Neuroscience 319 (2016) 233–245

INVESTIGATION OF A ROLE FOR GHRELIN SIGNALING IN BINGE-LIKE FEEDING IN MICE UNDER LIMITED ACCESS TO HIGH-FAT DIET

S. J. KING,* T. RODRIGUES, A. WATTS, E. MURRAY, A. WILSON AND A. ABIZAID

Department of Neuroscience, Carleton University, Ottawa, Ontario, Canada

Abstract—Binge eating is defined by the consumption of an excessive amount of food in a short time, reflecting a form of hedonic eating that is not necessarily motivated by caloric need. Foods consumed during a binge are also often high in fat and/or sugar. Ghrelin, signaling centrally via the growth-hormone secretagogue receptor (GHSR), stimulates growth hormone release and appetite. GHSR signaling also enhances the rewarding value of palatable foods and increases the motivation for such foods. As ghrelin interacts directly with dopaminergic reward circuitry, shown to be involved in binge eating, the current studies explored the role of GHSR signaling in a limited access model of binge eating in mice. In this model, mice received either intermittent (INT) or daily (DAILY) access to a nutritionally complete high-fat diet (HFD) for 2 h late in the light cycle, alongside 24-h *ad libitum* chow. In CD-1 mice, 2-h exposure to HFD generated substantial binge-like intake of HFD, as well as a binge-compensate pattern of 24-h daily intake. INT and daily groups did not differ in 2-h HFD consumption, while INT mice maintained stable intake of chow despite access to HFD. GHSR knock-out (KO) and wild-type (WT) mice both binged during HFD access, and exhibited the same binge-compensate pattern. INT GHSR KO mice did not binge as much as WT, while DAILY KO and WT were comparable. Overall, GHSR KO mice consumed fewer calories from HFD, regardless of access condition. GHSR KO mice also had reduced activation of the nucleus accumbens shell, but not core, following HFD consumption. These data support the ability of INT HFD in mice to induce a binge-compensate pattern of intake that emulates select components of binge eating in humans. There also appears to be a role for GHSR signaling in driving HFD consumption under these conditions, potentially via mediation of reward-related circuitry.

Key words: binge eating, intermittent access, high-fat diet, GHSR, ghrelin, nucleus accumbens.

INTRODUCTION

Binge eating is defined by the consumption of an excessive amount of food in a short time frame and is often associated with a sense of loss of control over the ability to cease eating once nutritional needs have been met (APA, 2013). Binge eating, therefore, reflects a form of hedonic eating that is not motivated by the necessity for calories or specific macronutrients, and is likely to be regulated in part by mesolimbic reward circuitry (Davis et al., 2009; for a review, see Bello and Hajnal, 2010). In support of this view, it is most often highly-palatable and energy-dense foods that are consumed during a binge episode (Kales, 1990; Elmore and DiCastro, 1991), and consumption of such foods are known to influence the short- and long-term plasticity of both homeostatic and brain reward circuitry (Bello et al., 2009; Johnson and Kenny, 2010). The precise mechanisms underlying the eating behavior seen in individuals that binge eat remain poorly understood. Identifying the factors that contribute to binge eating in both normal weight and obese binge eating disorder (BED) models, components of which may be dissociable, will aid in the understanding of the disorder and ideally improve treatment options for those that binge eat.

A number of animal models of binge-type eating have been developed (Corwin et al., 1998; Boggiano et al., 2007; Berner et al., 2008; Bello et al., 2009; Lardeux et al., 2013; Bake et al., 2014a). The limited access model, first demonstrated in rats by Corwin et al. (1998), is used to elicit binge-like consumption and alter patterns of fat intake that emulate select components of eating behaviors seen in humans with BED. This model is based on findings which show that restricting access to a substance can reliably enhance intake when that substance again becomes available (Wayner and Fraley, 1972; Pinel and Huang, 1976; Corrigall and Coen, 1989), and is thought to partially underlie the repeated failures of dieters who attempt to self-restrict intake of energy dense foods. In this model, rats placed under time restricted and sporadic access to fat demonstrate a binge-compensate pattern of feeding, such that they consume a greater number of calories on access days, and a reduced number of calories on non-access days relative to the control group who do not receive fat (Corwin et al., 1998). While this model does not accelerate the

*Corresponding author. Address: Life Sciences Research Building, Department of Neuroscience, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario, K1S 5B6, Canada. Tel: +1-613-720-4699.
E-mail address: sking3@connect.carleton.ca (S. J. King).

Abbreviations: BED, binge eating disorder; BSA, bovine serum albumin; DA, dopamine; GHSR, growth-hormone secretagogue receptor; HFD, high-fat diet; INT, intermittent; KO, knock-out; LHA, lateral hypothalamic area; NAcc, nucleus accumbens; SEM, standard error of the mean; TH, tyrosine-hydroxylase; VTA, ventral tegmental area; WT, wild type.

http://dx.doi.org/10.1016/j.neuroscience.2016.01.004

development of obesity in all studies, it can alter patterns of intake and dietary composition, which may be reflected in changes in endocrine function and central nervous system activation.

Recent studies examining the impact of intermittent (INT) but regularly scheduled, and therefore predictable, access to high-fat diet (HFD) found that rats with INT access exhibit a number of metabolic consequences, such as impaired glucose tolerance, increased insulin levels and increased fat mass, relative to controls (Bake et al., 2014b). Interestingly, the lack of alterations in both orexigenic and anorexigenic peptide gene expression in the arcuate nucleus of the hypothalamus (ARC) prior to scheduled HFD or sucrose access in mice and rats, suggests that alternate mechanisms may be involved in the regulation of such binge-eating behavior (Bake et al., 2013). The excessive intake under such access conditions may be driven by reward systems that supplant the hypothalamic homeostatic control of feeding. Rats allowed INT scheduled access to a sweet-fat mixture not only consumed more food, but also exhibited higher terminal ghrelin levels as compared to rats with continuous access (Bello et al., 2009), suggesting that ghrelin signaling may play a role in increasing consumption of the palatable food when access is restricted. Elevated ghrelin levels have also been found in rats that developed binge-like eating when exposed to chow followed by a sweet-fat mixture subsequent to 2 h of daily food deprivation (Cottone et al., 2008).

Ghrelin, is a 28-amino acid peptide that is produced primarily by endocrine mucosal cells in the stomach (Kojima et al., 1999; Date et al., 2000) and its predominant functions include the stimulation of growth hormone release and the regulation of food intake and energy balance, both in the short- and long-term (Cummings, 2006). Ghrelin plays a role in meal initiation by peaking just prior to a meal in schedule fed humans and rodents (Cummings et al., 2001; Drazen et al., 2006). Elevated levels of ghrelin also result in a greater accumulation of body fat stores and a concomitant reduction in physical activity, leading to increased weight gain and reduced basal metabolic rate (Tschop et al., 2000). Ghrelin binds to the growth-hormone secretagogue receptor of the 1A subtype (GHSR; Howard et al., 1996), present at appreciable levels in widespread regions of the rat and mouse brain as detected by *in situ* hybridization (Guan et al., 1997; Zigman et al., 2006). Outside of ghrelin's actions on homeostatic nuclei in the ARC (for review see Horvath et al., 2001; Abizaid and Horvath, 2008), ghrelin exerts a substantial influence on reward circuitry (for review see: Abizaid and Horvath, 2008; Abizaid, 2009; Dickson et al., 2011; Skibicka and Dickson, 2011a, Perello and Dickson, 2015). Both systemic and direct ventral tegmental area (VTA) ghrelin administration enhances the release of dopamine (DA) in the nucleus accumbens (NAcc) (Abizaid et al., 2006; Jerlhag et al., 2006, 2007) and increases excitatory inputs onto VTA DA producing cells that co-localize with GHSR (Abizaid et al., 2006). Ghrelin administration also increases the rewarding value of highly palatable food (Egecioglu et al., 2010; Perello et al., 2010) as well as the motivation to work for such foods (Skibicka et al., 2011b, 2012; King et al., 2011). Given the ability of ghrelin to interact directly with dopaminergic reward circuitry known to be involved in binge eating, it could potentially be involved in the development of binge eating behaviors generated by INT access to highly palatable foods in mice.

To date, the role of ghrelin in binge eating in humans has been equivocal. An early study implicated the presence of the Leu72Met variant of the ghrelin gene in the development of BED in a small cohort of human subjects (Monteleone et al., 2007). Additional studies have reported that plasma ghrelin is reduced in obese-BED individuals (Geliebter et al., 2005; Monteleone et al., 2005) as well as in those with a higher frequency and severity of binge-purge behaviors (Troisi et al., 2005). Interestingly, the post-prandial suppression of ghrelin is also attenuated in obese-BED populations (Geliebter et al., 2005), which may reduce the satiating effect of a meal and may contribute to over-eating in the short term. It is currently unknown whether ghrelin signaling in normal-weight populations of humans or rodents is important for the development of binge eating on high-fat foods.

We initially set out to establish whether the limited access model utilized in rats (Corwin et al., 1998; Davis et al., 2007) would be reproducible in mice using a nutritionally complete HFD. In Experiment 1, we hypothesized that subjecting adult male mice to a limited access model of HFD, allowing access for 2 h per day 3 times a week, would elicit a binge/compensate pattern of caloric intake, relative to both mice fed a HFD for 2 h daily and chow-fed controls. Given ghrelin's central role in driving food intake and enhancing food reward, in our second experiment we hypothesized that mice lacking intact ghrelin receptors (*GHSR-KO*) would exhibit an attenuated binge-like pattern of eating typically demonstrated under this access schedule in wild-type (WT) rodents. We also examined differences between the mice in terms of neuronal activation in reward-related brain regions using c-Fos immunohistochemistry.

EXPERIMENTAL PROCEDURES

Animals

Experiment 1. Twenty adult male CD-1 mice (Charles River, St. Constant, QC) weighing 35–45 g were housed individually in a temperature and humidity controlled vivarium with a 12:12 light/dark schedule (Lights on: 07:00 to 19:00). All mice had *ad libitum* access to water and nutritionally complete standard laboratory chow (Teklad Global 14% Protein Rodent Maintenance Diet 2014, Harlan Laboratories, Madison, WI, USA; percent of kilocalories derived from fat: 13, protein: 20, carbohydrates: 67; 2.9 kcal/g) for the duration of the study. After one week of acclimatization to the vivarium environment, bodyweight and food intake were measured for 7 days to serve as a baseline. At the end of the baseline period, mice were given access to a nutritionally complete HFD overnight (Open Source

Diets, D12492; Research Diets, Inc., New Brunswick, NJ; percent of kilocalories from fat: 60, protein: 20, carbohydrates: 20; 5.24 kcal/g). Mice were exposed to HFD overnight to prevent neophobia toward the diet, as well as to eliminate any mice that did not consume any HFD during the overnight exposure period. Two groups were matched by body weight and overnight HFD consumption and assigned to either (1) DAILY ($n = 9$); or (2) INT ($n = 10$) access to HFD for the remainder of the experiment.

Experiment 2. To determine whether a lack of GHSR signaling would either hinder the development or reduce the magnitude of binge-like consumption of a HFD under an INT access schedule, we used mice sustaining an in-frame deletion of the *ghsr* gene which was replaced with a LacZ reporter gene ($n = 18$; *GHSR KO*), and their WT littermates ($n = 18$; *GHSR* +/+), originally crossed onto a C57BL/J6 × DBA background, and subsequently backcrossed onto a C57BL/J6 background strain. Mice were originally obtained from a colony at Yale University as a kind gift from Dr. Tamas Horvath and derived from founder strains created at Regeneron Pharmaceuticals (Tarrytown, NY, USA). Baseline measurements and overnight HFD exposure were conducted as outlined in Experiment 1. However, two-thirds of the mice matched on 3-day chow consumption and overnight HFD intake were subsequently divided into 4 experimental groups: (1) KO DAILY and (2) WT DAILY, given access to the HFD for 2 h beginning at 15:30 each afternoon; (3) KO INT and (4) WT INT, given access to HFD for 2 h beginning at the same time as in the DAILY group, but only on Monday, Wednesday and Friday (M,W,F). The remaining mice formed two control groups, receiving HFD only during the initial overnight exposure period, but strictly *ad libitum* chow over the course of the experiment (1) Control KO ($n = 6$) and (2) Control WT ($n = 4$). All procedures were approved by the Canadian Council on Animal Care (CCAC) and the Carleton University Animal Care committee.

Limited access model

Mice were allowed *ad libitum* access to standard chow throughout the course of the experiments. Food intake and body weight were measured daily. For four weeks (Experiment 1) or six weeks (Experiment 2), mice were given either daily access to the HFD (DAILY) composed of 60% kilocalories (kcal) from fat for two hours beginning at 15:30, or were given access to the diet only 3 times per week, (INT; MWF) at the same time of day. HFD was placed in small glass flat-bottomed bowls in the home cage of the mice on the days that they were scheduled to receive the HFD. The amount of HFD consumed during the 2-h period was recorded. At the time of HFD removal, the bottom of the cage was examined to ensure that no spillage was left unrecorded, potentially inflating KCAL intake during this period. We used a 4-week paradigm in Experiment 1 to determine if this length of exposure would be sufficient to induce binge-like feeding in mice. In designing Experiment 2, we hypothesized that GHSR KO mice may binge less than WT within a 4-week period, but that given the opportunity, may eventually match intake seen in WT, and therefore Experiment 2 was designed to be 6-weeks in length.

Immunohistochemistry

Experiment 2. One hour following their last exposure to HFD, mice were injected with a lethal dose of Dorminal (1 mg/kg i.p.; CDMV, Quebec, Canada) and perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde. Brains were then extracted and post-fixed in 4% paraformaldehyde for 24 h, followed by submersion in a 30% sucrose solution (w/v) prior to sectioning for cryoprotection. Forty micrometer sections containing the NAcc core and shell regions (Figs 18–22, Paxinos and Franklin, 2001) were sliced at −21 °C on a Thermoscientific Cryostat. Sections were placed into wells containing 0.1 M phosphate buffer and every sixth section was subsequently processed for c-FOS IR using a standard immunohistochemical detection protocol similar to what has been outlined previously (Abizaid et al., 2005). Briefly, free-floating sections were washed in 0.1 M phosphate buffer (PB, pH 7.4), followed by incubation in a quenching solution (PB and 1% H_2O_2) at room temperature (RT) for 30 min. Sections were then rinsed in PB and incubated at RT for 1 h in a blocking solution comprised of 0.3% Triton-X and bovine serum albumin (BSA) in PB. Sections were then incubated for 48 h at 4 °C in a FOS-anti-rabbit primary antibody (Oncogene Science, Boston, MA, USA; 1:20,000) solution containing Triton-X and BSA. Sections were then rinsed in PB and incubated in a biotinylated IgG secondary antibody (donkey anti-rabbit; Jackson ImmunoResearch Laboratories Inc., Westgrove, PA, USA; 1:250) for 1 h, followed by an avidin–horseradish peroxidase complex (ABC Elite Kit, Vector laboratories, Burlington, ON, Canada; 1:500) and reacted with 0.05% diaminobenzidine and 0.03% H_2O_2 (DAB, Sigma Aldrich, St. Louis, MI, USA) for 10 min, resulting in a dark brown staining of c-FOS-positive nuclei. Sections were then mounted on gel-coated slides, dehydrated in a series of graded alcohol concentrations and submersed in Clearene (Surgipath). Slides were then cover-slipped using Permount (Fisher Scientific, Ottawa, ON, Canada). A control group of mice not receiving HFD throughout the experiment served as a comparison group.

Two to three coronal sections obtained from each mouse were examined to quantify c-Fos IR. All photos were taken under a 10× objective lens using standard bright-field microscopy on an Olympus BX51 microscope (Olympus Canada, Markham, ON, Canada) attached to an Olympus (DVC 2000C) camera, using Stereo Investigator 8 software (MBF Bioscience, Williston, Vermont, USA). The quantification of c-Fos IR was conducted using the Image J64 software (NIH freeware). C-Fos-positive cells were counted bilaterally in the NAcc. The counts reported are the mean (± standard error of the mean (SEM)) percent of control mice for each group.

Statistical analyses

Experiment 1. To examine the patterns of intake across time, food intake data were analyzed using a two-way repeated measures ANOVA (day × access group) followed by Bonferroni corrected post hoc tests, where overall differences were found. Independent sample t-tests were conducted on data examining overall means.

Experiment 2. Data were analyzed using either a two-way ANOVA (genotype × access schedule) or a repeated measures two-way ANOVA (day × genotype × access schedule), where appropriate. Where a significant overall difference was detected, follow up multiple comparisons using the Bonferroni correction were performed. All data are expressed as mean ± SEM. All statistical analyses were performed with SPSS 20.0.0 for Macintosh (IBM Corp., Armok, NY, USA) and statistical significance was set at $\alpha = 0.05$.

RESULTS

Experiment 1

High-fat diet (Fig. 1A, C). The total number of calories consumed from HFD during the 2-h access period did not differ between DAILY and INT groups on any day of the experiment (day × access group, $F_{(12,204)} = .826$, $p > 0.05$). However, a significant main effect of day ($F_{(1,17)} = 12.356$, $p < 0.05$) demonstrated that all mice increased the average amount of HFD that they consumed across the study period, starting at 5.5 ± .5 kcal on the first access day and increasing to 14.3 ± 1.1 kcal by the last access day. The overall average number of kcal consumed during the access period was equal to 10.9 ± 1.1 for the DAILY mice and 12.1 ± 0.7 for the INT mice ($t_{(17)} = 0.436$, $p > 0.05$), which in both cases came exclusively from HFD as the mice ate negligible amounts of chow during this period. While there were no group differences in HFD diet intake during the access period, CD-1 mice given either DAILY or INT HFD access schedules both exhibited binge-like eating behavior during the 2-h exposure to HFD late in the light cycle. Mice in the INT group consumed 85% ± 6% of their average daily baseline intake from HFD whereas the DAILY mice consumed 71% ± 7% within the same time period, however, the difference between groups was not statistically significant ($t_{(17)} = 1.375$, $p > 0.05$, see Fig. 1C).

Standard chow (Fig. 1B). In the 22-h period following access to the HFD, we found that the INT group consumed a significantly greater amount of calories from standard chow, relative to the DAILY group (day × access group, $F_{(12,204)} = 9.994$, $p < 0.05$). Post-hoc comparisons revealed that this difference emerged on the second access day and continued for the remainder of the access days ($p < 0.05$ for access days 2 through 13).

Total daily caloric intake (Fig. 1D). Total 24-h intake was significantly different between groups on both access days ($t_{(17)} = 10.140$, $p < 0.05$), upon which the INT group consumed a greater number of calories than DAILY mice (28.0 ± .6 vs. 20.1 ± .5), and on non-access days ($t_{(17)} = 9.871$, $p < 0.05$), upon which INT mice consumed fewer calories than DAILY mice (10.1 ± .5 vs. 17.4 ± .5).

Change from baseline daily intake (Fig. 1E, F). To determine if total daily intakes differed from average baseline intake, we calculated a change from baseline score for every day of the study. A two-way repeated measures ANOVA (day × group) demonstrated that mice consumed differing amounts of kcal (relative to their baseline) across access days, depending on their access condition (day × group interaction: $F_{(12,204)} = 4.235$, $p < 0.05$). On average, mice with INT access to HFD ate 11.2 ± 0.9 kcal whereas the group with DAILY access ate merely 2.6 ± 0.9 kcal above their average baseline intake on access days ($F_{(1,17)} = 41.887$, $p < 0.05$). With the exception of the first HFD access day, the INT group had higher intakes than the DAILY group on all access days ($p < 0.05$). Across non-access days, the INT group reduced their caloric intake to a greater extent than the DAILY group relative to their baseline (day × group interaction: $F_{(15,255)} = 4.100$, $p < 0.05$), and differences between groups were significant on non-access days 1,3,4,6,7,8,9,11,12,13,14,15, and 16 ($p < 0.05$). Therefore, restricting access of the HFD for two hours to only three days per week (M,W,F) induced a overeat-compensate pattern of consumption in the INT mice. Unexpectedly, this was not due solely to excessive consumption of HFD during the 2-h access period on the part on the INT mice, as both groups consumed binge-like amounts of HFD in 2 h. Rather, differences between groups appeared to emerge as a result of the INT mice showing greater chow consumption on HFD access days (see Fig. 1B). The overall mean change from baseline data for both access and non-access days can be seen in Fig. 1F. Both DAILY and INT groups increased caloric intake on days when they had access to HFD, relative to their baseline, but INT mice increased intake to a significantly greater extent than did DAILY mice ($t_{(17)} = 6.472$, $p < 0.05$). On non-access days, DAILY mice maintained a level of intake similar to baseline (1.1 ± .7 kcal), but the INT group reduced their intake (−5.1 ± .7 kcal; $t_{(17)} = 6.034$, $p < 0.05$) likely due to increased caloric consumption on the previous days.

Cumulative intake (Fig. 1G). We found that mice in the INT group exhibited greater cumulative 24-h caloric intake (comprised of both diets) across HFD access days, relative to the DAILY group (day × access group: $F_{(12,204)} = 89.53$, $p < 0.05$). Post-hoc tests revealed that significant differences emerged on the second access day, and persisted throughout the course of the experiment ($p < 0.05$ on all days). By the end of the experiment, INT mice had eaten 336.6 ± 7.3 kcal on

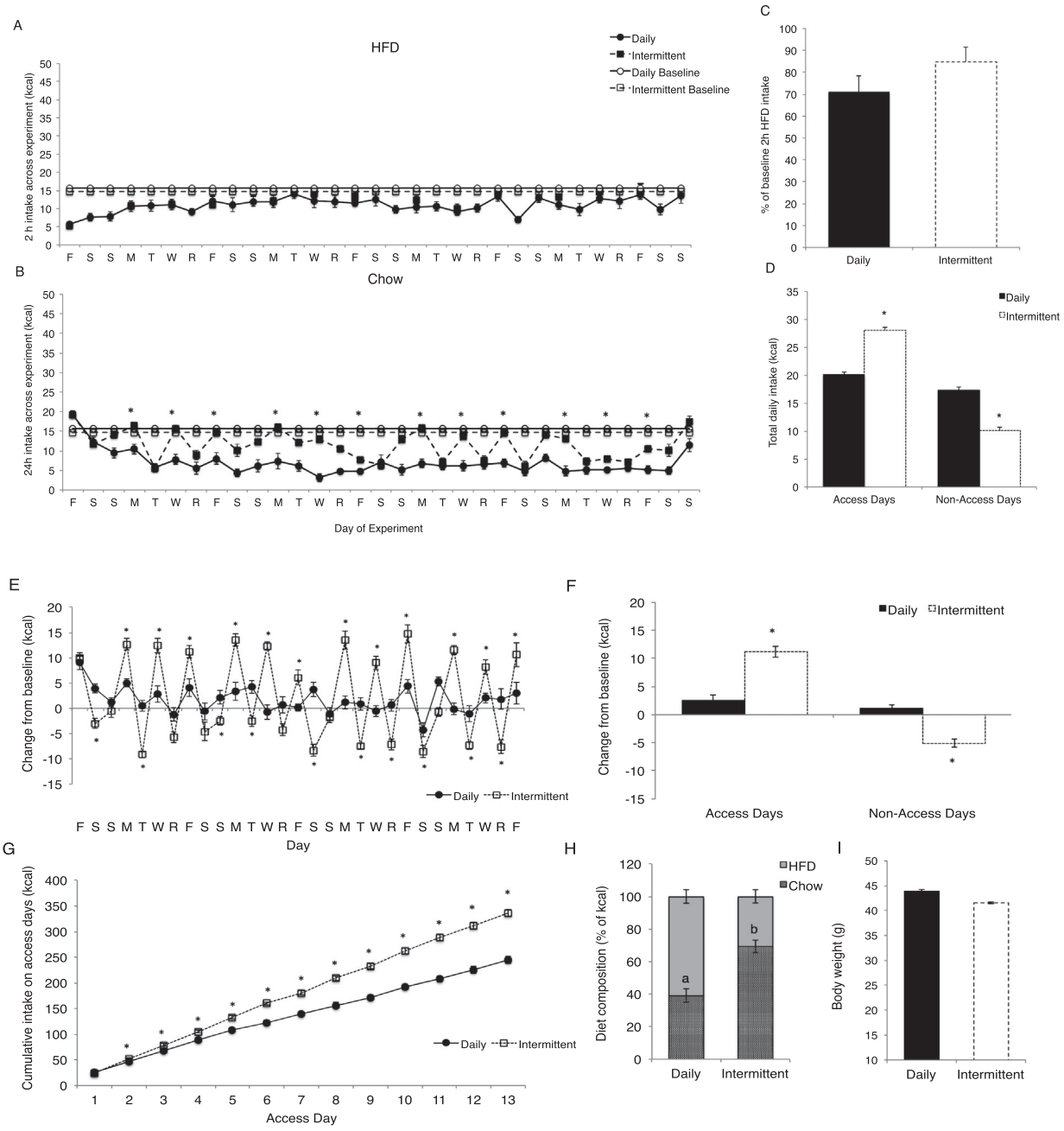

Fig. 1. (A–I) Feeding patterns, diet selection and body weight in CD-1 mice. Caloric intake from HFD (2 h) across the experiment (A). Intake of HFD on access days (M,W,F) did not differ between DAILY and INT mice, however, INT mice show greater chow intake within the same 24-h period. Percent of baseline intake consumed during 2 h of HFD access (C). All mice consumed $\geqslant$ 71% of their average daily intake within 2 h. Caloric intake from chow across the experiment, showing enhanced intake in INT mice on access days (C; $^{*}p < 0.05$). Total daily intake (24-h kcal) was higher in INT mice than in DAILY mice on access days and lower than DAILY mice on non-access days (D, $^{*}p < 0.05$). Change from baseline kcal across the experiment (E, $^{*}p < 0.05$), and average change from baseline intake on access and non-access days (F, $^{*}p < 0.05$). INT mice consumed a significantly greater amount of calories on days that they received access to HFD. They also reduced their intake relative to baseline on days following access to HFD, demonstrating a binge-compensate pattern of intake. DAILY mice did not significantly over- or under-consume relative to baseline on either access or non-access days. Cumulative 24-h intake on HFD access days (G). Mice in the INT group consumed a greater cumulative number of kilocalories on these days, relative to the DAILY mice. Statistically significant differences were found beginning on Day 2 of access and persisted over the course of the experiment. ($^{*}p < 0.05$). Percentage of kilocalories over the course of the experiment derived from either HFD or standard chow (H). Mice in the DAILY group consumed a greater percentage of their kilocalories from HFD than from chow, whereas mice in the INT group consumed a greater percentage from standard chow (a,b, $p < 0.05$). Body weight at the end of the experiment (I).

access days, whereas DAILY mice had consumed 244.8 ± 6.5 kcal on the same days.

Diet Composition (Fig. 1H). Over the course of the experiment, the INT group consumed a greater percentage of their daily calories from standard chow (69.4 ± 4.1) compared to the DAILY group (39.2 ± 4.2; $F_{(1,18)} = 27.5$, $p < 0.05$). The opposite pattern was found in the DAILY group, who consumed a greater percentage of their calories from HFD (60.8 ± 4.2) than

the INT group (30.6 ± 4.1). That the group with a greater frequency of access to HFD consumed a greater percentage of their overall calories from HFD is line with previously reported results (Corwin et al., 1998), even though the animals in the INT group also demonstrated binge-like consumption during HFD exposure. Despite consistently bingeing during the short exposure window 3 days a week, the INT animals did not consume as many overall calories from HFD as those that received the HFD every day for the same amount of time.

Body weight (Fig. 1I). Body weights did not differ between DAILY and INT mice fed HFD upon completion of the experiment ($t_{(17)} = 1.895$, $p > 0.05$).

Experiment 2

High-fat diet (Fig. 2A, B, G). GHSR KO and WT mice demonstrated differential patterns of intake when placed under the different schedules of HFD access. A two-way repeated measures ANOVA revealed that there was no effect of day over the course of the experiment, suggesting that HFD intake was stable across the 6-week period. However, significant main effects of both access ($F_{(1,23)} = 11.574$, $p < 0.05$) and genotype ($F_{(1,23)} = 13.076$, $p < 0.05$) were present. KO mice ate less of the HFD than did WT mice (6.1 ± .4 g vs. 7.9 ± .4 g, respectively) and DAILY MICE ate more HFD than INT mice (7.9 ± .4 g vs. 6.1 ± .4 g, respectively; see Fig. 2A).A two-way ANOVA (access × genotype) for overall average HFD kcal consumption on access days revealed similar results, with main effects of both genotype ($F_{(1,27)} = 16.211$, $p < 0.05$) and access ($F_{(1,27)} = 8.6753$, $p < 0.05$). KO mice consumed less of the HFD during access relative to WT mice, and DAILY mice consumed a greater amount of HFD than INT mice. However, a significant interaction between access schedule and genotype was also found ($F_{(1,27)} = 5.461$, $p < 0.05$), suggesting that the effect of genotype on HFD intake is dependent on access schedule. While both KO and WT mice receiving DAILY HFD ate a similar amount of HFD during the access period ($p > 0.05$), KO mice receiving HFD on an INT schedule failed to increase their intake of HFD to the extent seen in WT mice ($p < 0.05$), see Fig. 2B.

As in Experiment 1, placing mice under an INT or DAILY schedule of HFD access induced binge-like consumption during the HFD access period. A two-way ANOVA examining the mean percentage of control group kcal consumed from HFD during the 2-h HFD access revealed a main effect of genotype ($F_{(1,23)} = 7.236$, $p < 0.05$), with WT mice consuming a greater percentage of daily control intake from HFD (50.1% ± 3.4%) as compared to KO mice (35.4% ± 3.5%, see Fig. 2G). Main effect of access was also significant ($F_{(1,23)} = 11.499$, $p > 0.05$) with DAILY mice (53.9% ± 2.5%) consuming more than INT mice (41.9% ± 2.5%). A marginal interaction ($F_{(1,23)} = 4.129$, $p = 0.054$) suggested that there was a trend, showing that KO mice receiving HFD intermittently did not binge to the same extent as the rest of the groups, and this is likely to be contributing to the main effect of access, resulting in lower intake in INT mice.

Standard chow (Fig. 2C and D). A two-way repeated measures ANOVA for kcal derived from chow across access days revealed a day × access interaction ($F_{(34,510)} = 2.235$, $p < 0.06$), such that both INT and DAILY groups reduced their chow intake over the course of the experiment. A main effect of access was also found ($F_{(2,30)} = 15.307$, $p < 0.05$), with INT and DAILY mice both consuming less chow than control mice (see Fig. 2 C; M,W and F). Overall average chow intake on access days also differed between groups. A main effect of access ($F_{(2,36)} = 17.989$, $p < 0.05$) was revealed by a two-way ANOVA (genotype × access schedule). Both DAILY ($p = 0.000$) and INT ($p = 0.001$) mice consumed less chow than the control group, as control mice did not receive HFD and therefore calories were derived exclusively from chow. While DAILY mice consumed modestly less chow than the INT mice on access days (9.4 ± .6 kcal vs. 11.4 ± .6 kcal), this difference was not statistically significant (see Fig. 2D).

Two-way repeated measures ANOVA on non-access days revealed a day × access interaction ($F_{(2,660)} = 2.214$, $p < 0.05$), showing that DAILY mice reduced their chow intake on non-access days across the experiment, although these were not technically non-access days for this particular group as they were still receiving fat on these days. There was also a main effect of access ($F_{(2,30)} = 17.76$, $p < 0.05$). On most non-access days, both control and INT groups were consuming more chow than the DAILY groups (see Fig. 2C; T,R,S and S).

Change from baseline caloric intake (Fig. 2E, F). To examine whether the DAILY and INT access conditions induced changes in overall daily food consumption relative to the control chow-only group, we calculated the change from baseline kilocalories that all access groups consumed over a 24-h period (kcal consumed on access days-average baseline kcal). The means were calculated separately for days on which the mice had access to a HFD and for days on which they had access exclusively to chow (i.e. non-access days; only occurred within the INT and control groups). Within the DAILY group, consumption on alternating access and non-access days should not differ, theoretically, since these days are comprised of identical timing and food-related cues. On access days, there was a significant main effect of access condition ($F_{(2,35)} = 3.956$, $p < 0.05$). Mice receiving INT access consumed a greater number of calories above their average baseline intake (2.9 ± 0.5) compared to the control groups (0.7 ± 0.6). DAILY mice consumed an intermediate number of calories above baseline (1.3 ± 0.6), but did not differ from either the control ($p > 0.05$) or INT groups ($p > 0.05$). There was no main effect of genotype ($F_{(1,35)} = 9.66$, $p > 0.05$), nor a significant interaction ($F_{(2,35)} = 0.324$, $p > 0.05$, see Fig. 2E).

On *non-access* days, there was a main effect of access condition ($F_{(2,35)} = 6.314$, $p < 0.05$), wherein

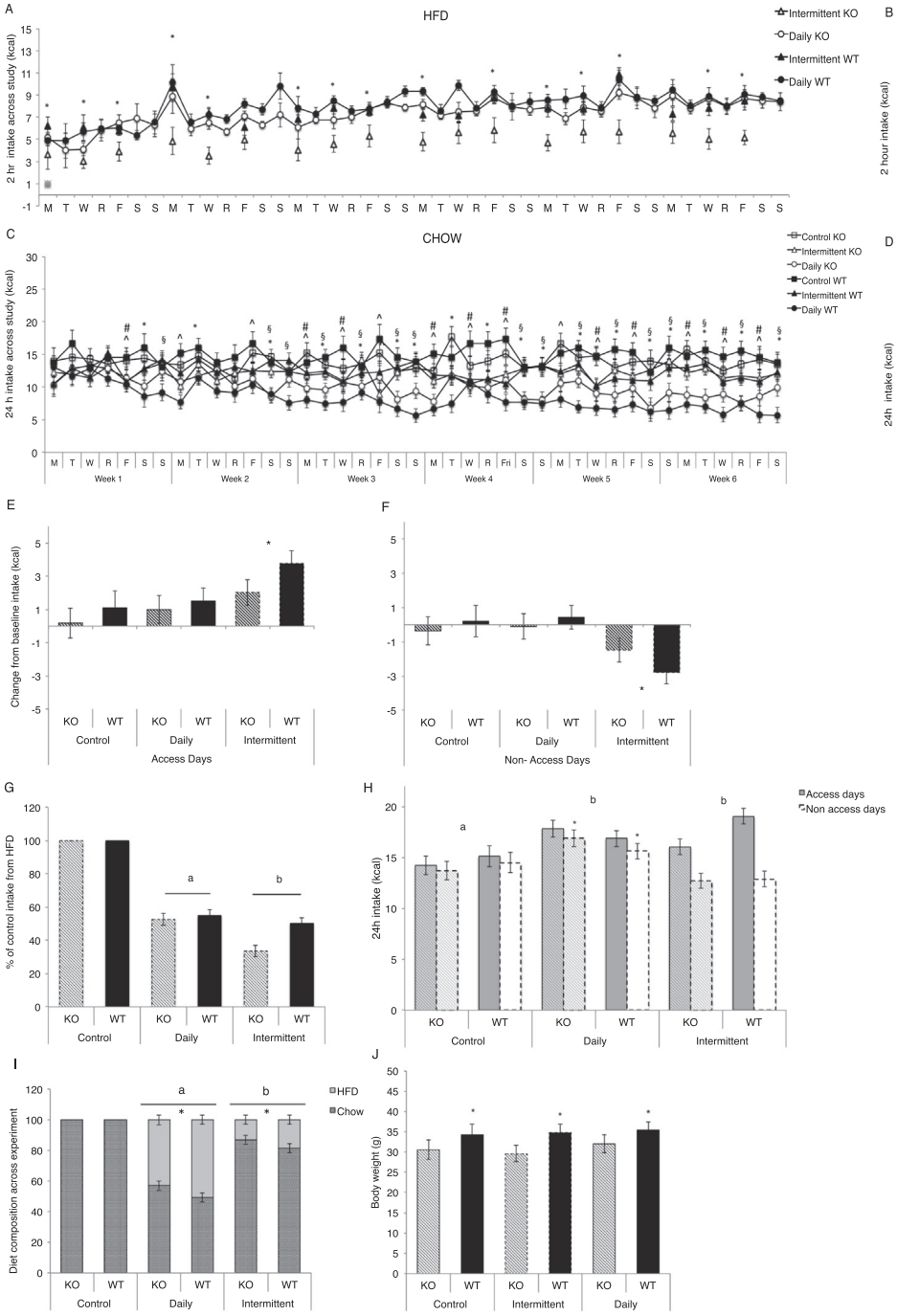

Fig. 2. (A–J) Feeding patterns, diet selection and body weight in C57-GHSR KO and WT mice. Caloric intake from HFD (2 h) across the experiment (A) and overall average HFD intake on access days (B; a,b: $p < 0.05$; denotes access group differences; $^{*}p < 0.05$, denotes differences between KO and WT mice within the INT group). Mice receiving DAILY access to HFD consumed modestly more HFD than those in the INT access group. KO mice in the INT group did not eat as much HFD as INT WT mice. Patterns of caloric intake from chow across the experiment (C; On access days: * denotes controls > daily mice, § denotes that INT > DAILY mice. On non-access days: # denotes that INT mice > DAILY mice, ^ denotes that DAILY > controls; all symbols reflect $p < 0.05$). Overall average chow intake on access days (D). Control mice ate the greatest amount of chow, followed by the INT and DAILY mice (a,b, $p < 0.05$). Change from baseline intake on access days (E) and non-access days (F). INT access to HFD resulted in an elevated caloric intake on access days, and a reduction on non-access days, relative to baseline intake (main effect of access, $^{*}p < 0.05$). Percent of average daily control group caloric intake consumed during 2-h HFD diet exposure (G). DAILY groups consumed more than INT groups (main effect of access, $^{*}p < 0.05$) and KO mice consumed less than WT mice (main effect of genotype, $^{*}p < 0.05$), however there was a trend toward KO INT mice consuming less than WT mice (marginal interaction: $p = 0.054$). 24-h intake on access and non-access days (H), showing that both INT and DAILY HFD access induced increases in total daily intake on access days (a,b, $p < 0.05$) and on non-access days DAILY mice ate the most, as they received HFD on these days. Diet composition across experiment (I). DAILY mice ate a greater percentage of their cumulative experimental calories from HFD relative to the INT mice (main effect of access condition, a,b: $p < 0.05$). GHSR KO mice consumed a lower percentage of their kcal from HFD regardless of access condition (main effect of genotype, $p < 0.05$). Body weight at end of the experiment (I). A main effect of genotype revealed that GHSR KO mice weighed less than WT mice ($^{*}p < 0.05$), but there was no main effect of access and no access by genotype interaction.

the INT groups reduced kcal intake to a greater extent than both control (INT: −2.1 ± 0.5 vs. Controls: −0.1 ± 0.6, $p < 0.05$) and DAILY groups (0.2 ± 0.5, $p < 0.05$). There was no significant main effect of genotype ($F_{(2,35)} = 3.627$, $p > 0.05$), nor a significant interaction between genotype and access condition for 24-h intake ($F_{(2,35)} = 1.096$, $p > 0.05$, see Fig. 2F).

Twenty four hour daily intake on access and non-access days (Fig. 2H). A two-way ANOVA examining average total 24-h intake on access days revealed a main effect of access ($F_{(2,35,)} = 6.065$, $p < 0.05$). Both DAILY and INT mice consumed more on access days than the control group. There was also a marginal interaction ($F_{(2,35)} = 3.156$, $p = 0.057$) showing that 24-h intake for KO INT mice tended to be lower, although this was not statistically significant. On non-access days, there was again a main effect of access ($F_{(2,35)} = 10.069$, $p < 0.05$). Daily groups consumed a greater number of kcal (16.3 ± .6) on these days than both controls (14.1 ± .6) and INT mice (12.8 ± .5).

Overall diet composition (Fig. 2I). The percentage of kilocalories consumed from HFD across the experiment differed between access conditions ($F_{(1,26)} = 106.463$, $p < 0.05$) and between genotypes ($F_{(1,26)} = 4.654$, $p < 0.05$). DAILY mice consumed a greater percentage of their total calories from HFD than did the INT mice (Daily: 46.7% ± 2.2% and Int: 15.7% ± 2.1%, $p < 0.05$) independent of genotype. In addition, the KO mice consumed a smaller percentage of calories from HFD, regardless of access condition (KO: 28.1% ± 2.2% vs. WT: 34.5% ± 2.1%, $p < 0.05$). No significant interaction was detected ($F_{(1,26)} = .158$, $p > 0.05$). With regards to chow intake, the main effects for access condition ($F_{(1,26)} = 106.463$, $p < 0.05$) and genotype ($F_{(1,26)} = 4.654$, $p < 0.05$) were once again significant, but with the opposite trend, as can be seen in Fig. 2I. Mice in the DAILY groups consumed less chow than those in the INT groups (Daily: 53.3% ± 3.0% vs. INT: 84.3% ± 2.1%, $p < 0.05$), and KO mice consumed a greater percentage of chow, independent of access condition (KO: 72.2% ± 2.2% vs. WT: 65.5% ± 2.1% $p < 0.05$). GHSR KO mice when placed under either a limited *or* limited + INT access to a HFD maintain a lower intake of HFD than do their WT littermates over the course of the experiment.

Body weight (Fig. 2J). As determined by a two-way ANOVA (genotype × access) on body weight at completion of the experiment, there was a significant main effect of genotype ($F_{(1,35)} = 5.048$, $p < 0.05$), such that KO mice weighed less than WT mice (30.7 ± 1.3 g and 34.8 ± 1.3 g, respectively). There was no main effect of access ($F_{(2,35)} = .313$, $p > 0.05$) and no interaction between genotype and access ($F_{(2,35)} = .102$, $p > 0.05$).

C-Fos IR in NAcc (Fig. 3). c-Fos IR was quantified after mice were sacrificed one hour subsequent to their last receipt of HFD. The data in Fig. 3 are expressed as the mean percentage of c-Fos-positive cells found in control mice. A two-way ANOVA revealed that there were no main effects of access ($F_{(1,24)} = 0.060$, $p > 0.05$) or genotype ($F_{(1,24)} = 1.31$, $p > 0.05$), and no interaction ($F_{(1,24)} = 0.231$, $p > 0.05$) in expression in the NAcc core region, with all mice showing similar levels of c-Fos expression following their final receipt of HFD. Alternatively, we found that expression differed between WT and KO in the shell region of the NAcc (main effect of genotype: $F_{(1,33)} = 8.003$, $p < 0.05$), with KO mice showing a comparable amount of expression to controls, but with WT mice showing nearly a threefold increase in c-Fos expression relative to control mice. Table 1 depicts c-Fos counts expressed as percent of controls in both the NAcc core and shell, as well as the lateral hypothalamic area (LHA) and the VTA. No main effect of access ($F_{(2,23)} = .479$, $p > 0.05$), genotype ($F_{(1,23)} = .002$, $p > 0.05$), nor an interaction ($F_{(2,23)} = .002$, $p > 0.05$) were found for expression in the LHA

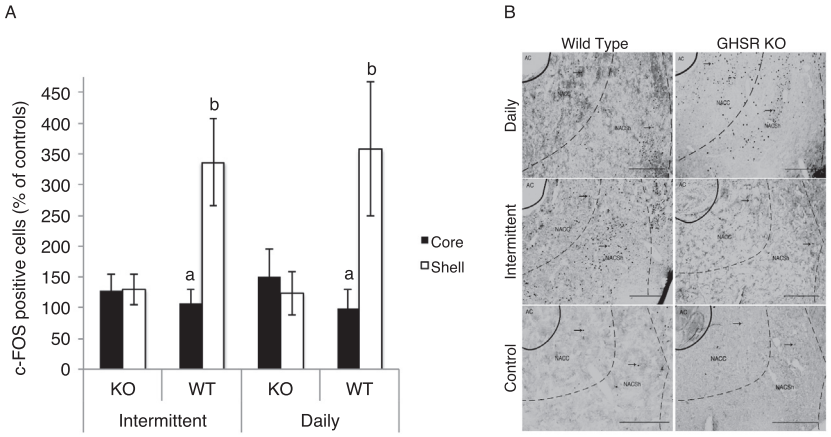

Fig. 3. c-Fos immunohistochemistry. (A) Number of c-FOS-positive nuclei expressed as a percentage of control mice in the NAcc shell and core regions. WT mice that received the HFD on either a DAILY or INT basis showed elevated c-FOS IR in the shell region, relative to the KO mice on both access schedules ($^{*}p < 0.05$). No significant differences in c-FOS IR were found in the core region between groups. (B) Representative photomicrographs of the shell region to visualize group differences (10× objective magnification; Bregma: +1.54 → +0.98; Scale bar = 250 μm).

Table 1. Percent of control c-Fos IR in select brain regions

Region	Daily		Intermittent	
	KO	WT	KO	WT
NAc shell	123.2 ± 34.9*	357.8 ± 109.5	129.5 ± 25.3*	336.5 ± 70.6
NAc core	150.7 ± 45.5	99.1 ± 29.6	127.7 ± 27.15	106.6 ± 22.6
LH	106.9 ± 26.24	81.36 ± 29.4	105.923 ± 29.4	134.99 ± 26.24
VTA	107 ± 41.8	210.3 ± 37.4	52.26 ± 48.2	130.8 ± 41.8

* Indicates $p < 0.05$.

nor in the VTA (access: $F_{(2,23)} = 1.493$, $p > 0.05$; genotype: $F_{(2,23)} = 2.646$, $p > 0.05$); interaction: $F_{(2,23)} = 1.493$, $p > 0.05$).

DISCUSSION

In the current experiments we examined the feeding patterns of mice subjected to a nutritionally complete HFD under limited access conditions. The aim of Experiment 1 was to attempt to replicate a rat model of binge eating in mice, originally developed by placing rats on a schedule of limited and sporadic access to fat (Corwin et al., 1998; Davis et al., 2007). Rats placed on this time-restricted (2 h/day) and sporadic (3×/week) access to either vegetable shortening (Corwin et al., 1998), or a nutritionally complete high-fat diet (Davis et al., 2007), display a binge/compensate pattern of feeding, typically evident by the second week of scheduled access. As this particular model has not yet been validated in mice, we sought to determine if mice subjected to a similar schedule of HFD access would exhibit a binge-like pattern of eating similar to that seen in rats. Furthermore, we were interested in determining whether a lack of functional ghrelin signaling in GHSR KO mice would hinder the development of binge eating in this model and examined post-HFD c-Fos activity in the NAcc, a key target of VTA DA neurons that play a substantial role in modulating both appetitive and consummatory responses to foods with high palatability (Castro and Berridge, 2014).

In Experiment 1, we found that male CD-1 mice exposed to an INT access schedule demonstrated a binge/compensate pattern similar to that seen in prior studies conducted in rats (Corwin et al., 1998; Davis et al., 2007). However, mice with INT access did not eat an amount of HFD that was consistently in excess of those mice on the DAILY access schedule, although they did binge, consuming 85% of their average baseline caloric intake during the 2-h HFD access period, meeting criteria for a binge episode (one example: consuming > 25% of average daily intake in a 1 h period; Halpern et al., 2013). Mice with DAILY access to the HFD consumed 71% of their average baseline calories during the HFD access period, though differences between access groups were not statistically significant. Therefore, mice exposed to HFD for 2 h, either on a DAILY or INT access schedule, binged on HFD when given access to it. These results do not meet the operationalization of binge eating outlined by Corwin et al. (2011), as the intake of the palatable food source in the INT access group was not consistently in excess of the DAILY group, nor did it escalate substantially over time. However, the number of kcals consumed was similar to the size of binges in mice given 2-h exposure to a HFD in the middle of the dark cycle, although these mice were exposed daily (i.e. predictably; Bake et al., 2013). The elevated total 24-h caloric intake seen in INT mice on access days in our study can be attributed to the fact that the INT access mice continued to eat a similar amount of chow as they did during baseline, while bingeing on the HFD during the access period. The binge-compensation pattern of feeding was not only evident when comparing across access conditions, but was also significantly different from the average baseline intake for each of the groups (see Fig. 1E, F). Further, as seen in previous studies (e.g. Corwin et al., 1998), the number of kcals consumed from HFD over the course of the experiment was highest in mice that received HFD every day.

While not fully in line with previous rat studies demonstrating binge-like intake of exclusively HFD, our findings from Experiment 1 suggest that mice exposed to a HFD under the INT schedule do not compensate by decreasing their intake of chow during the dark period. This phenomenon has been reported in alternative animal models of overeating, in history of dieting and stress models (Hagan et al., 2003), as well as in studies examining cue-potentiated feeding on chow (Boggiano et al., 2009). In addition, intake of a less palatable food increased when rats were placed back into the context in which they previously received a palatable food (Boggiano et al., 2009). Allowing the animal to see and smell a palatable food but not allowing them to eat it, (as in the INT groups) can also induce an above average increase in corticosterone (CORT), a response that appears to be exaggerated in mice that tend to binge eat (Cifani et al., 2009). The removal of HFD prior to the natural feeding cycle may also serve as a stressor, thereby inducing increased consumption of the food that remains available.

In Experiment 2 we used the limited access model on mice lacking GHSR in order to determine the role of ghrelin receptors in mediating binges by comparing them to their WT littermates. Mice in the DAILY access groups (both WT and KO) consumed modestly more HFD during the 2-h access compared to INT groups, demonstrating that both GHSR KO and WT mice increase their consumption of a HFD under this feeding paradigm. GHSR KO mice, however, consumed less of the HFD than their WT littermates when exposed to the INT HFD schedule. These results point to a role of

GHSR in mediating the overconsumption of calories seen under the INT HFD schedule.

Reduced caloric intake may involve a number of mechanisms that include an increase in the ability of satiety hormones to stop HFD feeding bouts in GHSR KO mice (Zigman et al., 2005). Alternatively, the lack of GHSR signaling may prevent ghrelin from interacting directly with dopaminergic neurons via GHSR in the midbrain and decrease overall motivation to eat the HFD on an INT HFD feeding schedule. The latter hypothesis is supported by our data examining cFoS IR in the NAcc. GHSR KO mice post-HFD showed lower levels of cFos activation in the NAcc, particularly in the shell region following consumption of HFD. Interestingly, although WT mice DAILY and INT groups did not differ in HFD intake, they did show elevated levels of cFos IR in this same region relative to control and GHSR KO mice. Therefore, our results support the notion that the NAcc is recruited following acute consumption of HFD in mice (Valdivia et al., 2014) and is an important component of the circuitry underlying food-seeking behaviors (Kelley, 2004; Di Chiara and Bassareo, 2007). Additionally, the importance of the glutamatergic system within the NAcc shell region, but not the core, in maintaining binge eating was also recently demonstrated in rats (Smith et al., 2014), suggesting a potentially greater role for the shell in regulating excessive or binge-like consumption of palatable foods.

Direct action of ghrelin on GHSR in the VTA can regulate the excitability of DA neurons, as shown by rapid induction of increased excitatory and reduced inhibitory inputs onto tyrosine-hydroxylase (TH) expressing neurons upon intra-VTA infusion. Intra-VTA ghrelin also increased TH neuron firing rates, as well as increased DA turnover in the NAc (Abizaid et al., 2006). Interestingly, we did not find exaggerated c-Fos expression in the VTA or in the LHA, nor differences between groups. Both of these regions have been shown to be activated in response to HFD and important for expression of food reward behaviors (e.g. Perello et al., 2010; Valdivia et al., 2014, 2015). It is possible that if we had looked at anticipatory rather than post-ingestive c-Fos profiles, we may have seen differences between groups, as mice that lack GHSR show an attenuated c-Fos profile in the VTA, as well as a reduced proportion of orexin neuron activation in the LHA (Lamont et al., 2012). It is possible that ghrelins direct action at the level of the VTA could become less prominent as the length of exposure to the palatable food increases, and that other ligand-independent interactions with alternate GPCRs in the NAc, such as the D1R, could facilitate the intake of HFD (for review see: Wellman and Abizaid, 2015). Lack of GHSR in this case would alter DA receptor activity in the NAc, without altering activity in the VTA or the LHA.

Activation of the NAc after chronic exposure to a palatable food may reflect how palatable the mice find the food, but may not require recruitment of VTA-DA after chronic repeated exposure. It has also been shown that direct action of ghrelin on the NAc is sufficient to induce a feeding response (Naleid et al., 2005) as well as to enhance locomotor responses to cocaine in rats (Jang et al., 2013), and therefore a lack of GHSR in NAc could suppress overeating of HFD that would typically be induced by ghrelin stimulation in this region. The higher levels of c-Fos seen in the shell region under both DAILY and INT access conditions only in WT mice, indicate that this region is activated to a greater extent following consumption of a HFD when animals have functional GHSRs. Although the precise relationship between c-Fos expression and transcription and/or depolarization is unknown (Hoffman et al., 1993), it is still a powerful method of detecting activation in response to a given stimuli, albeit with poor time-course precision. Determining the phenotype of the neurons exhibiting c-Fos IR in future studies would add to our knowledge which subpopulations of neurons in the NAcc are being activated in the context of this model.

With regard to change from baseline 24-h caloric intake, mice with INT access to HFD increased intake on access days, and reduced intake on non-access days, consistent with the binge-compensate pattern of intake seen in our first experiment, as well as in previous reports employing this model in rats. Interestingly, there was no effect of genotype on this measure, suggesting that KO mice are able to develop the same patterns of intake when placed under these access conditions, but do not binge on HFD to the same extent during HFD access periods.

Diet composition in Experiment 2 was similar to composition data found in Experiment 1, with mice receiving DAILY access to HFD deriving a greater percentage of their kilocalories from HFD than did the INT groups. Additionally, we found that KO mice, whether receiving DAILY or INT access did not consume as great a percentage of kcals from HFD as WT mice. Therefore, over a 6-week period, GHSR KO mice show reduced consumption of HFD when access to it is restricted, whether they receive access daily or only 3 days per week. Rodents lacking ghrelin signaling or receiving a GHSR antagonist have a reduced ability to form a conditioned place preference to HFD (Perello et al., 2010; Chuang et al., 2011), a reduction in cue-potentiated feeding (Walker et al., 2012) and reduced consumption of a high-fat ‘‘dessert” following a satiating meal (St.Onge and Abizaid, 2012). Therefore, it is clear that intact GHSR plays a role in increasing the consumption of a HFD, although we cannot comment here on a potential decrease in palatability of the HFD to mice lacking GHSR as we did not directly measure ‘‘liking” responses (Shin et al., 2011; Overduin et al., 2012).

The lack of two-hour consumption differences between access groups may suggest that the diet chosen, as it was a nutritionally complete HFD, may have led the groups to both consume such large amounts that differences between access groups could not be detected. The possibility exists that the HFD was too palatable and therefore drove excessive consumption in both access conditions. However, as previous experiments have demonstrated that a nutritionally complete HFD can induce a binge compensate pattern of intake in INT access comparable to vegetable shortening (Davis et al., 2007), we chose to use this diet in our current experiments. Further, there

is often overlap in fat intakes between INT and DAILY access groups, and in some cases, up to half of the animals from each group can be matched on short-term (1 h) fat consumption (Wojnicki et al., 2010). This can occur throughout the course of the experiment, and highlights the possibility of high variability within access groups, and could help to explain some of our discrepant findings in mice. Additionally, not all humans who are exposed to highly palatable fatty foods under an INT schedule, whether self-imposed or not, exhibit binge-like eating. Exploring the underlying mechanisms in animals and humans prone to binge eating those not prone, as well as the role of ghrelin in such models, may serve to highlight some of the underlying mechanistic differences between these groups.

In a recent study conducted by Valdivia et al. (2015), it was shown that 2-h HFD consumption over a 4-day period was sufficient to cause an escalation of intake and that a lack of GHSR eliminated this escalation across days. The HFD access group in this study was akin to our DAILY mice, in that they received HFD every day at the same time for 4 days in a row. We did not find an escalation of intake in our experiment, and it may be that GHSR KO mice given longer term predictable exposure to the HFD retain the ability to match the HFD intake of the WT mice after the first few days of intake. The unpredictability of the HFD access in the KO INT mice appears to contribute to the reduced binge-like consumption, potentially reflecting an inability of the GHSR KO mice to take advantage of limited availability of the palatable diet, as the WT mice do. It has been shown that mice administered a ghrelin antagonist during the acquisition phase of conditioned place preference for HFD failed to develop a preference, but that once acquired, the preference was not hindered by blockade of ghrelin on test day (Perello et al., 2010). Therefore, it is also possible that a lack of ghrelin signaling via GHSR during palatable food access that is more uncertain, GHSR KO mice lack the ability to orchestrate a full binge-like response. The anatomical and functional correlates of such behavior have yet to be explored in the context of this model.

Overall, we have shown that the two strains exhibited both similarities and differences in behavior when subjected to INT and DAILY access to palatable food. All mice over-ate on access days and under-ate on non-access days, as a result of adding 2-h access to palatable food to their *ad libitum* chow dietary regimen, showing that exposure to palatable foods in the absence of energy requirements enhances daily caloric intake. However, caloric consumption above and beyond baseline daily intake in CD-1 mice appeared to be a combination of consistent chow intake despite limited access to HFD, whereas in the C57BL6/J mice, intake of chow was not elevated in HFD access conditions, and in fact was lowered in mice that received DAILY HFD. Strain differences can affect the feeding behavior and metabolic parameters of mice given a HFD continuously (Nishikawa et al., 2007; Sims et al., 2013), however, there are only a few recent studies that examine binge-like feeding in mice and they used C57BL6/J mice (Czyzyk et al., 2010; Cao et al., 2014) and some examined sucrose intake (Yasoshima and Shimura, 2015) or chow intake (Razzoli et al., 2015). This makes it difficult to compare our CD-1 findings with those of other studies. However, as the few murine binge-like feedings paradigms utilize C57BL6/J mice, and as a number of genetically engineered mouse models are backcrossed onto this strain, we have continued to use these mice in our studies.

Upon examination of the limited access model in GHSR KO and WT mice, there appears to be a role for GHSR signaling in the amount of HFD consumed under these conditions, as GHSR KO mice fail to consume as much HFD across the study when access is restricted, and these results are enhanced when the access is scheduled in a less predictable manner (i.e. MWF). The mechanisms by which the lack of intact GHSR lessens the magnitude of the "binge" under INT access schedules in KO mice are unknown. It has been hypothesized that the interaction of ghrelin with mesolimbic reward circuitry serves to amplify the pleasure derived from a number of reinforcers (Lockie et al., 2015), and the lack of ghrelin-dependent GHSR signaling to reduce responding for stimuli such as cocaine (Abizaid et al., 2011) or nicotine (Jerlhag and Engel, 2011) supports this hypothesis. We are currently investigating the role GHSR signaling in various brain regions that may play a role in the development of binge-like behavior.

CONCLUSION

Taken together, data from both experiments support the ability of intermittently scheduled access to palatable food in mice to induce a binge-compensate pattern of intake that emulate select components of binge eating in humans. Additionally, there appears to be a role for GHSR signaling in the amount of HFD consumed under these conditions. The reciprocal relationship between INT feeding schedules and the neuroendocrine regulation of feeding behavior, as well as its underlying mechanisms remain poorly studied (for review see Murphy and Mercer, 2014), and the precise role of the ghrelin system in this process remains to be determined. Additional insight into these processes may aid in the understanding of binge eating and how this pattern of disordered eating develops and persists.

Acknowledgments—This work was supported by an NSERC discovery grant (A.A.) and an NSERC Alexander Graham Bell PGS (S.J.K.).

REFERENCES

Abizaid A, Mineur YS, Roth RH, Elsworth JD, Sleeman MW, Picciotto MR, Horvath TL (2011) Reduced locomotor responses to cocaine in ghrelin-deficient mice. Neuroscience 192:500–506.

Abizaid A, Horvath TL (2008) Brain circuits regulating energy homeostasis. Regul Pept 149(1–3):3–10.

Abizaid A (2009) Ghrelin and dopamine: new insights on the peripheral regulation of appetite. J Neuroendocrinol 21 (9):787–793.

Abizaid A, Liu ZW, Andrews ZB, Shanborough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Piccioto MR, Tschop MH, Gao XB, Horvath TL (2006) Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J Clin Invest 116:3229–3239.

Abizaid A, Mezei G, Thanarajasingam G, Horvath TL (2005) Estrogen enhances light-induced activation of dorsal raphe serotonergic neurons. Eur J Neurosci 21:1536–1546.

American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders. 5th ed. Arlington, VA: American Psychiatric Publishing.

Bake T, Murphy M, Morgan DGA, Mercer JG (2014a) Large, binge-type meals of high fat diet change feeding behavior and entrain food anticipatory activity in mice. Appetite 77C:60–71.

Bake T, Morgan DG, Mercer JG (2014b) Feeding and metabolic consequences of scheduled consumption of large, binge type meals of high fat diet in the Sprague-Dawley rat. Physiol Behav 128:70–79.

Bake T, Duncan JS, Morgan DGA, Mercer JG (2013) Arcuate nucleus homeostatic systems are not altered immediately prior to the scheduled consumption of large, binge-type meals of palatable solid of liquid diets in rats and mice. J Neuroendocrinol 25:357–371.

Bello NT, Hajnal A (2010) Dopamine and binge eating behaviors. Pharmacol, Biochem Behav 97(1):25–33.

Bello NT, Guarda AS, Terrillion CE, Redgrave GW, Coughlin JW, Moran TH (2009) Repeated binge access to a palatable food alters feeding behavior, hormone profile, and hindbrain c-Fos responses to a test meal in adult male rats. Am J Physiol Regul Integr Comp Physiol 297:R622–R631.

Berner LA, Avena HM, Hoebel BG (2008) Bingeing, self-restriction, and increased body weight in rats with limited access to a sweet-fat diet. Obesity 16(9):1998–2002.

Boggiano MM, Artiga AI, Pritchett CE, Chandler PC, Smith ML, Eldridge AJ (2007) High intake of palatable food predicts binge-eating characteristics independent of susceptibility to obesity: an animal model of lean vs. obese binge eating and obesity with and without binge eating. Int J Obes 31:1357–1367.

Boggiano MM, Dorsey JR, Thomas JM, Murdaugh DL (2009) The pavlovian power of palatable food: lessons for weight loss adherence from a new rodent model of cue-induced overeating. Int J Obes 33(6):693–701.

Cao X, Xu P, Oyola MG, Xia Y, Yan X, Saito K, Zou F, Wang C, Yang Y, Hinton Jr A, Yan C, Ding H, Zhu L, Yu L, Yang B, Feng Y, Clegg DJ, Khan S, DiMarchi R, Mani SK, Tong Q, Xu Y (2014) Estrogens stimulate serotonin neurons to inhibit binge-like eating in mice. J Clin Invest 124(10):4351–4362.

Castro DC, Berridge KC (2014) Advances in the neurobiological bases for food 'liking' vs 'wanting'. Physiology and Behavior. pii: S0031-9384(14)00299.

Chuang JC, Perello M, Sakata I, Osborne-Lawrence S, Savitt JM, Lutter M, Zigman JM (2011) Ghrelin mediates stress-induced food-reward behavior in mice. JCI 121(7):2684–2692.

Cifani C, Zanoncelli A, Tessari M, Righetti C, DiFrancesco C, Ciccocioppo R, Massi M, Melotto S (2009) Pre-exposure to environmental cues predictive of food availability elicits hypothalamic-pituitary-adrenal axis activation and increases operant responding for food in female rats. Addict Biol 14 (4):397–407.

Corwin RL, Wojnicki FHE, Fisher JO, Dimitriou SG, Rice HB, Young MA (1998) Limited access to a dietary fat option affects ingestive behavior but not body composition in male rats. Physiol Behav 63 (3):543–553.

Corwin RL, Avena NM, Boggiano MM (2011) Feeding and reward: perspectives from three rat models of binge eating. Physiol Behav 104(1):87–97.

Cottone P, Sabino V, Steardo L, Zorilla EP (2008) Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food. Neuropsychopharmacology 33(3):524–535.

Corrigall WA, Coen KM (1989) Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 99(4):473–478.

Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS (2001) A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714–1719.

Cummings DE (2006) Ghrelin in the short and long term regulation of appetite and body weight. Physiol Behav 89(1):71–84.

Czyzyk TA, Sahr AE, Statnick MA (2010) A model of binge-like eating behavior in mice that does not require food deprivation or stress. Obesity 18(9):1710–1719.

Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa, Nakazato M (2000) Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 141:4255–4261.

Davis CA, Levitan RD, Reid C, Carter JC, Kaplan AS, Patte KA, King N, Curtis C, Kennedy JL (2009) Dopamine for "wanting" and opioids for "liking": a comparison of obese adults with and without binge eating. Obesity 17(6):1220–1225.

Davis JF, Melhorn SJ, Shurdak JD, Heiman JU, Tschop MH, Clegg DJ, Benoit SC (2007) Comparison of hydrogenated vegetable shortening and nutritionally complete high-fat diet on limited access-binge behavior in rats. Physiol Behav 92:924–930.

Di Chiara G, Bassareo V (2007) Reward system and addiction: what dopamine does and doesn't do. Curr Opin Pharmacol 7:69–76.

Dickson SL, Egecioglu E, Landgren S, Skibicka KP, Engel JA, Jerlhag E (2011) The role of the central ghrelin system in reward from food and chemical drugs. Mol Cell Endocrinol 340:80–87.

Drazen DL, Vahl TP, D'Alessio DA, Seeley RJ, Woods SC (2006) Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. Endocrinology 147(1):23–30.

Egecioglu E, Jerlhag E, Salome N, Skibicka KP, Haage D, Bohlooly-Y M, Andersson D, Bjursell M, Perrissoud D, Engel JA, Dickson SL (2010) Ghrelin increases intake of rewarding food in rodents. Addict Biol 15(3):304–311.

Elmore DK, de Castro JM (1991) Meal patterns of normal, untreated bulimia nervosa and recovered bulimic women. Physiol Behav 49:99–105.

Geliebter A, Gluck ME, Hashim SA (2005) Plasma ghrelin concentrations are lower in binge-eating disorder. J Nutr 135 (5):1326–1330.

Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJS, Smith RG, Van der Ploeg LHT, Howard AD (1997) Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Mol Brain Res 48:23–29.

Hagan MM, Chandler PC, Wauford PK, Rybak RJ, Oswald KD (2003) The role of palatable food and hunger as trigger factors in an animal model of stress induced binge eating. Int J Eat Disord 34 (2):183–197.

Halpern CH, Tekriwal A, Santollo J, Keating JG, Wolf JA, Daniels D, Bale TL (2013) Amelioration of binge eating by nucleus accumbens deep brain stimulation in mice involves D2R modulation. J Neurosci 33(17):7122–7129.

Hoffman GE, Smith MS, Verbalis JG (1993) C-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. Front Neuroendocrinol 14(3):173–213.

Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M (2001) Minireview: ghrelin and the regulation of energy balance—a hypothalamic perspective. Endocrinology 142:4163–4169.

Howard AD et al (1996) A receptor in pituitary and hypothalamus that functions in growth hormone release. Science 273:974–977.

Jang JK, Kim WY, Cho BR, Lee JW, Kim J (2013) Microinjection of ghrelin in the nucleus accumbens core enhances locomotor activity induced by cocaine. Behav Brain Res 248:7–11.

Jerlhag E, Engel JA (2011) Ghrelin receptor antagonism attenuates nicotine-induced locomotor stimulation, accumbal dopamine

release and conditioned place preference in mice. Drug Alcohol Depend 117(2–3):126–131.
Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA (2006) Ghrelin stimulates locomotor activity and accumbal dopamine overflow via central cholinergic mechanisms: implications for its involvement in brain reward. Addict Biol 11:45–54.
Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, Engel JA (2007) Ghrelin administration stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. Addict Biol 12:6–16.
Johnson PM, Kenny PJ (2010) Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nat Neurosci 13(5):635–641.
Kales EF (1990) Macronutrient analysis of binge eating in bulimia. Physiol Behav 48(6):837–840.
Kelley AE (2004) Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. Neurosci Biobehav Rev 27:765–776.
King SJ, Isaacs AM, O'Farrell E, Abizaid A (2011) Motivation to obtain preferred foods is enhanced by ghrelin in the ventral tegmental area. Horm Behav 60(5):572–580.
Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656–660.
Lardeux S, Kim JJ, Nicola SM (2013) Intermittent access to sweet high-fat liquid induces increased palatability and motivation to consume in a rat model of binge consumption. Physiol Behav 114–115:21–31.
Lamont EW, Patterson Z, Rodrigues T, Vallejos O, Blum ID, Abizaid A (2012) Ghrelin-deficient mice have fewer orexin cells and reduced cFOS expression in the mesolimbic dopamine pathway under a restricted feeding paradigm. Neuroscience 218:12–19.
Lockie SH, Dinan T, Lawrence AJ, Spencer SJ (2015) Diet-induced obesity causes ghrelin resistance in reward processing tasks. Psychoneuroendocrinology 62:114–120.
Monteleone P, Fabrazzo M, Tortorella A, Martiadis V, Serritella C, Maj M (2005) Circulating ghrelin is decreased in non-obese and obese women with binge-eating disorder as well as in obese non-binge eating women, but not in patients with bulimia nervosa. Psychoneuroendocrinology 30(3):243–250.
Monteleone P, Tortorella A, Castaldo E, Di Fillippo C, Maj M (2007) The Leu72Met polymorphism of the ghrelin gene is significantly associated with binge eating disorder. Psychiatr Genet. 17 (1):13–16.
Murphy M, Mercer JG (2014) Intermittent feeding schedules-behavioral consequences and potential clinical significance. Nutrients 6:985–1002.
Naleid AM, Grace MK, Cummings DE, Levine AS (2005) Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides 26:2274–2279.
Nishikawa S, Yasoshima A, Doi K, Nakayama H, Uetsuka K (2007) Involvement of sex, strain and age factors in high fat diet-induced obesity in C57BL/6J and BALB/c mice. Exp Anim 56:263–272.
Overduin J, Figlewicz DP, Bennett-Jay J, Kittleson S, Cummings DE (2012) Ghrelin increases the motivation to eat, but does not alter food palatability. Am J Physiol Integr Physiol 303. R259-R69.
Paxinos G, Franklin BJ (2001) The mouse brain in stereotaxic coordinates. 2nd ed. San Diego, California: Academic Press.
Perello M, Sakata I, Birnbaum S, Chuang J, Osborne-Lawrence S, Rovinsky SA, Woloszyn J, Yanagisawa M, Lutter M, Zigman JM (2010) Ghrelin increases the rewarding value of a high-fat diet in an orexin-dependent manner. Biol Psychiatry 67:880–886.
Perello M, Dickson SL (2015) Ghrelin signaling on food reward: a salient link between the gut and the mesolimbic system. J Neuroendocrinol 27(6):424–434.
Pinel JP, Huang E (1976) Effects of periodic withdrawal on ethanol and saccharin selection in rats. Physiol Behav 16(6):693–698.
Razzoli M, Sanghez V, Bartolomucci A (2015) Chronic subordination stress induces hyperphagia and disrupts eating behavior in mice modeling binge-eating like disorder. Front Nutr 1(30). pii: 00030.
Shin AC, Zheng H, Pistell PJ, Berthoud HR (2011) Roux-en-Y gastric bypass surgery changes food reward in rats. Int J Obes 35 (5):642–651.
Skibicka KP, Dickson SL (2011a) Ghrelin and food reward: the story of potential underlying substrates. Peptides 32:2265–2273.
Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Dickson SL (2011b) Ghrelin directly targets the ventral tegmental area to increase food motivation. Neuroscience 180:129–137.
Skibicka KP, Shirazi RH, Hansson C, Dickson SL (2012) Ghrelin interacts with neuropeptide Y Y1 and opioid receptors to increase food reward. Endocrinology 153(3):1194–1205.
Smith KL, Rao RR, Velazquez-Sanchez C, Valenza M, Giuliano C, Everitt BJ, Sabino V, Cottone P (2014) The uncompetitive N-methyl-D-aspartate antagonist memantine reduces binge like eating, food-seeking behavior, and compulsive eating: role of the nucleus accumbens shell. Neuropsychopharmacology:1–9.
Sims EK, Hatanaka M, Morris DL, Tersey SA, Kono T, Chaudry ZZ, Day KH, Moss DR, Stull ND, Mirmira RG, Evans-Molina C (2013) Divergent compensatory responses to high-fat diet between C57BL6/J and C57BLKS/J inbred mouse strains. Am J f Physiol – Endocrinol Metabol 305(12):E1495–E1511.
St.Onge V, Abizaid A (2012). The role of ghrelin in reward based feeding. Endocrine Society's 94th annual meeting, June 2012.
Tschop M, Smiley DL, Heiman ML (2000) Ghrelin induces adiposity in rodents. Nature 407:908–913.
Troisi A, Di Lorenzo G, Lega I, Tesauro M, Bertoli A, Leo R, Iantorno M, Pecchioli C, Rizza S, Turriziani M, Lauro R, Siracusano A (2005) Plasma ghrelin in anorexia, bulimia, and binge-eating disorder: relations with eating patterns and circulating concentrations of cortisol and thyroid hormones. Neuroendocrinology 81(4):259–266.
Valdivia S, Cornejo MP, Reynalso M, De Francesco PN, Perello M (2015) Escalation in high fat intake in a binge eating model differentially engages dopamine neurons of the ventral tegmental area and requires ghrelin signaling. Psychoneuroendocrinology 60:206–216.
Valdivia S, Patrone A, Reynaldo M, Perello M (2014) Acute high fat diet consumption activates the mesolimbic circuit and requires orexin signaling in a mouse model. PLoS One 9(1):e87478.
Walker AK, Ibia IE, Zigman JM (2012) Disruption of cue-potentiated feeding in mice with blocked ghrelin signaling. Physiol Behav 108:34–43.
Wayner MJ, Fraley S (1972) Enhancement of the consumption of acclimated sapid solutions following periodic and prolonged withdrawal. Physiol Behav 9(3):463–474.
Wellman M, Abizaid A (2015) Growth hormone secretagogue receptor dimers: a new pharmacological target. eNeuro 2 (2):1–16.
Wojnicki FHE, Babbs RK, Corwin RLW (2010) Reinforcing efficacy of fat, as assessed by progressive ratio responding, depends upon availability not amount consumed. Physiol Behav 100:316–321.
Yasoshima Y, Shimura T (2015) A model for binge-like sucrose overconsumption: contribution of enhanced motivation for sweetener consumption. Physiol Behav 138:154–164.
Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, Williams TD, Lachey JL, Seeley RJ, Lowell BB, Elmquist JK (2005) Mice lacking ghrelin receptors resist the development of diet-induced obesity. J Clin Invest 115(12):3564–3572.
Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK (2006) Expression of ghrelin receptor mRNA in the rat and mouse brain. J Comp Neurol 494:528–548.

(Accepted 7 January 2016)
(Available online 11 January 2016)