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Nucleus accumbens cocaine-amphetamine regulated transcript mediates food intake during novelty conflict

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Abstract

Obesity is a persistent and pervasive problem, particularly in industrialized nations. It has come to be appreciated that the metabolic health of an individual can influence brain function and subsequent behavioral patterns. To examine the relationship between metabolic phenotype and central systems that regulate behavior, we tested rats with divergent metabolic phenotypes (Low Capacity Runner: LCR vs. High Capacity Runner: HCR) for behavioral responses to the conflict between hunger and environmental novelty using the novelty suppressed feeding (NSF) paradigm. Additionally, we measured expression of mRNA, for peptides involved in energy management, in response to fasting. Following a 24-h fast, LCR rats showed lower latencies to begin eating in a novel environment compared to HCR rats. A 48-h fast equilibrated the latency to begin eating in the novel environment. A 24-h fast differentially affected expression of cocaine-amphetamine regulated transcript (CART) mRNA in the nucleus accumbens (NAc), where 24-h of fasting reduced CART mRNA in LCR rats. Bilateral microinjections of CART 55–102 peptide into the NAc increased the latency to begin eating in the NSF paradigm following a 24-h fast in LCR rats. These results indicate that metabolic phenotype influences how animals cope with the conflict between hunger and novelty, and that these differences are at least partially mediated by CART signaling in the NAc. For individuals with poor metabolic health who have to navigate food-rich and stressful environments, changes in central systems that mediate conflicting drives may feed into the rates of obesity and exacerbate the difficulty individuals have in maintaining weight loss.

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Introduction

Regulation of food intake is complex, and involves interplay among homeostatic, hedonic, emotional, and cognitive circuitry to determine the behavioral drives for obtaining and consuming food. Beyond the complexity of managing energy balance at the individual level, there have been substantial changes in the food/activity environment of developed nations (1). These changes occurred over a very short time, and have been proposed to be an underlying factor contributing to the obesity epidemic by compromising the ability of individuals to avoid weight-gain or maintain weight-loss (2). The potential for changes in the food landscape to facilitate eating for reasons other than sustenance, is illustrated by studies that show stress and emotion influence food choice (3, 4), food consumption can alter mood (5, 6), and food consumption can be used as a coping strategy (7, 8). In obesogenic environments there are more frequent opportunities to use food beyond metabolic nourishment (i.e. for coping/comfort) (9). Therefore, identifying the neural correlates of non-homeostatic food intake, that mediate behavioral response to conflict, will be critical to understanding consummatory behaviors in complex environments.

The regulation of energy balance by neuropeptide systems in the hypothalamus has been a central aspect of researching homeostatic regulation of feeding (10). The arcuate hypothalamus (Arc) is a brain region that serves as an important interface for monitoring peripheral signals that indicate the status related to short (i.e. ghrelin) and long-term (i.e. leptin) energy states (for review see (11)). *In the Arc two main populations of neurons exist, an anorexigenic set of neurons that coexpress proopiomelanocortin/cocaine-and amphetamine- regulated transcript (POMC/CART), and an orexigenic set of neurons that coexpress neuropeptide-Y/Agouti-related peptide (NPY/AgRP)* (12). These first-order neurons in the arc interact with numerous other brain regions, including areas that regulate higher-order behaviors (13). A number of these neuropeptides are expressed in, and regulate, the response of brain regions associated with reward, emotion, and cognitive behavior to impact energy balance (14). The nucleus accumbens (NAc) is a forebrain region that has been implicated in the integration of internal states with salient positive and negative environmental cues (15) *and receives information from the hypothalamus directly or through relays in the midline thalamus* (16). Further, several of these neuropeptides expressed in the hypothalamus interact with systems traditionally implicated in reward and emotion like dopamine and opioids. In particular, CART is expressed in the NAc and delivery of the active peptide CART 55–102 to the NAc decreases responding for drug reinforcers (17, 18).

As the metabolic state of the individual can impact brain function (19, 20), and both the long and short-term status of energy balance in the individual can influence behavior to environmental challenges (21), we sought to determine how divergent metabolic endophenotypes impact behavior strategies during conflict. To this end we investigated the behavior of rats selectively bred for high and low intrinsic treadmill running capacity (High capacity runners; HCR and Low capacity runners; LCR) – *with LCR animals displaying dyslipidemia, elevated fasting and random glucose and insulin, abdominal adiposity, and hypertension* (22, 23) – in the novelty suppressed feeding paradigm (NSF) with different durations of fasting (24 and 48 hours). Treadmill running capacity was used as a surrogate for overall energy metabolism in the selection paradigm. Several groups have found

differences in central signaling pathways of HCR and LCR animals that include the melanocortin system (24), endogenous opioids (25), and monoamines (26). Although there were few basal differences in mRNA of peptides that regulate metabolism in the hypothalamus (27), food restriction is a potent perturbation eliciting changes in these systems. The NSF paradigm has been used to screen for anxiolytic and antidepressant compounds (28) and relies on the conflict between hunger and environmental novelty. We also examined expression of neuropeptide mRNA to fasting in brain regions regulating the hedonic and homeostatic response to feeding. Based on our findings in the NAc we bilaterally administered CART 55–102 into the NAc to determine its ability to regulate behavior during conflict between novelty and hunger.

Methods

Experiment 1: Behavioral response of HCR and LCR rats to 24 and 48 hours of fasting

Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility and all procedures were approved by the University Committee on Use and Care of Animals at The University of Michigan. Male HCR and LCR rats (5 months, generation 26, n=16/line; Bodweight HCR = 342–389g, LCR=398–525g) maintained on Purina 5001 chow were assigned in a counterbalanced design to a 24 or 48 hour fasting duration prior to being tested in the novelty suppressed feeding (NSF) paradigm (NSF1). *Animals were maintained on a reverse light/dark cycle (lights off 0700, lights on 1900) and all testing was done within the first 4 hours of the dark cycle.* Following the NSF paradigm animals were allowed free access to chow for 2 weeks and then assigned to the other fasting duration prior to testing in the NSF paradigm (NSF2). For example, a rat fasted for 48 hours in NSF1 would be assigned to the 24 hour fasting duration for NSF2, and vice versa. The same open field was used for both sessions however the location (testing room) for the NSF1 and NSF2 testing sessions was changed to maintain novelty to the testing paradigm and cleaning solutions were changed between NSF1 and NSF2 from 70% ethanol to 8% bleach, respectively.

For the experimental session, rats were transferred individually in their homecage from the colony to a distinct testing room. A 76×76×46cm open field arena under indirect lighting was used for the studies. A fresh stack (8 whole pellets) of chow (Purina 5001) was placed in the center square immediately before the rat entered the arena. Rats were placed in one corner of open field facing the center and a maximum of 15 minutes was allowed for exploration of the maze. The test was stopped and the animal returned to its homecage as soon as they began eating any of the chow in arena. This was assessed by visual and audible indications that the animal had bit off a piece of chow (as opposed to investigating or moving/carrying a piece with their mouth). If rats did not begin eating the food by the 15 minute timepoint the test was stopped and they were returned to their homecage. Following the test, each rat was immediately returned to the colony and given a premeasured amount of chow and the latency to begin eating in the homecage was measured. Food was weighed at 60min, 24h, 48h, and 1 week (168h) following refeeding and is expressed as the cumulative food consumption following the NSF paradigm. Body weight was also measured and expressed as a percentage of ad libitum body weight (weight before food restriction).

Behavior was analyzed using Noldus Observer (Noldus Information Technology, Leesburg, Virginia) by an observer blinded to the study. Number of line crosses in the center and periphery, the time spent in the center and periphery, and the time spent investigating (not eating) the chow was scored. As animals showed different latencies to begin eating (and therefore had differing durations of exposure to the open field) open field times are expressed as a percentage of the total time spent in the arena for each rat.

Experiment 2: Gene-expression and hormone response to fasting

Analysis of mRNA and hormonal response to fasting: Male HCR and LCR rats at (5 months, generation 27; Bodweight HCR = 320–421g, LCR=413–535g) maintained on standard chow were assigned ad libitum access to chow (n=9/line), and either a 24-h (n=8/line) or 48-h (n=8/line) fast. Food was removed just before the start of the dark cycle. Tissue collection occurred during the third to fourth hour following the beginning of the dark-cycle after 24 or 48-h fasting, in order to capture animals following typical ad libitum consumption (29). Animals were euthanized by rapid decapitation and brains were extracted and frozen in 2-methylbutane chilled to −30C and trunk blood was collected into pre-chilled tubes containing EDTA. Blood was spun at 3000rpm and 4C for 10 min and plasma was aliquoted and stored at −80C prior to the measurement of hormones.

In situ hybridization—Brains were sectioned at 10μm on a Leica cryostat at −16°C, thaw mounted onto SuperFrost Plus (Fisher, USA) slides and stored at −80°C until hybridization. Slides were processed as previously described (30). Separate sets of slides were hybridized overnight at 55°C with one of the following ^{35}S-labeled-antisense probes: proopiomelanocortin (POMC; J000759), cocaine and amphetamine regulated transcript (CART; NM017110), agouti-related peptide (AgRP; AF206017), neuropeptide Y (NPY; M20373). Following hybridization, slides were processed and air-dried before being exposed to Kodak Biomax MS Film (Rochester, NY). The resulting autoradiograms were digitized using a ScanMaker 1000XL Pro (Microtek, Carson, CA) and analyzed using ImageJ Analysis Software for PC (http://rsbweb.nih.gov/ij/) as previously described (31). Optical Density (OD) values were determined by averaging mRNA expression across the rostrocaudal extent of the arcuate hypothalamus. Expression of CART in the NAc core and shell were determined by averaging OD values from bregma = 2.20 to 1.60. The spectrum LUT included in the Image J package was used to psuedocolor representative autoradiogram images of CART expression in the NAc and an optical density scale (Kodak, Rochester NY) and representative sections (at the level between 2.2 to 1.8mm from Bregma) were cropped for display purposes (Figure 1B).

Circulating hormone levels—Blood was collected into K_2EDTA tubes (Vacutainer, Franklin Lakes, NJ) containing 10mg/ml of aprotinin (Sigma, St. Louis, MO) was added to a final concentration of 1000 KIU/ml. Plasma was isolated by centrifugation at 3000 rpm for 10 min and stored at −80°C. Samples were aliquoted to avoid multiple thaws, and aliquots were batched for each assay and completed in one run. Circulating levels of hormones were measured using commercially available kits for leptin (Assay Designs, Ann Arbor, MI; intra-assay variability = 8.36%), acylated-ghrelin (Cayman Chemical, Ann Arbor, MI; intra-assay variability =3.5%), and total adiponectin (Alplco Diagnostics, Salem, NH; intra-assay

variability = 6.0%). Corticosterone was measured by radioimmunoassay (MP Biomedicals, Orangeburg, NY; intra-assay variability =2.5%). A dilution of 1:2 was utilized to ensure all animals fell on the linear range of the curve for leptin based on previous assays in the HCR/LCR rats (27). For the other assays the manufacturer's specified protocol was followed.

Experiment 3: Influence of CART peptide microinjection into NAc on NSF behavior

Animals—Bilateral cannula were aimed at the NAc and implanted as previously described (32). Animals (7 months, generation 27; Bodweight HCR = 337–398g, LCR=443–492g) were anesthetized with sodium pentobarbital (75 mg/kg, i.p.). Animals were placed into a Kopf stereotaxic unit with skull flat, the incision site shaved and scrubbed with betadine wash, and a mid-sagittal incision was used to expose the skull. The coordinates for the NAc were A/P +2.0mm, M/L +/− 1.1mm, and −6.5mm from skull (incisor bar −4.0), as determined from bregma based on Paxinos and Watson (1998)(33). The tips of the 26-gague guide cannula were positioned in between the shell and core of the NAc to ensure diffusion into both regions. Cannulae were anchored to jewelers screws (Plastics One, Roanoke, VA) a using Ortho-Jet cold-setting dental acrylic (Lang Dental, Wheeling, IL). Flunixin (2.5 mg/kg, s.c.) was given postoperatively for pain management and moistened chow was provided.

Drugs and Microinjections—Rats were allowed ten days to recover from cannula surgery, and handled on days ten through fourteen following surgery. On day fifteen following surgery, animals were lightly restrained in a towel in order to remove the dust cap and dummy cannula and insert injector cannula. CART 55–102 (Phoenix Peptides, USA) was dissolved in isotonic sterile 0.9% saline at a concentration of 5μg/μl. Previous work demonstrated this dose was within a range that would not cause alterations in motor behavior (17, 34). Bilateral intra-accumbal injections of 0.3μl were administered by two-2ul Hamilton microsyringes (Hamilton Co., Reno, NV) controlled by a microinfusion pump (Harvard Apparatus, Holliston, MA) for a final delivery of 1.5ug per side. Microsyringes were connected to 33-gauge injector cannulae (PlasticsOne, VA) by polyethylene tubing. Displacement of an air bubble in the polyethylene tubing was used to monitor injection. Injections occurred over 90 seconds, with 30 seconds allowed after the injection to permit spread of the drug or vehicle. Immediately following the injection, dummy cannula and dust-caps were replaced and the animal was returned to its home-cage for 5 minutes prior to being introduced to the open field arena for assessment of novelty suppressed feeding. Treatment groups were HCR-Veh (n=6), LCR-Veh (n=6), HCR-CART (n=6), and LCR-CART (n=6).

Behavioral Testing—Food was removed 24-hours prior to microinjections. Five minutes following replacement of the dummy cannula and dust-caps, the NSF paradigm was conducted as described in experiment 1.

Verification of Cannula Placement—Placement was determined as previously described (32). Briefly, one week following the NSF paradigm, rats received an overdose of sodium pentobarbital (120 mg/kg, i.p.), and bilateral injections of India ink (25% v/v) were

administered with the same injection parameters used for drug injection (see above). Brains were collected and processed for enzymatic acetylcholinesterase staining, and placement mapped onto the Paxinos and Watson atlas (33).

Statistics—For behavioral, mRNA, and hormone measures, two-way Analysis of Variance (ANOVA) was used to compare the effects of line with diet (experiments 1&2) or drug treatment (experiment 3). For measures of change in body weight and cumulative food consumption a two-way ANOVA with repeated measures on time was utilized. In the case of cumulative food intake, Greenhouse-Geisser corrected (G-G corrected) p-values were used as the assumption of sphericity was violated. For all post-hoc comparisons, Tukey's Honestly Significant Difference was used to compare differences among means. Statistical significance was set at α=0.05.

Results

Experiment 1: Behavioral response of HCR and LCR rats to 24h and 48 hours of fasting

Significant main effect of fasting duration ($F_{1,60}$=7.38, p=0.009) was found with 48h of fasting resulting in a lower latency to begin eating in the open field compared to 24h of fasting. The main effect of line ($F_{1,60}$=7.04, p=0.010) was significant for latency to begin eating in the NSF paradigm, with LCR rats showing lower latencies compared to HCR (Figure 1). Post-hoc comparisons revealed that 24h and 48h fasted LCR animals had a lower latency to eat in the NSF paradigm compared to HCR-24h fasted animals. No differences were found between HCR and LCR rats following a 48-hour fast. There was no effect of testing order on latency to eat in the open field for the 24h ($F_{1,60}$=3.12, p=0.261) or 48h ($F_{1,60}$=1.08, p=0.571) fast.

A significant interaction of line x fasting duration ($F_{1,60}$=8.11, p=0.006) was found for the percentage of time spent exploring the center of the open field, with 24h fasted HCR rats spending a greater proportion of time exploring the center compared to 24h and 48h fasted LCR rats.

No significant interaction of main effects were found for the percent time spent investigating the chow or total line crosses in the open field.

Home cage feeding behavior—No significant interaction or main effects of line or duration of fasting were found for the latency to begin eating once rats were returned to their home cage in the vivarium (Figure 2).

There was a significant within subjects effect of time ($F_{3,180}$=2128, p<0.0001, G-G corrected) on cumulative food consumption following the NSF paradigm. No significant interactions were found for time x fasting, or line x time x fasting. No between subjects differences in cumulative food intake were found.

Significant line x time ($F_{3,58}$=4.88, p=0.0043) and fasting-duration x time ($F_{3,58}$=3.89, p=0.013) interactions were found for body weight in response to refeeding following the NSF paradigm. The three – way interaction for time x line x fasting-duration was not

significant. A significant interaction of line x fasting-duration was found between subjects for body weight ($F_{1,60}$=5.69, p=0.020). Following the NSF paradigm, HCR-48h fasted had the greatest percent weight loss compared to all other groups. Additionally, LCR-48h had greater weight loss compared to the 24-h fasted rats. After 24-hours of refeeding 48-h fasted animals still exhibited a greater decrease in % body weight from the ad libitum state compared to 24-h fasted rats of either group. No differences were found among groups after 48-hours of refeeding. After one week of refeeding body weight returned to pre-fasting levels for all groups, however LCR-24h rats had a higher percentage of pre-fasting body weight compared to all other groups.

Experiment 2: Neurohormonal response of HCR and LCR rats to fasting

Neurohormonal Response to Fasting—A significant line by fasting interaction ($F_{2,44}$=4.01, p=0.025) for CART expression in the core and shell of the NAc showed that in the ad libitum condition, LCR rats exhibited higher CART mRNA expression in the shell and core of the NAc compared to all other groups that was reduced by 24 and 48 hours of fasting (Figure 1). As the patterns of CART expression in the core and shell to fasting were identical, only data for the shell is shown.

Table 1 shows the mRNA response of orexigenic and anorexigenic neurpeptides in the Arc and circulating hormones. A significant main effect of fasting was found for AgRP and CART in the arcuate. AgRP increased with fasting, and CART decreased following a 24h fast in both HCR and LCR rats. There was no line x fasting interaction and no main effect of rat line for these transcripts. A significant interaction was found for POMC expression in the Arc with fasting inducing a slight elevation in mRNA expression in HCR compared to all other groups, however post-hoc comparisons did not survive Tukey multiple comparisons correction. No significant effects of line or fasting were detected for NPY expression in the Arc.

Figure 3 shows response of circulating hormones to fasting in HCR and LCR animals. Significant main effects of line were found for circulating leptin and adiponectin, with LCR rats having higher circulating leptin and lower circulating adiponectin compared to HCR rats. A significant line by fasting interaction was found for circulating levels of acylated-ghrelin. Fasting increased acylated-ghrelin in HCR rats, but induced a small decrease in LCR rats. There were no significant effects of line or fasting on circulating corticosterone. *It should be noted that the acylated-ghrelin levels are likely underestimated as the plasma preparation contained a protease inhibitor but samples were not acidified, which is known to result in degradation of acylated ghrelin* (35).

Experiment 3: Influence of CART peptide microinjection into NAc on NSF behavior

Verification of Cannula Placement—Following processing of tissue for acetylcholinesterase staining, sections were viewed under a light microscope and cannula tip placement was transcribed to the Paxinos & Watson plates (33). All cannula tips were localized between +1.60 and +2.70 from bregma, with the majority localized between +1.70 and +2.20 from bregma (Figure 4).

Behavior in NSF—A significant interaction ($F_{1,20}$=6.37, p=0.049) between line and drug was found for the latency to begin eating in the open field. Post-hoc analysis revealed that LCR-Veh animals had a lower latency to begin eating in the open field following a 24-hour fast compared to HCR-Veh and HCR-CART (Figure 4).

A significant interaction was found for activity in the open field ($F_{1,20}$=5.14, p=0.035). The total line crosses in the open field was greater in HCR-CART rats compared to LCR-CART rats.

There was no significant interaction or main effects for the percentage of total open field time spent exploring the center of the arena, or time spent investigating the chow in the open field.

Home cage feeding behavior—There was a significant line x drug interaction ($F_{1,20}$=6.86, p=0.017) for the latency to begin eating food after the rats were returned to their home cage in the vivarium (Figure 5). Post hoc comparisons revealed that HCR Veh-treated rats took longer to begin eating in their homecage following the NSF paradigm compared to all other groups.

A significant within subjects effect of time ($F_{3,60}$=932.4, p<0.0001, G-G corrected) on cumulative food consumption was found following the NSF paradigm. Additionally a significant line x time interaction was found ($F_{3,60}$=932.4, p=0.044, G-G corrected) with HCR rats having greater cumulative food consumption over the week following the NSF paradigm. No significant interactions were found for time x drug, or line x time x drug. No between subjects differences in cumulative food intake were found.

A within subjects effect of time ($F_{3,60}$=120.2, p<0.0001) on body weight in response to refeeding following the NSF paradigm was found. There were no significant interactions for time x line, time x drug or time x line x drug. Body weight response to the 24-hour fast and refeeding was similar among all rats regardless of phenotype or drug treatment.

Discussion

Behavioral responses to fasting differ in rats with divergent metabolic phenotypes

Here we report that rats who exhibit divergent metabolic profiles, exhibit distinct behavioral patterns for dealing with the conflict between hunger and environmental novelty. LCR animals carry substantial amounts of stored energy in the form of adipose tissue yet engage in consummatory behavior in novel environment following 24 hours of fasting. In contrast, HCR animals exhibited exploratory behavior but longer latencies to consume chow. A greater motivational load of hunger (48 hours of fasting) equilibrated the behavior of the two lines. Additionally, no differences between lines were found for latency to begin eating when animals were returned to their home cages. Fasting impacted neuropeptide mRNA expression in the arcuate hypothalamus in a similar fashion regardless of line. In contrast, CART expression in the NAc was found to be elevated in LCR rats under ad libitum conditions and reduced in those animals following a 24h fast. Bilateral microinjection of

CART 55–102 peptide into the NAc increased the latency to eat in the open field in LCR rats following a 24h fast.

In the novelty suppressed feeding paradigm, chronic antidepressant and acute anxiolytic drug administration are effective in decreasing the latency to eat in a novel environment (28). More recently the NSF paradigm has been used to assess behavior in response to a discrete stressor (36). Assessment of inherent differences in approaching this conflict, and the systems that might be mediating different behavioral strategies has not, to our knowledge, been made with models of divergent metabolism.

Following a 24-hour fast, LCR animals showed a lower latency to begin eating in the open field compared to HCR rats. The more commonly employed 48-hour duration of fasting utilized in studies investigating behavioral pharmacology in the NSF paradigm (28), equalized the latency to begin eating in open field in HCR and LCR rats. Therefore with a sufficient motivational load of hunger (48-hours) HCR and LCR animals exhibit the same latency to begin eating in the open field. *In other experiments HCR and LCR animals have not shown basal (i.e. undrugged/unstressed) behavioral differences in response to novelty* (37, 38).

Although the HCR animals took longer to begin eating in an anxiogenic environment following a 24 hour fast, it does not appear that they had lower levels of exploration, as the percentage of total time spent in the maze exploring the center of the arena was greater in HCR-24 animals (Figure1D). The HCR-24 animals spent more time in the testing arena, however this does not completely explain greater levels of center exploration as the data are represented as the percent of total time spent in the maze. These data suggest that the HCR rats are exploring the novel environment, but not completely willing to divert attention from risk-assessment to the act of consuming food in the face of potential danger (39). In contrast, LCR animals are more willing to divert attention from exploration/vigilance to consuming chow in a novel environment. With a greater motivational load of hunger following 48-h of food restriction, the behavioral patterns of HCR and LCR were indistinguishable.

The divergent behavioral patterns we observed in the NSF paradigm following 24-hours of fasting are intriguing as they suggest the resulting metabolic phenotypes associated with intrinsic aerobic capacity influence the behavioral strategies in a situation with conflicting drives between environmental novelty and hunger. The behavioral pattern of the LCR rats would also seem counterintuitive from an ethologic standpoint, as these animals have higher levels of body fat compared to HCR rats (22, 23, 40). Further, these differences are not solely attributable to hunger as homecage consumption was similar among groups (Figure2). Previous work has shown that LCR rats exhibit lower levels of defensive behavior in response to predator odor encountered in a familiar environment (38). Collectively, this suggests that the long-term metabolic phenotype of the animal impacts the neuropeptide and behavioral responses to short-term environmental challenges (e.g. fasting, predator odor).

CART in the NAc regulates behavioral response to conflict between hunger and novelty

Given the metabolic phenotype of the HCR LCR lines (22, 23) we initially investigated mRNA responses to fasting and feeding in the arcuate hypothalamus. The anorexigenic

POMC/CART, and orexigenic NPY/AgRP co-expressing neurons serve as an important interface for monitoring peripheral signals of energy states (for review see (11)). However in these studies, we did not see a substantial impact of metabolic phenotype (long-term) on the response of arc mRNA expression to acute fasting (Table 1). No differences in baseline expression were found between HCR and LCR rats in the arc. Twenty four hours of food restriction reduced mRNA expression of the anorexigenic peptide CART, while expression of mRNA for the orexigenic peptide AgRP was increased. The CART (41) and AgRP (42) responses in the arc are in line with previous reports in unselected rodent lines. This area plays a critical role as the interface between peripheral indicators of long-term and acute energy status (e.g. leptin and insulin, respectively) and disruptions in these systems can drastically influence energy balance; yet in animals with divergent metabolic phenotypes the mRNA response to fasting in a homeostatic region like the arc was not differential.

There has long been an appreciation for the ability of food to influence processes in the brain related to reward and cognition. Intriguingly the neuropeptides produced in first-order neurons of the arcuate hypothalamus have been implicated in a number of behaviors including depression-like behavior (43, 44), anxiety-like behaviors (45–47), and consummatory/appetitive (48) responses. The role for an anorexigenic neuropeptides (CART, POMC) to increase “depressive” and “anxious” behavior while orexigenic neuropeptides appear to have antidepressant/anxiolytic-like properties and enhance responding for rewarding stimuli (49) may have an ethological basis. Plainly, if an animal has recently eaten to satiety there should be less motivational drive (from a metabolic standpoint) for the animal to divert attention from exploration/vigilance to food consumption, potentially increasing risk of predation (39). Given the distribution of CART in limbic and reward regions we measured expression in response to fasting. We found that in the ad libitum state that LCR animals had greater CART expression in the NAc compared to HCR rats. Following a 24-hour fast, CART expression was significantly reduced in LCR rats, but did not change in HCR. A greater duration of fasting (48-hours) did not alter CART expression in the NAc beyond that seen after a 24h fast. The decrease in CART mRNA following fasting in these experiments is in line with previous work (34), and our results indicate that metabolic phenotype can impact the function of this system.

Two main isoforms of CART, 55–102 and 62–102, are present in regions of the brain that regulate feeding and emotion (for review see (50)). The longer isoform 55–102 is the most studied, and has been shown to impact both feeding (51) and anxiety-like behavior (48) in rodents through central mechanisms. The NAc has been heavily implicated in behavioral responding for rewarding and stressful stimuli (15, 52), and administration of CART directly to the NAc can reduce behavioral responses to drug-rewards (17, 18). It appears that the NAc is a critical region regulating the consumption of food (16, 53). Delivery of a N-methyl-D-aspartate (NMDA) receptor antagonist into the NAc increased latency to eat standard chow, while slightly decreasing latency to consume a novel food in the NSF paradigm (54). Inactivation of the NAc with gamma-Aminobutyric acid (GABA) receptor agonists reduced preference for a large, but uncertain, food reward (55). Given the differential CART mRNA response to fasting in the NAc among HCR and LCR animals, we sought do determine the impact of CART microinjection on behavioral responses in the face of competing drives of environmental novelty and hunger.

Microninjection of CART into the NAc increased the latency to eat in the open field in LCR-CART compared to LCR-Veh animals (Figure 4C). *No differences in behavioral patterns were present following an exploratory analysis of cannulae placement in to the core and shell of the NAc.* These results are consistent with work with drug reinforcers that show CART can decrease responding to reinforcing drugs (17, 18, 56). In contrast, CART administration to the NAc did not impact operant responding for sucrose pellets in fed rats (17), whereas a recent study suggests that CART enhances operant responding for food reward (57); however in the more recent report the CART isoform used and the consummatory behavior of the animals was not reported.

Based on these results CART appears to play a regulatory role in determining what aspects of the environment an animal is willing to devote its attention, potentially through enhanced "braking" of reward systems in the NAc in situations with competing drives; like hunger and novelty. Specifically, delivery of CART into the NAc increases the latency to consume food in an uncertain environment. Based on other measures of Open Field behavior, it does not appear that CART administration into the NAc impacts exploratory behavior as LCR and HCR animals showed comparable levels of exploration in the center of the open field and chow (Figure 4D & E). Further, it does not appear that this carries over to consummatory behavior in familiar environments like the animal's homecage (Figure 5). The trend for HCR-CART treated animals to exhibit a greater number of line crosses may be impacted by the duration of time in the arena between these two groups. The lack of differences in exploration and locomotion in a novel environment reported here are in agreement with previous work in these animals (37, 38), and combined with the similar latencies to eat upon return to their homecage suggest CART may help mediate which environmental factors are more salient (e.g. food or danger) for a given individual. The home-cage consumption is in contrast to previous work reporting decreased home cage consumption by NAc-CART over the short-term in food-deprived animals following a 48-h fast (34). In these experiments, however we utilized a 24-h fast which may account for the lack of impact of CART on homecage feeding (Figure 5B). These experiments are in line with previous work investigating the ability of CART microinjections to decrease responding for drug reinforcers (17, 18), however it will be important to examine these relationships in other models of metabolic dysfunction and to expand on the role CART is playing using molecular techniques to alter endogenous levels of the protein.

CART likely imparts its inhibitory influence on rewarding behavior by modulating dopaminergic systems, as microinjection of CART into the NAc reduces responding to drugs that enhance dopaminergic neurotransmission (17, 18, 56). LCR rats express lower levels of dopamine D2 receptors in the midbrain and striatum compared to HCR rats (26), and therefore we cannot rule out the possibility that regulation of behavior by CART is secondary to changes in DA tone as D3 activation results in downregulation of CART (58).

CART appears to be functionally and anatomically positioned to be a mediator of behavioral response to salient stimuli as mRNA and immunoreactivity are found across the brain in areas that mediate reward, homeostasis, and emotion (59). It is thought that CART increases inhibition potentially through a yet to be identified receptor coupled to Gi/Go signaling (60), or through interaction with GABAergic signaling (61). Within the NAc shell, the majority of

CART-containing cells are medium-sized projection neurons that contain the inhibitory neurotransmitter GABA (61, 62). This is functionally relevant as reducing excitatory neurotransmission in the NAc increases latencies for 'risky' behaviors (54, 55). The NAc also interfaces with other brain regions regulating homeostatic drives for feeding as it is innervated by CART projections from the hypothalamus, and also sends projections back to the hypothalamus (54, 61). Collectively this indicates that CART in the NAc is positioned to act as a braking mechanism for homeostatic and non-homeostatic modulators of food intake by modulating dopaminergic neurotransmission.

Additionally CART may also influence μ-opioid systems in the brain (63). Opioid systems have long been implicated in regulating responses at the interface of emotion and eating at various sites in the brain (64). Further, opioid neurotransmission in the NAc has been implicated in modulating the consumption of palatable food (53), and recent reports have implicated central opioid systems in regulating consumption in the face of environmental novelty (36, 65). This suggests that CART has the potential to influence multiple aspects of appetitive behavior (66), however this has yet to be tested directly.

The current epidemic of obesity has been particularly intractable due, in part, to the prevalence and availability of high-calorie palatable food. Here we provide evidence that beyond regulation at the homeostatic level, the internal systems mediating behavioral response to the conflict between hunger and environmental stress are influenced by the long- and short-term metabolic state of the individual. Functionality of these systems may be particularly relevant in situations where individuals need to balance competing drives like hunger and the novelty of the long-term implications of acute behavioral choices.

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Highlights

- Metabolic phenotype influences behavioral response to conflict
- Nucleus Accumbens (NAc) CART mRNA decreases after fasting in low capacity runners
- Latency to consume food in novel environment decreased by NAc CART microinjection

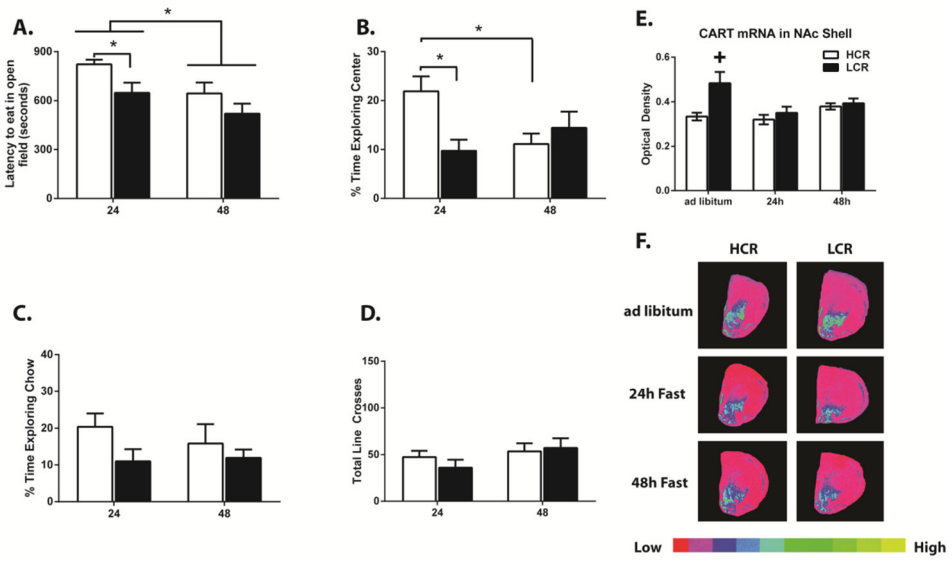

Figure 1.

Central mRNA response and behavioral response to fasting. Impact of duration of fasting on latency to eat (A), center exploration (B), chow exploration (C), and total line crosses (D) in the open field during the NSF paradigm. Asterisks and bars indicate pair-wise comparisons that survived Tukey's post-hoc multiple comparison correction. Quantification of CART mRNA expression in the shell of the NAc in the ad libitum state and following fasting (E); + indicates significant difference compared to all other groups. Representative autoradiograms of CART mRNA expression in the NAc and optical density scale (F). Data are represented as mean ± S.E.M.

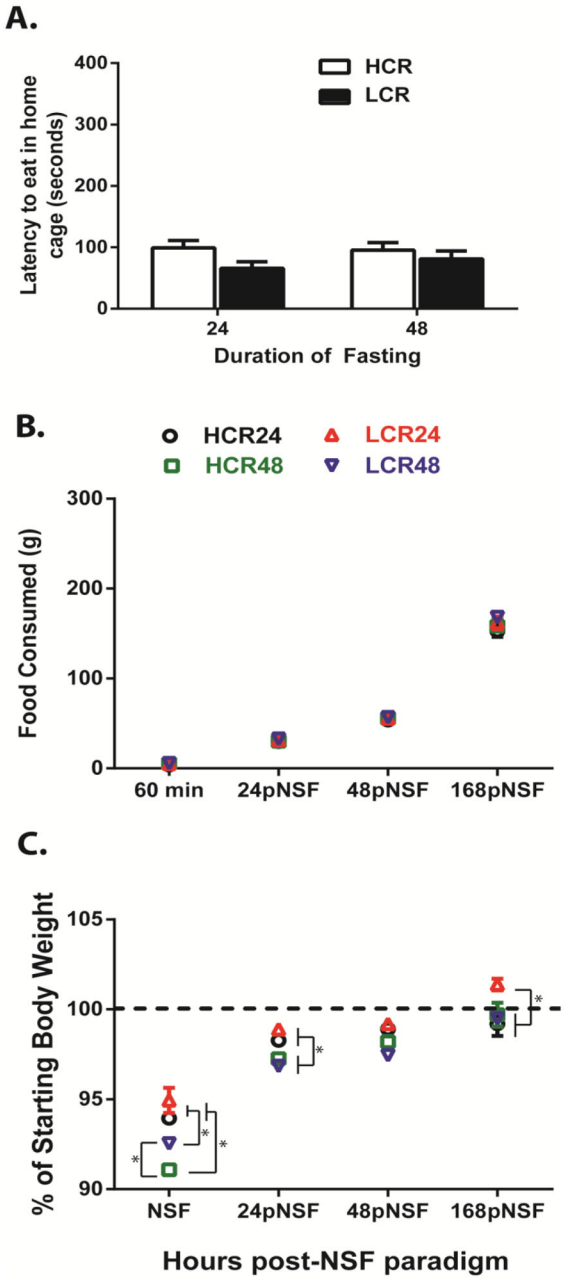

Figure 2.

Food intake and change in body weight following a 24h or 48h fast. Latency to begin eating in the homecage following fasting (A). Cumulative food consumption following return to the homecage (B). Body weight response to fasting and feeding as a percentage of pre-fasting body weight (C). Asterisks and bars indicate between subject pair-wise comparisons that survived Tukey's post-hoc multiple comparison correction. Data are represented as mean ± S.E.M.

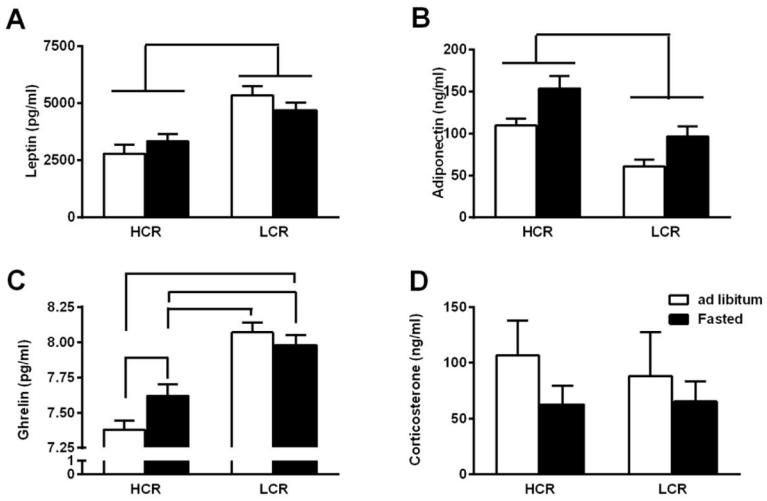

Figure 3.

Hormonal response to fasting in HCR and LCR rats for (A) leptin, (B) adiponectin, (C) ghrelin, and (D) corticosterone. Bars indicate post-hoc differences between groups that survived Tukey's correction for multiple comparisons. Data are presented as mean ± S.E.M.

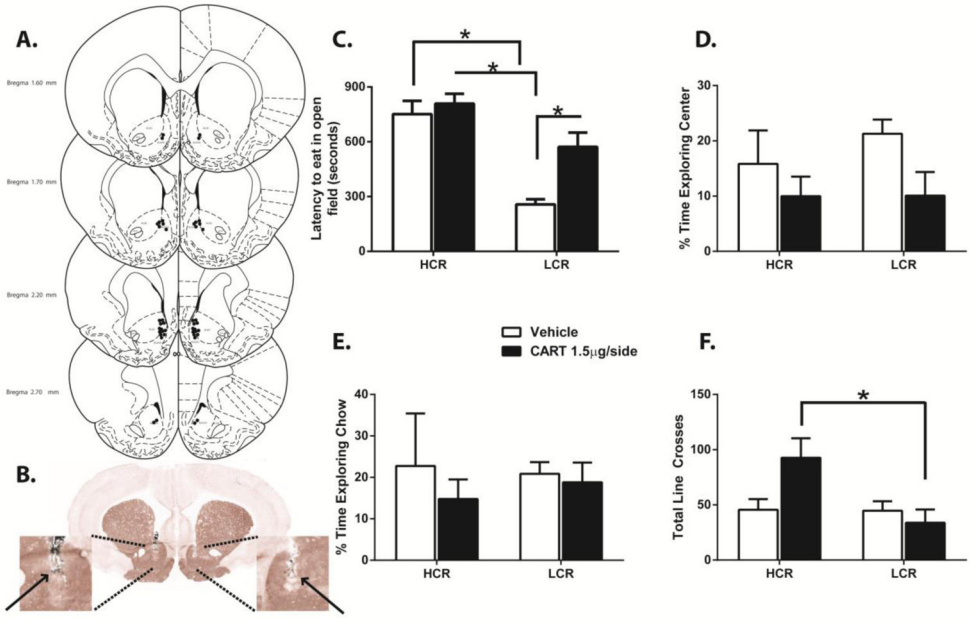

Figure 4.

Influence of bilateral microinjection of CART on behavioral response to a 24h fast. Map of cannula placement by brain level (A), and representative picture of placement in acetylcholinesterase stained tissue (B). Impact of duration of bilateral injection of CART 55–102 into the NAc on latency to eat (C), center exploration (D), chow exploration (E), and total line crosses (F) in the open field during the NSF paradigm. Asterisks and bars indicate pair-wise comparisons that survived Tukey's post-hoc multiple comparison correction. Data are represented as mean ± S.E.M.

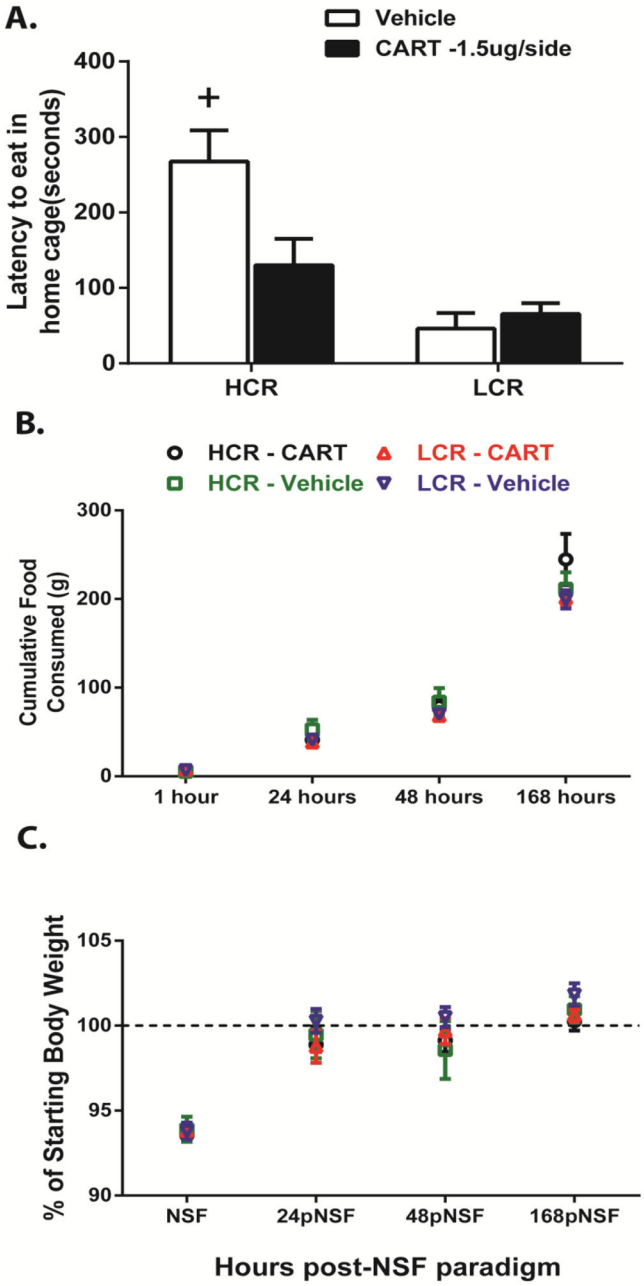

Figure 5.

Food intake and change in body weight following bilateral microinjection of CART after a 24h fast. Latency to begin eating in the homecage following fasting (A). Cumulative food consumption following return to the homecage (B). Body weight response to fasting and feeding as a percentage of pre-fasting body weight (C). Asterisks and bars indicate between subject pair-wise comparisons that survived Tukey's post-hoc multiple comparison correction. Data are represented as mean ± S.E.M.

Table 1

Response of mRNA expression in arcuate hypothalamus and circulating hormones to fasting. Data are represented as mean ± S.D., and significant p-values are indicated by **bold font**.

		HCR		LCR							
		ad libitum	fasted	ad libitum	fasted	Line x Feeding		Line		Feeding	
	n	10	10	9	12						
mRNA arcuate hypothalamus		**mean ± SD**	**mean ± SD**	**mean ± SD**	**mean ± SD**	**F**	**p**	**F**	**p**	**F**	**p**
	POMC	21 ± 6.3	26.9 ± 4.7	23.8 ± 7.5	21.1 ± 4.5	5.18	**0.029**	0.631	0.433	0.717	0.403
	CART	25.8 ± 3	22.1 ± 4.3	25.3 ± 3.5	22 ± 4.53	0.027	0.871	0.056	0.815	7.88	**0.008**
	NPY	27 ± 6.5	29.3 ± 8	25.5 ± 6.4	26.2 ± 4.8	0.154	0.697	1.28	0.266	0.542	0.466
	AgRP	13 ± 2.9	19.9 ± 9.9	10.2 ± 2.5	17.6 ± 3.9	0.052	0.822	5.38	**0.026**	42.28	**<0.0001**
Hormones											
	Leptin (pg/ml)	2871.57 ± 1341.73	3266.46 ± 925.76	5325.76 ± 886.10	4727.53 ± 1282.14	1.92	0.1746	29.78	**<.0001**	0.08	0.7784
	aGhrelin (pg/ml)	7.38 ± 0.20	7.62 ± 0.26	8.07 ± 0.21	7.98 ± 0.24	5.08	**0.0301**	53.62	<.0001	1.19	0.2816
	Adiponectin (ng/ml)	119.33 ± 37.50	144.42 ± 45.66	89.34 ± 41.67	74.79 ± 37.23	2.43	0.1272	15.37	**0.0004**	0.17	0.6807
	Corticosterone (ng/ml)	106.67 ± 98.38	62.69 ± 52.86	88.05 ± 118.48	65.38 ± 61.23	0.16	0.6924	0.09	0.7674	1.56	0.2204