University of Nevada, Reno

Conserved Molecular Mechanisms that Modulate Olfactory Information Processing and their relationship to Human Diseases

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

Bachelor of Science in Neuroscience and the Honors Program

by

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UNIVERSITY
OF NEVADA
RENO

THE HONORS PROGRAM

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Entitled

Conserved Molecular Mechanisms that Modulate Olfactory Information Processing and their relationship to Human Diseases

be accepted in partial fulfillment of the
requirements for the degree of

BACHELOR OF SCIENCE, NEUROSCIENCE

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May, 2018

Abstract

Integration of olfactory system information aids in understanding how olfaction can be studied for humans through model organisms. *Drosophila melanogaster* is useful in representing the basic circuitry of the olfactory system in studying foraging behavior, comparable to humans, through molecules such as insulin, Neuropeptide F, and short Neuropeptide F. The main question was what model organism most appropriately represents similar olfactory modulation to humans in studying diabetes and obesity. Rodents were found to be most representative of human disease states. The molecular importance of leptin and high-sugar diets for diabetes and of leptin and Neuropeptide Y for obesity presents rodents as effective model organisms to studying diabetes and obesity. Further studies will focus on performing rodent research with nasal insulin as a treatment for type 2 diabetes.

Acknowledgements

This literature-based thesis was supported by Undergraduate Research at the University of Nevada, Reno. The research was funded through the Honors Undergraduate Research Award. I am grateful to Dr. Dennis Mathew (Department of Biology, UNR) for providing valuable assistance towards the development of this thesis; I am also thankful to Honors thesis professor and director, Dr. Tamara Valentine (Honors Program, UNR).

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Chapter One: Introduction

A. Influence of Food on Organisms

In order to locate food, many animals predominantly rely on their olfactory neurons for survival. Olfactory neurons are signal transduction cells within the olfactory circuit that receive environmental cues, process and translate the signals into a behavioral response. This information processing is often modulated by the animal's internal state, such as hunger or stress (LeDue et al., 2016). While a considerable amount of research has been performed on sensory neuron responses to food odorants in various animal systems (Sasaki, Matsui, & Kitamura, 2016) and modulation of these responses by hunger (LeDue et al., 2016), less information is known about the molecular pathways that underlie such modulation. In today's Western societies, humans' eating habits vary widely compared to animals in the wild. Animals tend to eat to fullness but not excess since they have to remain fit in order to catch or prey on their next meals. Animals rely on their olfactory systems to find food and discriminate which foods are safe to eat versus which are poisonous. Humans rely less on smell to find food because markets and grocery stores provide these resources; food is, therefore, easily accessible in Western societies food (Kearney, 2010). However, most other species must rely on their sense of smell in order to eat. Regardless, understanding human olfaction is an important issue because disorders, such as diabetes and obesity, are linked to these modulatory pathways. For example, diabetes is a disease when an organism is incapable of making or responding to insulin and, instead, unnaturally digests the sugar in urine and the bloodstream. Obesity develops when an organism becomes overweight and turns into a problem due to negative health impacts. These consequences include elevated blood pressure, heart rate, and increased risk of heart attack, stroke, and diabetes (Center for Disease Control and Prevention, 2017). By the year 2050, the Center for Disease

Control expects 33% of the American population will have type 2 diabetes (T2D) and 42% will be obese (Trust for America's Health and Robert Wood Johnson Foundation, 2017; Soergel, 2014). The main focus of this thesis is to understand how modulation of olfactory circuits alter feeding behavior that could lead to human diseases.

This literature-based thesis will produce novel insights in the field of neurobiology[1] and pathology[2]. By collecting and organizing disparate information from various species, this research has potential to advance olfactory processing information in the following two ways: 1) by providing new information regarding molecular mechanisms that modulate olfactory neuron functions in an olfactory circuit; 2) by uncovering relationships between olfactory information processing and disease states in humans, specifically obesity and T2D. The separation of these objectives defines a clear distinction between literature review research on species that are mainly non-vertebrates, non-mammalian vertebrates, and mammals. The species examined include: *Drosophila melanogaster, Caenorhabditis elegans*, *Mus musculus,* and *Homo sapiens*. Stronger correlations can be found this way. Previously, researchers have studied how animal physiology is impacted after the development of diabetes. However, studies performed have not bridged these two conversations- model organism olfactory information processing and its relationship to diabetes and obesity in humans. Bringing pathology and neurobiology together will produce novel approaches to research laboratory models for these diseases. The central hypothesis for this study is that an animal's starved state modulates information transfer in the olfactory circuit via conserved signaling mechanisms, and disorders in these conserved mechanisms contribute to diabetes and obesity in both animals and humans.

[1] Neurobiology is the biology of an organism's nervous system.
[2] Pathology examines the cause of a disease.

B. Neural and Olfaction Anatomy

In order to introduce important terms, each neurobiological key word is explained below. These terms are vital to understanding the scientific terminology involved. For instance, biological refers to living organisms. A chemical is a matter form containing particular chemical composition and properties specific to the compound. Physiological relates to biological component pertaining to normal functions of a living system or organism. In addition, the following terms explain important keywords of this thesis. A mechanism establishes a particular process to send information by chemical molecules in a biological system. If a mechanism is conserved, the mechanism is common across multiple species. Many mechanisms aim to achieve homeostasis, or the preference for a biological system to be physiologically stable, through fluid, chemical, and internal temperature state balances.

Regarding the sense of smell, this main sensory modality will be, heretofore, acknowledged as olfaction. The olfactory bulb, part of the olfactory system, can be found in vertebrate forebrain neural structures to input olfactory information. Upon closer examination, the olfactory system contains many neurons. A neuron is a cell in the nervous system specialized to transmit nerve impulses and messages. Two types of olfactory neurons are specifically examined: first order neurons and second order neurons. A first order neuron, which expresses odor receptors that bind to incoming odorants, receives olfactory sensory information from the environment. The first order neuron converts the chemical interaction, which results from odorant binding to odor receptors into neural signals. The second order neuron receives information from the first order neuron and transmits it to the olfactory bulb, which relays messages to the brain as seen in Figure 1. Multiple features of a neuron aid in transmitting this information (National Center for Biotechnology Information, 2018). The dendrite initially receives neural impulses via chemical compounds released from the first neuron and sends the message to the second neuron's cell body. At the releasing end, known as the

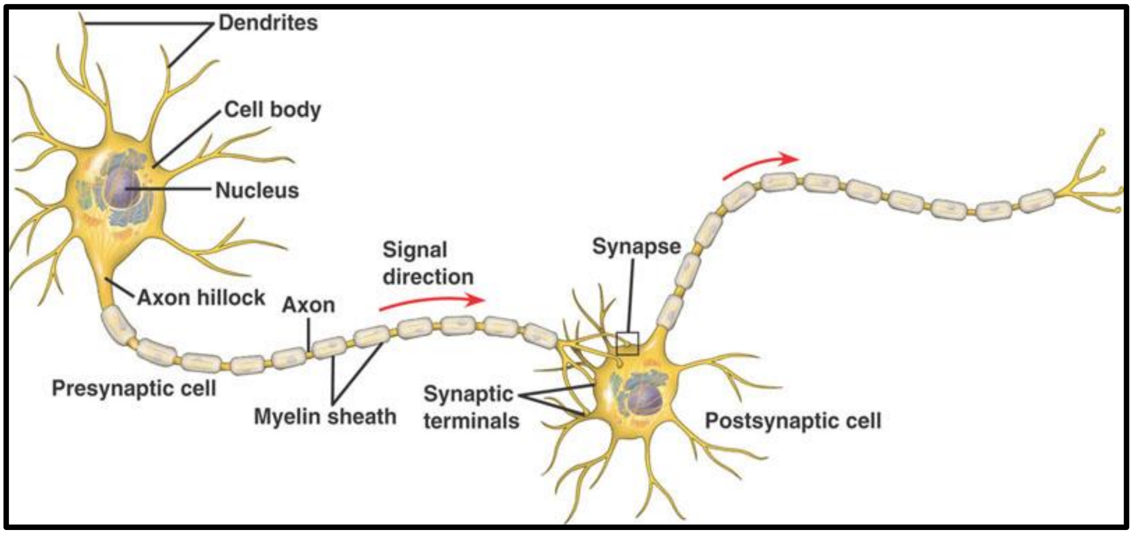

Figure 1: Anatomy of presynaptic and postsynaptic neurons. A presynaptic neuron receives an impulse at the dendrites, relays the message down the axon to the synapse to be sent to the postsynaptic neurons. Retrieved from Neurons, 2018.

synaptic terminal, chemical compounds are released out of the neuron. The synaptic cleft is the space where these chemical compounds move from one neuronal synaptic terminal to the next neuron's dendrite. The chemical compounds transported between neurons in the synaptic cleft are known as neurotransmitters. Neurotransmitters are ultimately released into the synaptic cleft due to a neural message sent to the synaptic terminal. These compounds are either excitatory, in that they enhance an impulse in the postsynaptic neuron or inhibitory during which neurotransmitters do not stimulate the postsynaptic neuron by blocking receptors and instead conflict with an excitatory message.

Neurotransmitters come in different chemical forms. Neuropeptides are short-chain polypeptides[3] that function as neurotransmitters. Signaling transmits a biological message involving multiple chemical compounds. Two specific signaling pathways will be discussed in

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[3] A polypeptide contains a chain of amino acids forming a protein.

this thesis: gamma-Aminobutyric acid (GABA) signaling and insulin receptor (IR) signaling. GABA signaling is affected by GABA, an amino acid[4] found in bacteria, yeast, and vertebrates acting as an inhibitory neurotransmitter in functionally mature neurons (Bouché, Lacombe, & Fromm, 2003). Insulin receptor signaling is activated by insulin, an important hormone[5] released by the pancreas into the bloodstream to regulate high sugar concentration levels in the blood and urine, attaching to an insulin receptor (Cell Signaling Technology, 2016).

Novel advancements have been made in analyzing animal behavior (Barsics et al., 2014; Jiang et al., 2017), often in the context of olfactory learning, and starvation dependent modulation of olfactory behavior (LeDue et al., 2016; Sasaki, Matsui, & Kitamura, 2016). Several molecular pathways including the short Neuropeptide F (sNPF) pathway, GABA signaling, and insulin signaling pathways have been implicated in starvation processes (Carlsson, Enell, & Nässel, 2013; Chen et al., 2013; Ignell et al., 2009). Regardless, current models provide an unclear molecular understanding of how the starved state impacts olfactory information processing and how they reliably predict animal behavior. Additional research, therefore, is needed to accomplish a greater understanding of olfactory information processing and how disruptions in these conserved modulatory pathways lead to disease states.

This study is founded on published research that is focused on olfaction and starvation. These studies suggest that the starved state of an animal modulates its olfactory sensitivity (Jiang et al., 2017; LeDue et al., 2016; Reisenman et al., 2013). Research also implicates the insulin- and GABA- signaling pathways to play a role in this modulation (Ignell et al., 2009; Root et al.,

[4] An amino acid can aid in neurotransmitter transport among cells (Reddy, 2018).
[5] A hormone is a molecule traveling through an organism's fluids regulating homeostasis (Barrington, 2017).

2008). However, conserved mechanisms must be compared to apply the similarities in the olfactory circuit to humans and diseases.

Chapter Two: Conserved Molecular Mechanisms

A. Conserved Olfactory Features

This thesis focuses on what conserved molecules are found across species and how these molecules apply to human diseases of diabetes and obesity. A comprehensive literature review based research aids in answering this question. This section is categorically based on olfactory molecules to explain conserved information. The literature presented in this section consists of literature review articles previously conducted, which were collated into tables or models by the reviewers.

Examining neuropeptide activity of diabetic larvae and obese flies reveals how modulatory[6] mechanisms result in the diseases the flies develop. Baker and Thummel (2007) identified that fruit flies can manage appropriate sugar levels throughout their bodies. This sugar control is to protect against any sudden changes to the environment is saved to be expended as energy when needed. The sugar is then taken up during food depletion or when the organism is starved. Baker and Thummel (2007) identified seven insulin-like peptides that are vital to the insulin-like receptor for the insulin pathway. However, activity of insulin was not explicitly stated in this data because there is little research so far. Further investigation is needed due to this disconnection with adult diabetic flies.

Although the olfactory system is effective in understanding hunger, the gustatory system is needed to provide additional information about metabolism. Studies conducted by Alcedo and Kenyon (2004) and Carleton, Accolla, and Simon (2010) have examined the relation of the olfactory system and gustation. Gustation helps detect specific food features- if it is safe to eat or not- and activates the reward pathway (Carleton, Accolla, & Simon, 2010). The reward pathway

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[6] Modulation examines adjustments at the neural level, for example.

was detected by neuronal firing in the non-human primate orbitofrontal cortex. Reward properties produce a full and satiated feeling in the organism. The gustatory system relates to the olfactory system based on this satiety, or feeling of fullness. Sensory-specific satiety occurs during eating when food becomes less rewarding but the taste remains unchanged. Carleton, Accolla, and Simon (2010) identified that sensory-specific satiety also can occur in humans. Gustatory chemosensitivity is susceptible to altering neuronal activity because of an organism's homeostasis. Carleton, Accolla, and Simon (2010) determined satiation and taste perception should be examined in terms of olfaction. However, identification of these sensory-specific foods is needed. Food preference can determine this olfactory-regulated behavior.

Olfaction is important in assisting control of homeostasis and energy uptake and usage. The perception of smell was correlated to indicate physiological activity (Riera & Dillin, 2016). Riera and Dillin (2016) found smell sensitivity decreases and satiety increases after eating. For example, rat olfactory sensory neurons, or OSNs, diminish odorant-induced activation. This neural activation reduction was revealed through patch-clamp recording[7] research before or after eating. Insulin and leptin, both hormones, increase OSN neural excitability even when odorants are not present. In addition, mice that lack leptin or leptin receptors take approximately ten times less to find food than wild-type mice (Riera & Dillin, 2016). Leptin is significant in determining obesity research because leptin aids in inhibiting appetite. Riera and Dillin (2016) also examined the influence of insulin, a hormone released by pancreatic Beta cells. Insulin enhances energy reserves in peripheral tissues and enhances energy usage in neural systems of the hypothalamus. This review article did provide a link to human disease. Olfactory activity is found to be a

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[7] Patch-clamp recording is a method to record electrochemical activation at the neural level.

warning of diseases such as obesity and diabetes (Riera & Dillin, 2016). The researchers, though, failed to examine if human diseases have the same causes.

Initially, literature review studies examined neuron receptors. Scientific understanding of $GABA_B$ receptors evolved by understanding the sensitivity of $GABA_B$ receptors (Billinton et al., 2001). Since these receptors are found to be responsive to extracellular Calcium ions, postsynaptic neuronal excitability is modulated by Calcium ions in the synapse. However, there are two forms of $GABA_B$ receptors which differ in levels during development: $GABA_{B(1a)}$ and $GABA_{B(1b)}$ (Billinton et al., 2001). When *Drosophila melanogaster* reach adulthood, there are twice as many $GABA_{B(1b)}$ receptors than $GABA_{B(1a)}$ receptors. Differing receptor levels across development may be due to splice variants during brain development, as the authors propose (Billinton et al., 2001). Although the study did examine the different GABA receptors, the activation of mutated binding pockets has yet to be fully understood.

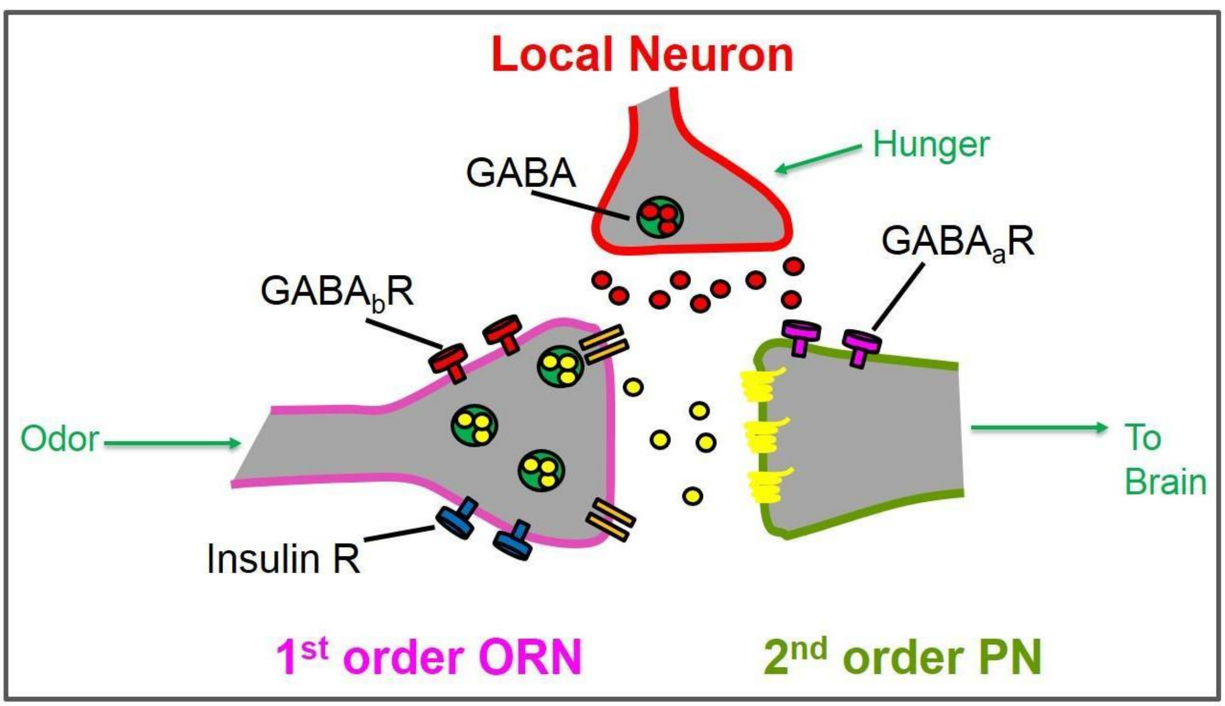

Figure 2: Neuronal signaling in the Drosophila antennal lobe. A working model of neuronal signaling between first order ORNs, second order PNs, and LNs, $GABA_b$ receptors and Insulin Receptors (noted as R) are found on the terminals of ORNs. $GABA_a$ receptors are found on PN dendrites. Adapted from Root et al., 2008. Olfactory receptor neuron is presented by ORN; projection neuron is presented by PN.

Investigating the various mutations will lead to knowledge of the whole receptor. Figure 2 presents a working model of a starvation-induced state affecting olfactory information processing at the neural level of *Drosophila melanogaster* larvae, which is comparable to the mammalian first and second neurons as well (Root et al., 2008). In this model, GABA, secreted by inhibitory local neurons (LNs), are received by $GABA_b$ receptors on the terminals of the first order olfactory receptor neurons (ORNs) or on the dendrites of the second order projection neurons (PNs). ORN terminals also have Insulin receptors (IRs) that sense insulin levels in a starved animal (Root et al., 2008). This adapted model aids in detecting the flow of olfactory conserved information in relation to the human disease of T2D.

Excess food intake exacerbates the development of human disease. Weight gain is due to increased intake of excessive energy beyond an organism's daily needs to maintain homeostasis (Sasaki, Matsui, & Kitamura, 2016). Obesity is endemic in the Western world because humans enjoy food (Kearney, 2010) and tend to overeat. Therefore, appetitive control is an important predictor of homeostatic activity (Sasaki, Matsui, & Kitamura, 2016). Eriksson et al. (2017) investigated these similar predictors. Specific neuromodulators were identified as factors of behavior, metabolism, and eating, all collectively impacting metabolism (Eriksson et al., 2017). One neuromodulator studied in *Drosophila melanogaster* was neuropeptide F, known as NPF. Silencing, or removing the NPF gene from the expressed cells reveal NPF as a neuromodulator on metabolism. The flies were starved for twenty-four hours; the data presented NPF flies eat 66% food than control flies, even though silencing suppressed some post-starvation eating (Eriksson et al., 2017). Therefore, the researchers discovered NPF is not responsible for controlling massive weight changes in one day. When NPF is silenced in *Drosophila* larvae, appetite lowers, but if the NPF gene is expressed, food intake increases, leading to extensive weight gain (Eriksson et al., 2017). Obese flies develop due to the impact of neuromodulators on metabolism on weight. Olfaction, thus, is the most viable sensory system to examine neuropeptides related to feeding and behavior.

Conserved olfactory features vary depending upon which model organisms are being examined. Particular molecules important to conserved olfaction is NPF of *Drosophila*, specifically. The influence of GABA on the first and second order neurons direct researchers to further examine what molecules may additionally be associated with olfaction and starvation because as of yet, starvation-induced GABA activity is unknown.

B. Different mechanisms that modulate sensory perception

By investigating different animals, we can explore the role of conserved olfaction similarities in disease development. The importance of identifying evolutionary conserved features in different model organisms is to aid in understanding how animals physiologically relate in their olfactory mechanisms. Although the sensory systems differ in relation to how each organism receives incoming information, we can trace the evolutionary development of diseases, particularity type 2 diabetes and obesity, by identifying molecular similarities.

Through evolutionary studies of senses, similarities have been established regarding insects, specifically flies, and worms. Simpson (2002) recognized that evolutionary studies typically rely upon the development process of the species individually. The researcher discovered that *Drosophila melanogaster* (flies) and *Caenorhabditis elegans* (worms) are so close evolutionarily that they can be known as satellite species. Satellite species can be compared on a molecular level rather than looking at a much larger system, such as organ systems. Acknowledging the genetic relation between two similar organisms provides the chance to look at a deeper level of comparison of each organism to comprehend what is distinctly similar.

Conserved molecular mechanisms are analyzed by identifying similar modulation of sensory perception. The three systems that are examined are vision, gustation, and olfaction. Audition and tactile sensation typically are not examined because *C. elegans* use a combination of hearing through bodily vibrations to understand stimuli which would be challenging to separate distinctly (“Life of an Earthworm”, 2018); whereas, *D. melanogaster* used different organs to identify sound and feeling (Galluzzo, 2015). Due to this inconsistency in sensations between worms and flies, the remaining sensations are more similar in comprehending conserved mechanisms across species. Visual, gustatory, and olfactory systems will be examined to identify why olfaction is the most viable sensory system to examine neuropeptides related to feeding and behavior.

i. Visual sensory pathway

Vision in both worms and flies relies upon light receptors. *C. elegans* do not have eyes as *D. melanogaster* do; however, these two satellite species have light receptors to analyze what is dark and light. Worms detect when they are above ground by the activation of light receptors compared to when they are underground in the dark (“Life of an Earthworm”, 2018). Light receptors are used for protection because worms are harder to find in the ground for predators than when worms are above ground. Light receptors are evolutionarily important for the survival of worms. Although a fly does have these same light receptors, a fly relies on its sense of vision more than worms to understand what a fly is approaching: predator or prey. Evolutionarily, worms developed a visual system to detect the difference between light and dark (“Life of an Earthworm”, 2018). Worms rarely go above ground but can detect when the worms are in order to warn them of potential predators. However, flies developed vision to detect what is the actual shape of objects in front of them. A fly’s visual preciseness is vital in survival to not land on or near a predator. Regardless, both animals use the visual system to prolong their lives. Rodents use their eyes in the same way- to survive. Priebe and McGee (2014) revealed information received at the rodent's retina is directed to the primary visual cortex, similar to that of the human's primary visual cortex. The primary visual cortex may be important for survival to help in detecting clearer edges, distinctions between objects, and how fast another object or organism is moving. The structure of the rodent visual system is similar to most mammals.

ii. Gustatory sensory pathway

Gustation provides a protective sensation to recognize if food is safe to eat. An organism identifies, through taste, if the organism is full. The gustatory system combines chemosensory, orosensory, and rewarding information about food (Carleton, Accolla, & Simon, 2010). An organism uses chemosensory information to appreciate the taste of a food item; orosensory associations include satiety perception of temperature and texture of the food (Carleton, Accolla, & Simon, 2010). However, the rewarding property is mostly associated with higher-order beings, such as humans, who understand the pleasure of food due to the frontal lobe executive function (Carleton, Accolla, & Simon, 2010). Carleton, Accolla, and Simon (2010) discovered that an organism's chemosensitivity to food is dynamic depending on how hungry the organism is. For example, if a fruit fly lands on spicy food and is hungry, its hunger will protect the organism from noticing the spice intensity which would normally prohibit it from eating the food. The reward pathway has been studied for both chemosensitivity and conditioning for food. Sasaki, Matsui, and Kitamura (2016) investigated the relationships between anti-reward, hunger, mood, and conditioning, which are the functions of neuronal circuit for reward consumption. The closest integrated areas include the frontal lobe of vertebrates, and the hippocampus, which is conserved for short-term memory, working memory, and conditioning. Memory assists in acknowledging quickly, for example, where food may be located or if food is safe to eat. Conditioning processes information regarding immediate responses involved in memory tasks, which often occur unconsciously for humans. Therefore, a species' reaction to incoming food affects the organism's activity to continue eating.

If an animal starves, essential functions are altered. Instead of sleeping, resting, or reproducing, an organism begins to search frantically for food (LeDue et al., 2016). Thus, an organism's inner controls of its metabolic state changes the organism's behavior (LeDue et al., 2016). LeDue et al. (2016) found starvation inhibits the bitter taste perception, permitting flies to eat bitter foods for survival. *Drosophila melanogaster* prefer sweet food in the wild, so in the laboratory, a fruit fly is fed carbohydrate-dense foods. LeDue et al. (2016), thus, investigated when a fruit fly would be most likely to eat bitter food for survival, which was determined during starvation. Bitter food is often considered an aversive food, indicating spoiling or rottenness. Sasaki, Matsui, and Kitamura (2016) examined this distinction by identifying what is most preferred to a fly during the appetitive phase, which is high-sugar foods. The appetitive phase collaborates all of the sensory stimuli, hormonal balances, and motivation to eat. This homeostatic appetite control depends on how many calories or nutrients the organism needs to remain fit and full. Additional research has been performed on *C. elegans* regarding bitter gustation. Conte et al. (2006) investigated bitter receptors by transgenically producing these receptors in *C. elegans* from mammals. The data indicated that due to the addition of these taste receptors in the worm's gustatory system, the worms were capable of tasting bitter tastants through G protein-coupled receptors (GPCR) (Conte et al., 2006). GABA receptor is a GPCR that was first introduced in Chapter One. Nevertheless, Conte et al. (2006) found that bitter tastants are detectable by transgenic *C. elegans* taste receptors. Further investigation includes examining GABA within the *C. elegans* gustatory pathway. Gustation can also extend to olfaction, which will be examined in the next section. Most importantly, however, is the application of gustation to rodents. Le Coutre and Montmayeur (2009) identified rodents taste to detect high-fat foods. Yet, a rodent can detect much more than just fat. A mouse can detect if it is a dietary fat, such as corn or mineral oil or fatty acid, such as linoleic or oleic acid (Le Coutre & Montmayeur, 2009). If a rodent tastes either of these two fats in a food, the rodent will increase its food intake because both fats are high in calorie, which may lead to weight gain. Further studies must be made to understand when the rodent will, in fact, stop from eating these dietary fats or fatty acids.

iii. Olfactory sensory pathway

The focused sensory system investigated in this thesis is olfaction. The fruit fly olfactory system contains peripheral neurons (PNs) that receive input from the olfactory receptor neurons (ORNs). Ko et al. (2015) researched *Drosophila melanogaster* olfactory input using two-photon microscopy to evaluate odor-specific activity. Starved flies suppressed their glomerular sensitivity when exposed to the cider vinegar odor; whereas, fed flies had a normal heightened sensitivity to cider vinegar as it is sweet to them (Ko et al., 2015). The suppression of glomerular sensitivity presents the importance of the glomerulus in detecting and relaying odorant information. The glomerulus detected was DM5, with “D” standing for information coming from the dorsal antennal lobe and 5 indicating the order of the glomeruli. Aversion, thus, to cider vinegar can be further tested through analyzing DM5 activity. A similar response was found in DM1, extending olfactory knowledge that DM1 and DM5 do regulate appetitive behavior (Ko et al., 2015). DM5 was also responsible in behavioral attraction to certain foods. Therefore, odor is lateralized to particular glomeruli for relaying information to the brain. However, apple cider vinegar produced an opposite response in DM1, DM2, and DM4 identified in a separate study (Root et al., 2011). The results conflicted with that of Ko et al. (2015). Regardless, the olfactory system is capable of controlling what a fruit fly, ultimately, consumes. Likewise, odor-dependent activity is conserved across species, particularly insects. The *Rhodnius prolixus*, or blood-sucking bug, senses the difference between safe and not safe food to consume. Reisenman et al. (2013) tested how the bug reacts to volatiles, feces-present pheromones, which are chemicals impacting behavior that are on the exterior of an organism, and attractive scents. Similar to findings by Ko et al. (2015), Reisenman et al. (2013) investigated how starvation impacts olfactory responses and, ultimately, the animal's behavior. Because of the intensity of the volatile odors, *Rhodnius prolixus* were significantly attracted to the volatile odors when starved compared to the other, less intense and safer odors (Reisenman et al., 2013). The researchers used an arm maze set-up to distinguish which odor the bug would prefer. An arm maze set-up contains the beginning point in the center of the maze where the bug is initially placed. Each arm contains a distinct odor or may not contain anything. The insect often circles to smell each odor for each arm before deciding where to go. A majority of the bugs in the Reisenman et al. (2013) study went to the less intense odors which are, to them, safe to consume. Olfaction can be, thus, an indication of decision-making often in a basic sense for survival. Volatile and extreme odors atypical for consumption become increasingly preferred when an organism is starved.

Regarding the insect antennal lobe of the olfactory circuit, the lobe transmits stimulus information into deeper areas of the system and, then, brain. The antennal lobe is a common neuromodulator target (Root et al., 2011). Projection neurons leaving the antennal lobe, or PNs, pass along neural messages to the mushroom body of the fruit fly, which is the main memory center (Root et al., 2011). Root et al. (2011) investigated how starvation affects antennal lobe

PNs and an organism's behavior. The researchers, resultantly, discovered that the odor is not the cause of particular behavior. In fact, the glomerulus is responsible for starvation modulation activity (Root et al., 2011). Due to many odors in the environment, glomeruli integrate the incoming stimuli to send particular information to the fruit fly brain fast enough for survival, which occurs in the first four hours of starvation (Root et al., 2011). Glomeruli integration helps in streamlining this process. Analyzing glomeruli from a larger perspective, the *Drosophila* antennal lobe contains approximately fifty glomeruli (Olsen & Wilson, 2008). Olsen and Wilson (2008) revealed through synaptic strength that lateral inhibition in the olfactory circuit is in fact due to ORN to PN inhibition. Through a separate nerve stimulation of *Drosophila*, $GABA_A$ and $GABA_B$ receptors are on similar ORN axon terminals (Olsen & Wilson, 2008). Axon terminals are located at the end of a postsynaptic neuron, found in Figure 1. These axon terminals are often referred to as synaptic bulbs, which is the region of neurotransmitter release to the synapse. Therefore, each GABA receptor is independent in regulating its distinct activity. However, Olsen and Wilson (2008) found specifically $GABA_B$ receptors are specific for delayed inhibition. Most importantly, ORN axon terminals are mediated by both receptors. Further research of how lateral excitation interacted with lateral inhibition must be performed.

a. Olfactory signaling pathways

The two signaling pathways further discussed include the following: GABA and insulin signaling pathways. The GABA signaling pathway for invertebrates is composed of GABA receptors. Of the research focused on by Billinton et al. (2001), the researchers investigated the structure of GABA receptors, particularly splice variants. Splice variants depend on the transcription of the receptor, because as the receptor is formed, the genetic material control the formation of different receptors from the same genes. The two main splice variants for synapse subtypes include $GABA_{B(1a)}$ and $GABA_{B(1b)}$ (Billinton et al., 2001). $GABA_{B(1a)}$ receptor is found at the presynaptic neuron; whereas, $GABA_{B(1b)}$ is found at the postsynaptic dendrite (Billinton et al., 2001). However, the exact locations have been contested. Both receptors, regardless, are highly sensitive to neural Calcium levels in both *C. elegans* and *D. melanogaster* (Billinton et al., 2001). Within the mammalian rat, $GABA_{B(1a)}$ and $GABA_{B(1b)}$ receptors are present but levels alter during development (Billinton et al., 2001). In fact, the most intense stage of development contains the highest concentration of both receptors (Billinton et al., 2001). Therefore, GABA receptors can indicate development progression. GABA is mostly an inhibitory neurotransmitter throughout the mammalian nervous system (Chen et al., 2007). $GABA_C$ receptors differ from $GABA_B$ receptors because $GABA_C$ receptors are Chloride ligand-operated channels (Chen et al., 2007). Due to immunoreactivity of the $GABA_{\rho 1}$ subunit receptor, Chen et al. (2007) discovered that within mitral cells of the olfactory bulb, the $GABA_{\rho 1}$ subunit receptors are involved in lateral inhibition. $GABA_A$ receptor is similar to $GABA_{\rho 1}$ subunit receptors. The $GABA_A$ receptor involves inhibition due to olfactory aversion (Okutani, 2002). Okutani (2002) investigated the rat olfactory response to an odor known as β-adrenoceptor agonist isoproterenol. The rat was averted to the shock-learning model when smelling the odor (Okutani, 2002). These specific receptors responsible for aversion can be found on the dendrites of mitral cells to form synapses with granule cells, which are contained in the granule layer of the cerebellum (Okutani, 2002). The mouse olfactory bulb contain glomeruli, which is comparable to the fruit fly olfactory bulb; whereas, mitral cells are specific to the mammals in their central nervous systems. Three GABA receptors of the GABA signaling pathway assist in regulating olfactory circuits across several organisms bridging across subphyla of vertebrates versus invertebrates.

Insulin signaling differs from GABA signaling by the compound receptors. The main receptors of *Drosophila melanogaster* for the insulin pathway include insulin-like receptors (InR) involved in starting an intracellular cascade (Baker & Thummel, 2007). An intracellular cascade involves a pathway of proteins or ligands interacting with receptors within a cell instead of between cells. This cellular signal pathway identifies what and how much protein needs to be transcribed and translated. Baker and Thummel (2007) determined these *Drosophila* insulin-like peptides (DILPs), which interact with the InRs, are involved in regulating sugar metabolism. An insulin-like peptide is involved in regulating growth and development; however, DILPs are completely distinct to the fruit fly. Glucose homeostasis is further investigated due to the research Baker and Thummel (2007) reported. The components that will be examined in this thesis include the similar features to GABA and insulin signaling found in Table 1: neuropeptide F, short neuropeptide F, neuropeptide Y, and insulin. Each compound is or is not expressed differently in either *D. melanogaster*, *C. elegans,* or *M. musculus.* Table 1 is vital for determining what the conserved molecular features in the olfactory circuit. Fruit flies expressed three of the four molecules in total of the olfactory system. Therefore, fruit flies may be most appropriate for discovering the functionality of the olfactory system in relation to foraging behavior. Although each *C. elegans* and *M. musculus* express less molecules in the olfactory system than *D. melanogaster*, *M. musculus* is the most effective model organism in comparing the olfactory system to that of a human’s. The two molecules rodents express as revealed in Table 1, insulin and NPY, are also expressed in human olfaction as Chapter Three will explain.

b. Conserved molecular features of the olfactory pathway

In order to identify conserved molecular mechanisms, the chemical compounds that are similar between species indicates the close relation. The main neurochemicals determined across the olfactory system in Table 1 are the following: neuropeptide F (NPF), short neuropeptide F (sNPF), neuropeptide Y (NPY), and insulin. Each of these compounds was initially identified as important for the olfactory circuit and was searched for in other organisms to find conserved mechanism features. Table 1 presents the compound that interacts with appetite and behavior. However, understanding how each of these compounds act normally and why they are significant aids in the importance of them for olfaction.

NPF and sNPF are neuropeptides regulating fruit fly appetite and development (Al-Anzi et al., 2009). Both interact directly with a particular G-protein-coupled receptor, which is often used in protein cascades to transmit information onto the surface of a cell, such as a postsynaptic neuron. Evolutionarily, NPFs may be closely related to the mammalian neuropeptide Y (NPY), which has also distinctly been identified for food intake regulation (Carlsson, Enell, & Nässel, 2013). When a fly is starved, *snpfr* (short neuropeptide F receptor) transcription is enhanced to receive sNPF due to an increased sNPF neuropeptide sensitivity and, thus, food sensitivity (Carlsson, Enell, & Nässel, 2013). However, Carlsson, Enell, and Nässel (2013) determined that in the developing fly, there is no expression of the regulatory olfactory circuit peptide, *snpfr*, or sNPF. In adult flies, though, sNPF is expressed (Carlsson, Enell, & Nässel, 2013). Therefore, the olfactory system develops during the fruit fly larval stage before metamorphosis. Root et al. (2011) investigated that sNPF may involve presynaptic activity as well. This was determined during starvation, which has increased ORN activity through sNPF signaling cascade. Root et al. (2011) tested which glomerulus sNPF significantly produces these results. Intraglomerular sNPF was found as necessary for olfactory sensitivity during starvation instead of intraglomerular sNPF (Root et al., 2011). Therefore, sNPF within an ORN drives the sensitivity to odors for a single ORN channel rather than sNPF between neurons.

Olfaction molecules have been determined as conserved across species. Table 1 reveals the most studied molecules in the olfactory system: insulin and Neuropeptide Y (NPY). However, the organism which was most studied for these molecules varied. Insulin in *D. melanogaster* regulated mostly foraging behavior; whereas, insulin in *M. musculus* controlled odorant responses. Additionally, NPY was detected in *C. elegans* and *M. musculus.* For *C. elegans*, NPY is most similar to leptin[8] because both assist in controlling food intake. Nevertheless, in *M. musculus*, NPY enhances odor responses. Through this research, *D. melanogaster* contains three of the four molecules involved in neurochemical signaling of the olfactory system. Therefore, for the basic olfactory system, *D. melanogaster* is the most appropriate animal in the laboratory setting to study foraging behavior in relation to odorants. Nevertheless, *M. musculus* is most valuable in studying olfactory epithelium development for insulin and neuropeptides in mammals.

[8] Leptin is an appetite-inhibiting hormone.

Neurochemical signaling components of the olfactory system	**Does *C. elegans* have the neurochemical? If yes, what are the distinct olfactory features?**	**Does *D. melanogaster* have the neurochemical? If yes, what are the distinct olfactory features?**	**Does *M. musculus* have the neurochemical? If yes, what are the distinct olfactory features?**
insulin	No research within the olfactory system.	Yes; insulin controls foraging behavior for insulin signaling (Root et al., 2011). Yes; insulin regulates inhibition in the presynaptic neuron and controls foraging behavior when starved for insulin signaling (Ko et al., 2015).	Yes; insulin regulates olfactory responses in the olfactory mucosa for insulin signaling (Negroni et al., 2012).
NPF	Yes; NPF was identified, yet no specific activity was determined (Clynen, Husson, & Schoofs, 2009).	Yes; when NPF signaling lowers, foraging behavior decreases (Shen & Cai, 2001)	No research within the olfactory system.
NPY	Yes; NPY controls food consumption (de Bono & Bargmann, 1998).	No research within the olfactory system.	Yes; microvillar cells of the olfactory epithelium produce NPY (Montani et al., 2006). Yes; NPY increases odorant responses (Negroni et al., 2012).
sNPF	No research within the olfactory system.	Yes; sNPF controls response intensity to food odors (Carlsson, Enell, & Nässel, 2013).	No research within the olfactory system.

Table 1: GABA and insulin signaling components of first and second order neurons in the olfactory system. The conserved mechanisms across *D. melanogaster, C. elegans, M. musculus* reveal *D. melanogaster* exhibits a majority of important neuropeptides and hormones. NPF stands for Neuropeptide F; NPY stands for Neuropeptide Y; sNPF means short Neuropeptide F.

C. Methods of Literature Research

The literature found was determined through the search engine for the University of Nevada, Reno library. Olfaction and starvation were individually searched with *D. melanogaster*, *C. elegans,* or *M. musculus*. Once the results were found, “journal article” and “available online” were selected as an advanced search. The first articles search was limited to the past ten years, and if more clarification was needed for scientifically and accepted data, the search was extended to within the past twenty years. To find review articles, the *Cell, Neuron,* and *Nature Neuroscience* journal search engines were used specified to review articles. This special search was due to a minimal number of articles found in the science field that are peer-reviewed articles as most research in the science field is focused on actual wet-laboratory data. Insulin and GABA signaling pathway data were separately found through a search in the University of Nevada, Reno online library with descriptions of insulin or GABA and starvation or olfaction. The insulin articles were often closely related to diabetes, so no additional research articles were needed for animal diabetes. For obesity, the same online search engine was used to find articles describing obese *D. melanogaster, C. elegans,* or *M. musculus*. Each search aided in finding the most applicable and recent research data.

D. Conclusion

Although *C. elegans* and *D. melanogaster* may have the important signaling components of detecting food odors, *M. musculus* is most important in insulin signaling for specific development of insulin. Due to evolution studies, all three species have developed gustatory, visual, and olfactory sensory pathways in order to help them survive their own environments in the wild. However, to study olfaction in terms of specific peptides and hormones, rodents are most appropriate.

Chapter Three: Relationship to humans and diseases

A. Diseases associated with olfactory and starvation pathways

Studying human diseases are researched in both humans and animals. Animals are excellent models to understand how human diseases physiologically develop or function. The two diseases examined in this thesis, as previously examined in Chapter Two, are diabetes and obesity because of the potential connection between the insulin receptors on the first and second order olfactory neurons to metabolic diseases.

Not only does olfaction have a significant impact on behavior, but starvation does as well. The two pathways interact to alter how an animal responds to its internal appetite state. For example, Reisenman et al. (2013) revealed if a *Rhodnius prolixus*, a small insect capable of flying, is starved, the animal's internal metabolism influences the olfactory behavior completely. Therefore, starvation affects olfaction, instead of in the opposite direction. Feeding changes how an animal smells and what it smells. Additionally, animals may be used to represent human eating disorders, such as binge eating disorder. Obesity is, in fact, a symptom of binge eating disorder in humans (Kim, 2012). Over-intake of food in humans can be represented in animals, although the psychological component cannot be controlled because the animals are forced to overeat in a laboratory setting (Kim, 2012). Therefore, studying overconsumption of food in the research setting is most appropriately examined by providing highly-concentrated fat or carbohydrate foods for extended periods of time to increase an organism's weight.

Although the olfactory systems between most nonhuman mammals and humans are comparable, particular differences must be noted to understand what denotes the distinction between these systems. For example, the olfactory region in animals is, in fact, larger in relative size compared to a human's olfactory region (Djupesland, Mahmoud, & Messina, 2013). This may be because animals rely more on their sense of smell for food quality than humans do. Regardless of the relative size, Djupesland, Mahmoud, and Messina (2013) identify that the surface area of the olfactory nerve in humans is larger than in animals, which aids in transport of molecules, particularly medications. Similarities between species' olfactory systems include, particularly, how molecules are received in insects, mammals, and worms. Su, Menuz, and Carlson (2009) identify the glomeruli of the insect olfactory system is comparable to the olfactory bulb of mammals. Odorants are received by olfactory receptor neurons in both insects and mammals; the glomerulus or olfactory bulb processes the odorants which are then sent to higher order processes in the brain (Su, Menuz, & Carlson, 2009). However, *C. elegans* are not insects. Therefore, the comparison must be made between mammals and roundworms. Though *C. elegans* have a small amount of chemosensory cells in the olfactory system, these worms detect a comparable number of odorants as mammals detect (Ache & Young, 2005). The fly, mouse, and worm olfactory systems are, thus, successful in representing the human olfactory system when studying all three systems together to compare.

i. Diabetes

Diabetes is a disease that can manifest in two distinct ways. Type 1 diabetes (T1D) occurs when the human body makes its own insulin but does not effectively respond to it. Type 1 symptoms include extensive fluctuation in weight, fatigue, and change in appetite. Type 2 diabetes (T2D) occurs when the body does not make enough of its own insulin and has, therefore, difficulty digesting carbohydrates, or sugars. T2D manifests in excessive urination, increased thirst, and is typically associated with being overweight or obese. T1D most commonly develops among children; whereas, T2D develops more often in adulthood. Since T1D is not associated with being overweight, this literature-based thesis will focus on T2D because T2D is diagnosed in obese individuals.

Although insulin is produced in the pancreas, insulin does travel throughout the human body in the circulatory system. Gray, Meijer, and Barrett (2014) proposed a pathway of how insulin reaches the brain interstitial fluid, which reaches the olfactory system. From arteries, insulin travels through the choroid plexus[9] (Gray, Meijer, & Barrett, 2014). Insulin then is transported through glial cells to the brain's interstitial fluid and finally to the veins if in excess to be returned to the body (Gray, Meijer, & Barrett, 2014). Although insulin is a large peptide hormone, insulin can cross many barriers throughout the body to reach the brain and then the olfactory system.

Understanding how diabetes works links how diabetes is best treated. For humans, T2D is treated by a lifestyle change. Lifestyle changes may include eating healthier foods, increasing physical exercise, bariatric surgery, and taking diabetes medicine, such as pills or injectable insulin (National Institute of Diabetes and Digestive and Kidney Diseases, 2016). Thus far, bariatric surgery is the most effective form of treatment for obesity (Hussain & Bloom, 2013). However, the surgery does have fatal risks. Thus, more research is needed to provide alternative treatments. Injectable insulin, currently used by many, controls glucose in the circulatory system (National Institute of Diabetes and Digestive and Kidney Diseases, 2016). An alternative method to injectable insulin is an inhaler (National Institute of Diabetes and Digestive and Kidney Diseases, 2016). The insulin reaches the patient's lungs and is transported into the blood (National Institute of Diabetes and Digestive and Kidney Diseases, 2016). However, many of these methods are costly, and alternative methods should be produced. Fourlanos et al. (2011)

[9] The choroid plexus is located in the ventricles of the brain where cerebrospinal fluid and endothelial cells are produced.

revealed nasal insulin may be an approach to examine since the medication breathed in through the nose has shown to be effective in T1D patients. The nasal insulin restored immune system tolerance to insulin for these individuals (Fourlanos et al., 2011). Further treatment of T2D with nasal insulin, thus, should be performed in the laboratory setting with nonhuman mammals.

a. Conserved molecular features

Comparing between species, two vertebral species were selected in studying how olfactory system molecules influence diabetes and odorant response activities. Rodents represent one of the species because rats, *Rattus norvegicus*, and mice, *Mus musculus*, are similar due to their anatomy and physiology. *Homo sapiens*, or humans, are the other species compared to in this thesis. Table 2 presents the data from primary research articles on either rodents or humans in relation to T2D. Since the molecules GLUT4, insulin, leptin, and sugar were detected in the olfactory systems of both rodents and humans, both *Rattus norvegicus* and *Mus musculus* are excellent predictors of how the olfactory system interacts with the development or regulation of diabetes. Four out of the six molecules revealed through the literature in relation to diabetes and olfaction are expressed in both rodents and humans. In addition, the activity of leptin and sugars in both rodents and humans was comparable. Although insulin receptors (IRs) and NPY were not detected specifically in the human olfactory system influencing behavior or neural activity, further research will aid in understanding if the function of IRs and NPY are the same for both rodents and humans. Therefore, the effects of insulin on the olfactory system may be examined more in humans, specifically, because insulin is not capable, as the literature revealed so far, of being received by an IR in the olfactory system. Instead, the studies revealed that insulin is received by the trigeminal nerve in the brain, directly going to the blood brain barrier (Shpakov, 2017). Nevertheless, because 66% of the molecules revealed are present in rodents and humans, rodents are an appropriate model in studying potential treatments, such as nasal insulin in a laboratory setting, or processes of T2D within the olfactory system.

Molecule	**Organism that Manifests the Molecule**	**Effect**	**Reference**
GLUT4	Humans	GLUT4 crosses neural membrane with enhanced insulin.	Shpakov, 2017
	Rats	GLUT4 mRNA discovered in olfactory bulb granule cells.	Vannucci et al., 1998
insulin	Humans	An increase in insulin enhances GLUT4 movement across neuron membrane; intranasal insulin received at nasal mucosa, crosses cavity to olfactory bulb and to the hippocampus and other brain regions; if insulin is received by the trigeminal nerve[10], insulin will go to various parts of the brain.	Shpakov, 2017
	Rats	Insulin in satiated rats inhibit OSN responses.	Negroni et al., 2012
	Rats	Intranasal insulin, with IGF-1, is capable of being transported to the blood-brain barrier through the olfactory pathway.	Thorne et al., 2004
	Mice	Mice with diabetes cannot distinguish between odorants.	Rivière et al., 2016
IR	Humans	No research within the olfactory system.	
	Rats	Insulin binding to IRs in olfactory mucosa was greater than olfactory bulb.	Lacroix et al., 2008
leptin	Humans	Leptin modulates identification of odors.	Trellakis et al., 2011
	Mice	Leptin reduces sensitivity in the olfactory epithelium.	Rivière et al., 2016
NPY	Humans	No research within the olfactory system.	
	Rats	NPY modulates odorant detection in a rat's olfactory mucosa within the odorant-induced olfactory sensory neurons.	Negroni et al., 2012
sugar (fructose or sucrose)	Humans	Diabetic patients ate less sucrose than control patients but 3.5 times more sweeteners that did not contain sucrose; diabetes influences alternative sugar intake.	Tepper, Hartfiel, & Schneider, 1996
	Mice	Fructose decreases responses of OSNs.	Rivière et al., 2016

Table 2: Diabetic mutations influence eating behavior. In both animals and humans, mutations of proteins and proteases affect an appetitive state for the organism. GLUT4 stands for glucose transporter member 4; OSN stands for olfactory sensory neurons; IGF-1 stands for insulin-like growth factor 1[11]; IR stands for insulin receptor; NPY stands for neuropeptide Y.

[10] The trigeminal nerve accepts olfactory sensory information.
[11] IGF-1 is a neurotrophic protein growth factor.

ii. Obesity

A person's weight is medically categorized as underweight, normal, overweight, and obese by certain scaling factors. BMI, or body mass index, calculates a person's weight and height to determine which of the four categories, underweight, normal, overweight, or obese, an individual's weight is in. To calculate one's own BMI, convert weight from pounds to kilograms and height from total inches to meters, then divide weight by height two times (National Health Services England, 2016). The resulting BMI[12] can be simpler in detecting through Figure 3. Since 2013, obesity has been defined as a disease since the problems are not associated with simply lack of self-discipline to eat less or exercise more (Covington, 2017). Obesity is a disease because it impairs normal body functioning, lowers one's lifespan, and has been linked to genetics. For instance, an obese individual may pass down the disease to a child. Obese individuals have an increased tendency for cardiovascular problems compared to those of a normal weight (Obesity Society, 2015). Obesity has become an epidemic[13] that approximately 35% of people in the United States have (Barlow, Durand, & Hofmann, 2018); whereas, 70% of Americans are overweight (Barlow, Durand, & Hofmann, 2018). Figure 3 reveals overweight individuals have a BMI of 25 to less than 30; an obese individual has a BMI of greater than 30. Therefore, understanding how to study obesity is important in helping resolve the commonly-diagnosed disease.

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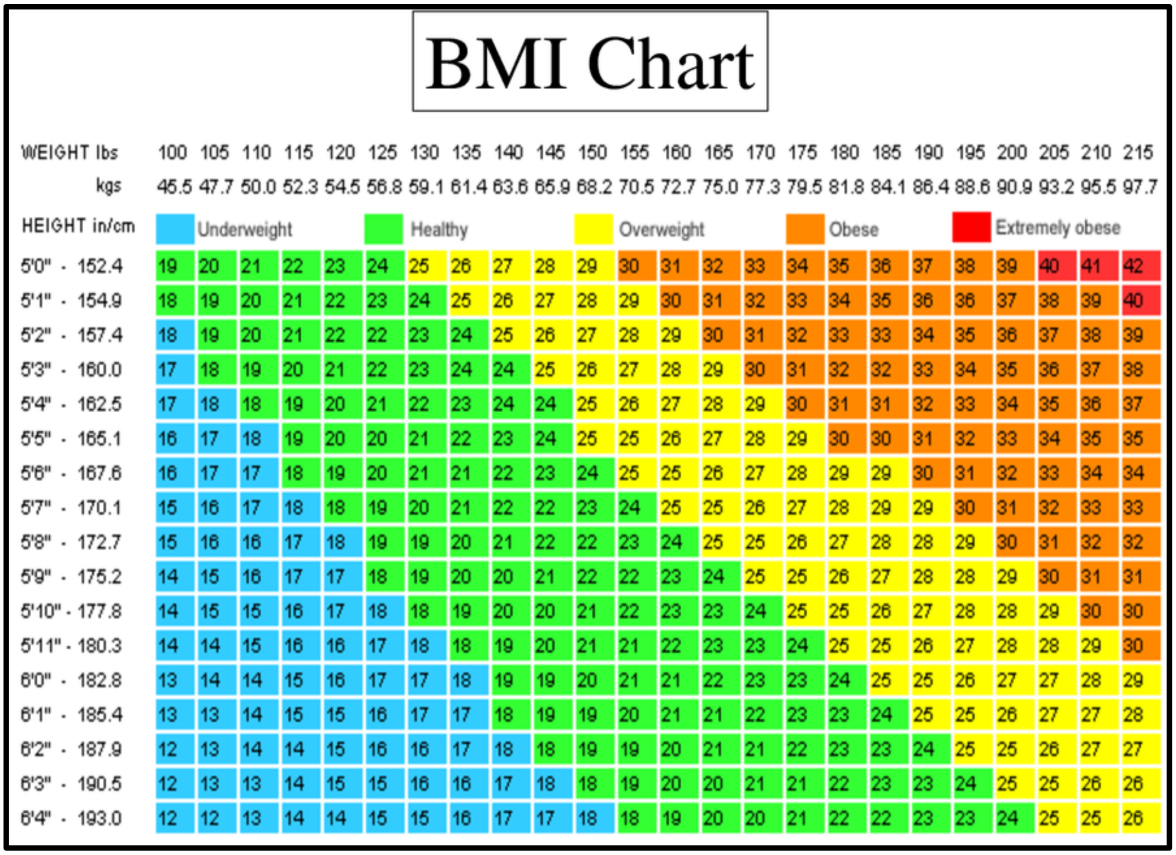
[12] However, BMI is not entirely accurate in detecting if an individual is normal weight, overweight, or obese because muscle mass is not taken into account.

[13] An epidemic is an outbreak of a particular disease in a community during a certain time.



BMI Chart

WEIGHT lbs	100	105	110	115	120	125	130	135	140	145	150	155	160	165	170	175	180	185	190	195	200	205	210	215
kgs	45.5	47.7	50.0	52.3	54.5	56.8	59.1	61.4	63.6	65.9	68.2	70.5	72.7	75.0	77.3	79.5	81.8	84.1	86.4	88.6	90.9	93.2	95.5	97.7
HEIGHT in/cm	Underweight					Healthy					Overweight					Obese				Extremely obese				
5'0" - 152.4	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
5'1" - 154.9	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	36	37	38	39	40
5'2" - 157.4	18	19	20	21	22	22	23	24	25	26	27	28	29	30	31	32	33	33	34	35	36	37	38	39
5'3" - 160.0	17	18	19	20	21	22	23	24	24	25	26	27	28	29	30	31	32	32	33	34	35	36	37	38
5'4" - 162.5	17	18	18	19	20	21	22	23	24	24	25	26	27	28	29	30	31	31	32	33	34	35	36	37
5'5" - 165.1	16	17	18	19	20	20	21	22	23	24	25	25	26	27	28	29	30	30	31	32	33	34	35	35
5'6" - 167.6	16	17	17	18	19	20	21	21	22	23	24	25	25	26	27	28	29	29	30	31	32	33	34	34
5'7" - 170.1	15	16	17	18	18	19	20	21	22	22	23	24	25	25	26	27	28	29	29	30	31	32	33	33
5'8" - 172.7	15	16	16	17	18	19	19	20	21	22	22	23	24	25	25	26	27	28	28	29	30	31	32	32
5'9" - 175.2	14	15	16	17	17	18	19	20	20	21	22	22	23	24	25	25	26	27	28	28	29	30	31	31
5'10" - 177.8	14	15	15	16	17	18	18	19	20	20	21	22	23	23	24	25	25	26	27	28	28	29	30	30
5'11" - 180.3	14	14	15	16	16	17	18	18	19	20	21	21	22	23	23	24	25	25	26	27	28	28	29	30
6'0" - 182.8	13	14	14	15	16	17	17	18	19	19	20	21	21	22	23	23	24	25	25	26	27	27	28	29
6'1" - 185.4	13	13	14	15	15	16	17	17	18	19	19	20	21	21	22	23	23	24	25	25	26	27	27	28
6'2" - 187.9	12	13	14	14	15	16	16	17	18	18	19	19	20	21	21	22	23	23	24	25	25	26	27	27
6'3" - 190.5	12	13	13	14	15	15	16	16	17	18	18	19	20	20	21	21	22	23	23	24	25	25	26	26
6'4" - 193.0	12	12	13	14	14	15	15	16	17	17	18	18	19	20	20	21	22	22	23	23	24	25	25	26

Figure 3: BMI chart of height versus weight. A BMI of 30 or more, in orange, indicates obese, and a BMI of 40 or more, in red, indicates extremely obese (Retrieved from Brodwin, 2018).

a. Conserved molecular features

Due to the molecules detected in the relation of diabetes and olfaction of Chapter Two, the same molecules were focused on in the study of obesity and olfaction. Several molecules were added, though, because more studies have been performed regarding obesity compared to diabetes due to the primary articles detected in the literature search. These molecules in Table 3 include: azelaic acid[14], ghrelin[15], Neuropeptide Y Receptor 2 (NPY2R), Olfactory receptor 544 (Olfr544), and orexin[16]. The molecules not examined, but were studied regarding diabetes instead in Table 2, are GLUT4, insulin, IR, and sugar. Upon a search for these molecules, within

[14] Azelaic acid is a dicarboxylic acid used biologically after infection.
[15] Ghrelin is a hormone involved in hunger.
[16] Orexin is a neuropeptide that assists in controlling appetite.

rodents and humans in relation to olfaction and obesity, revealed GLUT4, insulin, IR, and sugar are not focused for primary research experiments. Table 3 presents the activity of the molecules examined. Therefore, rodents are an effective model in studying how the olfactory system interacts with the development of obesity and overeating in general because 5/7 of the molecules presented in humans are also in rodents regarding olfaction and obesity. Of all the molecules, only aza and Olfr544 were not found in the research on humans. The molecules most similar between rodents and humans are leptin, due to odorant behavior, and NPY, which regulates hunger and weight. NPY2R, however, acts oppositely in humans versus rodents. In humans, enhancing activity of NPY2R increases weight gain; whereas, in rodents, activation of NPY2R decreases food consumption. Although rodents do differ in some molecular activity compared to humans, overall, rodents resemble humans in the molecules involved in obesity and overeating. Therefore, as Table 2 also revealed, both *Rattus norvegicus* and *Mus musculus* are effective model organisms in studying the interaction of the olfactory system with obesity.

The data collected for diabetes and obesity reveal rodents are an appropriate model organism to represent the human olfactory system. Examining how NPY and leptin can be altered in the rodent olfactory system would help combat obesity, specifically. However, to understand T2D in humans, nasal insulin shall be the first molecule studied in the laboratory setting with either rats or mice.

Molecule	Organism that produces the molecule	Effect	Reference
Aza	**Humans**	No research within the olfactory system.	
	Mice	Aza activates Olfr544 and then CREB; intake of Aza in food activates Olfr544.	Wu et al., 2017
ghrelin	**Humans**	Ghrelin decreases with overeating.	Fernandez-Aranda et al., 2016
	Humans	Ghrelin increased sniffing of odorants without changing reward pathway.	Tong et al., 2011
	Rats	Ghrelin lowered thresholds of odorant detection and instead enhanced sniffing.	Tong et al., 2011
leptin	**Humans**	Increased leptin increased odor identification correctness however obese individuals had the lowest levels of leptin.	Trellakis et al., 2011
	Mice	Leptin modulates olfactory behavior in nasal mucosa.	Getchell et al., 2006
NPY	**Humans**	Inhibiting NYP leads to loss of weight as an effective treatment to overweight patients.	Tilan et al., 2007
	Rats	NPY is important in neuromodulation of vertebral olfactory systems but only in hungry vertebrates.	Negroni et al., 2012
NPY2R	**Humans**	Inhibiting NPY2R leads to loss of weight as an effective treatment to overweight patients.	Tilan et al., 2007
	Mice	Activation of NPY2R inhibits food intake.	Loktev & Jackson, 2013
Olfr544	**Humans**	No research within the olfactory system.	
	Mice	Olfr544 activates lipolysis; Olfr544 inhibits growth of adipose tissue and activates lipolysis; Olfr544 can be a focus in treating obesity.	Wu et al., 2017
orexin	**Humans**	Orexin revealed obesity should be treated as an addiction but to food.	Kenny, 2011
	Rats	Orexin increased sensitivity and activated GABAergic neurons or mitral cells.	Julliard et al., 2007
	Rats	Olfactory sensitivity increased in fasted animals.	Aimé et al., 2007

Table 3: Obesity mutations influence eating behavior. Protein and hormonal mutations within both animals and humans change how an organism behaves in reaction to available food. Aza means Azelaic acid; NPY means neuropeptide Y; NPYR2 stands for neuropeptide Y receptor 2; Olfr stands for olfactory receptor.

iii. GABA signaling

As explained in Chapter Two, GABA is an inhibitory neurotransmitter released from the local neurons into the synapse between the first and second order neurons. Although $GABA_BR3$, R standing for receptor, has been found in insects, $GABA_BR1$ and $GABA_BR2$ have specifically been detected in mammals (Jamal et al., 2012). These two subunits, R1 and R2, combine to form the $GABA_B$ receptor in mammals. The importance, regardless of $GABA_B$ receptors on the second order neurons is if GABA binds to the receptors, regulation of information transmission occurs (Kuriyama, Hirouchi, & Kimura, 2000). Without GABA binding, the second order neurons are increasingly fired with the message. Therefore, GABA acts as a gateway messenger. Nakamura et al. (2011) discovered if $GABA_BR1$ is deficient in rat adipocytes[17], leptin production decreases. Therefore, a link of $GABA_BR1$ to obesity may be made but not in the olfactory system. Opposingly, if $GABA_B$ receptors are overstimulated on rat nociceptors[18], as Laffray et al., (2007) revealed, the two subunits will eventually dissociate. $GABA_B$ receptors are, thus, delicate in nature. Although research on these subunits has been composed on mice, the research found was not in the olfactory system (Jamal et al., 2012). Therefore, GABA signaling is not focused on in Chapter Three due to olfaction in mammals.

B. How diabetes and obesity relate

Because T2D and obesity are often diagnosed simultaneously, both of these diseases may be examined in tandem. T2D and obesity are, thus, statistically related through diagnoses. T2D, 90% of patients diagnosed with T2D are also obese (Obesity Society, 2015). If diabetes increases in prevalence, obesity will, too. However, obesity is commonly the first to be diagnosed if the diseases are not diagnosed simultaneously because obesity is the most common risk factor for

[17] Adipocytes are fat cells that store energy.
[18] Nociceptors are pain receptors on the dendrites of sensory neurons.

developing T2D (Obesity Society, 2015). Targeting both diseases is critical in treating and preventing this epidemic. As of 2012, the National Institutes of Health predicted an estimated 8.37% of the American population have T2D (Obesity Society, 2015). Diabetes and obesity have both negatively impacted the health of many people in the United States. Thus, determining rodents as effective model organisms to study both diabetes and obesity is critical for the health of Americans. Due to obesity as the primary risk factor of T2D, preventing weight gain, overall, is the single most effective determinant to inhibit the development of T2D.

C. Conclusion

Due to the results found, rodents are reliable model organisms to represent the olfactory system of humans. In order to understand the development of both diabetes and obesity, dependable treatments are needed. Both *Rattus norvegicus* and *Mus musculus* can be used in laboratory research to gather knowledge about these diseases and rely that the results will accurately apply to humans.

Conclusions

T2D and obesity impair the health of many Americans. In order to target how to control this epidemic at the neural circuit level, *Drosophila melanogaster* will be the most efficient in studying foraging behavior as representative of overeating. The studies examined focus on how the fruit flies respond to altered molecules within the olfactory system. Insulin is the most appropriate molecule in examining relation to humans since NPF and sNPF have yet to be discovered in the human olfactory system. *Drosophila melanogaster* can aid further research of insulin activity in the olfactory system beyond solely foraging behavior.

Additionally, *Rattus norvegicus* and *Mus musculus* will be the most reliable mammalian and vertebral model organisms to study how the olfactory system interacts with the biological functions of T2D and obesity. Further studies to be conducted in the laboratory include examining how nasal insulin affects T2D in either *Rattus norvegicus* or *Mus musculus*. Because insulin has a direct pathway through the olfactory system, nasal insulin may be effective in treating T2D to avoid injectable insulin for a greater variety of treatments for patients and possibly to prevent obesity as well.

T2D and obesity are highly comorbid in humans. In order to decrease the expected number of diagnoses in the coming thirty years, reducing obesity may help in decreasing the cases of T2D. Understanding the influence of insulin, leptin, and NPY will likely aid researchers in finding alternative treatments to injectable insulin for T2D patients or effective preventions of the development of obesity and, thus, T2D. Nasal insulin may be found to be such an alternative treatment.

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