Melanocortin activity in the amygdala controls appetite for dietary fat

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Running Title: Amygdala melanocortin activity controls food selection

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ABSTRACT

The amygdala is rich in melanocortin 4 receptors. Since the reduction in dietary fat intake after enterostatin is injected into the central nucleus of the amygdala (CeA) is blocked by a melanocortin 4 receptor antagonist, we investigated the role of melanocortin activity in the CeA in regulating food intake and macronutrient choice. Sprague-Dawley (SD) rats, fitted with CeA cannulas, were fed either chow, a high fat diet or adapted to a 2-choice High fat (HF) or Low Fat (LF) diet. Injections of the MC4R agonist Melanotan II (MTII) into the CeA had a dose dependent inhibitory effect on food intake that lasted for at least 24 hours. This response was greater in rats fed a high fat diet. The inverse agonist AgRP and antagonist SHU9119 increased food intake in a dose dependent manner, the hyperphagia lasting for 60 hours. In rats adapted to a 2 choice HF/LF diet, MTII decreased HF consumption but had no effect on LF consumption resulting in a long lasting decrease in total calorie intake (-35.5% after 24h, p<0.05). Total calorie intake increased in both AgRP and SHU9119 treated rats (32% and 109% after 24h respectively) as the result of increased intake of HF diet. There was no modification of LF consumption with AgRP treatment and a transient non significant decrease with SHU9119 treatment. Amygdala BDNF expression was increased by AgRP in fed rats. These results identify the amygdala as a site of action for the melanocortin system to control food intake and dietary preferences.

Keywords: Agouti related protein, Melanotan II, SHU9119, Brain derived neurotrophic factor, food intake.
INTRODUCTION

For years, hypothalamic centers have been considered as the major brain centers regulating food intake and/or macronutrient selection (45). The neural circuits involved in the regulation of feeding behavior are complex and more recent studies implicate other parts of the brain in the regulation of ingestive behaviors. Areas such as the nucleus accumbens, the amygdala, the brain stem and many others, form together with the hypothalamic areas a complex circuitry regulating all levels of feeding behavior (6, 8, 55). Indeed, the paraventricular nucleus is not essential for either NPY-induced feeding or the anorexic response to melanocortin activity (16) since both are evident in PVN-lesioned rats. Further, the anorectic response to MTII is evident in the dorsovagal complex of the brain stem (52, 58).

A number of neuropeptides and compounds are known to selectively affect macronutrient intake when administered centrally. For example, the orexigenic neuropeptide Y (NPY) (47, 48, 50), norepinephrine (NE) (30, 50) or agouti-related protein (AgRP) (23) have been reported to induce specific selection of macronutrients. Enterostatin, a satiety factor secreted in the GI tract and expressed also in the central nervous system (CNS), induces an immediate reduction in food intake and inhibits appetite specifically for dietary fat when injected peripherally or centrally (31, 37, 39).

It has been hypothesized that the role of the amygdala in the overall complex feeding behavior is to mediate responses that modulate the behavior. Multiple laboratories have investigated the role of the amygdala on different aspects of the feeding behavior including the role in food reward (4, 18, 20, 25) and food aversion (43, 54). There is also growing evidence for the role of the amygdala in the regulation of macronutrient selection. Lesions of this part of the brain alter macronutrient selection (29). A specific
reduction in dietary fat intake has also been observed after enterostatin administration into the central bed nucleus of the amygdala (CeA) in fasted rats adapted to a macronutrient choice paradigm (34). Mu-opioid stimulation of the amygdala can induce voracious eating of fat (51) while intra-amygdala NPY reduces the preference for dietary fat but doesn’t influence total caloric intake in rats given a choice of diets (41). The melanocortin system has a central role in regulation of energy homeostasis (44, 46); blockage or loss of MC3 and MC4 receptors induces hyperphagia, reduced energy expenditure and obesity (2, 3, 10). MC4-R deficient mice exhibit obesity, hyperphagia and hyperinsulinemia. Melanocortin 4 receptors are expressed in the amygdala and there are NPY/AgRP axonal projections from the arcuate region of the hypothalamus to the amygdala (24, 35) questioning the role of the melanocortin system within the amygdala. The purpose of the experiments presented here was to explore the role of the melanocortin system within the amygdala to regulate food intake and macronutrient selection. We provide evidence that melanocortin signaling in the CeA can regulate feeding behavior by showing strong effects of the MC3R/MC4R antagonist SHU9119 and the MC3R/MC4R agonist Melanotan II (MTII), as well as the inverse agonist AgRP (1, 11, 14), to influence food intake and dietary preferences when administered onto the CeA.
MATERIALS AND METHODS

**Animals:** Male Sprague-Dawley rats (body weight 220-240 g; Charles River) were individually housed in hanging wired mesh cages in a temperature (22-24°C) and light controlled (lights off 1900-0700 h) room. Food and water were available *ad libitum*. The Institutional Animal Care and Use Committee of Utah State University approved the animal protocol.

Rats anaesthetized with pentobarbital sodium (Nembutal; 0.1 ml/100 g body weight, ip) were stereotaxically implanted with one unilateral or two bilateral stainless steel cannula(s) (Plastics One, Roanoke, VA) aimed to the CeA: [coordinates (AP/L/DV to bregma) -2.4/-3.8/-6.0 mm according to Paxinos and Watson (40) and previous experiments (34). Each cannula was secured in place with 3 anchor screws and dental acrylic and occluded with a 26 gauge wire stylet. The injector was designed to project 2 mm beyond the guide cannula tip. Each rat received an injection of the analgesic drug Carprofen (Rimadyl 5 mg/kg, s.q.) before returning to their home cage. Adaptation to high fat (HF) or two choice HF/low fat (LF) diets began after rats had regained their preoperative weight (about 7 days). Cannula placement was checked after sacrifice of the animals by histology after injection of the water soluble methylene blue dye.

**Peptides and drugs administration:** All drugs and peptides were administered in 0.5 µL saline solution over a 1 minute time period. The injector was left in place for an additional 1 minute to prevent backflow. AgRP was purchased from Phoenix Pharmaceuticals (Belmont, CA), SHU9119 and Melanotan II (MTII) where purchased from Sigma Aldrich (St Louis, MO).

**Experimental Design:** After recovery from the cannula implant, some rats were adapted to a HF diet over a 10 day period. Other rats were progressively adapted to a 2-choice
HF/LF diet paradigm over a 20 day period prior to experimentations by providing each diet on successive days for 14 days before providing both diets together. The HF diet (% energy as fat/protein/carbohydrate 45/20/35; 4.73 kcal/g D12451 Research Diets, New Brunswick, NJ) and LF diet (% energy as fat/protein/carbohydrate 10/20/70; 3.85 kcal/g D12450B; Research Diets) were provided in individual feeding cups whose position in the cage was changed daily. Body weight and food intake were recorded daily during the adaptation period.

Experimental Protocols:

**Experiment 1: Dose response effects of MTII on food intake.** SD rats fed either dietary chow (n=8) or adapted to the HF diet (n=8) were fasted for 24h. One hour before lights off, they were injected with vehicle or MTII (0.05, 0.1 or 0.5 nmol) into the CeA. Fresh food was reintroduced after injection and food intake was recorded after 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours allowing for spillage. All rats received all treatments in a random order with at least 7 days between each treatment.

**Experiment 2: Dose response effects of SHU9119 on food intake.** 28 SD rats fed chow were injected one hour before lights out with either saline vehicle or SHU9119 (0.01, 0.1 or 1.0 nmoles) (n=7/group) over a one minute period and immediately returned to their home cages. Food intake was then measured at intervals over the next 24 hour period and daily for the subsequent 3 days.

**Experiment 3: Dose response effects of AgRP on food intake.** 28 SD rats adapted to a HF diet received injections of either saline vehicle or AgRP (0.01, 0.03 and0.1 nmoles)(n=7/group)into the CeA one hour before lights out. The rats were returned to their home cages and intake of the HF diet measured at specific time intervals over the next 5 days.
**Experiment 4: Effect of bilateral amygdala AgRP on food intake.** 21 satiated rats with bilateral CeA cannulas that were either fed regular chow or had been adapted to the HF diet were injected with either saline vehicle bilaterally, unilateral saline and AgRP (0.03nmoles) or bilaterally with AgRP (0.03nmoles)(n=7/group). Food intake was measured at intervals over the next 120 hours.

**Experiment 5: Effect of MTII on dietary selection.** 13 rats adapted to the 2-choice HF/LF diet were fasted for 24h. One hour before lights off, they were injected with vehicle (n=6) or MTII (0.5 nmoles; n=7) into the CeA. Fresh food was reintroduced after injection and food intake was recorded after 0.5, 1, 2, 4, 8, 12, 24, 48 and 72 hours allowing for spillage.

**Experiment 6: Effect of SHU9119 and AgRP on dietary selection.** The rats used in experiment 4 were kept on the 2 choice-diet for another 7 days to allow them to recover. They were then randomized with another 15 rats of the same age that had been adapted to the HF/LF diet choice and reassigned to 3 new groups based on body weight and diet selection. One hour before lights-off, they were injected with either vehicle (n=10), AgRP (0.1 nmoles; n=9) or SHU9119 (1 nmoles; n=9). Food was removed at the time of injection and fresh food was reintroduced 1 hour after the injection. Food intake was recorded after 1, 2, 4, 8, 12, 24 and daily during the following 7 days allowing for spillage.

**Experiment 7: The effect of AgRP on expression of brain-derived neurotrophic factor in the amygdala.** After completion of the feeding studies in Experiment 4, 5 rats with bilateral cannulas were each injected unilaterally with saline and AgRP (0.03nmoles), returned to their cages and food and sacrificed 2 hours later by decapitation. Brains were
rapidly removed and the both whole amygdala regions dissected from each rat, frozen in liquid nitrogen and stored at -80°C until processed for RNA extraction.

**RNA extraction and quantitative PCR of BDNF gene expression.** RNA was extracted from the amygdalas using TriReagent (Molecular Research, Cincinnati, OH). RNA was treated with DNase (Qiagen, Valencia, CA) and cleaned by RNeasy columns (Qiagen, Valencia, CA). Purified total RNA from each individual tissue was primed using oligo (dT) and reverse transcribed using the superscript First-Strand Synthesis system for RT-PCR (Invitrogen, Carlsbad, CA). Transcript expression levels of BDNF were quantified using SYBR® Green (Quanta BioSciences, Inc., Gaithersburg, MD) and normalized to the expression of cyclophilin B. The following primers for rat genes were used: BDNF: Forward, 5’AATGTTCGGTTGA GAAGAG-3’, Reverse 3’TGCAACCGAAGTATAAGAATAACCA;; cyclophilin Forward, 5’- GGTGGAGAGCACCAAG ACAGA, Reverse, 3’GTAGTAACCAGCT GAGGCCG was used as control.

**Statistical Analyses.** The food intake data are expressed as mean ± SEM. Body weight and food intake were analyzed using either two-way analysis of variance (ANOVA) with Neumann-Keuls post hoc tests or t-tests when appropriate. BDNF expression data was analyzed by a paired “t” test as each animal had unilateral injections of saline and AgRP. Significance was set at p<0.05 for all analyses.

**RESULTS**

**Effect of administration of MTII into the CeA on food intake.** Figure 1 shows the temporal anorexic response to several doses of MTII in rats fed either dietary chow (1A)
or a HF diet (1B). MTII reduced food intake in rats on both diets in a dose related manner. The effective dose on the chow diet was <0.05nmoles and on the HF diet between 0.01 and 0.05 nmoles. However, the magnitude of the inhibitory effect appeared to be greater in rats fed the HF diet (36% versus 19% inhibition at the 0.5nmole dose (Figure 1C).

**Effect of SHU9119 administration into the CeA on food intake.** The MC4/MC3R receptor antagonist SHU9119 caused a dose dependent stimulation of food (rat chow) intake in satiated rats (Figure 2). The effective dose was around 0.1n mole; the stimulation of intake was evident within one hour and lasted for more than 48 hours. The highest dose used (1.0n mole) doubled food intake after 24 hours. This orexigenic effect declined slowly over subsequent days but had not completely disappeared after 96 hours.

**Effect of AgRP administration into the CeA on intake of HF diet.** The MC4R inverse agonist AgRP produced a pronounced stimulation of food intake with an effective dose of < 0.01 nmoles (Figure 3). All 3 doses of AgRP had similar orexigenic effects within the first 4 hours after which a dose related stimulation of food intake became apparent. Once again the feeding response was prolonged, food intake only approaching the level of that in the control animals after 5 days.

Bilateral injections of AgRP enhanced intake of both the chow (Figure 4A) and HF diet (Figure 4B) compared to unilateral injections in the first 24 hours, after which there were no differences in food intake between the two groups. Food intake remained elevated compared to the control vehicle injected rats in both the unilateral and bilateral AgRP injected rats for 72 hours.

**Effect of MTII administration into the CeA on macronutrient selection.** When given the choice of diets, the rats in this experiment showed a distinct preference for dietary fat
after an overnight fast. Amygdala MTII (0.5nmole) inhibited intake of the HF diet but no effect on intake of the LF diet caloric consumption (Figure 5). The inhibitory effect on intake of HF diet was evident as early as 30 minutes after the injection (-83.9% compared to saline injected rats, p<0.05), maximal at 24h (-35.5% compared to saline group, p<0.05) but was still evident on day 2 (-17.3 % compared to saline group, p<0.05). As a result of the decrease in intake of HF diet, MTII also reduced total caloric intake of the rats over the first 2 days. The MTII treated rats recovered caloric intake to control levels after 72 h.

**Effect of SHU9119 and AgRP injections into the CeA on macronutrient selection.**

For this study, we chose doses of SHU9119 (1.0nmole) and AgRP (0.1nmole) that had maximal effects in our dose response studies. In satiated rats, administration of the MC3/4R antagonist SHU9119 into the CeA induced an increase in caloric intake as early as 2 hours after the injection (Figure 6). This effect was potent (two-fold at 24h, p<0.05) and long lasting, the orexigenic effect of SHU9119 lasting for 4 days, food intake only returning to control levels at day 5. In this experiment, the rats showed only a slight preference for the HF diet over the low fat diet as shown by the intake on the day prior to the experiment (This is shown as Day 0 in lower panels of figure 6). Notwithstanding that, SHU9119 induced a profound stimulation of intake of the HF diet that was maximal in the first 24 hours but only slowly returned to control levels by day 6. In contrast, there was a small but non-significant inhibitory effect of SHU9119 on intake of the low fat diet. Injection of AgRP into the CeA had a similar but less profound effect than that seen with SHU9119. The stimulation in caloric intake was smaller than with SHU9119 and lasted for a shorter time (2 days) (32% at 24H; p<0.05 and 21.2% at 48h; p<0.05) but was again entirely due to a stimulation of intake of the HF diet. Although the numbers of
animals are small, there did not appear to be any relationship between the magnitude of
the response to AgRP and SHU9119 and the basal level of preference for the HF diet
(data not shown).

**Effect of AgRP injections into the CeA on expression of BDNF.** The effect of AgRP
on BDNF gene expression relative to cyclophilin was compared in individual animals.
Figure 7 shows that AgRP injections into the CeA increased expression of AgRP, as
measured in the whole amygdala, by over 2-fold compared to the saline injected animals.
This increase was observed in 4 of the 5 rats that were tested.
DISCUSSION

The amygdala, a brain region traditionally studied for its role in behaviors such as fear and anxiety, also influences ingestive behaviors (18, 20, 51). It plays an important role in the development of conditioned taste aversion and the reward response to ingested food (19, 54). Previous research from our group has shown the importance of the amygdala in modulating food preferences whether it was through the administration of enterostatin (32, 33), or NPY (41) in rats given a 2-choice diet paradigm. In the study presented here, we have examined the role of the melanocortin system in the amygdala control of food intake and macronutrient selection using the melanocortin agonist MTII, the melanocortin antagonist SHU9119 as well as the reverse agonist AgRP.

We showed that MTII, SHU9119 and AgRP all had dose related effects to influence food intake and that the doses required were very similar to those that elicit responses at the hypothalamic sites (12, 22, 27, 38, 46, 59). Bilateral injections of submaximal doses of AgRP had additive effects in the initial 24 hour period. As with the effects identified at the arcuate/PVN level (23), CeA melanocortin activity was associated with changes in the intake of dietary fat; the melanocortin 3/4 receptor agonist Melanotan II (MTII) administered into the CeA decreased the consumption of calories solely through a reduction in the intake of the HF diet. There was no compensatory increase in intake of the LF diet and the inhibitory effect was prolonged, normal food intake levels not being fully restored until 72 hours after the MTII administration. Conversely, both the MC3/4 receptor antagonist SHU9119 and the inverse agonist AgRP had a prolonged orexigenic effect that lasted between 2-4 days. The increase in caloric intake was again just related to the increase in consumption of the HF diet. The response to SHU9119 was particularly
pronounced in its magnitude and duration. This rapid onset, long lasting effect of AgRP has been described by others (21, 23, 26, 42) after administration of AgRP into the third ventricle. Increased hypothalamic levels of AgRP (13, 49) have been associated with an obesity phenotype. Its selective orexigenic action on dietary fat in animals given a choice of diet is believed to reflect actions on the melanocortin system as AgRP acts as an inverse agonist to melanocortin receptors MC3-R and MC4-R (9). The results presented in this paper suggest that the melanocortin system is functioning in the CeA, as it does in the hypothalamus (23), to modulate selectively the appetite for dietary fat.

Alternative explanations for the apparent selectivity towards dietary fat should be considered. It is possible that this is not a specific effect on fat intake but that it is related to the basic preference of the animal. It had previously been suggested (38) that AgRP mainly affected appetite rather than food preferences on the basis of paraventricular AgRP enhancing the intake of dietary chow rather than a palatable sucrose solution. Kim and colleagues (28), reported that AgRP injections in the CeA, at the same dose level we used in our studies, induced a delayed and weak increase of food intake, and suggested that the amygdala was less significant to the control of food intake compared to other hypothalamic areas such as paraventricular or dorsomedial nuclei. However, we observed comparable responses at comparable doses in the CeA to those reported for the hypothalamus. Indeed, in our experiments using a 2-choice diet paradigm, we found a very large and fast increase in food consumption due to an increase of the HFD consumption when either AgRP or SHU9119 was administered into the CeA. The response elicited by AgRP was less dramatic than the response to SHU9119 at the doses used. This difference may reflect differences in clearance or differences in access to or
binding to the receptor. In the currently reported studies there was a clear fat preference of the animals tested in the MTII experiment. However, in the studies of the response to AgRP and SHU9119, there was only a small imbalance in the number of calories of HF diet eaten compared to LF (grams of LF eaten were greater than HF) and still there was only a stimulation of dietary fat intake in response to AgRP and SHU9119. Further we could find no relationship between basal fat preference and the increase in fat intake. Even individual rats with a LF diet preference only increased the HF diet intake. Secondly it is possible that differences in the temporal sequence of eating the HF and LF diets might bias the results. However, the time courses provide no evidence that HF diet is eaten preferentially before low fat diet in any of the experiments. Further, the stimulation of fat intake by SHU9119 and AgRP and the inhibition by MTII can be observed over a long time period extending over several days. Finally, we have only demonstrated that melanocortin signaling in the CeA can affect intake of the specific diets that we presented to the rats. Whether a similar response would be observed with diets of differing fat composition, texture or flavor needs to be assessed before we can be conclusive in our interpretation of the data.

The pathways and neural sites activated or inhibited in response to changes in CeA melanocortin activity are currently unknown. The CeA has connections to all of the hypothalamic regions that affect food intake as well as to other parts of the limbic system and neocortical regions of the brain (17) that allow it a central role in integrating feeding behavior and allowing the cognitive and emotive aspects of food intake (8, 15, 18, 22, 34, 51, 56) to override the homeostatic controls within the hypothalamus (5).
MTII had a potent and fast effect on caloric consumption. Thirty minutes after receiving MTII in the CeA, the rats already exhibited a significant reduction in caloric intake, resulting from loss of appetite for the diet rich in fat. The size and speed of the response to the administration of MTII, and to AgRP and SHU9119, suggest that the responses are not the result of diffusion of the drug through the brain to other areas (27). We have shown that the small injection volumes used in these studies do not diffuse outside of the amygdala within the first 4 hours after injection (7). Thus rapid diffusion to the hypothalamic centers cannot be an explanation of our observations.

The response to MTII was greater in rats fed a HF diet compared to rats fed the low fat regular chow diet. This fat sensitive increase in response to MTII has been previously reported for icv injection of MTII (12) targeting the arcuate/PVN regions but in this case it was only evident in rats resistant to the development of obesity. Whether the CeA response to MTII likewise differs between obesity resistant and obesity sensitive animals is not known at this time.

The long lasting (several days) responses to single intra-amygdala injections of MTII, AgRP and SHU9119 are similar to those observed after intracerebroventricular administration. Considering the short half-life of these compounds, this suggests that acute changes in melanocortin 4 receptor activity induce long term changes in downstream signaling or effector activity. The observation that the pattern of fos expression differs between the acute and longer term responses to intracerebroventricular AgRP was proposed as evidence that the short term and long term responses to AgRP have different mechanisms (22). It could also be evidence of the activation of neural circuits to counteract the long term effects of AgRP as suggested by Berthoud and colleagues (57). The underlying mechanism(s) responsible for the prolonged responses to
changes in melanocortin receptor modulation remain unclear. However, the observation that bilateral injections of submaximal AgRP doses induced a greater acute (0-24 hours) feeding response than a unilateral injection but had no additional effect on the long term feeding response (24-72 hours) could be indicative of two differing mechanisms.

The increase in BDNF gene expression within the whole amygdala in response to the AgRP injections into the CeA was unexpected since the inhibitory effect of MC4R agonists is dependent upon BDNF expression (36, 53). Since we assayed BDNF gene expression in the whole amygdala, we cannot at this time be sure if the changes in BDNF we observed were localized to the CeA or evident in other amygdala regions. If the changes were localized to the CeA, the magnitude of the response would have been underestimated by our analysis of the whole amygdala. This observation requires further investigation. We had anticipated a down regulation of BDNF gene expression in response to AgRP. However, in our study, the rats did have access to food in the 2 hours between AgRP injections and sacrifice so it is possible that this increase in BDNF expression was indirect in response to the AgRP induced hyperphagia.

**Perspectives and Significance:** There is now considerable evidence to support a role of the amygdala in regulating dietary choice. Dietary fat intake can be reduced by injections of either enterostatin (33, 34) or NPY (41) while Mu-opioid agonists will enhance fat intake (51). In this manuscript, we provide evidence for melanocortin signaling to affect dietary fat intake in a similar manner to that previously demonstrated within the hypothalamus (21, 23, 26, 42). The importance of this CeA system and how it may interact with other systems within the amygdala or with the melanocortin network within
the hypothalamus to affect fat intake remains to be evaluated. Likewise, it remains to be seen if this apparent selectivity towards dietary fat reflects a specific macronutrient response or is more related to the reward or aversive aspects of feeding that are affected by the amygdala.
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REFERENCES:


LEGENDS TO FIGURES

**Figure 1:** Time course of the anorexic response to different doses of MTII injected into the CeA of rats fed either dietary chow (1A) or a HF diet (1B). The relative inhibition compared to the intake of the saline injected control animals is shown in panel C. Letters a, b, c show statistical difference (p<0.05) for 0.5, 0.1 and 0.05 nmole MTII respectively compared to saline controls. * p<0.05 compared to LF diet group.

**Figure 2:** Time course of the response to various doses of SHU9119 injected into the CeA on food intake of rats fed dietary chow over the initial 24 hours (panel A) and for 4 days (panel B) after the injection. Panel A shows cumulative intake during day 1. Panel B shows daily food intake. *p<0.05 compared to saline controls at that time point.

**Figure 3:** Time course of the response to various doses of AgRP injected into the CeA on food intake of rats fed a High Fat diet. Panel A shows cumulative intake over the initial 24 hour period. Panel B shows the daily intake for 5 days after a single AgRP injection. a: p<0.05 versus saline for each of the 3 AgRP doses; b, p<0.05 versus saline for the 0.1 and 0.03 nmole AgRP doses.

**Figure 4:** The effect of bilateral injections of AgRP in the amygdala on food intake. Rats were fed either regular chow diet (A) or a high fat diet (B). Cumulative food intake during the initial 24 hours is shown in the upper panels. The lower panels show daily food intake over the 5 day experimental period. a, p<0.05 vs saline/saline group, b, p<0.05 compared to Saline/AgRP group.

**Figure 5:** The effect of amygdala MTII (0.5 nmoles) on macronutrient selection in male Sprague-Dawley rats adapted to a 2-choice diet. Panels represent the cumulative consumption of High Fat diet (HFD), Low Fat Diet (LFD) and total calories (Total Kcal)
with in the first 24h after administration (top panels) and daily average for 3 days following injection (bottom panels). * p<0.05 compared to saline group at same time point.

**Figure 6:** The effect of amygdala AgRP (0.1 nmoles) and SHU9119 (1nmole) on macronutrient selection in male Sprague-Dawley rats adapted to a 2-choice diet. Panels represent the consumption of High Fat diet (HFD), Low Fat Diet (LFD) and total calories (Total Kcal) cumulated within the first 24h after administration (top panels) and daily average for 3 days following injection (bottom panels). * p<0.05 compared to saline group at same time point.

**Figure 7:** Effect of AgRP on expression of BDNF in the amygdala. Bilaterally cannulated rats received AgRP (0.1nmoles) into one amygdala and saline vehicle to the other amygdala. Values are shown as Means ± SEM. The insert shows BDNF expression in the individual amygdala of each rat. * p<0.05 compared to saline control.
Figure 1

A

Food intake (kcal)

Time (hours)

B

Food intake (kcal)

Time (hours)

C

% Inhibition from Saline

MTII dose (nmol)

LFD

HFD

ND

*
Figure 3

(A) Food intake (Kcal) over time (hours) for different saline doses. The graph shows a significant increase in food intake with time, with the highest intake observed in the 0.1 nmole group. The error bars indicate the standard deviation.

(B) Food intake (Kcal) over time (days) for different saline doses. The graph shows a gradual increase in food intake with time, with the highest intake observed in the 0.1 nmole group. The error bars indicate the standard deviation.
Figure 4
Figure 5

**HFD**

- Food Intake (kcal vs. Time (hours))
- Legend: 
  - Saline
  - MTII 0.5 nmol

**LFD**

- Food Intake (kcal vs. Time (hours))

**Total Kcal**

- Food Intake (kcal vs. Time (hours))

- Legend: 
  - Saline
  - MTII 0.5 nmol
Figure 6
Saline

AgRP

Animal #

Relative Expression

BDNF

Saline
AgRP

*