FEEDING MICROSTRUCTURE APPROACHING CESSATION IN LARVAL *MANDUCA SEXTA*:

EFFECTS OF SATIATION, IMMUNE CHALLENGE, MOLTING, AND PREDATOR STRESS.

by

Dylan William Miller

Submitted in partial fulfilment of the requirements
for the degree of Master of Science

at

Dalhousie University
Halifax, Nova Scotia
October 2018

DEDICATION PAGE

I dedicate my work to all the other students struggling with their own writing right now.

If I managed to pull it off, I'm confident that you can too.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

As animals approach satiation, their motivation to feed decreases. However, there are a variety of factors other than satiation that can affect feeding motivation. The tobacco hornworm caterpillar, *Manduca sexta*, is an ideal system for studying feeding motivational physiology. It shows no circadian rhythm in its feeding and has a relatively simple nervous system. It reduces feeding when ill, exposed to model predators, and during certain developmental periods. It reduces feeding when approaching a developmental molt, as in other larval insects. The stress from a predator can also reduce feeding. The force with which an animal bites during feeding has been linked to feeding motivation in many other species. By noninvasively measuring the force applied to food during feeding, changes in *M. sexta* motivation can be examined across a feeding period and between different conditions. Understanding how this caterpillar terminates a self-generated behaviour such as feeding, provides insight into the regulation of motivation.

ACKNOWLEDGEMENTS

I extend my sincerest thanks Dr. Shelley Adamo for her patience, guidance, understanding, and support since joining her lab. I came here for the sole reason of working under her, and it's been even more worthwhile than I imagined. I would also like to thank my committee, Dr. Vin LoLordo and Dr. Nathan Crowder for their aid and patience. This also would have been far more difficult, if not impossible, without the help and support of my lab mates, especially Laura McMillan. I would also like to thank my parents, Christine and William, for their lifelong understanding, support, and kindness towards me. I would be nowhere without it. Lastly I would like to thank my cat Shilo, who daily ensures that my sanity remains intact.

CHAPTER 1 INTRODUCTION

Feeding behaviour in insects is plastic. It can be influenced by external factors such as predator presence (McPeek, 1989) and internal factors such as blood sugar concentration (Thompson and Redak, 2000). The factors responsible for the initiation of feeding in insects have been studied, but the factors that lead to the cessation of feeding (i.e. satiation) are less well understood (Hanson and Dethier, 1973; Dethier, 1976). Satiation typically occurs when food is still available, suggesting that an internal regulatory process suspends feeding, despite the presence of food cues (Kupfermann, 1974; Bellisle and Blundell, 2013).

1.1 FEEDING MOTIVATION: Initiation & Satiation

1.1.1 Feeding initiation: Drosophila

The neural circuity regulating feeding has been best studied in fruit flies (*Drosophila melanogaster*). When a fruit fly is starved, a cascade of events results in food-seeking behaviours. Food deprivation leads to a reduction in energetic sugar supplies (i.e. glucose and trehalose) as well as amino acids. The lowered energy and nutrient availability are sensed through a TOR signaling pathway by the fat body of flies (Géminard et al., 2009). The fat body is analogous to a combination of the vertebrate liver and adipose fat tissues (Branch and Shen, 2017). The fat body releases the cytokine 'Unpaired 2' into the hemolymph, where it binds to cells in the *pars intercerebralis* (PI), the primary neurosecretory region of the *D. melanogaster* brain and considered similar in function to the vertebrate hypothalamus (Scharrer and Scharrer, 1944; Nässel, 2002; Nässel et al., 2013). The cells signaled by Unpaired 2 inhibit the PI's systemic secretions of *D. melanogaster* insulin-like peptides (Dilp), which are similar in structure to vertebrate insulins.

These molecules serve a highly conserved function between insects and mammals to signal energy availability in the animal (Yin Peng Zhan et al., 2016). Decreased Dilp

levels in turn increase sensitivity of *D. melanogaster* olfactory receptor neurons to attractive food scents (Root et al., 2011). The reduced Dilp levels also promote food-seeking motor activity through signaling in the Mushroom Bodies (MB), multimodal integration centers in the brain of fruit flies that play a role in learning and memory. Reduced levels of Dilp modulate the circuits of the MB leading to increased valence of food odors and tastes (Aso et al., 2014; Yu et al., 2016).

The exact mechanism by which the MB induces hyperactivity is still unclear. Activating the relevant MB outputs alone does not induce motor hyperactivity, but their ablation also fully prevents the increased food-seeking response (Tsao et al., 2018). For my purposes motivation is defined as 'the energizing of behavior in pursuit of a goal' (Simpson and Balsam, 2016), and the goal I will focus on is feeding. The combination of the increased odorant sensitivity, motor activity, and increased food odor value all increase the likelihood of the flies sensing and encountering suitable food, satisfying the definition for a motivated state.

The MB is important for integrating signals to control motivational states (Tsao et al., 2018) and it is important to note that there are signals beyond Dilp that act on the MB to modulate food-seeking behaviour. The endogenous compounds Neuropeptide F (NPF), short Neuropeptide F (sNPF), and serotonin are all compounds that act on the MB to increase food-seeking behaviour, while suppression of feeding can result from Dilp and Allatostatin-A (Tsao et al., 2018). These act in combination on different cells in the MB regulate foraging and the ingestion of food (Figure 1.1).

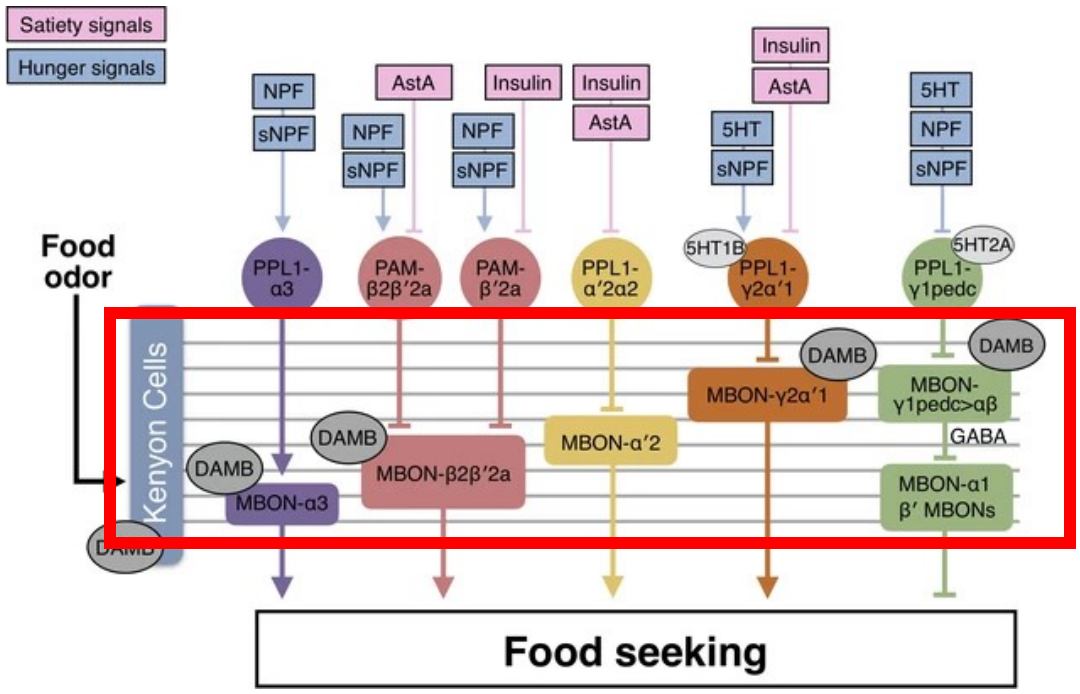


Figure 1.1 Figure modified from (Tsao et al., 2018), demonstrating how the different hunger and satiety signals interact and combine their effects on separate cell lines in the Mushroom Body to modulate responses to odors and food seeking behaviour in *Drosophila*. The red rectangle indicates cells present in the MB.

1.1.2 Feeding Initiation: Vertebrates

For many vertebrates, including humans and rodents, feeding motivation and initiation are similar to *D. melanogaster* at a theoretical level. Peripherally located receptors can sense a reduced energy or nutritional state and send signals (e.g. via insulin) to the central nervous system (Camilleri, 2015), increasing odorant sensitivity (Prud'homme et al., 2009), and food-seeking behaviour (e.g. hyperactivity, (Pirke et al., 1993)). This food seeking motivational state ultimately results from the brain's integration of a diverse array of molecular signals with many of the signals acting via distinct neural circuits and representing different factors contributing to feeding motivation (Berthoud et al., 2017). Among the best studied of these compounds in rodents and humans are insulin, ghrelin, and serotonin (Gruninger et al., 2007). Insulin informs on the energy availability of the organism (Roh et al., 2016), ghrelin on the volume of stomach contents (Powley and Phillips, 2004), and serotonin signaling can provide information on potential competing motivational states (Gruninger et al., 2007). As in *D. melanogaster,* these diverse, multimodal signals for feeding motivation follow different neuronal pathways to integrate into the brain, however the mechanics of this integration remain poorly understood (Berthoud et al., 2017).

1.1.3 Satiation: Drosophila

Once food is discovered and feeding is initiated, much of the behaviour is governed by the *D. melanogaster*'s Subesophageal Zone (SEZ) which is directly connected to the motor neurons involved in feeding (McKellar, 2016). The SEZ contains Central Pattern Generators (CPGs) that control the rhythmic, stereotyped movements involved in feeding (Sebastian Hückesfeld et al., 2015). Feeding is maintained by neurons in the PI that induce further feeding in response to nutrient ingestion, creating a positive feedback loop and perpetuating the rhythmic feeding activity of the flies on nutritional food over non-nutritional, but sweet, food. (Dus et al., 2015) Activation of these neurons alone can directly induce the full cycle of proboscis extension, the motor feeding activity of adult flies. The PI also induces gut movement in response to this ingestion, ensuring that as more food is ingested it moves through the digestive tract and is excreted (Dus et al.,

2015). There are also negative feedback mechanisms that accumulate to eventually terminate feeding.

Satiation is the cessation of nutritionally motivated feeding from the accumulated effects brought about by the act of feeding, including energy and nutritive content of food, volumetric feedback from the digestive system, and hormonal signals (adapted from (Bellisle et al., 2012). It is less well understood than a positive feeding motivational state. Feeding initiation is easier to induce noninvasively (via food deprivation) compared to the cessation of feeding, and this probably explains why feeding initiation is better understood.

In *D. melanogaster* satiation signals from the foregut are transmitted to the feeding center of the SEZ via the recurrent nerve. (Pool et al., 2014). Severing the recurrent nerve in both fruit flies (Pool et al., 2014) and blowflies (Dethier and Gelperin, 1967) leads to significantly increased food consumption irrespective of the carbohydrate, protein, or salt content of the food, and its connection between the foregut and feeding motor centers allows for rapid sensing of ingested contents and regulation of feeding motor behaviour.

However, the rest of the nervous system is still key for overall governance of feeding behaviour. The SEZ, and specifically its feeding motor CPGs, receive input from other brain regions, including the MB, and these connections can modulate the rhythm of firing for the motor CPGs and the feeding movements they control (Sebastian Hückesfeld et al., 2015). Specifically, the MB's influence is necessary for healthy feeding behaviour, as temporary inactivation of the MB significantly reduces food intake while they are inactive (Zhao and Campos, 2012), yet the mechanism of this influence is still unknown. As shown above, there are multiple endogenous neuropeptides and cellular pathways whose input is ultimately integrated within the MB's to influence feeding motivation.

1.1.4 Satiation: Vertebrates

For many vertebrates, feeding satiation is similar to *D. melanogaster* at a theoretical level. Feeding motor movements in vertebrates are governed in by central nervous system CPG's controlling mouthpart muscles (Lund et al., 1998). Once feeding begins, it

is maintained through a positive feedback loop generated by mechanoreceptors in the mouth (Lavigne et al., 1987). As food moves into the digestive system, stretch receptors in the stomach respond to the volume of the food and nutrient sensing receptors in the intestines respond to the nutrition and energy content of the food, and both begin to send satiation signals to the brain (Powley and Phillips, 2004). The brainstem, which houses the CPG's for feeding motor behaviour in many vertebrates, also has neurons capable of directly sensing circulating nutrient content in blood and subsequently inhibiting feeding (Blouet and Schwartz, 2012). The blood nutrient content and gut-derived satiation signals accumulate, leading to the end of feeding. Like *D. melanogaster*, several endogenous signals influencing feeding motivation and satiation have been identified in many vertebrates, but the mechanics of this integration and how this could influence individual aspects of their feeding behaviour remains poorly studied. Feeding motivation is suppressed under conditions other than nutrient sufficiency, however, and learning more about those could give insight into how this vital process occurs in the brain.

1.2 ENDOGENOUS FEEDING INHIBITION COMPOUNDS IN INSECTS

In addition to those detailed above, several more endogenous anorectic hormones have been identified and connected to physiological states or contexts for *D. melanogaster*. Allatostatin-A (AstA in Figure 1.1) has a role in regulating developmental hormone's synthesis and release (Stay and Tobe, 2007) signals feeding inhibition (Chen et al., 2016; Hergarden et al., 2012). Leucokinin signals the volume of gut contents and is expressed in neurons in the SEZ and those projecting to the MB, inhibiting its synthesis causes overeating (Al-Anzi et al., 2010; Itskov and Ribeiro, 2013). Hugin is a protein involved in developmental and food chemosensory signaling in *D. melanogaster*, is expressed in the SEZ and receptors are expressed in the MB, and overexpressing hugin reduces feeding (Melcher and Pankratz, 2005; Bader et al., 2007). Dopaminergic circuits have a well-conserved but complex role in motivation for *D. melanogaster* (Wise, 2004), and

increasing systemic dopamine concentration can inhibit or increase feeding depending on the nutritional status of the fly (Burke et al., 2012).

In the honey bee *Apis mellifera*, endogenous anorectic hormones have also been identified. Feeding inhibition can be induced through application either Queen Mandibular Pheromone, released by queen bees to ensure proper colony formation (Slessor et al., 2005), or Brood Pheromone, which coordinates social behaviours within a colony (Pankiw, 2004; Pankiw and Page, 2003). As with *D. melanogaster*, increasing systemic dopamine concentration will also inhibit feeding in bees (Scheiner et al., 2002). As well, increasing systemic insulin concentration can inhibit feeding, however in the honey bee whether or not this does so depends on the bee's age (Mengoni Goñalons et al., 2016). Juvenile Hormone, a hormone involved primarily in control of developmental state in insects (Riddiford, 1994), can also reduce feeding depending on the age of the bee (Pankiw and Page, 2003).

More endogenous anorectic hormones have been identified in the larvae of two model *Lepidopteran* species. In the silkworm *Bombyx mori* the hormone Myosuppressin has a developmental role and inhibits muscle cell activation (Tanaka, 2016), and increasing systemic concentration of it will reduce feeding (Nagata et al., 2011). Bombyxin, a peptide in *Bombyx* that is structurally and functionally analogous to insulin, also reduces feeding in the silkworms as its concentration is increases (Masumura et al., 2000). In the tobacco hornworm *Manduca sexta* increased concentration of systemic octopamine inhibit feeding (Ismail and Matsumura, 1992). Octopamine is a neurohormone for invertebrates thought to be analogous to norepinephrine for vertebrates (Pflüger and Stevenson, 2005), and it is elevated in response to stress (Harris and Woodring, 1992) or illness (Adamo, 2005).

1.3 NON-NUTRITIVE & VOLUMETRIC FEEDING CESSATION

1.3.1 Illness Induced Anorexia

D. melanogaster reduces or ceases feeding while mounting an immune response, referred to as 'illness-induced anorexia'. Though it may seem counterintuitive to limit energy intake during infection, this behavioural response has repeatedly been shown to increase their ability to fight off infections (Bashir-Tanoli and Tinsley, 2014; Ayres and Schneider, 2009). Illness-induced anorexia has been identified across other insect phyla as well, such as the caterpillar *Manduca sexta* (Adamo et al., 2006), crickets (Sullivan et al., 2016) and honey bees (Kazlauskas et al., 2016), though notably bumblebees increase their feeding in response to infection (Tyler et al., 2006). This behavioural response in insects appears to be mediated in part by an increase in the systemic concentration of hormones that suppress feeding. In *D. melanogaster* infection can increase concentration of some Dilps (Sung et al., 2017), which reduces feeding as discussed above, and in *M. sexta* there is an increase in octopamine levels (Adamo, 2005), which decreases their feeding (Ismail and Matsumura, 1992). Evidence in *M. sexta* suggests this behaviour is adaptive, because it helps resolve a conflict between food detoxification and the immune system's shared use of a limited molecular resource, which for *M. sexta* is glutathione (GSH) (McMillan et al., 2018). The immune system's utilization of GSH reduces *M. sexta*'s capacity to detoxify food, making feeding potentially dangerous-force feeding lipids to caterpillars experiencing a bacterial infection reduces their survival (Adamo et al., 2006). However, a neuronal circuit mediating the specific illness-induced behaviour has not been identified in any insect. Illness-induced anorexia is also present across vertebrate species, and appears in every vertebrate species tested for the behaviour thus far (Adelman and Martin, 2009; Murray and Murray, 1979). For vertebrates, this behaviour is in part mediated by the immune system's release of cytokines, which bind to target neurons in the brain to inhibit feeding (Dantzer, 2004).

1.3.2 Molting

D. melanogaster and other insect larvae also cease feeding when approaching the start of their developmental molt between larval instars (Nijhout et al., 2014). Molting is when insect larvae shed the exoskeleton of their previous instar to grow a new, larger one for the next instar. The behavioural and physiological of the molting process are mediated by a group of hormones known as ecdysteroids (i.e. ecdysone) (Yamanaka et al., 2013). Ecdysteroids bind to ecdysteroid receptors on neurons located throughout the body and central nervous system of insect larvae (Truman et al., 1994b). Specifically, there are ecdysteroid receptors in the mushroom body (Lee et al., 2000) and subesophageal zone (M. Schubiger et al., 1998) of *D. melanogaster* larvae, regions respectively involved in feeding motivation and feeding motor behaviour. In larva of the silkworm moth *Bombyx mori*, feeding them an endogenous ecdysteroid is enough to reduce feeding (Tanaka et al., 1994). Pre-molt feeding cessation has been studied in additional insect model organisms such as *M. sexta* (MacWilliam et al., 2015) and honey bees (Michener, 1974), as well as many lepidoptera (Barbehenn and Keddie, 1992). Feeding cessation arises because during the molt they shed most of the inner gut lining (i.e. peritrophic layer), and this is safest for the larva if the gut is empty of food (Waldbauer, 1968). For example, in *M. sexta* it's been shown that shedding the peritrophic layer exposes the layers beneath it, like the epithelial layer, which are more vulnerable to infection and if infected present a higher risk of spreading the infection to the rest of the organism (Russell and Dunn, 1996; Russell and Dunn, 1991). To reduce the risk of infection, the *M. sexta* larvae cease feeding prior to beginning the molt, emptying their gut via defecation and reducing the potential for any lingering pathogens once the loss of gut lining takes place. In *M. sexta* larvae this feeding cessation is confirmed as a behavioural change and not a consequence of any physical inability to eat. Exoskeleton shedding in *M. sexta* starts by displacing their previous head capsule and mandibles, which physically prevents feeding, however the cessation behaviour occurs prior to this and while their mandibles are still functional (MacWilliam et al., 2015; Bestman and Booker, 2003). Vertebrate species do not have an equivalent to the molting behaviour present in larval insects. Compared to satiation or

illness-induced anorexia in insects, the pre-molt reduction in feeding motivation empties their gut to reduce likelihood of infection when shedding the inner gut lining.

1.3.3 Predator Stress

Another condition that can induce feeding cessation in insects is the stress resulting from a predator's presence or perceived presence. Feeding in invertebrates is a very risky activity when a predator is nearby-*M. sexta* larvae are at threefold greater risk of being killed by a wasp predator while feeding compared to resting (Bernays, 1997), and in another caterpillar *Uresephita reversalis*, feeding increased the risk of predation a hundred-fold (Bernays, 1997). In the cricket *Gryllus texensis*, exposure to a mock predator can induce freezing, or even flight if the attack is perceived as imminent (Adamo et al., 2013). In addition to this immediate cessation of feeding, predator stress can induce a long-term reduction in feeding for insects. For *M. sexta*, the presence of a predator can reduce leaf consumption by up to 32% over the course of 24 hours in young larvae (1st to 3rd instar) (Thaler et al., 2014). This has been particularly well studied in the A*plysia* sea slugs (Kupfermann and Weiss, 1981) where a tail pinch leads to a reduced consumption feeding period, and the same response to a tail pinch has been found and studied in rodents (Antelman et al., 1975). However, in larger *M. sexta*, food consumption is not significantly reduced by predator stress, although weight gain is (Adamo et al., 2017), suggesting a possible complex effect on feeding. In insects the reduction in feeding during an immune challenge is partly mediated by the neurohormone octopamine, with systemic levels rising in response to predator stress for both *M. sexta* and *G. texensis*, (Adamo and Baker, 2011; Adamo et al., 2013; Adamo et al., 2017).

1.4 MANDUCA SEXTA

1.4.1 Neuroanatomy

To study the states of feeding cessation in insects, this experiment will use the larvae, also known as caterpillars, of the hawkmoth *M. sexta,* which possess a variety of

advantages for the study of feeding behaviour and cessation. As with *D. melanogaster*, several relationships between certain neuronal regions and aspects of feeding behaviour have already been characterized in *M. sexta*. Three specific regions' relationships to feeding behaviour have been focused on: the suboesophageal ganglion (SEG), analogous to the *D. melanogaster* SEZ, the frontal ganglion (FG), and the brain (Br, referred to as the supraesophageal ganglion in some studies). A simplified version of the neuronal connections between these 3 regions is shown in Figure 1.2.

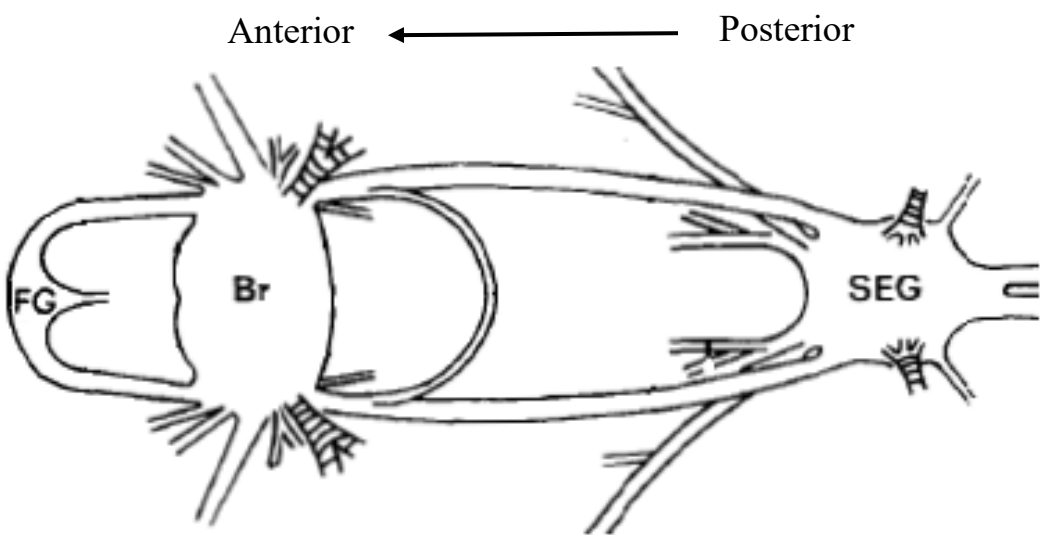


Figure 1.2 Connections between key nervous system areas in M. sexta feeding behaviour. Frontal Ganglion (FG), Brain/Supraesophageal Ganglion (Br), and Suboesophageal Ganglion (SEG), (Griss et al., 1991). Scale bar is 100 um

The SEG is connected to and directly controls *M. sexta's* mandibular muscles and is responsible for biting. So long as the SEG remains connected to the mandible muscles and peripheral nerve inputs, even if every other connection is severed, the caterpillar is still fully capable of chewing, though no actual ingestion occurs (Griss et al., 1991). When isolated, the SEG can generate a spontaneous firing rhythm, however the timing of this rhythm does not match the timing of biting, so this rhythm alone is not responsible for biting in healthy caterpillars (Bowdan and Wyse, 2000). The addition of some neurohormones like octopamine can modify this firing pattern. The FG is connected to the buccal (swallowing) muscles of the caterpillar, as well as muscles in the foregut, as severing its connections prevents both food swallowing and foregut peristalsis (Miles and Booker, 1998; Miles and Booker, 1994; Griss et al., 1991). The caterpillar will still bite food if the FG is removed, but it can't swallow the food or move it through the foregut, making its activity necessary for feeding, even if it doesn't control biting itself. The brain in *M. sexta* is thought to provide a regulatory role, allowing or inhibiting the activity of other feeding regions. When its connections to these regions are cut, the caterpillars are still fully capable of feeding and ingesting, however they will eat less mass and take longer to do so (Griss et al., 1991).

There is also evidence of a similar role for the larval *Manduca sexta* mushroom bodies that were discussed for *D. melanogaster* (Figure 1.1). Interneurons in the larval *M. sexta* mushroom bodies appear to integrate multiple different sensory inputs (Itagaki and Hildebrand, 1990) and other cells in the mushroom bodies receive ecdysteroid hormones signaling developmental state (Truman et al., 1994a). However, much about the mushroom bodies in larval *M. sexta* remains unknown, so it is uncertain how far the similarities or differences extend between their mushroom bodies and those of *D. melanogaster.*

1.4.2 Feeding Behaviour

The drive to eat predominates *M. sexta* behaviour. Feeding lacks a circadian rhythm in this species, and they feed frequently throughout the day (Reynolds et al., 1986; Bernays and Woods, 2000). The caterpillars feed often, increasing nearly 10,000-fold in mass

from hatching until, an average of 19 days later, the larval stage ends (Grunert et al., 2015; Kingsolver, 2007). *M. sexta* are also specialist feeders, feeding almost exclusively on the *Solanaceae* family of plants (Yamamoto, 1974; Madden and Chamberlin, 1945) and often spending their entire larval stage feeding on the plant their egg was laid on (Bernays and Woods, 2000).

Elements of *M. sexta* larval feeding behaviour have already been studied, particularly with a focus on the 'microstructure' of their feeding. Feeding microstructure are the details that best represent the motor processes underlying them, such as the force, time in the feeding period, and the time to the next bite for each bite in the meal. This contrasts with the feeding's 'macrostructure' which includes the total duration of a feeding period, or the total mass consumed in a certain amount of time, useful information but not sufficiently detailed to provide insight on the moment-to-moment neuronal events occurring throughout a feeding period. Previous characterization of feeding microstructure demonstrates a positive relationship between the mass of a caterpillar and their frequency of bites within a feeding period, as well as the duration of feeding (Bowdan, 1988a). Their feeding microstructure is also influenced by food deprivation, with food deprived caterpillars biting more frequently and having shorter pauses between groups of bites during the first post-deprivation feeding period (Bowdan, 1988b; Timmins and Reynolds, 1992). Addition of a phagostimulant (sucrose) to the caterpillar's food increased bite frequency, total feeding duration, and reduced the duration of pauses between groups of bites, but a deterrent (quinine) only reduced the total feeding duration and did not have an impact on any microstructure elements (Bowdan, 1995). While these studies provide a useful basis for understanding some aspects of *M. sexta* feeding, no study to date has focused on how this feeding microstructure may change as the caterpillar approaches feeding cessation.

1.4.3 Satiation

M. sexta are also advantageous for studying feeding cessation behaviour, particularly compared to other insects (e.g. *D. melanogaster*), because of the few physiological feedback signals determining their satiation (Simpson and Bernays, 1983). In a prior

study *M. sexta* appetite was not reduced by injecting wax into their foregut suggesting that, in contrast to other insects, they do not utilize volumetric feedback as a cessation signal (Timmins and Reynolds, 1992; Simpson and Bernays, 1983). The same study showed their feeding was not reduced through altering the osmotic balance of the gut contents, again eliminating that as a cessation signal, and again this contrasts with previous findings on other insects (Simpson and Bernays, 1983). It was ultimately shown that only adding diet extract directly into the gut of the caterpillars would make them wait longer before feeding again and decreased the amount eaten (Timmins and Reynolds, 1992). This suggests that nutrient content alone in the gut provides the feedback signal to induce *M. sexta* feeding cessation, in contrast to the complex, multimodal signaling found in many other animals (Simpson and Bernays, 1983; Toates, 1983).

1.5 MEASURING FEEDING MOTIVATION

Animal feeding is separated into groups of individual bites called bouts, which are then separated by a slightly longer pause before biting resumes. In general as animals show reduced motivation to feed, determined by how close to feeding cessation they are, the average length of time between feeding bouts increases. Satiation is often determined experimentally by a certain amount of time the animal spends not feeding. Inter bout interval has been negatively correlated with feeding motivation in animals as diverse as cows (Tolkamp et al., 1998; Greter, 2012), zebra finches (Slater, 1974), *Aplysia* (Susswein et al., 1978), and fortunately for my experiment, larval *Manduca sexta* (Bowdan, 1988a).

In several vertebrate species, feeding motivation has been linked to the biting force applied by their feeding, with higher force representing higher motivation to feed and under the assumption that food deprived animals have higher feeding motivation. In animals as diverse as geckos (Anderson et al., 2008), horn sharks (Huber et al., 2005), Komodo dragons (D'Amore et al., 2011), finches (van der Meij, M A and Bout, 2006), and in humans. (Frecka et al., 2008) While bite force has been linked to aggression hierarchy (Lailvaux et al., 2011; Condon et al., 2016) and the hardness of the food being

eaten (Weihmann et al., 2015) in some invertebrates, no study has yet examined it in the context of feeding motivation. A review of the literature has in fact found no study on any animal examining bite force over the course of a feeding period, to see how it may change as the animal approaches cessation. Bite force will be included in the studies of *M. sexta* feeding microstructure as an additional means of testing motivational differences between the cessation contexts.

Further study of feeding cessation is necessary for a complete picture of feeding behaviour and motivation (Toates and Booth, 1974; Toates, 1983). Feeding motivation in invertebrates, specifically insects, could form a simple and accessible model of motivated behaviours in animals (Dethier, 1964; Kupfermann, 1974; Toates, 1983; Simpson and Bernays, 1983). Reduced feeding motivation for insects occurs in satiation, illness, or when approaching a molt, and the motivational changes for each of these conditions are partially mediated by distinct molecular and neuronal signaling pathways. The objective of this research is to determine whether these different motivational states create observable changes in feeding microstructure relative to one another. This can provide a window into the mechanism of motivational control of behaviour in insects (Kuslansky et al., 1987; Bowdan, 1992).

1.6 PREDICTIONS

I predict that in larval *M. sexta* the average time between feeding bouts and average force applied from biting their food will change over the course of a meal. I also predict that this change in inter-bout-interval and average force while feeding will be different depending on the feeding motivation state of the caterpillar.

In healthy caterpillars approaching nutritive satiation, I predict that food consumption will increase gut and systemic nutrient content and lead to a decrease in feeding motivation as feeding progresses. From this, I predict that the average bite force applied within a meal will be highest at the beginning of the meal because of the increased motivation that accompanies feeding initiation. I further predict that the average bite

force will decrease as the meal progresses, until the average bite force reaches its lowest point at the end of the meal, reflecting the decrease in feeding motivation that accompanies satiation. I also predict that as a consequence of the changing feeding motivation throughout the meal, the nonfeeding interval between bouts will increase as the caterpillars approach satiation.

In immune-challenged caterpillars, I predict that the need to conserve resources shared between their immune response and food detoxification put them in a state of decreased feeding motivation even at the beginning of a meal. From this, I predict that the average bite force will begin at a lower level compared to that observed at the beginning of the meal in healthy caterpillars. Bite force will continue to be a low throughout the meal with little variance, reaching its lowest average bite force as the meal is ending. This low variance in average bite force over the course of the meal, and overall lower average bite force compared to healthy caterpillars approaching satiation represent the animal's overall reduced feeding motivation. I further predict that the change in motivation of immune challenged caterpillars will lead to a longer inter-bout-interval at the start of feeding compared to the beginning of the meal in healthy caterpillars approaching satiation. I predict that the average interval will continue to be longer throughout the meal with little variance, reaching the longest average time between bouts as the meal is ending.

In caterpillars approaching their developmental molt, I predict the combination of developmental signals to ensure timely gut emptying and the feedback from nutritional intake leads to a sharper and more sudden decline in feeding motivation. From this, I predict that the average bite force within a meal will begin at a higher level, similar to the beginning of the meal for healthy caterpillars, but then the average bite force will decrease more rapidly as the meal progresses compared to healthy caterpillars approaching satiation, at the end reaching a lower average bite force than the end of the meal for the healthy caterpillars. This difference compared to healthy, but sated caterpillars would reflect the high feeding motivation as the meal is initiated, but rapid reduction in motivation as the caterpillar approaches the point where its gut must be cleared in order to molt. I further predict that the change in motivation of caterpillars

approaching their molt will induce a shorter inter-bout-interval at the start of feeding, like the beginning of the meal in caterpillars approaching satiation. I predict that the average interval will lengthen more rapidly as the meal progresses compared to healthy caterpillars reaching the longest average time between bouts as the meal is ending.

Lastly, in caterpillars exposed to acute predator stress, I predict there will be an initial reduction in motivation when they begin feeding to avoid predator detection, but that feeding motivation will rise as feeding continues without another predator strike, then fall at the end of the meal because result of nutritional feedback signals. From this I predict that in caterpillars feeding immediately after acute predatory stress, the average bite force applied by feeding will begin at a lower value compared to the non-stressed equivalent group. I predict bite force will then increase towards the middle of feeding to a similar average bite force as the non-stressed equivalent, before decreasing to the same ending value as the non-stressed group. This difference compared to non-stressed groups would reflect the initial hesitation to attract predator attention by feeding. I further predict that in caterpillars feeding immediately after acute predatory stress, the average inter-bout-interval will be longer compared to the non-stressed equivalent group. I predict that it will shorten towards the middle of feeding to a similar interval as the non-stressed equivalent, before lengthening to the same ending value as the non-stressed group.

CHAPTER 2 MATERIALS AND METHODS

2.1 ANIMALS

All experiments utilized 4th instar larval *Manduca sexta* caterpillars from the Adamo lab colony. The colony was established from eggs from Great Lakes Hornworm (https://www.greatlakeshornworm.com, MI, USA). Prior to experimentation, caterpillars were fed ad libitum on an artificial wheat germ diet specifically designed for *M. sexta* supplied by Great Lakes Hornworm.

For all experiments, except the molt-sleep experiment (described below), 4th instar, day 2 (4-2) caterpillars were massed and placed into groups based on weight. This was done to ensure that none of the groups differed on average initial weight, which can influence feeding microstructure (Bowdan, 1988a).

I assessed the effect of four distinct types of feeding inhibition on the microstructure of feeding. I tested the effect of: 1) satiation, 2) illness induced anorexia, 3) molt sleep, and 4) predatory stress, on the detailed structure of feeding.

2.2 EXPERIMENT 1: Effects of Satiation on caterpillar feeding force and microstructure

2.2.1 Prior to Meal Recording

After being assigned to the satiation group, caterpillars were initially observed for up to 1 hour or until they finished a meal to ensure that they were healthy (e.g. deep blue colour, not flaccid) and currently feeding, then returned to their container. Caterpillars were left to feed ad-libitum on their food for 2 hours, and then food deprived for 1 hour to normalize their gut contents going into the feeding trial. Differences in gut nutritional content can affect feeding microstructure (Bowdan, 1988b). Deprivation longer than 1 hour can have long-lasting effects on feeding and feeding microstructure. At the end of

the hour with no food (3 hours post-treatment), caterpillars were placed before an $8mm^3$ block of wheat germ food attached to a force transducer (described below). Each time the caterpillar bit into the food, it would displace the force transducer, making an automatic recording of each bite. This is a novel method for measuring feeding behaviour that I developed for this experiment.

2.2.2 Meal Recording

The caterpillars were left to feed until 2 full meals had been recorded. A meal was defined from previous studies on *M. sexta* feeding microstructure as continuous feeding, with no nonfeeding period greater than 2 minutes long (Bowdan, 1984). Once 2 minutes of nonfeeding were recorded, a meal was considered over, and voluntary meal termination is confirmation of the caterpillar's nutritionally satiated state. Two meals were recorded because prior research and personal observation found that the microstructure of a meal immediately following one hour of food deprivation has microstructure differences compared to meals recorded during ad-lib feeding (Bowdan, 1988b). Data for comparison between caterpillars were derived from this second meal for all groups in the study, except for those in the molt-sleep condition (described below).

2.3 EXPERIMENT 2: Effects of Illness Induced Anorexia on Caterpillars Feeding Force and Microstructure

2.3.1 Prior to Meal Recording

After being assigned to the immune-challenged group, caterpillars were initially observed for up to 1 hour or until they finished a meal to ensure that they were healthy (e.g. deep blue colour, not flaccid) then an immune challenge was applied. Immune challenge injections were performed with a sterile 10 µL Hamilton Syringes (Model 701, Hamilton Company, Reno, NV). Immune challenge animals were injected with a 10 µL mixture of heat-killed *Bacillus cereus* (Gram-positive bacterium, Microkwik culture, Carolina Biological, Burlington, NC, USA), *S. marcescens* (Gram-negative bacterium, Microkwik

culture, Carolina Biological), and *Beauveria bassiana* (strain GHA, fungus, BotaniGard 22WP; Laverlam, Butte, MT, USA), with a final concentration of each at approximately 1/10 the LD50 of the live pathogens. The injection was made parallel to the body wall between the 6th and 7th abdominal segments. This mixture at this dosage and injection site has previously been shown to activate immune behavioural responses, including illness induced anorexia, in *M. sexta* caterpillars (McMillan et al., 2018).

As above, following treatment application, the caterpillars were fed ad-libitum for 2 hours, then were food deprived for 1 hour. At the end of that hour (3 hours post-treatment), caterpillars fed on an 8mm^3 block of wheat germ food placed on the force transducer (described below), as 3 hours following the initiation of an immune challenge is the peak of illness-induced feeding reduction in *M. sexta* (Adamo et al., 2006).

The separate sham injected group of caterpillars were treated the same as the immune challenged animals, except instead of injection of heat-killed pathogens, sham animals were poked with a sterile 10 uL Hamilton Syringe until cuticle was penetrated to mimic the immune challenge injection.

2.3.2 Meal Recording

As with the satiation experiment animals, data from the force transducer was recorded using Chart acquisition software (described below). The immune challenged and sham animals were similarly left to feed until 2 full meals had been recorded.

2.4 EXPERIMENT 3: Effects of Molt-Sleep on Caterpillar Feeding Force and Microstructure

2.4.1 Prior to Meal Recording

4th instar molt-sleep caterpillars were assigned to this group by estimating their proximity to molting from 4th to 5th instar larvae. By measuring the extent of cuticle apolysis around their 7th abdominal spiracle, a prediction for their time until head capsule slippage (HCS)

can be made (Langelan et al., 2000). HCS is a visually obvious event in the molting process in which the current head capsule is displaced to allow room for the new, larger head capsule of the next instar (Curtis et al., 1984). It has been shown that *M. sexta* show reduced feeding in hours approaching HCS, a period known as "molt sleep", after which they cease to eat until ecdysis (MacWilliam et al., 2015). Caterpillars were selected if apolysis measurements predicted HCS within 8 hours of measurement.

2.4.2 Meal Recording

The caterpillar's behaviour was recorded with the same software, force transducer and food block setup as the previous experiments. The force transducer data was recorded until no meals had occurred for 1 hour, at which point the caterpillar was removed and checked for mouthpart responsiveness to tactile stimulation. Unresponsive mouthparts are a marker for the final cessation of feeding that occurs before HCS (Bestman and Booker, 2003). The Chart recording was stopped regardless of whether the caterpillar's mouthparts were responsive. Those with responsive mouthparts at this point were not included in any analysis. Those with unresponsive mouthparts were placed in a separate container with food and observed until HCS occurred to ensure their feeding had ceased.

Data for the molt-sleep caterpillars were derived from the second-to-last meal recorded before the caterpillars were confirmed as entering molt-sleep. Personal observation showed significant variation in the microstructure of the final meal before entering molt-sleep, making it likely to be unreliable for comparison with other groups. During this time of this second-to-last meal prior to molt-sleep, the caterpillars still show both the physiological and behavioural signs of approaching molt-sleep (Langelan et al., 2000; MacWilliam et al., 2015).

2.5 EXPERIMENT 4: Effects of Acute Predator Stress on Caterpillar Feeding Force and Microstructure

Predatory stress appears to reduce feeding in M. sexta (Adamo et al., 2016). I tested whether the reduction in feeding results in a different microstructure compared to those without predator stress.

2.5.1 Prior to Meal Recording

4th instar, day 2 caterpillars were massed and, based on weight, placed into one of 6 treatment groups: immune challenged (IC), sham poked (Sham), handling control (Control), immune challenged and given acute predator stress (IC + Predator Stress), sham poked and given acute predator stress (Sham + Predator Stress), and handling control and given acute predator stress (Control + Predator Stress). As in the above experiments, following group placement they were observed for up to 1 hour to ensure that they were feeding, then had an initial treatment (IC, Sham, Control) applied. The IC and Sham caterpillars were respectively given heat-killed pathogen injection and sterile cuticle poke (both as above), while the Control caterpillars were handled the same as the Experiment 1 satiation caterpillars. Then they were fed ad libitum for 2 hours, food deprived for 1 hour, and behavioural recording began.

2.5.2 Meal Recording

To emulate a predatory attack the left 4th proleg of caterpillars in the predator stressed groups were gently squeezed with curved forceps (Fisherbrand™ Curved Medium Point General Purpose Forceps, Fisher Scientific, Waltham, Massachusetts, US.) 8 times consecutively, and of approximately equal force, over 20 seconds. This method has been previously validated as causing both behavioural (defensive strike) and physiological (increase in hemolymph octopamine levels) predator stress responses. (Adamo et al., 2017) The stimulus was applied following the caterpillar's first confirmed meal. The caterpillars in non-predator stressed groups were not handled following their first

confirmed meal. All caterpillars were recorded until a second full meal had been confirmed.

2.6 EQUIPMENT

Force measurements were made using an $8mm^3$ block of the caterpillar's food placed on a force transducer (MLT050/A, ADInstruments, Grand Junction, CO) connected via a bridge pod amplifier (ML110, ADInstruments) to a digital data acquisition system (ML760, ADInstruments). The force transducer's range was 0-50g with a post amplification sensitivity of 6.2 μV and LabChart range of -20 to +20 mV.

Caterpillars were placed on a plasticine ramp allowing them access to the food on the transducer without the need to climb up to it or on it. Plasticine walls and a clear plastic ceiling ensured the caterpillar stayed in position and reduced motion artifacts.

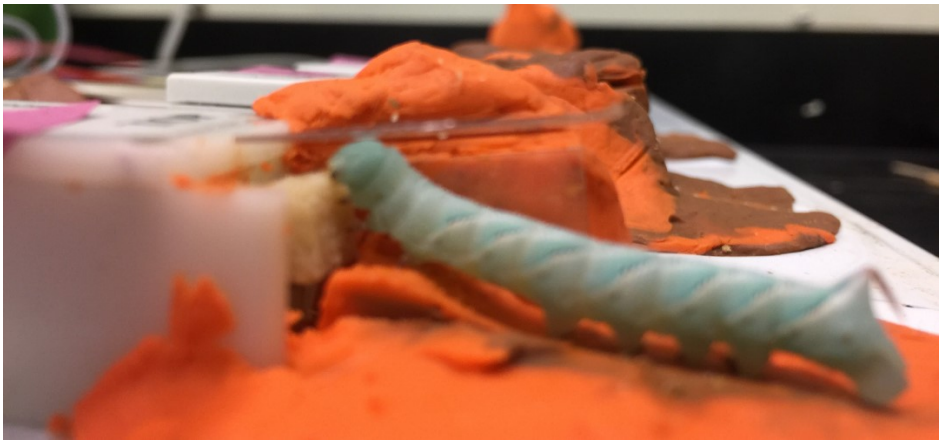


Figure 2.1 Picture of the force transducer setup utilized in the caterpillar feeding experiments. Half of the plastercine normally around the setup has been removed to show the position of the caterpillar.

2.7 DATA ACQUISITION

Data were acquired from behavioural recordings using the LabChart (Chart5 v5.5.6, ADInstruments). After trial-and-error on pilot data, the event detection settings best matching observed biting events were those using the 'Cyclic Measurements-Height' function with a minimum peak height of 1 Standard Deviation, an auto-leveling window of 1.15 s, and noise floor of 0.005. These settings accurately identified bites as they were visually observed during the LabChart recording, as well as line up with bites when compared to video recordings of the feeding behaviour. These settings, combined with the R code I developed for this purpose, described below, also did not erroneously identify force artifacts in the LabChart recording as bites.

This software produced data for the time between bites (in seconds) and the peak force amplitude (in mV) for each individual bite in a meal. I made the custom R code to group the bites into bouts, which are periods of continuing biting without pauses longer than a certain threshold. This minimum pause threshold was determined using previously established methods for determining bouts in feeding and other behaviours (Slater and Lester, 1982; Slater, 1974; Bowdan, 1988a). Briefly, the time between events (bites, in this case) from several recordings are graphed as a log survivor curve, and the inflection point of the curve is the minimum interval between bouts. The caterpillars for this study were found to have a minimum pause between bouts of 1.15s.

The R code searched chronologically through the bites to find any bites with a time until the next of 1.15 seconds or greater. That bite become the last in a bout including all previous bites that hadn't yet been grouped into a bout. The code then eliminated any bouts having only a single bite as feeding bouts always contain at least two bites.

Lastly to account for potential variation between food cubes' physical quality as a force conductor, as well as variation in individual caterpillar mass, the R code then standardized the bite amplitude data to within the individual meal, giving the lowest amplitude bite an arbitrary value of 10, and the highest amplitude bite a value of 100, and giving all other bite amplitudes a new value based on this scale.

CHAPTER 3 DATA ANALYSIS

3.1 INITIAL COMPARISON

Analysis was performed on the feeding microstructure elements. I did examine the broader elements describing meals (Time, Bite, and Bout totals), but no further analysis was performed. For example, a sample size calculation for Experiment 4 found that Time, Bite, and Bout would have required individual group sizes of 212, 186, and 199, respectively, to reach significance at an alpha of 0.05 and 80% power.

Table 3.1 Total number of bites summary for meals in Experiment 4. Minimum, average, maximum, and the standard deviation for the number of bites for all meals of a given condition within experiment 4 are represented.

Group	Minimum	Average	Maximum	SD	N
Satiation	43	465.53	1802	452.58	15
Sham	60	431.93	1522	415.54	15
IC	87	370.33	1746	417.06	15
Satiation + Pred Stress	64	456.80	3953	974.42	15
Sham + Pred Stress	78	306.53	1106	266.19	15
IC + Pred Stress	85	249.93	754	174.87	15

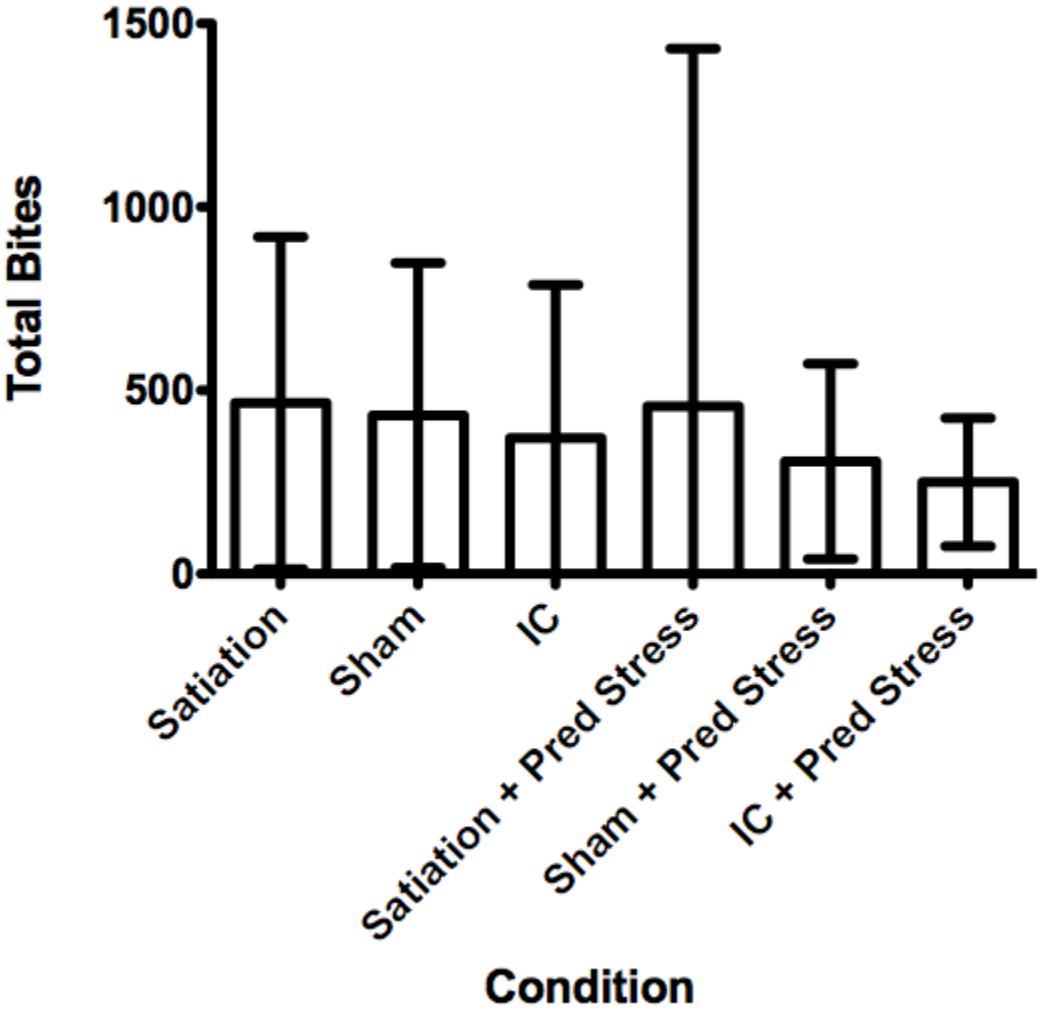


Figure 3.1 Effects of Satiation, Sham Injection, Immune Challenge, and the Predator Stress conditions on average total number of bites in a meal (n = 15 per group). The error bars represent the standard deviation for that condition.

Table 3.2 Total number of bouts summary for meals in Experiment 4. Minimum, average, maximum, and the standard deviation for the number of bouts for all meals of a given condition within experiment 4 are represented.

Group	**Minimum**	**Average**	**Maximum**	**SD**	**N**
Satiation	13	86.87	344	92.21	15
Sham	10	68.07	170	58.57	15
IC	10	59.13	287	69.63	15
Satiation + Pred Stress	12	65.07	442	21.69	15
Sham + Pred Stress	14	41.93	94	21.18	15
IC + Pred Stress	12	39.60	113	24.17	15

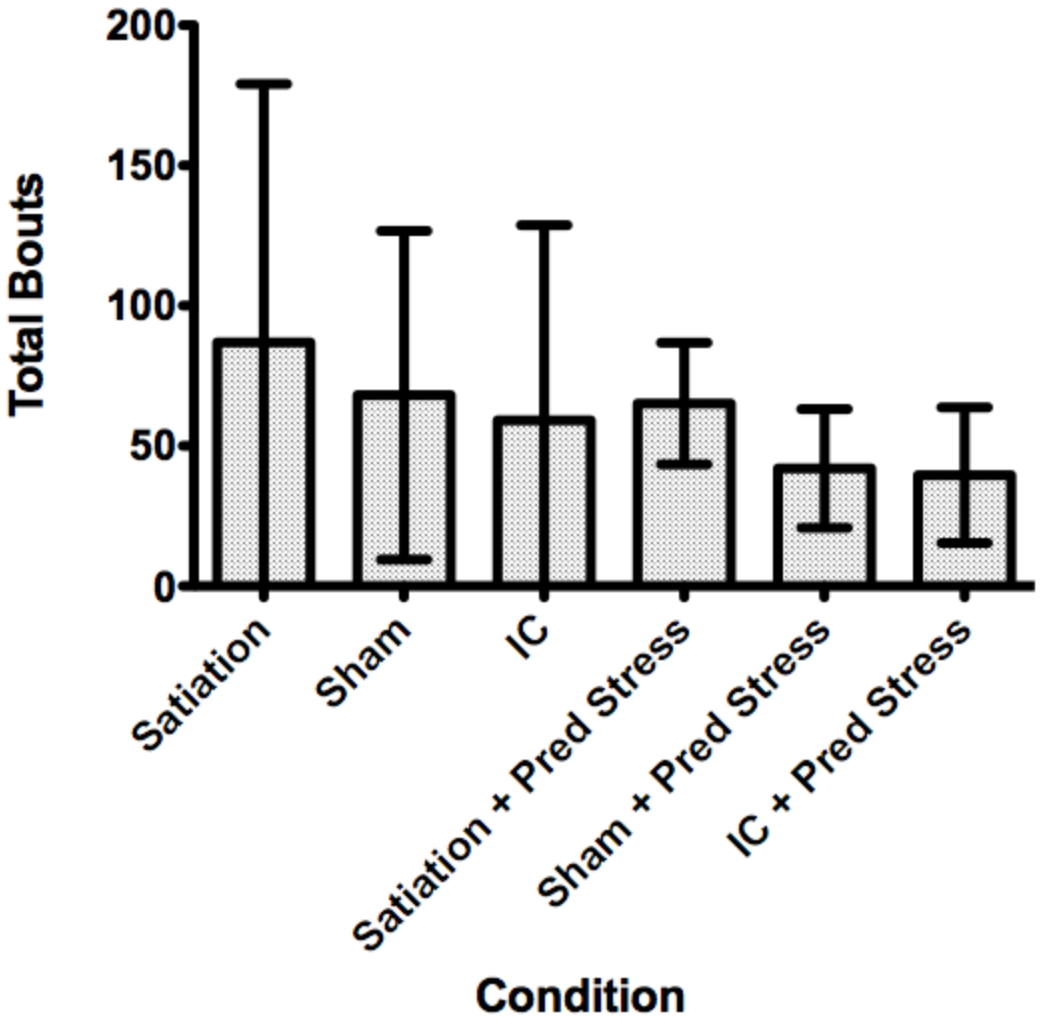


Figure 3.2 Effects of Satiation, Sham Injection, Immune Challenge, and the Predator Stress conditions on average total number of bouts in a meal (n = 15 per group). The error bars represent the standard deviation for that condition.

Table 3.3 Total meal duration (in seconds) summary for meals in Experiment 4. Minimum, average, maximum, and the standard deviation for the number of bouts for all meals of a given condition within experiment 4 are represented.

Group	**Minimum (s)**	**Average (s)**	**Maximum (s)**	**SD**	**N**
Satiation	99.65	506.10	1699.74	429.45	15
Sham	66.63	450.89	1312.51	409.07	15
IC	91.30	470.52	2305.47	551.76	15
Satiation + Pred Stress	73.51	506.56	3548.14	385.51	15
Sham + Pred Stress	66.30	347.78	1212.08	280.24	15
IC + Pred Stress	129.45	320.40	791.96	183.46	15

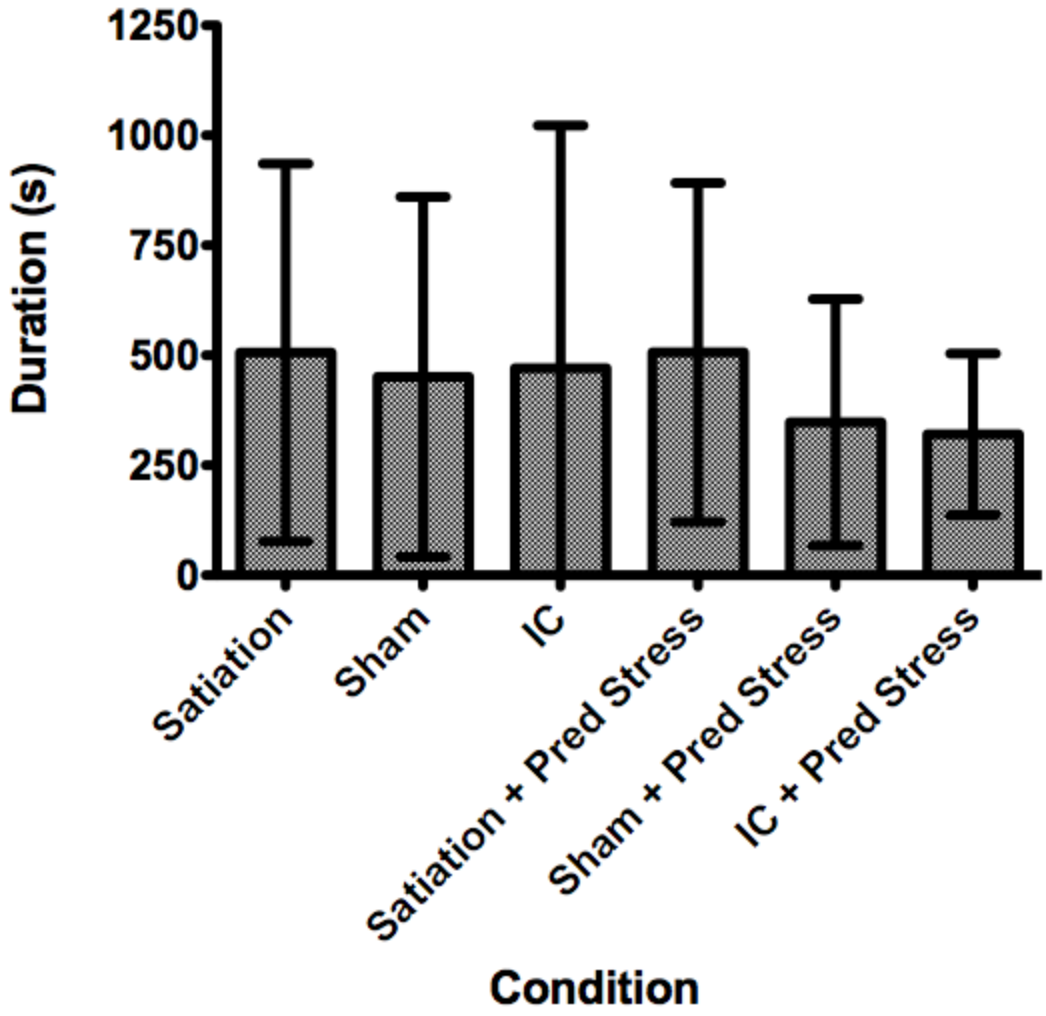


Figure 3.3 Effects of Satiation, Sham Injection, Immune Challenge, and the Predator Stress conditions on average total duration of a meal in seconds (n = 15 per group). The error bars represent the standard deviation for that condition.

The variability of these measures and high group total required for analysis suggests low accuracy in using them to identify which condition a meal came from. Examining the microstructure of a meal could provide more accuracy and nuance for the effect of each condition on feeding behaviour.

3.2 BOUT MICROSTRUCTURE AND VARIABLES

The timing of bites within a meal (in seconds), the time between bites (in seconds), the and transformed amplitude of force applied by the caterpillar bites (in arbitrary units) were used to describe this microstructure. Using a custom MatLab code I developed for this experiment, the following variables were generated by the calculations described:

Bites per second: Number of bites in the bout divided by the duration of the bout

Bout duration: Time between first bite in a bout and last bite in a bout.

The above variables have been utilized before to characterize feeding in *Manduca sexta*. (Bowdan, 1988b)

A review of the literature found no studies comparing changes in feeding force over the course of a meal, and therefore no previously used variables to describe it. Therefore, the following were developed for this study:

Minimum: Amplitude of the lowest amplitude bite in the bout

Mean: Average amplitude of all bites in the bout

Maximum: Amplitude of the highest amplitude bite in the bout

Range: Maximum amplitude of the bout minus the minimum amplitude of the bout

Max – Mean difference: Maximum amplitude of the bout minus the mean amplitude of the bout

Max – Min ratio: Maximum amplitude of the bout divided by the minimum amplitude of the bout

Max – Mean ratio: Maximum amplitude of the bout divided by the mean amplitude of the bout

An objective method was needed to compare these variables across the time course of a meal and how they may or may not change, as well as comparing between meals from different animals. From pilot data, the lowest number of bouts in a meal was found to be 9. Therefore the "start" time section, representing microstructure at the initiation of a meal, was defined as the first 3 bouts in the meal. The "end" time section, representing microstructure at the termination of a meal, was defined as the final 3 bouts in the meal. To determine the "middle" time section, representing microstructure at a section between the meal's initiation and termination, I would take the total number of bouts in the meal and divide it by two. The resulting bout number would become the centermost bout. The "middle" time section would be this bout, the bout before it, and the bout after it. In cases where the total number of bouts was odd, making the total divided by two a non-integer, an arbitrary rule was put in place beforehand where the centermost bout was determined by rounding this number up.

3.3 EXAMPLE OF TIME SECTION CALCULATION

If a meal has 18 bouts

"Start" is bouts 1, 2, and 3

"End" is bouts 16, 17, and 18

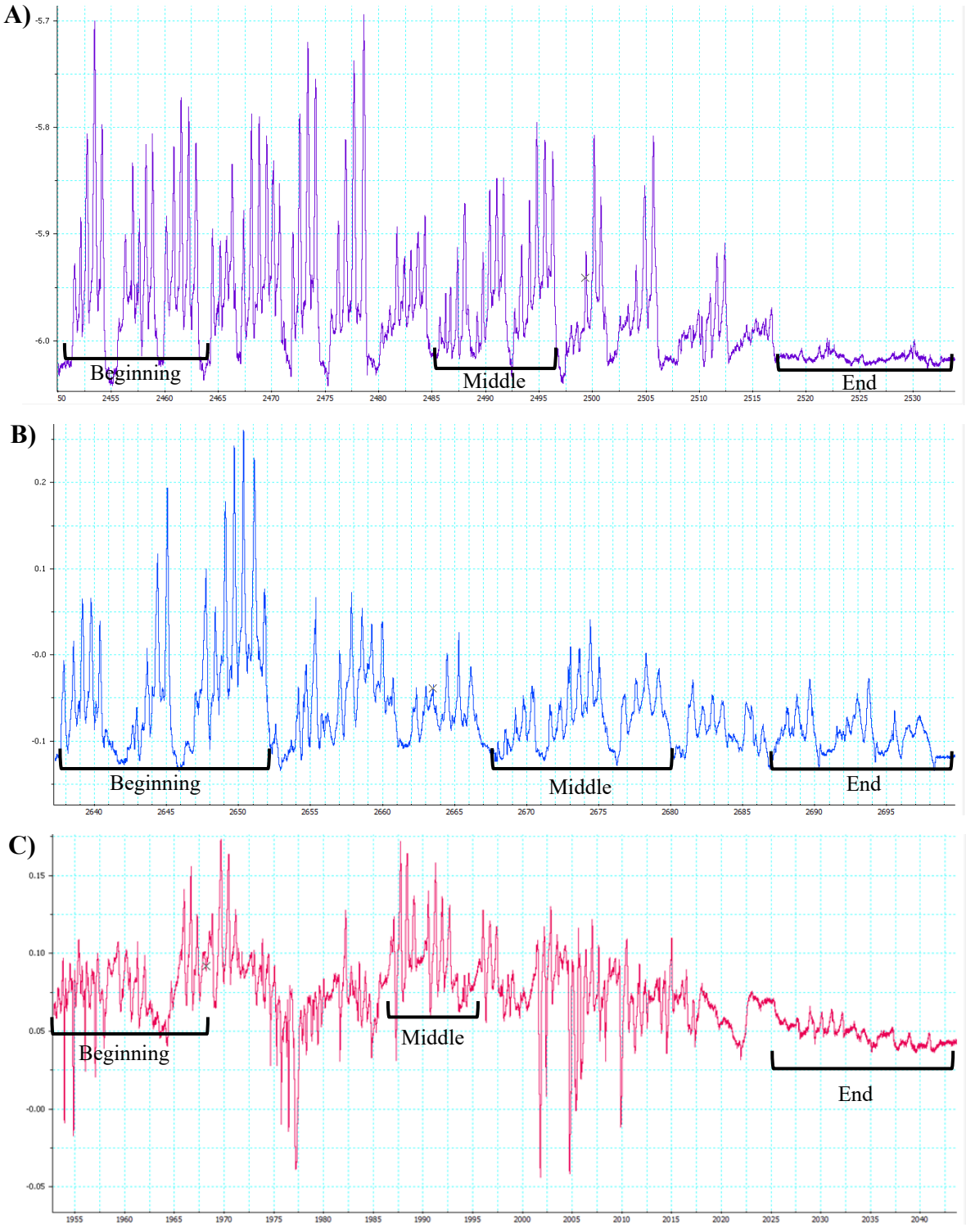
"Middle" is found by: 18/2 = 9, not changed since the total number of bouts is even. Therefore, the "middle" is the centermost bout, bout 9, the bout before it (8), and the bout after it (10), so bouts 8, 9, and 10

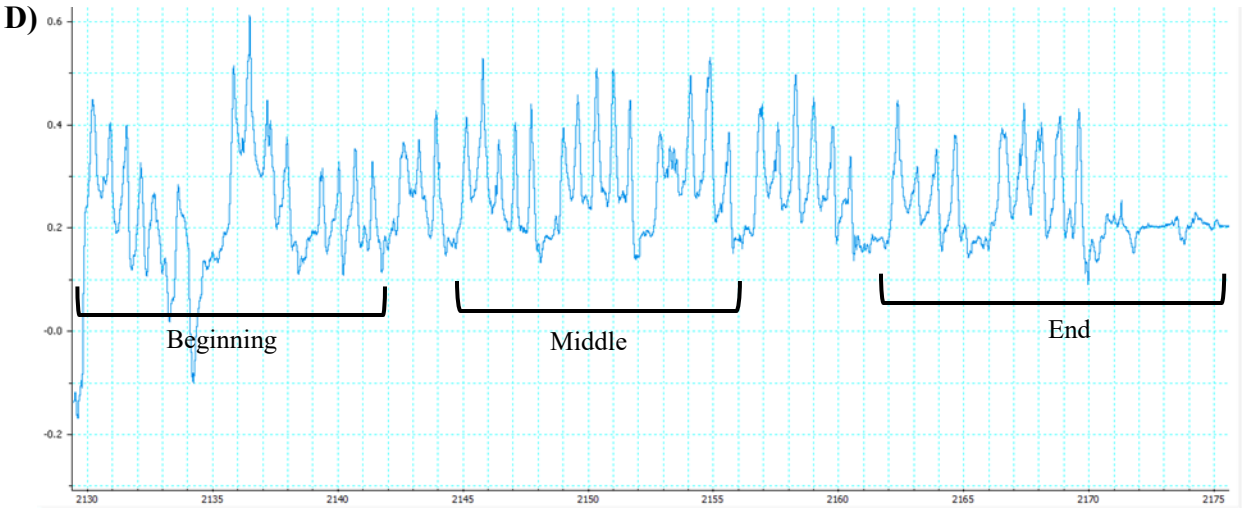
If a meal has 15 bouts

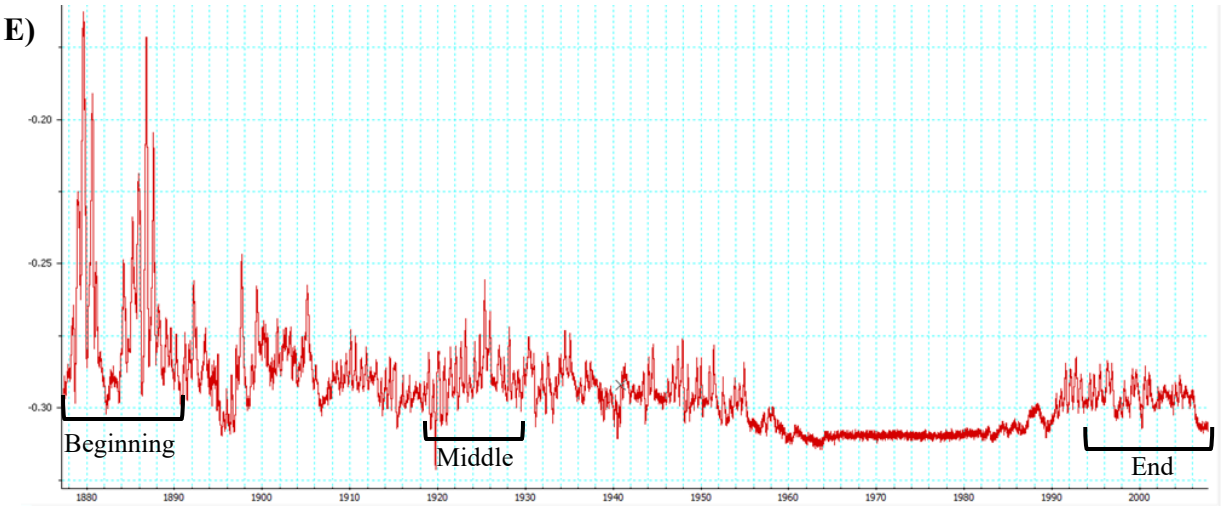
"Start" are bouts 1, 2, and 3

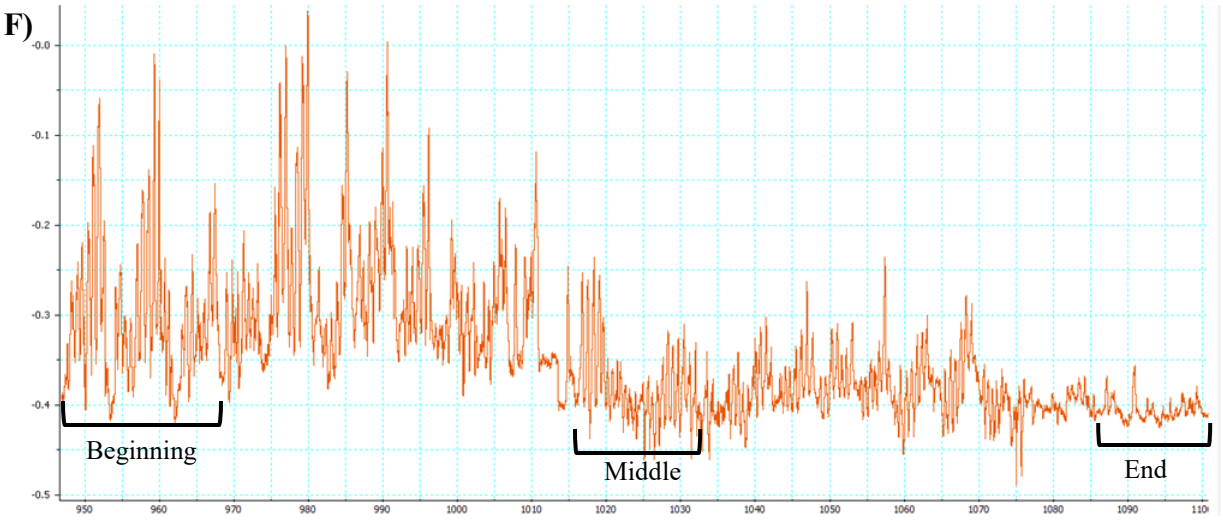
"End" are bouts 13, 14, and 15

"Middle" is found by: 15/2 = 7.5, rounded up to 8 since the total number of bouts is odd. Therefore, the "middle" is the centermost bout, bout 8, the bout before it (7), and the bout after it (9), so bouts 7, 8, and 9


A)
-5.7
-5.8
-5.9
-6.0
Beginning
Middle
End
50
2455
2460
2465
2470
2475
2480
2485
2490
2495
2500
2505
2510
2515
2520
2525
2530
B)
0.2
0.1
-0.0
-0.1
Beginning
Middle
End
2640
2645
2650
2655
2660
2665
2670
2675
2680
2685
2690
2695
C)
0.15
0.10
0.05
-0.00
-0.05
Beginning
Middle
End
1955
1960
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
2030
2035
2040


D)
0.6
0.4
0.2
-0.0
-0.2
Beginning
Middle
End
2130
2135
2140
2145
2150
2155
2160
2165
2170
2175


E)
-0.20
-0.25
-0.30
Beginning
Middle
End
1880
1890
1900
1910
1920
1930
1940
1950
1960
1970
1980
1990
2000


F)
-0.0
-0.1
-0.2
-0.3
-0.4
-0.5
Beginning
Middle
End
950
960
970
980
990
1000
1010
1020
1030
1040
1050
1060
1070
1080
1090

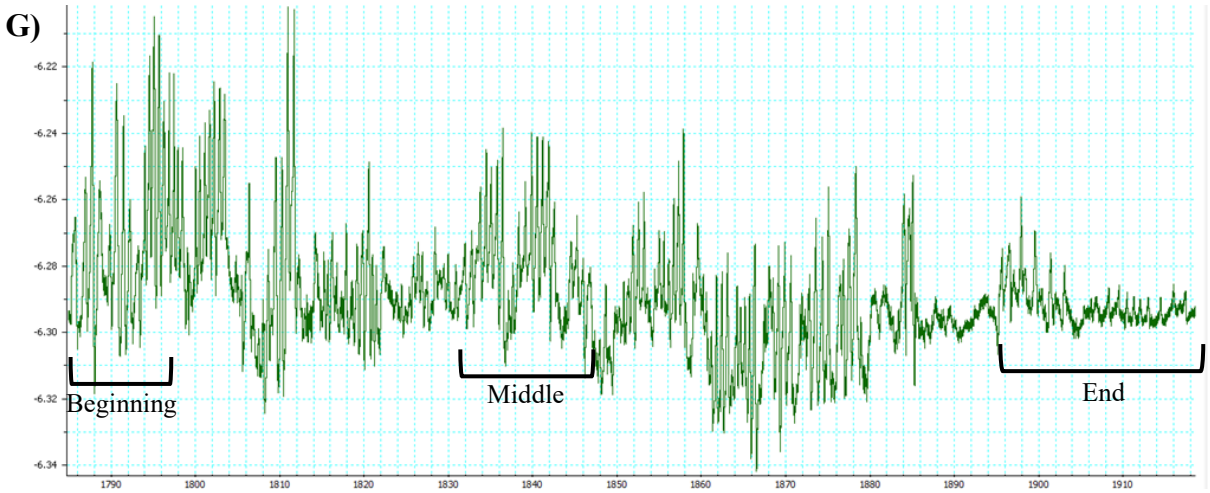


Figure 3.5 Examples from each condition of force transducer recordings with the beginning, middle, and end time sections of each meal indicated. Y axes indicate time in seconds, and X axes indicate bite amplitude in mV. A) Satiation, B) Sham Injection, C) Immune Challenged, D) Molt Sleep, E) Predator Stress + Satiation, F) Predator Stress + Sham Injection, G) Predator Stress + Immune Challenged.

3.4 MEAL MICROSTRUCTURE VARIABLES

With these time sections for objective comparison established, a new set of variables were generated describing each time section and allow direct comparison within and between meals. Each time section in each meal was described with:

Section Duration: Duration, in seconds, from start of first bout to end of third bout in the time section

Time Feeding: Total duration, in seconds, of the three bouts themselves, disregarding time between the bouts, in the time section

Percent spent feeding: Time Feeding divided by Section Duration, and multiplied by 100

Bites per bout: The average number of bites in each of the 3 bouts in the time section

Bout Duration: The average of the Bout Duration of each of the 3 bouts.

Bites Per Second: The average of each bout's bite total divided by the average of each bout's duration.

Inter Bout Interval: The average time between each of the 3 bouts.

The above variables have also been utilized before to characterize feeding in *Manduca sexta*. (Bowdan, 1988b)

From the variables describing feeding force in the individual bouts, the following were developed to describe the three time sections in each meal:

Minimum**:** Average of the Minimum value for each of the 3 bouts

Mean**:** Average of the Mean value for each of the 3 bouts

Maximum**:** Average of the Maximum value for each of the 3 bouts.

Amplitude Range**:** Average of the Amplitude Range value for each of the 3 bouts.

Max – Mean difference**:** Average of the Max-Mean difference value for each of the 3 bouts

Max – Min ratio: Average of the Max-Min difference value for each of the 3 bouts

Max – Mean ratio: Average of the Max-Mean ratio value for each of the 3 bouts

3.5 DIMENSION REDUCTION

To reduce the number of variables for analysis, a correlation matrix was generated for the dependent variables giving a Spearman's correlation coefficient for each relationship. Variables with a Spearman's coefficient outside of the -0.8 to 0.8 range are normally considered 'very highly correlated', and therefore too closely related to justify including both in the analysis for this experiment. (Swinscow, 1997). I removed one of the very highly correlated variables, with a preference towards first removing those variables that had the largest number of highly correlated relationships. The exception to this was that I did not remove the 'Mean bite amplitude' variable, even though it was highly correlated with more variables than the Minimum bite amplitude'. I judged that the Mean value would be more indicative of trends than the Minimum, and therefore the mean value was retained, instead of the minimum value. The reduced dataset contained the following variables: Section Duration, Bites per bout, Bites Per Second, Inter Bout Interval, and Mean bite amplitude.

With the most highly correlated variables removed, further dimension reduction was attempted through a Principal Components Analysis (PCA). PCA was chosen because of the low number of restrictions it places on data distribution, such as not requiring a normal distribution, and its potential to reveal more subtle explanations for the variance in the remaining variables.

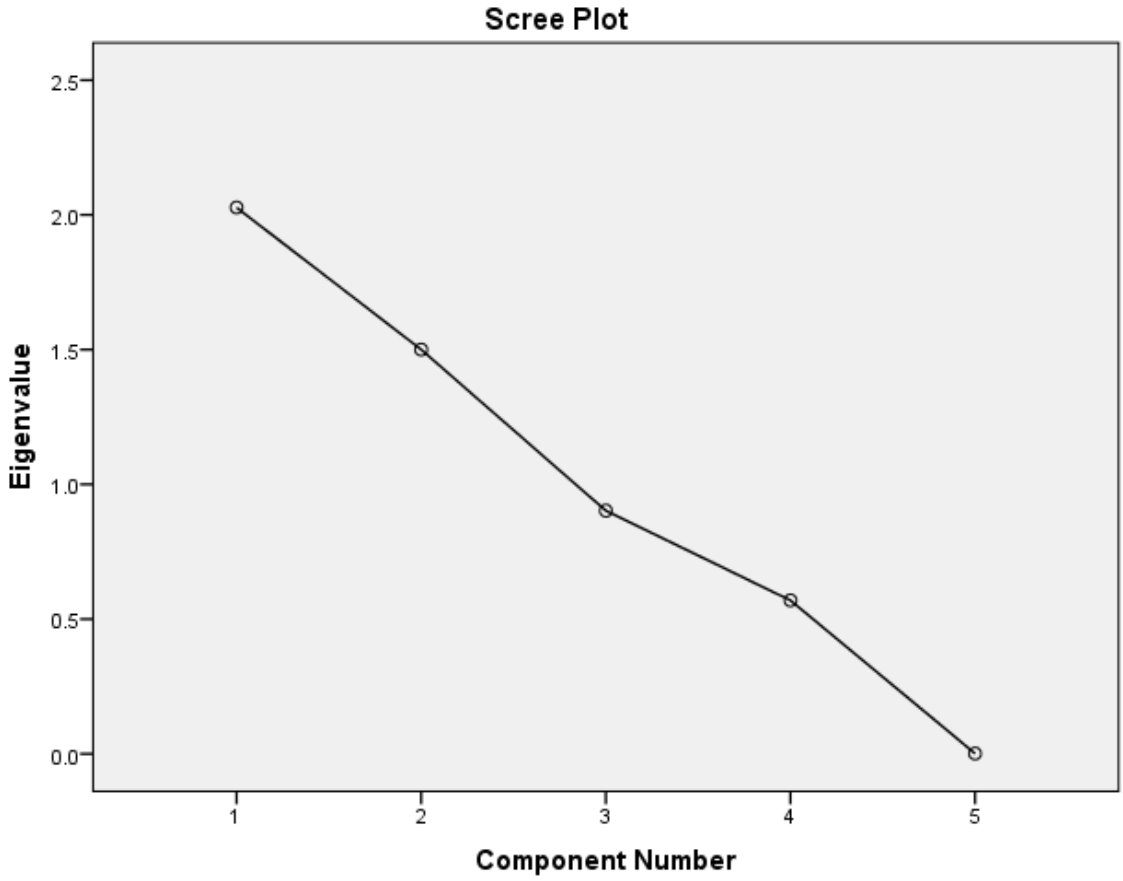


Figure 3.6 Scree Plot PCA on microstructure variables. Component 1 Eigenvalue: 2.027, Component 2: 1.5, Component 3: 0.902, Component 4: 0.569, Component 5: 0.001.

The principal components analysis returned 3 components with Eigenvalues near or above 1, the rule of thumb threshold for a component being significant. As well, the first three components together were required to explain at least 80% of the variance (88. 59%) across all 5 variables, another rule of thumb threshold. This does not substantially reduce the number of variables for analysis, so another method was utilized.

3.6 MIXED MANOVA

To compare the interaction effects between conditions, predator stress (for Experiment 4), and section the five measured variables at each time section, a mixed MANCOVA was utilized. The group sizes across Experiments 1, 2, and 3 (N = 9) differ from those in Experiment 4 (N = 15), so a pair of mixed MANOVA analyses were performed. For the groups in Experiments 1, 2, and 3, a mixed MANOVA was performed on the 5 variables between the four groups and three time sections. For the groups in Experiment 4, a 2 x 3 x 3 mixed MANOVA examined potential interaction effects on the variables across the two Predator Stress conditions (stressed or not), the three base conditions (Satiation, Sham, and Immune Challenge) and the meal section (beginning, middle or end).

One assumption in performing a MANOVA is that the dependent variable data are normally distributed. Prior to running the above analyses, a Shapiro-Wilk test on each variable and at each time section showed that almost none of the variables followed a normal distribution, normal defined as a Shapiro-Wilk statistic value above 0.001. As the mixed MANOVA analysis requires use of normally distributed inputs, the data were all log-scaled prior to analysis. The log-transformed data showed a normal distribution (visualized using Q-Q plots, Ghasemi and Zahediasl, 2012), so the analyses were performed on these transformed datasets.

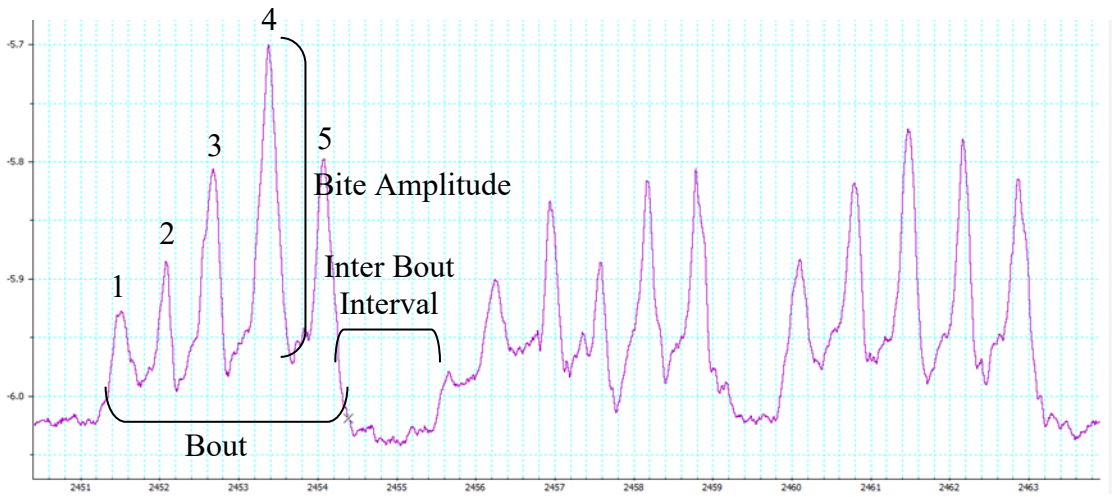


Figure 3.7 Examples of microstructure element identification in the Beginning section of the above Satiation sample recording.

CHAPTER 4 RESULTS

4.1 EXPERIMENT 1: Satiation

A repeated measures MANCOVA using animal mass as a covariate found statistically significant feeding microstructure changes over time in a meal ($F(10,24) = 2.286$, $p = 0.047$; Wilk's $\Lambda = 0.262$, partial $\eta 2 = 0.488$) in the first Satiation group trial from Experiment 1 (Figure 4.1). Univariate analysis with a Greenhouse Geisser correction for sphericity showed that the Mean Bite Amplitude ($F(10,24) = 5.648$, $p = 0.015$, partial $\eta 2 = 0.414$) had statistically significant differences over time in a meal. Time did not have a statistically significant effect on any other feeding microstructure variables (Table 4.1).

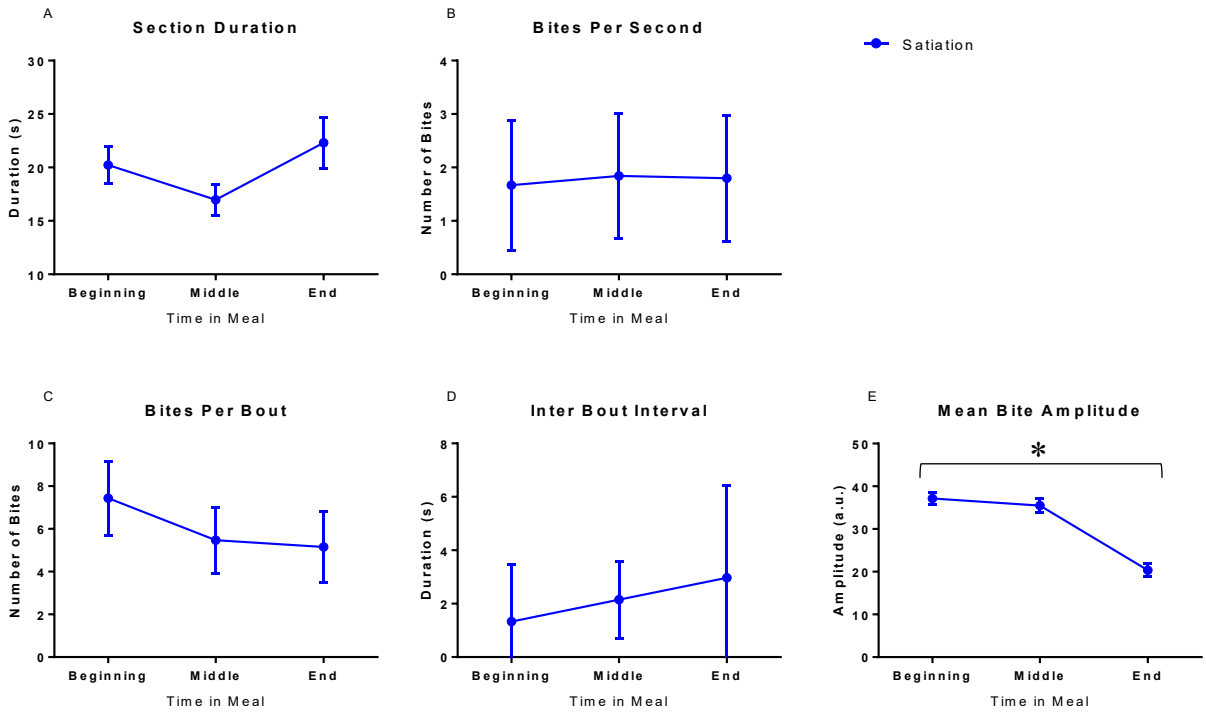


Figure 4.1 Effects of Satiation on caterpillar feeding microstructure. The graphs show the mean A) Section Duration, B) Bites Per Second, C) Bites Per Bout, D) Inter Bout Interval, and E) Mean Bite Amplitude at each of the 3 measured time segments for Satiation group (n = 9) caterpillars. The error bars represent the standard deviation at that time segment. A (*) indicates a statistically significant main effect of time in meal on the variable as determined by the repeated measures MANCOVA, $\alpha = 0.05$

Table 4.1 Univariate analysis of how feeding microstructure variables are affected by time in the first Satiation condition (n = 9).

Group	Variable	df	F	p	Partial η2
Satiation	Section Duration	2	0.51	0.591	0.06
	Bites Per Second	2	0.688	0.498	0.079
	Bites Per Bout	2	1.493	0.258	0.157
	Bout Interval	2	1.784	0.21	0.182
	Mean Bite Amplitude	2	5.648	0.015*	0.414

(*) represents a statistically significant effect of time on the variable as determined by a repeated measures MANCOVA, $\alpha = 0.05$.

4.2 EXPERIMENT 2: Immune Challenge

A repeated measures MANCOVA with a Bonferroni corrected $\alpha = 0.025$ and using animal mass as a covariate found statistically significant feeding microstructure changes over time in a meal ($F(10,88) = 3.132$, $p = 0.002$; Wilk's $\Lambda = 0.544$, partial $\eta 2 = 0.263$) in the first trials of the Satiation, Sham Injected, and Immune Challenged groups of animals (Figure 4.2). Univariate analysis with a Greenhouse Geisser correction for sphericity showed that the Bites Per Bout ($F(10,88) = 4.648$, $p = 0.018$, partial $\eta 2 = 0.162$), Bout Interval ($F(10,88) = 6.854$, $p = 0.003$, partial $\eta 2 = 0.222$), and Mean Bite Amplitude ($F(10,88) = 11.456$, $p < 0.001$, partial $\eta 2 = 0.323$) had statistically significant differences over time in a meal. Time did not have a statistically significant effect on any other feeding microstructure variables (Table 4.2). The interaction of time and caterpillar's group did not have a statistically significant effect on microstructure variables (Table 4.3).

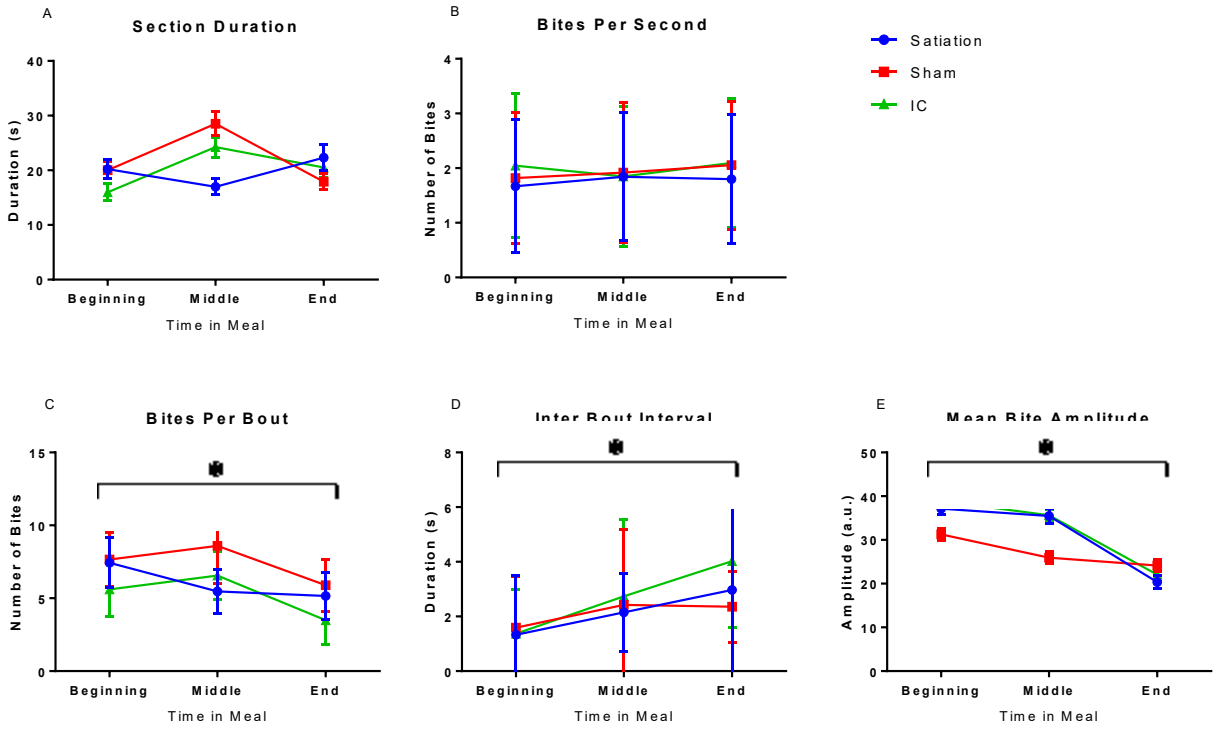


Figure 4.2 Effects of Satiation, Immune Challenge, and Sham Injection on caterpillar feeding microstructure. The graphs show the mean A) Section Duration, B) Bites Per Second, C) Bites Per Bout, D) Inter Bout Interval, and E) Mean Bite Amplitude at each of the 3 measured time segments for the groups (n = 9 each). The error bars represent the standard deviation for that group at that time segment. A (*) indicates a statistically significant main effect of time in meal on the variable as determined by the repeated measures MANCOVA, corrected $\alpha = 0.025$.

Table 4.2 Univariate analysis of how feeding microstructure variables are affected by time in the first Satiation, Sham Injection, and Immune Challenge conditions (n = 9 for each group).

Source	Variable	df	F	p	Partial η2
Time	Section Duration	2	1.025	0.363	0.041
	Bites Per Second	2	1.159	0.321	0.046
	Bites Per Bout	2	4.648	0.018*	0.162
	Bout Interval	2	6.854	0.003*	0.222
	Mean Bite Amplitude	2	11.456	<0.001*	0.323
Time x Group	Section Duration	2	1.572	0.201	0.116
	Bites Per Second	2	0.804	0.525	0.063
	Bites Per Bout	2	0.939	0.443	0.073
	Bout Interval	2	0.479	0.746	0.038
	Mean Bite Amplitude	2	1.109	0.363	0.085

(*) represents a statistically significant effect of time on the variable as determined by a repeated measures MANCOVA, corrected $\alpha = 0.025$.

Table 4.3 Multivariate analysis of how feeding microstructure variables are affected by time and by the interaction of time and group condition in the first Satiation, Sham Injection, and Immune Challenge conditions (n = 9 for each group).

Effect	Wilk's Λ	F	Hypothesis df	Error df	p	Partial η2

Time	0.544	3.132	10	88	0.002*	0.263
Time X Group	0.63	1.1	10	88	0.356	0.439

(*) represents a statistically significant main effect on the variables as determined by a repeated measures MANCOVA, corrected $\alpha = 0.025$.

4.3 EXPERIMENT 3: Molt Sleep

A repeated measures MANCOVA with a Bonferroni corrected $\alpha = 0.0167$ and using animal mass as a covariate found statistically significant feeding microstructure changes over time in a meal ($F(10,56) = 2.905$, $p = 0.005$; Wilk's $\Lambda = 0.434$, partial $\eta 2 = 0.342$) in the Molt Sleep and first Satiation group of animals (Figure 4.3). Univariate analysis with a Greenhouse Geisser correction for sphericity showed that the Mean Bite Amplitude ($F(10,56) = 5.973$, $p = 0.008$, partial $\eta 2 = 0.272$) had statistically significant differences over time in a meal. Time did not have a statistically significant effect on any other feeding microstructure variables (Table 4.4). The interaction of time and caterpillar's group did not have a statistically significant effect on microstructure variables (Table 4.5).

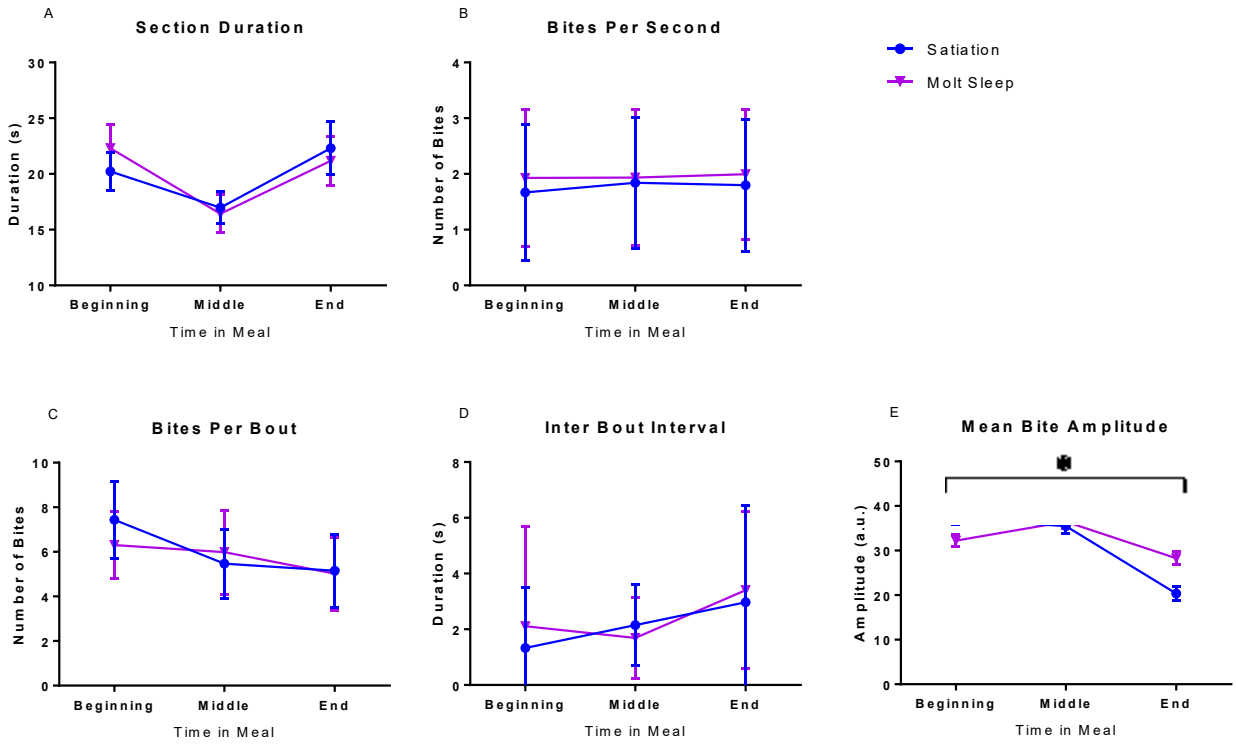


Figure 4.3 Effects of Satiation and approaching Molt Sleep on caterpillar feeding microstructure. The graphs show the mean A) Section Duration, B) Bites Per Second, C) Bites Per Bout, D) Inter Bout Interval, and E) Mean Bite Amplitude at each of the 3 measured time segments for the groups (n = 9 each). The error bars represent the standard deviation for that group at that time segment. A (*) indicates a statistically significant main effect of time in meal on the variable as determined by the repeated measures MANCOVA, corrected $\alpha = 0.0167$.

Table 4.4 Univariate analysis of how feeding microstructure variables are affected by time in the Molt Sleep and first Satiation conditions (n = 9 for each group).

Source	Variable	df	F	p	Partial η2
Time	Section Duration	2	0.898	0.409	0.053
	Bites Per Second	2	0.432	0.618	0.026
	Bites Per Bout	2	1.634	0.218	0.093
	Bout Interval	2	2.342	0.132	0.128
	Mean Bite Amplitude	2	5.973	0.008*	0.272
Time x Group	Section Duration	2	0.071	0.916	0.004
	Bites Per Second	2	0.267	0.728	0.016
	Bites Per Bout	2	0.296	0.671	0.018
	Bout Interval	2	0.63	0.488	0.038
	Mean Bite Amplitude	2	1.682	0.205	0.095

(*) represents a statistically significant effect of time on the variable as determined by a repeated measures MANCOVA, corrected $\alpha = 0.0167$.

Table 4.5 Multivariate analysis of how feeding microstructure variables are affected by time and by the interaction of time and group condition in the first Satiation and Molt Sleep conditions (n = 9 for each group).

Effect	Wilk's Λ	F	Hypothesis df	Error df	p	Partial η2
Time	0.434	2.905	10	56	0.005*	0.342

Time x Group	0.763	0.81	10	56	0.62	0.126

(*) represents a statistically significant main effect on the variables as determined by a repeated measures MANCOVA, corrected $\alpha = 0.167$.

4.4 EXPERIMENT 4: Predator Stress

A 2 x 3 x 3 repeated measures MANCOVA using animal mass as a covariate found statistically significant feeding microstructure changes over time in a meal ($F(10,328) = 48.015$, $p < 0.001$; Wilk's $\Lambda = 0.165$, partial $\eta 2 = 0.594$) for the second trials of the Satiation, Sham Injected, and Immune Challenged groups of animals, and Predator Stressed iterations of those groups (Figure 4.4). Univariate analysis with a Greenhouse Geisser correction for sphericity showed that the Section Duration ($F(10,328) = 6.207$, $p = 0.003$, partial $\eta 2 = 0.069$), Bites Per Bout ($F(10,328) = 9.704$, $p < 0.001$, partial $\eta 2 = 0.104$), Bout Interval ($F(10,328) = 17.995$, $p < 0.001$, partial $\eta 2 = 0.176$), and Mean Bite Amplitude ($F(10,328) = 305.662$, $p < 0.001$, partial $\eta 2 = 0.784$) had statistically significant differences over time in a meal. Time did not have a statistically significant effect on any other feeding microstructure variables (Table 4.6). The interactions of time and caterpillar group, separated by both 'Condition' (Satiation, Sham Injected, Immune Challenged) and 'Predator Stress' (No Predator Stress, presence of Predator Stress) did not have a statistically significant effect on microstructure variables (Table 4.7).

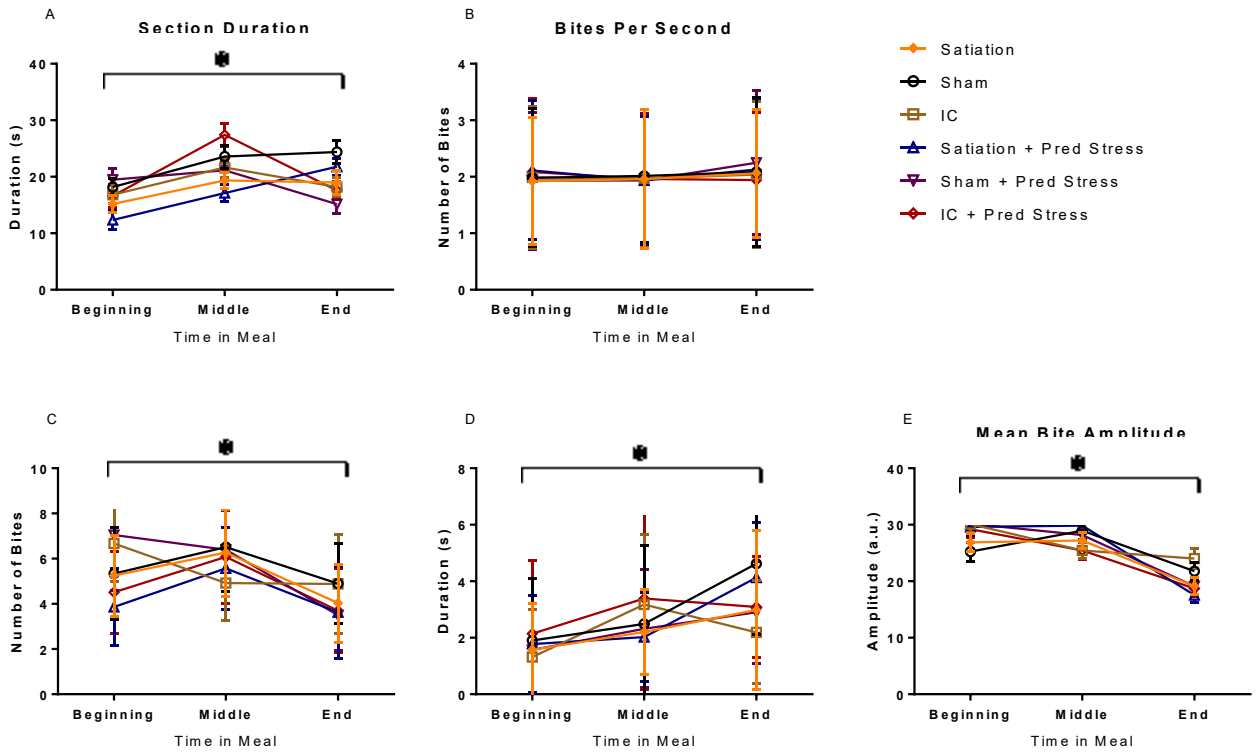


Figure 4.4 Effects of Satiation, Immune Challenge, Sham Injection, and the Predator Stress iterations of those conditions on caterpillar feeding microstructure. The graphs show the mean A) Section Duration, B) Bites Per Second, C) Bites Per Bout, D) Inter Bout Interval, and E) Mean Bite Amplitude at each of the 3 measured time segments for the groups (n = 15 each). The error bars for represent the standard deviation for that group at that time segment. A (*) represents a statistically significant main effect of time in meal on the variable as determined by the repeated measures MANCOVA, $\alpha = 0.05$.

Table 4.6 Univariate analysis of how feeding microstructure variables are affected by time in the second Satiation, Immune Challenge, and Sham Injection conditions, and the Predator Stress iterations of those conditions on caterpillar feeding microstructure (n = 15 for each group)

Source	**Variable**	**df**	**F**	**P**	**Partial η2**
Time	Section Duration	2	6.207	0.003*	0.069
	Bites Per Second	2	2.217	0.112	0.026
	Bites Per Bout	2	9.704	<0.001*	0.104
	Bout Interval	2	17.955	<0.001*	0.176
	Mean Bite Amplitude	2	305.662	<0.001*	0.784
Time x Treatment	Section Duration	4	1.73	0.148	0.04
	Bites Per Second	4	0.848	0.496	0.02
	Bites Per Bout	4	0.662	0.615	0.016
	Bout Interval	4	2.337	0.065	0.053
	Mean Bite Amplitude	4	1.291	0.28	0.03
Time x Predator Stress	Section Duration	2	0.318	0.721	0.004
	Bites Per Second	2	0.54	0.583	0.006
	Bites Per Bout	2	1.15	0.318	0.013
	Bout Interval	2	0.302	0.715	0.004
	Mean Bite Amplitude	2	3.431	0.067	0.039

Time x Condition x Predator Stress	Section Duration	4	1.604	0.178	0.037
	Bites Per Second	4	0.674	0.61	0.016
	Bites Per Bout	4	1.672	0.161	0.038
	Bout Interval	4	0.754	0.543	0.018
	Mean Bite Amplitude	4	0.403	0.669	0.01

(*) represents a statistically significant effect of time on the variable as determined by a repeated measures MANCOVA, $\alpha = 0.05$.

Table 4.7 Multivariate analysis of how feeding microstructure variables are affected by time and by the interaction of time and group condition in the second Satiation, Immune Challenge, and Sham Injection conditions, and the Predator Stress iterations of those conditions on caterpillar feeding microstructure (n = 15 for each group)

Effect	**Wilk's Λ**	**F**	**Hypothesis df**	**Error df**	**p**	**Partial η2**
Time	0.165	48.015	10	328	<0.001*	0.594
Time x Condition	0.831	1.584	20	668	0.051	0.126
Time x Predator Stress	0.942	0.987	10	328	0.455	0.029
Time x Condition x Predator Stress	0.888	0.989	20	668	0.474	0.029

(*) represents a statistically significant main effect on the variables as determined by a repeated measures MANCOVA, $\alpha = 0.05$.

CHAPTER 5 DISCUSSION

5.1 PREDICTIONS AND OUTCOMES

Table 5.1 Predictions and their outcomes of average bite force progression for each group in all experiments.

Groups	**Predicted changes in bite force during meal**	**Outcomes**
Satiation	Decrease over time until meal's end	Matched predictions, bite force decreased over time.
Immune Challenge	Compared to Satiation: Lower starting value, decrease further over time until meal's end, lower ending value than Satiation	Values not different from Satiation or Sham Injected for any time segment
Molt Sleep	Compared to Satiation: Start at same value, but decrease more rapidly over time until meal's end, lower ending value than Satiation	Values not different from Satiation for any time segment
Predator Stress	Compared to equivalent non-stressed group: Start at lower value, increase to same value as non-stressed (middle section), then decrease to same ending value as non-stressed	Values not different from equivalent non-stressed group, and no values different between stressed or non-stressed groups for any time segment

Table 5.2 Predictions and their outcomes of inter-bout-interval progression for each group in all experiments.

Groups	Predicted changes in inter-bout-interval during meal	Outcomes
Satiation	Increase over time until meal's end	Did not match predictions-no change over course of meal
Immune Challenge	Compared to Satiation: Higher starting value, increase further over time until meal's end, higher ending value than Satiation	Significant increase over course of meal, but not different from Satiation or Sham Injected for any time section
Molt Sleep	Compared to Satiation: Start at same value, but increase more rapidly over time until meal's end, higher ending value than Satiation	Values not different from Satiation for any time section
Predator Stress	Compared to equivalent non-stressed group: Start at lower value, increase to same value as non-stressed (middle section), then decrease to same ending value as non-stressed	Values show significant increase over course of meal, but not different from equivalent non-stressed group, and no values different between stressed or non-stressed groups for any time segment

The average bite force applied by *M. sexta* in feeding significantly changed over the course of a meal in all treatments, which was in line with my predictions for the satiation group caterpillars (Table 5.1). Contrary to my predictions, the direction and magnitude of these changes were the same for all treatments and conditions (Table 5.1). The inter-bout-interval duration increased as feeding approached cessation in almost all conditions,

but contrary to my predictions there was no difference across conditions and the trends followed the predicted trend for satiation caterpillars (Table 5.2).

5.2 PUTATIVE CPG INTERNEURONS

Feeding microstructure in *M. sexta* is the result of the rhythmic activity in a putative central pattern generator (CPG) located in the suboesophageal ganglion (SEG) (Rohrbacher, 1994a). The cells in this CPG directly innervate the mandible muscles controlling feeding, which suggests that the CPG's neuronal activity was the same for all treatments and conditions as well. The stereotypic nature of the changes in bite force as feeding motivation declined suggests that in *M. sexta*, the different pathways leading to feeding cessation converge on neurons in the CPG in a way that ultimately produces the same change in its motor activity.

The putative feeding CPG in *M. sexta* is a collection of motor neurons, pre-motor neurons, and interneurons, all ultimately directing the muscle activity involved in biting (Rohrbacher, 1994a). Some cells within the CPG can modify its firing rhythm, and one identified cell, interneuron 101 (IN 101), could have a role in the microstructure feeding cessation I observed. IN 101 directly synapses onto motor neurons for the muscles that close the mandibles during a bite (Figure 5.1), and the force applied by this mandible closing is responsible for the observed bite force (Rohrbacher, 1994b).

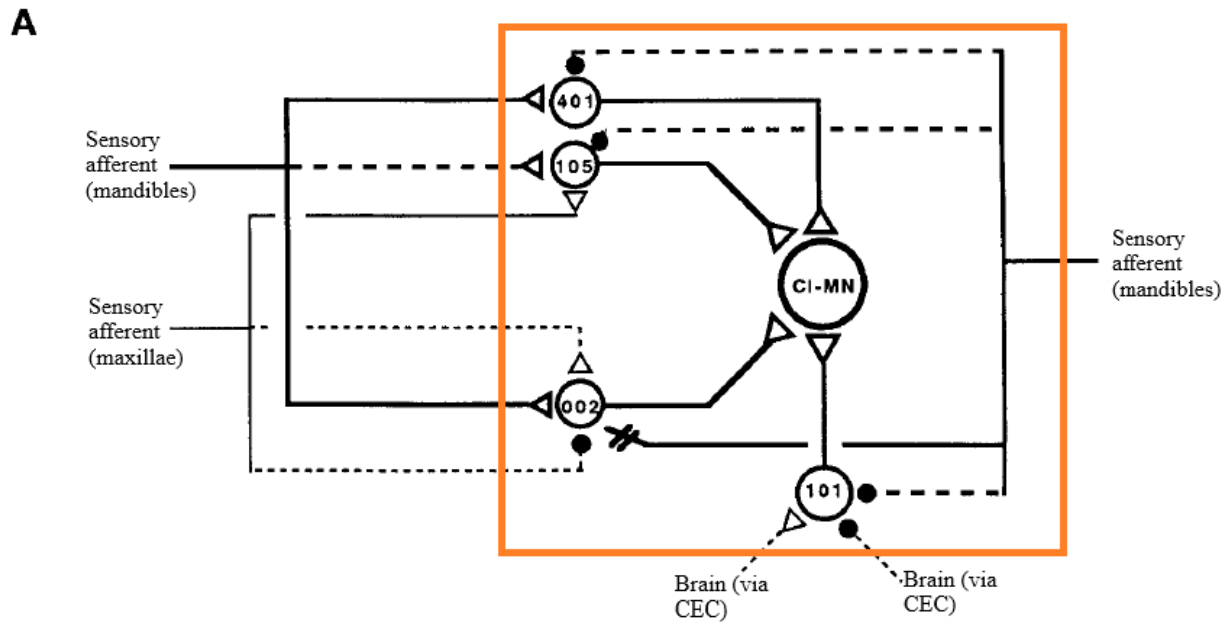


Figure 5.1 Circuit diagram of network involving connections between premotor neurons (numbered) and closer motor neurons (Cl-MN). Inside **orange square** are cells in putative CPG, **solid lines** are direct connections, **dashed lines** are polysynaptic connection. **Filled circles** are inhibitory synapses, **open triangles** are excitatory synapses, and the **slashed connection** is one with no observed effect. Adapted from Figure 8A of (Rohrbacher, 1994b).

When depolarized, IN 101 induces a firing rate increase for the CPG, and when hyperpolarized it is the only identified neuron for this CPG thus far that can directly inhibit the motor neurons for the closing muscles (Rohrbacher, 1994b; Rohrbacher, 1994a) in addition to decreasing the firing rate for the CPG. IN 101 receives both inhibitory and excitatory projections from the caterpillar's brain (Figure 5.2) via the circumoesophageal connective (CEC), so it is capable of endogenous depolarization and hyperpolarization. This makes IN 101 well positioned to mediate feeding cessation and the subsequent reduction in the force applied by bites. The bite force microstructure similarities across feeding cessation conditions could be a result of the different feeding cessation signals converging on and inhibiting the activity of IN 101.

*D. melanog*aster has 2 interneurons called the 'Feeding' (Fdg) neurons that have a similar role as that proposed for IN 101, and whose function supports the idea of converging inputs influencing feeding motor behaviour (Flood et al., 2013). The Fdg interneurons are in *D. melanogaster's* feeding motor CPG, which, as in *M. sexta,* is in the SEG. Although there hasn't been a direct examination of the effects of exciting or inhibiting Fdg neurons on rhythmic CPG firing, it can be inferred through the feeding behaviour. Excitation of the Fdg neurons induces the Proboscis Extension Reflex (PER), which is a full cycle of the feeding motor behaviour and akin to a bite in *M. sexta* larvae. Conversely, inhibition of the neurons prevents a feeding motor response, even in starved flies with ready access to food (Flood et al., 2013; Pool et al., 2014). The extensive dendritic arbor of the Fdg neurons is similar to neurons integrating state and sensory inputs that influence the motor patterns of fruit fly mating behaviour (Kimura et al., 2008). The activity and anatomy of the *D. melanogaster* Fdg neurons suggest a role for them as integration points for inputs to converge and affect the feeding motor circuit along a common final neuronal pathway, similar to the role proposed for IN 101 neurons.

5.3 CONCLUSIONS

Despite the variety of both internal and external factors capable of modifying *M. sexta* feeding behaviour, this study demonstrates that this variety is not reflected in the feeding

microstructure of the caterpillar. Even simultaneously applying acute predator stress and an immune challenge, both of which reduce feeding motivation in *M. sexta*, did not show a difference in how the animal approached feeding cessation. There are a number of ways in which feeding can terminate (Table 5.1), but satiation, illness-induced anorexia, and molting all have the same effect on feeding microstructure. This similarity suggests that at the neuronal level there may be a single pathway inducing feeding cessation, and past work on the *M. sexta* putative feeding CPG has identified an interneuron, IN 101, that is likely a part of that single pathway. The microstructure similarity could be the result of feeding cessation inputs converging on IN 101, as it is the only interneuron identified thus far capable of directly inhibiting motor neurons. However, I cannot exclude the possibility of multiple independent inputs inducing feeding cessation by individually inhibiting feeding motor neurons, pre-motor neurons and interneurons. Given the similar behavioural effect at the microstructure level, I believe this option is unlikely. Further studies are still needed.

REFERENCES

Adamo SA (2005) Parasitic suppression of feeding in the tobacco hornworm, manduca sexta: Parallels with feeding depression after an immune challenge. Archives of Insect Biochemistry and Physiology (Hoboken) 60:185-197.

Adamo SA, Baker JL (2011) Conserved features of chronic stress across phyla: The effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket, gryllus texensis. Hormones and Behavior 60:478-483.

Adamo SA, Kovalko I, Mosher B (2013) The behavioural effects of predator-induced stress responses in the cricket (gryllus texensis): The upside of the stress response. Journal of Experimental Biology (England) 216:4608-4614.

Adamo SA, Fidler TL, Forestell CA (2006) Illness-induced anorexia and its possible function in the caterpillar, manduca sexta. Brain, Behavior, and Immunity (United States) 21:292-300.

Adamo SA, Easy RH, Kovalko I, MacDonald J, McKeen A, Swanburg T, Turnbull KF, Reeve C (2017) Predator exposure-induced immunosuppression: Trade-off, immune redistribution or immune reconfiguration? Journal of Experimental Biology (Cambridge) 220:868-875.

Adelman JS, Martin LB (2009) Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. Integrative and Comparative Biology (Oxford) 49:202-214.

Al-Anzi B, Armand E, Nagamei P, Olszewski M, Sapin V, Waters C, Zinn K, Wyman RJ, Benzer S (2010) The leucokinin pathway and its neurons regulate meal size in drosophila. Current Biology 20:969-978.

Anderson RA, McBrayer LD, Herrel A (2008) Bite force in vertebrates: Opportunities and caveats for use of a nonpareil whole-animal performance measure. Biological Journal of the Linnean Society 93:709-720.

Antelman SM, Szechtman H, Chin P, Fisher AE (1975) Tail pinch-induced eating, gnawing and licking behavior in rats: Dependence on the nigrostriatal dopamine system. Brain Res 99:319-337.

Aso Y et al (2014) Mushroom body output neurons encode valence and guide memory-based action selection in drosophila. eLife (England) 3:e04580.

Ayres JS, Schneider DS (2009) The role of anorexia in resistance and tolerance to infections in drosophila. PLoS Biology (San Francisco) 7:e1000150.

Bader R, Wegener C, Pankratz M (2007) Comparative neuroanatomy and genomics of hugin and pheromone biosynthesis activating neuropeptide (PBAN). Fly 1:228-231.

Barbehenn RV, Keddie A (1992) Gut contents in molting lepidopteran larvae: A source of error in nutritional studies. Entomologia Experimentalis Et Applicata (Oxford, UK) 62:87-91.

Bashir-Tanoli S, Tinsley MC (2014) Immune response costs are associated with changes in resource acquisition and not resource reallocation.

Bellisle F, Blundell JE (2013) Satiation, satiety: Concepts and organisation of behaviour. In: Satiation, satiety and the control of food intake Satiation, satiety: Concepts and organisation of behaviour. pp3-11.Elsevier Ltd.

Bellisle F, Drewnowski A, Anderson GH, Westerterp-Plantenga MS, Martin CK (2012) Sweetness, satiation, and satiety. Journal of Nutrition (United States) 142:54s.

Bernays EA (1997) Feeding by lepidopteran larvae is dangerous. Ecological Entomology 22:121-123.

Bernays EA, Woods HA (2000) Foraging in nature by larvae of manduca sexta—influenced by an endogenous oscillation. Journal of Insect Physiology 46:825-836.

Berthoud H, Münzberg H, Morrison CD (2017) Blaming the brain for obesity: Integration of hedonic and homeostatic mechanisms. Gastroenterology 152:1728-1738.

Bestman JE, Booker R (2003) Modulation of foregut synaptic activity controls resorption of molting fluid during larval molts of the moth manduca sexta. Journal of Experimental Biology (England) 206:1207-1220.

Blouet C, Schwartz G (2012) Brainstem nutrient sensing in the nucleus of the solitary tract inhibits feeding. Cell Metabolism (United States) 16:579-587.

Bowdan E (1995) The effects of a phagostimulant and a deterrent on the microstructure of feeding by manduca sexta caterpillars. Entomologia Experimentalis Et Applicata (Oxford, UK) 77:297-306.

Bowdan E (1992) Behavior as a window on physiology: A simple apparatus for recording caterpillar feeding. Advances in Physiology Education (UNITED STATES) 262:9.

Bowdan E (1988a) Microstructure of feeding by tobacco hornworm caterpillars, manduca sexta. Entomologia Experimentalis Et Applicata (Oxford, UK) 47:127-136.

Bowdan E (1988b) The effect of deprivation on the microstructure of feeding by the tobacco hornworm caterpillar. Journal of Insect Behavior 1:31-50.

Bowdan E (1984) Short communications about bowdan apparatus. (Warsaw) 15:203-208.

Bowdan E, Wyse GA (2000) Temporally patterned activity recorded from mandibular nerves of the isolated subesophageal ganglion of manduca. Journal of Insect Physiology 46:709-719.

Branch A, Shen P (2017) Central and peripheral regulation of appetite and food intake in drosophila. In: Appetite and food intake: Central control (Harris RBS, ed), Boca Raton (FL): CRC Press/Taylor & Francis.

Burke CJ, Huetteroth W, Owald D, Perisse E, Krashes MJ, Das G, Gohl D, Silies M, Certel S, Waddell S (2012) Layered reward signalling through octopamine and dopamine in drosophila.

Camilleri M (2015) Peripheral mechanisms in appetite regulation. Gastroenterology (United States) 148:1219-1233.

Chen J, Reiher W, Hermann-Luibl C, Sellami A, Cognigni P, Kondo S, Helfrich-Förster C, Veenstra JA, Wegener C (2016) Allatostatin A signalling in drosophila regulates feeding and sleep and is modulated by PDF. PLOS Genetics 12:e1006346.

Condon C, Lailvaux SP, Patek S (2016) Losing reduces maximum bite performance in house cricket contests. Functional Ecology (London) 30:1660-1664.

Curtis AT, Hori M, Green JM, Wolfgang WJ, Hiruma K, Riddiford LM (1984) Ecdysteroid regulation of the onset of cuticular melanization in allatectomized and black mutant manduca sexta larvae. Journal of Insect Physiology 30:597-606.

D'Amore DC, Moreno K, McHenry C, Wroe S (2011) The effects of biting and pulling on the forces generated during feeding in the komodo dragon (varanus komodoensis). PLoS One (San Francisco) 6:e26226.

Dantzer R (2004) Cytokine-induced sickness behaviour: A neuroimmune response to activation of innate immunity. European Journal of Pharmacology (Netherlands) 500:399-411.

Dethier VG (1976) The hungry fly: A physiological study of the behavior associated with feeding. Oxford, England: Harvard U Press.

Dethier VG (1964) Microscopic brains. Science 143:1138-1145.

Dethier VG, Gelperin A (1967) Hyperphagia in the blowfly. Journal of Experimental Biology 47:191.

Dus M, Lai J, Gunapala K, Min S, Tayler T, Hergarden A, Geraud E, Joseph C, Suh GB (2015) Nutrient sensor in the brain directs the action of the brain-gut axis in drosophila. Neuron (United States) 87:139-151.

Flood TF, Iguchi S, Gorczyca M, White B, Ito K, Yoshihara M (2013) A single pair of interneurons commands the drosophila feeding motor program. Nature (London) 499:83-87.

Frecka JM, Hollis JH, Mattes RD (2008) Effects of appetite, BMI, food form and flavor on mastication: Almonds as a test food. European Journal of Clinical Nutrition (England) 62:1231-1238.

Géminard C, Rulifson EJ, Léopold P (2009) Remote control of insulin secretion by fat cells in drosophila. Cell Metabolism 10:199-207.

Greter AM (2012) Towards understanding feeding motivation and management factors affecting feeding behaviour in limit-fed dairy heifers.

Griss C, Simpson SJ, Rohrbacher J, Rowell CHF (1991) Localization in the central nervous system of larval manduca sexta (lepidoptera: Sphingidae) of areas responsible for aspects of feeding behaviour. Journal of Insect Physiology 37:477-482.

Grunert LW, Clarke JW, Ahuja C, Eswaran H, Nijhout HF (2015) A quantitative analysis of growth and size regulation in manduca sexta: The physiological basis of variation in size and age at metamorphosis: e0127988. PLoS ONE 10:e0127988.

Gruninger TR, LeBoeuf B, Liu Y, Garcia LR (2007) Molecular signaling involved in regulating feeding and other motivated behaviors. Mol Neurobiol 35:1-20.

Hanson FE, Dethier VG (1973) Role of gustation and olfaction in food plant discrimination in the tobacco hornworm, manduca sexta. Journal of Insect Physiology (ENGLAND) 19:1019-1031.

Harris JW, Woodring J (1992) Effects of stress, age, season, and source colony on levels of octopamine, dopamine and serotonin in the honey bee (apis mellifera L.) brain. Journal of Insect Physiology 38:29-35.

Hergarden AC, Tayler TD, Anderson DJ (2012) Allatostatin-A neurons inhibit feeding behavior in adult drosophila. Proceedings of the National Academy of Sciences of the United States of America (Washington) 109:3967-3972.

Huber DR, Eason TG, Hueter RE, Motta PJ (2005) Analysis of the bite force and mechanical design of the feeding mechanism of the durophagous horn shark heterodontus francisci. Journal of Experimental Biology (England) 208:3553-3571.

Ismail SMM, Matsumura F (1992) Studies on the biochemical mechanisms of anorexia caused by formamidine pesticides in the tobacco hornworm manduca sexta. Insect Biochemistry and Molecular Biology 22:713-720.

Itagaki H, Hildebrand JG (1990) Olfactory interneurons in the brain of the larval sphinx moth manduca sexta. Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology (Germany) 167:309.

Itskov PM, Ribeiro C (2013) The dilemmas of the gourmet fly: The molecular and neuronal mechanisms of feeding and nutrient decision making in drosophila. Frontiers in Neuroscience (Switzerland) 7:12.

Kazlauskas N, Klappenbach M, Depino AM, Locatelli FF (2016) Sickness behavior in honey bees. Frontiers in Physiology (Switzerland) 7:261.

Kimura K, Hachiya T, Koganezawa M, Tazawa T, Yamamoto D (2008) Fruitless and doublesex coordinate to generate male-specific neurons that can initiate courtship. Neuron (United States) 59:759-769.

Kingsolver JG (2007) Variation in growth and instar number in field and laboratory manduca sexta. Proceedings of the Royal Society B: Biological Sciences (England) 274:977-981.

Kupfermann I (1974) Feeding behavior in aplysia: A simple system for the study of motivation. Behavioral Biology 10:1-26.

Kupfermann I, Weiss KR (1981) Tail pinch and handling facilitate feeding behavior in aplysia. Behavioral and Neural Biology 32:126-132.

Kuslansky B, Weiss KR, Kupfermann I (1987) Mechanisms underlying satiation of feeding behavior of the mollusc aplysia. Behavioral and Neural Biology 48:278-303.

Lailvaux SP, Zajitschek F, Dessman J, Brooks R (2011) Differential aging of bite and jump performance in virgin and mated teleogryllus commodus crickets. Evolution (Malden, USA) 65:3138-3147.

Langelan RE, Fisher JE, Hiruma K, Palli SR, Riddiford LM (2000) Patterns of MHR3 expression in the epidermis during a larval molt of the tobacco hornworm manduca sexta. Developmental Biology 227:481-494.

Lavigne G, Kim JS, Valiquette C, Lund JP (1987) Evidence that periodontal pressoreceptors provide positive feedback to jaw closing muscles during mastication. J Neurophysiol 58:342-358.

Lee T, Marticke S, Sung C, Robinow S, Luo L (2000) Cell-autonomous requirement of the USP/EcR-B ecdysone receptor for mushroom body neuronal remodeling in drosophila. Neuron 28:807-818.

Lund JP, Kolta A, Westberg K, Scot G (1998) Brainstem mechanisms underlying feeding behaviors. Current Opinion in Neurobiology 8:718-724.

M. Schubiger, A.A. Wade, G.E. Carney, J.W. Truman, M. Bender (1998) Drosophila EcR-B ecdysone receptor isoforms are required for larval molting and for neuron remodeling during metamorphosis. Development (England) 125:2053-2062.

MacWilliam D, Arensburger P, Higa J, Cui X, Adams M (2015) Behavioral and genomic characterization of molt-sleep in the tobaccohornworm, manduca sexta. Insect Biochemistry and Molecular Biology (Amsterdam) .

Madden AH, Chamberlin FS (1945) Biology of the tobacco hornworm in the southern cigar- tobacco district. Washington: U.S. Dep. of Agriculture.

Masumura M, Satake S, Saegusa H, Mizoguchi A (2000) Glucose stimulates the release of bombyxin, an insulin-related peptide of the silkworm bombyx mori. General and Comparative Endocrinology 118:393-399.

McKellar CE (2016) Motor control of fly feeding. Journal of Neurogenetics (England) 30:101-111.

McMillan LE, Miller DW, Adamo SA (2018) Eating when ill is risky: Immune defense impairs food detoxification in the caterpillar manduca sexta. Journal of Experimental Biology (Cambridge) 221:jeb173336.

McPeek MA (1989) Predation risk and the foraging behavior of competing stream insects. Ecology (Brooklyn) 70:1811-1825.

Melcher C, Pankratz MJ (2005) Candidate gustatory interneurons modulating feeding behavior in the drosophila brain. PLoS Biology (United States) 3:e305.

Mengoni Goñalons C, Guiraud M, de Brito Sanchez, María Gabriela, Farina WM (2016) Insulin effects on honeybee appetitive behaviour. The Journal of Experimental Biology (England) 219:3003-3008.

Michener CD (1974) The social behavior of the bees: A comparative study. Harvard University Press.

Miles CI, Booker R (1998) The role of the frontal ganglion in the feeding and eclosion behavior of the moth manduca sexta. Journal of Experimental Biology 201:1785.

Miles CI, Booker R (1994) The role of the frontal ganglion in foregut movements of the moth, manduca sexta. Journal of Comparative Physiology, A 174:755-767.

Murray MJ, Murray AB (1979) Anorexia of infection as a mechanism of host defense. The American Journal of Clinical Nutrition (United States) 32:593-596.

Nagata S, Morooka N, Matsumoto S, Kawai T, Nagasawa H (2011) Effects of neuropeptides on feeding initiation in larvae of the silkworm, bombyx mori. General and Comparative Endocrinology 172:90-95.

Nässel DR (2002) Neuropeptides in the nervous system of drosophila and other insects: Multiple roles as neuromodulators and neurohormones. Progress in Neurobiology 68:1-84.

Nässel DR, Kubrak OA, Liu Y, Luo J, Lushchak OV (2013) Factors that regulate insulin producing cells and their output in drosophila. Front Physiol 4:.

Nijhout HF, Riddiford LM, Mirth C, Shingleton AW, Suzuki Y, Callier V (2014) The developmental control of size in insects. Wiley Interdisciplinary Reviews: Developmental Biology (Hoboken, USA) 3:113-134.

Pankiw T, Page RE (2003) Effect of pheromones, hormones, and handling on sucrose response thresholds of honey bees (apis mellifera L.). Journal of Comparative Physiology A 189:675-684.

Pankiw T (2004) Brood pheromone regulates foraging activity of honey bees (hymenoptera: Apidae). J Econ Entomol 97:748-751.

Pflüger H, Stevenson P (2005) Evolutionary aspects of octopaminergic systems with emphasis on arthropods.

Pirke KM, Broocks A, Wilckens T, Marquard R, Schweiger U (1993) Starvation-induced hyperactivity in the rat: The role of endocrine and neurotransmitter changes. Neuroscience & Biobehavioral Reviews 17:287-294.

Pool A, Kvello P, Mann K, Cheung S, Gordon M, Wang L, Scott K (2014) Four GABAergic interneurons impose feeding restraint in drosophila. Neuron (United States) 83:164-177.

Powley TL, Phillips RJ (2004) Gastric satiation is volumetric, intestinal satiation is nutritive. Physiology & Behavior 82:69-74.

Prud'homme MJ, Lacroix MC, Badonnel K, Gougis S, Baly C, Salesse R, Caillol M (2009) Nutritional status modulates behavioural and olfactory bulb fos responses to isoamyl acetate or food odour in rats: Roles of orexins and leptin. Neuroscience 162:1287-1298.

Reynolds SE, Yeomans MR, Timmins WA (1986) The feeding behaviour of caterpillars (manduca sexta) on tobacco and on artificial diet. Physiological Entomology 11:39-51.

Riddiford LM (1994) Cellular and molecular actions of juvenile hormone I. general considerations and premetamorphic actions. In: Advances in insect physiology (Evans PD, ed), pp213-274.Academic Press.

Roh E, Song DK, Kim M (2016) Emerging role of the brain in the homeostatic regulation of energy and glucose metabolism. Experimental & Molecular Medicine (United States) 48:e216.

Rohrbacher J (1994a) Fictive chewing activity in motor neurons and interneurons of the suboesophageal ganglion of manduca sexta larvae. Journal of Comparative Physiology, A 175:629-637.

Rohrbacher J (1994b) Mandibular premotor interneurons of larval manduca sexta. J Comp Physiol A .

Root C, Ko K, Jafari A, Wang J (2011) Presynaptic facilitation by neuropeptide signaling mediates odor-driven food search. Cell 145:133-144.

Russell VW, Dunn PE (1996) Antibacterial proteins in the midgut of manduca sexta during metamorphosis. Journal of Insect Physiology 42:65-71.

Russell VW, Dunn PE (1991) Lysozyme in the midgut of manduca sexta during metamorphosis. Archives of Insect Biochemistry and Physiology (United States) 17:67-80.

Scharrer B, Scharrer E (1944) Neurosecretion vi. a comparison between the intercerebralis-cardiacum-allatum system of the insects and the hypothalamo-hypophyseal system of the vertebrates. The Biological Bulletin 87:242-251.

Scheiner R, Plückhahn S, Öney B, Blenau W, Erber J (2002) Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. Behavioural Brain Research (Netherlands) 136:545-553.

Sebastian Hückesfeld, Andreas Schoofs, Philipp Schlegel, Anton Miroschnikow, Michael J Pankratz (2015) Localization of motor neurons and central pattern generators for motor patterns underlying feeding behavior in drosophila larvae. PLoS One (San Francisco) 10:e0135011.

Simpson SJ, Bernays EA (1983) The regulation of feeding: Locusts and blowflies are not so different from mammals. Appetite (England) 4:313-346.

Slater PJB (1974) The temporal pattern of feeding in the zebra finch. Animal Behaviour 22:506-515.

Slater PJB, Lester NP (1982) Minimising errors in splitting behaviour into bouts. Behaviour 79:153-161.

Slessor K, Winston M, Le Conte Y (2005) Pheromone communication in the honeybee (apis mellifera L.). J Chem Ecol (New York) 31:2731-2745.

Stay B, Tobe SS (2007) The role of allatostatins in juvenile hormone synthesis in insects and crustaceans. Annual Review of Entomology 52:277-299.

Sullivan K, Fairn E, Adamo SA (2016) Sickness behaviour in the cricket gryllus texensis: Comparison with animals across phyla. Behavioural Processes (Netherlands) 128:134-143.

Sung EJ, Ryuda M, Matsumoto H, Uryu O, Ochiai M, Cook ME, Yi NY, Wang H, Putney JW, Bird GS, Shears SB, Hayakawa Y (2017) Cytokine signaling through drosophila Mthl10 ties lifespan to environmental stress. Pnas 114:13786-13791.

Susswein AJ, Weiss KR, Kupfermann I (1978) The effects of food arousal on the latency of biting in aplysia. Journal of Comparative Physiology 123:31-41.

Swinscow T (1997) Statistics at square one. BMJ Publishing Group.

Tanaka Y, Asaoka K, Takeda S (1994) Different feeding and gustatory responses to ecdysone and 20-hydroxyecdysone by larvae of the silkworm, bombyx mori. Journal of Chemical Ecology (United States) 20:125-133.

Tanaka Y (2016) Chapter 66 - myosuppressin. In: Handbook of hormones (Takei Y, Ando H, Tsutsui K, eds), pp13. San Diego: Academic Press.

Thaler JS, Contreras H, Davidowitz G (2014) Effects of predation risk and plant resistance on manduca sexta caterpillar feeding behaviour and physiology. Ecological Entomology 39:210-216.

Thompson SN, Redak RA (2000) Interactions of dietary protein and carbohydrate determine blood sugar level and regulate nutrient selection in the insect manduca sexta L. BBA - General Subjects 1523:91-102.

Timmins WA, Reynolds SE (1992) Physiological mechanisms underlying the control of meal size in manduca sexta larvae. Physiological Entomology 17:81-89.

Toates FM (1983) Control theory and feeding. Transactions of the Institute of Measurement and Control .

Tolkamp BJ, Allcroft DJ, Austin EJ, Nielsen BL, Kyriazakis I (1998) Satiety splits feeding behaviour into bouts. Journal of Theoretical Biology (England) 194:235-250.

Truman JW, Talbot WS, Fahrbach SE, Hogness DS (1994a) Ecdysone receptor expression in the CNS correlates with stage-specific responses to ecdysteroids during drosophila and manduca development. Development 120:219-234.

Truman JW, Talbot WS, Fahrbach SE, Hogness DS (1994b) Ecdysone receptor expression in the CNS correlates with stage-specific responses to ecdysteroids during drosophila and manduca development. Development 120:219-234.

Tsao C, Chen C, Lin C, Yang H, Lin S (2018) Drosophila mushroom bodies integrate hunger and satiety signals to control innate food-seeking behavior. eLife (Cambridge) 7:.

Tyler ER, Adams S, Mallon EB (2006) An immune response in the bumblebee, bombus terrestris leads to increased food consumption. BMC Physiology (England) 6:6.

van der Meij, M A, Bout RG (2006) Seed husking time and maximal bite force in finches. Journal of Experimental Biology (England) 209:3329-3335.

Waldbauer GP (1968) The consumption and utilization of food by insects. In: Advances in insect physiology The consumption and utilization of food by insects. pp229-288.Elsevier Science & Technology.

Weihmann T, Reinhardt L, Weißing K, Siebert T, Wipfler B (2015) Fast and powerful: Biomechanics and bite forces of the mandibles in the american cockroach periplaneta americana. PLoS One (San Francisco) 10:e0141226.

Wise RA (2004) Dopamine, learning and motivation. Nature Reviews Neuroscience (England) 5:483-494.

Yamamoto RT (1974) Induction of hostplant specificity in the tobacco hornworm, manduca sexta. Journal of Insect Physiology 20:641-650.

Yamanaka N, Rewitz KF, O'Connor MB (2013) Ecdysone control of developmental transitions: Lessons from drosophila research. Annu Rev Entomol 58:497-516.

Yin Peng Zhan, Li Liu, Yan Zhu (2016) Taotie neurons regulate appetite in drosophila. Nature Communications (London) 7:13633.

Yu Y, Huang R, Ye J, Zhang V, Wu C, Cheng G, Jia J, Wang L (2016) Regulation of starvation-induced hyperactivity by insulin and glucagon signaling in adult drosophila. eLife (Cambridge) 5:.

Zhao XL, Campos AR (2012) Insulin signalling in mushroom body neurons regulates feeding behaviour in drosophila larvae. Journal of Experimental Biology (England) 215:2696-2702.