and inhibition of this local signaling event blocks sham feeding of fats. Dietary lipids are comprised principally of triglycerides, which are hydrolyzed, at least in part, by lingual lipases that release their free-fatty acid (FFA) component, and mounting evidence suggests that FFAs are essential for fat "taste" and preference. Indeed, our work reveals that, similar to corn oil, oral exposure to the FFAs, oleic acid (18:1) or linoleic acid (18:2), robustly increases jejunal eCB levels. This effect is absent for stearic acid (18:0), linolenic acid (18:3), or mineral oil–which contains no FFAs but has similar textural properties to nutritive oils (e.g., corn oil). Furthermore, rats display strong preferences for solutions containing 18:2 versus mineral oil. Importantly, this preference is attenuated by pretreatment with peripherally-restricted cannabinoid 1 receptor antagonists. Collectively, our work supports the classification of fat as a primary taste quality and suggests that the gut eCB system is a major contributor to the preferences displayed by mammals for fatty foods based on their orosensory properties.

154
Gastro-intestinal vagal afferent satiety signals.
A J PAGE[1,2]
[1]*University of Adelaide Adelaide, Australia,* [2]*Royal Adelaide Hospital Adelaide, Australia*

Vagal afferent nerves are a major pathway by which food related signals, from the stomach and small intestine, access the brain to modulate food intake and associated behaviour. Our cognitive perception of fullness following food intake depends on activation of these vagal afferents via two principle routes 1) mechanical distension of the stomach and 2) the presence of luminal nutrients which trigger endocrine and paracrine secretions from the mucosa of both the stomach and small intestine. The fundamental mechanisms involved in the activation of these afferents are just beginning to be understood but it is clear they are not in a fixed state and can be modulated by a number of factors including diet, gastrointestinal hormones, adipokines and circadian variations. Our laboratory has obtained evidence that the modulatory effect of various appetite regulating hormones, including ghrelin and leptin, are altered in high fat diet conditions in such a way as to promote food intake and weight gain. In addition, upon return to a 'normal' diet the alterations, that occur in high fat diet conditions, in vagal afferent sensitivity to both direct activation and modulation are resistant to change and likely to defend the increased weight gain.

155
Nutrient-induced plasticity of gut-brain signaling
G DE LARTIGUE
Dept. of Anatomy, Physiology and Cell Biology, UC Davis School of Veterinary Medicine Davis, CA,

The vagus nerve connects peripheral organs with the central nervous system. Vagal afferent neurons, located in the nodose ganglia, have processes terminating within the lamina propria of the gut. These neurons express a plethora of receptors that sense hormones released from the gut epithelium, and provide information about the quantity and type of nutrients throughout the gut to the brain. The neurochemistry of vagal afferent neurons changes in response to nutrient availability. Prolonged food withdrawal increases expression of orexigenic peptides, while decreasing anorectic peptide expression. Conversely, refeeding reverses the expression of these proteins. The plasticity of these neurons in response to nutrient availability is dependent on the presence or absence of CCK acting at CCK1 receptors and is modulated by leptin and ghrelin. Ingestion of a high fat diet prevents the dynamic changes in expression in vagal afferent neurons, resulting in constitutive expression of receptors and transmitters associated with increased food intake. In diet-induced obesity, vagal afferent neurons become leptin resistant, resulting in reduced sensitivity of these neurons to CCK. Using a conditional knockout mouse lacking leptin receptor expression in afferent neurons, the absence of leptin signaling in vagal afferent neurons reduces the sensitivity of these neurons to CCK, and prevents nutrient induced plasticity. As a result, these conditional knockout mice become hyperphagic and weigh more than wild type controls. Thus, nutrient-induced plasticity of vagal afferent neurons is required for stringent control of food intake.

156
Synaptic plasticity and the nature of information transfer at vagal afferent to NTS synapses.
JH PETERS
Washington State University Pullman, WA, USA

Primary vagal afferent neurons provide a direct neural pathway through which the ongoing status of visceral organ systems (including the heart, lungs, and gastrointestinal tract) is conveyed to the brain. Vagal afferents contact neurons within the nucleus of the solitary tract (NTS) and initiate homeostatic reflex pathways as well as inform the relevant forebrain projecting neurocircuitry on the appetitive drive to eat, or not. The range of information types, time-frames, and relative physiological urgencies conveyed by vagal afferents is remarkable. As a result information transfer must be at one time reliable and precise while maintaining plasticity to match autonomic function to the physiological state. The talk aims to briefly discuss the functional and anatomical properties of vagal afferents which enable them to span these opposing properties. Three topics will be covered including: 1. the multiple pathways for fast neurotransmitter release (synchronous, asynchronous, and spontaneous) and their contribution to information transfer, 2. the anatomical segregation of afferent innervation at the first synapse and its relevance for signal identity and coding, and 3. the impact physiological 'state' (ex. fed vs. fasted) has on incoming visceral afferent information. Central to all three topics is the presence of vanilloid receptor 1 (TRV1) and related temperature sensitive TRP channels (TRPV3 and TRPM3). The intersection of these properties and presence of these TRP channels conveys both the rigidity and plasticity of the vagal afferent to NTS synapse.
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157
Pavlovian Conditioning of Hedonic Food Cues in Overweight and Lean Individuals
M MEYER[1], *V RISBROUGH*[2], *K BOUTELLE*[2]
[1]*Alliant University San Diego, CA, USA,* [2]*UCSD San Diego, CA, USA*

Pavlovian conditioning is one mechanism implicated in the development of heightened food cue reactivity.This study examined differences between overweight and lean

young adults in their acquisition and extinction of conditioned salivary responses to visual cues paired with different stimuli (chocolate milkshake, tasteless water and no taste stimulus.Thirty-three young adults (ages 18-26; 67% female;16 overweight (BMI>28);17 lean (BMI<24)) participated and swallowing frequency was measured using electromyograph (EMG) recordings to measure salivation.Acquisition consisted of 27 pairings of cues with taste stimuli; during extinction visual cues were presented alone. During the acquisition phase, overweight subjects exhibited differential learning of chocolate vs. water associated cues, exhibiting greater swallowing in response to a cue paired with chocolate shake than a cue paired with water ($t(15)=2.71$, $p=.016$). Lean participants did not show this differential learning ($t(16)=0.78$, $p=.489$). After acquisition, overweight subjects also exhibited more swallow responses to the cue predicting chocolate shake compared to normal weight subjects ($t(31)=-2.09$, $p=.008$). Among overweight participants, there was a trend toward significance of the extinction of responding ($t(15) = 1.77$, $p = .098$). These are the first results to show differential acquisition of Pavlovian conditioned responding in overweight individuals, as well as differential conditioning to hedonic versus neutral cues.

158

Impaired glucose utilization underlies early, but not later, hippocampal-dependent learning deficits.

SL HARGRAVE, KP KINZIG
Department of Psychological Sciences, Purdue University West Lafayette, IN, USA

High-fat/dextrose diet (HFD) consumption induces an obesity phenotype and impairs hippocampal-dependent discrimination ability after short and long (10 and 90 day) but not intermediate exposure durations (Kanoski, et al., 2010). Although 90-day exposure was associated with increased BBB permeability, the basis for the short-term deficit is unspecified. In the present study, rats (n = 10/group at each time point) were tested on a spontaneous alternation (SA) task following 10, 40, and 90 days on a chow, HFD, high-fat/sucrose or ketogenic diet. At 10 days, HFD rats were not only impaired at SA ($p < 0.05$), but analysis of gene expression revealed that they had significantly ($p < 0.05$) lower levels of hippocampal glucose (GLUT1) and monocarboxylase (MCT1) transporter mRNA compared to the other groups. However, these differences were not observed at 40 or 90 days. A separate study assessed *place* (PL) and *response* (RES) learning (only PL is hippocampal-dependent) by HFD and chow-fed rats (n = 10/group at each time point) that were trained to criterion on a food-rewarded Y-maze task. At 8 days, HFD- and chow-fed rats utilized opposite strategies (RES and PL, respectively). RES strategy was associated with elevated blood glucose levels ($p < 0.05$). Interestingly, by 40 days, this effect had dissipated. Taken together, these results suggest that hyperglycemia and impairments in hippocampal energy utilization may underlie the transient cognitive impairments that develop after brief HFD exposure.

159

Dose-dependent effects of leptin on learning and memory: can the detrimental effects of high leptin levels help explain obesity-associated cognitive impairments

AL TRACY, M SALGADO FERRER, JK BRYNILDSEN
Grinnell College Grinnell, IA, USA

One of the established consequences of obesity in human and non-human animals is impaired learning and memory (L&M). The adipose hormone leptin acts via the hippocampus to facilitate learning in a number of tasks and is involved in physiological processes that are associated with L&M. Yet, in obesity, cognitive declines are observed despite increases in circulating leptin levels. With respect to ingestive behavior, obese subjects display "leptin resistance" (LR) in which leptin fails to normally increase food intake or induce hypothalamic signaling. It has been hypothesized that physiological LR occurs in the hippocampus of obese animals and that this may contribute to deficits in L&M. To test this idea, we used a diet-induced obesity model (ad lib 45% fat diet for 10 weeks) in rats. These rats were significantly impaired in learning the Morris water maze (MWM) following dietary manipulation. However, we observed no evidence of physiological hippocampal LR in response to central leptin injection. An alternative hypothesis to hippocampal LR is that high levels of circulating leptin are directly detrimental to L&M. Supporting this, we have found a significant dose-dependent effect of leptin on hippocampal-dependent L&M. Specifically, high doses of leptin impaired MWM performance in lean mice and rats. Collectively, our behavioral and physiological evidence supports the hypothesis that excess leptin directly impairs L&M in obesity rather than the idea that hippocampal LR accounts for obesity related cognitive deficits.

160

The medial prefrontal cortex is necessary for the motivational control of intake

MA PARENT[1,2], MS CAETANO[1,2], L HARENBERG[1], R WEBSTER[3], B LIU[3], M LAUBACH[1,2]
[1]The John B. Pierce Lab New Haven, CT, USA, [2]Yale Univ Sch of Med New Haven, CT, USA, [3]Yale Univ New Haven, CT, USA

We examined the role of medial prefrontal cortex (mPFC) in control of food intake. Operant behavioral studies led to the view that mPFC has an inhibitory role in the control behavior. To study mPFC function in the context of food intake, we used a simple licking task in which rats had access to alternating concentrations of liquid sucrose in 30s epochs. Rats reduced intake when the available concentration of sucrose was low and engaged in

sustained bouts of licking with access to a higher level. Test sessions using only low-level sucrose revealed a negative contrast effect. Pharmacological (muscimol,cholinergics) and optogenetic strategies (ArchT) were used to manipulate mPFC during the licking task. Based on our hypothesis that mPFC has an inhibitory role in the control of behavior, we expected inactivation mPFC to eliminate negative contrast by increasing intake of low sucrose. Instead, rats consumed less high sucrose due to failure to maintain sustained licking bouts and initially consumed equal amounts of the two levels; only later in the session showing differential intake. Testing with intra-mPFC infusions of scopolamine revealed similar effects that were opposed by infusion of XE-991. Our results implicate mPFC in control of food-intake, suggesting: 1) the role of mPFC is not solely inhibitory, 2) the ability of mPFC to regulate consummatory behavior is modulated by cholinergic tone 3) mPFC is necessary for maintaining sustained licking bouts and 4) expression of learned behavioral strategies.

161

Sodium selenate and conditioned taste aversion learning

T.A. HOUPT, J.G. BROWN, A. KIMBROUGH
Biological Science, Neuroscience, Florida State Univ. Tallahassee, FL, USA

CTA learning is constrained by phosphatase activity and is enhanced by, e.g., okadaic acid (Oberbeck 2010) or calcineurin knockdown (Baumgartel 2008). Conversely, phosphatase activation should attenuate CTA. Recently, sodium selenate was found to be a specific activator of protein phosphatase 2A (PP2A; Corcoran 2010, van Eersel 2010). To characterize the effects of selenate on CTA, we determined 1) the dose of selenate which induces a CTA and 2) if a subthreshold dose of selenate would attenuate a LiCl-induced CTA. Water-restricted rats were given 10-min access to 0.125% saccharin, then injected with selenate (0, 0.5, 1, or 2 mg/kg ip, n=6 /dose). The next day, 2-bottle 24-h preference extinction tests of water vs. saccharin were begun and continued for 14 days. Rats that received saccharin paired with 1 or 2 mg/kg selenate acquired a CTA with reduced saccharin preference across extinction, but rats receiving 0 or 0.5 mg/kg showed a high preference and thus no CTA. A separate group of rats were injected on conditioning day with the low dose of selenate (0.5 mg/kg) or saline. 2h later, the rats were given 10-min access to saccharin, then injected with either NaCl or LiCl (0.15 M, 6 ml/kg ip, n= 6/group). Preference tests were run for 14 days. Vehicle-LiCl rats showed a persistent decrease in saccharin preference. While selenate-pretreated rats showed some reduction in saccharin preference, their preferences were higher than vehicle-LiCl rats. This suggests that selenate interfered with the rats' ability to acquire a CTA, consistent with phosphatase activation.

162

Obesity is associated with impaired working memory and negative outcome learning

G. COPPIN[1,2], S. NOLAN-POUPART[1,2], M. JONES-GOTMAN[3], D.M. SMALL[1,2]
[1]John B. Pierce Laboratory New Haven, CT, USA, [2]Yale University New Haven, CT, USA, [3]McGill University Montreal, QC, Canada

We examined performance on implicit, explicit, and working memory tasks in lean (N= 16), overweight (N= 16), and obese (N= 17) individuals matched for age, gender, and education. The Abstract Design List Learning (ADLL) task measures explicit learning and memory by asking participants to learn and recall a list of abstract designs. In the Conditioned Cue Preference Test (CCPT), participants search for red balls on a computer screen by clicking on boxes, after which an abstract design is briefly flashed before the emergence of either a red or a black ball. Correct responses are rewarded with a pleasant sound and a snack food (Nerd, raisin or M&M's ®). Unbeknownst to the participants one design is rewarded 90%, one 50%, and a third only 10% of the time. Working memory is employed to tally the number and position of red balls while post task preference judgments are used to probe the formation of associations between designs and positive (90% design) or negative (10% design) outcomes. Upon debriefing participants that report an awareness of the association are excluded. The groups performed similarly on the ADLL. In contrast to the lean group, the obese and overweight groups were impaired in the working memory and preference formation tasks. Intriguingly, and contrary to our prediction, obese, but not lean individuals displayed a preference for the negative outcome design. These findings are consistent with an emerging literature demonstrating impaired working memory and negative outcome learning in obesity.

163

High-energy (HE) diet impairs the ability to use energy state signals to control appetitive behavior

CH SAMPLE, CD HUGHES, M PATT, MB GYIMAH, S JOHNSON, TL DAVIDSON
American University Washington, DC, USA

Interoceptive cues arising from food deprivation and satiation serve as powerful discriminative cues that compete with external cues (e.g., tones, lights) for the control of behavior. Hippocampus-lesioned rats show deficits in using interoceptive cues, and a diet high in saturated fat and processed sugars has been shown to interfere with hippocampal function. The current study investigated whether HE diet selectively impairs the ability to use deprivation cues as discriminative stimuli. Male Sprague-Dawley rats were trained to use cues produced by 0- and 24-hour food deprivation as signals for the delivery of sucrose pellets. Group 24+ received sucrose under 24-hour but not 0-hour food deprivation,

while Group 0+ received the opposite contingency. When performance (indexed by magazine approach) reached asymptote, half of the rats in each group were placed on a HE diet, while the rest remained on chow. Following 45 days of ad libitum exposure to the diets, discriminative performance was impaired for the HE diet-fed rats compared to chow-fed controls. Tone and light cues were then presented with deprivation cues as compound signals for sucrose delivery. HE diet-fed rats were able to discriminate after the introduction of tone and light cues. Subsequent tests examined the basis of the discrimination for both groups. The findings suggest that HE diet interferes with the ability to use energy state cues but not food-related external cues like those that promote intake in our obesogenic environment. This work was supported by NIH grant RO1 HD29792.

164

Hippocampal synaptic rescue by corticosteroid inhibition occurs independently of leptin receptor activation in db/db mice

M WOSISKI-KUHN[1], JR ERION[1], SP WILSON[2], AM STRANAHAN[1]
[1]Georgia Regents University Augusta, GA, USA, [2]University of South Carolina School of Medicine Columbia, SC, USA

Impaired leptin receptor signaling influences metabolic homeostasis and hippocampal synaptic plasticity. Leptin receptor deficiency elevates adrenal steroids, but the relative contributions of increased corticosterone levels and impaired leptin receptor signaling to deficits in hippocampal plasticity have yet to be resolved. We manipulated corticosterone levels in the hippocampus and periphery to address whether corticosterone acts locally to induce cognitive deficits in leptin receptor deficient db/db mice. These experiments revealed that inhibition of adrenal steroidogenesis attenuates synaptic structural and functional impairments in db/db mice, while intrahippocampal corticosterone treatment recapitulates cognitive and synaptic deficits. db/db mice also exhibit corticosterone-mediated reductions in hippocampal brain-derived neurotrophic factor (BDNF), and lentivirus-driven reinstatement of BDNF expression normalizes hippocampal synaptic plasticity in a leptin receptor-independent manner. These observations support a central role for elevation of hippocampal corticosterone and suppression of BDNF expression as a mechanism for synaptic dysfunction in the absence of leptin receptor signaling.

165

LOW INTENSITY SWIMMING EXERCISE INDUCES SODIUM APPETITE, URINARY EXCRETION AND REDUCTION ON HEART RATE VARIABILITY IN WISTAR RATS

BZ BOSCHETTI-VALDO, BO COSTA, VC VALDO, IH SAVIOLLI, CA OGIHARA, MA SATO, RL ALMEIDA
FACULDADE DE MEDICINA DO ABC - FMABC SANTO ANDRE, Brazil

This study aimed to investigate the effects of swimming exercise on sodium appetite, renal excretion and heart rate variability in rats. Male Wistar rats (n=6/group) were divided in sedentary and swimming exercised (1 h/day, 5 days/wk, 6 wks; 2% body weight load) groups. Water and 0.3 M NaCl daily intake and urine were measured each 24 h in individually metabolic cages. Arterial pressure and heart rate (HR) were recorded at the end of 6 wks of exercise. Exercised rats had an increased sodium intake from the 1st to the 6th wk (8.4±1.6; 14.5±2.2; 14.3±2.0; 13.9±2.2; 16.1±2.7; 13.2±3.4 mL) compared to the sedentary group (3.4±0.5; 4.0±0.6; 5.2±1.1; 6.1±0.9; 6.8±1.7; 6.5±2.2 mL). There were no differences in water intake between groups. Exercised rats also showed an increased urinary volume excretion measured at 1st, 3rd and 6th wks when compared to the sedentary rats. Exercised rats showed a decrease in the natriuresis at 1st, 3rd and 6th wks in comparison to the sedentary rats. No difference was observed in the kaliuresis between groups. Exercised rats showed a diminished Low Frequency (LF) component of the HR (3.44±0.71 ms2/Hz) in the frequency domain compared to sedentary rats (8.53±2.91 ms2/Hz). The HR variability in the time domain was also reduced in exercised (6.10±0.50 ms2) in comparison to sedentary rats (11.16±2.94 ms2). The data suggest that exercise induces an increase in the sodium appetite and diuresis, a decrease in natriuresis and reduces the sympathetic modulation of the HR.

166

Purinergic receptor blockage reduces the facilitation of NaCl intake induced by alpha$_2$-adrenergic activation in the lateral parabrachial nucleus

A MOREIRA DE FARIA[1], SP BARBOSA[2], MF MENEZES[2], GMF ANDRADE-FRANZé[2], S GASPARINI[2], LA DE LUCA JR[2], JV MENANI[2], PM DE PAULA[2], CAF ANDRADE[1,2]
[1]Programa Multicêntrico de Pós Graduação em Ciências Fisiológicas - Unifal-MG Alfenas/MG, Brazil, [2]Dept. of Physiol. and Pathology, Sch. of Dent. - FOAr - UNESP Araraquara/SP, Brazil

Alpha$_2$-adrenergic and P2X purinergic receptor activation in the lateral parabrachial nucleus (LPBN) facilitates hypertonic NaCl intake. In the present study, we investigated the effects of the blockage of purinergic P2X receptors of the LPBN on the facilitation of 0.3 M NaCl intake induced by bilateral injections of moxonidine

(alpha$_2$-adrenoceptor/imidazoline receptor agonist) into the LPBN. Male Holtzman rats (n=5) with cannulas implanted bilaterally in the LPBN were submitted to sodium depletion (furosemide, 20 mg/kg b.wt. subcutaneously combined with sodium deficient diet for 24 h). Bilateral injections of moxonidine (0.5 nmol/0.2 µl) into the LPBN increased sodium depletion-induced 0.3 M NaCl intake (29.7±4.2, vs. vehicle: 18.9±0.7 ml/120 min). The P2X purinergic antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, 4 nmol/0.2 µl) alone into the LPBN did not change 0.3 NaCl intake (18.5±4.6 ml/120 min), however, the pretreatment with PPADS into the LPBN abolished the effects of moxonidine on 0.3 NaCl intake (14.3±4.1 ml/120 min). These results suggest a possible interaction between alpha$_2$-adrenergic and P2X purinergic receptors in the LPBN to facilitate sodium intake.

167

Effect of fourth ventricle injection of ghrelin on angiotensin II-induced neuronal activation

KS PLYLER, D DANIELS
Dept. Psychology, Univ. Buffalo Buffalo, NY, USA

Lateral ventricle (LV) injection of ghrelin attenuates fluid intake after various dipsogenic stimuli including injections of angiotensin II (AngII). Our recent studies found that fourth ventricle (4V) injection of ghrelin also reduces AngII-induced fluid intake, suggesting that ghrelin-responsive hindbrain structures interact with forebrain systems that underlie AngII-mediated fluid intake. To identify potential sites of interaction between these substrates, we used Fos immunohistochemistry to test for differences in neural activity in the brains of rats given 4V ghrelin and LV AngII. To this end, rats were given two consecutive 1µl injections, one into the LV (10ng AngII or vehicle) and a second into the 4V (0.5µg ghrelin or vehicle). Ninety minutes after the injections, rats were killed and their brains were processed for Fos immunoreactivity (Fos-ir). The number of Fos-ir cells was quantified in a number of sites relevant to the response to AngII including the paraventricular nucleus (PVN) and the subfornical organ (SFO). Preliminary analysis on a small group of rats (n=3-5 per group) found that AngII treatment increased the number of Fos-ir cells in the PVN by 88% and the addition of ghrelin reduced this increase by 47%. In the SFO, AngII caused an 89% increase in the number of Fos-ir cells, but this was unaffected by ghrelin. The studies are ongoing and other brain areas require examination, but these initial results highlight the PVN as a potential site for the interaction between hindbrain effects of ghrelin on AngII-induced fluid intake.

168

The morphological and functional alteration induced by continuous sodium deficiency on taste organs of rats.

K UEDA[1], C INUI-YAMAMOTO[1], M NAKATSUKA[1], C AN[1], S KUMABE[1], S WAKISAKA[2], Y IWAI[1]
[1]Osaka Dental University Hirakata, Japan, [2]Osaka Univ.Grad.Sch.of Dent. Suita, Japan

It is needless to say that appropriate nutrition intake is very important for proper physical development. Of course, sodium, one of the minerals that are important nutrition, is necessary for a healthy life. It has already reported that dietary sodium restriction (DSR) during early developmental stage brings certain changes of the central gustatory system. However the alterations in the peripheral gustatory system caused by DSR are still unclear. In this study, we performed immunohistochemistry on taste buds of rats with the treatment of DSR through embryonic day 3 to adulthood by using some antibodies against molecules participating in taste transmission in taste buds such as AbH, NCAM and PKD2L1 etc. to investigate the effect of DSR on the peripheral gustatory system. We have preliminarily performed the two-bottle taste preference test of DSR rats for salty, sour, sweet, bitter and umami taste. We found a decrease in the number of NCAM immunoreactive cells in DSR rats compared with control rats. In addition, we found that the preference for 0.1% NaCl solution and avoidance for 0.3% NaCl solution of DSR rats decreased. In contrast, the reactions for sour, bitter and umami taste were not significantly changed. This study reveals that sodium deficiency brings some disadvantage in the development of taste buds and the formation of taste sensitivity, and suggests the possibility that NCAM may participate in salty taste transmission.

169

Temporal Effects of Acute Stress on Sodium Consumption in Wistar Rats

A.M. WILLIAMS
Laurentian University Sudbury, ON, Canada

Stress has been shown to effect consumption. For example, acute stressors and chronic stressors have been found to result in increased and decreased consumption respectively. (Krebs et al., 1996) Sodium for example increases during stress because sodium attenuates the stress response and is thus rewarding. (Krause et al., 2011) The purpose of this study was to examine the effect of a 95 dB stressor on the consumption of various sodium liquids (sodium chloride mixed with water). To this end, 12, food deprived, female Wistar rats were each placed in a test cage for six 30 minute trials of both a control and a stress session; during which they had access to four sodium concentrations (0%, 0.15%, 0.5%, and 1.0%). The analyses involved two repeated measures ANOVA's; One

testing the main effects of stress and sodium concentration, and their interaction; The other testing the main effects of trial and stress, and their interaction. The independent variables were stress (stress vs. control), sodium concentration (0%, 0.15%, 0.50%, 1.0%), and trial (six trials). The dependent variable was consumption in milliliters. The stress by day interaction was significant (F (5, 7) = 6.95, $p<.05$, $\eta^2=0.83$), revealing that consumption on stress trial 1 (M=4.20, SE= 0.16) and consumption on control trial 5 (M=4.24, SE=0.29) were significantly greater than consumption on other trials. This may result from the acute stressor becoming a chronic stressor through repeated exposure during the experiment, resulting in larger consumption first during the stress trial and then later during the control trial. This suggests that stress affects sodium liquid intake; acute stress increases sodium consumption and chronic stress decreases sodium consumption.

170

Lesions in Insular Cortex Differentially Affect Taste-Guided Behaviors in Rats

GD BLONDE, MB BALES, AC SPECTOR
Dept. of Psychology, Florida State University
Tallahassee, FL, USA

While the gustatory cortex (GC) is implicated in expression of conditioned taste aversions (CTA), little is known regarding its necessity in other fundamental taste-guided behaviors. Here, rats with excitotoxic lesions targeting GC (n=26) or sham lesions (n=14) were assessed in the gustometer for retention of a presurgically LiCl-induced CTA to 0.1M sucrose in a brief access generalization test. The same animals were then postsurgically trained in a two-response operant detection task testing water v. NaCl then water v. KCl, and psychometric functions were assessed. Next, the rats were trained and tested for their ability to discriminate between NaCl and KCl concentrations. Lesions meeting our GC lesion criterion (resulting in a group averaging 80% damage to GC and involving surrounding regions) led to impaired performance in the CTA (LiCl-injected, n=9) and detection tasks and in learning the discrimination (n=18), but degree of deficit between tasks correlated weakly, if at all. Further lesion analysis including those not meeting the lesion criterion suggests that different areas within insular cortex are critical to maintain normal function for distinct taste-guided behaviors. These data and others from our laboratory suggest that areas posterior in GC and/or insular cortex are more involved in CTA retention, whereas areas more squarely encompassing GC are involved with taste sensitivity and discrimination, and even these latter regions can be further distinguished across tasks. Additional studies using selective lesions targeting these hypothesized “hot spots” are planned.

171

Obesity interferes with the oro-sensory detection of long-chain fatty acids in Human

M CHEVROT[1], P PASSILLY-DEGRACE[1], G ENDERLY[2], D ANCEL[1], C MARTIN[1], M GOMES[1,2], I ROBIN[3], S ISSANCHOU[2], B VERGES[3], S NICKLAUS[2], P BESNARD[1]
[1]U866 Team 6 Dijon, France, [2]INRA Dijon, France, [3]U866 Team 8 Dijon, France

Existence of a negative correlation between the oro-sensory detection of dietary lipids and body mass index (BMI) was recently reported in Human. It was extrapolated that obesity might promote overconsumption of fatty foods by decreasing the detection of lipids in oral cavity. Psychophysical and physiological approaches were used to explore this hypothesis. Linoleic acid (LA) detection threshold was determined in lean (1930, n=30) volunteers using a 3-AFC test. Mean detection thresholds were 0.053% (w/v) and 0.075% (w/v) in lean and obese subjects, respectively and did not differ across groups. No relationship between LA detection threshold and BMI was observed but 5 obese subjects detect only the highest concentration (5%) or failed the test. Interestingly, these 5 subjects have a higher consumption of fatty foods. Oral stimulation with 1% LA emulsion induced a rise in plasma triglycerides level ([TG]pl), as compared to control emulsion in lean subjects. This [TG]pl up-regulation, considered as a biomarker for oral fat detection, was not retrieved in obese subjects. Altogether these data support that obesity may interfere with the oro-sensory detection of free fatty acids in Human.

172

The Effect of Product Information on Flavor Perception and Consumption

CA FORESTELL, KV CAVANAGH, B KRUJA
The College of William & Mary Williamsburg, VA, USA

In an increasingly obesogenic food environment, making healthful food decisions can be complex. Because consumers are often bombarded with excessive information about foods they often rely on simple heuristics to decide whether to purchase or consume a food. In the present study we tested females who were either restrained or unrestrained eaters to determine whether their perception and intake of a food would be affected by the presence of a brand name. All participants were provided with the same ad libitum snack. For half of the participants, the snack was labeled with a brand typically associated with healthful foods or a brand associated with unhealthful foods. Results indicated that all participants rated the snack with the healthful brand label as more satisfying and as having a better taste and flavor. Furthermore, restrained eaters consumed more of the snack if it was paired with a healthful brand than the unhealthful brand, whereas unrestrained eaters’

consumption did not differ. Thus it appears that food-related beliefs influence consumers' intake, especially that of restrained eaters. Further research is warranted to investigate these beliefs in order to improve recommendations for healthful eating in a society facing an increased prevalence of overeating and obesity.

173

Posterior Insular Cortex Damage is Associated with Conditioned Taste Aversion Deficits

K HASHIMOTO[1, 2], LA SCHIER[1], MB BALES[1], GD BLONDE[1], AC SPECTOR[1]
[1]Dept. of Psychology, Florida State University Tallahassee, FL, USA, [2]Faculty of Life Sciences, Kumamoto University Kumamoto, Japan

Bilateral lesions of gustatory cortex (GC), an area of insular cortex where taste-responsive neurons reside, reportedly eliminate a presurgically conditioned taste aversion (CTA). The effectiveness of GC lesions to impair expression of a postsurgical CTA is equivocal in the literature. To examine this further, rats were injected ip with LiCl (0.15M, 2 mEq/kg, n=38) or saline (0.15M, n=17) after ingesting 0.1M NaCl (15-min) on 2 trials. Separate groups of rats then received bilateral ibotenic acid infusions targeting the anterior GC (n=9), posterior GC (n=10), both anterior and posterior GC (n=20), or PBS infusions in anterior and posterior GC (SHAM, n=15). Extensive bilateral damage to GC (≥50%/side, mean damage = 88%, GCX, n=11) did not disrupt CTA retention in postsurgical 15-min 1-bottle and 48-h 2-bottle preference tests (0.1M NaCl versus dH_2O). GCX also failed to attenuate the expression of a CTA to 0.1M sucrose acquired postsurgically, but was associated with more rapid extinction across repeated 48-h 2-bottle tests. Interestingly, more comprehensive analyses of lesion topography of all LiCl-injected rats revealed that the extent of damage to posterior GC and especially areas surrounding posterior GC were best associated with CTA impairments. These results suggest that the regions more dorsal and caudal to the GC, which have been previously implicated in visceral function, may be more integral to CTA retention and acquisition than the GC itself.

174

Taste preference changes in different life stages of rats.

C INUI-YAMAMOTO[1], T YAMAMOTO[2], K UEDA[1], M NAKATSUKA[1], C AN[1], S KUMABE[1], Y IWAI[1]
[1]Dept. of Oral Anatomy, Osaka Dental Univ. Hirakata, Japan, [2]Faculty of Health Science, Kio Univ. Nara, Japan

Taste preferences are known to be affected by aging which causes changes in the dietary and energy requirements. However, the mechanisms of shift in taste preference by aging still remain unclear. Therefore, to elucidate differences in taste preference among the life stages, firstly we investigated preferences to several taste solutions in different ages of rats. Secondly, we recorded responses of the chorda tympani nerve to several taste stimuli. We used juvenile (3-6 weeks), young-adult (8-11 weeks), adult (17-20 weeks), middle-aged (34-37 weeks) and old-aged (69-72 weeks) Sprague-Dawley male rats. All rats were fed ad libitum during all tests. Taste solutions were NaCl (0.1, 0.3 M), HCl (10, 50 mM), sucrose (0.3, 0.5 M), saccharin-Na (5, 50 mM), quinine-HCl (0.3, 0.03 mM), and monosodium glutamate (MSG) (0.1, 0.3 M). The preference ratios for the 0.5 M sucrose and 0.1 M MSG in the middle-aged group were lower than those in the juvenile and young-adult groups. On the other hand, the preference ratio for the 0.03 mM quinine-HCl in the middle-aged group was higher than that in the juvenile and young-adult groups. There were no significant differences in the preference for HCl and NaCl among the groups. In the responses of the chorda tympani nerve to taste stimuli, there were no significant differences among the groups. These results suggest that aging changes taste preferences, but does not affect the neural activity of the chorda tympani nerve.

175

Functional knockout of forebrain 14-3-3 attenuates conditioned taste aversion.

A. KIMBROUGH, Y. WU, Y. ZHOU, T.A. HOUPT
Biological Science and College of Medicine, Neuroscience, Florida State Univ. Tallahassee, FL, USA

14-3-3 proteins are scaffolding proteins that sequester signaling proteins in the cytoplasm. 14-3-3 functional knock out (FKO) mice express a YFP-fused R18 peptide in brain neurons, which blocks 14-3-3 binding. Previously we found that while FKO mice can form a conditioned flavor preference and have normal unconditioned preferences for saccharin, polycose, quinine, HCl, and NaCl, the FKO mice were unable to form a CTA after a single pairing of CS (saccharin) and US (0.15M LiCl, 20 ml/kg or 40 ml/kg). We determined if FKO mice could acquire a CTA after repeated CS-US pairings. Water-restricted FKO or wildtype (wt) mice were conditioned on alternate days with 10-min access to 0.125% saccharin followed by LiCl or NaCl injection (0.15M, 20 ml/kg, ip). Mice received a total of 6 CS-US pairings. A 30-min 2-bottle preference test was given after conditioning days 3 and 6. Extinction was measured with separate groups of mice given either 30-min or 24-h preference tests of saccharin vs. water for 8 days. LiCl-injected wt mice acquired a significant CTA which persisted throughout 30-min/day extinction or slowly extinguished during 24-h/day extinction. LiCl-treated FKO mice formed a CTA after multiple pairings. The CTA in FKO mice extinguished after 1 day of 2-bottle testing (either 30 min or 24 h). Thus, 14-3-3 FKO mice can acquire a CTA after multiple CS-US pairings, but it extinguishes within 30min-24h. 14-3-3 is critical for CTA learning. The

timing, locus, and identity of proteins binding to 14-3-3 and contributing to CTA remain to be determined.

176

Individual variation in taste phenotype predicts susceptibility to weight gain on a highly palatable diet

GC LONEY, LM POLISCHUCK, LA ECKEL
Program in Neuroscience, Florida State University Tallahassee, FL, USA

The perceived taste of a food is a major determinant of its associated pleasantness and the willingness of an organism to consume it. As such, taste is a major factor governing diet choice and caloric intake. Recent studies have demonstrated that rats express individual differences in the taste perception of the artificial sweetener sucralose such that animals can be unambiguously categorized as either sucralose avoiding (SA) or sucralose preferring (SP). Here, we examined whether this taste phenotype influences caloric intake, diet choice, and weight gain. Food intake and body weight were recorded daily in SP and SA rats (n = 21) given 4 weeks access to a highly palatable diet (chow supplemented with Borden's sweetened condensed milk, diluted 1:2 with water). Total caloric intake was greater in SP rats, relative to SA rats, on 25 of the 27 days of palatable diet exposure. SP rats gained more weight while consuming this diet than SA rats (155 ± 9.6 g vs. 172 ± 5.5 g, respectively, $P < 0.05$). To investigate whether SP rats may be less responsive to satiety cues than SA rats, we monitored 1-h intake of a palatable .8M sucrose solution offered immediately after a chow meal in rats adapted to a restricted-feeding schedule. Preliminary analysis (n = 11) of this "dessert" test, revealed that SP rats consumed more sucrose than SA rats (10.5 ± 1.5 g vs. 7.5 ± 0.8 g, respectively, P = 0.08). These findings suggest that SP rats are more susceptible than SA rats to the drive to consume palatable food beyond that needed for homeostatic regulation.

177

Comparative immunolocalization of the lipid-receptors CD36 and GPR-120 in taste buds cells from mouse circumvallate papillae

D ANCEL[1], P PASSILLY-DEGRACE[1], C SCHNEIDER[2], C MARTIN[1], P BESNARD[1]
[1]*INSERM U866 Dijon, France,* [2]*AgroEcology Dijon, France*

The lipid-receptors CD36 and GPR120 have been shown to be involved in the preference for fat. However, their respective role in the lipid taste reception remains a matter of debate. Although they share similar specificity for long-chain fatty acids (LCFA), their affinity greatly differs, the magnitude of CD36 binding affinity being greater than that of GPR120. Recent data suggests a role of *"signaling bridge"* played by CD36 or related-receptors in the recognition and transfer of lipid ligands to other lipid receptors (*e.g*. Toll-like receptors or olfactory receptors). Consistently with this model, it is tempting to speculate that lingual CD36 might also serve as an essential co-receptor in taste buds by transferring LCFA to GPR120. To address this question, a computer program allowing an automatic quantification of the fluorescence volume per taste bud cell (TBC) and /or per taste bud, produced by CD36 and GPR120 labeled using a conventional immunohistochemistry method for confocal microscopy was produced. We found that CD36 and GPR120 are generally co-expressed in the same TBC from mouse circumvallate papillae (CVP). Nevertheless, whether GPR120-positive cells always express CD36, the converse is not true. This finding suggests that lingual CD36 and GPR120 might have different but, likely, complementary function in the orosensory detection of dietary lipids in the mouse.

178

Taste alters the disposition of fat by modulating gastric emptying in rats

K SAITO[1,2], J LEES[1], M TORDOFF[1]
[1]*Monell Chemical Senses Center Philadelphia, PA, USA,* [2]*Kao Corporation Tokyo, Japan*

Oral sensations guide the disposition as well as the selection of nutrients. Here, we investigated how preferred and nonpreferred tastes influence the disposition of fat. Adult male Sprague Dawley rats were infused with 5 ml of 20% intralipid through an intragastric catheter and with 0.3 ml of a taste solution through an intraoral catheter. Tail vein blood was sampled. Concentrations of fat fuels (plasma triglycerides and nonesterified fatty acids) were slightly higher at 2 and 4 h after rats tasted a sweet solution (0.125% saccharin + 3% glucose) than after they tasted water. They were markedly lower after rats tasted a non-preferred solution—either 0.15% quinine hydrochloride or a sweet solution that had previously been the conditioned stimulus for lithium-induced taste aversion. The disposition of ^{14}C-triolein mixed with the gastric load was determined. At 4 h postinfusion, rats that received a nonpreferred taste had significantly more ^{14}C remaining in the stomach than did those that received water to taste. The simplest explanation of these findings is that taste—particularly bad taste—influences fat disposition by altering gastric emptying.

179

Caloric Intraduodenal Preloads Modulate Taste Reactivity to an Intraoral Taste Stimulus Associated with LiCl-induced Malaise

LA SCHIER, AC SPECTOR
Dept. of Psychology, Florida State University Tallahassee, FL, USA

Stimulation of the GI tract inhibits taste-responsive neurons in the brainstem. Given that taste receptors also

reside in GI cells, it is of interest whether postoral *taste-like* signals influence oral taste processing in a chemospecific manner to ultimately impact taste-guided behaviors. Here we assessed if affective responses to an IO sweet stimulus (0.3M sucrose) were best modulated by an ID preload (3ml) of: a) a matching chemical stimulus (0.3M sucrose, 0.4kcal/ml), b) an isocaloric non-matching stimulus (Intralipid), or c) a non-caloric control (0.15M NaCl) in a serial taste reactivity (TR) test (0.5ml/30s IO infusions once every 3min for a total of 15min). Prior to the test, rats were given two 15min sessions to ingest 0.3M sucrose in the home-cage, each directly followed by either LiCl injection (2 mEq/kg, ip) to condition aversions to sucrose (CTA, n=22) or saline injection (unconditioned, n=19). Our earlier work indicated that ID sucrose curbed ingestive TR while increasing aversive TR to an IO sucrose stimulus previously rendered aversive via conditioning. Here, both ID sucrose and Intralipid significantly suppressed ingestive TR in the CTA group; aversive TR increased but was variable and not signficant. ID sucrose and Intralipid did not differentially affect TR to IO sucrose, relative to ID NaCl, in unconditioned rats. These results suggest that ID caloric stimuli may augment taste-driven behaviors under some conditions (e.g., CTA). Ongoing tests are further exploring the extent of chemospecificity in such taste-visceral integrative processing.

180

Experimentally reducing CD36 mRNA on the circumvallate papillae decreases fat preference in obesity-resistant rats, not obesity-prone rats

AL SCHREIBER[1], KP ARCENEAUX[2], SD PRIMEAUX[1,2]
[1]Joint Diabetes, Endocrinology, & Metabolism Program, LSU Health Science Center New Orleans, LA, USA,
[2]Pennington Biomedical Research Center Baton Rouge, LA, USA

The detection of dietary fat mediates the intake of high fat foods and may be regulated by the oral cavity. Differences in fatty acid sensors in the oral cavity have been proposed as mechanisms contributing to the susceptibility in becoming obese. The fatty acid receptor, CD36, is expressed on the circumvallate papillae (CV) of the tongue and is a potential taste receptor for fat. The goal of the current study was to examine the role of CV CD36 on fat preference in obesity-prone Osborne-Mendel (OM) rats and obesity-resistant S5B/Pl (S5B) rats. OM and S5B rats were habituated to a non-pelleted high fat or low fat diet using a two-choice paradigm. RNA interference techniques were used to reduce the expression of CD36 on the CV. CD36 siRNA or non-targeting control siRNA were applied to the CV for 5 days. Food intake and fat preference were determined during the application of siRNA and for 6 days following application. On Day 2 of siRNA application, CD36 siRNA transiently decreased fat preference in OM and S5B rats. Immediately following siRNA application, CD36 siRNA decreased fat preference in the obesity-resistant S5B rats, but not in the obesity-prone OM rats. These data indicate that CD36 on the tongue mediates fat preference in obesity-resistant S5B rats. A reduction in CD36 on the tongue of obesity-prone OM rats was not sufficient to decrease fat preference in this strain.

181

Taste-evoked Arc expression in the mouse brainstem

J TÖLE, F STÄHLER, W MEYERHOF
Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbrücke Nuthetal, Germany

The purpose of taste is to evaluate the nutritive value of food and to prevent the organism from ingesting noxious substances. While many bitter tastants have adverse effects and therefore justify rejection behavior, our daily diet contains numerous compounds that are recognized as bitter and yet are harmless or even have beneficial effects. Thus, a mechanism to discriminate harmful from advantageous bitter compounds could be beneficial. Differential neuronal processing would be a prerequisite for a putative discrimination among bitter substances. To analyze processing of bitter information in the first gustatory relay station, the NTS, we established the Arc catFISH method for the taste system. Based on the strict temporal pattern of intracellular distribution of the Arc RNA, this method allows to conclude by which of two subsequent stimuli a neuron was activated. To this end, we applied taste stimuli to the oral cavity of mice either 30 or 5 minutes prior to sacrifice, or at both timepoints. Arc expression was analyzed using fluorescent in situ hybridization and Arc-positive neurons were quantified. Interestingly, only about a third of the Arc-expressing neurons were activated by both of two sequentially applied bitter stimuli. Furthermore, the fraction of neurons activated twice was higher when the same bitter stimulus was applied as opposed to when two different bitter compounds were used. This indicates that bitter information in the NTS is processed by distinct, yet overlapping neuron populations providing the basis for discrimination of bitter tastants in the brainstem.

182

A possible role for salivary proteins in bitter food acceptance

A-M. TORREGROSSA, L.V. NIKONOVA, L.E. MARTIN
Florida State University Tallahassee, FL, USA

Few studies have examined the influence of salivary proteins on diet acceptance, but it has been demonstrated that salivary proline-rich proteins (PRPs) bind to bitter-tasting tannins and may function to increase the acceptability of tannin-containing diets. The role of salivary PRPs, however, has not been well explored with other bitter taste stimuli. To address this question, we collected saliva samples and measured spontaneous

feeding behavior in rats (n=8) fed a control diet (16 days) followed by a diet containing 0.4% quinine (14 days). PRPs were upregulated during quinine exposure ($p<0.01$) and were expressed maximally by the sixth day of quinine exposure. Total food intake was reduced while rats were fed the quinine diet ($p<0.01$). Meal size (g/meal), a measure of postingestive feedback, and rate of feeding (g consumed/sec), a measure of palatability, were reduced during the first 3 days on the quinine diet ($p's<0.01$) but returned to near-baseline levels by the sixth day of the diet presentation. Rats increased the number of meals (in 24-h) consumed during the first 3 days of the quinine diet, but decreased the number of meals over time ($p<0.01$). The increase in PRPs, in coincidence with alterations in feeding behavior, suggests that PRPs are a compensatory response to quinine adulteration and may play a role in altering the acceptability of a quinine-adulterated diet.

183

Modulation of sweet taste sensitivities by leptin and endocannabinoids

R YOSHIDA, M NIKI, M JYOTAKI, T OHKURI, N SHIGEMURA, Y NINOMIYA
Section of Oral Neuroscience, Graduate School of Dental Sciences, Kyushu University Fukuoka, Japan

Leptin (Lep) and endocannabinoids (EDs) have reciprocal effects on sweet taste sensitivities of mice, suppression and enhancement, respectively. However the endogenous action of Lep and EDs on taste responses is not fully understood. In this study, we examined expression of related molecules, the effect of leptin on taste cell responses and the effect of antagonists for Ob-Rb (leptin L39A/D40A/F41A : LA) and CB_1 (AM251) on the chorda tympani (CT) responses in mice with different serum Lep levels. About 40 % of taste cells expressing T1r3 coexpressed Ob-Rb and a subset of taste cells expressed biosynthesizing enzyme (DAGL) and degrading enzyme (MAGL) of ED (2-AG). In about half of sweet sensitive taste cells, 20 ng/ml leptin suppressed responses to sweeteners. The effect of leptin was concentration dependent, reached maximal level at 10-20 ng/ml and was inhibited by LA. Administration of LA significantly increased CT responses to sweeteners in lean mice, whereas administration of AM251 did not affect. Moreover the effect of LA on CT responses to sweeteners gradually decreased with increasing plasma leptin levels, whereas the reverse is true for ECs. These results suggest a possibility that circulating Lep may act as a modulator in mice that tonically influence basal sweet sensitivity, while ECs may become more effective with defects in Lep system. Supported by JSPS KAKENHI Grant Number 18077004, 18109013, 23249081 (YN) and 21791808, 23689076 (RY).

184

Caffeine increases salivary habituation to olfactory food cues

AM ZIEGLER, N KASAD, K LYNCH, C OLVIDA, JL TEMPLE
SUNY University at Buffalo Buffalo, NY, USA

Habituation is a basic property of the nervous system whereby responses to stimuli decrease with repeated exposure. Salivary habituation to food cues is a reliable predictor of energy intake. Furthermore, the rate of habituation is correlated with energy intake, body weight, and weight gain and/or loss. Thus, finding ways to increase the rate of habituation to food may reduce energy intake and prevent weight gain. We tested the hypothesis that caffeine administration would increase the rate of salivary habituation to olfactory food cues and reduce post-testing energy intake compared to placebo. Adult participants visited the laboratory on two occasions and consumed a beverage containing a placebo on one visit and caffeine (2 mg/kg) on another. Thirty minutes later, participants had saliva collected after eight, one-minute exposures to olfactory and visual cues from pizza with two minute intertrial intervals to establish habituation. These trials were followed by three, one-minute exposures to olfactory and visual cues from brownies to demonstrate dishabituation to the novel food. At the end of the saliva collection, participants were given *ad libitum* pizza and brownies to eat. We found that caffeine administration increased the rate of salivary habituation compared to placebo. In addition, energy intake from pizza was reduced after caffeine consumption, but not energy intake from brownies. When taken together, these results suggest that caffeine can increase the rate of salivary habituation to food cues and that this reduced habituation may be a mechanism by which acute caffeine reduces energy intake.

185

Acute modulation of sympathetic nervous system (SNS) activity does not alter energy expenditure (EE), diet-induced thermogenesis (DIT) or brown adipose tissue (BAT) temperature after Roux-en-Y gastric bypass (RYGB) surgery in rats

K ABEGG[1], M BUETER[2], TA LUTZ[1]
[1]Inst Vet Physiol, Univ Zurich Zurich, Switzerland, [2]Dept Visc Transpl Surg, Univ Hosp Zurich Zurich, Switzerland

The compensatory decrease in EE in response to body weight loss is attenuated after RYGB surgery, potentially due to increased DIT. Because increased postprandial gut hormone levels after RYGB could increase DIT by stimulating SNS activity in the BAT, we investigated if acute modulation of SNS signaling alters EE, DIT and BAT temperature (T) differently after RYGB and sham surgery. Adult male Wistar rats were randomized for RYGB (n=6), sham surgery (n=5) or sham surgery body

weight-matched (n=5). T sensors were implanted into BAT of all rats. The effects of acute intraperitoneal administration of the b3-agonist CL316,243 (CL, 0.1 mg/kg) on fasting EE and BAT T and the b-adrenergic antagonist propranolol (P, 20 mg/kg) on DIT and BAT T after a test meal were tested using indirect calorimetry and telemetry. Rats were fasted for 12 hours and injected in the mid light cycle. BAT T was higher in sham compared to BWm and RYGB rats during light and dark phase in both fasted and ad libitum fed state, but there was no difference between BWm and RYGB. CL increased EE and BAT T in all rats. The increase in EE, but not in BAT T, was higher in sham compared to BWm and RYGB rats. P treatment did not alter DIT or BAT T differently in RYGB, sham or BWm rats. DIT of control treated animals did not differ between surgery groups. We conclude that altered EE in RYGB rats is not caused by increased DIT or differences in BAT SNS activity.

186

The Hypothalamic Chemoarchitecture Project: High Resolution Mapping of Neuronal Populations Involved in Pre-autonomic, Neuroendocrine, and Feeding Control

BE PINALES[1], GA LEAN[2], CE WELLS[1,3], ND SILVA[1], EM WALKER[1,3], P ROJAS[1], N DOMINGUEZ[1], A MARTINEZ[1,3], RH THOMPSON[2], AM KHAN[1,2,4]
[1]Dept. of Biological Sciences, Univ. of Texas at El Paso (UTEP) El Paso, TX, USA, [2]Border Biomedical Research Center, UTEP El Paso, TX, USA, [3]Graduate Program in Pathobiology, UTEP El Paso, TX, USA, [4]Dept. of Biological Sciences, University of Southern California Los Angeles, CA, USA

Several neurochemical systems help control complex motivated behaviors and neuroendocrine and autonomic functions, but little is known about how these systems are spatially interrelated within the brain. We are conducting a large scale, wide field multi-fluorescence mapping project for the hypothalamus of the adult male rat, in which we are identifying nearly two dozen chemical systems within the same brain at high resolution. We have identified many peptidergic neuronal populations, including those for acetylcholinesterase, Agouti-related peptide, calbindin, co-peptin, enkephalin, hypocretin/orexin, melanin concentrating hormone, alpha melanocyte stimulating hormone, neuronal nitric oxide synthase, neurotensin, parvalbumin and Substance P. Several of these peptides are also being visualized in relation to hypothalamic angioarchitecture. Our analyses are revealing novel interrelationships for these chemical systems which are being mapped within the Swanson rat brain atlas. This dataset should provide a greater understanding of how the hypothalamus coordinates autonomic, neuroendocrine and behavioral responses to changes in metabolic status and behavioral state.

187

Short and Long SNS-Sensory Feedback Loops in White Fat

V RYU, TJ BARTNESS
Dept. of Biology, Georgia State University Atlanta, GA, USA

We previously demonstrated white adipose tissue (WAT) innervation using the established retrograde sympathetic nervous system (SNS)-specific transneuronal viral tract tracer, pseudorabies virus (PRV) and showed its role in the control of lipolysis. Conversely, we demonstrated WAT sensory innervation using the established anterograde sensory system (SS)-specific transneuronal viral tracer, the H129 strain of herpes simplex virus-1, with sensory nerves showing responsiveness with increases in SNS drive to WAT. Between these studies, several brain areas were part of both the SNS outflow and SS inflow from WAT suggesting SNS-SS feedback loops. Therefore, we injected both PRV and H129 into inguinal WAT (IWAT) of Siberian hamsters. Animals were perfused on Days 5 and 6 post inoculation after H129 and PRV injections, respectively, and brains and spinal cords were processed for immunohistochemical detection of each virus across the neuroaxis. The presence of H129+PRV colocalized neurons in the spinal segments innervating IWAT suggested short SNS-SS loops with significant co-infections (>60%) in discrete regions of several brain areas, notably the medial preoptic area, paraventricular nucleus, lateral hypothalamus, deep mesencephalic nucleus, periaqueductal grey, intermediate reticular nucleus and the nucleus of the solitary tract. Collectively, these results strongly indicate the neuroanatomical reality of the central SNS-SS feedback loops with short loops in the spinal cord and long loops in the brain, both likely involved in the control of lipolysis.

188

The impact of macronutrients on brown adipose tissue thermogenesis

A STEFANIDIS, D NGUYEN, MJ WATT, BJ OLDFIELD
Department of Physiology, Monash University Melbourne, Australia

The identification of brown adipose tissue (BAT) in adult humans and importance in the determination of levels of obesity has led to a renaissance in this field, particularly in relation to the potential for it to be targeted as an anti-obesity therapy. Furthermore, dietary macronutrients have been shown to be unequal in terms of their impact on *food intake*; however, the outstanding question is how different macronutrients drive *energy expenditure*. These experiments aim to characterize the impact of specific macronutrient groups on energy expenditure in BAT and gain an insight into the underlying mechanism(s). Adult male Sprague Dawley rats were surgically implanted with a cannula directed into the stomach and exteriorized subcutaneously in the dorsal region of the neck. At the

same time, a telemetric device was implanted between the interscapular lobes of the BAT to measure shifts in local temperature, indicative of thermogenic activity. Macronutrients (glucose, lipid, protein) were matched for calories and volume and infused 1) directly into the stomach or 2) towards the brain (via carotid artery). The administration of glucose, lipid or protein, both peripherally or towards the brain causes a differential activation of BAT thermogenesis. There was a significant impact of all macronutrients, with the most profound effect on BAT activity derived from lipid, followed by protein and glucose. These data provide a framework for the formulation of "smart diets" that will allow the effective control of body weight through modulation of energy expenditure.

189

Diet-induced obesity decreases the preference for lipids in the mouse

A BERNARD, M CHEVROT, D ANCEL, M BUTTET, S ABDOUL-AZIZE, C MARTIN, JF MERLIN, H POIRIER, I NIOT, NA KHAN, P PASSILLY-DEGRACE, P BESNARD
AgroSup Dijon, UMR U866 INSERM DIJON, France

Recent data suggest the existence of a relationship between oro-sensory sensitivity to dietary lipids, regulation of fat intake and body mass index. However, the mechanisms by which changes in taste sensitivity take place remain poorly understood. Whether obesity by itself can affect the preference for fatty foods is unknown. To address this question, mice were subjected to obesogenic diets mainly composed of saturated lipids alone or added with sucrose. Using a combination of biochemical, physiological and behavioral approaches, we observed that: 1°) the preference for lipids, determined by using the two-bottle preference test paradigm, is decreased in diet-induced obese (DIO) mice whatever the composition of the obesogenic diets, 2°) this behavioral change has an oro-sensory origin since it is retrieved when post-oral cues are minimized (1 min licking test), 3°) this phenomenon is reversible, attraction for lipid being restored in calorie-restricted DIO mice, 4°) the lipid preference is inversely correlated to adipose tissue size, 5°) the decrease in the level of the lipid-receptor CD36 usually found in gustatory circumvallate papillae from fasted control mice refed a lipid diet is altered in DIO animals, 6°) the CD36-dependent calcium signaling in taste bud cells is disturbed in DIO mice. Altogether, these data bring the first demonstration that obesity alters the oral lipid detection system leading to a decrease in the preference for dietary lipids in the mouse.

190

Does brief repeated access to sugar-sweetened water induce obesity?

M JOURNEL, G FROMENTIN, C CHAUMONTET, N DARCEL, D TOME
AgroParisTech, INRA, UMR914 Nutrition Physiology and Ingestive Behavior Paris, France

Introduction: Sugar-sweetened beverages are an important contributory factor to the obesity epidemic, and the development of animal models is necessary to understand the underlying mechanisms. Our goal was to observe, in the adult mouse, the effects of 14% w/v sucrose-sweetened water (SSW) on weight gain, feeding behavior and central pathways involved in homeostasis and reward. **Methods**: Over 5 weeks, 42 C57BL/6 male adult mice (7wks old at arrival) on a 9.30am-9.30pm dark cycle with time-limited (9.30am-5pm) access to a normal fat (NF) or a high-fat (HF) diet, were given the same time-limited access to water, SSW, or "2hSSW" (time-limited water plus SSW for a randomly-positioned 2h period within 9.30am-5pm), giving 6 diet-drink groups (n=7 each). We measured body weight and composition, diet and drink intake, and (by qRT-PCR) the relative expression of various peptides and receptors in the hypothalamus and nucleus accumbens. **Results:** NF-SSW and HF-2hSSW groups did not compensate for the energy contained in the SSW, gaining more weight than mice drinking water and eating the same diet, respectively. 2hSSW access also caused intra-day hyperphagia of the HF diet after removal of SSW, and these mice increased their SSW intake over 5 weeks. Finally, mice drinking 2hSSW under HF had decreased signaling in the hypothalamus, and an increased signaling in the nucleus accumbens under NF. **Conclusion:** These results demonstrate that, depending upon diet and access, SSW has differential effects on body weight, food behavior and neuronal signaling.

191

Peripubertal high fat diet (HFD) exposure impairs central information processing

MA LABOUESSE, W LANGHANS, U MEYER
Physiology and Behavior Laboratory, ETH Zurich Zurich, Switzerland

HFD consumption is suspected to lead to impairments in basic cognitive functions such as learning and memory, but the consequences of HFD on central information processing remain elusive. Here we fed male mice a HFD (60% kcal from fat) or a control diet (CD) for 8 weeks (starting postnatal day P28) and studied central information processing using the paradigms of prepulse inhibition (PPI) and latent inhibition (LI). PPI is an operational measure of sensorimotor gating, which reflects the ability to filter intrusive sensory-motor information. LI is a form of selective associative learning,

considered to index an organism's capacity to ignore irrelevant stimuli. HFD mice displayed an overall 25% reduction in PPI scores as compared to CD mice ($p<0.05$). CD mice displayed a robust LI effect ($p<0.05$) that was fully abolished in HFD mice. Also, HFD feeding led to presynaptic dopaminergic abnormalities in the form of increased tyrosine hydroxylase density in the nucleus accumbens core ($p<0.05$) and shell ($p<0.001$), and the HFD-induced disruption of PPI was restored ($p<0.01$) by systemic administration of the dopamine (DA) receptor antagonist haloperidol, highlighting a potential contribution of the accumbal DA system to HFD-induced PPI deficits. Finally, our results indicate that short-term HFD exposure in peripuberty (P28-56) ($p<0.05$), but not in adulthood (P70-98), leads to impairments in PPI. In summary, our findings add further weight to the emerging role of dietary influences on brain functions and draw particular attention to peripuberty as a vulnerable period to the deleterious effects of HFD.

192

DIFFERENT REGULATION OF PRODYNORPHIN AND PRONOCICEPTIN GENES EXPRESSION IN RESISTANCE TO DIET-INDUCED OBESITY

MV MICIONI DI B.[1], M PUCCI[2], R CICCOCIOPPO[1], M MACCARRONE[3], S GAETANI[4], C D'ADDARIO[2], C CIFANI[1]

[1]University of Camerino Camerino, Italy, [2]University of Teramo Teramo, Italy, [3]Campus Bio-Medico Univ Rome Rome, Italy, [4]Sapienza University Rome Rome, Italy

Obesity, one of the major global health issues is a complex disease strongly influenced by our genetic background. Among the different mechanisms leading to inter-individual differences in obesity, epigenetic regulation of gene expression recently emerged as a potentially important contributor. We used the Diet-induced obesity (DIO) rat model to analyze the differences in the epigenetic regulation of prodynorhin (PDYN) and pronociceptin (PNOC) genes between outbred Sprague-Dawley rats placed on a high-fat diet becoming obese (DIO), compared their diet resistant (DR) counterparts. Gene expression analysis revealed in the hypothalamus (HY) of DR rats a significant reduction of PNOC mRNA when compared to DIO rats as well as to control chow-fed group. Moreover, in the nucleus accumbens (NA) of DR rats a significant increase in PNOC counteracted by a decrease in PDYN gene expression was observed. Consistently, DNA methylation at PDYN promoter resulted to be increased in DR rats in the NA. No changes in DNA methylation status at PNOC promoter were observed in both HY and NA. We thus show selective changes in opioid peptides gene expression in DR rats key brain regions in homeostatic (HY) and hedonic (NA) eating, confirming a major role for PNOC in body weight gain in the HY. Moreover, in the NA, PDYN epigenetic regulation provides new insight for possible interventions, either through nutrition or specific drugs, to modify obesity risk.

193

Effects of Diet-Induced Obesity and Reversal of Circadian Rhythms on Body Weight, Metabolism, and Activity in Rats

HM MURPHY, CH WIDEMAN

John Carroll University University Heights, OH, USA

Obesity in America has risen sharply in the past three decades. Maintenance of the balance between diet and activity depends upon the body's naturally occurring circadian rhythm, a pattern of rest and activity. When this pattern is disrupted, as seen in people on shift work schedules, many systems cannot adjust and debilitating effects are seen in body weight, food intake, adipose tissue deposition, metabolism, and activity. Utilizing a rodent model, the present experiment sought to understand the effects of a high-fat diet and circadian de-synchronization on body weight, caloric intake, feed efficiency, adiposity, and activity. Animals were fed a high-fat diet or a control diet during the dark phase or light phase of the circadian cycle. Not surprisingly, rats fed a high-fat diet gained more body weight and had significantly more adiposity than those fed a control-diet, whether fed during the normal dark cycle or an altered cycle. Of particular interest, however, were the findings that light-fed animals consumed significantly fewer calories than dark-fed animals and feed efficiency was significantly more positive in light-fed animals, suggesting that metabolic conditions were compromised under altered circadian conditions. There was also a dramatic decrease in activity levels in those animals with altered circadian rhythms. Results indicate that a high-fat diet and circadian misalignment lead to significant disruptive changes in the variables examined. These changes mimic those observed in humans working on shift work schedules.

194

Increased Fat Consumption Exacerbates Metabolic and Behavioral Effects of PCOS in a Rodent Model

IB RESSLER, BE GRAYSON, YM ULRICH-LAI, RJ SEELEY

University of Cincinnati Cincinnati, OH, USA

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting women of reproductive age. Though a comorbidity of PCOS is obesity, some women are lean. We hypothesized that increased saturated fat consumption would exacerbate metabolic and stress indices in a rodent model of PCOS. Female rats were implanted with dihydrotestosterone (DHT) or placebo pellets and half of each group was maintained *ad libitum* on either a high-fat diet (HFD) or nutrient-matched low-fat diet (LFD). DHT-treated animals gained more body

weight, had irregular cycles and were glucose intolerant in comparison to controls, irrespective of the diet consumed. Furthermore, HFD-fed, DHT-treated animals resulted in the highest levels of fat mass and insulin resistance. With regard to stress behaviors, DHT–treated animals demonstrated increased anxiety in the elevated plus maze by decreased distance travelled and time in the open arms. In contrast, HFD consumption increased immobility during the forced swim test. DHT-treatment suppressed diurnal corticosterone measurements in both the LFD and HFD groups. In parallel, DHT-treatment significantly dampened stress responsivity during a mild stressor. The brains of these animals showed attenuated cFos activation in the VMH and ARC; irrespective of DHT-treatment, however, all HFD animals had elevated PVN cFos activation. In conclusion, while hyperandrogenism drives overall body weight gain, glucose intolerance, anxiety behaviors and stress responsivity, HFD consumption exacerbates adiposity, insulin resistance and depressive behaviors.

195

Salience resting state network integrity is increased in obesity and predicts orbitofrontal cortex activation to high-calorie food cues

JA STARKE[1], MA ANJARI[1], CG PRECHTL[1], S SCHOLTZ[1], AD MIRAS[1], N CHHINA[1], G DURIGHEL[1], ML SLEETH[2], GS FROST[2], JD BELL[1], AP GOLDSTONE[1]

[1]Metabolic and Molecular Imaging Group, MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital London, United Kingdom, [2]Division of Diabetes, Endocrinology and Metabolism, Imperial College London, Hammersmith Hospital London, United Kingdom

Obesity and fasting may alter food hedonics and brain reward systems. Brain activity in the resting state (in absence of a task) may also be functionally important. Identified resting state networks include a salience network (SALN) involving insula, ventral anterior cingulate (vACC) and orbitofrontal (OFC) cortex, regions also activated by food cues. We hypothesised that the SALN is altered in obesity and by feeding state. 83 adults (64% male, mean ± SD age 33.2 ± 10.7y, BMI 19.1-53.1 kg/m^2, 29 lean, 28 overweight, 26 obese) had 10min resting state fMRI after an overnight fast. 22 of the non-obese subjects (17 male) re-attended twice, remaining fasted or receiving a 730kCal breakfast, and 85min later had resting state fMRI followed by task fMRI while rating the appeal of food pictures. Obesity was associated with increased resting SALN integrity including OFC, vACC and insula ($P<0.05$). In the sub-cohort, there was no significant effect of fasting on SALN integrity ($P=0.70$-0.75 vs. fed). When fasted, resting SALN integrity in the OFC was positively correlated with task OFC activation to high-calorie foods ($r=+0.52$, $P=0.02$), but this was not significant to low-calorie foods or when fed ($P=0.2$-0.8). In conclusion, the salience network at rest may encode aspects of food reward given the acute and chronic influences of nutritional state.

196

Sucrose intake measured in a 23-h two-bottle test, decreases following several days maintained on a high fat diet

Y TREESUKOSOL, T.H. MORAN

Department of Psychiatry & Behavioral Sciences, School of Medicine, Johns Hopkins University Baltimore, MD, USA

Prior orosensory and ingestive experience influences food selection and intake. The hyperphagia observed when animals are presented a calorically-dense diet may be driven by enhanced orosensory stimulation and/or reduced inhibitory cues. Here, intake to an array of sucrose concentrations was measured across 23-h sucrose vs. water 2-bottle intake tests. After the first test series, rats were switched to a 45% high fat diet (HF) or maintained on standard chow (CHOW). Following 5 days on the respective diets, the sucrose concentration array was re-tested. The CHOW group displayed increased sucrose intake to the mid-range concentrations, but unexpectedly, the HF animals failed to increase intake to sucrose. In fact, the HF group showed decreased intake at the higher concentrations. Additionally, during sucrose testing both groups reduced diet intake as sucrose solution intake increased. The increase in sucrose intake and decrease in diet intake were less pronounced in HF animals such that total caloric intake across sucrose testing days was comparable across the two groups. There was no significant group difference in body weight thus the main effect of group revealed when comparing sucrose intake appears to be attributed to changes associated with high fat diet exposure. Possible explanations for these changes may be related to alterations in orosensory responsivity, conditioned preferences, satiety signaling or reward circuits. These possibilities are not mutually exclusive.

197

Weight Suppression as Sole Predictor of Weight Gain in a Sample of College Age Students

SR WINTER[1], ML BUTRYN[1], E STICE[2], N MARTI[3], MR LOWE[1]

[1]Drexel University Philadelphia, PA, USA, [2]Oregon Research Institute Eugene, OR, USA, [3]University of Texas Austin, TX, USA

Dieting and other measures of restrained eating have been discussed as potential predictors of weight gain in young adults. This study aimed to examine measures often utilized as predictors of weight gain and the relationship with actual weight changes over time in 199 female weight gain prone college students. Despite great variability in weight among individuals in the sample, the

sample as a whole did not experience significant weight change through a two-year follow-up. As a result, separate univariable logistic regression analyses were performed for the hypothesized risk factors (which included self-report assessments of dieting, restrained eating, disinhibition and emotional eating) and revealed that none of these traditional self-report measures predicted weight gain among those who gained at least 10% of their body weight in this sample. However, weight suppression (the numeric discrepancy between highest historical adult weight and current weight) did emerge as the sole predictor of significant weight gain, $b = .07$, $SE_b = .03$, $p = .02$, 95% CI [.01, .13]. These results indicate that among many theoretically viable risk factors, weight suppression alone predicted weight gain in this atypical sample of college students. Thus, interventions targeting a much younger demographic should be assessed in order to prevent weight suppression and ultimately sizable weight gain.

198

Altered Flavor Preference Formation in Overweight Individuals

AE WRAY[1], RK BABBS[1,2], G COPPIN[1,2], MG VELDHUIZEN[1,2], IE DE ARAUJO[1,2], DM SMALL[1,2]
[1]John B. Pierce Laboratory New Haven, CT, USA, [2]Yale University New Haven, CT, USA

Flavor preferences are thought to be dependent upon associative learning. One critical associative process is flavor-nutrient conditioning (FNC) whereby preference increases for a flavor paired with positive post-ingestive consequences (Yeomans, 2007). This form of learning depends critically on dopamine signaling (Sclafani, 2011). Since dopamine signaling is blunted in obesity we predicted that FNC would be impaired in those with higher BMI. 31 healthy weight (HW, BMI=21.3±0.4) and 19 overweight (OW, BMI=27.1±0.5) non-dieting healthy subjects were enrolled. Fasted subjects rated their liking for non-caloric novel flavored beverages before and after consuming these beverages paired with either 112.5 kcals from maltodextrin (CS+) or 0 kcals (CS-). Triangle tests ruled out subjects who were able to detect maltodextrin. Following the pre-test subjects underwent 4 exposure sessions in which, on separate days, they drank either the caloric or non-caloric beverage in a fasted state (lunch and dinner). Stimulus ratings of the non-caloric versions of the beverages were reassessed at post-test. All subjects rated all flavors as slightly liked at pre-test. As predicted, liking ratings increased significantly to "like moderately" for the CS+, but not CS- flavor in the HW group following exposure. In striking contrast, liking ratings for the CS+, but not CS- flavor decreased significantly to "moderately disliked" in the OW group after exposure. These results demonstrate that flavor preference formation is disrupted in OW individuals.

199

Leptin and cannabinoids modulate sweet sensitivities of enteroendocrine STC-1 cells.

M JYOTAKI, R YOSHIDA, K SANEMATSU, N SHIGEMURA, Y NINOMIYA
Section of Oral Neuroscience, Graduate School of Dental Sciences, Kyushu University Fukuoka, Japan

Sweet-sensing taste receptor cells are very important for animal to be able to detect carbohydrate sources of calories. Previously, we have demonstrated that sweet taste sensitivity in taste receptor cells would be affected by leptin and endocannabinoids [ECs: 2-arachidonoly glycerol (2-AG) and anandamide] in mice. Leptin, an anorexigenic mediator, selectively suppresses sweet taste responses. In contrast, ECs, orexigenic mediators, selectively enhance sweet responses. Recent studies have demonstrated that a sweet taste receptor is expressed in endocrine cells of the gastrointestinal tract and is involved in the secretion of gastrointestinal satiation peptides and glucose absorption. In this study, we examined intracellular Ca^{2+} responses to sweet compounds and modulation of leptin and ECs on the responses in mouse endocrine cell line STC-1. Ca^{2+} responses to sweet compounds (glucose, sucrose, sucralose and SC45647) were suppressed by gurmarin, a selective inhibitor for mouse sweet taste receptor (mT1R2/T1R3), suggesting that responses to sweet compounds in STC-1 cells occurred through mT1R2/T1R3. Leptin selectively suppressed the responses to these sweet compounds, and the suppression disappeared by a leptin antagonist. Conversely, 2-AG selectively enhanced the responses to these sweet compounds, and the enhancement disappeared by a cannabinoid receptor antagonist. These results suggest new roles for leptin and ECs as key molecules in the regulation of intestinal sensitivity to dietary sugars.

200

Leptin Receptor neurons co-expressing the neuropeptide galanin, control orexin neurons and mediate sucrose preference

A.I. LAQUE[1], K. REZAI- ZADEH[1], K. BUI[1], C. SCHWARTZENBURG[1], C. MORRISON[1], B. RICHARDS[1], C.J. RHODES[2], H. MUENZBERG[1]
[1]Pennington Biomedical Reseach Center Baton Rouge, LA, USA, [2]University of Chicago Chicago, IL, USA

Leptin, an adipose derived hormone that negatively regulates energy balance, has also been shown to regulate reward function via the mesolimbic dopaminergic system. However, the exact mechanism how leptin mediates these effects are largely unknown. Here, we focus on a subset of leptin receptor (LepRb) neurons co-expressing galanin (Gal-LepRb neurons). We show that Gal-LepRb neurons in the lateral hypothalamus (LH) are inhibitory neurons, stimulated by leptin and directly innervate orexin neurons. We developed mice with a deletion of LepRb in galanin

neurons (Gal-LepRb^{KO} mice). In line with the hypothesized lack of the inhibitory input from Gal-LepRb neurons, Gal-LepRb^{KO} mice indeed show enhanced activation of orexin neurons. Orexin acts in the VTA and modulates reward behavior, thus, in a two-bottle choice test we evaluated differences in the consumption of palatable food rewards (isocaloric 25%sucrose & 10% intralipid solutions). Interestingly, Gal-LepRb^{KO} mice elicit a robust sucrose preference over lipids in contrast to wild-type mice exhibiting no preference. Similarly, Gal-LepRb^{KO} mice showed enhanced motivation to obtain a sugar-rich treat in an incentive runway paradigm. Our data suggests that leptin inhibts orexin neurons via Gal-LepRb neurons, to decrease the rewarding value of food.

201

Leptin regulates the trafficking of mu opioid receptors

DM MCDOUGAL[1], LJ STEWART[1], MJ BARNES[1]
[1]Pennington Biomedical Research Center Baton Rouge, LA, USA, [2]Pennington Biomedical Research Center Baton Rouge, LA, USA, [3]Pennington Biomedical Research Center Baton Rouge, LA, USA

MOR are located in several locales in the central nervous system responsible for regulating energy intake, specifically the arcuate nucleus. The arcuate nucleus is of particular interest because neurons located here play a pivotal role in regulating energy homeostasis. Given that neurons located in the arcuate nucleus are regulated by the anorexic hormone leptin, we sought to determine if leptin can regulate the expression and trafficking of MOR. Intraperitoneal injection of leptin (5ug) into ob/ob mice significantly decreased the gene expression of MOR in the arcuate nucleus. Utilizing immunocytochemistry, we observed that activation of the leptin signaling cascade resulted in MOR being internalized within five minutes of activation and this response was observed for sixty minutes. Internalization of mu opioid receptors by leptin is proposed to occur because of the phosphorylation of SER375 that was observed. Data obtained with live cell calcium imaging demonstrated that activating leptin's signaling cascade reduced the percentage of cells that respond to bath applied DAMGO (mu opioid receptor agonist)(500nM) compared to control (i.e. no activation of leptin's signaling cascade). These observations suggest that leptin can regulate the expression and trafficking of mu opioid receptors. Supported by National Institute of Health, NIDDK, grant number DK078588-01A2.

202

Dose dependent inhibition of weight gain and body fat by fourth ventricle leptin infusions.

M. VAILL, B.N. DESAI, R.B. HARRIS
Georgia Regents University Augusta, GA, USA

We previously reported that 12 day 4th ventricle infusions of low doses of leptin increase body fat of rats (Harris, AJP 304, 2013). By contrast, higher dose 4th ventricle injections of leptin inhibit food intake, cause weight loss and increase hypothalamic PSTAT3 (Ruiter et al. Endocrinology 151, 2010). This study tested whether infusion of higher doses of leptin inhibited food intake and weight gain and activated hypothalamic STAT3. Male Sprague Dawley rats fitted with 4th ventricle cannula were infused with 0, 0.1, 0.3, 0.6, 0.9, 1.2 or 2.0 ug leptin/day for 12 days. There was a dose dependent inhibition of 12 day food intake with a significant effect at 0.3 ug/day. Weight gain was inhibited by 0.3 and 0.6 ug/day and weight loss occurred with higher doses. There was a dose dependent reduction in body fat with a maximum effect at 0.9 ug/day and the three highest doses of leptin reduced lean mass. Hindbrain PSTAT3 was increased by all but the lowest dose of leptin. Hypothalamic PSTAT3 was stimulated by 1.2 or 2.0 ug leptin/day and SOCS3 was inhibited by doses of 0.6 ug/day and higher. There were no differences in activation of hypothalamic ERK1/2 or PI3K. In a second study, CSF leptin was found to be increased 10 fold by a five day infusion of 0.3 ug leptin/day and 100-fold by 1.2 ug leptin/day. Thus weight loss associated with 4th ventricle leptin infusion may depend upon simultaneous activation of the hypothalamus. Supported by NIH grant DK059303

203

Dietary Modulation of Cognitive Behavior

RB KANAREK
Tufts University Medford, MA, USA

Scientific and commercial interest in the role of dietary supplements and functional foods has risen dramatically over the past decade. Individuals more and more are turning to dietary supplements and functional foods as a means of shaping their own health, although many of the health claims for these products remain unproven. This presentation will highlight recent research assessing the possible role of these nutritional variables in mediating brain function and cognitive behavior. More specifically, studies exploring the effects of omega-3-fatty acids, caffeine, theanine and creatine on cognitive performance and mood-related behaviors will be reviewed. Additionally, this presentation will examine the complicated nature of studying nutrient intake on brain function and behavior.

204

Bidirectional regulation of hippocampal neuroplasticity following caloric restriction or overnutrition

AM STRANAHAN
Georgia Regents University Augusta, GA, USA

While it may seem counterintuitive to look for changes evoked by diet outside of hypothalamic feeding circuits,

mounting evidence suggests that brain regions involved in cognition respond to energetic challenges with structural and molecular adaptations that ultimately lead to changes in learning and memory. Hippocampal neurons contribute to learning by modifying the number and strength of synaptic connections. The continuum between impairment and enhancement of hippocampal synaptic function is determined, in part, by metabolic and neuroendocrine signals that compromise or support neuroplasticity. Data from rodent models suggests that caloric excess reduces hippocampal neuroplasticity, and although the precise signaling mechanism(s) are still being resolved, deficits in neurotrophic factor expression likely mediate learning impairment in the context of overnutrition. By contrast, caloric restriction evokes molecular and structural adaptations at hippocampal synapses that are consistent with improved memory performance. Intermittent fasting evokes physiological adaptations similar to caloric restriction, but results in comparable patterns of intake over the course of feeding and fasting cycles, and data from this dietary regimen suggests that extending the interval between meals also promotes neuronal resilience across multiple disease models. Taken together, these data indicate that hippocampal neuroplasticity is bidirectionally regulated by caloric abundance or scarcity, implicating dietary energy intake as a central determinant of memory and cognition.

205

High Fat Diet-Induced Injury to the Brain: Investigating What, Where, and How.

A.J. BRUCE-KELLER[1], J.K. PEPPING[2], D.K. INGRAM[1], J.N. KELLER[1]
[1]Pennington Biomedical Research Center/LSU Baton Rouge, LA, USA, [2]Department of Pathobiological Sciences, School of Veterinary Medicine/LSU Baton Rouge, LA, USA

Consumption of high fat diet (HFD) causes neurologic in addition to metabolic impairment. This study was designed to understand the degree to which, and the mechanisms by which, HFD disrupts brain function in mice, with an emphasis on the pro-inflammatory/pro-oxidant enzyme NADPH oxidase. C57Bl/6 (WT) mice and mice deficient in the NAPDH oxidase subunit NOX2 (NOX2KO) were administered different diets and evaluated for metabolic and neurologic function. While diet-induced weight gain in WT and NOX2KO mice was similar, NOX2KO mice had smaller visceral adipose deposits, attenuated visceral adiposopathy, and diminished visceral adipose macrophage infiltration. Moreover, NOX2KO mice had improved glucose regulation. Brain injury was assessed using markers of cerebrovascular integrity, synaptic density, and reactive gliosis; and data show that HFD caused injury in WT, but not NOX2KO mice. Finally, biochemical analyses of brain redox signaling and oxidative injury revealed that mice respond to HFD with significantly enhanced oxidative stress localized primarily to neuronal populations in key areas of the cerebral cortex and hypothalamus. Collectively, these data indicate that NOX2 is a significant contributor to the pathogenic effects of HFD, and reinforce a key role for visceral adipose inflammation in metabolic and neurologic decline. Development of NOX-based therapies could accordingly preserve both metabolic and neurologic function in the context of HFD

206

What makes the "obesogenic" environment "obesogenic"? Integrating physiological and cognitive perspectives to understand why energy regulation fails.

TL DAVIDSON
American University Washington, DC USA

It has often been suggested that the current obesity epidemic is attributable to an obesogenic environment that is characterized by the widespread availability of low cost, energy-dense, highly-palatable, foods and beverages, and an abundance of external cues that keep thoughts of these foods and beverages almost constantly in mind. It has also been claimed that this combination environmental factors overwhelms the physiological controls that normally operate to maintain energy balance and body weight. The purpose of this talk is to consider how components of the current obesogenic environment may produce this undesirable effect. The talk will describe how environmental cues and physiological signals are integrated in the learned control of energy regulation and will show how this integrated system can generate a mechanism for the inhibitory control of feeding behavior. This will set the stage for a review of recent findings which shows how certain dietary factors that are pervasive the current food environment, including some that are used to control energy intake, may promote overeating and obesity by interfering with specific components of this inhibitory mechanism. The talk will conclude with a discussion of how this type of interference could result in a vicious-cycle of obesity and a progressive decline in cognitive health.

207

Insulin detemir has increased transport from blood to cerebrospinal fluid and prolonged anorectic action relative to regular insulin

DP BEGG, A MAY, DA D'ALESSIO, RJ SEELEY, M LIU, SC WOODS
Metabolic Diseases Institute, University of Cincinnati Cincinnati, OH, USA

Insulin detemir (DET) is a long-acting insulin analogue with an attached fatty-acid side chain. DET improves glycemic outcomes in diabetic patients similarly to regular-insulin (RI) formulations. However, unlike RI,

DET often causes weight loss. Given that centrally administered insulin is catabolic, we aimed to determine if weight loss associated with chronic DET treatment results from increased insulin transport into the central nervous system. We compared transport of DET and RI into the cerebrospinal fluid (CSF) of rats using different doses (0, 0.1, 0.3, 1, 3 and 10 U/kg ip at 30 min) and sampling times (0.5 U/kg at 0, 5, 10, 20, 40, 80, 160 and 320 min). We also compared the effects of acute third-ventricular (i3vt) administration of DET with that of RI, and vehicle, on food intake and body weight over a 48-h period. Both DET and RI had comparable saturable, receptor-mediated transport into the CSF. Thirty min after an ip dose of 1 U/kg, DET resulted in greater CSF insulin than RI despite no difference in plasma insulin levels. CSF insulin remained elevated significantly longer following ip DET than following ip RI. Acute i3vt infusion of DET caused a similar reduction of food intake and body weight at 24 h as RI. However, at 48 h, food intake and body weight remained lower following DET but not RI administration. Overall, these data support the hypothesis that DET improves weight management by an enhanced and prolonged centrally-mediated reduction of energy intake

208

Leptin Receptor Neurons in the Dorsomedial Hypothalamus Regulate BAT Thermogenesis and Energy Expenditure

KP REZAI-ZADEH[1], A LAQUE[1], C SCHWARTZENBURG[1], C MORRISON[2], H MUNZBERG[1]
[1]Central Leptin Signaling, Pennington Biomedical Research Center (PBRC) Baton Rouge, LA, USA,
[2]Neurosignaling, PBRC Baton Rouge, LA, USA

Leptin acts on its central receptors (LepRb) to regulate energy homeostasis by controlling food intake and energy expenditure. Leptin's action on energy expenditure involves sympathetic control of brown adipose tissue (BAT) thermogenesis, but the central circuits involved are not well understood. Here, we show that LepRb neurons in the dorsomedial hypothalamus (DMH) regulate body temperature, energy expenditure, and body weight, with no impact on food intake. Intra-DMH leptin injections correct hypothermia and improve body weight in *ob/ob* mice without inducing anorexia. Also, leptin directly triggers neuronal activation of DMH LepRb neurons. Accordingly, direct pharmacogentic neuronal activation of DMH LepRb neurons robustly increased energy expenditure that lead to significant weight-loss without affecting food intake. Interestingly, induction of energy expenditure indeed resulted from β_3 adrenoreceptor-dependent BAT thermogenesis, but also involved a β_3-independent rise in locomotor activity, highlighting skeletal muscle metabolism as an important contributor to thermoregulatory effects. Finally, direct optogenetic manipulation of DMH LepRb neurons suggested a high excitatory stimulus is required to evoke energy expenditure. Taken together, our data reveal a neuronal substrate for the physiological control of metabolism and implicate a promising new target for obesity therapies.
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209

Prolonged intragastric delivery of bitter agonists induces weight loss in obese mice

B AVAU, F AKOR, J TACK, I DEPOORTERE
Gut Peptide Research Lab, Targid, University of Leuven Leuven, Belgium

Background and aim: Gut bitter taste receptors, TAS2R, may regulate gastrointestinal responses to ingested food. We previously showed acute inhibitory effects after gavage of bitter agonists on food intake and gastric emptying in mice (PNAS 108:2094,2011). This study aimed to investigate the effects of prolonged bitter treatment in obese mice. Methods: Mice, fed a high fat diet for 3 months, were gavaged with phenylthiocarbamide (PTC, 1.44 µmol/kg), denatonium benzoate (DB, 2.88µmol/kg) or water for 2 weeks. Food intake and weight were monitored and mice were sacrificed after an overnight fast. mRNA levels were quantified using real-time PCR and ghrelin levels were determined via radioimmunoassay. Results: PTC or DB treatment reduced body weight with 4.9% ($P<0.01$) and 3.1% ($P<0.05$) respectively. Food intake did not change in DB-treated mice, but was reduced ($P<0.05$) with 5.4% in PTC-treated mice. Fat pad masses did not differ significantly after bitter treatment but UCP2 mRNA expression was increased ($P<0.005$) in fat from PTC (1.33-fold) and DB-treated (1.34-fold) mice. mRNA expression of the gustatory G-protein, a-gustducin, was decreased 1.6-fold ($P<0.001$) and 1.2-fold ($P=0.07$) in the duodenum of DB and PTC-treated mice, respectively. Ghrelin and GLP-1 mRNA expression in duodenum was lower ($P<0.05$) in DB-treated mice, while ghrelin and CCK mRNA tended ($P=0.09$) to be increased in PTC-treated mice. No changes in mRNA expression were observed in the stomach. Total but not octanoyl plasma ghrelin was higher ($P<0.001$) in PTC-treated mice. Blood glucose and insulin levels were not affected by the bitter agonists. Conclusion: Long-term intragastric delivery of bitter tastants induces weight loss possibly due to increased UCP-2-induced lipid oxidation. Downregulation of a-gustducin in the duodenum and bitter tastant selective changes in the balance of (an)orexigenic hormone levels imply an important role for the duodenum in bitter taste perception which may contribute to the observed metabolic changes.

210

Optogenetic Activation of the Orexin Neurons Stimulates Spontaneous Physical Activity

CE PéREZ-LEIGHTON[3], DB BUSHEY[1,3], MK GRACE[2], CJ BILLINGTON[1,2,3], CM KOTZ[1,2,3]
[1]University of Minnesota, Department of Food Science and Nutrition Saint Paul, MN, USA, [2]Department of Veterans Affairs Minneapolis, MN, USA, [3]Minnesota Obesity Center Minneapolis, MN, USA

The hypothalamic orexin/hypocretin neurons modulate arousal, food intake, reward and spontaneous physical activity (SPA). Orexin injections in multiple brain regions increase SPA and individual orexin responsiveness predicts susceptibility to obesity. It is unknown whether activation of endogenous orexin using optogenetics affects SPA. To test this, C57/BL6J mice (n = 12) were injected in the orexin neuronal field with a lentivirus expressing either channelrhodopsin or a control protein (enhanced green fluorescent protein) under control of the orexin promoter. The mice were also implanted with fiber optic cannulae. SPA was measured using three 16-beam sets of infrared activity sensors. Three weeks after virus injection, mice were acclimated to the beam-break cages for 3 consecutive days. On d4 and d5, SPA was measured during a 1.5 h acclimation followed by 2 h of light stimulation, and 1 h post stimulation. The light stimulation protocol consisted of 15 sec of a 20 Hz 5 ms pulse followed by 5 sec of rest. This train of stimulation shows that SPA, corrected for baseline activity, is significantly increased (P = 0.02) by optogenetic activation of the orexin neurons. This finding is consistent across days of testing. Additional stimulation parameters and measurements of food intake are in progress. These data provide the first evidence that stimulation of caudal lateral hypothalamic orexin neurons using optogenetics drives SPA in mice.

211

Restrictive Feeding Practices Promote Daughters' Early Dieting

KN BALANTEKIN[1,2], JS SAVAGE[1,2], LL BIRCH[1,2]
[1]Nutritional Sciences, Penn State University Park, PA, USA, [2]Center for Childhood Obesity Research, Penn State University Park, PA, USA

Controlling feeding practices, including the use of restriction, have unintended consequences on eating behavior and weight status. Further, parental encouragement to diet has been shown to promote dieting before adolescence. However, less is known about how exposure to restrictive feeding practices influences early dieting among pre-adolescent girls. The sample included 169 non-Hispanic white families. Mothers completed the Child Feeding Questionnaire and Parent Encouragement of Child Weight Loss Scale at daughter age 9y and 11y to assess maternal restriction and encouragement to diet. Daughters were asked "Have you ever dieted?" when they were 11y. Using logistic regression, maternal encouragement to diet predicted daughters' early dieting ($p < 0.05$), adjusting for BMI percentile at 9y. However, when restriction was added to the model, restriction ($p < 0.01$) but not encouragement, was significant. With each unit increase in exposure to restriction, daughters were 1.88 (95% CI: 1.18, 2.98) times more likely to report early dieting (by 11y). Additional analyses include examining the influence of restriction and maternal weight concerns over time on early dieting. Findings suggest that restrictive feeding practices, over and above encouragement to diet, increase the likelihood that daughters will diet early, before adolescence. Parents who are concerned about their child's weight may need guidance with setting limits, responsive feeding, and being a positive role model for healthy eating rather than using controlling feeding practices.

212

Conditioned sweet-paired stimuli elicit potentiated feeding in humans.

TL RIDLEY-SIEGERT, H CROMBAG, MR YEOMANS
UNIVERSITY OF SUSSEX BRIGHTON, United Kingdom

Past research in animals suggests that environmental food cues that predict food delivery can potentiate subsequent consumption in the absence of immediate nutrient needs. The present study extends this to ask whether more proximal cues that predict the experience of a hedonic reward (sweet taste) potentiate eating in humans. Forty-five normal-weight volunteers who liked the taste of 10% sucrose completed a disguised conditioning procedure during which novel visual cues were paired with 10% sucrose or a neutral taste (artificial saliva), with additional distracter cues and tastes used to disguise these associations. Thirty-minutes post training participants were given free-access to sweet and savoury snacks that were labelled with either the sweet-paired or the neutral-paired cues. Participants presented with food and drink stimuli labelled with the sweet-paired cue ingested significantly more than those given the same products labelled with the neutral-paired cue ($F(1, 40)=5.40$, $p = .025$). The increase in consumption was seen with both savoury and sweet items suggesting that cues potentiated feeding by activation of a general motivational state rather than sensory-specific expectation. There was no evidence that participants' level of explicit knowledge of the cue-taste associations affected consumption. These data provide evidence that a visual stimulus associated with a palatable sweet taste can elicit a generalised increase in intake, and suggest that learned associations between environmental cues and hedonic food rewards could be an important feature of the obesogenic environment.

213

The Effect of Achievement Stress and Attachment Stress on Stress Eaters and Stress Under-Eaters

MH EMOND[1], A STILLAR[1], A ROBINSON[1], S VAN BLYDERVEEN[2], S KOSMERLY[1]
[1]Laurentian University Sudbury, ON, Canada, [2]McMaster University Hamilton, ON, Canada

Stress has a bidirectional effect on eating behaviour, with the potential to elicit a **hyper**phagic or **hypo**phagic response depending on the individual and the stress. These differential responses to stress have led to the classification of two distinct stress-eating populations: stress eaters and stress under-eaters. This study's purpose was to examine the effects different types of stress (achievement and attachment) have on eating behaviour in stress eaters (SE) and stress under-eaters (SU). Participants were assigned to one of three conditions: achievement stress, attachment stress, or control group. Video stress-induction techniques were employed. Participants were provided a variety of food options, varying in nutritional content, to eat while watching their video. Self-report questionnaires and total food consumption were measured following the stress-induction. Results showed SE consumed significantly more Calories than SU in the achievement stress condition (SE: 244.5; SU 67.8 Cal). There was no difference between SE and SU in the attachment stress condition. However, both groups consumed more in the attachment condition (SE: 310; SU 296.1 Cal) than the control condition (SE: 126.78; SU: 80.5 Cal). In conclusion, attachment stress and achievement stress affect stress-eating behavior and stress-related food preferences differently, with Achievement stress producing an increase in consumption for SE and Attachment stress causing an increase in consumption for both SE and SU.

214

Roux-en-Y Gastric Bypass Increases Intravenous Alcohol Self-Administration in Dietary Obese Rats

A HAJNAL[1,2], JE POLSTON[1], CE PRITCHETT[1], JM TOMASKO[2], AM ROGERS[2], ND VOLKOW[3], PK THANOS[3,4]
[1]Penn State College of Medicine, Dept. of Neural & Behavioral Sciences Hershey, PA, USA, [2]Penn State College of Medicine, Dept. of Surgery Hershey, PA, USA, [3]3Laboratory of Neuroimaging, NIAAA Intramural Program, NIH Bethesda, MD, USA, [4]Behavioral Neuropharmacology and Neuroimaging Lab, Brookhaven National Laboratory Upton, NY, USA

Concerns have been raised by clinical reports of an increased risk for alcohol abuse following Roux-en-Y gastric bypass (RYGB) surgery. Our recent studies in rats corroborated this notion (Hajnal et al., 2012; Thanos et al., 2012). A hypothesis is that increase in alcohol absorption following RYGB underlies the enhancement in alcohol rewarding effects. To control for differential alcohol absorption, in the present study high fat (60% kcal from fat) diet-induced obese Sprague-Dawley male rats with RYGB or sham-surgery (SHAM) self-administered alcohol (1%) intravenously (IV). Compared to SHAM, RYGB rats made significantly more operant lick responses to earn IV alcohol infusion (+30-50%) on a fixed ratio (FR-5) schedule, and achieved higher breakpoints (+25-40%, $p<0.05$) during a progressive ratio (PR-2) schedule of reinforcement task. These findings indicate that the enhanced alcohol reward after surgery is not just due to changes in alcohol absorption but may reflect direct effects in the sensitivity to alcohol's rewarding effects in the brain. Future studies on underlying mechanisms and susceptibility factors that increase alcohol reward in some bariatric patients are warranted to allow for more customized personalized interventions and treatments.

215

The Profound Effect of Social Stress on Health

CA SHIVELY, TC REGISTER
Wake Forest School of Medicine Winston-Salem, NC, USA

Coronary heart disease (CHD) is the leading cause of death, and depression and CHD are the two leading causes of disability in the US. Coronary artery atherosclerosis (CAA) and its complications cause CHD. Cynomolgus monkeys are a well-established model of CAA. Psychosocial stress doubles the risk of CHD in people, and social subordination stress doubles CAA in female monkeys. The mechanisms through which stress increases CAA/CHD are not well understood. Abdominal obesity is prevalent and often accompanied by the metabolic syndrome, which increases risk of CHD. Chronic stress increases cortisol and NPY which promote visceral obesity. We hypothesized that social stress may cause visceral fat deposition and the metabolic syndrome, which, in turn increase CAA. Social instability stress increases visceral fat deposition in male monkeys, and stressed subordinate females deposit more fat in the viscera. Females with high visceral: subcutaneous fat ratios (VAT:SAT) are socially isolated, receive more aggression and less grooming, are desensitized to cortisol, have impaired ovarian function, higher heart rates late in the day, and more CAA than low VAT:SAT females. Social stress causes depression and depression is highly comorbid with CHD. Subordinate females are more likely to become depressed than dominants. Depressed monkeys have dyslipidemia, poor ovarian function, high heart rates, and low activity levels all of which are CAA risk factors. Depressed females develop four times the CAA as nondepressed females. Thus, social stress may increase CAA/CHD by increasing visceral obesity and the metabolic syndrome, and by increasing depression.