University of Nevada, Reno

Obesity: Regulatory Mechanisms of Feeding Behavior and Energy Homeostasis

A thesis submitted in partial fulfillment of the requirements for the degree of

Bachelor of Science in Biology and the Honors Program

by

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We recommend that the thesis
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Abstract

The mechanisms which regulate feeding behavior and energy balance are evaluated in order to gain new ideas and insight. The importance of this regulatory system within society, which faces the issue of obesity, is discussed. A better understanding of the feeding behavior regulatory system is sought in order to allow for the development of clinical treatments that promote health. Thorough analysis of the literature about the regulatory mechanisms of feeding behavior is performed, to develop new approaches and insight into obesity. The brain/gut communication network, hormone signaling, microbiota, and non-hypothalamus/gut communication systems are evaluated using an integrated approach. This knowledge could have potential value for clinical therapies to resolve the obesity issue. Current knowledge, unanswered questions, and new insight/answers are provided, using figures and text.

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I would like to extend my gratitude to my thesis advisor, Dr. Alexander van der Linden. His support and hard work throughout the process have allowed me to develop my knowledge and carry out this thesis project. He has made the experience intellectually rewarding and satisfying.

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Introduction

Humans must consume food in order to obtain the energy necessary to carry out biological processes. This food energy allows us to perform the metabolic processes that are essential for life and function. It is, therefore, quite important to achieve a balance between energy intake and energy utilization, through regulated eating behavior. Insufficient food consumption and nutrition is harmful, but the consumption of excess food is also harmful. The consumption of excess food causes increases in body weight, as the excess energy must be stored. This energy is stored, generally in the form of adipose or fat tissue, which can lead to obesity. Obesity, in which body fat levels are above healthy standards and the Body Mass Index is greater or equal 30, presents a variety of health risks and issues. Obesity is a world-wide epidemic that has major effects on people's health and quality of life, with some estimates showing over 30% of people in America are considered obese (Nguyen & El-Serag, 2010). Thus, for understanding the relationship between feeding behavior and body weight regulation, it is important to understand the mechanisms through which feeding behavior and energy balance are regulated at the cellular and molecular level.

Feeding behavior is regulated through a complex system of interactions between the brain and gut, which utilizes neural and hormonal signaling mechanisms. The stomach and gut must communicate with the brain so that food intake is regulated in a way that provides sufficient, but not excess caloric intake. Therefore, appropriate feeding must correspond to its physiological metabolic energy needs. The interactions between the digestive system and the brain determine how food products are processed once consumed. Given the fundamental importance of this brain-gut metabolic network, and

its great relevance to the issue of obesity, knowledge of the mechanisms of this system is quite valuable. The brain/gut network shown in Figure 1, depicts the brain and gastrointestinal components, which communicate through neural and hormonal connections. This network depends on strong communication between the organs within the abdominal region and the brain region, through hormone and neural signals. Information is recognized in a variety of ways, including through G-protein coupled receptors, such as GPR 119, as shown. Great study has been conducted to evaluate this powerful biological system, but many of the details about the neuronal and molecular pathways as well as metabolic processes that regulate feeding behavior and energy homeostasis are not fully understood (Morton *et al*. 2006).

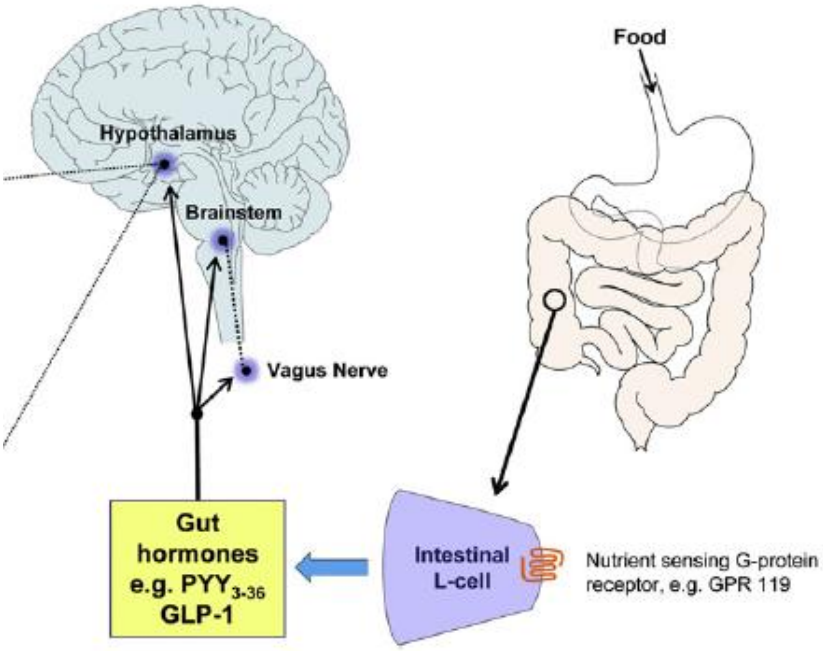

Figure 1. Gut-brain communication. The hypothalamus, located in the brain, together with the gastrointestinal system, including the stomach (which stores food) and the intestines are important to regulate feeding behavior. These organ systems are connected via nerves (such as the vagus nerve) and hormonal interactions.
Adapted from Sam *et al*. (2011)

The neural and hormonal components of the feeding behavior regulatory system have been evaluated through scientific experimentation, which includes chemical, pharmacological, and other methods. Neuronal cells within the brain and nervous system recognize and transmit signals indicating energy status. The brain, and particularly the hypothalamic region, recognizes hormonal and peptide signals (Belgardt *et al* 2009), which provide the means through which the stomach and brain can communicate hunger or satiety conditions, and thus govern eating behavior. In response to differing food energy levels within the body, different chemical signals provide messages to the brain to regulate eating. The peptide hormone ghrelin is produced in the stomach and pancreas, and acts on cells within the hypothalamus to stimulate hunger (Dietrich & Horvath, 2011). Another hormone called leptin produces a satiety signal through its binding to cells in the hypothalamus's arcuate nucleus (Dietrich & Horvath, 2011). Additionally, the hormone insulin works to store food-derived products, such as glucose. Through hormone interactions and cell signaling such as these, the brain and gut communicate energy needs and regulate feeding behavior. A more complete understanding of these complex interactions between hormones, neurons, and gastrointestinal cells could have real clinical and academic value.

The other component of the gastrointestinal system that provides increased complexity to the energy homeostasis regulatory system is the presence of intestinal microbiota. These microbiota, which provide organisms with a unique bacterial microbiome, have a large role in the processing of food products and energy metabolism (Backhed *et al*. 2004). It has been shown through bacterial compositional studies that the microbiota found in obese individuals is different compared to the microbiota found in

the intestines of lean individuals (Ley *et* al. 2006), which suggests a function for them in the body-weight regulatory process. Specifically, lean individuals have a higher proportion of *Bacteroidetes* than obese individuals (Ley, 2006). With these roles, and their relationship with biological hormones, microbiota may also be involved in the regulation of feeding behavior. This relationship is uncertain, but insight into these interactions is important and useful.

An additional dimension to the feeding behavior regulatory system is provided by the influence of factors outside of the main brain/gut communication system. These factors include the sensory and physiological mechanisms that affect animals' behavior and metabolism. Organisms exist within the context of their environment, and so the sensory signals obtained by organisms from the environment, such as olfactory, visual, and taste cues, have effects on feeding behavior. Eating and body weight regulation is also affected by biological clocks, which has a large impact on the metabolic processes. This clock is located in the suprachiasmatic nucleus (SCN), which is outside of the hypothalamus of the brain that is important in brain/gut communication (Mistlberger, 2011). This study shows the functional importance of other brain regions, outside of what are considered the main brain/gut communication pathways, in affecting feeding behavior. It has been shown that obese individuals are more stimulated to overeat in response to certain external cues, such as food portion size or appearance, displaying the importance of external factors in controlling eating (Herman & Polivy, 2008). Therefore, the feeding behavior regulatory system must be evaluated within the context of external cues and brain regions outside of the direct brain/gut communication system.

This thesis project will provide a more complete understanding of the mechanisms through which feeding behavior is controlled in mammas. The thesis seeks to evaluate the complex metabolic pathways and biological processes through which hormones provide communication between the brain and gut to coordinate appropriate feeding behavior, and the possible role of gut microbiota within this framework. This system of interactions acts through an integrated system of biological mechanisms and pathways. This system works within a context of external sensory signals, as well as physiological signals outside of the main brain/gut communication system. A solid understanding of the regulatory system that controls feeding behavior, using this integrated and coordinated approach, has not been well developed. By viewing the entire synchronized system within the context of a whole organism, a more practical and valuable understanding of the feeding behavior regulatory system can be obtained. This understanding can provide knowledge that is relevant to the societal issues of obesity and body weight control (Morton *et al*. 2006). Gaining insight into the unanswered questions regarding the neural and hormonal mechanisms of feeding behavior regulation, and developing a cohesive and integrated understanding of this regulatory system are the goals of this thesis.

This thesis will be conducted using a literature based research approach. A thorough collection of research will be obtained, and examined to gain new insight and provide an integrated perspective about feeding behavior regulation within mammals. This integrated approach is distinctive and different from the literature, and is most useful to provide clinically relevant knowledge. Knowledge about the neuronal circuits, gastrointestinal cells, hormone signals, gut microbiota, etc. will be gathered and

assimilated into a more complete view of the system as an interacting whole, rather than separate components. The writing of the thesis is organized into chapters to first discuss the current knowledge of each subject, then introduce an unanswered question, and finally to provide insight/answers about the question. The knowledge provided by this thesis project will add to the current understanding of the regulation of feeding behavior and metabolic disorders, such as obesity, which are important for society.

Literature Review

Given the importance of feeding behavior and maintaining a proper energy balance in body weight regulation and overall health, many scientific studies have been conducted to explore the underlying neural and molecular mechanisms. Moreover, recent studies (Backhed *et al*. 2004) in humans and rodents have shown that communication between the brain and microbiota present in the digestive tract is important for fat storage, adding more complexity to the system of body weight control. These factors work within the context of external and sensory cues, and in combination with mechanisms outside the main brain/gut communication mechanism, in order to produce a comprehensive system of feeding behavior regulation.

Hormones and the Feeding Behavior Regulatory System-

The hormone leptin, was discovered in 1994 (Zhang *et al.* 1994) and signals the nutritional status of mammals. Leptin circulates throughout the body, and acts on the hypothalamus by inhibiting excessive food intake (Farooqi & O'Rahilly 2009). Issues with leptin, such as leptin deficiency and leptin resistance have been attributed to obesity within humans (Farooqi & O'Rahilly 2009). While some of the pathways through which

leptin act to regulate feeding behavior are known, studies have not been conducted to determine all of these pathways or to develop the complete range of therapies to combat obesity using leptin-related treatments.

In addition to leptin, other hormones, such as ghelin and insulin have active roles within the feeding behavior and energy balance control systems. In a study by Tschop *et al*. (2000) the hormone ghrelin was found to promote adiposity within rodents. This study involved the introduction of ghrelin into mice by injection, and determined the role of ghrelin within the energy balance control system. Thus, metabolic hormones such as ghrelin and leptin signal hunger and satiety signals, respectively, and stimulate synaptic processes within neuronal circuits in the hypothalamus to control feeding behavior. However, although these hormones play important roles in regulating metabolic processes in fat tissues (leptin is among others secreted from fat deposits) and the stomach (ghrelin is secreted from the stomach), there are neurons receiving these hormonal signals and responsible synaptic changes that occur in these neurons. These neurons that express *Agouti-related protein* (*Agrp*) are a population of neurons in the hypothalamic arcuate nucleus (Arc), and increasing their activity is sufficient to induce voracious feeding behavior (Aponte *et al.* 2011). While another population of neurons that express *propiomelanocortin* (*Pomc*) and form a neuronal circuit with *Agrp*-containing neurons, inhibits feeding behavior.

An important recent study by Yang *et al*. (2011) recognized how neuron memory circuits can be flip-flopped between these feeding states (voracious feeding, and inhibition of feeding) based on physiological signals (hunger and satiety). This study was performed *in vitro* using electrophysiological, pharmacological, and optogenetic

techniques. The study determined that in response to hunger and ghrelin, a pre-synaptic pathway involving an AMP-activated kinase (AMPK)-dependent pathway, regulates the neural activity of *Agrp*-containing neurons. However, this study does not determine if such results are consistent with *in vivo* studies, which is currently a major question in the field. Moreover, the study did not identify the pre-synaptic cells that undergo AMPK-dependent changes to stimulate *Agrp*-containing neurons.

Insulin, a hormone secreted by the pancreas, has key roles in the metabolism of glucose within the body and is affected by an integration of factors, including nutritional status and neural factors (Seino *et al*. 2011). Insulin has a large role in the removal of glucose from blood, and obese individuals often have hyperinsulinemia, in which blood insulin levels are too high (Kissebah *et al*. 1982). A study done by Polonsky *et al*. (1988) showed that higher Body Mass Index (BMI) measurements corresponded with higher levels of insulin secretion, rather than weaker insulin clearance. These findings suggest a role for insulin in the regulation of body weight, and potentially in the control of feeding behavior.

There are additional components involved with the complex system of feeding behavior and energy homeostasis regulation. Peptide tyrosine tyrosine (PYY), Glucagon-like peptide-1 (GLP-1), and Oxyntomodulin (OXM) have been shown to control appetite and/or reduce food consumption (Batterham *et al*. 2002; Tang-Christensen *et al*. 2001; Dakin *et al*. 2001). Additionally, cholecystokinin (CKK) has been shown to reduce food intake in rodents upon peripheral administration (Antin *et al*. 1975), and pancreatic peptide (PP) injection has been shown to reduce food intake and reduce body weight gain (when administered for six days) (Asakawa *et al*. 2003). Glucagon, which increases

energy expenditure and increases satiety, has interesting clinical potential in the treatment of obesity (Nair, 1987; Schulman *et al*. 1957). Overall, there is a variety of signals that work together to control feeding behavior and energy homeostasis, and it is important to understand how they all interact.

It can be seen that these studies leave much room for expanded evaluation to answer these questions. In addition, as communication must occur between the digestive and diverse neuronal systems, it is very important that the neurons and the pathways performing the communication functions are defined accurately. The interactions between the various hormones, such as ghrelin, leptin, and insulin, are also not completely understood. Each of these hormones has major implications for feeding behavior and energy homeostasis, and understanding precisely where and how these hormones act is a subject of much interest.

Microbiota and the Regulation of Feeding Behavior and Energy Homeostasis-

There is potential to improve the understanding of how microbiota act on fat storage and body weight control. Gut microbiota, which are the bacterial microbiome within the gastrointestinal tract, have been found to have important roles in the processing and metabolism of food products. A recent study by Backhed *et al*. (2004) determined the importance of gut microbiota in determining the energy processing and storage of consumed items by rodents. This study found that the levels of fat deposition within mice were affected by the levels and variety of microbiota within the gut. The study involved the manipulation of mice (and their microbiota) and the analysis of metabolic and physical composition effects. Interestingly, not only obese mice, but also obese humans were shown to have a different diversity of bacteria in their intestine in

comparison to lean individuals (Ley *et al.* 2006). The study was important, but left questions about the potential for modifying microbiota by diet or exercise. The important discovery that microbiota can be modified by a mice's physical condition was made by Ley *et al.* (2005). This study revealed the potential for obesity therapies that involved modification of gut microbiota. However, the question remains how such modifications occur at the molecular and neural level and how well such results could be translated to humans. Moreover, normal microbiota in mice has been associated with the development of the brain and anxiety-like behaviors (Neufeld *et al*. 2011), and thus it would be interesting to establish if feeding behavior is directly controlled by microbiota as well. If it is expected that microbiota do affect feeding behavior, through what mechanisms (hormonal, molecular, etc.) would they be carried out? Altogethere these fundamental questions still need to be answered, and this thesis project will provide possible answers and valuable insights into these questions.

Influence of Factors Outside of the Hypothalamus/Gut Communication System-

While a main communication network exists between the brain's hypothalamus and the gut (with a variety of related hormone signaling mechanisms), the regulation of feeding behavior exists within a context of sensory cues and additional physiological mechanisms. Eating decisions are subject to sensory cues received by the environment, which can include olfactory, visual, and taste cues. It has been shown that overweight individuals respond to sensory (olfactory, visual, taste, etc.) cues to food without regulating or limiting consumption, while normal-weight individuals decreased food intake (Jansen *et al*. 2003). However, the implications of external sensory cues and their

interactions within the physiological mechanisms of feeding behavioral control are not completely understood.

Feeding behavior is also affected by physiological processes that are not strictly within the hypothalamus/gut communication system. The circadian clock has important effects on the metabolism of food and energy. This clock is located in the brain's suprachiasmatic nucleus (SCN), and affects the rhythm and timing of many biological processes. However, the exact location and extent of cells involved with a food anticipatory clock is largely uncertain (Mistlberger, 2011). These cells, which likely could work through oscillations, may correspond with the mammal's hunger status and help coordinate feeding behavior. This clock may also interact with the metabolism of hormones, including the metabolism of ghrelin and leptin (Mistlberger, 2011), indicating the importance of the circadian clock in feeding behavior and energy homeostasis.

The knowledge that has been obtained about the mechanisms of feeding behavior and energy homeostasis regulation is significant. However, an integrated understanding of the entire system has not been adequately developed. A lack of understanding of the whole-organismal and interacting system of feeding behavior and energy homeostasis regulation has been an obstacle in the field and an obstacle towards the development of health care applications using such knowledge (Morton *et al*. 2006). This thesis seeks to answer the questions that have come about and provide an integrated understanding of the regulatory system of feeding behavior and energy homeostasis.

Chapter 1- Understanding the Brain/Gut Network

Current Knowledge About the Brain/Gut Network

In order to understand the mechanisms through which feeding behavior is regulated in organisms, it is necessary to understand the details about the organ systems involved. The regulatory system utilizes brain and gut neuroendocrine communication signals to promote or reduce eating. Peripheral signals from the gut and signals from the central nervous system interact to coordinate eating and maintain body weight.

Within the central nervous system, the brain's hypothalamus and brainstem are of particular importance, as they function as centers that receive peripheral hormone signals depicting the body's nutritional status (Murphy & Bloom, 2006). The hypothalamus's arcuate nucleus (ARC) has a major role, being composed of medially located appetite stimulating neurons (orexigenic) (Bewick *et al*., 2005) and laterally located appetite inhibitory neurons (anorexigenic) (Elias *et al*., 1998). These neurons release their signals, such as neuropeptide Y and Agouti-related protein (AgRP) (orexigenic), or alpha-melanocyte stimulating hormone (alpha-MSH) derived from pro-opiomelanocortin (POMC) and other precursor polypeptides. The ARC nucleus can receive circulating hormone signals directly, as its neurons have axon terminals located near the median eminence of the brain, which does not have a complete blood-brain barrier (Peruzzo *et al*., 2000). Within the brainstem, the nucleus tractus solitaries (NTS) receive gastrointestinal signals from the vagal nerves, which carry such signals to the hypothalamus. The NTS recognize gut hormones, which can reach the NTS through the circumventricular organs (which do not have complete blood-brain barriers) (Baraboi *et al*., 2010). Additional signals can reach the brainstem from the gut through the ascending vagal nerve pathways (Jobst *et al*., 2004). Thus, the hypothalamic ARC receives hunger and satiety signals through several tracks, which can be processed and used to

communicate intra-hypothalamic (such as the hypothalamic paraventricular nucleus) and extra-hypothalamic energy homeostasis actions.

It has been found that the brain's hypothalamic region, primarily through AGRP and POMC neuron activity, utilizes a process of synaptic plasticity, in which the neurons are set to their appropriate state based on the hormones signals that they receive (Yang *et al*. 2011). In this neuronal circuit, an Amp-activated kinase (AMPK) positive feedback loop in response to ghrelin, plasticity was induced to persistently activate AGRP neurons (inducing a memorized feeding state). However, in response to leptin, this feeding activity was switched off, through an opioid receptor-dependent pathway (suggested opioid release from POMC neurons) (Yang *et al*. 2011). The hypothalamic circuit, seen in Figure 2, depicts the AgRP neurons which receive the glutamate signal, along with the surrounding neurons and signals, such as calcium and opioids. It is thus seen that a memory circuit system is utilized by the hypothalamus in response to physiological state, with hunger status being switched by a flip-flop mechanism (Yang *et al*. 2011).

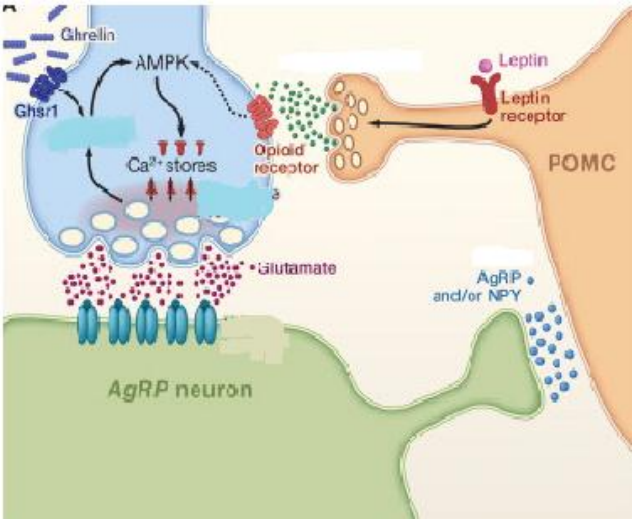

Figure 2. Hypothalamic feeding circuit. Ghrelin is recognize by Ghsr1 receptors on neurons, which then release glutamate to AgRP neurons. This process stimulates

feeding, which is reversed by the hormone leptin. Feeding is induced by AgRP neurons and NPY, while POMC neurons are involved with satiety. It is the identity of the neurons that release glutamate that is unknown.
Adapted from Dietrich & Horvath (2011).

Consumed food enters through the mouth, and upon transport through the esophagus, enters the stomach to be further digested. Food is then largely processed within the stomach and small intestine. The functions of the gastrointestinal tract must include digestion as well as the corresponding function of communicating the hunger/energy needs of the body. Cells within this system, enteroendocrine cells, compose a major endocrine complex within the body, releasing many different hormones and peptide signal types (Sjolund *et al*., 1983). From stem cells located within the intestines, enteroendocrine cells form into different varieties to express their different products. It seems that enteroendocrine cells are differentiated from two branches, from which different product types (substance P/serotonin vs. GLP-1/PYY/NT/CCK) are produced (Roth *et al*., 1992). While these gastrointestinal enteroendocrine cells release signals, they also obtain information about the gut's contents and food needs. The gut's cells have been shown to have receptors that recognize food related signals, such as amino acids (Jang *et al*., 2007; Rozengurt *et al*., 2006). This ability is extremely important to allow the gastrointestinal system to sense the presence of food contents, and coordinate the appropriate feeding behavior and appropriate metabolic response. Various nutrient molecules are thus chemically recognized by the various enteroendocrine cells, which then can release the corresponding signals to the brain. It is recognized that these enteroendocrine cells can be activated through mechanisms similar to taste receptor cells in the tongue. Additional signals are received by these gastrointestinal cells through

physical and neural mechanisms. The stomach is equipped with neural sensory mechanisms that react to the tension, stretch, and/or volume of the stomach in response to food (Cummings and Overduin, 2007). It is generally accepted that the stomach's main methods of recognizing satiety are more volumetric, while those of the intestines are chemical. These mechanisms provide the gastrointestinal system with the powerful and necessary ability to recognize food contents and energy needs within the body. In turn, the cells of the gastrointestinal tract coordinate such information into appropriate metabolic and feeding behavior responses through subsequent communication with the brain using hormones and peptides. Through this integrated communication system between the brain and gut, involving chemical, neural, and physical mechanisms, food can be detected, metabolic needs can be determined, and proper eating behavior can be promoted.

Unanswered Questions About the Brain/Gut Network

By understanding this brain/gut system more completely, clinical and pharmacological treatments for obesity can be developed. There are questions that exist in the study of this system, whose answers could be valuable for the scientific community and society as a whole. The exact nature of the neural cells from which the ghrelin induced glutamate release originated is still unknown (Yang *et al*. 2011). From which neurons these glutamate signals are released in order to activate AgRP neurons at their ionotrophic glutamate receptors in response to ghrelin, is an important piece of knowledge. The identity of these pre-synaptic cells is unknown, but can likely be identified through evaluation of the known information about the brains' neurons' structures and functions. Gaining insight into this question is one focus of this thesis.

Additionally, it is known that gastrointestinal cells can recognize food components using mechanisms similar to those used by taste receptor cells on the tongue. Specifically, it is known that intestinal cells contain sweet and bitter taste receptors, which means the gastrointestinal tract can sense nutrients (sweet and bitter type nutrients) through mechanisms similar to the tongue's mechanisms of taste sensation. However, it is not known whether or not the salty, sour, or umami type taste receptors of the tongue are also utilized by the gastrointestinal tract in a nutrient sensing capacity. Whether the enteroendocrine cells can recognize the signals from these salty, sour, and umami taste molecules is an important question that should be answered. By evaluating the literature dealing with the gastrointestinal cell receptors and tongue taste receptor cells, insight into this major question can be gained, which could allow for therapeutic treatments that manipulate these mechanisms to be developed. Gaining this type of insight about enteroendocrine cell functioning is another focus of this thesis.

Providing insight into the question about the pre-synaptic cells involved in the ghrelin response and the question of gastrointestinal cell receptor mechanisms should advance the field of knowledge and improve the potential for clinical obesity treatments.

Insight/Answers About the Brain/Gut Network

The identity of the neural cells that carry out the glutamate release in response to ghrelin in order to activate the AgRP neurons is not known. These neural cells must be able to both recognize ghrelin, and respond to ghrelin with a glutamate release. Additionally, these glutamate-releasing neurons must be able to reach the AgRP neurons in order to promote feeding behavior. With these criteria, the identity of these neurons can be better understood and determined. The major cell receptor type within mammals

that recognizes the hormone ghrelin as the endogenous ligand is the growth hormone secratagogue (GHSR) class of receptors (Martin-Pastor *et al*., 2010). This means that the neurons that are recognizing ghrelin, according to the model by Yang *et al*. (2011), likely contain these receptors (See Figure 2). The presence of these receptors has been found in different regions of the brain, including in the important hypothalamic neurons (Zigman *et al*., 2006). Interestingly this study by Zigman *et al*. (2006) also found the presence of GHSR mRNA within nuclei of the vagus nerve. These locations for the ghrelin receptors, especially those within the hypothalamus, could make them excellent neurons used for the activation of AgRP neurons. These ghrelin receptor containing neurons must additionally have the ability to activate AgRP neurons through an AMPK-mediated calcium release. This implies a location for these cells that is relatively close to the AgRP neurons of the hypothalamic arcuate nucleus, if they are to be identified as responsible for the ghrelin induced feeding response. Different neural inputs have been determined to connect with the arcuate nucleus of the hypothalamus. It has been found that neurons within the forebrain and the brainstem (including the lateral parabrachial nucleus, ventrolateral medulla, etc.) have extensions that enter the hypothalamic arcuate nucleus (Li *et al*., 1999). It is possible that these connections allow for corresponding cells from within the brainstem to stimulate AgRP neurons to induce feeding in response to ghrelin. However, it seems likely that the unidentified cells have a closer proximity to the hypothalamus and the AgRP neurons within it. An understanding of which neurons contain ghrelin receptors, which also have connectivity to the hypothalamus is key to the identification of the neurons that mediate the ghrelin induced feeding response via a release onto AgRP neurons. Visual mapping of AgRP neurons within the hypothalamus

show the presence of astrocyte cells and POMC neurons in the area around AgRP neurons (Dietrich & Horvath, 2011). Perhaps, it is possible that one of these cell types is responsible for the ghrelin induced release (if they have ghrelin receptors and the ability to activate AgRP neurons). However, additional studies which provide a detailed view of the area around and the synaptic geography of the AgRP neurons could be useful in determining the precise identity of these pre-synaptic cells that stimulate AgRP and induce feeding. An interesting approach that has been used to map connections and inputs into the hypothalamus has involved the virus mapping method, which allows for retrograde tracing of inputs from specific neurons (DeFalco *et al*., 2001). Perhaps this approach could be used to determine the exact neurons that connect to the hypothalamus AgRP neurons. Other potential experiments could potentially involve the selective inhibition of certain neural cell types, in order to view the resulting effects on ghrelin sensitivity and feeding behavior. With the knowledge of the identity of these neuronal cells, therapies that reduce their ghrelin sensitivity or reduce their glutamate release could potentially be developed, which would reduce the strength of AgRP neuron activation and should reduce feeding behavior. Additionally, these types of studies could be utilized to understand the mechanisms and pathways used by the leptin and POMC neuron system to flip the feeding circuit to the opposite position (satiety).

Determining the use of salty, sour, and umami taste cell receptors within the gastrointestinal system for nutrient sensing could also have clinical importance. Since it is known that sweet and bitter taste receptors, which use G protein-coupled receptors (GPCR), such as GPR 119 (see Figure 1), are found both within the mouth/tongue and the gastrointestincal cells (Sternini *et al*., 2008), it seems possible that this oral to

gastrointestinal parallel extends to the use of salty, sour, and umami taste receptors as well. It would seem that there would be an advantage to the utilization of similar receptor types within the gastrointestinal tract compared to the oral cavity with respect to salty, sour, and umami, just as there is for sweet and bitter receptors. These receptor types are fast acting, as can be seen with their quick activation within the mouth, having near immediate recognition of tastes within the mouth. It seems logical that such taste receptors within the gut would then be advantageous for each type of taste as well. However, these connections do not prove that these types of receptors are utilized within the gut to sense salty, sour, and umami nutrient types, making experimental observations quite valuable.

It has been shown that taste chemosensing within the gastrointestinal tract is largely carried out by GCPRs (Geraedts *et* al., 2011). There is evidence that umami taste receptors also use G protein-coupled receptors (Behrens & Meyerhof, 2011). This could mean that it is very possible that umami may be sensed in the gut using mechanisms similar to those used by the mouth and tongue, similar to sweet and bitter tastes. It is important to note though that umami is a unique taste, which is produced by the glutamate (amino acid related), and so it utilizes multiple receptor types (Yasumatsu *et* al., 2009), which could make it unique compared to sweet and bitter tastes. However, the sensing mechanisms used by for sour and salty tastes do not utilize GPCRs (Roper, 2007). Interestingly, sour taste recognition is conducted largely through acidification and acid sensitive membrane proteins (Roper, 2007). Salty taste recognition has been connected to epithelial sodium channels, which is unique compared to sweet, bitter, and umami tastes (Roper, 2007). Given the different mechanisms used by the salty and sour

taste, compared to the GPCR mechanism of the other tastes, the parallel between oral and gastrointestinal sensing for these tastes may not be quite as strong. The gut and oral environments are quite different, and perhaps the mechanisms (more ion related) used to receive salty and sour signals within the oral system do not translate directly to the gastrointestinal system, as they more or less do for sweet, umami, and bitter tastes. Testing methods which knock out the genes encoding for the different oral receptor types, and then allow for the resulting gastrointestinal sensing of the different tastes to be observed could be useful. If tests show that the knockout of a certain receptor type reduces taste sensing in the oral system and the gastrointestinal system, then it would be suggested that similar mechanisms are used in sensing that corresponding taste at each level (oral and gastrointestinal).

Nutrient and taste sensing within the gastrointestinal system is important in coordinating the feeding behavior of mammals. The sensing of each nutrient and taste type within the stomach enables hormonal and neural signals to be sent to the brain in order to promote behaviors. It is important that food products are sensed properly so that satiety signals are produced at the appropriate time, in order to prevent overeating. Therapies which can manipulate nutrient sensing, perhaps via taste receptor mechanisms, could be of value in promoting proper feeding behavior.

Chapter 2- Hormones and Chemical Mechanisms of Communication

Current Knowledge About the Hormones and Chemical Mechanisms of Communication

A primary method utilized for communication purposes and cell signaling within the brain/gut axis is gut hormone and peptide release (See Figure 1). A variety of

peptides are released by the gastrointestinal tract, which contribute to physiological processes in different ways. These peptides have been found to regulate the body's feelings of hunger or satiety, and food nutrient processing. In response to the presence of nutrients and the distension of the stomach, gastrointestinal cells release these peptides which can signal the central nervous system (Sam *et al*. 2011). Of central importance are the gut hormones Peptide tyrosine tyrosine (PYY), Glucagon-like peptide-1 (GLP-1), and oxyntomodulin (OXM), which are important mediators of feeding behavior (Sam *et al*. 2011). Additionally, the hormones ghrelin and leptin play major roles in regulating feeding behavior and the body's physiological status as satisfied or hungry. These hormones are further discussed in Chapter 1 of this thesis, as they are the major hormones involved with the proposed flip-flop mechanism (Yang *et* al. 2011). The gut peptides PYY, GLP-1, and oxyntomodulin are key components of the body's regulatory system of feeding behavior, and so an understanding of these hormones and their mechanisms of action could have real clinical and therapeutic value.

Current Knowledge about PYY

Peptide tyrosine tyrosine (PYY) is a 36-amino acid peptide that exists in two forms, which vary slightly due to an enzymatic cleavage at the peptide's amino terminal location (Medeiros and Turner, 1994). PYY is located in enteroendocrine cells in the stomach and intestines, with high levels of PYY found in the colon and rectal areas (Adrian *et al*. 1995). PYY is released from gastrointestinal enteroendocrine cells into circulation in a proportional response to caloric intake (Adrian *et al*. 1985). The presence of PYY within circulation has been shown to have anorectic effects, causing reduced food intake and weight gain (Vrang *et al*. 2006). PYY is thought to reduce feeding

behavior via its action on the hypothalamic ARC's Y2 receptor. PYY has been found to bind to these Y2 pre-synaptic inhibitory receptors that are found on NPY neurons of the ARC, which prevents NPY activation and reduces feeding behavior (Batterham *et al*. 2002). Additionally, PPY seems to exert its effects on the hypothalamus through a vagal brainstem pathway, in which PYY is detected by Y2R receptors of the vagus nerve (Koda *et al*. 2005). Other anorectic effects from PYY may be due to its ability to delay gastric emptying, decrease secretions from the pancreas, and increase absorption of electrolytes and fluids by the ileum (Savage *et al*. 1987; Symersky *et al*. 2005). It is important to understand these mechanisms through which PYY exhibits its reducing effects on feeding behavior, to allow for obesity and feeding control pharmacological therapies.

Unanswered Question about PYY

The major issue faced by the scientific community regarding PYY as an anti-obesity therapy is developing an effective way to deliver the peptide to the body. The peptide must be delivered in a way that promotes its anorectic effects, while not inducing nausea or other side effects (Zac-Varghese *et al*. 2011). Subcutaneous delivery of PYY was tried, but provided no real effect on food intake (for unclear reasons). The administration of PYY utilizing a nasal spray approach is a possibility, but getting through the mucociliary barrier is difficult for some larger peptides (Zac-Varghese *et al*. 2011). Additionally, when delivered nasally, PYY was found to induce nausea and other abdominal side effects, perhaps due to rapid absorption (Zac-Varghese *et al*. 2011). Given that PYY works naturally within the gut through action on the gut vagal afferents, the use of orally delivered PYY has seemed appropriate. However, it is difficult to deliver the PYY orally in a way that is not disrupted by the acidic gastric environment or

by degradative enzymes from the pancreas. This means that PYY must be somehow modified or facilitated in order to allow intestinal absorption, while maintaining the integrity of the peptide and its desired effects (Zac-Varghese *et al*. 2011). It can be seen that PYY has potential as an anti-obesity treatment, but faces some hurdles that must be overcome regarding its method of therapeutic delivery. Therefore, a goal of this thesis research is to provide insight into how PYY can best be administered to induce its desired effects on feeding behavior.

Insight/Answers about PYY

Oral intake of PYY allows for increases in the blood concentrations of the PYY peptide, occurring through gastrointestinal absorption. The efficacy of absorption through the gastrointestinal tract and the fact that PYY works and is released naturally within the gut, likely make oral intake the most appropriate delivery method (as opposed to nasal, intravenous, or some other method) (Beglinger *et al*., 2008). It is through this absorption that PYY's feeding reduction effects occur. To be effective therapeutically, the PYY administered must be in an appropriate dose. Specifically, it seems important that the plasma PYY concentrations after oral intake would need to be higher than the PYY concentrations that occur naturally with a meal. Otherwise, the effects of therapeutic PYY would be comparable to PYY's natural effects, and feeding behavior regulation would show little real change. However, side effects of PYY administration (nausea, abdominal discomfort, etc.) are dose dependent, specifically in regards to the plasma concentrations of the drug. To avoid these side effects, a proper dosage that provides for reduced feeding behavior, while not exceeding the general range that induces side effects (plasma concentration of about >350 pg/ml) could be found. It

seems quite possible that an intermediate dosage value between the minimum needed to induce desired results and the maximum dosage value possible without producing unwanted side effects could be found. Additionally, perhaps there could be potential to administer the PYY in fractional dosages, rather than all at once, as peak concentrations within the blood were reached after about 15-35 minutes (Beglinger *et al*., 2008). Perhaps, by introducing PYY at different times, the plasma concentration levels could be kept within the acceptable range, while allowing for sustained controlled feeding behavior. As low blood PYY concentrations have been associated with obesity and increased eating, it is important to maintain adequate PYY in circulation (Karra *et* al., 2009). Another approach could possibly involve simultaneous administration of the PYY, along with other drug(s) (such as antiemetic drugs or others) meant to minimize the side effects of the PYY. These approaches could help combat the issue of side effects related to PYY intake.

The issue of degradation of PYY within the gastrointestinal tract also affects the clinical potential of PYY to reduce feeding behavior and help treat obesity. Orally administered PYY is degraded largely by proteolytic enzymes within the stomach. One approach to solving this degradation problem could be additional administration of substances that effectively prevent the formation or release of these digestive enzymes within the stomach and intestines. However, shutting off an entire class of enzymes simply to administer PYY would be much too intrusive and extreme, with great potential for unintended consequences. It, therefore, seems that the best approach would be to manipulate the PYY delivery mechanism itself, rather than the body's natural response to the PYY. By coating the PYY with an enteric coating, its full release could be delayed

until the intestine is reached, where proteolytic activity is milder. The main issue with this approach is the delay in the absorption of the PYY, which could make its clinical effects less meaningful. To solve this issue, it seems possible that the PYY could be delivered with the enteric coat earlier than it would otherwise be delivered. By timing the delivery of the PYY and allowing early oral intake of enteric coated PYY (perhaps at some point prior to feeding), the peptides' clinical effects could be achieved at a suitable an appropriate time. The other PYY delivery approach utilizes an agent-based method, in which peptides are noncovalently interacted with small organic molecules that can act as carriers (Beglinger *et al*., 2008). By utilizing specific organic molecules that can most effectively interact with the PYY peptide, allow absorption into the bloodstream, and then dissociate from the peptide, PYY could be delivered effectively. To do so, organic molecules that noncovalently bond to the PYY and increases PYY lipophilicity could be utilized. An evaluation of the organic molecules that are most suitable delivery agents for specific peptides, such as PYY, would therefore be quite useful. By understanding these pharmacological principles related to PYY, the peptide can be delivered effectively through the oral pathway.

The additional degradation of PYY within the blood stream, largely by metalloendopeptidases, gives PYY a short half-life (Addison *et al*., 2011). Degradation makes it more difficult to achieve sustained results from PYY. This could perhaps be combatted with multiple deliveries of PYY. Additionally, inhibitors of these degradative metalloendopeptidases could be further studied. By further testing the effects of these inhibitors, PYY's half-life and therapeutic sustainability could be enhanced.

The salivary region (tongue, etc.) has been found to contain PYY hormone and receptors, which can promote decreased feeding behavior (Acosta, *et al*., 2011). By utilizing this approach to delivery, perhaps in combination with the oral approach, PYY can be delivered effectively. This salivary approach would avoid the issue of degradation within the gastrointestinal tract by enzymes. By combining salivary delivery with an oral delivery (such as enteric coated PYY), a sustainable reduction in feeding could be produced. This combination approach has potential, and evaluation of possible combinations between the two delivery methods could be of value.

Current Knowledge about GLP-1

Glucagon-like peptide-1 (GLP-1), a 30 amino acid peptide released from gastrointestinal cells, is also an important gut hormone involved with the regulation of feeding behavior. GLP-1 is released into circulation in response to glucose consumption, and acts as both an incretin (MacDonald *et al*. 2002) and as an inhibitor of glucagon (Willms *et al*. 1996), allowing it to reduce blood glucose levels after a meal. GLP-1 also acts to delay gastric emptying and induce satiety effects (MacDonald *et al*. 2002). GLP-1, similar to PYY, acts on the hypothalamic ARC, PVN, and supraoptic nucleus (Shughrue *et al*. 1996), allowing it to reduce food intake significantly. These effects seem to be fairly dependent on the dosage level, and reach the hypothalamus through vagal and brainstem pathways (Imeryuz *et al*. 1997). GLP-1 is degraded in circulation by the DPP-IV enzyme, and so native GLP-1 has not been suitable for clinical use. Mimetics of native GLP-1 have been developed, including exenatide (a synthetic version of exendin-4), which have been shown to last longer in circulation and have weight loss effects (in addition to reduction in blood glucose effects). However, while GLP-1

variants may provide clinical value in the treatment of obesity, they have been associated with nausea and abdominal side effects (Astrup *et al*. 2011). It is therefore seen that GLP-1 has potential clinical value for obesity, but there are issues that must be resolved.

Unanswered Question about GLP-1

In order for GLP-1 to be an effective obesity treatment, it must be delivered in a way that allows it to be long acting (not degraded), and not nausea inducing. To deal with the issue of GLP-1 degradation in circulation, longer lasting analogues have been developed. However, some of these analogues (such as liraglutide) have been shown to have nausea side effects, in addition to their weight loss effects (De Silva & Bloom, 2012). Interestingly, nausea seemed to decline as the duration of the therapy was increased. The other solution to the issue of degradation was to inhibit the enzyme DPP-IV, which caused GLP-1 degradation. However, this method allowed for little overall effects on weight loss, which was likely due to DPP-IV's role in activating other anorectic hormones (De Silva & Bloom, 2012). Therefore, it seems that some form of GLP-1, administered with an effective and careful dosage, provides a valuable potential for obesity treatment. Therefore, a goal of this thesis is to provide insight into how GLP-1 can be delivered in a way that provides effective results (longer lasting), with minimal side effects.

Insight/Answers about GLP-1

The issue of rapid GLP-1 degradation and inactivation can be achieved through the use of inhibitors against the degradation enzymes or through the usage of resistant GLP-1 incretin analogues. As GLP-1 is largely degraded by dipeptidyl peptidase IV (DPP IV), inhibitors of this peptidase seem like a good target to enhance GLP-1's activity.

However, this brings the potential issue of enhanced activation for additional peptides such as NPY and others, which are also degraded by DPP IV (Mentlein, 2009). To combat this effect, it is possible to additionally administer specific inhibitors for these other peptides, which could prevent the unwanted effects associated with some of these other peptides. Therefore, further study about what types of molecules can be used to specifically inhibit each of the gastrointestinal peptides could be conducted. By determining specific inhibitors which can bind to only specific peptides with unwanted side effects, DPP IV inhibitors can be used to maximize the therapeutic effects of GLP-1.

The use of GLP-1 receptor agonists, such as liraglutide and exentatide has been shown to promote weight reduction (Spellman, 2012). However, the usage of such agonists was associated with nausea, vomiting, and diarrhea (Spellman, 2012). Interestingly, the side effects of the injected drugs seemed to be fairly transient, with about 10% or less percentages of patients who exhibited nausea after 8-10 weeks of treatment with the GLP-1 analogues (Madsbad, 2009). This could allow for the dosage level of the drugs could be adjusted over time, with milder dosages to begin the treatment. This could possibly allow patients to begin the use of the drugs in low dosages to avoid side effects and increase the dosage over time as the body becomes more acclimated. It may be possible to administer drugs that combat such side effects along with the administration of the liraglutide and exenatide. However, careful consideration is needed to ensure that such combination remain effective in promoting weight loss, do not develop toxicities when combined, and are suitable for the individual patient. Additionally, it has been found that GLP-1 receptors are found throughout the body, within the periphery and central nervous system (Hayes *et al*., 2010). Therefore, it may

be possible to direct GLP-1 at specific receptors in order to avoid possible side effects. Perhaps it is only certain GLP-1 receptors, which due to their location or other characterstics, are susceptible to the induction of adverse side effects. A useful experiment could try to target specific GLP-1 receptors of distinct locations and type, and evaluate the resulting effects of food intake and adverse side effects. If there are different levels of feeding reduction and/or side effects associated with each GLP-1 receptor group, then this strategy of specific GLP-1 receptor targeting could have real importance and value. In addition to beneficial effects on body weight, the GLP-1 agonists, such as exenatide, also can produce beneficial effects on the cardiometabolic system of obese individuals (Kelly *et al*., 2012). It seems therefore that in addition to weight loss, the GLP-1 agonists have additional benefits to the risks associated with obesity. Therefore, strategies such as these described, which can reduce the side effects of GLP-1, while maintaining efficacy, should be of real interest to the health community.

Current Knowledge about OXM

Oxyntomodulin (OXM) is a 37 amino acid peptide released by enteroendocrine cells in proportional response to caloric consumption, which is known to delay gastric emptying, reduce gastric acid secretion, and decrease food intake (Sam *et al*. 2011). OXM has been found to coordinate its anorectic effects through hypothalamic pathways, specifically through the GLP-1 receptor (Baggio *et al*. 2004). Its anorectic effects using this receptor are similarly powerful to GLP-1, despite its lower affinity for the GLP-1 receptor (Dakin *et al*. 2001), and its usage of different hypothalamic pathways. Given its

strong anorectic effects, OXM has potential for usage as a therapeutic agent to treat obesity within the population.

Unanswered Question about OXM

While oxyntomodulin's anorectic effects have been shown, its exact mechanisms of action are uncertain. Its use of the GLP-1 receptor is suggested, but it seems likely that its action may involve a distinct population of receptors or a subpopulation of the GLP-1 receptor (Sam *et al*. 2011). This is because the presence of a GLP-1 antagonist within the ARC, blocked OXM's anorectic effects, but not those of GLP-1 (Dakin *et al*. 2004). In order to realize OXM's value as an anti-obesity treatment, its mechanism of action must be determined and understood. Therefore, providing insight into OXM's mechanism of action is a goal of this thesis research.

Insight/Answers about OXM

The mechanism of action of OXM likely utilizes some distinct class of GLP-1 type receptors or some OXM specific receptor class. Interestingly, OXM has been found to have cardiovascular effects (increased heart rate), which occur without the use of GLP-1 receptors (Sowden *et al*., 2007). This was suggested by the fact that mice lacking the GLP-1 receptors exhibited heart rate increases in response to OXM just as strongly as mice with GLP-1 receptors. Additionally, it has been shown through magnetic resonance imaging tests, that OXM and GLP-1 have distinct hypothalamic activation patterns (Chaudhri *et* al., 2006). Interestingly, both OXM and GLP-1 were found to both increase neuronal activity within the brainstem (Parkinson *et* al. 2009). Perhaps, this could mean that a common GLP-1 type receptor is present within the brain stem, while independent receptors are used to recognize the different peptides within the hypothalamus. Given

that OXM contains the whole glucagon structural sequence, it seems likely that some type of glucagon receptor is utilized by OXM (Sowden *et al*., 2007). With these results related to the cardiovascular effects of OXM, it seems quite possible that similar principles are found for OXM's feeding behavior effects. Interestingly, it has been found that the structural manipulation of OXM could produce analogues with enhanced binding to GLP-1 receptors, larger decreases in feeding, and reduced enzyme degradation (Druce *et al*., 2009). Additionally, other manipulations were found to decrease receptor affinity with different effects on OXM's anorectic capabilities. Interestingly, it has been shown that OXM can exert its gastrointestinal effects even with its structure minimized to its C-terminal octapeptide fragment (Carles-Bonnet *et al*., 1996). This could suggest that in addition to GLP-1 receptors which bind OXM, other receptors with affinity for the OXM C-terminal fragment may be involved with promoting its anorectic effects. Further study could involve determining the exact types of receptors that have such affinity, which could promote a gastrointestinal response. By determining the presence of other such receptors, particularly within or near the brain/gut communication axis, OXM's mechanism of action could be better understood. Given the OXM induced reduction in activity within the arcuate, supraoptic, and paraventricular nuclei of the hypothalamus, it seems likely that various forms of OXM receptors may exist within the hypothalamus (Chaudhri *et* al., 2006). The development of an OXM analogue that can be recognized by the full extent of potential OXM receptors could have the ability to promote increased, sustained, and more direct anorectic effects.

Chapter 3- Gut Microbiota and Feeding Behavior

Current Knowledge About Gut Microbiota and Feeding Behavior

Within the gastrointestinal tract of humans live large populations of microbes, which have important roles in the processing and digestion of food products. The two major groups of bacteria within the human gut are the *Bacteroidetes* and *Firmicutes*, and their relative proportions have major effects on a person's body weight and composition (Ley *et al*. 2006). More specifically, it has been found that lean individuals have a higher relative proportion of *Bacteroidetes* than obese people. Additionally, with weight loss, and low calorie consumption, this proportion of *Bacteroidetes* increases (Ley *et al*. 2006). This evidence shows a very important role for gut microbiota in the regulation of body weight, and suggests its potential role in the regulation of feeding behavior. It can, therefore, be seen that manipulation of gut microbiota may have clinical importance as an anti-obesity treatment, through regulation of feeding behavior or energy metabolism.

It has been shown that the microbiota within the gut has profound implications for individuals' susceptibility towards obesity and individuals' food energy processing. Additionally, it has been shown that microbiota have profound effects on the central nervous system, with particular behavior implications (Grenham *et al*. 2011). Figure 3 depicts the gut/brain network with respect to the gut microbiota. This system is connected via neural and immunity system based connections. Specifically, microbiota within the gut have been associated with neurochemical changes within the brain, and have been shown to correlate with anxiety-like behavior (Neufeld *et al*. 2011). This evidence suggests a role for microbiota in regulating behavior.

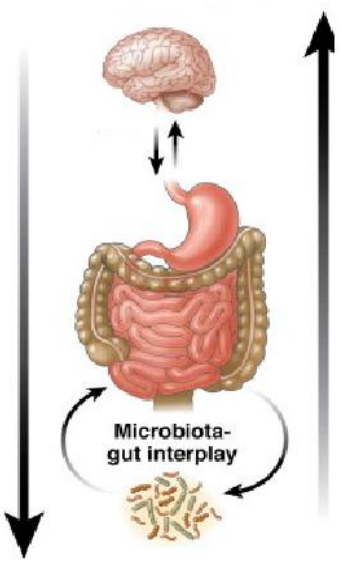

Figure 3. Microbiota and the brain/gut network. There is communication between the brain and gut, which allows the gut microbiota to have behavioral implications. These connections are mediated through neural and immune system mechanisms.
Adapted from Collins & Bercik (2009)

Unanswered Question About Gut Microbiota and Feeding Behavior

The influence of gut microbiota specifically on feeding behavior is relatively uncertain. There is evidence that gut microbiota may be able to communicate with the brain through vagal neurons, and evidence suggests gut microbiota is able to affect brain development (Tehrani *et al*. 2012). Given this evidence and these roles for gut microbiota, it seems possible that the gut microbiota may have a role in regulating feeding behavior. This could enhance the manipulation of gut microbiota as a potential anti-obesity treatment. Therefore, providing insight into whether the effects of the gut microbiota on the central nervous system and behavior extend to feeding behavior is a goal of this thesis. By evaluating the gut microbiota within the context of the central nervous system and the brain/gut communication axis, insight into its role in feeding behavior regulation can be gained.

Insight/Answers About Gut Microbiota and Feeding Behavior

There is strong evidence that the microbiota within a mammal's gastrointestinal tract has real influences on behavior. Studies have shown that variations within the microbiota, such as with the introduction of new bacterial types, are able to exert influences on behavior (such as with the induction of anxiety like behavior) (Collins & Bercik, 2009). Additionally, variable behavioral responses and stress responses were shown in comparisons of germ free mice and mice with microbiota (Collins & Bercik, 2009). Given that the microbiota is located within the gastrointestinal tract, and the control of behavioral function is conducted by the brain, some type of communication must be occurring between the two in order to allow microbiota to affect feeding behavior (See Figure 3). In particular, it can be proposed that through some mechanism, microbiota likely has influence on the neurons within hypothalamus, where feeding behavior is largely controlled. It seems quite likely, that the primary mechanisms of this communication are through neural and immune-system mediated mechanisms. Neuronal mechanisms are the major communication source of the nervous system, making their role within this microbiota-brain network quite likely. Given the immune system's importance in regulating non-self cell types (such as microbiota) and the importance of the immune system within the gut, an immune mediated mechanism is also quite likely to play a role in allowing microbiota to control feeding behavior.

The major neural pathway connecting the microbiota and the central nervous system is likely the vagus nerve. It has been shown that microbiota induced behavior changes were not exhibited by mice that had been vagotomized (Bravo *et al*. 2011). Through neural branches, information from the vagus nerve is able to enter the brainstem,

and in turn reach the hypothalamus. It seems likely that vagal signaling pathways are able to communicate the presence and activity of the different microbiota population. Interestingly, the vagus nerve has also been found to play a role in transmitting immune function information from the gastrointestinal tract to the brain (Wang *et* al., 2002). As the microbiota is sensitive to the presence of food molecules within the gastrointestinal tract, feeding behavior regulation is a likely extension of this vagal mediated microbiota-brain communication network.

An immune system mediated connection between the gut and the brain is likely a key player in the regulation of feeding behavior. The gastrointestinal tract contains a great deal of very active and important gut-associated lymphoid tissue (GALT), which is responsible for regulating the contents of the gut, monitoring non-self molecules, and inducing an appropriate immune response. This GALT system is meant to process antigen signals, in order to distinguish the different molecules as requiring or not requiring an immune response. Interestingly, it has been shown that some microbiota molecules mimic important neuropeptides (Fetissov & Dechelotte, 2011). The immune system, including the GALT, utilizes cytokine (protein signal molecules), to send messages and promote immune responses. It has been shown that cytokines from the immune system, such as interleukin-1, are able to influence the neuroendocrine functions of the hypothalamic-pituitary-adrenal axis (Turnbull & Rivier, 1999). This means that there is bi-directional communication between the immune system and the nervous system, which allows each to have influences on the activities of the other. Perhaps it is possible that the presence or activity of certain microbiota could induce an immune response that affects neuropeptides that regulate feeding behavior, such as neuropeptide

Y and Agouti-related peptide (AgRP). An immune response that stimulates neuropeptide Y or AgRP release, or inhibits satiety neuropeptides, could in turn stimulate feeding. This makes possible the idea that the lymphoid tissue of the gut is able to exert influences on feeding behavior in response to gut microbiota.

A key factor that allows an immune mediated mechanism of feeding behavior regulation via the microbiota-brain communication is the potential for mimicry between microbiota molecules and neurons and neuropeptides (Fetissov & Dechelotte, 2011). This means that the immune system can recognize certain microbiota as foreign and potentially develop an immune response to such molecules. However, with mimicry, the immune system can become primed to respond to molecules of neurons or neuropeptides (due to their similarity to microbiota proteins). This means that the immune system could greatly change the activity and presence of key neuropeptides and neurons within the nervous system. It has been found that auto-antibodies have been able to bind selectively to alpha-melanocyte stimulating hormone (alpha-MSH) neurons (Fetissov *et al*., 2002). This was found in patients with anorexia nervosa and/or bulimia nervosa patients (who exhibit minimized eating/ thin body composition). Therefore, it seems possible that such an auto-immune response to these neurons may have been correlated or a cause of the reduced eating behavior and eating disorders. By connecting these facts, it seems possible that gut microbiota could prime the immune system to carry out an auto-immune response against neurons or neuropeptides that could result in excessive feeding behavior. Perhaps excessive feeding behavior could result from an auto-immune response against neurons that produce neuropeptide Y, AgRP, or other appetite stimulatory neuropeptides. This excessive eating would occur if the immune response against such cells had a

stimulatory effect. On the other hand, perhaps an immune response could damage and/or inhibit the activity of appetite controlling neurons and neuropeptides, such as POMC neurons. The inhibition of these neurons could also result in increased eating. Through these mechanisms, the immune response from the GALT, resulting from priming by the gut microbiota, could affect the neurons that regulate feeding behavior and in turn influence eating. Further evaluation into what types of immune responses (cytokine, chemokine, antibodies, etc.) can target hypothalamic neurons could be useful. It is important to recognize which gut microbiota molecules are potentially able to promote auto-immune responses against these neurons. This understanding could allow for medical therapies that manipulate such immune responses, in order to promote proper eating and body weight regulation within humans.

Chapter 4- Feeding Behavior Regulation Outside of the Main Hypothalamus/Gut Network

Current Knowledge About Sensory Factors in Feeding Behavior Regulation

While communication along the main network between the hypothalamus and the gut plays a central role in the regulation of feeding behavior, there are factors outside of this axis that are also important. The gut/hypothalamic axis works within a physiological context with many different interacting processes and pathways. Therefore, one must recognize the presence of factors outside of this gut/hypothalamic axis in the system of feeding behavior regulation. These important factors include sensory cues (visual, olfactory, taste) and the circadian clock. These factors affect behavior in relation to food

and affect hormone metabolism, respectively, making them quite relevant to the study of feeding behavior regulation.

Mammals make decisions regarding feeding within a context of environmental cues that are sensed through taste, olfaction, and visual mechanisms. The brain has sensory mechanisms that recognize taste, visual, and olfactory signals, independently from the individual's hunger or satiety status (Rolls, 2005). However, the behavioral response to these sensory cues is largely modulated by the hunger/satiety status of the individual (Rolls, 2007). Thus, there is an interactive relationship between sensory cues and hunger status, relating to the regulation of eating.

The sensory mechanisms related to feeding are primarily located within the brain's cerebral cortex, and their signals help determine an individual's response to food and corresponding behavior. Within the primary taste cortex, primates have neurons that recognize the different tastes (sweet, salty, bitter, sour, umami), and also factors such as food temperature, viscosity, etc. (Rolls, 2007). Additionally, primates have an additional secondary cortex that responds to more specific tastes (Rolls, 2007). It is important to note that the primary taste cortex mechanism does not control appetite or the reward value of food, but only recognizes the presence of these tastes. The reward value provided to the different taste sensations is greatly affected by hunger, with hunger promoting the reward value and pleasantness of the food (Rolls, 2007). With increased consumption of food, the reward value and pleasure associated with such food and its corresponding tastes begin to decrease. This occurrence seems to be quite food specific, as consumption to satiety of one food decreases the neuron response towards that food, with less of a neuron response decrease in other food/taste types (Rolls, 2007). It is

believed that the decrease in pleasure provided by a food stimulus is regulated by decreased neuronal activity within the caudolateral orbitofrontal cortex taste area and the lateral hypothalamus, rather than a decrease in neuronal activity within the frontal opercular cortices, insular taste cortices, or the solitary tract nucleus (Rolls, 2007). This shows the separation between sensory and reward systems in response to food and taste cues. From these sensory signals and other satiety signals however, the orbitofrontal cortex receives signals about the value of food, which can allow for a corresponding decision about feeding behavior (Rolls, 2007). Interestingly, reduced orbitofrontal cortex volume, has been associated with disinhibited eating and obesity within individuals (Maayan *et al*. 2011). It is seen that the orbitofrontal cortex has a major role as a center of convergence between olfactory, taste, and visual sensory cues (and satiety information), and thus is of great importance to the treatment of obesity and excessive food consumption.

Olfactory sensory inputs seem to have a strong relation to taste inputs, with both inputs having a combined effect on taste/flavor of foods. Olfactory neurons are located within the orbitofrontal cortex area of the brain and other regions, and help recognize odor signals, while affecting taste signals (Rolls, 2007). Olfaction has been linked to an organism's hunger status, with specific neurons having decreased activity following consumption of specific corresponding foods. It has been found that individuals receive less olfactory pleasure from foods that have been eaten to satiety, compared to other foods and compared to the same food prior to consumption (Rolls, 1997). An implication of this finding is that the presence of a variety of foods and flavors can promote increased feeding behavior, and thus obesity. Therefore, there is a connection between the

olfactory response to food and an individual's opinion of a food, which thus affects eating behavior decisions (Rolls, 2007). In a study by Jansen *et* al. (2003), it was shown that overweight children responded to the odors of pleasant food by overeating, compared to lean children. This shows the importance of sensory cues in promoting or decreasing eating behavior. With the specific olfactory sensitivity adjustment made in response to specific foods, it can be seen that foods' olfactory sensory cues can strongly influence eating behavior.

Visual sensory cues about food are processed by visual neurons within the orbitofrontal cortex and other cortex areas of the brain. These neurons additionally show olfactory and taste sensing capabilities, with visual sensing being most active in response to food in the presence of hunger (Critchley & Rolls, 1996). With these functions, visual sensory neurons play a role in the selection of food and flavor, and visual cues can influence the perceived flavor of a food. This is due to the convergence of sensory information that occurs largely within the orbitofrontal cortex of the brain, which allows interaction between sensory neuron signals. For example, with the visual presence of cheese, the corresponding flavor and odor of cheese would generally be perceived as pleasant. However, without the visual stimulus from cheese, the same odor or flavor could likely be considered unpleasant (Rolls, 2007). Therefore, visual stimuli and the visual sensory neuron system have an important role in the control of feeding behavior and have potential clinical value in the treatment of obesity in society.

Unanswered Question About Sensory Factors in Feeding Behavior Regulation

Sensory mechanisms that control feeding behavior could have great clinical value in the treatment of obesity. Over time, sensory responses to food have increased (at a

society level), while satiety signals in response to eating have been unchanged, which has led to increased pleasure received from food, increased overeating, and increased obesity (Rolls, 2007). The importance of the brain's orbitofrontal cortex as a center of information convergence between sensory cues and satiety signals is recognized. However, the mechanisms through which the orbitofrontal cortex processes satiety and sensory cues in order to induce a behavior response towards food are uncertain. A method of ensuring that satiety signals are not overridden by sensory cues (within the orbitofrontal cortex) would have great clinical value in the treatment of obesity. Therefore, it is a goal of this thesis to provide insight into how to ensure that satiety signals are not overridden by sensory or reward cues within the orbitofrontal cortex, to allow effective control of feeding behavior.

Insight/Answers About Sensory Factors in Feeding Behavior Regulation

A major determinant of feeding behavior is the relative strength of satiety signals compared to the reward signals in response to food. It is, therefore, important that satiety signals can overcome the influences of food reward cues, in order to avoid excessive eating and obesity. It has been shown that obese individuals are similar to substance dependent individuals in that their reward response towards food cues is elevated compared to lean individuals (Gearhardt *et al*., 2011). This means that obese individuals' show greater activation within the orbitofrontal cortex, amygdala, etc. when presented with palatable food cues. Interestingly, while these individuals have increased brain reward activity in response to anticipatory food cues, this reward activity and the associated dopaminergic release was decreased and lower compared to lean individuals during the actual process of eating (Gearhardt *et al*., 2011; Stice *et al*., 2008). For these

reasons, similar to a drug dependent individual, an obese individual has an increased desire to eat, a reduced reward while eating, and in turn a problem with overeating to compensate for such effects. Therefore, it seems that one approach to obesity treatment could parallel the types of methods used to treat drug dependent individuals.

It is important to recognize that sensory mechanisms from the environment (such as food smell, food taste, etc.) are largely processed within the orbitofrontal cortex, as are satiety signals (Rolls, 2011). Individually, satiety/hunger is processed in different neural regions compared to olfactory, taste, and other external sensory cues. The orbitofrontal cortex and the amygdala were the only areas of convergence between the different signals (Hinton *et al*., 2004). This makes the orbitofrontal cortex very important in the decision making process for feeding behavior. In order to allow for proper control of eating, it would seem that the orbitofrontal cortex would need to receive satiety signals that are stronger than the reward signals from food. This would allow the orbitofrontal cortex to promote control of eating, resulting in decreased feeding behavior. These principles allow for the potential for treatments that could help individuals control feeding behavior. It seems that a drug that inhibits the sensory receptors within the orbitofrontal cortex that respond to food reward cues (good smells, good tastes, etc.) could promote reduced eating. Alternatively, a drug that could potentially enhance the sensitivity of satiety signals within the orbitofrontal cortex could also help regulate feeding behavior. Information within the orbitofrontal cortex is flows through multiple pathways (Barbas, 2007), making multiple methods of manipulation of these signals seem possible. While different precise mechanisms could be used, the key factor of this approach would be to

ensure that the orbitofrontal cortex is more strongly activated and influenced by satiety signals than by reward signals.

Another more simple approach to allow individuals to better control eating (based on sensory cue processing) could be to reduce the variety of food given to individuals. It has been shown that sensory neuron responses to food cues (taste, sight, etc.) are food specific (Rolls *et al*., 1986). This means that if an individual reaches satiety with the consumption of one food, then he/she will show reduced hypothalamic neural actitivity in response to that food's sensory cues. However, hypothalamic neural activity is practically unchanged in response to sensory cues from other food types which were not eaten to satiety (Rolls *et al*., 1986). This means that an individual will have continuous sensory motivations to eat when presented with a great variety of food types. On the other hand, when presented with only one food type, the individual can reach satiety, have a reduced hypothalamic sensory response to the food, and in turn stop eating appropriately. This could have potential clinical relevance, as individuals susceptible to obesity could better control their eating if they are presented with a smaller variety of food to potentially eat. The study of sensory cues in the control of feeding behavior could allow for more invasive drug therapies for obesity, or more simple techniques such as reductions in the variety of foods served, which are both important.

Current Knowledge About Feeding Entrained Circadian Clocks

In addition to sensory neuron mechanisms, the circadian clock, also has important effects on the regulation of feeding behavior. While a circadian clock that responds to light entrainment conditions is located within the suprachiasmatic nucleus (SCN), the exact location and identity of the cells involved with a feeding centered circadian clock

are largely uncertain. However, these different types of circadian clocks are interrelated and affect one another. It is thought that the rhythms of feeding behavior are regulated largely by circadian oscillators within hypothalamic, corticolimbic, brainstem, and other cells (even cells outside of the brain). It has been shown that the genetic circadian oscillators become entrained by feeding time (Mistlberger, 2011). This means that cells within organisms shift their circadian rhythm in coordination with feeding. For example, when rats were fed only in light periods (different from their typical behavior pattern), the circadian rhythm of neural, gastrointestinal, and other cells was shifted to correspond with the feeding time. A shift in the circadian rhythm such as this corresponded with behavioral changes and modified food appetitive activity (seeking food, etc.) (Mistlberger, 2011). Thus, perhaps an individual's predisposition to eat or not to eat may be largely influenced by the function of the circadian clock. It is known that circadian clock and metabolism are bi-directionally coupled, which means that each influences the other and allowing them to correspond with each other (Mistlberger, 2011). Thus, hormonal and physiological functions vary throughout a time period based on a circadian rhythm. This means that feeding behavior and its effects on body weight would differ depending on the status of the circadian clock. Therefore, clinical therapies that utilize knowledge of the circadian biological feeding time clock could provide value in the treatment of obesity.

Unanswered Question About Feeding Entrained Circadian Clocks

Through a strong understanding of how feeding behavior coordinates the circadian clock, and the corresponding physiological responses to the circadian clock, valuable clinical treatments for obesity can be developed. However, the field has not determined

the real identity of the cells involved with the food-entrained circadian clock. There is uncertainty about the extent of which cells, (neural or other), are completely responsible for this circadian clock and its physiological implications. The distribution or centralization of circadian oscillator cells and their location is uncertain. Gaining understanding to this question provides real clinical and academic value. Therefore, it is a goal of this thesis to provide insight into which cells are responsible for coordinating feeding behavior regulated by the circadian clock.

Insight/Answers About Feeding Entrained Circadian Clocks

It has been shown that feeding has profound effects on the clock genes of mammals, which can induce oscillations that affect hormone and metabolic activity (Escobar *et al*., 2009). Evidence suggests that the food entrained oscillator system is a product of oscillating cell types within both the central nervous system and the periphery. It appears likely that this food entrainable circadian clock is composed of a distributed network of oscillators, rather than one centralized oscillator complex. There are numerous types of clock genes, which seem to have different levels of influence on the food-entrainable clock system (Feillet *et al*. 2006). This study by showed that mutations within some clock genes, such as the *Per2* gene found in the SCN, decreased the expression of food anticipatory activities, while other mutation types had little influence on food anticipatory activities. These food anticipatory activities include body temperature increase, activity, and hormone releases prior to the predictable consumption time (Verwey & Amir, 2009). One can recognize the importance of specific oscillator types within the food-entrainable clock, rather than a generalized importance of all circadian oscillators for the control of feeding behavior.

It is likely that the food entrainable clock oscillators are distributed throughout the body. This is evidenced by the fact that lesions in different brain nuclei regions affected food anticipatory activity, but did not eliminate such activity completely (Verwey & Amir, 2009). Additionally, it has been found that circadian clock genes are located within the abdominal region organs (Davidson *et* al., 2003). It seems possible that such oscillators could be distributed within the gastrointestinal system, as this is the system that coordinates feeding and processes food products. Interestingly, a study by Davidson *et al*., 2003) suggested that the *Per1* clock gene within the gastrointestinal tract was not likely responsible for coordinating the food entrainable clock. However, this gene codes for only one type of circadian clock, and so it is possible that other oscillators within the gastrointestinal help coordinate the food entrainable clock. Evidence such as this suggests that the coordinated study of oscillators within the periphery as well as in the central nervous system is the best approach to understanding the food entrainable circadian clock system. Importantly, the food entrainable circadian clock is likely coordinated independently of the SCN, as feeding and behavioral rhythms can be maintained even without the function of the SCN oscillator system (Mistlberger & Antle, 2011). With many different potential oscillator types, encoded by different genes, it is difficult to determine which oscillators are most responsible for circadian rythms of feeding behavior. Experimentation in which different combinations of oscillators are inhibited or oscillator genes are knocked out, and the resulting effects on feeding are observed, could be useful to identify the most important oscillators responsible for the food entrainable clock within mammals. Perhaps special focus could be placed on oscillators which have neural or hormonal connections to the hypothalamus or

gastrointestinal tract, as these are the primary sites of feeding behavior regulation. Just as there are medications that affect sleep, which is coordinated largely by the SCN's circadian system, it seems possible that feeding and the food entrained circadian system can be also be manipulated medically. These types of experiments could help determine the role of unique oscillator cells that are distributed throughout the body, and could allow for clinical treatments that manipulate the food entrained circadian system to produce desired health outcomes, such as healthy feeding and body weight.

Conclusion

With the major issue of obesity within human society, it is quite important that the mechanisms that control feeding behavior are well understood. The proper regulation of feeding behavior is absolutely necessary in order for mammals to achieve a healthy body weight. Feeding must be carried out in a controlled fashion to achieve a sufficient intake of energy for metabolic processes, while avoiding excessive intake that could promote obesity. By understanding the interacting mechanisms that allow for the regulation of feeding behavior, valuable health and clinical treatments can be developed that promote proper eating and reduce obesity within society.

The major components of the feeding behavior regulatory system, which include the central nervous system and gastrointestinal system, along with hormones, microbiota, and nutrient receptors, are coordinated in a sophisticated and interacting system. There is a large amount of knowledge that has been gained about this system, but many questions remain unanswered. This thesis has discussed the feeding behavior regulatory system and evaluated many of its unanswered questions. Through research and analysis of the

known literature about the feeding behavioral regulatory system, and the brain/gut network in general, good insights have been gained. This thesis has provided insight into the neural mechanisms that regulate eating, and can provide evaluations that promote further evaluation. Using the known information about the feeding behavior regulatory system, along with the information of this thesis, it may be possible to further the pursuit of improved obesity treatments. It is the goal of this thesis to evaluate the known information and provide new and profound answers and insight. The issue of obesity and the role of the feeding behavior regulatory system in human health are very important for society. It was this importance that was expressed by this thesis project. The study of the mechanisms of feeding behavior regulation is a field of great importance for society, with amazing clinical and academic potential. An integrated approach (as used in this thesis), which evaluates the entire interacting regulatory system within the context of the whole human or mammal, is the most realistic and fitting for clinical application. For these reasons, this thesis was carried out, to help promote the progress of the scientific community and the well-being of society.

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