Melanocortin Administration to the Ventral Tegmental Area Alters Homeostatic and Hedonic Feeding

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doi: https://doi.org/10.57709/4474650

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MELANOCORTIN ADMINISTRATION TO THE VENTRAL TEGMENTAL AREA ALTERS HOMEOSTATIC AND HEDONIC FEEDING

by

Haw-Han Yen

Under the Direction of Aaron G. Roseberry

ABSTRACT

Dopamine neurons of ventral tegmental area (VTA) are critical for control of homeostatic feeding, which relates to food intake necessary for maintaining normal physiological function. It also plays an important role in hedonic or ‘reward’ feeding. Alteration of mesolimbic dopamine pathways can change both baseline homeostatic and reward feeding. The melanocortin system of the hypothalamus also is important for control of feeding, and recently it has been shown that melanocortins can modulate the food intake by acting at dopamine pathways. Here, we tested whether injection of melanocortin receptor agonists into the VTA also affected reward feeding. Injection of melanocortin receptor agonists into the VTA decreased consumption of rewarding sucrose solutions (hedonic feeding), in addition to the expected reductions in normal chow intake (homeostatic feeding). These studies will give a better understanding of the mechanisms regulate acutely food intake and may have implication for treating obesity.

INDEX WORDS: Obesity, Melanocortin, Homeostatic, Hedonic, Reward, MTII, Dopamine, VTA
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by

Haw-Han Yen

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science
in the College of Arts and Sciences
Georgia State University
2013
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HOMEOSTATIC AND HEDONIC FEEDING

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August 2013
ACKNOWLEDGEMENTS

This thesis could not be done without the guidance and the help of so many people who paid much concern. I would like to appreciate my colleagues, Danielle Mankin, Dereka Moore, Katherine Stuhrman, Dorkas Wu and Radhakishen Jayanti, for encouraging me during this hard time. I am so thankful to have Dr. Bartness and Dr. Xue been my thesis committees, gave me a lot of valuable comments that benefit me in my life. I am also grateful to Dr. Blaustein, Dr. Carruth, LaTesha D. Warren and Moneka Jones for helping me not be bothered by some problems other than thesis, such as finance and recommendations. At the last, it is my great pleasure to have my mentor, Dr. Aaron G. Roseberry, whose trust, patience, encouragement, guidance, and tolerance from the begging to the end, makes me not only know how to become a real scientist but also be successful in my future.
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1. INTRODUCTION

Obesity has recently been designated as a disease by the American Medical Association in 2013. It is a physical disease that may be induced by behavioral and genetic problems. In this modern society, the population of obesity is booming and it causes huge medical expenses. Obesity (defined as a BMI value is above 30 kg/m$^2$ (the BMI above 25 kg/m$^2$ is considered as overweight), or waist circumference is greater than 40 inches (male)) population is increasing during the past three decades, although the increasing rate of prevalence tend to be slight (U.S. adult obesity: 14.5%- 30.5% during 1976- 2000, around 35% in 2010 and no significant difference compare with 2003-2008), it still keeps 31.8% of U.S. children and 68.8% of U.S. adults in either obesity and overweight (the prevalence of obesity is 16.9% and 35% individually) (Flegal et al., 2012, Ogden et al., 2012). Obesity is not only a prevalent disease in U.S. but also around the world, both developed and developing countries have increased the budget for the treatment of various illnesses, such as diabetes, cardiovascular diseases and certain types of cancer that are higher risk of diseases associated with obesity. It is not an issue for people to obtain food in most countries today but finding the balance between energy consumption and expenditure has become a major challenge and burden for our current and future generations. The control of homeostatic and reward feeding is critical in regulation of body weight and food intake, but it is still not clear in which factors or mechanisms to maintain energy homeostasis of body or cause the obesity. Individuals “need” to eat in order to maintain energy requirements for normal function, thus the need-based eating is the homeostatic feeding which helps keep the energy homeostasis of the body. Additionally, people would “like or want” to finish the appetizing plate even in satiety due to the pleasure effect of eating. Hedonic, the want-based feeding makes people tracing the reward and it may be the reason to cause the overweight.
1.1. Homeostatic Control in Brain Circuit

The body weight is regulated by that the central nervous system by its control of food intake, locomotion and metabolism, however, the interaction among the different parts of the nervous system to control these factors is still unclear. Homeostatic feeding is regulated by different parts of brain besides the peripheral factors such as the concentration of glucose in blood or some hormones such as CCK, ghrelin, leptin and insulin that are secreted by digestive system or adipose tissue.

Through controlling feeding and body weight, the ARC (hypothalamic arcuate nucleus), VMH (ventromedial hypothalamus), VLH (ventrolateral hypothalamus) and brainstem had been considered centers of homeostatic feeding (DeFalco et al., 2001). The subjects with bilateral lesions of VMH and VLH result in opposite effects, hyperphagia (which cause obesity at later) and aphagia (which lead to death by starvation without awareness), suggested VMH and VLH are responsible to satiety and hunger respectively (Harrell et al., 1975, King and Frohman, 1985, Williams et al., 2000). More recent evidence indicates that other parts of the brain, such as the ARC are important for homeostatic feeding. There are two different sets of neuropeptides released by two distinct groups of neuron in ARC where integrates most of signals from periphery, controls appetite hormones and be regulated by positive and negative feedback (Schwartz et al., 2000). First, catabolic peptides, such as proopiomelanocortin (POMC)- a precursor of melanocyte stimulating hormone (MSH) corticotropin (ACTH), β-endorphin and encephalin released from POMC neurons in ARC when obtain leptin and insulin from adiposity or blood, can reduce the food intake and increase energy expenditure that would result the loss of body weight. Second, the anabolic neuropeptides, the AgRP and NPY are released when other part of ARC receiving signals from adiposity, have the effect of accumulation of body fat by
increasing food intake and reducing the energy expenditure. The nucleus of solitary tract (NTS) of brainstem integrates the satiety signals such as CCK from digestive tract, sensory input from viscera and the catabolic or anabolic projection from paraventricular nucleus (PVN) or LH where are afferent through ARC, that may send either signals to periphery for regulating the food intake (Schwartz et al., 2000). Anatomically, it is clear that there are several major parts of CNS control homeostatic feeding. The hypothalamus, ARC and brainstem receive signals of food intake and adiposity from periphery and affect energy homeostasis via the autonomic nervous system, however, the mechanisms in control of eating are still not fully understood and need to be further investigated.

1.2. Melanocortin in Homeostatic Feeding

Melanocortin system, includes distinct groups of neurons which release neuropeptides POMC and AgRP, is critical involving caloric homeostasis and relating to the changing of body weight by its catabolic and anabolic effects respectively. The melanocortin receptors in afferent neurons, regulates homeostatic feeding and body weight. Melanocyte stimulating hormones (MSH), such as α, β, and γ-melanotropin, are derived from proopiomelanocortin (POMC). MSH and its analog such as melanotan II (MTII) are anorexigenic agents that suppress the feeding behavior and agonizing melanocortin receptors (Klusa et al., 1999, Hamilton and Doods, 2002, Pierroz et al., 2002). Previous study demonstrated that two hours following intracerebroventricular (ICV) injection with MTII, mice had significantly decreased food intake (Fan et al., 1997, Azzara et al., 2002). Thus, the POMC neuron produced MSHs acts as an appetitive suppressor in brain that may alter feeding behavior through agonizing its receptors (Bertolini et al., 1986, Roselli-Rehfuss et al., 1993, Millington, 2007). Furthermore, the administration of MSHs had been identified to not only modulate feeding behavior, but also is a useful treatment to improve
cardiovascular function (Bertolini et al., 1986).

Another set of neuron, AgRP neuron, which projection to POMC neuron had proved able to regulate eating through melanocortin system. AgRP are endogenous antagonists/inverse agonists of melanocortin receptors that are not only able to stop decrease food intake but also stimulate eating. With specifically stimulating to AgRP neurons in hypothalamic ARC by channelrhodopsin, the optogenetic tools, mice exhibit profound eating (Aponte et al., 2011, Krashes et al., 2011, Atasoy et al., 2012, Cansell et al., 2012). The brainstem administration of SHU9119, an exogenous antagonist of melanocortin receptor, has same effect of feeding with AgRP (Grill et al., 1998). The control of satiety and hunger are supposed to involve with the feedback of melanocortin system that can be agonized and antagonized by various factors. Fasting rats have increased AgRP/ NPY neuron mRNA and decreased POMC neuron mRNA comparing with fed ad libitum rats (Korner et al., 2001). Korner and colleagues found hypothalamic leptin injections in rats was nearly completely reversal of the AgRP/ NPY and POMC neuron mRNAs expression when in fasting state.

There are 5 types of melanocortin receptor, type 1 through 5. The MC3R and MC4R are expressed in central nervous system and the functions to homeostatic feeding had been tested by using MSH, MTII, AgRP or SHU9119 in previous studies (Cone, 1999, Kishi et al., 2003, Cone, 2005, 2006, Fani L, 2013). Melanocortin receptor is a g-protein coupled receptor which activation within cAMP/ PKA pathway not only stimulating feeding but also improving long term potentiation in hippocampus (Shen et al., 2013). Generally, MC3R is likely to control physiological metabolism, such as energy expenditure, oxygen consumption and locomotion. For MC4R, it plays a critical role in food intake and energy balance. In Butler and Cone’s review study, MC3RKO subjects exhibited increased adipose mass and decreased lean mass despite the
absence of hyperphagia when exposed to high fat diet; however, MC4RKO subjects were hyperphagic, had increased both lean and fat mass, hyperglycemia, hyperinsulinemia and hyperleptinemia, which resulted in type 2 diabetes. MC4RKO mice had higher energy intake during moderate fat chow was applying and had significant body weight increase. MC3R and MC4R alter appetite and metabolism independently in different way, the MTII administration to either MC3R or MC4R knockout mice indicates melanocortin receptors are required for regulating energy homeostasis by MSH (Butler et al., 2001, Butler and Cone, 2002). Previous study shows the overexpression of POMC neurons in ventral tegmental area (VTA) can significantly ameliorates the high-fat induced dietary obesity (Andino et al., 2011), suggested an important role about reward is also responsible to food intake and body weight due to the motivation may change the caloric consumption such as fat and sugar which is parallel to obesity.

1.3. Dopamine and Hedonic Feeding

Although there are various studies and reviews discuss the anatomy of homeostatic pathway and its regulation in feeding behavior, the complete mechanisms to cause obesity are still unclear because there is another critical role, which makes further food intake when in satiety, the reward system. Food can also be a natural reward in certain brain circuits such as mesolimbic pathway. Some previous studies in several decades ago had shown the feeding, especially the acute feeding following starvation, could marked induced the dopamine turnover in several parts of brain such as hypothalamus, nucleus accumbens and amygdala (Heffner et al., 1980, Hernandez and Hoebel, 1988). Hedonic effect can be caused by high fat and appetizing sugar food that gives pleasure feeling to consumer as a reward through the brain circuit. Dopamine released in certain brain circuit such as mesolimbic pathway that plays a critical role of reward eating.

Food is a type of energy for homeostatic balance to maintain body weight and functions.
Palatable food, such as those high in fat and sugar has hedonic effect that arouse feeding behaviors by alerting specific brain circuits (Saper et al., 2002). Animals also experience failed reproduction, impaired immune system and have chronic stress when the food intake is short whereas the pleasurable rewarding is able to relieve the stress through mesolimbic pathway (Ulrich-Lai et al., 2010). Neurologists have found the behavior of obese animal model to be similar with drug addiction (Kenny, 2011). Previous studies demonstrated that addictive animals would work hard, such as lever press, nose poke or endure distress like electric foot shock for rewards such as drugs and food (McFarland et al., 2004). Food is a natural reward within brain that can cause motivation, reinforcement and addiction consequently. The potential problem is that reward effect may result in compulsion, reinforcement and addiction, however, the mechanism is still unclear (Berridge, 2004). The obese people have decreased density of D2 receptors, which has the similar mechanism with the drug addicts (Wang et al 2001).

Dopamine as neurotransmitters are critical factors involve in reward system and give animals hedonic effect (Adamantidis et al., 2011). There are several pathways regulating reward system in brain. The mesocortical and mesolimbic dopamine pathway, which is made of dopamine neurons in VTA that project to frontal cortex, amygdala, hippocampus and nucleus accumbens, are reward circuits controlling hedonic effect (Wise, 2004, Britt et al., 2012). The nigrostriatal pathway which neurons project to caudate putamen from substantia nigra compacta is about the feeding motivation and also partially involved in the production of movement, as a part of basal ganglia motor system (Szczytpka et al., 2001). Parkinson’s disease is characterized by apoptosis of dopamine neurons in the substantia nigra and cause lower body weight compare with healthy people (Beyer et al 1995). Patients that are given levodopa treatments, a dopamine precursor that can easily cross blood brain barrier, have significant weight loss suggesting the medication may
be responsible and dopamine can be a important factor in regulation of body weight (Bachmann et al 2009).

The food reward and reward- predictive stimuli can evoke phasic dopamine signaling throughout the striatum (Brown et al., 2011). Dopamine deficient mice do not only lose the preference of appetite, but also decrease regular food intake that cause decreased body weight and death during the experiment (Zhou and Palmiter, 1995, Palmiter, 2008), but reversed the hypophagia when nigrostriatal dopamine was restored (Hnasko et al., 2006). Dopamine in reward is a key factor that motivates subjects to alter their eating (Palmiter, 2007). The D2R knock-out mice also increases hypothalamic leptin sensitivity; thus, the injection of leptin into D2R knock-out mice will cause hypophagia and higher locomotion. The role of VTA has been well demonstrated in drug addiction, and the addictive mechanism can also be applied for natural food reward that makes human tend to accumulate more energy as body fat (Kelley and Berridge, 2002, Volkow and Wise, 2005, Sulzer, 2011).

Dopaminergic neurons arise from VTA in the mesocorticolimbic dopamine pathway were broadly discussed in reward, learning and memory, prediction and incentive salience (want-based behavior)(Wise, 2004, Cacciapaglia et al., 2011). The projection from VTA is not only given reward but also the aversion via distinct groups of neuron (Zhang and Kelley, 1997, Lammel et al., 2012). Current study used optogenetic tools to investigate the role of VTA that plays different roles in both feeding and reward through different types of afferent neurons, such as glutamatergic afferent can activate dopamine neuron and stimulate the dopamine release in VTA whereas the GABAergic neuron has the opposite effect for controlling the reward and aversion. These finding give a possibility to change the appetizing by inducing different sets of

1.4. Melanocortin Regulates Dopaminergic Neuron Related Feeding

The roles of mesolimbic dopaminergic and melanocortin systems in regulating feeding behavior are well known; however, whether melanocortin and dopamine neuron act together or independently to regulate food intake and reward has not been highly studied. Evidence shows not only the MC3R and MC4R are found in VTA (Butler and Cone, 2002) but also the administration of α-MSH into VTA causes increased dopamine level in NAc and stimulates grooming behavior which can be blocked by MC4R antagonists (Lindblom et al., 2001). In chronic stress, the activation of MC4R decreases the strength of excitatory synapses of D1R in NAc neurons (Lim et al., 2012). The POMC overexpression in ARC or VTA leads to TH (tyrosine hydroxylase) level increase in both area and tempered increasing body weight (Andino et al., 2011). Furthermore, Roseberry recently showed that administering MC3R and MC4R agonist and antagonist in VTA acutely and chronically affect food intake and body weight (Roseberry, 2013). This suggests melanocortin can act on dopamine pathway to regulate behaviors including homeostatic feeding. This raises the question of whether the melanocortin system can also affect reward related food intake through dopamine system of the VTA.

1.5. Hypothesis

In this study, we will test whether melanocortins act on dopamine neuron to regulate reward feeding by testing whether the activation of MC3R and MC4R in VTA affects intake of pleasurable and rewarding sucrose solutions. These studies will provide better understanding of mechanisms controlling food intake, especially appetizing foods. The results also could
contribute to identifying a novel tool for investigating the appetitive behavior and effective treatment to human obesity.
2. MATERIALS AND METHODS

2.1. Animals

Young adult male Sprague–Dawley rats (Harlan Laboratories, Madison, WI, USA), weighing around 250 grams at the beginning of experiment, were used for all experiments. Rats receive one-week acclimation after delivery and after surgery. Rats were housed with ad libitum food and water under a 12 hour light-dark phase. All protocols and procedures were approved by the Institutional Animal Care and Use Committee at Georgia State University and conformed to the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Cannula Implantation

For all experiments, indwelling cannulae were implanted bilaterally targeting the VTA. Rats were anesthetized with isoflurane (1–5 %). Rats received implantation of stainless steel cannula (26 gauge) (Plastics One) at bilateral VTA (A/P $-5.6$, M/L +/- $-2.2$, and D/V $-7$ relative to bregma; angle of $12^\circ$ to the midline) on a stereotactic apparatus (David Kopf Instruments, Tujunga, CA, USA) using standard flat-skull techniques. Cannulas were fixed by dental cement with anchoring to the skull using 4 stainless steel screws. Stainless steel wire stylets were placed in the cannula to prevent occlusion. The injectors extended 1 mm beyond the end of cannula. Rats were given ibuprofen (~15 mg/kg) in their drinking water for 3 days prior and post surgery for pain relief.

2.3. Microinjection

Rats were gently restrained by hand for processing microinjection. Stainless steel injectors (33 gauge) extending 1 mm beyond the tip of the cannula were inserted bilaterally. Injections done in volume of 0.2 $\mu$l were injected into VTA over 1 minute by using Hamilton syringes connected to microinfusion pump. Injectors were left in one additional minute after injection to limit backflow.
up the cannula. Stylets were placed back to cannulas after removing injectors. Rats were immediately moved back to testing cages for testing. Injections were spaced a minimum of three days apart to allow for full recovery between injections. Rats received the mock injection prior to injection for acclimating to inject procedure. MTII was dissolved in sterile saline, which was used for all control injections.

2.4. Two-Bottle Choice Test

For two-bottle choice test, rats were set in individual testing cages (acute tests) or in their home cages (prolonged access tests) with two bottles filled with water or sucrose solutions (1%, 2% or 10%). During acute tests rats did not have access to food. Testing was done during the early light phase for the short-term 4 hour acute intake (1% or 10% sucrose solution) and at the onset of the dark phase for prolonged access, 24 hours long-term intake (2% sucrose). Bottle positions were switched on every trial to prevent habit. Rats were acclimated to test procedure until the intake of sucrose and water was stable prior to testing with injection of MTII.

![Two-Bottle Choice Test](image)

**Figure 1. Diagram of two bottle choice test**
2.5. Measurement of Food Intake

Rats were given access to normal chow (Purina rodent diet #5001, PMI Nutrition International) throughout all experiments, except during the short-term access 2 bottle choice tests, when no food was present. Food intake was measured by providing rats a set amount of food and then weighing the food at specific intervals (e.g. 24 hours). Both food intake and sucrose solution in every trial were converted total caloric intake measured in each experiment using caloric values of 3.36 kcal/g for chow and 3.94 kcal/g for sucrose.

2.6. Histology

For confirming the location of injection site, 0.2 µL Neurotrace, a fluorescence Nissl stain, was injected at the end of all experiments, 24 hours before perfusion. All rats were completely anesthetized with ketamine/ xylazine (93/7 mg/kg) and were transcardially perfused with cold saline and 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde, at 4 °C overnight. Coronal brain slices (100 µm) containing the VTA were prepared by using vibrating blade microtome. The slices were mounted to glass slides and the location of injected Neurotrace was observed and captured the images by Nikon fluorescence microscope. Brain sections were then stained with cresyl violet, and the locations of the injectors were determined by light microscopy. The locations of all injection sites are indicated in diagrams of rat brain atlas (Paxinos and Watson, 1998) (Figure 2).

2.7. Data Analysis and Statistics

All data are presented as means ± SEM. Data were plotted using Office Excel (2010 Microsoft, Inc), and statistical analysis was performed using SigmaPlot (v12.0; Systat Software, Inc). All data were analyzed using repeated measures ANOVA as appropriate, followed by
Tukey’s post hoc tests for individual comparisons. All analyses were performed with a significance level of $p<0.05$ set a priori.
3. RESULTS

3.1. The Conformation of Injector Sites

The location of each injection site is indicated on the diagrams of rat brain atlas (Paxinos and Watson, 1998) shown in Figure 2. Sample images of injection site including Fluorescence Nissl stain are also shown in Figure 2. One rat was excluded due to the injection outside of VTA and 4 rats’ injection sites could not be confirmed due to the death during experiments.

![Figure 2. The confirmation of injector site. A. Diagram of rat coronal sections demonstrating injector locations in VTA. The locations of the injectors are indicated by dots. Numbers next to each section indicate the location of the section relative to bregma (Paxinos and Watson 1998). B, C, D, Sample images (bregma -5.30) of the same brain section containing the injector locations. B and D show in bright field, C shows the injection of Neurotrace](image-url)
3.2. Does VTA MTII Affect Intake of Low Concentration (2%) Sucrose Solution in Long-Term Access Tests?

We previously show MTII injection to VTA could decrease food intake (reference). Here, we initially tested whether injection of MTII into the VTA also affected hedonic feeding by testing whether administration of MTII to the VTA reduced amount of sucrose intake in 2 bottle choice tests. As control, we also measured the food intake.

Following the MTII injection to bilateral VTA just prior to the onset of the dark cycle, there is significantly decreased 2% sucrose intake at 24th hours in dose-dependent manner of 50 pmol and 10 pmol but not 1 pmol, compared with the saline injection group (Figure 3). The MTII injection also caused decreased sucrose preference that was from ~80% of total solution consumption to ~65% without affecting the intake of water. In agreement with previous study (Roseberry, 2013), MTII also caused decreasing of both chow and total caloric intake in these tests (Figure 3).
Figure 3. MTII injection to VTA suppressed sucrose, food and total energy intake in prolonged access tests. A. 24 Hr sucrose intake in 2 bottle choice tests B. 24 Hour water intake in 2 bottle choice tests. C-D. 24 Hour food intake (C) and total caloric intake (sucrose + chow; D) after MTII injection to the VTA in the 2 bottle choice tests. n=7. **p<0.01
3.3. Does VTA MTII Affect Short-Term Intake of High Concentration (10%) Sucrose?

In order to understand the hedonic feeding, rats were given choice tests in satiety during the early light phase. MTII was injected to bilateral VTA and rats were given 2 bottle choice tests between water and 10% sucrose solution to test whether the melanocortin receptor agonist is sufficient for blocking acute intake of sucrose solution.

In the early light phase, MTII 50 pmol injection significantly blocked sucrose intake starting at second hour (Figure 4). Rats keep highly preference to 10% sucrose solution following all injections with relatively low water intake when comparing with 10% sucrose solution. In acute 10% sucrose test, MTII also reduced the 24 hour food and caloric intake as expected but this was only observed for 50 pmol dose and with no effect for 10 pmol or less (Figure 4).
Figure 4. MTII injection to VTA suppressed acute intake of high concentration sucrose in 2 bottle choice tests. A-C Sucrose intake at 1 (A), 2 (B), and 4 (C) hours post-MTII injection in 2 bottle choice tests. D-F. Water intake at 1 (D), 2 (E), and 4 (F) hours post-MTII injection in 2 bottle choice tests. G-H. 24 hour intake of chow (G) and total calories (H) following MTII injection in the 2 bottle choice tests. n=11, **p<0.01
3.4. Does VTA MTII Affect Short-Term Intake of Low Concentration (1%) Sucrose?

At last, we also test whether MTII acutely affect intake of a low concentration of sucrose solution that is only marginally preferred and test if MTII injection acutely change this consumption pattern during the early light phase. Water and 1% sucrose solution were given and measured at 1<sup>st</sup>, 2<sup>nd</sup> and 4<sup>th</sup> hour following the injection of saline or MTII.

The 1% sucrose drinking in early light phase was dose-dependently decreased by MTII injections at 1<sup>st</sup>, 2<sup>nd</sup> and 4<sup>th</sup> hours (Figure 5). The 50 pmol MTII injection to bilateral VTA had caused significantly decreased drinking of 1% sucrose since the second hour. 10 pmol MTII decreased sucrose intake but was not statistically significant (p=0.06). No significant difference was found in water drinking (Figure 5 d-f). MTII injection also decreased 24 hour food and caloric intake in these experiments as expected (Figure 5).
Figure 5. MTII injection to VTA suppressed acute intake of low concentration sucrose in 2 bottle choice tests. A-C Sucrose intake at 1 (A), 2 (B), and 4 (C) hours post-MTII injection in 2 bottle choice tests. D-F. Water intake at 1 (D), 2 (E), and 4 (F) hours post-MTII injection in 2 bottle choice tests. G-H. 24 hour intake of chow (G) and total calories (H) following MTII injection in the 2 bottle choice tests. n=9, **p<0.01
4. DISCUSSION

In this study, we test whether melanocortin system is critical to alter either homeostatic or reward eating by MTII agonizing central melanocortin receptors that express in part of mesolimbic dopamine pathway, the VTA. It is known that feeding can alter the dopamine level in mesolimbic pathway, in addition, we ask whether the animals consume appetizing sucrose solution in satiety and this reward-related feeding can be suppressed by melanocortins injection. Thus, in this study, the principle examination is to ask whether the injection of melanocortin receptor agonist to VTA affect the intake or preference of appetizing sucrose solutions and find the clue if melanocortins can affect hedonic feeding by directly inducing dopamine neuron.

In all of the experiments in this study, the 50 pmol dose of MTII to VTA was able to suppress both chow and total caloric intake in 24 hours, which demonstrate the suppression of homeostatic feeding, as we have observed before (my paper reference). 50 pmol MTII also decreased the sucrose intake in both chronic and acute tests that suggested MTII injection to VTA did change the hedonic feeding. Additionally, the result suggests long-lasting stability of MTII to alter feeding.

We found that the lower 10 pmol dose of MTII decreased the intake of food and total calories in 24 hours in 1% sucrose, but not 10% sucrose testing, although the suppression of 10 pmol MTII was not statistically significant. When comparing acutely high and low sucrose intake in lower MTII injection, it suppressed 24 hours food and total caloric intake in low sucrose tests but not in high sucrose, this suggests high concentration sucrose might stimulates more reward in mesolimbic pathway which result in subsequently higher food intake that lower dose MTII could not suppress. The 24 hours energy intake between both acute feedings, which shows about 10
Kcal difference in either lower dose MTII injections, gives a clue that different level of reward may be regulated by melanocortins in dose dependent manner.

At first, we conclude the MTII injection to bilateral VTA is able to alter homeostatic feeding, which decreases the total energy intake that consistent with the results of MC3R and MC4R knock-out study (Butler and Cone, 2002). The MTII injected rats had less consumption of food that indicates the mechanism via MC4R not MC3R, because MC4R regulate the food intake and MC3R likely control the physiological metabolism (Butler and Cone, 2002). The role of MC3R in this study may be needed to have test, such as the locomotion and the energy expenditure. In addition, we also suggest that melanocortins may have the effect to dopamine neurons in VTA to alter the feeding, especially the hedonic feeding. We already show the MTII injection to VTA suppress intake of appetizing sucrose when rats were in satiety and sucrose is also known as a highly reward to mesolimbic pathway, furthermore, melanocortin receptors had been found on D1R neurons (Cui and Lutter, 2013), thus, we likely believe that melanocortins can regulate hedonic feeding by directly or indirectly affect dopamine neurons in mesolimbic pathway.

In summary, through the administration of melanocortin receptor agonist into bilateral VTA, the study concludes that the hedonic feeding can be altered and the melanocortin receptor may be related to dopaminergic neuron, which had been proven to change the motivation of eating. Previous study demonstrated that i.c.v. injection of MTII to 3rd or 4th ventricle can decrease the meal size and glucose intake which confirms the result of our study (Williams et al., 2002, De Jonghe et al., 2012). This specific injection to VTA with MTII helps us getting better understanding about the relation between homeostasis and motivation. It opens up the potential idea that more researches can be done to apply the finding on other parts of the brain circuit such as NAc and CPu in various addictions, such as ethanol and drugs (Szczypka et al., 2001, Zhang
and Kelley, 2002, Navarro et al., 2011, Lim et al., 2012). Although there are more mechanisms of homeostatic and hedonic pathways that need to be worked on, the finding of the effect from melanocotin receptor to mesolimbic DA pathway could be a useful start to treat obese people in the future.
5. REFERENCES


