Role of the Gustatory Thalamus and Medial Amygdala in Taste Learning

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THESIS

Submitted as partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Psychology
in the Graduate College of the
University of Illinois at Chicago, 2017

Chicago, Illinois

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ACKNOWLEDGMENTS

I would like to thank my thesis committee for their insightful feedback and assistance in shaping this project. I owe a special debt of gratitude to my advisor, Steve Reilly, for his guidance throughout my graduate career.

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AAV	adeno associated virus
ANOVA	analysis of variance
ARFID	avoidant restrictive food intake disorder
BLA	basolateral amygdala
BNST	bed nucleus of the stria terminalis
CNA	central nucleus of the amygdala
CNO	clozapine-N-oxide
CS	conditioned stimulus
CTA	conditioned taste aversion
DREADD	designer receptor exclusively activated by designer drugs
η_p^2	partial eta squared
EGFP	enhanced green fluorescent protein
GABA	γ –amino-butyric acid
GC	gustatory cortex
GT	gustatory thalmus
hM3D(Gq)	mutated human muscarinic receptor coupled to the excitatory G-protein G_q
hM4D(Gi)	mutated human muscarinic receptor coupled to the inhibitory G-protein G_i
LH	lateral hypothalamus
LiCl	lithium chloride
MeA	medial amygdala
NMDA	n-methyl-d-aspartic acid
NTS	nucleus tractus solitarius
PBN	parabrachial nucleus
US	unconditioned stimulus
VPPC	ventroposteriormedial thalamus parvicellular division

SUMMARY

Three experiments were conducted to examine the function of the gustatory thalamus and medial amygdala in taste neophobia. In Experiment 1A, the gustatory thalamus was infused with GABA agonists (i.e., baclofen and muscimol) to cause a temporary neuronal inactivation immediately before and during the presentation of a novel tastant. This experiment was designed to replicate, extend, and refine our understanding of the attenuation of taste neophobia caused by permanent lesions of the gustatory thalamus. Experiment 2A employed the same approach but infusions were targeted to the medial amygdala to test two competing hypotheses. First, inactivation of medial amygdala neurons might replicate the effect of permanent lesions and cause an attenuation of taste neophobia. Alternatively, neurons in the medial amygdala might normally be inhibited during taste neophobia, in which case the infusion of GABA agonists may cause an enhanced taste neophobia response. In Experiment 3, neurons within the medial amygdala were transfected with designer receptors exclusively activated by designer drugs (DREADDs) that produced, in separate groups of animals, either neuronal excitation or inhibition upon systemic injection of an otherwise inert ligand. Thus, Experiment 3 tested the hypothesis that exogenous excitation or inhibition of neurons within the MeA would have opposing effects of, respectively, attenuating or enhancing the expression of taste neophobia.

Infusion of GABA agonists into the gustatory thalamus prior to the presentation of a novel taste solution caused an attenuation of taste neophobia observed as elevated intake of the novel tastant relative to control subjects. Therefore, the hypothesis that neuronal excitation in gustatory thalamus is critical for the normal expression of taste

neophobia was supported. GABA agonist infusions in the medial amygdala prior to novel taste access had little effect on the expression of taste neophobia contrary to the predicted result of either hypothesis. However, in Experiment 3 the inhibition of neurons in the medial amygdala via DREADDs produced a heightened neophobic response supporting the hypothesis that medial amygdala inhibition drives the expression of taste neophobia. Medial amygdala excitation via DREADDs did not produce the predicted attenuation of taste neophobia. There are some indications that DREADDs excitation of MeA neurons during the initial exposure to the novel tastant attenuated the rate of taste neophobia habituation and may have long lasting effects on taste palatability.

Overall, my results indicate that inactivating neurons in the gustatory thalamus with GABA agonists can attenuate the expression of taste neophobia. Thus, it seems that neuronal excitation within the GT is critical for the expression of taste neophobia, and this excitation can be modulated by GABA signaling. Inhibiting MeA neurons with DREADDs, but not GABA agonists, enhances taste neophobia, which is a behavioral effect that has not been previously observed in response to any neural manipulation. Also, I found that GABA agonist infusions into either the GT or MeA after access to a novel tastant was sufficient to cause a conditioned taste aversion.

From a broader perspective, these studies have highlighted the differences that seemingly similar neural manipulations can have, that is, temporary pharmacological lesions do not necessarily replicate the effects of permanent lesions and can produce novel effects, like CTA. And, neuronal inhibition via pharmacological manipulations of

endogenous signaling systems (i.e., GABA) or exogenous manipulations (i.e., DREADDs) can have different effects on behavior. We see the comparison of effects produced by various techniques as a beneficial means to learn about a system from many different angles.

I. INTRODUCTION

On the first encounter with a novel edible animals face a dangerous, maybe lethal, choice: is this a safe nutritious food or is it poisonous? To defend against self-poisoning, powerful feeding system defense mechanisms exist within the central nervous system, specifically taste neophobia and conditioned taste aversion (CTA). Taste neophobia, the fear of novel tastes, limits the palatability, and thus consumption, of unknown edibles that may be poisonous (e.g., Garcia & Hankins, 1975; Hobbs & Roberts, 1993; Janzen, 1977; Lin, Amodeo, Arthurs, & Reilly, 2012a). If this fear proves unfounded (i.e., no aversive internal effects emerge following ingestion), taste neophobia habituates allowing increased consumption of the food on subsequent exposures (e.g., Lin et al., 2012a). However, when suspicions of toxicity are encouraged by the experience of aversive post-ingestive effects, a CTA develops (Garcia, Kimeldorf, & Koelling, 1955; Garcia & Koelling, 1966; for a book length review see Reilly & Schachtman, 2009). CTA renders the taste of the toxic food disgusting so that it will not be consumed in future encounters (e.g., Arthurs, 2012; Arthurs, Lin, Amodeo, & Reilly, 2012; Arthurs & Reilly, 2013; Breslin, Spector, & Grill, 1992; Dwyer, 2012; Spector, Breslin, & Grill, 1988). So, when an animal has ingested a novel food that contains poison, taste neophobia both increases the likelihood of survival (by limiting the initial dose of poison) and primes the rapid development of a CTA (that prevents repeated self-poisoning). Thus, taste neophobia and CTA are interrelated. Following the habituation of taste neophobia (i.e., when the food has become familiar and safe), if the food subsequently becomes toxic, a CTA will be slower to develop (a phenomenon termed latent inhibition; Lubow, 1989; 2009). On the other hand, CTAs to multiple foods can enhance taste neophobia to the point that an animal abandons

feeding and starves to death (Richter, 1953). The current focus is on the neural substrates of taste neophobia, but it is critical to bear in mind the relationship between these two feeding system defense mechanisms.

To frame the current investigation, we will begin by reviewing taste neophobia as a behavioral phenomenon in human and non-human animals, and how taste neophobia is assessed in the laboratory. Then, we will discuss some salient features of palatability as a psychological construct, as taste neophobia is an ingestive behavior closely linked with taste palatability. Having oriented ourselves to the psychological aspects of taste neophobia, we will turn to neuroanatomical matters reviewing the basic structure of the central gustatory system and the current base of knowledge regarding the neural substrates of taste neophobia. With this broader background in place we can address (1) some of the recent findings that prompted the current investigation, (2) the specific hypotheses and predictions thereof, and (3) the rationale and designs of the particular experiments.

1.1 Taste Neophobia as a Behavioral Phenomenon

Taste neophobia is an important influence on food selection in both human and non-human animals expressed as a hesitation to sample unfamiliar foods (Barnett, 1958; Birch & Marlin, 1982; Corey, 1979; Domjan, 1977; Lin et al., 2012a; Lin & Reilly, 2012; Pliner & Salvy, 2006; Rozin, 1976). However, interest in researching taste neophobia is motivated by very different factors across these populations. In humans, the major focus in taste neophobia research has been on children (Cooke, Wardle & Gibson, 2003; Dovey, Staples, Gibson, & Halford, 2008; Falciglia, Couch, Gribble, Pabst, & Frank, 2000). Taste

neophobia has a major influence on diet selection in children, and exaggerated taste neophobia can lead to 'picky eating' (i.e., a difficulty or even refusal to integrate new foods into the diet), which is troublesome to parents and other caretakers, or can develop into avoidant restrictive food intake disorder (ARFID) a childhood eating disorder that can persist even into adulthood (Fisher et al., 2014; Forman et al., 2014; Kenney & Walsh, 2013; Kreipe & Palomaki, 2012; Nicely, Lane-Loney, Masciulli, Hollenbeak, & Ornstein, 2014; Norris et al., 2014). In the non-human animal literature, taste neophobia plays a prominent role in controlling animal populations. That is, whether attempting to reduce a pest population (e.g., rodents; Barett & Spencer, 1949; Elton, 1954) or increase commercial livestock production (e.g., dairy cows; Launchbaugh, Provenza, & Werkmeister, 1997) it is necessary to account for taste neophobia.

In humans, taste neophobia can be viewed as a personality trait, with considerable individual variability, that governs the willingness to sample unknown foods (e.g., Fallon & Rozin, 1983; Pliner & Salvy, 2006; Rozin & Fallon 1980). Taste neophobia expression can be influenced by a variety of factors including direct and indirect information about the taste and effects of the food, social modeling of parents or peers, type of food (e.g., plant versus animal), overall novelty of the feeding situation, and arousal (for a review see Pliner & Salvy, 2006). Taste neophobia is a primary factor in what is commonly referred to as 'picky eating' in children and can hamper the development of a well-rounded nutritious diet (Dovey et al., 2008; Falciglia et al., 2000; Johnson, Davies, Boles, Gavin, & Bellows, 2015). Evidence suggests that food/taste neophobia is influenced by age, with neophobia appearing minimal in infants, peaking between the ages of 2 to 5 years old, and then gradually declining thereafter (e.g., Cashdan, 1994; Pliner & Loewen, 1997).

Neophobia is exhibited for all manner of foods, but is particularly prominent for vegetables in children (Cashdan, 1998) but shifting to animal products in adults (Pliner & Pelchat, 1991). Cooke, Wardle, & Gibson (2003) conducted a survey of 564 mothers with children between 2-6 years old. High levels of neophobia were associated with lower consumption of vegetables, fruit, and meat, but not eggs, fatty foods, starches, or sweets. Cooke et al. forward the account that this pattern of neophobia represents a now obsolete adaptive mechanism geared to avoid the most likely sources of poisoning: endogenous plant toxins (e.g., Fowler, 1983) and spoiled animal foods (e.g., Hobbs & Roberts, 1993).

Current evidence suggests that repeated exposures to foods that trigger a neophobic response in children is an effective strategy in overcoming neophobia-induced food rejection during normal behavior as well as in 'picky eaters' (Birch & Marlin, 1982; Kaar, Shapiro, Fell, & Johnson, 2016; Pliner, Pelchat, & Grabski, 1993; Wardle, Herrera, Cooke, & Gibson, 2003). However, once novel food avoidance has reached clinical criterion for ARFID treatment shifts to individualized plans involving a combination of behavioral strategies, cognitive behavioral therapy, and family interventions (Forman et al., 2014; Lock, 2015).

The study of taste neophobia in the non-human animal laboratory became prominent with the advent of the academic study of rodent pest control, which began around the onset of World War II. Researchers in the Bureau of Animal Population at Oxford University undertook the task of conducting basic research on rodent population control methodologies to aid in the war effort (Elton, 1954; for reviews see Freeman & Riley, 2009; Keiner, 2005). In initial attempts to test various poisons, baits and delivery methods these researchers experienced minimal success due to a phenomenon they

termed ‘bait-shyness’ or what we refer to as taste neophobia. That is, rodents were hesitant to sample the novel poisoned baits, and consumed a small (non-lethal) amount on initial encounters and thereafter avoided the bait (i.e., a CTA was acquired; Elton, 1954). In practical applications, this issue was addressed by a method called prebaiting in which non-poisoned baits were deployed until they were reliably consumed and then a poisoned bait was substituted (e.g., Barnett & Spencer, 1949). The early failures of the Bureau of Animal Population studies and the necessity of prebaiting highlight the importance of taste neophobia as a feeding system defense mechanism and the integration of taste neophobia and CTA.

In commercial livestock applications, animals (e.g., cows, mink, pigs, sheep) are submitted to feed changes (e.g., weaning, pasture changes) and any taste neophobia-induced delay in adapting to the novel feed adversely impacts production (e.g., Launchbaugh, Provenza, & Werkmeister, 1997; Malmkvist, Herskin, & Christensen, 2003; Oostindjer, Munoz, Van den Brand, Kemp, & Bolhuis, 2011; Simitzis, Feggeros, Bizelis, & Deligeorgis, 2005). Perhaps the most critical feeding shift occurs during weaning as animals establish consumption of solid foods. Young animals develop feeding strategies by both social modeling and experimentation (Provenza & Balph, 1988). So, for example, an effective strategy to reduce neophobia in piglets has been to maintain maternal contact and provide enriched environments promoting social interaction (Oostindjer et al., 2011). Also, novel food intake can be increased in lambs by mixing novel and familiar foods or providing repeated exposures to several novel foods, which leads to an overall decline in neophobia (Launchbaugh et al., 1997).

Regarding psychological models of taste neophobia, I am aware of only one model posited by Nachman and Ashe (1974), stating that taste neophobia must involve a memory process and a perceptual alerting process. A disruption of the memory process would likely render animals incapable of forming, retaining, or accessing safe taste memories. That is, animals would be unable to habituate taste neophobia. On the other hand, disrupting the perceptual alerting mechanism might cause animals to fail to respond appropriately to the perception of a novel taste. That is, animals might recognize that the taste is novel but fail to suppress intake, thus failing to respond to the danger implied by the novel taste.

1.2 Assessing Taste Neophobia in the Laboratory

Typically, taste neophobia is assessed in the laboratory by examining the intake of aqueous taste solutions (Barnett, 1958; Corey, 1979; Domjan, 1977; Garcia & Hankins, 1975; Miller & Holzman, 1981; Rozin, 1976). Taste neophobia can be judged to have occurred if a taste is consumed in smaller quantities when novel, hence dangerous, than when familiar and safe. However, when assessing taste neophobia in the laboratory an appropriate standard by which to judge the occurrence of taste neophobia must be used. Two main standards are employed in the literature, a single trial definition of taste neophobia and a multi-trial definition of taste neophobia. A single trial procedure involves comparing the intake of a novel tastant to the intake of water on the previous day (e.g., Morris, Frey, Kasambira, & Petrides, 1999; Yamamoto, Fujimoto, Shimura, & Sakai, 1995). By this standard, if thirsty animals consume less of a novel tastant than water on the previous day, then, taste neophobia has occurred. This definition of taste neophobia

is typically incorporated into CTA experiments to examine taste neophobia during the first taste trial before any aversive stimulus has been paired with the novel taste. This definition is problematic for several reasons. First, comparing intake of water and a tastant is rarely a valid comparison, typically a tastant is either more (e.g., 0.9% NaCl, 5% sucrose; Miller & Holzman, 1981) or less (e.g., 1% citric acid; Miller & Holzman, 1981) preferred than water, thereby yielding a faulty comparison (see Reilly & Bornovalova, 2005). Furthermore, without additional taste exposures in the absence of contingent aversive consequences the habituation of taste neophobia cannot be examined. Therefore, a clear assessment of taste neophobia can only be obtained by using a multi trial design in which the tastant is repeatedly presented in the absence of aversive consequences such the tastant becomes familiar and safe (e.g., Lin et al., 2012a). In this design, the magnitude of the taste neophobia response can be determined by comparing intake when the tastant is either novel or familiar. The habituation of taste neophobia can be assessed across trials as intake increases until asymptotic performance is reached. In CTA experiments, a multi-trial assessment of taste neophobia can be achieved by including a saline treated control group that is presented the tastant on enough trials to allow for asymptotic intake to be achieved (e.g., Arthurs, 2012; Arthurs & Reilly, 2013).

To assess the influence of a neural manipulation on taste neophobia it is necessary to rule out potential alternative explanations for behavior. When examining the role of structures within the central gustatory system a primary concern is that neural manipulations will alter the perception of the taste properties of a given stimulus. Such a disruption in taste perception can be tested for in a couple of ways. First, tastants are consumed in a concentration dependent matter (e.g., Flynn, Grill, Schwartz, & Norgren,

1991; Scalera, Grigson, & Norgren, 1997; Spector, 1995a; Spector, Grill, & Norgren, 1993; Spector, Scalera, Grill, & Norgren, 1995). These sorts of concentration curves can be used to determine if lesioned animals perceive tastes normally, and if not, whether the lesion-induced deficit in taste perception attenuates the intensity of taste perception, observed as a rightward shift in the concentration curve seen in lesioned animals as compared to normal controls. Second, as an internal control, asymptotic performance in the lesion and non-lesion saline treated control groups can be compared. If, the lesion has influenced taste perception, then asymptotic intake should be different. Notably, while reviewing the literature on the neural substrates of taste neophobia we will see that if a lesion has been shown to cause a deficit in taste neophobia it has always been an attenuation, that is, lesions that influence taste neophobia cause a novel tastant to be consumed in greater quantities than normal. In these cases, it is important to determine if the lesion has merely caused the tastant to be perceived as less intense yielding a rightward shift in the intake curve. If a perceptual deficit can be ruled out, then, we are left to conclude that the lesion is disrupting some aspect of taste neophobia.

What is the underlying nature of the taste neophobia response? Traditionally, taste neophobia has been conceptualized as the fear-induced avoidance of a novel food until the post-ingestive effects become known (Barnett, 1958; Corey, 1978; Domjan, 1977; Garcia & Hankins, 1975; Rozin, 1976). Avoidance can be contrasted with aversion during which foods are rejected because they are disgusting (Parker, 2003; Pelchat, Grill, Rozin, & Jacobs, 1983). Therefore, the distinction between avoidance and aversion hinges on palatability. Taste avoidance involves a decrease of intake without a change in palatability, whereas, both intake and palatability are suppressed in taste aversion. Since

these phenomena are identical in terms of intake we must assess palatability to differentiate avoidance from aversion. Surprisingly, the role of palatability in the occurrence and habituation of taste neophobia was only recently assessed by Lin, Amodeo, Arthurs, and Reilly (2012a; but see Neath, Limebeer, Reilly, & Parker, 2010).

1.3 What is Palatability?

Palatability is the affective value of a taste/food, which ranges along a continuum from positive to negative (Berridge, 2000; Breslin et al., 1992; Steiner, Glaser, Hawilo, & Berridge, 2001). One methodology for assessing palatability in non-human animals (e.g., rats) is the taste reactivity test (e.g., Grill & Norgren, 1978a, 1978b; for review see Berridge, 2000). The taste reactivity test involves the analysis of orofacial and somatic responses to taste stimuli. These responses can be defined as either ingestive or aversive. Ingestive responses include mouth movements, tongue protrusions, face washing and paw licking, while aversive responses include a single orofacial response, gaping, which is analogous to retching or vomiting (Parker, 2014; Travers & Norgren, 1986). While gaping gates all aversive taste reactivity sequences, additional somatic responses can occur to extremely aversive tastes including chin rubbing, headshakes, and face washing with paw flailing (e.g., Grill, 1985). Typically, taste stimuli are delivered in small volumes directly into the oral cavity via an indwelling cannula, which (1) affords a great deal of experimental control in the presentation of stimuli, (2) allows discreet time periods for the analysis of the behavioral response, and (3) limits the opportunity for post-ingestive feedback to influence behavior.

Attempts have been made to analyze taste reactivity during voluntary consumption of an aqueous tastant (e.g., Brown, Penney, Skinner, & Martin, 2011; Pelchat et al., 1983). However, this approach presents several technical challenges in that licking of the solution represents a competing response with ingestive taste reactivity measures (e.g., mouth movements, tongue protrusions) and once a tastant is no longer voluntarily sampled, then, there is no opportunity to observe aversive taste reactivity responses (e.g., gapes). Furthermore, Wilkins and Bernstein (2006) reported that delivery method—intraoral versus voluntary—altered the expression pattern of the immediate early gene c-Fos, which is widely used as an indicator of neuronal activation (e.g., Morgan & Curran, 1991). Thus, the tastant delivery method may influence the neural circuitry involved in behavior. For present purposes, we are interested in understanding the neural substrates of taste neophobia underlying normal voluntary ingestive behavior; therefore, all stimuli were administered by voluntary consumption in thirsty rats, an approach for which taste reactivity is poorly suited.

I employed lick pattern analysis to measure palatability because this approach allows the simultaneous measurement of palatability and voluntary intake. Lick pattern analysis involves monitoring the temporal structure of licks on a spout during voluntary intake (e.g., Davis, 1973; 1989; 1998; Davis & Levine, 1977; Davis & Smith, 1992). Several dependent measures can be extracted from lick patterns, but two have been shown to track taste palatability: lick cluster size and initial lick rate (for reviews see Dwyer, 2012; Lin, Arthurs, & Reilly, 2014). A lick cluster is defined as a run of licks separated by brief pauses (e.g., < 0.5 sec) and initial lick rate is the number of licks occurring during a given time period at the beginning of the tastant access period (e.g.,

3-min after the first lick). For quinine, an unconditionally aversive taste, initial lick rate and lick cluster size decrease as a linear function of increasing concentration (Hsiao & Fan, 1993; Spector & St. John, 1998). Conversely, for unconditionally preferred stimuli such as sucrose initial lick rate and lick cluster size increase monotonically as a function of increasing concentration (Davis & Smith, 1992). Importantly, measures of palatability such as initial lick rate and lick cluster size can be dissociated from intake, which traces out a parabolic concentration curve with peak intake at moderate concentrations of sucrose with intake falling off for low or high concentrations of sucrose (e.g., Davis & Smith, 1992). Given these patterns of results initial lick rate and lick cluster size are used as measures of palatability (i.e., hedonic value) for aqueous taste stimuli (for reviews see Davis, 1989, 1998; Davis & Levine, 1977; Dwyer, 2012; Lin et al., 2014).

If taste neophobia, and the habituation thereof, were an avoidance response, then, one should expect palatability to be stable across all exposures as the stimulus transitions from novel to familiar. However, as shown by Lin, Amodeo, Arthurs, and Reilly (2012a), both intake and palatability (i.e., lick cluster and initial lick rate) for a novel tastant (0.5% saccharin) were low on Trial 1. Over repeated exposures, each measure increased substantially thereby revealing the true magnitude of palatability suppression produced by the taste neophobia mechanism on the first taste exposure. Thus, perhaps taste neophobia is more accurately defined as an unconditioned taste aversion response, rather than an unconditioned taste avoidance.

If aversive internal consequences are perceived following the initial encounter with a novel taste the CTA mechanism reduces palatability further and prevents intake on future encounters (e.g., Arthurs et al., 2012). From a basic science perspective, taste

neophobia now becomes of critical interest since it acts as a gate for the feeding system: taste neophobia serves a primary role in the consumption of food and rejection of poison. From a clinical perspective, understanding the neural substrates of taste neophobia will advance our basic understanding of a system central to issues faced by clinical populations suffering from maladaptive unconditioned and conditioned aversions such as, for example, ARFID or chemotherapy-induced CTA.

ARFID recently emerged as a clinical diagnosis with the publication of the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 2013), which significantly revised the diagnosis of childhood eating disorders (e.g., Fischer et al., 2014). ARFID manifests in childhood (but can persist into adulthood), and is expressed as persistently disordered eating that can lead to (1) significant weight loss (or, in children, failure to grow), (2) nutritional deficiency, (3) dependence on feeding tubes or oral nutritional supplements, or (4) interference with psychosocial functioning (American Psychiatric Association, 2013). Furthermore, these feeding disturbances are all in the absence of disturbed body image. Several mechanisms are thought to contribute to ARFID including inadequate food intake based on a restricted range of accepted foods (Bryant-Waugh, Markham, Kreipe, & Walsh, 2010). That is, ARFIDs can be attributed, in part, to extreme levels of taste neophobia such that the palatability of the novel food is so low that it is disgusting, thereby precluding future sampling, and thus preventing the habituation of taste neophobia. For some of these individuals, the palatability of novel foods may become trapped in a kind of limbo: the novel food is not sampled because it is disgusting and it is disgusting because it is not sampled. Research into the neural underpinnings of taste neophobia will provide a base of knowledge from which to pursue

translational studies to allow the attenuation of neophobia sufficient to overcome the inertia of neophobia-induced malnutrition.

CTAs are an important feeding system defense mechanism in most animals, but in humans they can be maladaptive. For example, maladaptive CTAs are a debilitating consequence of some widely used medical therapies (e.g., chemotherapy). For patients undergoing chemotherapy, maladaptive CTAs can have such a negative impact that life-saving treatments are discontinued (Carey & Burish, 1988; Miller & Kearney, 2004). Repeated bouts of chemotherapy-induced illness can cause even extremely familiar preferred foods to be rendered disgusting (Bernstein, 1985). As previously noted, latent inhibition delays CTA acquisition to these familiar foods, but the strength and frequency of the chemotherapy agent eventually wins out (Scalera & Bavieri, 2009). By developing a comprehensive understanding of (1) taste neophobia, (2) the habituation of taste neophobia, and (3) how the latent inhibition caused by the attenuation of taste neophobia delays CTA it will become possible to manipulate the interactions of these feeding system defense mechanisms to develop treatments that further delay the acquisition of chemotherapy-induced CTA, perhaps indefinitely.

1.4 Central Gustatory System

The rat has proven to be a highly successful model system to analyze the neural substrates that govern taste learning, and one that I will employ in the current set of experiments. Therefore, an overview of the central gustatory system is required (see Figure 1; for reviews see Lundy & Norgren, 2004; Spector, 2009). In the rat, taste information ascends from the oral cavity (e.g., tongue, palate, pharynx, and epiglottis) via

branches of three cranial nerves: facial (VII), glossopharyngeal (IX), and vagus (X), which terminate in the rostral portion of the nucleus tractus solitarius (NTS; Barraco, El-Ridi, Ergene, Parizon, & Bradley, 1992; Halsell, Travers, & Travers, 1993; Hamilton & Norgren, 1984; Whitehead & Frank, 1983). Taste neurons from the rostral NTS terminate in the pontine parabrachial nucleus (PBN) primarily in the medial sub-nucleus (mPBN; Herbert, Moga, & Saper, 1990; Norgren & Leonard, 1971; 1973). Taste responsive neurons in the mPBN project to diverse forebrain targets along two pathways. The ascending dorsal pathway involves reciprocal projections with the ventroposteriormedial parvicellular region of the thalamus, henceforth referred to as the gustatory thalamus (GT; Cechetto & Saper, 1987; Emmers, 1977; Halsell, 1992; Norgren, 1974; Ogawa, Hayama, & Ito, 1984), which, in turn, is reciprocally connected with the gustatory region of the insular cortex (GC; Cechetto & Saper, 1987; Kosar, Grill, & Norgren, 1986; Norgren & Wolf, 1975; Wolf, 1968). The ventral pathway ascends from the PBN and consists of a network of connections with several nuclei including the central nucleus of the amygdala (CNA), bed nucleus of the stria terminalis (BNST), lateral hypothalamus (Alden, Besson, & Bernard, 1994; Fulwiler & Saper, 1984; Halsell, 1992; Krukoff, Harris, & Jhamandas, 1993; Moga et al., 1990; Norgren, 1974, 1976; Saper & Loewy, 1980), and substantia innominata (Block & Schwartzbaum, 1983; Fulwiler & Saper, 1984). Furthermore, the ascending and descending projections of the central gustatory system are largely ipsilateral, although some minor contralateral projections do exist (Lundy & Norgren, 2004; Magableh & Lundy, 2014; Tokita, Inoue, & Boughter, 2009).

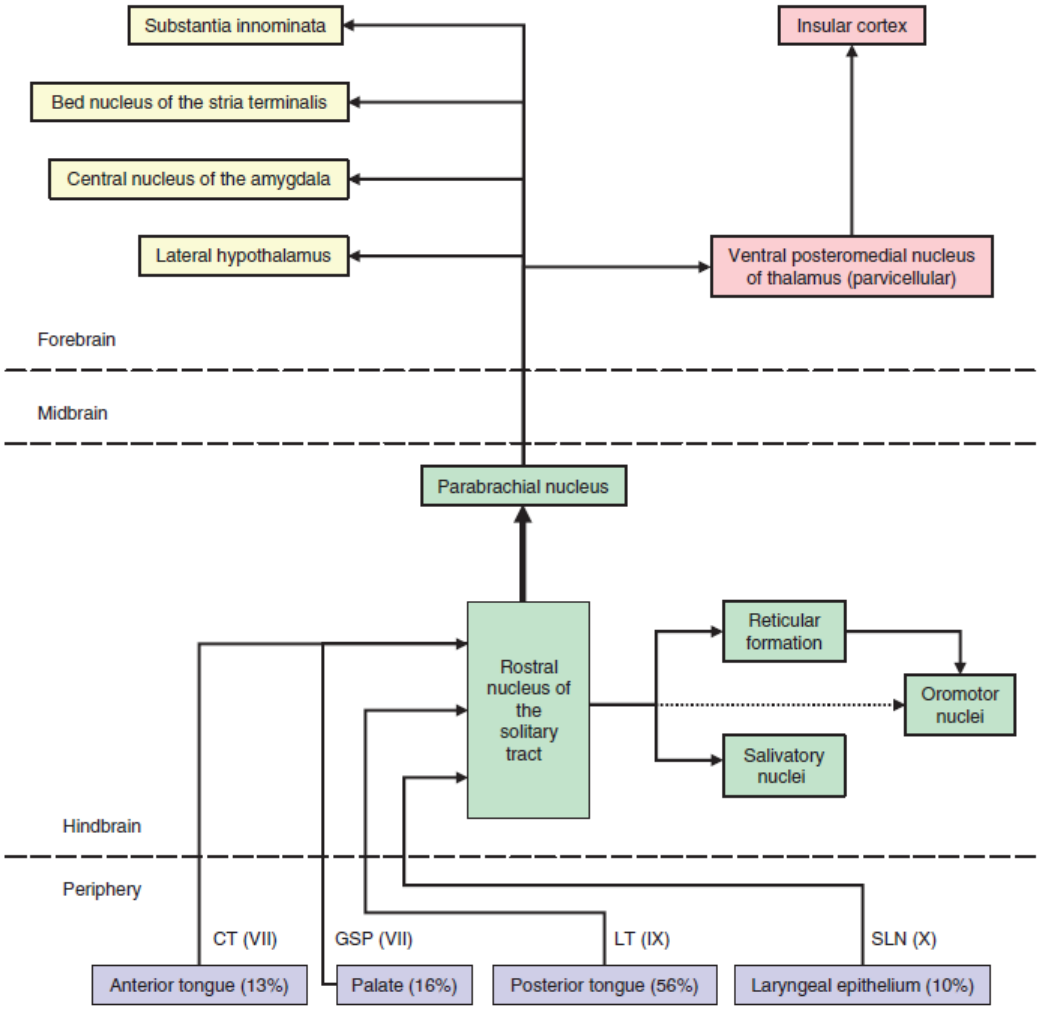

Figure 1. Schematic representation of the ascending pathways of the central gustatory system (Spector, 2009).

It should be noted that, there are dorsal-ventral pathway interconnections not included in Figure 1, as they have not been definitively implicated in the processing of taste information per se. However, these interconnections may serve an important role in the interface of the canonical taste system with other systems concerned with hedonic value assessments or fear-related behavior. For example, the GC is reciprocally

connected with the basolateral amygdala (BLA), a connection implicated in hedonic processing (e.g., Grossman, Fontanini, Wieskopf, & Katz, 2008). The GT sends a projection to the medial amygdala (MeA; Nakashima et al., 2000; Ottersen & Ben-Ari, 1979; Turner & Herkenham, 1991), a region implicated in a variety of fear-related behaviors (e.g., predator odors, fear-potentiated startle; Li, Maglinao, & Takahashi, 2004; Takahashi, Hubbard, Lee, Dar, & Sipes, 2007; Walker, Paschall, & Davis, 2005). The focus of the present work is on the GT and MeA.

The GT appears in coronal section as a narrow medial projection of the ventral posteromedial thalamic nucleus (Paxinos & Watson, 2007) and is wedged between the parafasicular nucleus and the medial lemniscus. The GT is anatomically continuous with what Paxinos and Watson (2007) term the ventral posterior nucleus of the thalamus, parvicellular part (VPPC). Taste-responsive neurons are concentrated in the medial aspect of the VPPC rapidly decreasing in frequency laterally where thermally-responsive neurons predominate (Emmers, 1977; Liu & Fontanini, 2015; Lundy & Norgren, 2004). Continuing laterally, VPPC neuronal responsivity shifts from thermal to tactile. As such, the lateral half of the VPPC contains few taste-responsive neurons. Thus, the GT is defined as the medial half of the VPPC as depicted in Paxinos and Watson (2007). Traditionally, the GT has been thought of as a simple relay for passing sensory information from the PBN to the GC (e.g., Ables & Benjamin, 1960; Andersson & Jewell, 1957; Blum, Walker, & Ruch, 1943). Three deficits traditionally attributed to GT lesions (1) innate taste preferences and aversions, (2) salt appetite, and (3) CTA learning are likely the result of large electrolytic lesions that damage structures adjacent to the GT, including fibers of the ventral taste pathway, since these deficits are not found with

circumscribed GT lesions (for a review see Reilly, 1998). However, as reviewed by Reilly (1998), small electrophysiologically-guided electrolytic lesions of GT neurons revealed a different set of behavioral deficits in taste-attenuated odor aversion learning (Reilly & Pritchard, 1996b), autoshaping (Reilly & Pritchard, 1997), and anticipatory negative contrast (Reilly & Pritchard, 1996b). Thus, the GT does not serve as a simple taste relay from hindbrain to cortex, but is involved in several more complex taste-guided behaviors.

The amygdala is a heterogeneous grouping of structures in the ventral temporal lobe that can be subdivided into a variety of nuclei (Janak & Tye, 2015; Reilly & Bornovalova, 2005). In coronal sections the MeA appears as an oblong group of four contiguous subnuclei (anterior dorsal, anterior ventral, posterior dorsal, posterior ventral) within the most ventromedial portion of the amygdala bordered medially by the optic tract and laterally by the basomedian and central nuclei of the amydgala, as well as the BNST interamygdaloid division and intercalated nuclei (Paxinos & Watson, 2007). The MeA is perhaps best understood for its role in odor-guided behaviors such as social interaction (Arakawa, Arakawa, & Deak, 2010; Maras & Petrulis, 2010), and fear of predator odor (Li et al., 2004; Takahashi et al., 2007; Walker et al., 2005). To the best of my knowledge, the MeA does not receive direct taste projections from the PBN, but it is a recipient of a descending projection from GT (Nakashima et al., 2000; Ottersen & Ben-Ari, 1979; Turner & Herkenham, 1991), although the role, if any, of this projection in taste guided behavior is not understood.

1.5 Neural Substrates of Taste Neophobia

To identify taste system nuclei involved in taste neophobia a useful approach has been the analysis of the expression of immediate early genes such as c-Fos, a marker of neuronal excitation (Morgan & Curran, 1991). Permanent brain lesions can be used to determine if a given brain region is critical for taste neophobia in general. Then, temporary neuronal inactivation can be used to ask whether a brain region is critical to the expression of taste neophobia, the habituation of taste neophobia, or the retention of safe taste memory. Once a brain area is implicated in behavior, then, the role of specific neurochemical systems can also be addressed with the use of targeted intracranial microinjections of pharmacological agents. Over the last 10 years or so an increasing number of sophisticated techniques have been developed to manipulate defined populations of cells with the use of genetically targeted expression of effector genes, such as designer receptors exclusively activated by designer drugs (DREADDs; e.g., Roth, 2016). To my knowledge, DREADDs have not been used to study taste neophobia.

In evaluating the literature examining the neural substrates of taste neophobia it is important to bear in mind the methods used to assess the occurrence and habituation of taste neophobia. As mentioned previously, to assess the occurrence and habituation of taste neophobia it is necessary to compare the consumption of the tastant when novel, and potentially dangerous, to consumption when the tastant has become familiar and safe. However, in many cases, attempts have been made to examine lesion effects on taste neophobia during a CTA experiment. Typically, these studies impose a one-trial definition of taste neophobia comparing novel tastant intake with water intake on the

previous day. This approach can offer some hints about potential lesion effects on taste neophobia, but are not definitive and must be viewed with caution.

Koh, Wilkins, and Bernstein (2003) conducted the first study that used c-Fos expression to explore the neural substrates of taste neophobia. In the Koh et al. (2003) study, a series of four experiments were conducted to assess differential levels of c-Fos expression in response to novel versus familiar 0.5% saccharin in a variety of structures including BLA, CNA, GC, NTS, and PBN. Saccharin is an artificial sweetener, which at this concentration elicits a strong neophobic response, but after becoming familiar 0.5% saccharin is readily consumed. In the key experiment, animals were either given voluntary access to saccharin (Group Bottle) or had saccharin infused directly into the oral cavity via an intraoral cannula (Group Intraoral). Intraoral animals were not water restricted, but rats in Group Bottle were maintained on a fluid restriction schedule allowing 30-min access to either saccharin or water each morning and then water only for an additional 7.5 hours of access immediately after the 30-min morning access period. For six days, half of the rats in Group Bottle and Group Intraoral were given, respectively, 30-min daily access or a 5-ml intraoral infusion of 0.5% saccharin and the other half were given equivalent exposure to water. On Day 7, all rats were exposed to 0.5% saccharin, to equate saccharin exposure intake in Group Bottle was capped at 5 ml. Two hours after the test trial on Day 7 all rats were killed and the brains were processed for c-Fos expression in BLA, CNA, GC, NTS, and PBN.

Saccharin naïve rats in Group Bottle showed elevated levels of c-Fos in the CNA and GC, relative to familiar controls, there was not a statistical difference in levels of c-Fos expression in either the BLA, NTS, or PBN. In Group, Intraoral only the CNA showed

a significant elevation of c-Fos in response to the novel saccharin. This difference in expression patterns may serve as another example of how the neural underpinnings of voluntary and involuntary intake can be somewhat different (see also Wilkins & Bernstein, 2006).

In a later study of similar design to that of Group Bottle in Koh et al. (2003), thirsty rats were allowed to drink 5-ml of a saccharin solution (0.5%) that was either novel or familiar (Lin, Roman, Arthurs, & Reilly, 2012). The expression of c-Fos keyed off novel saccharin was significantly higher in several nuclei including BLA, CNA, GC, and GT, but not mPBN, MeA, or BNST. Thus, based on c-Fos expression four areas of the forebrain have been implicated in the processing of taste neophobia BLA, CNA, GC, and GT.

In addition to c-Fos studies, permanent lesions have been widely used to identify nuclei involved in taste neophobia. Research examining the neural substrates of taste neophobia has, for the most part, focused in the GC and amygdala, primarily the BLA, but also CNA and MeA. In reviewing this literature, I will adopt a quasi-neuroanatomical order moving in order caudal to rostral and dorsal to ventral to cover the broader background material while reserving some studies with direct relevance to the current investigation for a more focused exposition.

To my knowledge, there is no literature directly addressing a potential role for the NTS in taste neophobia. Surprisingly few studies have examined a role for the NTS in CTA and have consistently concluded that NTS lesioned animals show normal CTA acquisition (Flynn, Grill, Schulkin, & Norgren, 1991; Grigson, Shimura, & Norgren, 1997a, 1997b). However, NTS lesions have been shown to cause a rightward shift in concentration dependent intake functions suggesting a lesion-induced reduction in

perceived stimulus intensity for a range of tastants (Shimura, Grigson, & Norgren, 1997). None of these CTA studies indicate a NTS lesion-induced effect on taste neophobia. The most likely explanation for the surprising conclusion that lesioning the first component of the central gustatory system has no effect on either taste neophobia or CTA seems to be that some spared population of NTS taste neurons were sufficient to preserve normal behavior (for additional discussion see Reilly, 2009). Another concern in all studies using permanent lesions is behavioral compensation. Compensation consequent to brain damage, typically unilateral, can be a beneficial outcome in clinical cases of human brain injury (e.g., Filli & Schwab, 2015; Lacour, Helmchen, & Vidal, 2016), but the potential for compensation is always a concern when a permanent lesion study yields null results. However, in the case of the NTS, compensation would have to be occurring via the relay of taste information from the cranial nerves to the PBN, or other nuclei in the central gustatory system, via a path that did not involve NTS neurons. There is no evidence for such an alternate taste pathway.

As reviewed by Reilly (1999; 2009), mPBN lesions appear to influence taste perception, but do not render animals taste-blind, (Flynn, Grill, Schwartz, & Norgren, 1991, Spector, 1995a, 1995b; Spector, Grill, & Norgren, 1993; Spector, Scalera, Grill, & Norgren, 1995) and disrupt the associative mechanism necessary for CTA learning (Grigson, Reilly, Scalera, & Norgren, 1998; Reilly, Grigson, & Norgren, 1993; Spector, Norgren, & Grill, 1992; but see also Aguero, Gallo, Arnedo, Molina, & Puerto, 1996, 1997; DiLorenzo, 1988; Flynn, Grill, Schulkin, & Norgren, 1991). Regarding taste neophobia, mPBN lesions have been shown to increase intake of a novel tastant (DiLorenzo, 1988; Grigson et al., 1998; Reilly et al., 1993; Yamamoto et al., 1995), which could be

interpreted as an attenuation of taste neophobia. However, each of these studies was focused on CTA learning allowing only a single trial assessment of potential lesion effects on taste neophobia.

Voluntary intake tests and taste reactivity analysis have shown that mPBN lesion animals exhibit a blunted taste reactivity and taste concentration intake curves that are shifted to the right (Flynn, Grill, Schulkin, & Norgren, 1991; Spector, 1995a, 1995b; Spector, Grill, & Norgren, 1993; Spector, Scalera, Grill, & Norgren, 1995), that is, they treat a variety of taste stimuli (e.g., sucrose, quinine) as if they were less concentrated leading to higher levels of intake and a potential indirect influence on taste neophobia. That is, taste neophobia, like CTA, is a concentration dependent phenomenon: rats show more neophobia to highly concentrated taste stimuli (Domjan & Gillan, 1976; Miller & Holzman, 1981) and will acquire stronger CTAs to more concentrated tastants (Barker, 1976; Dragoin, 1971). Therefore, determining any role of the mPBN in taste neophobia maybe confounded by lesion effects on taste perception. Importantly, this confound does not seem to apply to lesions of downstream taste nuclei. For example, lesions of the GT do not alter concentration dependent responding (e.g., Flynn, Grill, Schwartz, & Norgren, 1991, Reilly & Pritchard, 1996a, Scalera et al., 1997). To my knowledge, no such analysis of gustatory perception consequent to MeA lesions (or other manipulations) has been undertaken, as the MeA is not a recipient of ascending taste projections from the mPBN.

While most pontine taste responsive neurons are found in the mPBN, taste responsive neurons are scattered throughout other parabrachial regions, including the waist region of the brachium conjunctivum, and within some lateral PBN subnuclei such as the ventrolateral (Fulwiler & Saper, 1984; Halsell & Frank, 1991; Herbert et al., 1990;

Norgren & Leonard, 1971; 1973; Norgren & Pfaffmann, 1975; Ogawa et al., 1984; Perrotto & Scott, 1976; Rosen, Victor, & Di Lorenzo, 2011; Van Buskirk & Smith, 1981; for reviews see Lundy & Norgren, 2004; Reilly, 1999). Reilly and Trifunovic (2001) examined the effect of ibotenic acid lesions of the lateral PBN on taste neophobia to a variety of taste (i.e., alanine, saccharin, and quinine) and non-taste (i.e., capsaisin and almond-odor) stimuli. Lateral PBN lesions disrupted taste neophobia to both alanine and saccharin, but not almond odor, capsaisin, or quinine. This selective disruption of taste neophobia to sweet taste stimuli (i.e., alanine and saccharin) while sparing neophobia to a bitter tastant (e.g., quinine) as well as odor and trigeminal stimuli is curious and is not fully understood. Examining the histology figures in the Trifunovic and Reilly report it seems clear that lesions were mostly confined to the lateral PBN including the ventrolateral PBN, and that there was some encroachment on the waist region of the PBN, which also contains taste responsive neurons. Thus, one possibility is that these lesions selectively damaged taste neurons involved in sensing sweet tastes found in the ventrolateral and waist region of the PBN, while sparing taste neurons in the medial PBN that respond to bitter tastes. However, this is an empirical question that should be addressed by future research.

Another report from our laboratory attempted to examine the role of protein synthesis within the whole PBN in taste neophobia (Lin, Amodeo, Arthurs & Reilly, 2012b). Anisomycin is a drug that acts as a broad-spectrum protein synthesis inhibitor and has been widely used in neuroscience to test the hypothesis that memory formation is a protein synthesis dependent phenomenon (Bruning, Breitfeld, Kahl, Bergando-Acosta, & Fendt, 2016; Pedroza-Llinas, Ramirez-Lugo, Guzman-Ramos, Zavala-Vega, & Bermudez-Rattoni, 2009; Rodriguez-Ortiz, De la Cruz, Gutierrez, & Bermudez-Rattoni,

2005; Schiffino & Holland, 2016). However, microinjections of anisomycin into the PBN caused a CTA, and this anisomycin-induced CTA was attenuated by pretreatment with lidocaine (Lin et al., 2012b). That is, anisomycin can cause the release of neurotransmitters (Canal, Chang, & Gold, 2007; Qi & Gold, 2009) and even trigger apoptosis (Rudy, 2008), but lidocaine counteracts anisomycin-induced neurotransmitter release (Sadowski, Canal, & Gold, 2011). Therefore, it seems that anisomycin-induced neurotransmitter release from some part of the PBN caused an aversive US effect.

Few studies have examined a functional role for the GT in taste neophobia. Therefore, most information concerning a potential role of the GT in taste neophobia has been gathered from CTA studies (e.g., Flynn, Grill, Schulkin, & Norgren, 1991; Grigson, Lyuboslavsky, & Tanase, 2000; Mungardee, Lundy, & Norgren, 2006; Reilly, Bornovalova, Dengler, & Trifunovic, 2003; Reilly & Pritchard, 1996b; Scalera, Grigson, & Norgren, 1997). Only one of these studies has suggested a potential attenuation of taste neophobia consequent to GT lesions (Reilly et al., 2003); however, these lesions did not delay CTA acquisition. Since, to date, any lesion that attenuated taste neophobia also delayed CTA acquisition the statistically significant but numerically small (~2 ml) elevation of novel tastant intake in the GT-lesioned rats of the Reilly et al. study was attributed to sampling error rather than an attenuation of taste neophobia. As for a potential role for the GT in CTA most studies have found no effect of GT lesions on CTA acquisition (Flynn, Grill, Schulkin, & Norgren, 1991; Mungardee et al., 2006; Reilly et al., 2003; Reilly & Pritchard, 1996b; Scalera et al., 1997). The one exception employed unusually short tastant access periods and found that GT lesions prevented morphine-induced, but not LiCl-induced, CTA (Grigson et al., 2000).

Prompted by the c-Fos results of the Lin, Roman, Arthurs, and Reilly (2012) study, I used excitotoxic lesions to study the role of the GT in taste neophobia as part of my Master's thesis (Arthurs, 2012; Arthurs & Reilly, 2013). Notably, my study was the first to explicitly focus on a potential role of the GT in taste neophobia, rather than examining taste neophobia as a secondary concern to CTA learning. I found that GT lesioned animals consumed significantly more novel 0.15% saccharin (~18 ml) than neurologically intact controls (~11 ml). Unexpectedly, this substantial taste neophobia attenuation had no influence on the acquisition of a CTA induced by either amphetamine, morphine, or LiCl. Based on these results, we concluded that the GT-lesion induced ablation of morphine-induced CTA reported by Grigson et al. (2000) was the result of a ceiling effect imposed by the brief tastant access period. Thus, two novel findings emerged from my Master's research (1) a role for the GT in taste neophobia and (2) a GT lesion-induced attenuation of taste neophobia that did not delay CTA acquisition. Extending these novel findings is one goal of the current investigation.

Turning now to the GC, neuronal excitation within the GC has been implicated in taste neophobia by c-Fos studies (e.g., Koh et al., 2003; Lin, Roman, Arthurs, & Reilly, 2012), and studies using excitotoxic lesions have demonstrated a critical role for the GC in taste neophobia. For example, Lin, Roman, St. Andre, and Reilly (2009) demonstrated that excitotoxic GC lesions cause a massive disruption of taste neophobia with lesioned animals consuming more than twice the volume of novel 0.5% saccharin (~12 ml) as intact control animals (~5 ml). Furthermore, lesions of the GC produce a latent inhibition like delay in CTA learning (e.g., Lin, Arthurs, & Reilly, 2011; Roman, Lin, & Reilly, 2009; Roman, Nedieridze, Sastre, & Reilly, 2006; Roman & Reilly, 2007), and lesions of the GC

after the acquisition of a CTA attenuate the retention of a previously acquired CTA (Lin, Arthurs, & Reilly, 2015). Therefore, the GC may play an important role in recognizing a dangerous stimulus, whether that danger stems from novelty or experience. This danger processing account of GC function is supported by work in other labs such as a recent report by Stehberg and colleagues. Moraga-Amaro, Cortes-Rojas, Simon, and Stehberg (2014) used a combination of excitotoxic lesions and pharmacological inactivations to examine the role of the GC in (1) taste neophobia, (2) the habituation of taste neophobia, and (3) latent inhibition-like effects on CTA acquisition. They conclude that lesions of the GC diminish taste neophobia, but do not influence the habituation of any residual taste neophobia or the retention of a safe taste memory. Thus, the GC may be critical for responding to dangerous taste stimuli, and when this danger response is damaged by permanent lesions rats treat a novel taste as though it were safe and familiar.

So, we can see that lesions to structures in the dorsal taste pathway involving mPBN→GT→GC disrupt taste neophobia, but this disruption is somewhat different depending on the region. mPBN lesions appear to influence taste perception but do not render animals completely ageusic, and may cause an attenuation of taste neophobia. GT lesions attenuate taste neophobia but do not cause a latent inhibition like delay of CTA acquisition, as is the case for GC lesions. However, the exact role of each structure is less clear. Now we will examine the literature on ventral taste pathway targets of PBN taste projections to the BNST, LH, and CNA, as well as two additional subnuclei of the amygdala the BLA and MeA, which have been implicated in taste neophobia.

The literature on a potential role for the BNST in taste neophobia is rather small. Roman et al. (2006) examined the effect of excitotoxic lesions of the BNST on CTA. In

this study, thirsty rats with BNST or SHAM lesions were given 15-min access to a bottle of 0.15% saccharin and were then injected 15-min later with LiCl. Both BNST lesion and SHAM animals acquired a CTA with no between groups differences on either the two conditioning trials or the final taste-only test trial. Thus, based on equivalent Trial 1 intake of novel saccharin it appears that BNST lesions do not disrupt taste neophobia. However, since this study focused on CTA acquisition, and not taste neophobia, there were no saline treated control animals to assess potential lesion effects on the habituation of taste neophobia. When Lin, Roman, et al. (2012) compared c-Fos in the BNST evoked by saccharin that was either novel or familiar there was not a statistically significant difference in c-Fos in the BNST after novel saccharin consumption. Thus, there is little evidence for a role of the BNST in taste neophobia.

As for the LH, Roman et al. (2006) found that excitotoxic lesions of the LH had the problematic, but anticipated, effect of decreasing overall fluid intake (i.e., both water and saccharin) preventing a between groups (SHAM vs. LH-lesion) comparison of Trial 1 intake to test a potential lesion effect on taste neophobia. However, due to the anticipated difference in fluid intake, saline treated controls were included in this experiment. In the saline treated control subjects, comparing relative shifts in the pattern of intake between neurologically intact SHAM-lesioned and LH-lesioned animals it appears that LH lesions did not influence the attenuation of taste neophobia. That is, SHAM-saline rats consumed ~19 ml on Trial 1 and increase slightly on Trial 2 consuming ~21 ml (a net increase of ~2 ml), and a similar relative net increase can be seen in LH-lesioned animals injected with saline (~2 ml; Trial 1 ~12 ml, Trial 2 ~14 ml). Both saline treated groups appear to maintain asymptotic performance similar to the level of intake observed on Trial 2. Overall, then,

there may have been a slight amount of taste neophobia in each group on Trial 1, but the comparable Trial 2 increase in each group does not suggest any lesion effect on the occurrence or habituation of taste neophobia. Also, LH lesions did not appear to influence the acquisition of a LiCl-induced CTA (Roman et al., 2006). However, LH-lesion induced hypodipsia remains a major confound for clearly assessing a role for the LH in taste neophobia. However, Kesner and Berman (1977) found no effect of low level electrical stimulation of the LH on taste neophobia, which supports the idea that the LH is not involved in taste neophobia.

In contrast to the literature on the BNST and LH there is a larger body of literature addressing the role of the amygdala in taste neophobia. Most of the literature examining the effects of excitotoxic lesions of the amygdala on taste neophobia has been thoroughly reviewed by Reilly and Bornovalova (2005). Many of the early studies on amygdala lesions and taste learning involved electrolytic (i.e., non-specific) lesions of multiple subnuclei within the amygdala and are therefore of limited utility in understanding amygdala function; however, these studies did provide general evidence that the amygdala has a role in taste-guided behavior (e.g., Lasiter, 1982; McGowan, Hankins, & Garcia, 1972). Three subnuclei in the amygdala have been examined by selective lesions for a role in taste-guided behavior: BLA, CNA, and MeA.

The BLA is the most well studied of the amygdala subnuclei within the context of taste-guided learning with a wide range of both lesion and pharmacological studies indicating a role for the BLA in both taste neophobia and CTA. A number of studies have looked at the effect of electrolytic BLA lesions on both taste neophobia and CTA (Aggleton, Petrides, & Iversen, 1981; Aja, Sisouvong, Barrett, & Gietzen, 2000; Borsini &

Rolls, 1984; Fitzgerald & Burton, 1981, 1983; Kolakowska, Larue-Achagiotis, & Le Magnen, 1984; Lasiter & Glansman, 1985; Nachman & Ashe, 1974; Shimai & Hoshishima, 1982;). These studies report a mix of outcomes with most reporting normal taste neophobia, some reporting attenuated taste neophobia (Aggleton et al., 1981; Fitzgerald & Burton, 1983; Kolakowska et al., 1984; Shimai & Hoshishima, 1982), and one report of persistent taste neophobia in lesioned animals (Borsini & Rolls, 1984). Outside of our laboratory, only two studies have examined the effects of excitotoxic lesions of the BLA on taste-guided behavior (Morris et al., 1999; Sakai & Yamamoto, 1999). Unfortunately, each study assessed taste neophobia relative to water intake on the previous day, a definition that is problematic as described previously. Morris et al. used a 2% sucrose taste solution to examine taste neophobia in animals with ibotenic acid lesions of the BLA and found normal taste neophobia. However, taste neophobia was assessed relative to baseline water consumption, which is problematic when the tastant is 2% sucrose that is likely to be preferred to water. Also, the focus of the Morris et al. study was CTA learning and, as such, a relatively non-neophobic stimulus was used, which may cast doubt on their results. Sakai and Yamamoto (1999) examined the effect of ibotenic acid lesions of the BLA on taste neophobia evoked by a 0.1% saccharin solutions, again a tastant the produces minimal neophobia, and found no lesion induced attenuation of taste neophobia. In both cases, there is a strong possibility that ceiling effects precluded the detection of a lesion-induced attenuation of taste neophobia. That is, as mentioned previously, taste neophobia is a concentration dependent phenomenon, and if control animals show little, or no, taste neophobia, then, there is little hope of detecting a lesion-induced attenuation of taste neophobia. While the older literature

reviewed in this section offers little evidence of a role for the BLA in taste neophobia we will see in Section 1.6 that this is not the case.

As an aside, the BLA and GC have been the focus of many studies attempting to determine the cellular and molecular underpinnings of taste learning (e.g., Adaikkan & Rosenblum, 2015; Barki-Harrington, Belelovsky, Doron, & Rosenblum, 2009; Bermudez-Rattoni, 2004; Stehberg, Moraga-Amaro, & Simon, 2011). This level of analysis, determining neurotransmitter systems and intracellular mechanisms, is a natural progression once a brain region has been identified as critical to behavior. However, for many brain regions, including the GT and MeA, the nature of their involvement in taste neophobia or CTA is not well understood or was only recently recognized. Therefore, it is necessary to adopt a systems level analysis of these brain regions before proceeding to more fine-grained analyses of cellular and molecular mechanisms.

As for the CNA, only a single study has reported a CNA lesion-induced attenuation of taste neophobia (Morris et al., 1999). However, this result is called into question because (1) no data was presented to quantify the attenuation and (2) neophobia was assessed relative to water intake. This single report is contrasted with several reports of normal taste neophobia in CNA lesioned rats (Aja et al., 2000; Kemble, Studelska, & Schmidt, 1979; Lasiter & Glanzman, 1985; Sakai & Yamamoto, 1999). Each of these studies used electrolytic lesions except for the ibotenic acid lesions used by Sakai and Yamamoto. Recall that in each c-Fos study of taste neophobia (Koh et al., 2003; Lin, Roman et al., 2012) there were increased levels of c-Fos expression the CNA of animals exposed to novel, relative to familiar, saccharin. As previously noted, increased levels of neuronal excitation in response to a novel tastant does not prove that the CNA has a

functional role in taste neophobia. But, it is worthwhile to note that a well-designed experiment examining the functional role of the CNA in taste neophobia has yet to be conducted. Thus, the lesion literature suggests that an intact CNA is not necessary for the expression of taste neophobia; however, I do not consider this view to be conclusive.

Only a handful of studies have been conducted to examine the role of the MeA in CTA with some potential information on taste neophobia (Aggleton et al., 1981; Meliza, Leung, & Rogers, 1981; Rollins, Stines, McGuire, & King, 2001). Each of these studies found no effect of MeA lesions on taste neophobia. Unfortunately, this small literature also contains many flaws which preclude confident interpretation of the reported results. Each of these studies used electrolytic lesions. Of these, only the Meliza et al. study used a discreet taste stimulus (0.2% saccharin), while Aggleton et al. used a 15% sucrose solution and Rollins et al. used a 33% milk solution each of which possess stimulus features other than taste (e.g., Cheslock, Varlinskaya, Petrov, & Spear, 2000; Moio, Rillo, Ledda, & Addeo, 1996; Rhinehart-Doty, Schumm, Smith, & Smith, 1994). Therefore, it seems that insufficient research has been conducted to allow a meaningful assessment of a potential role for the MeA in taste neophobia.

1.6 Background for the Current Study

Recalling that the habituation of taste neophobia delays CTA acquisition due to latent inhibition (Lubow, 1989; 2009), one might reasonably expect a lesion-induced attenuation that causes animals to treat a novel taste as if it were familiar and safe (elevating intake of a novel taste) would also delay the acquisition of CTA (i.e., require more taste-illness pairings for CTA acquisition). In fact, this is the case for both the BLA (St. Andre & Reilly,

2007) and GC (Lin, Arthurs, & Reilly, 2011; Roman, Lin, & Reilly, 2009; Roman & Reilly, 2007). In both nuclei, the lesions evidently cause rats to treat a novel taste as if it were familiar, evidenced by attenuated taste neophobia and a knock-on latent inhibition-like delay of CTA acquisition to a genuinely novel taste.

Bilateral lesions of the MeA cause an attenuation of taste neophobia observed as an overconsumption of a novel tastant, an attenuation qualitatively similar to that seen with bilateral lesions of the BLA or GC (Lin et al., 2009). The Lin et al. study is a good example of the taste neophobia design that has become standard in our laboratory. Animals are placed on a schedule allowing 15-min water access each morning. Once water intake is stable for three days (i.e., no between-groups differences) taste neophobia trials can start. A taste neophobia trial consists of replacing morning water with a novel taste stimulus, typically 0.5% saccharin. Trials occur in a three-day cycle with a trial day followed by two water only days. The habituation of taste neophobia is measured as increased intake across trial days until asymptotic intake is reached. Then, the magnitude of the initial taste neophobia response can be appreciated by comparing consumption of the tastant when either novel (Trial 1) or familiar (asymptote).

Unlike neurons in BLA and GC, neurons in the MeA did not show elevated levels of c-Fos expression in rats exposed to a novel, rather than familiar, saccharin solution (Lin, Roman, et al., 2012). As noted previously, c-Fos is a marker of neuronal excitation (Kovacs, 2008) and as such cannot detect if a neuronal population is inhibited during a behavioral response, such as taste neophobia. Also, while MeA lesions cause an attenuation of taste neophobia they do not appear to delay the acquisition of a CTA (for a review see Reilly & Bornovalova, 2005). Therefore, it seems that neuronal excitation in

BLA and GC may be critical to taste neophobia and normal CTA acquisition, while neuronal inhibition in MeA may underlie taste neophobia and with no influence on CTA. From the perspective of understanding neural circuits, the next logical step was to ask if these regions form functional subunits that mediate these behaviors. For example, do the BLA and GC form a functional unit critical for assessing taste familiarity while the MeA and GC form a functional unit critical for the evaluation of taste safety?

Lin and Reilly (2012) used an asymmetrical-disconnection approach to analyze the connectivity of the BLA, GC, and MeA in taste neophobia. The taste system is highly lateralized (e.g., Lundy & Norgren, 2004); although contralateral projections do exist, ipsilateral projections are far more prominent. So, if two nuclei are components of an ipsilateral circuit, destroying one, or the other, of the two nuclei in contralateral hemispheres should produce a deficit like bilateral lesions of either nucleus alone (e.g., Clark & Bernstein, 2009; Leung & Balleine, 2013). Following this logic, Lin and Reilly prepared three sets of experimental animals with contralateral lesions of (1) BLA-GC, (2) BLA-MeA, or (3) GC-MeA. Following testing on our laboratory's standard taste neophobia procedure, only the BLA-GC animals showed a taste neophobia attenuation qualitatively similar to that seen with bilateral lesions of either nucleus; the MeA did not appear to functionally interact with either the BLA or GC during taste neophobia testing. These results were interpreted as showing that the BLA and GC form an amygdala-cortical functional unit critical to taste neophobia that does not include the MeA.

In the c-Fos study of Lin, Roman, et al. (2012) the GT was also activated during taste neophobia. So, my Masters research (Arthurs, 2012; see also Arthurs & Reilly, 2013) examined the role of the GT in taste neophobia and CTA. To determine if GT lesions

influenced either taste neophobia or CTA I used a hybrid experimental design essentially identical to our standard taste neophobia design except a 0.15% saccharin solution was used and separate groups of animals were administered either saline or a CTA-inducing US. This design was conceived to provide a level of saccharin intake on trial 1 that would allow the detection of (1) a lesion-induced attenuation of taste neophobia, (2) the normal occurrence and attenuation of taste neophobia, or (3) decreased intake caused by a CTA.

In the first experiment animals had saccharin paired with either saline (to allow assessment of taste neophobia) or morphine (to allow the assessment of CTA learning) over 4 conditioning trials and were then presented with saccharin on a taste only test trial. On Trial 1 animals with GT lesions consumed significantly more novel saccharin (~ 17.5 ml) than neurologically intact controls (~11 ml). On subsequent trials, all saline treated animals increased saccharin intake to the same asymptotic level; although, due to the substantially higher Trial 1 intake, this increase was smaller in the GT lesioned animals than in control subjects. Morphine treated animals in each group (GT-Lesion and Control) decreased intake across trials at a rate that was evenly matched. That is, each group decreased intake of saccharin across trials in essentially parallel lines maintaining the separation established by the higher Trial 1 intake of the GT lesioned animals. This result was unexpected. Previously, any neural manipulation that caused an attenuation of taste neophobia had the additional consequence of delaying CTA acquisition through a latent inhibition-like effect (e.g., Lin, Arthurs, & Reilly, 2011). However, this was not the case for GT-lesioned animals in Experiment 1 of my Master's research.

This initial experiment provided evidence that GT lesions attenuated taste neophobia (overconsumption of novel tastant) without delaying CTA acquisition.

However, the significantly different intake levels on Trial 1 for GT-Lesion versus Control groups prevented a proper comparison of CTA acquisition between these groups. Therefore, in a follow-up study the same cohort of animals was redistributed into new groups, counterbalanced for prior experience, and the acquisition of a LiCl-induced CTA was examined. To prevent differences on Trial 1 complicating interpretation, half of the rats in each lesion condition had their intake restricted to ensure equivalent intake on Trial 1. Thus, Lesion and control animals were divided into two groups: Uncapped that had access to an unlimited quantity of the quinine taste for 15-min, or Capped with intake capped at 5 ml to provide an equivalent starting point for CTA acquisition. This arrangement yielded four groups Uncapped-Control, Uncapped-Lesion, Capped-Control, and Capped-Lesion. Animals were given two conditioning trials and a single taste only test trial. The Uncapped-Control animals consumed significantly less quinine (~7.5 ml) than Uncapped-Lesion animals (~11.5 ml) on the first conditioning trial. Replicating the lesion-induced overconsumption of a novel tastant found in Experiment 1. On the second conditioning trial and test trial there were no between-groups differences in quinine consumption and intake was suppressed to essentially zero on the test trial, again suggesting no difference in CTA acquisition. In the Capped groups, there were no significant differences between the Control and Lesion groups in intake across trials and by the test trial both groups had acquired a strong CTA with intake levels of essentially zero. Thus, the capping procedure was effective in equating Trial 1 intake and there was still no difference in the rate of CTA acquisition. The results from the Capped groups indicate that GT lesions do not influence the rate of CTA acquisition.

In another cohort of animals (Experiment 3), I examined the effect of GT lesions on saccharin taste neophobia and amphetamine CTA using the same design as Experiment 1. In this experiment, animals were tested in drinking chambers which allowed the collection of lick data. A similar pattern of results emerged. GT lesioned animals consumed significantly more novel saccharin and this elevated intake was characterized by increased lick cluster size. Furthermore, GT lesions did not delay the acquisition of an amphetamine-induced CTA. Therefore, I replicated the GT lesion-induced attenuation of taste neophobia observed in both previous experiments and showed that this increase intake was accompanied by elevated palatability. Notably, we had previously demonstrated that the habituation of taste neophobia is accompanied by increased taste palatability (Lin, Amodeo, Arthurs, & Reilly, 2012a). So, both the habituation of taste neophobia and GT lesions increase palatability and intake, but GT lesions do not seem to alter the perception of familiarity known to delay CTA acquisition. Thus, perhaps the GT plays a role in neophobia via the initial estimation of taste safety but not familiarity.

Overall, in the literature two patterns of deficits can be discerned from lesions of forebrain nuclei involved in taste neophobia. First, the BLA and GC, working as a functional unit, produce an attenuation of taste neophobia that has a knock-on effect manifest as a latent inhibition-like delay of CTA acquisition. Second, lesions of either the GT or MeA result in attenuated taste neophobia that does not influence CTA acquisition.

1.7 Experiment Justification and Approach

The goal of my doctoral research is to better understand the roles of the GT and MeA in taste neophobia. There are two aims of the project. First, I used intracranial pharmacological inactivations as a temporary lesion to refine our understanding of the

involvement of the GT and MeA in taste neophobia (Aim 1) and, second, DREADDs were used to determine if the expression of taste neophobia can be influenced by modulating neural activity in the MeA (Aim 2).

Concerning Aim 1, permanent lesions have implicated both the GT and MeA in taste neophobia. Permanent lesions provide a powerful means to determine if a brain region is critically involved in a given behavior, and provide the benefit of not being temporally restricted. That is, no matter when the region is involved in the behavior a permanent lesion will reveal that involvement thereby vastly simplifying the process of determining whether a given region plays a critical role in behavior. Once a region is determined to be critical for a given behavior, it is often desirable to determine the temporal nature of that involvement. A useful strategy to determine the temporal aspect of a regions involvement in behavior is to employ inhibitory pharmacological manipulations, such as targeted infusions of inhibitory drugs, to cause, in effect, a temporary brain lesion (e.g., Koh & Bernstein, 2005; Majchrzak & Di Scala, 2000; Martin & Ghez, 1999). γ–amino-butyric acid (GABA) is the primary inhibitory neurotransmitter in the brain (Dutar & Nicoll, 1988; Kornau, 2006; Mohler, 2006; Ulrich & Bettler, 2007). By infusing a GABA agonist cocktail consisting of muscimol ($GABA_A$) and baclofen ($GABA_B$) each target brain area can be manipulated immediately prior to and during novel taste exposure on a single trial. The use of pharmacology to manipulate endogenous neurotransmitter systems has the additional benefit of biological reliance. That is, if GABA agonists infusion affect behavior, then, perhaps GABAergic receptors play a role in the normal modulation of behavior.

Thinking of pharmacological inactivation as a straightforward temporary lesion effect should lead me to predict that temporary lesions of the GT or MeA will replicate the

attenuation of taste neophobia observed with permanent lesions (e.g., Arthurs, 2012; Arthurs & Reilly, 2013; Lin et al., 2009). However, considering the results of the taste neophobia c-Fos study of Lin, Roman, et al. (2012) led me to predict a potential alternative outcome. Recall that Lin, Roman, et al. found elevated c-Fos expression in the GT, but not MeA, in response to a novel taste. These results suggest that neuronal excitation in the GT correlates to the occurrence of taste neophobia. Infusing GABA agonists prior to the presentation of a novel taste tests the hypothesis that neuronal excitation in the GT is necessary for the expression of taste neophobia and that this excitation can be modulated by GABAergic signaling. Conversely, there is no evidence that neuronal excitation in the MeA is correlated with the expression of taste neophobia. However, we know that permanent lesions of the MeA attenuate the expression of taste neophobia. So, the MeA is involved in taste neophobia, but this role does not seem to involve the excitation of neurons in the MeA. The simplest alternative hypothesis is that neurons in the MeA are critically inhibited during taste neophobia. If this is the case, then, infusing GABA agonists in the MeA might increase the inhibition of MeA neurons and result in an enhanced taste neophobia response (i.e., lower Trial 1 intake than control subjects). However, my hypothesis that neuronal inhibition in the MeA is critical to taste neophobia is merely an informed guess, and the GABA infusion might result in a temporary lesion effect. Thus, in Experiment 2, I set out to test competing hypotheses about the role of GABAergic signaling in the MeA. On one hand, I predicted that GABA agonist infusions will enhance taste neophobia (decrease Trial 1 intake relative to controls), or, alternatively, GABA infusions in the MeA might act as a temporary lesion and cause an attenuation of taste neophobia.

Concerning the pharmacological effects of the GABA agonist cocktail, $GABA_A$ receptors directly gate a Cl^- channel with activation allowing an influx of chloride into the cell causing hyperpolarization (Dutar & Nicoll, 1988; Mohler, 2006). The $GABA_B$ receptor is metabotropic acting via the $G_{\beta\gamma}$ subunit and second messenger systems to inhibit presynaptic Ca^{2+} or postsynaptic K^+ channels (Kornau, 2006; Ulrich & Bettler, 2007), depending on receptor localization. Furthermore, presynaptic $GABA_B$ activation can affect vesicle priming thereby dampening neurotransmitter release (Sakaba & Neher, 2003). Thus, the effect of GABA agonists depends on the nature of the cell expressing the receptor (e.g., excitatory or inhibitory) as well as receptor localization. By infusing GABA agonists into a brain region, we expect to activate $GABA_A$ and $GABA_B$ receptors both pre- and post-synaptically. The targeted infusion of these agonists into a given brain region has the advantage of causing a temporary state of pre- and post-synaptic neuronal inhibition through diverse mechanisms.

Intracranial pharmacology has been used in our laboratory (e.g., Figueroa-Guzmán, Kuo, & Reilly, 2006; Figueroa-Guzmán & Reilly, 2008; Lin, Amodeo, Arthurs, & Reilly, 2012b) to provide a temporally specific manipulation of neural structures. The value of this technique is that the specificity of the inactivation allows better understanding of the function of a target structure at different moments in the processes that underlie a behavior. Also, temporary pharmacological lesions are thought to be less prone to the development of compensatory mechanisms than permanent lesions due to their acute nature (e.g., Koh & Bernstein, 2005; Majchrzak & Di Scala, 2000; Martin & Ghez, 1999). However, systemically administered baclofen has been shown to induce a CTA (Wilson,

Biesan, Remus, & Mickley, 2011), necessitating that we screen for potential CTA-inducing effects of centrally administered baclofen.

Chemogenetic techniques (i.e., DREADDs; Armbruster, Li, Pausch, Herlitze, & Roth, 2007) have increased in popularity over the last few years (e.g., Roth, 2016; Smith, Bucci, Luikart, & Mahler, 2016; Urban & Roth, 2015). Traditional pharmacological approaches are limited by the pattern of expression of endogenous receptor; DREADDs, on the other hand, allow experimental control (i.e., activation or inhibition) of cells via exogenous means. That is, exogenous excitatory or inhibitory receptors lacking an endogenous ligand can be expressed in cells and then activated by an otherwise inert ligand.

In a chemogenetic experiment, a viral vector (e.g., adeno associated virus; AAV) is used to infect neurons of interest (via a specific promoter sequence; e.g., human synapsin; hSyn) with genetic material encoding a designer receptor (e.g., a mutated human muscarinic receptor [e.g., hM4D(Gi)]) lacking an endogenous ligand. After stereotaxically guided injection of the viral vector several weeks (2-6 weeks; e.g., Smith et al., 2016) are required to allow receptor expression. Then, a normally inert designer drug (e.g., clozapine-N-oxide; CNO) can be delivered systemically to exclusively activate the designer receptors. DREADD receptor activation can either excite or inhibit the previously infected neurons, depending on the nature of the expressed receptor. To allow visualization of, respectively, expressed receptors of infected cells additional genetic material can be delivered by the virus including fluorescent proteins such as mCherry and EGFP. Thus, implanting a DREADDs virus is like a lesion surgery, but the receptors are dormant until activated. During testing, transient receptor activation involves a simple IP

injection rather than a complex and delicate process of intracranial pharmacological infusions.

1.8 Hypotheses and Predictions

In the present set of studies pharmacology and chemogenetics will be used to extend our understanding of the role of the GT and MeA in taste neophobia, an understanding that is currently based on studies examining excitotoxic lesions or immediate early gene expression. The use of excitotoxic lesions and immediate early gene expression have been a very successful approach in identifying brain regions that are critical for taste neophobia and CTA. We see the current studies as part of a transition leading to a more nuanced understanding of the function of these critical brain regions individually and as ensembles forming circuits.

In Aim 1, I used temporary pharmacological lesions of the GT (Experiment 1A) or MeA (Experiment 2A) to refine the temporal involvement of these areas in taste neophobia. That is, excitation in GT neurons seems critical for the expression of taste neophobia; thus, pharmacologically inhibiting this response should attenuate the expression of taste neophobia (i.e., increase Trial 1 intake; see Table 1). In the MeA the predicted result is less clear. On one hand, the temporary pharmacological lesion may replicate the attenuation of taste neophobia seen with permanent lesions. Alternatively, I have hypothesized that inhibition of MeA neurons is critical to the normal expression of taste neophobia. Additional inhibition of this population via pharmacology may enhance the expression of taste neophobia. In Aim 2, I used DREADDs to either inhibit or excite MeA neurons predicting an opposing effect on the expression of taste neophobia (Experiment

3), that is, if inhibition of MeA neurons is causally involved in the normal expression of taste neophobia, then, DREADDs inhibition should enhance taste neophobia and DREADDs excitation should attenuate taste neophobia (see Table 1).

Table 1. Hypothesized Results for Each Experiment

Experiment	Group	Novel Taste Response Relative to Controls
1A	**GT-GABA**	Increased Intake
2A	**MeA-GABA**	Increased or Decreased Intake
3	**MeA-Excitation**	Increased Intake and Palatability
	MeA-Inhibition	Decreased Intake and Palatability

Note. Predicted performance in the experimental group of each experiment relative to performance in the control group.

1.9 Rationale and Experimental Designs

1.9.1 Aim 1. To maintain comparability with previous studies we used our standard taste neophobia design with minimal modifications to accommodate the use of intracranial microinjections. Animals were adapted to a water deprivation schedule allowing 15-min access each morning and 15-min access in the afternoon. Also, each experiment was conducted in two replications and on a rotating daily schedule such that animals were tested in a 3-day cycle consisting of a test day, and two recovery days. Animals were

divided into squads of three such that only one animal in each squad had a test trial on a given day.

In Experiment 1A and 2A, the GABA agonist cocktail was infused 20-min prior to tastant access. Typically, microinfusions of GABA agonists are thought to take effect within minutes of injection and last for at least 40-min (e.g., Baker & Ragozzino, 2014a, 2014b). Thus, infusion of GABA agonists 20-min prior to a 15-min test session should allow the GABA agonist cocktail time to take full effect and provide peak inhibition of the GT neurons during the tastant access period with effects beginning to expire after the tastant access period has ended.

Experiment 1B and 2B were CTA experiments pairing a quinine (0.0001 M) taste conditioned stimulus (CS) with the GABA agonist unconditioned stimulus (US) injected into either the GT or MeA, respectively, after a 5-min interstimulus interval. In the past (Arthurs, 2012; Arthurs & Reilly, 2013) we have successfully used 0.0001 M quinine during follow-up experiments when saccharin was used as the CS in the first experiment. Therefore, we used the same two stimuli (saccharin and quinine) as earlier studies to maintain comparability between studies. The GABA infusion occurred 5-min after the end of the tastant access period as would be typical in a standard CTA experiment using LiCl. While CTAs can be formed over very long CS-US intervals, on the order of hours, we wanted to optimize the detection of even weak CTA-inducing properties of the GABA infusion, and CS-US intervals on the order of minutes typically yield strong CTAs. Thus, with the 5-min delay between the end of the CS access period and the GABA agonist infusion coupled with some time for the infusion to take effect we should be within a functional CS-US interval of 5-25-minutes.

1.9.2 Aim 2. We used our standard taste neophobia design to investigate the influence DREADDs excitation or inhibition of MeA neurons on taste neophobia. Since I predicted that MeA excitation would increase novel saccharin intake and MeA inhibition would decrease novel saccharin intake, we needed a concentration of saccharin that would offer an intermediate level of intake in the control group on Trial 1. During my Master's thesis experiments, 0.15% saccharin provided an intermediate level of Trial 1 intake in the control group and was selected for this experiment on that basis.

The experiments of Aim 1 were conducted in the home cage for two main reasons. First, conducting the experiments in drinking chambers would have required 3 or more replications rather than the two needed for conducting the experiments in the home cage. Each additional replication would have lowered the statistical power available to detect potential replication effects on behavior. Second, we were concerned about the integrity of any lick data we might have collected. Rats are extremely sensitive to noise, and for this reason we always minimize activity in the testing room during tastant access periods. If the pharmacological infusion experiments had been conducted in drinking chambers personnel would have needed to enter and exit the testing room while animals were in the drinking chambers. Any noise generated during this time may have compromised lick data. While testing in the home cages we could avoid entering the testing room during tastant access periods. However, in Aim 2 the simplicity of CNO injections, as opposed to intracranial infusions, allowed me to conduct this experiment in the drinking chamber and collect lick data to analyze palatability (i.e., initial lick rate and lick cluster size).

2. METHOD

2.1 Aim 1: Pharmacological Manipulation of GT or MeA and Taste Learning

Two sets of animals were prepared with bilateral intracranial cannula targeting either the GT (Experiment 1) or the MeA (Experiment 2). These animals were infused with a cocktail of GABA agonists before access to novel saccharin (Experiments 1A and 2A) or after the presentation on novel quinine (Experiments 1B and 2B). In Experiment 1A and 2A, the influence of temporary inactivation of, respectively the GT or MeA on taste neophobia was examined. Experiments 1B and 2B tested whether the infusion of GABA agonists after access to a novel quinine solution influenced quinine intake on subsequent trials.

2.1.1 Experiment 1: Effect of GABA agonists in the GT and taste learning

2.1.1.1 Subjects. Thirty-three experimentally naïve male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA) and housed in a climate controlled (~22°C) vivarium maintained on a 12:12 hr light:dark cycle (lights on at 7:00 AM).

2.1.1.2 Surgery. All rats were anesthetized with a mixture of ketamine and xylazine (100:10 mg/kg, IP) and secured in a stereotaxic frame. Twenty-three rats were implanted with bilateral guide cannula (22 gauge; Plastics One, Roanoke, VA) targeted 2 mm dorsal to the GT (AP -3.7, ML 0.8, DV -4.3). The remaining animals formed an anesthesia only Control group (n = 10). To implant cannula, a midline incision was made in the scalp allowing visualization of cranial sutures as a reference for stereotaxic coordinates. Trephine holes were drilled in the skull overlying each coordinate. Then, the guide cannula was lowered into position, and secured with dental acrylic anchored to the skull via implanted jeweler's screws. After the dental acrylic hardened, guide cannulae

were fitted with obturators that occupied the lumen of the guide cannula, but did not project beyond the end of the cannula. Obturators were loosened daily to ensure patency and habituate subjects to handling procedures. Animals were maintained on ad libitum food and water for at least 7 days following surgery to allow for recovery.

2.1.1.3 Apparatus. All behavioral testing occurred in the home cage. Intracranial infusions were conducted in a room adjacent to the vivarium.

2.1.1.4 Procedure.

2.1.1.4.1. Experiment 1A. After recovery from surgery (7-10 days), rats were adapted to a water access schedule allowing 15-min access in the morning and 15-min access four hours later. Water intake was measured to the nearest 0.5 ml. Once water intake was stable over a three-day period (i.e., no main effect of Group and no significant Group x Day interaction), taste neophobia trials began. Taste neophobia trials consisted of presenting 0.5% saccharin in place of morning water. Intracranial infusions were made 20-min before the first taste neophobia trial. For intracranial infusions, cannulated rats were removed from the home cage for 5-min during which time they received, according to group assignment, an infusion of saline (Group Saline; n = 9), or a cocktail of GABA agonists (0.5 µl/side, 1.0 mM baclofen hydrochloride and 0.1 mM muscimol hydrobromide; Group GT-GABA; n = 14). Infusions were made through an injector cannula (26 gauge; Plastics One) attached by polyethylene tubing (PE20; Braintree Scientific) to a microsyringe (10 µl; Hamilton) controlled by a syringe pump (KD Scientific). The infusion cannula remained in place for 2-min before and after the infusion, which was made at a rate of 0.25 µl/min. Rats in Group Normal (n = 10) received a mock infusion

and were then returned to the home cage. Each of the five taste neophobia trials were separated by two water only days.

2.1.1.4.2 Experiment 1B. As in Experiment 1A, except cannulated rats (from Experiment 1A) were assigned to Group Saline (n = 9) or Group GT-GABA (n = 14) in a quasi-random fashion counterbalancing for prior experience. Additionally, the taste stimulus was 0.0001 M quinine (instead of saccharin), and intracranial infusions occurred 5-min after the first quinine presentation. This follow-up experiment was conducted 7 days after the termination of Experiment 1A, during which the animals were maintained on the same water access schedule of 15-min each morning and afternoon.

2.1.2 Experiment 2: GABA agonists in the MeA and taste learning

2.1.2.1 Subjects. Thirty-four rats were acquired as in Experiment 1.

2.1.2.2 Surgery. As in Experiment 1, except 27 rats had cannula implanted targeted 2 mm dorsal to the MeA (AP -2.5, ML 3.2, DV -6.1). The remaining 7 rats formed an anesthesia control group (Group Normal).

2.1.2.3 Apparatus. As in Experiment 1.

2.1.2.4 Procedure.

2.1.2.4.1 Experiment 2A. As in Experiment 1A, except Group Saline contained 12 subjects and Group MeA-GABA contained 15 subjects.

2.1.2.4.2 Experiment 2B. As in Experiment 1B. Group Saline contained 15 subjects and Group MeA-GABA contained 12 subjects.

2.1.3 Histology. All cannulated rats were deeply anesthetized with a mixture of ketamine and xylazine (120:15 mg/kg, IP) and perfused transcardially with phosphate

buffered saline followed by a 10% formalin solution. Injector cannula were inserted throughout the perfusion to establish a clear injector cannula tract in the fixed tissue. Subsequently, brains were extracted, stored in 10% formalin at 4°C, and transferred to a 20% sucrose solution 48-hours prior to coronal sectioning (50 μm) on a cryostat. Slices through the cannula tract were mounted on slides and stained for nissil bodies using cresyl violet. Cannula placements were evaluated by examining the tracts made by the guide and injector cannulae with the aid of a light microscope.

2.1.4 Data Analysis. Intake was analyzed via mixed designs analysis of variance (ANOVA) using *Statistica* software (version 13, Dell Inc., 2015) and, where necessary, planned comparisons (i.e., simple main effects with adjusted error term taken from overall ANOVA) or Tukey HSD post-hoc tests. The α level was set at .05. For significant main effects the effect size is reported as a partial eta-squared (η_p^2).

2.2 Aim 2: Chemogenetic inhibition or excitation of the MeA in taste neophobia

It was hypothesized that inhibition of neurons in the MeA is critical to the expression of taste neophobia. Thus, increasing this inhibition might produce an enhanced expression of taste neophobia, while excitation of these neurons might attenuate taste neophobia, thus reproducing the behavioral effect produced by excitotoxic lesions of MeA. To evaluate these predictions rats were prepared by infecting MeA neurons with a viral vector encoding either an excitatory DREADD, an inhibitory DREADD, or for control subjects, EGFP. An additional control group consisted of animals that were anesthetized and placed in the stereotaxic frame, but did not receive any surgical manipulation.

2.2.1 Experiment 3: Chemogenetic inactivation or excitation of the MeA in taste neophobia

2.2.1.1Subjects. Thirty-five experimentally naïve rats were acquired as in Aim 1.

2.2.1.2 Surgery. General surgical procedures were identical to Aim 1 except isoflurane was used for anesthesia. Isoflurane was not used in previous studies because it was not available in the lab at the time they were conducted. Isoflurane was used in this experiment due to several advantages over ketamine and xylazine with respect to the duration of these surgeries as compared to those of Aim 1. That is, the surgeries in Aim 1 required approximately 45-min each to complete and as such did not typically require additional injections of ketamine and xylazine to maintain a surgical plane of anesthesia. On the other hand, surgeries in the current aim required appropriately 90-min to complete, which, if ketamine and xylazine had been used, would have required booster injections to maintain depth of anesthesia and these booster injections carry a risk of overdose. Therefore, isoflurane offered a significant advantage over ketamine and xylazine. Also, each experiment includes a control group that was anesthetized and placed in the stereotaxic frame controlling for exposure to anesthesia and potential nerve damage induced by ear bars. The chorda tympani nerve runs very close to the ear, carries taste information from the tongue to the rostral NTS, and could have been damaged by the ear bars of the stereotaxic frame. Rats were randomly assigned to three groups per surgical condition. A virus construct was infused bilaterally into the MeA (0.5 µl/site; Site 1, AP -2.0, ML 3.1, DV -8.3; Site 2, AP -3.0, ML 3.4, DV -8.5). Experimental groups were defined by the nature of the genetic material contained in each virus. Group Excitation (n = 12) was transfected with an excitatory DREADD (AAV-hSyn-hM3D(Gq)-mCherry; UNC

Vector Core, Chapel Hill, NC). Group Inhibition (n = 12) was transfected with an inhibitory DREADD (AAV-hSyn-hM4D(Gi)-mCherry; UNC Vector Core). Finally, the Control group (n = 11) contained 6 animals (Group EGFP) infected with a benign fluorescent reporter virus (AAV-hSyn-EGFP; UNC Vector Core) to control for non-specific effects of neuronal viral infection, as well as 5 animals (Group Normal) that were anesthetized and placed in the stereotaxic frame but received no surgical manipulation. Animals in Group Normal serve as a control for potential damage to the chorda tympani nerve and as a reference for normal behavior in the absence of any viral infection whatsoever. Viral infusions were made as in Aim 1 except for the infusion rate being changed to 0.05 µl/min to minimize the spread of the virus beyond the target structure.

2.2.1.3 Apparatus. Behavioral testing was conducted in drinking chambers (Med Associates ENV-008) equipped with a retractable drinking spout and lickometer circuitry (e.g., Arthurs et al., 2012; Arthurs & Reilly, 2013). Drinking chambers were 30.5 cm long x 24.1 cm wide x 29.2 cm high with modular aluminum sidewalls and clear polycarbonate doors, ceilings, and back walls. Steel bar floors were electrically connected through a lickometer circuit to a retractable spout which in the extended was positioned ~3mm outside the drinking chamber, but was accessible for licking via an oval access hole (1.3cm wide x 2.6 cm high at 6.0 cm above the floor) in the right-side chamber wall. The lickometer circuit (0.3 µA) was used to monitor individual licks with a temporal resolution of 10 milliseconds. Each chamber was housed within a sound attenuating cubicle equipped with a ventilation fan, white noise generator (~80 dB), and a shaded light bulb (100 mA, 28V) providing diffuse illumination. Chambers were connected to a computer (Med Associates) in an adjacent room that controlled all events and recorded data using

custom programs written in the Medstate notation language.

***2.2.1.4 Procedure*.** Following 3 weeks to recover from surgery the rats were placed on a deprivation schedule that allowed 15-min access to water in both the morning (in the drinking chambers) and the afternoon (in the home cages). Animals spent 3 weeks acclimating to the water deprivation schedule and reaching stable water intake baselines in the drinking chambers. Thus, DREADDs were allowed 6 weeks after surgery to reach peak expression before taste neophobia trials commenced. Each trial involved 15-min access to 0.15% saccharin once every third day during the morning drinking period. Forty-five minutes before Trial 1 all rats were injected with CNO (4 mg/ml/kg, IP), and then on each subsequent trial with vehicle (0.5% DMSO in physiological saline, 1 ml/kg, IP). Volume consumed and lick times were recorded. CNO was injected 45-min prior to saccharin access as this time point is well within the estimated duration of CNO efficacy (e.g., Roth 2016). CNO doses in the range of 1-3 mg/kg are common (Roth, 2016). We intentionally used a high dose of CNO to ensure that parameters were optimized for the detection of an effect, which, if present, could be refined in later experiments by titrating the CNO dose.

2.2.1.5 Histology. As in Aim 1, except tissue was examined for fluorescence (i.e., mCherry in groups Excitation and Inhibition or EGFP in group Control) marking infected cells.

2.2.1.6 Data Analysis. In addition to intake, lick pattern measures (i.e., lick cluster size and initial lick rate) were analyzed using the same statistical approach employed in Aim 1.

3. RESULTS

3.1 Experiment 1: GABA agonist in the GT and taste learning

3.1.1 Anatomical. Four subjects were dropped from statistical analyses due to misplaced cannula tips. In each case, misplaced cannula tips were located caudally to the GT and thus any infusion was unlikely to affect GT neurons. Guide cannulae caused damage to a number of structures dorsal to the GT including several thalamic nuclei (i.e., central medial thalamic nucleus, medial dorsal thalamic nucleus), the habenula (medial habenular nucleus, lateral habenular nucleus), hippocampus (granular layer of the dentate gyrus, fasciola cinereum), the corpus callosum, and cortex (retrosplenial dysgranular cortex, retrosplenial granular cortex). In addition, injection cannulae caused limited damage within the GT. However, any behavioral effect of damage to these structures would be expected to produce a difference between cannulated control animals (Group Saline) and neurologically intact control animals (Group Normal)—no such differences were found. Figure 2 depicts the position of injection cannula tips (inverted triangles) targeting the GT of all subjects included in statistical analyses.

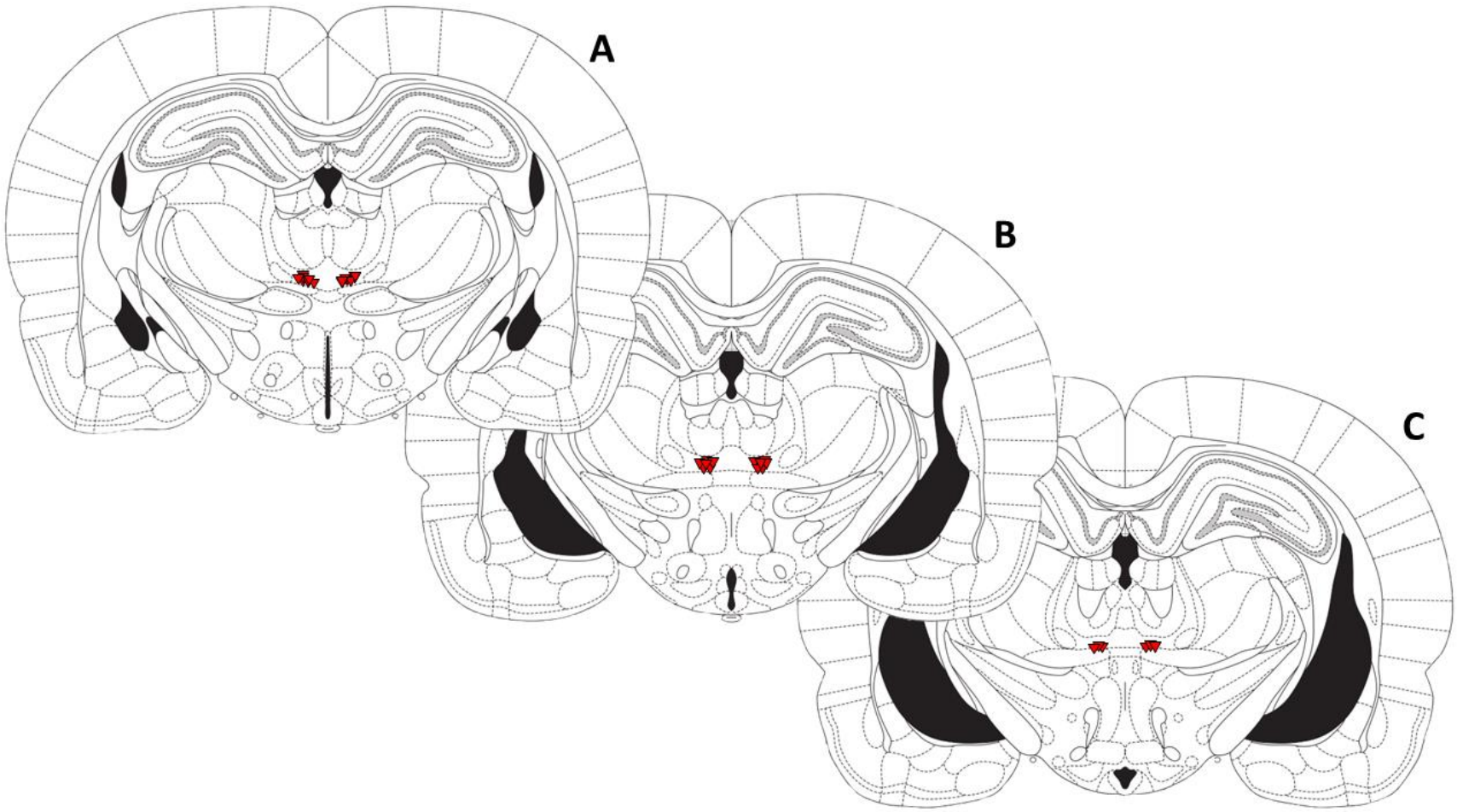

Figure 2. Experiment 1 Histology. Cannula placements for all subjects included in statistical analyses. Plate levels relative to bregma: A = -3.48, B = -3.72, and C = -3.96.

3.1.2 Behavioral. On Trial 1, two subjects in Group GT-GABA failed to consume any saccharin thus failing to engage in the experiment and precluding the observation of taste neophobia. The reason for this problem, despite stable water intake, was not apparent. Therefore, these animals were dropped from the experiment. Statistical analyses of baseline water and experimental saccharin intake revealed no difference between the control groups Normal (n = 10) and Saline (n = 8), which were collapsed to form group Control (n = 18) used in all subsequent analyses. As previously noted, the experiment was conducted in two replications. A three-way ANOVA of saccharin intake with replication and groups as between subject factors and trials as a within-subject factor revealed no significant main effect of replication, $F(1, 23) = 1.274$, $p > .05$. Thus, for subsequent analyses the datasets from the two replications were collapsed. For the three days preceding Trial 1, a mixed-design ANOVA revealed no statistical difference in water intake (see Table 2) between Group Control and Group GT-GABA during the morning drinking session (*ps* > .05).

Table 2. Experiment 1A Water Baseline

Experiment 1	**-3**	**-2**	**-1**
Control	15.67 (0.75)	16.81 (0.78)	15.81 (0.65)
GT-GABA	14.39 (1.05)	15.78 (1.11)	15.06 (0.92)

Note. Mean (ml±SE) water intake for the three days prior to Trial 1.

As shown in Figure 3, group Control, displaying the normal pattern of taste neophobia performance, consumed relatively little of the novel saccharin solution on Trial 1, and intake increased on subsequent trials reaching asymptote across Trials 3 to 5. This pattern contrasts with the performance of the GT-GABA group (n = 9) that consumed twice as much saccharin on Trial 1, a level that they maintained on Trials 2 and 3. Thus, Group GT-GABA consumed less saccharin than the Control group on Trials 2 and 3. On Trial 4 Group GT-GABA increased saccharin intake but remained somewhat lower than the Control group, a pattern that persisted on Trial 5. These impressions were confirmed by statistical analyses. A mixed design ANOVA with Group as the between-subjects variable and Trial as the within-subjects variable revealed a main effect of Group, $F(1,25) = 4.41$, $p < .05$, $\eta_p^2 = 0.149$, Trial $F(4,100) = 35.83$, $p < .05$, $\eta_p^2 = 0.589$, and a significant Group x Trial interaction, $F(4,100) = 10.88$, $p < .05$, $\eta_p^2 = 0.303$. Planned comparisons of the significant interaction revealed that on Trial 1 saccharin intake in Group GT-GABA was significantly higher than Group Control ($p < .05$). On Trials 2-5 Group GT-GABA consumed significantly less saccharin than Group Control ($ps < .05$). Regarding within-group differences across Trials, Group Control significantly increased intake from Trial 1 to 2 ($p < .05$) and from 2 to 3 ($p < .05$) but reached asymptote from Trials 3-5 ($ps > .05$). In Group GT-GABA, intake was not statistically different across Trials 1-3 ($ps > .05$), increased from Trial 3 to Trial 4 ($p < .05$) and was stable from Trial 4 to Trial 5 ($p > .05$). It is noted that during the conduct of this experiment (i.e., before subjects were dropped from the analysis for misplaced injector cannulae) Group Control and Group GT-GABA had reached the same asymptotic level of intake on Trials 4 and 5, at which time the experiment was terminated.

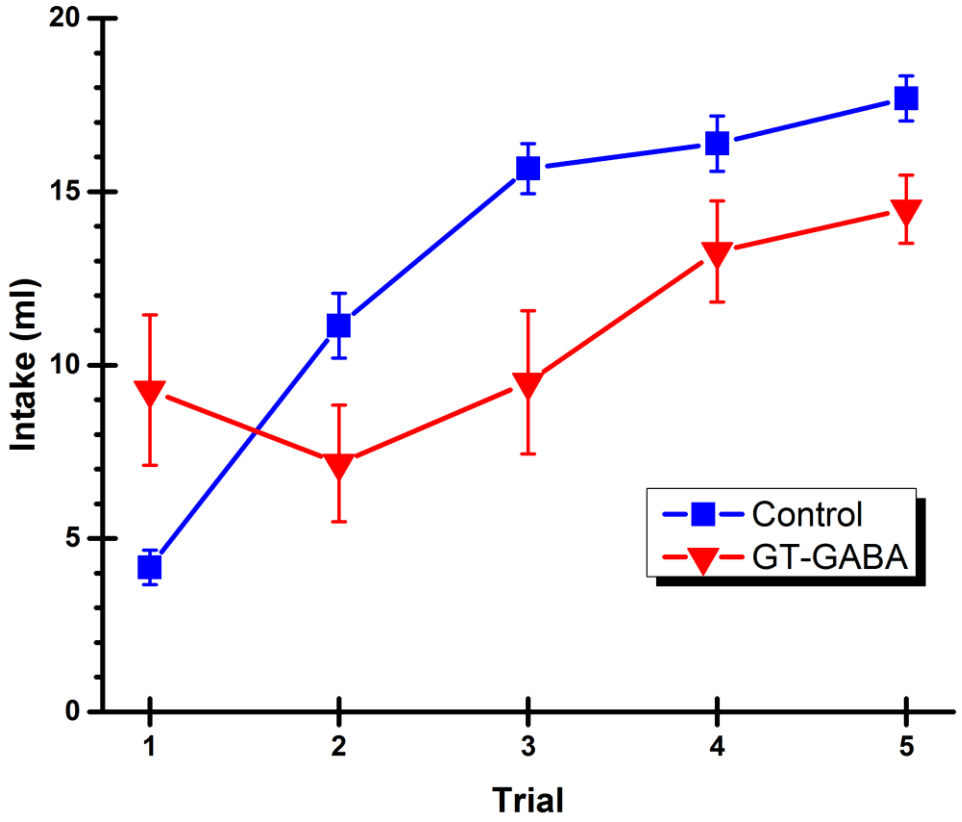

Figure 3. Experiment 1 Results. Mean (ml±SE) 0.5% saccharin intake for Control (n = 18) and GT-GABA (n = 9) subjects.

3.1.3 Experiment 1B. A 3-way ANOVA with factors for replication, group, and trial revealed no main effect of replication ($F < 1$); thus, the data sets from each replication were collapse for all analyses. There were no statistical differences between the two control groups Normal (n = 10) and Saline (n = 7) so they were collapsed into Group Control (n = 17) for all analyses. Baseline water intake is summarized in Table 3; a two-way ANOVA of the data from which these means were derived showed that performance was stable over the 3 days prior to Trial (*ps* > .05).

Table 3. Experiment 1B Water Baseline

Experiment 1B	-3	-2	-1
Control	16.56 (0.65)	16.59 (0.63)	16.24 (0.63)
Treatment	14.46 (0.78)	15.58 (0.75)	16.33 (0.75)

Note. Mean (ml±SE) water intake on the three days preceding Trial 1.

An inspection of the results of Experiment 1B shown in Figure 4 indicates equivalent Trial 1 performance between groups with a Trial 2 increase in Group Control but a substantial decrease in Group GT-GABA (n = 12). These impressions were confirmed by statistical analyses. A repeated measures ANOVA revealed a significant Group main effect, $F(1, 27) = 16.44$, $p < .05$, $\eta_p^2 = 0.378$, Trial main effect, $F(2, 54) = 4.07$, $p < .05$, $\eta_p^2 = 0.131$, and Group x Trial interaction, $F(2, 54) = 12.60$, $p < .05$, $\eta_p^2 = 0.318$. The significant interaction was followed up with planned comparisons. On Trial 1 (i.e., prior to any intracranial infusion), there was no statistical difference in quinine intake between groups Control and GT-GABA ($F < 1$). However, on Trials 2 and 3 Group GT-GABA consumed significantly less quinine than Group Control ($ps < .05$). In Group Control, quinine intake increased on Trial 3 relative to Trial 1 ($p < .05$). On the other hand, in Group GT-GABA, quinine intake decreased from Trial 1 to Trial 2 ($p < .05$) and was not statistically different on Trials 2 and 3 ($p > .05$).

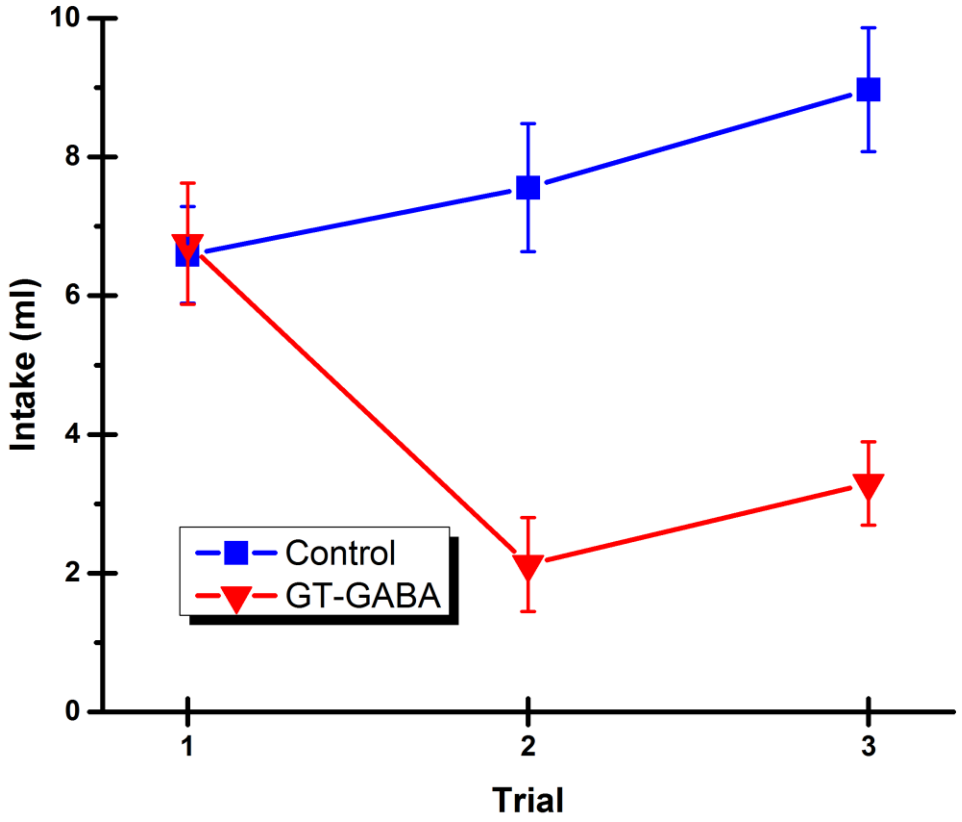

Figure 4. Experiment 1B Results: Mean (ml±SE) 0.0001 M quinine intake for Control (n = 17) and GT-GABA (n = 12) subjects.

3.2 Experiment 2. MeA GABA agonist infusion before novel taste.

3.2.1 Anatomical. Seven subjects were dropped from statistical analyses due to misplaced cannula tips. Misplaced cannula tips tended to be unilateral with one tip located dorsal medial to the MeA leaving the optic tract between the cannula tip and the MeA preventing the delivery of infused drugs. Guide cannulae caused damage to a number of regions dorsal to the MeA including internal capsule, optic tract, several thalamic nuclei

(e.g., laterodorsal thalamic nucleus ventrolateral part, reticular thalamic nucleus, ventral posteriolateral thalamic nucleus, ventral posteriomedial thalamic nucleus), hippocampus (CA2, CA3, fimbria of the hypothalamus, stratum lucidum of the hippocampus, subiculum transition area), and cortex (e.g., lateral parietal association cortex). Injector cannulae also caused some damage within the MeA. If damage to any of these regions influenced the behavior of interest such an effect would be observed as a difference between the Normal and Saline control groups. Figure 5 depicts the placement of injector cannula tips (inverted triangles) targeting the MeA of all subjects included in statistical analyses.

Concerning the efficacy of the GABA infusions in effecting the MeA it is worthwhile to note the MeA is considerably larger than the GT in terms of overall volume, particularly the rostrocaudal axis. Placement of the injector cannula within the MeA results in mechanical damage to a small subset of MeA neurons, but maximizes the likelihood that the infusion will diffuse to most MeA neurons. Furthermore, due to random placement variance, injector cannulae were distributed along most of the rostrocaudal axis of the MeA, which may serve as a control for the diffusion of GABA agonists.

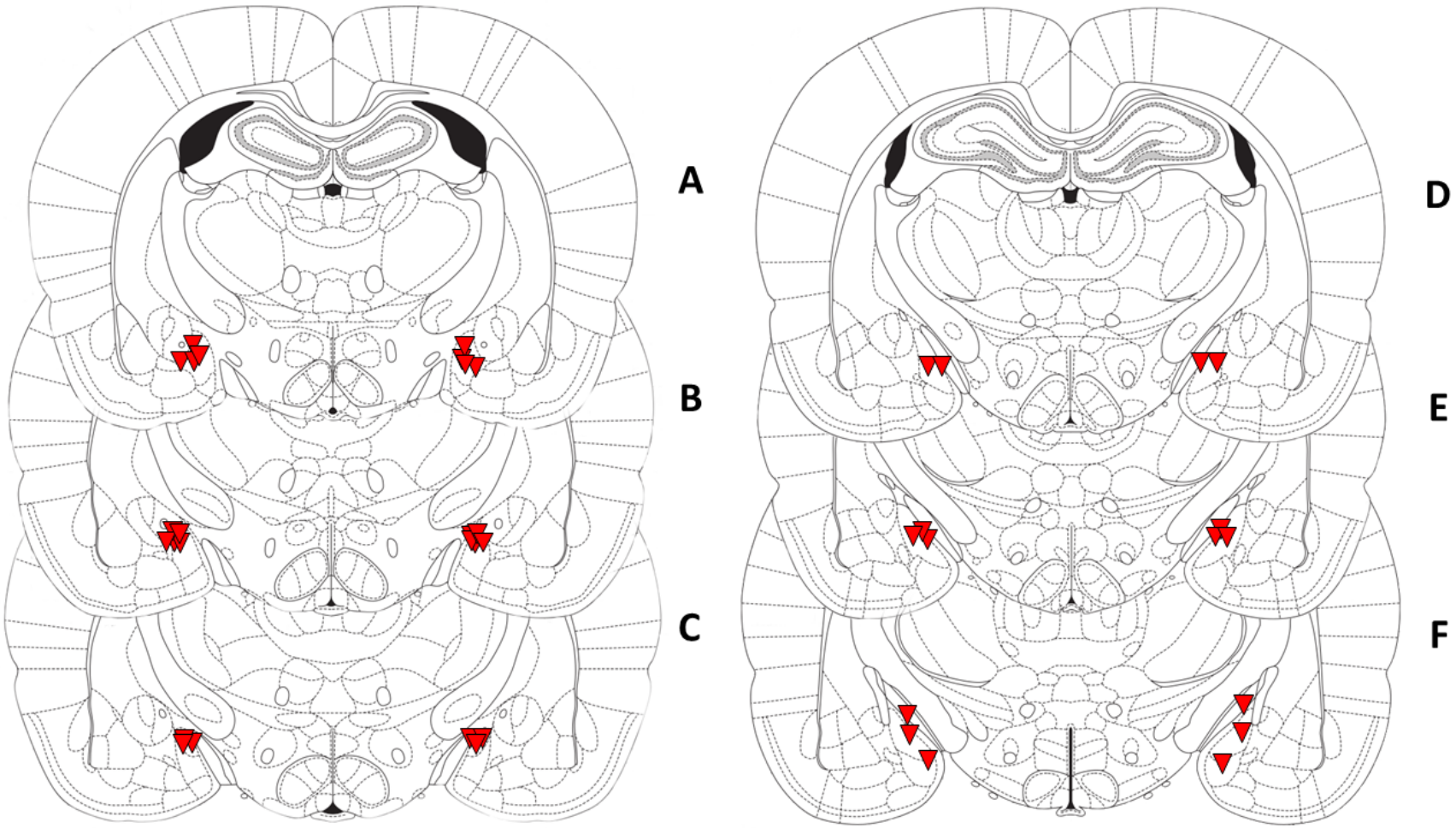

Figure 5. Experiment 2 Histology. Red triangles denote the placement of cannula tips included in the final statistical analyses of Experiment 2A and 2B. Plate levels relative to bregma: A = -2.04, B = -2.28, C = -2.52, D = -2.76, E = - 3.00, and F = -3.24.

3.2.2 Behavioral. One subject did not consume any saccharin on Trial 1. As with the two animals in Experiment 1A, failing to consume saccharin on Trial 1 precluded the observation of taste neophobia. Therefore, this rat failed to engage in the experiment and was accordingly dropped from the statistical analysis. Preliminary analysis of water baseline and experimental saccharin intake via a 3-way ANOVA revealed no main effect of replication ($F < 1$), as such the data sets from each replication were collapsed for all additional analyses. Analyses of the Normal (n = 7) and Saline (n = 9) groups revealed no differences in behavior regarding either water or saccharin; thus, these groups were collapsed to form Group Control (n = 16). Stable baseline water performance (see Table 4) was indicated by a lack of statistical difference between Group Control and Group MeA-GABA (*ps* > .05).

Table 4. Experiment 2A Water Baseline

Experiment 2A	**-3**	**-2**	**-1**
Control	13.94 (0.55)	14.78 (0.66)	14.08 (0.62)
Treatment	13.81 (0.83)	13.56 (0.99)	14.25 (0.93)

Note: Mean (ml±SE) water intake during the three days preceding Trial 1.

The results of the taste neophobia trials are summarized in Figure 6. Surveying the figure conveys the impression that there were no between-group differences in the occurrence and subsequent habituation of saccharin taste neophobia. Statistical analyses support these impressions. A mixed-design ANOVA revealed a significant main

effect of Trial, $F(3, 75) = 85.56$, $p < .05$, $\eta_p^2 = 0.774$, but no effect of Group ($F < 1$) and a trend toward a significant interaction ($p = 0.067$). Post-hoc tests of the Trial effect indicate that intake significantly increased from Trial 1 to Trial 2 ($p < .05$) and from Trial 2 to Trial 3 ($p < .05$) but not Trial 3 to Trial 4 ($p > .05$). So, each group expressed neophobia on Trial 1 that significantly habituated by Trial 4. The trending interaction term may warrant some additional discussion. While not statistically significant it seems that the most likely driver for this trending interaction is the between group difference on Trial 1 where Group MeA-GABA consumed a numerically greater amount of novel saccharin. A significant elevation of novel saccharin intake in Group MeA-GABA would have been a replication of the effect observed with permanent lesions. Also, it is worth noting that, in the pre-histology data set Group MeA-GABA consumed less saccharin than the Control group on Trial 2, this encouraged the view that a follow-up experiment was necessary to determine if infusing GABA agonists into the MeA would induce a CTA.

3.2.3 Experiment 2B. As previously noted, the CTA follow-up experiment was conducted because systemic injections of baclofen have been shown to induce CTAs (Wilson et al., 2011), and in the pre-histology dataset of Experiment 2A there was a significant difference in Trial 2 intake such that Group MeA-GABA consumed significantly less saccharin than Group Control, indicative of a possible CTA. However, as seen in Figure 6, after animals were dropped for misplaced cannula there was no longer a between-groups difference in saccharin intake on Trial 2 of Experiment 2A.

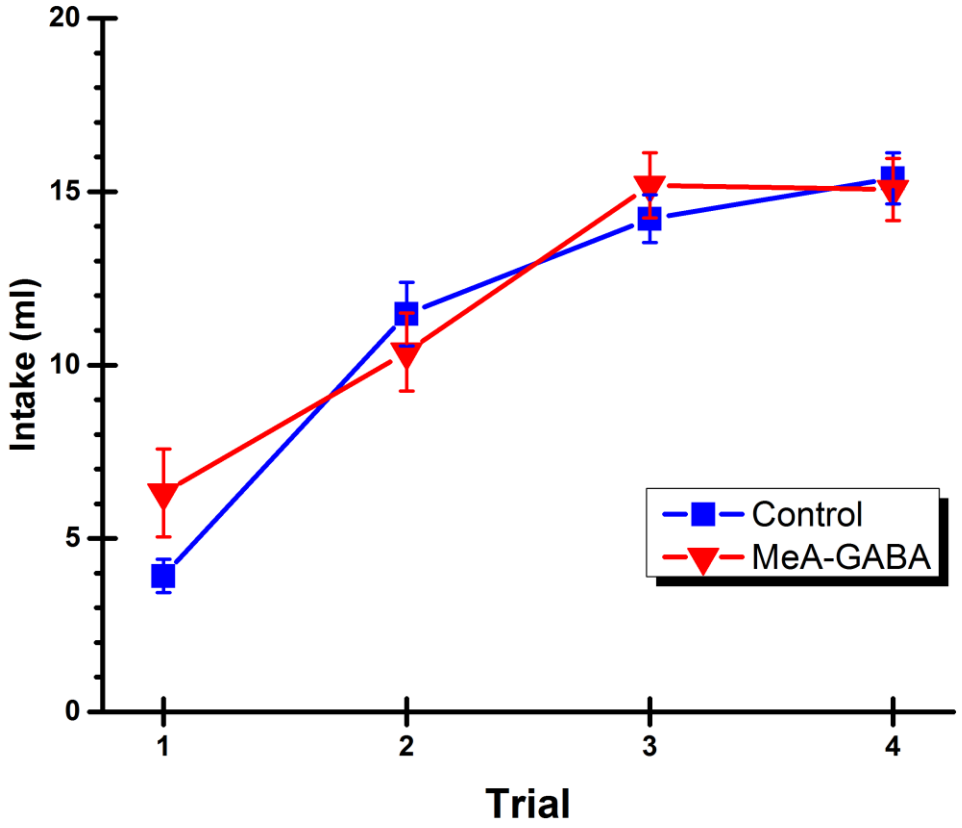

Figure 6. Experiment 2A Results. Mean (ml±SE) 0.5% saccharin intake for Control (n = 16) and MeA-GABA (n = 10) subjects.

Initial data analysis of the post-histology Experiment 2B data for both the water baseline and quinine intake showed that there was no effect of replication ($F < 1$). Thus, groups Normal (n = 7) and Saline (n = 11) were collapsed into Group Control (n = 18) due to statistically equivalent performance. Concerning baseline water performance (see Table 5), morning water intake was not statistically different between Group Control and Group MeA-GABA across the 3 days preceding Trial 1.

Table 5. Experiment 2B Water Baseline

Experiment 2B	-3	-2	-1
Control	13.96 (0.94)	13.47 (0.80)	14.74 (0.94)
Treatment	12.34 (1.13)	13.27 (0.96)	12.79 (1.13)

Note. Water Mean (ml±SE) intake for three days prior to Trial 1.

Inspection of Figure 7 shows equivalent Trial 1 performance between groups with a gradual habituation of taste neophobia in Group Control across the remaining trials, whereas Group MeA-GABA (n = 11) decreased intake on Trial 2 and maintains this level of intake on Trial 3, an interpretation supported by statistical analysis. A mixed-design ANOVA found a significant main effect for Group, $F(1,25) = 8.69$, $p < .05$, $\eta_p^2 = 0.257$, and a significant Group x Trial interaction, $F(2,50) = 6.60$, $p < .05$, $\eta_p^2 = 0.209$, but no significant effect of Trial ($F < 1$). Post-hoc analysis of the interaction revealed that Group Control significantly increased intake from Trial 1 to Trial 3 ($p < .05$), and that the MeA-GABA subjects consumed significantly less quinine than Controls on Trial 2 and Trial 3 ($ps < .05$).

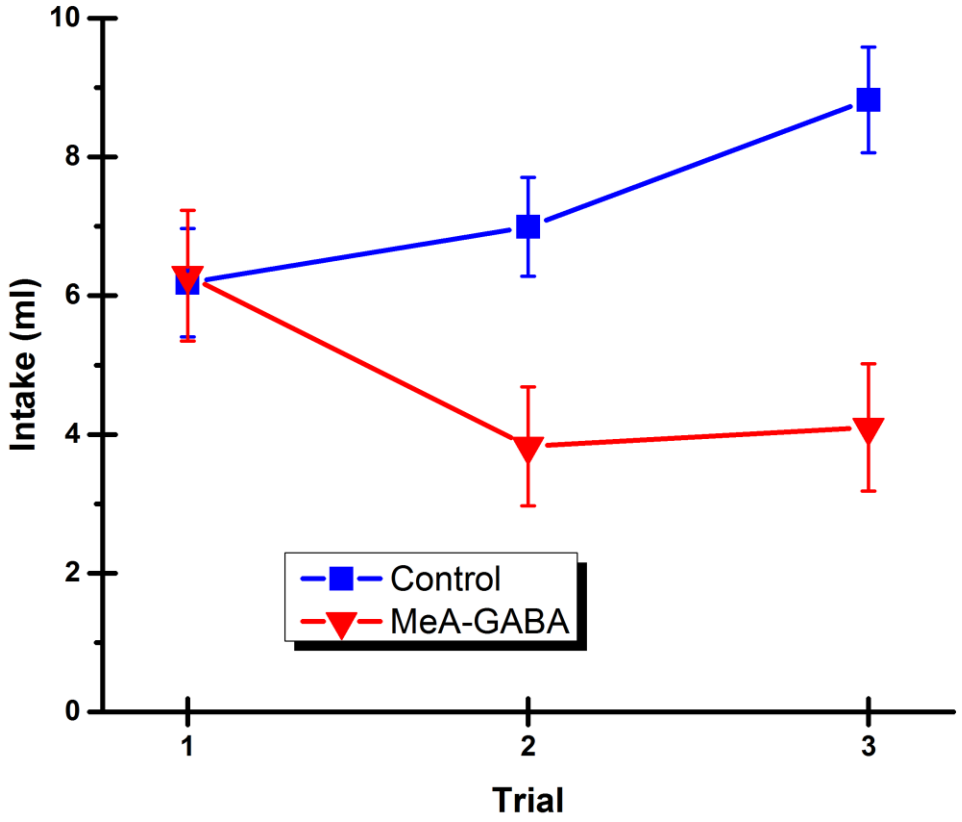

Figure 7. Experiment 2B Results. Mean (ml±SE) 0.0001 M quinine intake for Control (n = 16) and MeA-GABA (n = 11) subjects.

3.3 Experiment 3: Chemogenetic inhibition or excitation of the MeA in taste neophobia

3.3.1 Anatomical. Figure 8 illustrates a typical bilateral MeA DREADDs infection for MeA-Excitation or MeA-Inhibition subjects included in the statistical analyses. Eight subjects were excluded from statistical analyses due to misplaced viral infections. As in Experiment 2, misplaced infusions tended to be unilateral with the misplaced infusion occurring dorsomedial to the MeA in which case the fibers of the optic tract prevented

delivery of the virus to most MeA cells. These misplaced viral injections were most likely due to improper leveling of the skull in the mediolateral plane resulting in a tilted brain causing one infusion to be deep within the ventromedial aspect of the MeA and the other to be a dorsomedial miss centered within the fibers of the optic tract and internal capsule. Gross damage to structures overlying the MeA was not observed since the guide and injector cannula were only in place for a few minutes during the viral injections and the brain had approximately 10 weeks to heal. Also, as in the experiments of Aim 1, if damage to these overlying structures had any influence on taste neophobia such an effect would have been detected as a between-groups difference in behavior between the EGFP-infected rats and the non-infected (Normal) animals.

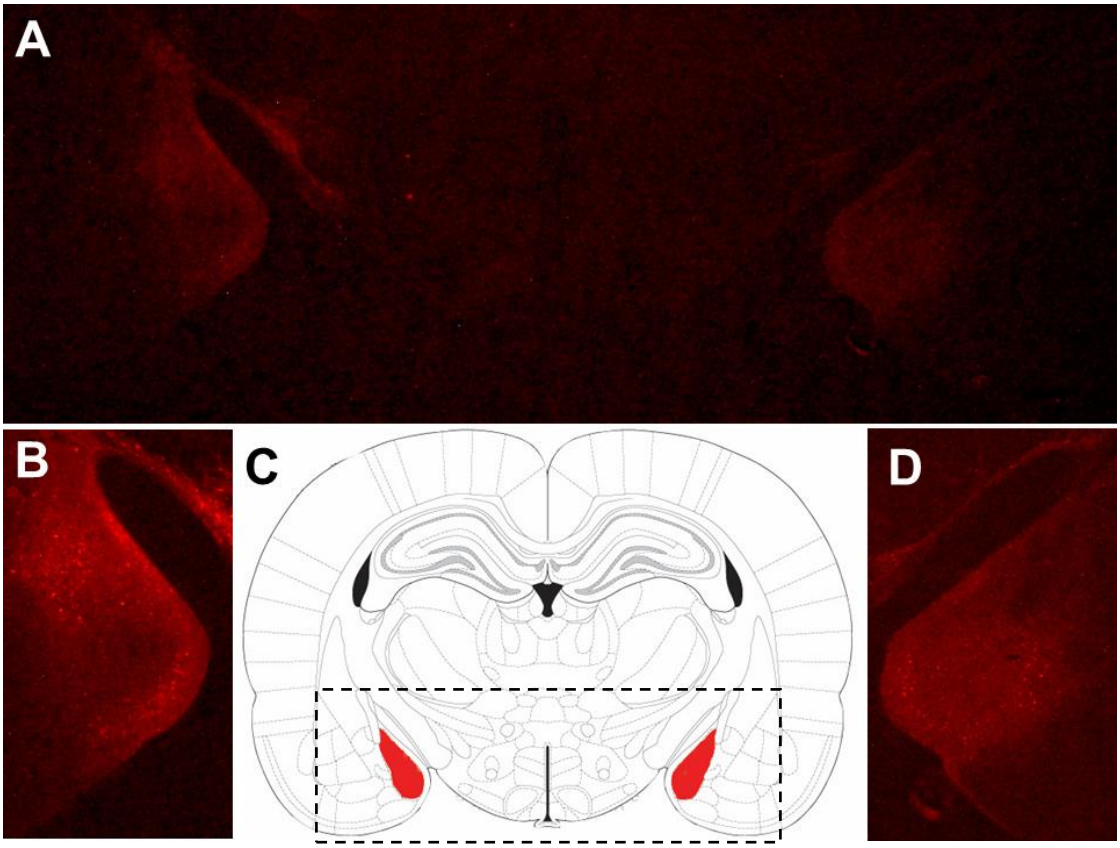

Figure 8. Experiment 3 Histology. Panel A shows a bilateral DREADDs infection (1.25X) representative of those included in statistical analyses. Panels B and D are a higher magnification (2X) view of the medial amygdala regions from Panel A. Panel C shows a schematic representation of the brain in coronal section at the level of the infection (bregma – 3.24), the medial amygdala has been highlighted in red and dashed lined indicate the region pictured in Panel A.

3.3.2 Behavioral. The two control groups Normal (n = 5) and EGFP (n = 6) were not statistically different (*ps* > .05) on any of the dependent measures and were thus collapsed into Group Control (n = 11). To establish a stable baseline of performance between groups, morning water intake in the drinking chamber (see Table 6) was analyzed for the three days preceding Trial 1 (e.g., Arthurs & Reilly, 2013). Repeated measures ANOVAs of each dependent measure found no significant main effects of

Group and no Group x Trial interaction (*ps* > .05). However, there was a main effect of Trial for both intake, $F(2,62) = 24.92$, $p < .05$, and initial lick rate, $F(2,62) = 5.87$, $p < .05$, but not lick cluster size ($p > .05$). Thus, there were no significant between-groups differences in water intake prior to the initiation of experimental procedures.

Table 6. Experiment 3 Water Baseline

	Intake			Initial Lick Rate			Lick Cluster Size		
Group	**-3**	**-2**	**-1**	**-3**	**-2**	**-1**	**-3**	**-2**	**-1**
Control	13.24 (0.87)	13.64 (0.91)	12.08 (0.86)	1000.45 (34.62)	1028.18 (34.93)	956.27 (33.53)	80.58 (7.11)	81.50 (12.34)	80.00 (6.74)
MeA-Excitation	11.95 (0.87)	12.29 (0.91)	10.17 (0.86)	951.09 (34.62)	950.36 (34.93)	942.18 (33.53)	59.23 (7.11)	67.41 (12.34)	59.77 (6.74)
MeA-Inhibition	12.49 (0.83)	12.42 (0.87)	11.05 (0.82)	1039.50 (33.15)	1020.33 (33.45)	950.58 (32.10)	69.65 (6.80)	74.91 (11.81)	71.03 (6.46)

Note. Mean (±SE) performance for water on the three days prior to the beginning of Experiment 3 for all subjects included in statistical analyses.

3.3.2.1 Intake. As noted previously, 0.15% saccharin was used in this experiment to allow the detection of both increases and decreases in the Trial 1 performance of the experimental rats. In Figure 9, we can see the normal occurrence and habituation of taste neophobia in Group Control evidenced by low Trial 1 intake relative to asymptotic performance on Trials 2, 3 and 4. Relative to Trial 1 performance in the Control group, Group MeA-Excitation (n = 8) appears to have shown normal taste neophobia, and Group MeA-Inhibition (n = 8) displayed exaggerated taste neophobia. The Trial 1 exaggeration

of taste neophobia in Group MeA-Inhibition is expressed as lower amount of saccharin intake. On Trial 2 Group MeA-Excitation consumed less saccharin than Group Control. Group MeA-Inhibition increased intake on Trial 2, relative to Trial 1, with performance equivalent to Group MeA-Excitation. On Trials 3 and 4 there appear to be no between groups differences on intake. These impressions are generally confirmed by statistical analyses.

A repeated measures ANOVA of the intake (Figure 9) data revealed a significant main effect of Group, $F(2,24) = 5.02$, $p < .05$, $\eta_p^2 = 0.295$, Trial, $F(3,72) = 29.13$, $p < .05$, $\eta_p^2 = 0.548$, and a significant Group x Trial interaction, $F(6,72) = 3.06$, $p < .05$, $\eta_p^2 = 0.203$. Planned comparisons of the interaction were conducted to clarify the pattern of results. Turning first to the Control group, there was a significant increase in saccharin intake from Trials 1 to 2 ($p < .05$), but there was no difference between Trials 2 and 3 or 3 and 4 ($Fs < 1$). Intake in Group MeA-Excitation was not significantly different from the Control group on Trial 1 ($F < 1$). However, on Trial 2 Group MeA-Excitation consumed significantly less saccharin than Group Control ($p < .05$). Then, on Trials 3 and 4 intake was not significantly different in Groups Control and MeA-Excitation ($Fs < 1$). Thus, Group MeA-Excitation showed normal taste neophobia on Trial 1, but, surprisingly, a delay in the habituation of taste neophobia on Trial 2 relative to the Control group. Group MeA-Inhibition consumed significantly less saccharin than Group Control on Trial 1 ($p < .05$). On Trial 2 there was no statistical difference between Groups Control and MeA-Inhibition ($p = .052$), although intake was numerically lower and this p-value does border on significance. On Trials 3 and 4 there was no significant difference between Groups Control and MeA-Inhibition, *p*

= 0.30 and 0.14, respectively. Thus, Group MeA-Inhibition consumed significantly less saccharin than the Control group on Trial 1 but not at asymptote.

Overall, using the standard measure of taste neophobia, intake, the Control Groups produced the quintessential pattern of performance, lower intake on Trial 1 relative to subsequent asymptotic intake. The performance of Group MeA-Excitation was comparable to that of Group Control, except on Trial 2 when the MeA-Excitation subjects drank less saccharin than Controls. The inhibitory DREADD exaggerated the expression of taste neophobia on Trial 1 but returned performance to normal levels thereafter.

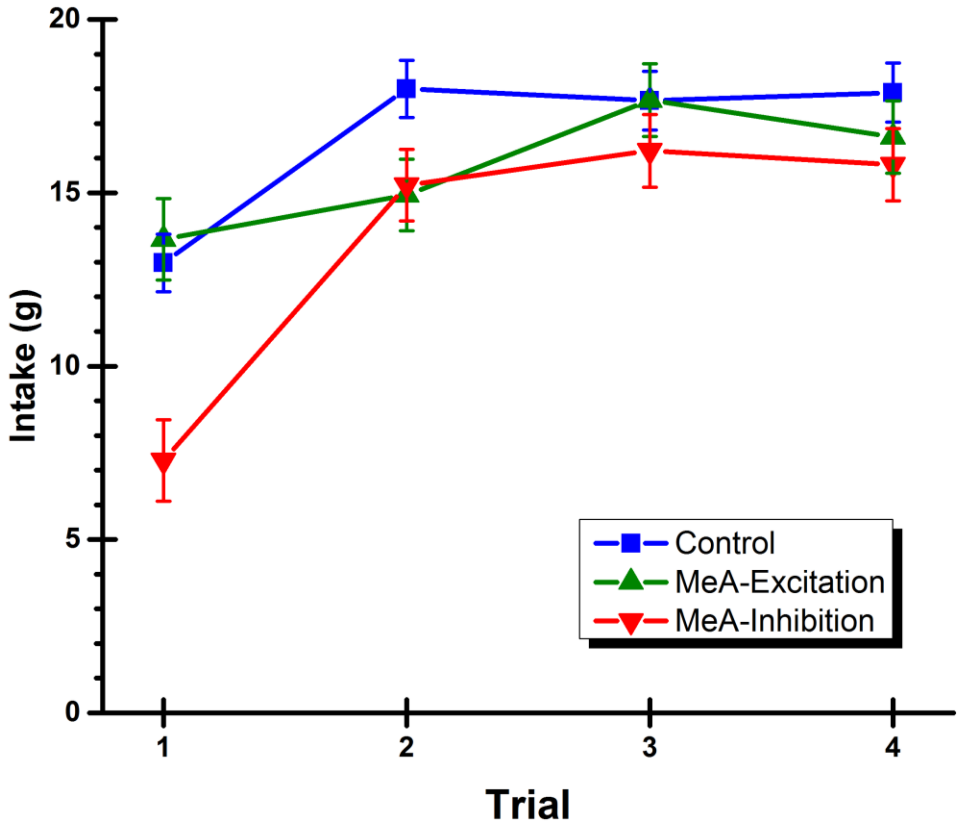

Figure 9. Experiment 3 Intake. Mean (g±SE) 0.15% saccharin intake for the three groups in Experiment 3. CNO was injected 45-min prior to novel saccharin access on Trial 1, and vehicle was injected 45-min prior to each of the subsequent trials.

3.3.2.2 Initial Lick Rate. Moving on to the measures of palatability a somewhat different pattern of performance was revealed. As can be seen in Figure 10, the initial lick rate (i.e., total licks in the 3-min after the first lick) in Group Control appears to be constant across Trials 1-4, perhaps indicative of a ceiling effect. Performance in Group MeA-Excitation appears to parallel that of Group Control but at a slightly lower level. Group MeA-Inhibition had a lower Trial 1 initial lick rate than the other groups, but performance increases to match that of Group Control on Trials 2-4.

An ANOVA of initial lick rate data confirmed a significant main effect of Trial, $F(3,72) = 3.18$, $p < .05$, $\eta_p^2 = 0.117$, and a significant Group x Trial interaction, $F(3,72) = 3.60$, $p < .05$, $\eta_p^2 = 0.248$, but no main effect of Group ($p > .05$). Planned comparisons of the interaction term revealed that performance in Group Control was not statistically different across trials suggesting a ceiling effect for initial lick rate at this concentration of saccharin (*ps* > .05). Across all trials Group MeA-Excitation showed numerically lower initial lick rates relative to Group Control but this difference was only statistically significant on Trial 2 ($p < .05$). On Trial 1, Group MeA-Inhibition licked at a lower rate than Group Control ($p < .05$). Across trials 2-4 there was no statistical difference between Groups Control and MeA-Inhibition (*Fs* < 1). In sum, on Trial 1 MeA-Inhibition, but not MeA-Excitation, showed significantly lower initial lick rates than Group Control, and all three groups reached similar asymptotic performance on later trials.

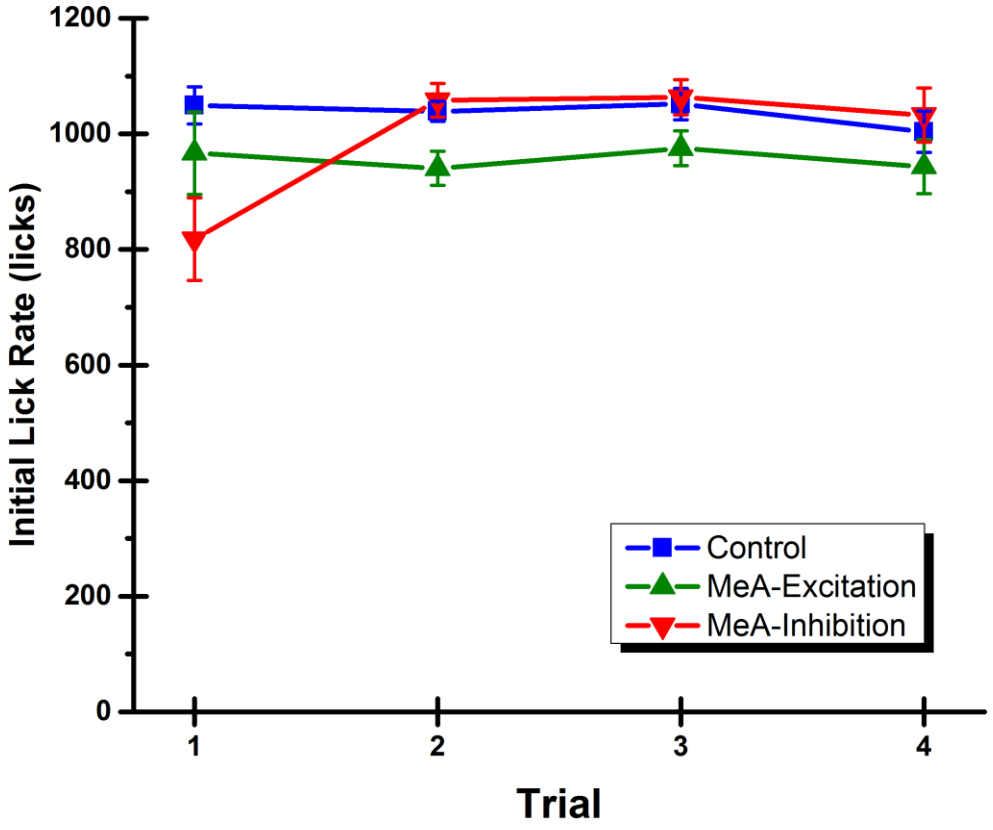

Figure 10. Experiment 3 Initial Lick Rate. Mean (licks±SE) saccharin initial lick rate for the three groups in Experiment 3. CNO was injected 45-min prior to novel saccharin access on Trial 1, and vehicle was injected 45-min prior to each of the subsequent trials.

3.3.2.3 Lick Cluster Size. Unconstrained by ceiling effects, the second measure of palatability clearly revealed the expected pattern of behavior in Control subjects, increased palatability previously shown to accompany the habituation of taste neophobia (e.g., Arthurs, 2012; Arthurs & Reilly, 2013; Lin et al., 2012a). In Figure 11 we can see that Group Control had a neophobic reaction to saccharin in terms of lick cluster size

demonstrated as low lick cluster size on Trial 1 relative to performance on Trials 2-4. Group MeA-Excitation had an equivalent lick cluster size to that of Group Control on Trial 1, but, unlike the Control Group, lick cluster size did not increase across Trials 2-4. Strikingly, Group MeA-Inhibition displayed a lick cluster size on Trial 1 approximately half that of Group Control; performance increased on Trial 2 but did not reach the same level as Group Control at asymptote, Trials 2-4.

A mixed design ANOVA of lick cluster size (Figure 11) showed a significant main effect of Group, $F(2, 24) = 4.50$, $p < .05$, $\eta_p^2 = 0.273$, Trial, $F(3, 72) = 6.20$, $p < .05$, $\eta_p^2 = 0.205$, and a Group x Trial interaction, $F(6,72) = 3.27$, $p < .05$, $\eta_p^2 = 0.214$. Planned comparisons of the interaction revealed that Group Control significantly increased lick cluster size from Trial 1 to 2 ($p < .05$), with asymptotic performance occurring between Trials 2-4 ($Fs < 1$). Thus, Group Control demonstrate the taste neophobia, on Trial 1, and the habituation thereof, Trials 2-4. Turning to Group MeA-Excitation, lick cluster size was not significantly different between Group Control and Group MeA-Excitation on Trial 1 ($F < 1$). Furthermore, in Group MeA-Excitation there were no significant within-group differences in lick cluster size across all 4 trials ($ps > .05$). Therefore, Group MeA-Excitation appears to display taste neophobia on Trial 1 evidenced by equivalent performance to the Control group; however, Group MeA-Excitation does not appear to habituate from this neophobic reaction evidenced by failing to increase lick cluster size (i.e., palatability) on subsequent trials. In Group MeA-Inhibition, lick cluster size on Trial 1 was significantly lower (i.e., less than half) that of Group Control ($p < .05$). In Group MeA-Inhibition, lick cluster size significantly increased from Trial 1 to Trial 2 ($p < .05$), and

was not statistically different across Trials 2-4 indicating asymptotic performance (*ps* > .05).

Concerning asymptotic performance, it appears both DREADDs groups have lower asymptotic lick cluster sizes than Group Control. For Group MeA-Excitation this impression is supported by statistical analyses with a significant difference between the Control and MeA-Excitation groups on Trials 2-4 (*ps* < .05). In the case of Group MeA-Inhibition, lick cluster size is significantly lower than Group Control only on Trial 3 (*p* < .05).

Turning to between-groups comparisons, on Trial 2 Group MeA-Excitation, but not MeA-Inhibition (*p* > .05), was significantly lower than Group Control (*p* < .05). On Trial 3 both experimental groups had significantly lower lick cluster size than Group Control (*ps* < .05). On Trial 4, as in Trial 2, only Group MeA-Excitation was significantly lower than Group Control (*p* < .05). So, in terms of lick cluster size, relative to asymptotic intake, both Groups Control and MeA-Inactivation exhibited low Trial 1 performance that increased across subsequent trials to asymptote, indicative of the occurrence and habituation of taste neophobia. Group MeA-Excitation showed no statistical differences in lick cluster size across trials. However, on Trial 1 there was no between groups difference for Groups Control and MeA-Excitation, suggesting that Group MeA-Excitation had a normal neophobic response, and the failure to significantly increase lick cluster size across trials indicates a failure to habituate taste neophobia in terms of palatability. Taken in combination with the similarity of Trial 1 performance in Groups Control and MeA-Excitation it appears that Group MeA-Excitation failed to habituate taste neophobia. Also, it appears that asymptotic performance in Group MeA-Inhibition was lower than Group

Control. That is, there was no within-groups difference across Trials 2-4 in Group MeA-inhibition, and on Trial 3 lick cluster size was significantly lower than Group Control. So, Group MeA-Inhibition displayed enhance taste neophobia on Trial 1, and habituated taste neophobia on Trial 2, but this habituation never reached the same level as the Control group, indicating a lower asymptotic level of palatability after the initial increase in taste neophobia.

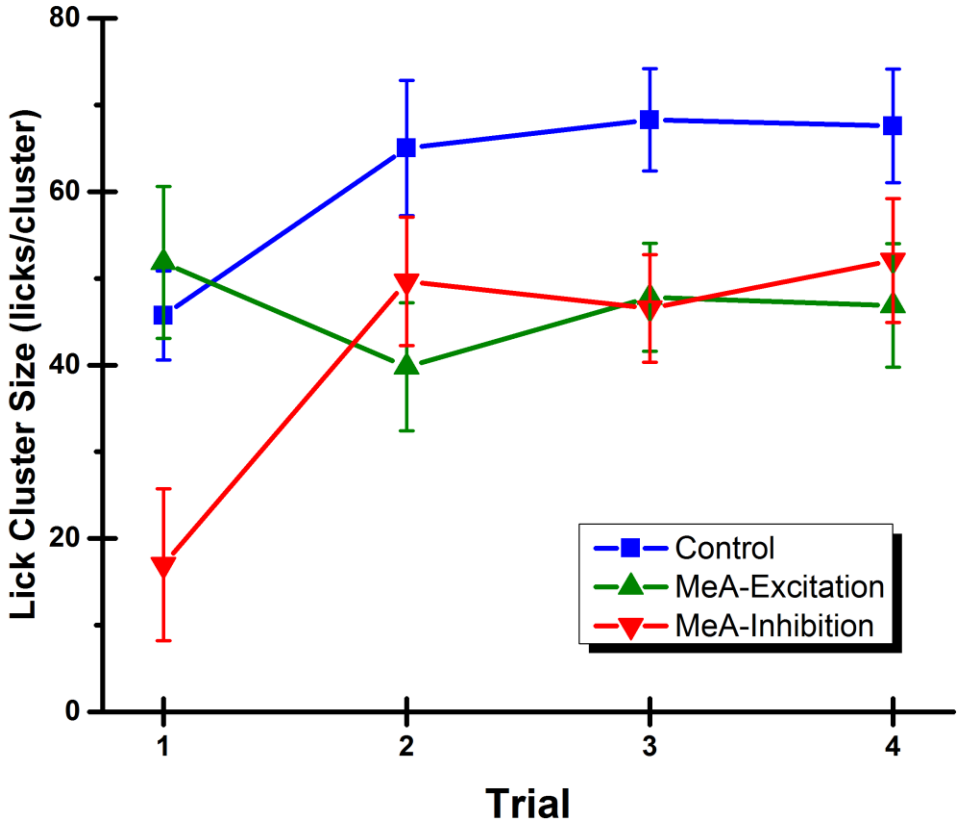

Figure 11. Experiment 3 Lick Cluster Size. Mean (lick cluster size±SE) 0.15% saccharin lick cluster size from Experiment 3. CNO was injected 45-min prior to novel saccharin access on Trial 1, and vehicle was injected 45-min prior to each of the subsequent trials.

4. DISCUSSION

The current set of studies was undertaken based on work in which I demonstrated that permanent lesions of the GT attenuated the expression of taste neophobia, and that this deficit had no effect on subsequent CTA acquisition (Arthurs, 2012; Arthurs & Reilly, 2013). This study demonstrated that the GT was necessary for taste neophobia, and, for the first time, that a lesion could attenuate taste neophobia without delaying CTA acquisition. An examination of the literature revealed a potentially similar pattern of results for MeA lesions: an attenuation of taste neophobia (Lin et al., 2009) that did not produce a delay in CTA acquisition (e.g., Aggleton et al., 1981; Meliza et al., 1981; Rollins et al., 2001). This pattern of deficits, attenuated taste neophobia and normal CTA, contrasts sharply with the pattern of deficits observed consequent to lesions of either the GC or BLA, which cause an attenuation of taste neophobia and a latent inhibition-like delay of CTA acquisition (Lin et al., 2009; 2011; 2015; Lin & Reilly, 2012; Roman & Reilly, 2007; Roman, Lin, & Reilly, 2009; St. Andre & Reilly, 2007). The fact that lesions of different structures can cause a similar attenuation of taste neophobia while having a differential impact on CTA suggests that taste neophobia has multiple behavioral components. Thus, the present study examined whether pharmacological inactivation of either the GT or MeA would replicate the attenuation of taste neophobia generated by permanent lesions of either structure. Additionally, I tested the hypothesis that neuronal inhibition in the MeA drives the expression of taste neophobia.

To refine the understanding of the role of the GT and MeA in taste neophobia, I used intracranial microinfusions of GABA agonists to create temporary lesions of the GT (Experiment 1) or MeA (Experiment 2). This approach provides a constrained time

window for the silencing of neurons and synaptic terminals in the target region occurring immediately before and during the presentation of a novel tastant. My experimental predictions were that temporary pharmacological lesions of the GT or MeA prior to novel taste access should result in the attenuation of taste neophobia (i.e., overconsumption of a novel tastant) similar to that seen with excitotoxic lesions (Arthurs, 2012; Arthurs & Reilly, 2013; Lin et al., 2009). However, a prior study using c-Fos to examine neuronal excitation in response to taste neophobia revealed elevated neuronal excitation in the BLA, GC, and GT but not MeA (Lin, Roman, et al., 2012). The combination of results from lesions and c-Fos suggested to me that inhibition of neurons in the MeA may be critical for taste neophobia expression. So, in the case of the MeA, it was predicted that if neuronal inhibition drives taste neophobia, then, GABA agonists may cause an enhanced taste neophobia response (i.e., lower intake relative to control subjects). But, GABA agonist infusions influenced neuronal cell bodies in the MeA as well as presynaptic terminals. So, I used DREADDs (Experiment 3) to either excite or inhibit MeA neurons during the presentation of a novel taste stimulus predicting, respectively, an attenuation or enhancement of taste neophobia.

During the first presentation of 0.5% saccharin in Experiment 1A Control subjects displayed taste neophobia by consuming a small volume of novel saccharin (see Figure 3). The Control subjects then demonstrated the habituation of taste neophobia by increasing intake on Trials 2 and 3 before reaching a level of asymptotic intake on Trials 3-5 approximately 3 times higher than Trial 1. During Trial 1, GT-GABA rats consumed significantly more of the novel solution than Control subjects. Thus, GABA agonists infused into the GT prior to the presentation of a novel tastant attenuated taste neophobia

(i.e., increased saccharin intake relative to controls), supporting my hypothesis. So, this experiment has demonstrated that neuronal excitation in the GT is necessary to the normal expression of taste neophobia, and that GABA can modulate this excitation.

As for performance on later trials, GT-GABA rats did not significantly change saccharin intake across Trials 1, 2, and 3. Therefore, despite consuming more saccharin than Control animals on Trial 1, GT-GABA animals consumed significantly less saccharin than Control animals on Trials 2 and 3. Saccharin intake in the GT-GABA group began to approach the level of the Control group on Trials 4 and 5 but remained somewhat lower. Notably, during the experiment (i.e., before animals were dropped for misplaced cannulae) both groups had reached the same level of asymptotic performance. However, in the final pattern of results, the GT-GABA animals expressed a significant delay in the habituation of taste neophobia and low asymptotic intake of saccharin.

Why did GT-GABA animals fail to increase saccharin intake on Trials 2 and 3, and why was asymptotic intake lower than Controls? Permanent lesions of the GT do not appear to influence the habituation of taste neophobia (Arthurs, 2012; Arthurs & Reilly, 2013). The most likely explanation is that GT-GABA animals acquired a CTA to saccharin on Trial 1. A weak CTA would explain consuming less saccharin than Control animals on Trials 2 and 3, and these trials would also serve as CTA extinction trials because GABA agonists were no longer being infused into the GT. Extinction could account for increased saccharin intake on Trial 4, and the fact that asymptotic intake between Trials 4 and 5 was lower than the Controls. Alternatively, the delay in the habituation of taste neophobia could be attributed to a disruption of taste memory formation. However, if Trial 2 performance were due to a memory deficit induced by the treatment administered on Trial

1, then, we might expect an increase in saccharin consumption in Group GT-GABA from Trial 2 to Trial 3 of a similar magnitude to that observed between Trial 1 and 2 in the Control group. Such an increase was not observed. These alternative hypotheses were tested in Experiment 1B.

In Experiment 1B (see Figure 4), I infused the GABA agonist cocktail after Trial 1 to determine if GABA agonist infusions into the GT induced a CTA or caused a taste memory deficit. The Control group, which in different subgroups received either a saline infusion or a mock infusion, showed taste neophobia and the habituation thereof over the three taste trials, while GT-GABA animals reduced quinine intake from Trial 1 (~7 ml) to Trial 2 (~2 ml), and intake remained low on Trial 3 (~3 ml). The alternative hypothesis, that GABA infusions into the GT cause a taste memory deficit, receives no support. A disruption of taste memory formation on Trial 1 would predict equivalent intake on Trial 2, not a decrease, and then a significant increase of intake on Trial 3. Therefore, it seems that GABA agonist infusions into the GT caused a CTA. Understanding how and why infusing these drugs into the GT induces a CTA will be an important issue for future research.

In Experiment 2A (see Figure 6), the Control group showed the expected occurrence and habituation of taste neophobia. The infusion of GABA agonists into the MeA prior to the presentation of a novel saccharin solution caused a small increase in saccharin intake (~2 ml) relative to the Control group. While this effect was not statistically significant it is trending in the direction of replicating the attenuation of taste neophobia seen with permanent MeA lesions and not toward enhanced taste neophobia, as predicted by the alternative hypothesis (GABA-induced enhancement of taste neophobia). The numerical

difference on Trial 1 and the trending interaction encourage another experiment to test for potential effects of GABA agonist in the MeA. Perhaps, infusions of GABA agonists into the MeA might cause a significant attenuation of taste neophobia if some parameters of the study were changed.

In Experiment 2B (see Figure 7), the Control group demonstrated neophobia on Trial 1 that habituated across Trials 2 and 3. In Group MeA-GABA, infusing GABA agonists into the MeA after the presentation of a novel quinine stimulus produced a significant reduction of intake, which suggests that a CTA was acquired after a single taste drug pairing. Notably, these were the same animals included in the Experiment 2A analysis, but counterbalanced for prior condition such that half of the animals in the MeA-Saline and MeA-GABA groups switched conditions between experiments. Thus, the absence of aversion in Experiment 2A and the aversion in Experiment 2B cannot be attributed to differences in cannula placement.

The aversion in MeA-GABA animals in Experiment 2B may be less severe than that observed in Group GT-GABA during the Experiment 1B. Therefore, is seems that the infusion of GABA agonists in ether structure triggers some CTA inducing effects, but these effects are stronger in the GT than the MeA. Unsurprisingly, the aversive effects of either infusion are more clearly expressed when occurring in typical CTA experimental arrangement (i.e., CS-US), as in Experiments 1B and 2B, than during the backward pairing (i.e., US-CS) that occurred in the primary experiments (i.e., 1A and 1B). Backward pairings can produce CTAs, but these tend to be weaker than CTAs produced when the taste stimulus precedes the onset of aversive effects (e.g., Boland, 1973).

Why GABA agonist infusions into either the GT or MeA can produce a CTA will be an important question for future research. As discussed previously (see sections 1.5 and 1.6), lesions of the GT or MeA do not appear to influence CTA acquisition suggesting that these regions are not necessary for the acquisition of CTA. But, we see here that infusions of GABA agonists into either structure are sufficient to produce a CTA. Of course, these CTA experiments were originally included due to concerns that central infusions of baclofen might mimic systemic injections of baclofen and induce a CTA (Wilson et al., 2011). Perhaps, systemically administered baclofen induced CTA via actions in the central nervous system including both the GT and MeA.

Turning now to Experiment 3, relative to the neophobic response in the Control group on Trial 1 we can see that MeA-Inhibition subjects consumed significantly less novel saccharin (see Figure 9), whereas Group MeA-Excitation consumed a volume similar to the Control group. Thus, the hypothesis that MeA neuronal inhibition enhances taste neophobia was supported, but the converse prediction that MeA excitation would attenuate taste neophobia was not supported. Control subjects increased intake on Trial 2, indicating the habituation of taste neophobia. The MeA-Excitation animals drank about the same amount on Trial 2 as they did on Trial 1, resulting in lower Trial 2 intake than the Control group. On Trial 2, MeA-Inhibition animals doubled saccharin intake relative to Trial 1 reaching a level equivalent to MeA-Excitation Trial 2 performance such that both DREADDs groups were lower than Control subjects. Therefore, it appears that the DREADDs manipulations of MeA neurons had a differential effect on the habituation of taste neophobia: MeA-Excitation on Trial 1 appears to have delayed the attenuation of taste neophobia, whereas MeA-Inhibition on Trial 1 did not appear to delay the

attenuation of taste neophobia. On Trials 3 and 4 there were no between-group differences in saccharin intake indicating a common asymptote for familiar saccharin intake. In contrast to Experiments 1 and 2, in Experiment 3 there is no evidence, either pre- or post-histology, of decreased intake on Trial 2, suggesting that the DREADDs manipulations did not possess the aversive effects seen with GABA agonist infusions.

The Control group in Experiment 3 failed to show taste neophobia in terms of initial lick rate (see Figure 10). So, in terms of initial lick rate, palatability was constant in the Control group as the saccharin stimulus transitioned from novel to familiar. This pattern of results is most likely due to a ceiling effect in initial lick rate with this concentration of saccharin. Licking in rodents functions off a central pattern with licking occurring at a frequency of between 6-8 licks per second (e.g., Travers, Dinardo, & Karimnamazi, 1997). Therefore, in 3-min the maximum number of licks, assuming no pauses in licking, is between 1,080 and 1,440. With initial lick rates in the Control group hovering around 1,050 it seems very likely that this level of performance is near the maximum level, accounting for some brief pauses in licking. With higher concentrations of saccharin (e.g., 0.5%) the occurrence and habituation of taste neophobia can be observed in initial lick rate data (e.g., Lin et al., 2012a). Group MeA-Excitation essentially parallels the Control group with a somewhat lower level of performance failing to show any evidence of the occurrence or habituation of taste neophobia. Turning to Group MeA-Inhibition we see a significant suppression of initial lick rate on Trial 1 that then rapidly increases to match the other two groups over the subsequent trials (see Figure 10). That is, Group MeA-Inhibition demonstrated an enhanced taste neophobia response which rapidly attenuates on subsequent trials. So, the enhancement of taste neophobia caused by DREADDs

inhibition of MeA neurons seen in the suppression of intake (see Figure 9) is also observed in the suppression of initial lick rate (i.e., palatability).

In terms of lick cluster size (see Figure 11), Group Control exhibited the expected pattern of results with lower lick cluster size on Trial 1, which increased on subsequent trials as taste neophobia habituated. In Group MeA-Excitation we see no effect on Trial 1 with performance matching the Control group. However, on subsequent trials it seems that the Trial 1 manipulation prevented the increase in palatability that normally accompanies the habituation of taste neophobia. Since, palatability is lower in Group MeA-Excitation than the Control group this may indicate the formation of a taste aversion on Trial 1. However, the consistency of performance argues against a CTA account for these results. That is, lick cluster size does not decrease on Trial 2, which might indicate CTA acquisition, and lick cluster size does not increase during later trials, which might indicate the extinction of a weak CTA. In any case, further experiments will be necessary to evaluate the nature of the MeA-excitation effect. The most striking effect is seen in Group MeA-Inhibition where the inhibition of MeA neurons prior to novel saccharin access produced a dramatic reduction in lick cluster size (i.e., palatability) to a level less than half (~18 licks per cluster) that of the other two groups (~ 50 licks per cluster). So, we can see that inhibiting MeA neurons decreases the palatability and intake of a novel saccharin solution, but does not suppress intake on later trials, indicating that MeA neuronal inhibition transiently increases taste neophobia without inducing a CTA. This confirms my hypothesis about the function of MeA neurons in taste neophobia and demonstrates a novel effect: a neural manipulation that enhances taste neophobia has never been observed before, at least to my knowledge.

Overall, the pattern of results in the MeA-Inhibition group seems clear—inhibiting MeA neurons enhances taste neophobia in terms of both palatability and intake. The subtler effects observed in the MeA-Excitation group are more difficult to interpret. Across each dependent measure the excitation of MeA neurons on Trial 1 did not influence Trial 1 behavior (i.e., the expression of taste neophobia), but seemed to produce a transient (intake) or sustained (i.e., initial lick rate and lick cluster size) delay in the habituation of taste neophobia. This is curious since, in general, palatability tracks intake, but in this experimental group taste neophobia eventually habituates in terms of intake but there is no concomitant increase in palatability. This pattern of results does not fit any of the previously forwarded accounts for delayed attenuation of taste neophobia such as a taste memory disruption or CTA acquisition. A taste memory deficit would predict the habituation of taste neophobia on Trial 3 for all dependent measures. As for a CTA, If the CTA was not strong enough to cause a decrease on Trial 2, then, it ought to have habituated quickly (i.e., increased intake on Trial 3 or 4). It is as if exciting MeA neurons during the presentation of a novel tastant locked taste palatability at that level on all subsequent trials. If so, this is a novel and intriguing result that will require additional experiment to understand.

Importantly, the DREADDs approach differs in the scope of effects from the pharmacological manipulation. That is, infusions of baclofen and muscimol cause both pre- and post-synaptic inhibition thereby influencing MeA neurons as well as synaptic terminals within the MeA. AAV serotype 8 viral vectors like the ones used in Experiment 3 to deliver the DREADDs have not shown any retrograde activity (Aschauer, Kreuz, & Rumpel, 2013). Therefore, the DREADDs manipulations performed in Aim 2 were isolated

to cell bodies within the MeA. The infusion of GABA agonists in the MeA prior to Trial 1 caused a small numerical increase in the consumption of novel saccharin; however, this effect was not statistically significant. Thus, there was a non-significant trend for the temporary lesion to replicate the permanent lesion effect. This pattern of results contrasts with those from Experiment 3 in which MeA neuronal inhibition with DREADDs had the opposite effect from permanent lesions, enhancing rather than attenuating taste neophobia. This suggests an important difference between the pharmacological inactivation by GABA agonists, acting both pre- and post-synaptically, and inhibitory DREADDs restricted to inhibiting neuronal cell bodies within the MeA. Teasing apart the differences seen between these manipulations will be an important goal for future research.

In sum, there are three main findings: (1) neuronal excitation within the GT is necessary for the normal expression of taste neophobia, (2) inhibiting neurons within the MeA with DREADDs, but not GABA agonists, enhances taste neophobia, (3) infusions of GABA agonists into either the GT or the MeA is sufficient to cause a CTA. Additionally, using DREADDs to excite MeA neurons prior to the presentation of a novel taste did not influence the expression of taste neophobia, but rather appears to influence the habituation of taste neophobia. Interestingly, this effect is manifest as a short delay as measured by intake, but a longer—maybe permanent—delay in terms of palatability (i.e., initial lick rate and lick cluster size).

4.1 Future Directions

The results of Experiment 1A demonstrate, for the first time, that excitation of GT neurons is necessary for the expression of normal taste neophobia. This result leads to several new questions. What specific population of neurons in the GT are excited during taste neophobia, and what are the afferent and efferent connections of these neurons? What is the precise function of neuronal excitation in the GT? Does this neural circuit, the GT and its afferent and efferent connections, underlie the estimation of taste safety, or do they serve another purpose? What are the neurochemical mechanisms of this circuit?

The results of Experiment 1B demonstrate that infusions of GABA agonists into the GT are sufficient to produce a CTA. Why do GABA agonists infused into the GT cause a CTA? Lesion studies show that an intact GT is not necessary for CTA acquisition, but the results of Experiment 1B show that pharmacological manipulation of the GT is sufficient to induce a CTA. Parsimony suggests that GABA agonists in the GT cause an aversive US effect that produces a CTA. If, and how, this aversive effect occurs could provide valuable information on the role of the GT in taste learning.

In Experiment 2A, GABA infusions in the MeA failed to replicate the overconsumption of novel saccharin seen with permanent lesions of the MeA. However, it might be useful to conduct a similar experiment with somewhat different procedures such as a different saccharin concentration or GABA agonist dosing. Also, considering Experiment 3, we know that inhibiting MeA neurons can enhance the expression of taste neophobia. GABA agonists in the MeA may have inhibited the same neurons inhibited by DREADDs in Experiment 3 resulting in enhanced taste neophobia and simultaneously inhibited another population of cells or synaptic terminals within the MeA that replicated the lesion effect of

attenuating taste neophobia. Thus, one possibility is that in Experiment 2A GABA agonists in the MeA had opposing effects on different neural substrates of taste neophobia resulting in a net effect that appeared normal.

Moving forward, it will be important to determine the specific neuronal population in the MeA that, when inhibited, enhances the expression of taste neophobia. Identifying these neurons will in turn lead to questions about the source of inhibitory input, and what downstream effects inhibiting these neurons has on other neuronal populations. Furthermore, the different patterns of effects seen with GABAergic and DREADDs inhibition of the MeA suggest that there is a complex underlying circuitry in the MeA that will need to be teased apart.

In Experiment 2A, there was some indication in the pre-histology dataset that Group MeA-GABA consumed less saccharin on Trial 2 than Group Control. In the final dataset (see Figure 6) this pattern was no longer present, suggesting that the MeA GABA infusions were not aversive. Then, during Experiment 2B when GABA infusions occurred after taste consumption, as in a typical CTA experiment, the infusions appeared to act as an effective CTA inducing stimulus. Although, the severity of the CTA in MeA-GABA animals seems somewhat less than that seen for the GT in Experiment 1B. Therefore, it seems that infusing GABA agonists into the MeA is aversive but this stimulus may be less severe that if the infusions occur in the GT. As with the GT, understanding the nature of the MeA GABA induced CTA will be an important future direction that will inform out understanding of the neural circuitry underlying taste learning.

An important future direction will be to determine whether manipulations that enhance taste neophobia also affect consumption of a familiar tastant. For instance, will DREADDs

inhibition of the MeA decrease intake of a familiar food? If so, is this decrease occurring because the tastant is perceived as novel? Does the MeA plays a role in the estimation of taste safety? If so, then inhibition of MeA neurons may cause a taste to be perceived as less safe (i.e., dangerous) and thus drives lower consumption. However, if the role of the MeA is constrained to estimating the safety of unfamiliar stimuli, then perhaps inhibiting MeA neurons would have no effect on a familiar food. That is, the estimation of taste safety may be unnecessary in the presence of a safe taste memory, which presumably governs decisions about consuming a familiar safe food. If MeA neuronal inhibition were to decrease the intake of a familiar safe food this might indicated that the MeA plays an important role in ongoing decisions about food safety that is not limited to the initial encounter with novel foods.

4.2 Translational Issues

Developing an understanding of the fundamental neuroanatomical and neurochemical underpinnings of taste learning will provide a foundation for translational studies to develop treatments for maladaptive taste learning. And, rodent models can be used to investigate potential clinical treatments for patients suffering from ARFIDs or maladaptive CTAs. Demonstrating that pharmacologically inactivating neurons and synaptic terminals within the GT can cause the overconsumption of a novel tastant, while, conversely, inhibiting neurons in the MeA can suppress consumption of a novel taste offers hope that with a comprehensive understanding of the neural circuits underlying taste neophobia we could modulate the response to novelty as needed to either counteract ARFIDs or delay the acquisition of maladaptive CTA.

Translational studies could pursue several approaches to treating aberrant taste learning in humans. First, deep brain stimulation has become a powerful tool to treat illnesses ranging from Parkinson's disease (e.g., Hickey & Stacy, 2016) and obsessive compulsive disorder (e.g., Castle, Bosanac, & Rossell, 2015) and is being tested in clinical trials for a range of neuropsychiatric disorders (e.g., anorexia nervosa, Lipsman et al., 2013; depression; Kubu et al., 2016). While the mechanisms underlying deep brain stimulation remain poorly understood (e.g., Chiken & Nambu, 2016; Torres-Sanchez, Perez-Caballero, & Berrocoso, 2016) the approach is simple and can be easily tested in rodent models. Regions implicated in the control of taste neophobia (or CTA) could be implanted with stimulating electrodes and tested for effects on taste neophobia (or CTA). Any promising targets could then be tested in human clinical trials.

Identifying specific cellular populations and neurochemical messenger systems involved in taste neophobia and CTA would open the opportunity for the application, or development, of pharmacological treatments for maladaptive taste learning. Currently, there are no FDA approved pharmacological treatments for ARFID. Considering the broad range of pharmaceuticals already available it seems likely that a currently available drug may have some efficacy in treating maladaptive taste neophobia (i.e., ARFIDs). If the neurochemical underpinnings of taste neophobia were well characterized, then, drugs could be selected or designed to treat ARFID. Also, there has been some early success in rodent models combining pharmacological treatment and deep brain stimulation, which are ineffective individually, to effectively treat neurological changes thought to underlie drug addiction (Creed, Pascoli, & Luscher, 2015). An analogous pharmacological approach could be taken to developing interventions to prevent, or delay, the acquisition

of maladaptive CTAs, which are currently only managed by treating the symptoms of illness with antiemetic drugs (Basch et al., 2011; Perwitasari et al., 2011).

A caveat to the pharmacological approach outlined above is the reliance on a somewhat unique neurochemical substrate that can be altered via pharmacology. If, however, such a discrete target is not present this approach would be either ineffective or so non-specific as to cause a debilitating host of side effects. For these reasons, it may be that the clinical application of DREADDs technology could offer the best treatment option for clinical populations suffering for aberrant taste learning. This approach is currently in development (Urban & Roth, 2015). AAV viruses have been used successfully in gene therapy procedures to treat a variety of conditions (Guedon, et al., 2015; Kaplitt et al., 2007; Mitchell, Nicolson, Warischalk, & Samulski, 2012). The ability to use DREADDs in humans would allow the specific manipulation of a population of cells via oral drug dosing that could be titrated as needed over time and discontinued entirely if the patient were to recover normal function.

Eating can be one of life's greatest pleasures. In the end, it is my hope that we will eventually understand enough about the neural substrates of taste learning to offer relief to people suffering from debilitating illnesses that limit their ability to meet basic nutritional requirements much less enjoy food fully.

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6. ACC approval

All experiments were approved as part of ACC protocol 14-210.

2. CURRICULUM VITAE

Joseph W. Arthurs

Department of Psychology
University of Illinois at Chicago
1007 W. Harrison St. (M/C 285)
Chicago, IL 60607

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EDUCATION

Ph.D. *expected* 2017 — University of Illinois at Chicago
Dissertation: Role of the gustatory thalamus and medial amygdala in taste learning
Major: Behavioral Neuroscience
Minor: Statistics, Methods, and Measurement
Advisor: Steve Reilly

M.A. 2012 — University of Illinois at Chicago
Thesis: Role of the Gustatory Thalamus in Taste Learning
Advisor: Steve Reilly

B.S. 2007 — University of Idaho
Major: Psychology

CURRENT RESEARCH SUPPORT

2015-2016 UIC Provost's Award for Graduate Research — Award: $2,250
Medial and Central Amygdala in Taste Neophobia and CTA
J. Arthurs, Principal Investigator

ACADEMIC AWARDS AND HONORS

Graduate Student Paper Award—Midwestern Psychological Association 2016 Annual Meeting
Behavioral Neuroscience Division Research Assistant, Summer 2015
Nancy Hirschberg Grant for Undergraduate Research to **mentee Simon Komar**, 2014
Department of Psychology Certificate of Achievement, 2010-2015 academic years

RESEARCH TECHNIQUES

Behavioral:

- Consummatory intake testing
- Microstructural lick analysis
- Place preference conditioning
- Taste reactivity testing
- Intracranial self-stimulation

Neural/Physiological:

- Stereotaxic placement of cannulae, electrodes, and glass micropipettes
- Insertion of designer receptors exclusively activated by designer drugs (DREADDs)
- Intracranial pharmacological microinfusions
- Induction of discrete excitotoxic and electrolytic brain lesions
- Intraoral cannulation surgery
- Gastric cannulation surgery

Histology:

- Immunohistochemical staining (e.g., NeuN, cFos)
- Nissil staining (e.g., cresyl violet)
- Fluorescent microscopy (e.g., mCherry)

PUBLICATIONS

Lin, J.Y., Arthurs, J., and Reilly, S. (2011). Role of the insular cortex in morphine-induced conditioned taste avoidance. *Brain Research*, *1384*, 80-88.

Arthurs, J., Lin, J. Y., Amodeo, L. R., and Reilly, S. (2012). Reduced palatability in drug-induced taste aversion: II. Aversive and rewarding unconditioned stimuli. *Behavioral Neuroscience, 126,* 433-444.

Lin, J. Y., Amodeo, L. R., Arthurs, J., and Reilly, S. (2012). Taste neophobia and palatability: The pleasure of drinking. *Physiology and Behavior, 106,* 515-519.

Lin, J. Y., Arthurs, J., Amodeo, L. R., and Reilly, S. (2012). Reduced palatability in drug-induced taste aversion: I. Variations in the initial value of the conditioned stimulus. *Behavioral Neuroscience, 126,* 423-432.

Lin, J.Y., Roman, C., Arthurs, J., and Reilly, S. (2012). Taste neophobia and c-fos expression in the rat brain. *Brain Research, 1448,* 82-88.

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Arthurs, J., and Reilly, S. (2013). Role of the gustatory thalamus in taste learning. *Behavioural Brain Research, 250,* 9-17*.*

Lin, J-Y., Arthurs, J., and Reilly, S. (2013). Reduced palatability in pain-induced conditioned taste aversions. *Physiology and Behavior, 119,* 79-85.

Lin, J.-Y., Arthurs, J., and Reilly, S. (2014). Conditioned taste aversion, drugs of abuse and
palatability. Neuroscience and Biobehavioral Reviews, 45, 28-45.

Lin, J.-Y., Arthurs, J., & Reilly, S. (2015). Gustatory insular cortex, aversive taste memory and taste neophobia. *Neurobiology of Learning and Memory, 119,* 77-84.

Manuscripts in Preparation

Arthurs, J., Lin, J.-Y., Ocampo, R., & Reilly, S. Reduced palatability in lactose-induced conditioned taste aversions.

Arthurs, J., Lin, J.-Y., Ocampo, R., & Reilly, S. Influence of chemogenetic inactivation of gustatory insular cortex on taste neophobia.

Arthurs, J., Lin, J.-Y., and Reilly, S. Effects of temporary inactivation of medial amygdala and the gustatory thalamus on expression of taste neophobia.

Lin, J.-Y., Arthurs, J., and Reilly, S Conditioned taste aversions: From poisons to pain to drugs of abuse.

Lin, J.-Y., Arthurs, J., and Reilly, S. Reduced palatability in anesthesia-induced conditioned taste aversions.

Lin, J.-Y., Arthurs, J., and Reilly, S. Effects of temporary inactivation of the basolateral amygdala and the gustatory insular cortex on taste neophobia.

PROFESSIONAL PRESENTATIONS

Lin, J.Y., Arthurs, J., and Reilly, S. (November, 2010). Morphine-induced conditioned taste avoidance: Effects of insular cortex lesions and taste preexposure. Society for Neuroscience, San Diego, California.

Arthurs, J., Lin, J.Y., and Reilly, S. (May, 2011). First impressions matter in morphine-induced conditioned taste avoidance learning, Midwestern Psychological Association, Chicago, Illinois.

Arthurs, J., Lin, J. Y., and Reilly, S. (November, 2011). The gustatory thalamus and taste avoidance learning. Society for Neuroscience, Washington D.C., Washington D.C.

Arthurs, J., Lin, J. Y., and Reilly. (March, 2012). The role of the gustatory thalamus in taste learning. Chicago chapter of the Society for Neuroscience, Chicago, Illinois.

Arthurs, J., Lin, J. Y., and Reilly, S. (May, 2012). The gustatory thalamus in taste neophobia. Midwestern Psychological Association, Chicago, Illinois.

Arthurs, J., Lin, J. Y., Amodeo, L. R., and Reilly, S. (May, 2012). Conditioned taste aversions induced by morphine and amphetamine. Midwestern Psychological Association, Chicago, Illinois.

Lin, J. Y., Arthurs, J., Horn-Amodeo, L., and Reilly, S. (May, 2012). Amphetamine-induced conditioned taste aversions reduce palatability. Midwestern Psychological Association, Chicago, Illinois.

Lin, J. Y., Arthurs, J., Horn-Amodeo, L., and Reilly, S. (May, 2012). Palatability increases with taste familiarity. Midwestern Psychological Association, Chicago, Illinois.

Arthurs, J., Lin, J.-Y., and Reilly, S. (October, 2012). The gustatory thalamus and drug-induced conditioned taste aversions. Society for Neuroscience, New Orleans, Louisiana.

Lin, J.-Y., Arthurs, J., and Reilly, S. (October, 2012). Pain and palatability: A microstructural analysis of licking pattern. Society for Neuroscience, New Orleans, Louisiana.

Arthurs, J., Lin, J.-Y., and Reilly, S. (November, 2013). Effects of inactivations of the amygdala on taste neophobia. Society for Neuroscience, San Diego, California.

Komar, S., and Arthurs, J. (April, 2014). Effects of lesions to nucleus accumbens shell on taste learning. Student Research Forum, Chicago, Illinois.

Arthurs, J., Reilly, S., and Lin, J.-Y. (November, 2014). Effects of temporal inactivation of gustatory insular cortex and gustatory thalamus on taste neophobia. Society for Neuroscience, Washington D.C., Washington D.C.

Arthurs, J., Lin, J.-Y., and Reilly, S. (October, 2015). Anesthesia-induced conditioned taste aversions. Society for Neuroscience, Chicago, Illinois.

Arthurs, J., Lin, J.-Y., Ocampo, R., and Reilly, S. (May, 2016). Gastrointestinal pain-induced taste suppression: Avoidance or aversion? Midwestern Psychological Association, Chicago, Illinois.

COLLOQUIOUM PRESENTATIONS

Arthurs, J., (March, 2011) The gustatory thalamus and taste avoidance learning. Behavioral Neuroscience Seminar. University of Illinois at Chicago, Chicago, IL.

Arthurs, J., (February, 2012) The gustatory thalamus and taste learning. Behavioral Neuroscience Seminar. University of Illinois at Chicago, Chicago, IL.

Arthurs, J., (November, 2012) Survey of recent work by Dr. Laura Huxley in preparation for her Laboratory of Integrative Neuroscience Seminar. University of Illinois at Chicago, Chicago, IL.

Arthurs, J. (April, 2012) Reward and aversion in conditioned taste aversion. University of Illinois at Chicago, Chicago, IL.

Arthurs, J., (September, 2013) Glucocorticoids interact with noradrenergic arousal system in the shell of the nucleus accumbens to enhance memory consolidation of both appetitive and aversive taste learning, a presentation of Wichmann, Fornari, and Roozendaal (2013). University of Illinois at Chicago, Chicago, IL.

Arthurs, J., (February, 2014) Brain stimulation reward and conditioned taste aversion learning. University of Illinois at Chicago, Chicago, IL.

Arthurs, J., (March, 2015) Role of the medial amygdala in taste learning. University of Illinois at Chicago, Chicago, IL.

RESEARCH IN PROGRESS

- Forebrain circuits involved in taste neophobia and conditioned taste aversion
- Chemogenetic manipulation of taste learning circuits
- Role of aversive and rewarding properties in drug-induced taste aversions
- Pharmacological manipulation of the parabrachial nucleus during taste learning
- Pharmacological and neuroanatomical underpinnings of drug-induced taste aversions

MENTORING OF UNDERGRADUATE RESEACH ASSISTANTS

Coordinating volunteer activities within the lab, instruction in laboratory skills (e.g., animal handling, behavioral testing, and histology,), and mentorship concerning post-graduate career options especially graduate school.

- Kim Johnson (Spring 2010)
- Liz Gutierez (Spring 2010)
- Christina Demitro (Fall 2010)

- Rene Bayley (Fall 2010-Spring 2011)
    - *Graduate student University of Louisville Clinical Psychology*
- Jo Emely Ramirez (Fall 2010- Fall 2011)
- Brittany Hunter (Fall 2010-Fall 2011)
    - *Medical student, Chicago School of Medicine*
- Marco Martinez (Summer 2011-Spring 2013)
    - *Medical student, University of Illinois at Chicago*
- Genia Wat (Spring 2011-Spring 2012)
- Gary Garhammer (Spring 2011-Fall 2011)
- Parth Shah (Fall 2011-Spring 2012)
- Simon Komar (Fall 2013-Fall 2015)
    - *2013-2014 Nancy Hirschberg Memorial Grant for Undergraduate Research*
    - *2014-2015 LAS Undergraduate Research Initiative Award*
- Roberto Ocampo (Spring 2015-Spring 2016)
- Emma Bono (Fall 2015-Present)
- Jessica Poskus (Fall 2015-Spring 2016)

TEACHING EXPERIENCE

Courses Taught

Spring 2014 Class: PSCH 343 Statistical Methods in Behavioral Science

Guest Lectures

Fall 2013 Class: PSCH 343 Statistical Methods in Psychology

Lab and Recitation Teaching Assistant

Fall 2010 Class: PSCH 361 Laboratory in Learning and Conditioning
Instructor: Dr. Steve Reilly
Spring 2011 Class: PSCH 361 Laboratory in Learning and Conditioning
Instructor: Dr. Steve Reilly
Spring 2012 Class: PSCH 361 Laboratory in Learning and Conditioning
Instructor: Dr. Steve Reilly
Spring 2013 Class: PSCH 361 Laboratory in Learning and Conditioning
Instructor: Dr. Steve Reilly
Fall 2013 Class: PSCH 343 Statistical Methods in Psychology
Instructor: Andrew Jarosz, ABD
Spring 2015 Class: PSCH 361 Laboratory in Learning and Conditioning
Instructor: Dr. Steve Reilly
Spring 2016 Class: PSCH 361 Laboratory in Learning and Conditioning
Instructor: Dr. Steve Reilly

Lecture Teaching Assistant

Fall 2011 Class: PSCH 360 Lectures in Learning and Conditioning
Instructor: Dr. Steve Reilly

Fall 2012	Class: PSCH 360	Lectures in Learning and Conditioning
	Instructor:	Dr. Steve Reilly
Fall 2013	Class: PSCH 343	Statistical Methods in Psychology
	Instructor:	Andrew Jarosz, ABD
	Class: PSCH 262	Behavioral Neuroscience
	Instructor:	Dr. Eric Gobel
Fall 2014	Class: PSCH 360	Lectures in Learning and Conditioning
	Instructor:	Dr. Steve Reilly
Fall 2015	Class: PSCH 360	Lectures in Learning and Conditioning
	Instructor:	Dr. Steve Reilly

MEMBERSHIP IN PROFESSIONAL ORGANIZATIONS

Midwestern Psychological Association
Society for Neuroscience

REFEREE EXPERIENCE (Ad hoc)

Behavioral Processes
Toxicology and Applied Pharmacology

REFERENCES:

Dr. Steve Reilly, Professor, Department of Psychology, University of Illinois at Chicago, sreilly@uic.edu office: 312-413-2625.

Dr. Michael Ragozzino, Professor, Department of Psychology, University of Illinois at Chicago, mrago@uic.edu Office: 312-773-1366.

Dr. Mitchell Roitman, Associate Professor, Department of Psychology, University of Illinois at Chicago, mroitman@uic.edu Office: 312-996-3113.