Role of the Gustatory Thalamus in Taste Learning

BY

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<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>AMY</td>
<td>amygdala</td>
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<tr>
<td>ANC</td>
<td>anticipatory negative contrast</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>BLA</td>
<td>basolateral amygdala</td>
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<td>BNST</td>
<td>bed nucleus of the stria terminalis</td>
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<td>C</td>
<td>Celsius</td>
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<td>CGS</td>
<td>central gustatory system</td>
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<td>CNA</td>
<td>central nucleus of the amygdala</td>
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<td>CS</td>
<td>conditioned stimulus</td>
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<td>CTA</td>
<td>conditioned taste aversion</td>
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<td>GT</td>
<td>gustatory thalamus</td>
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<td>GTX</td>
<td>gustatory thalamus lesion</td>
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<td>hr</td>
<td>hour</td>
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<tr>
<td>IC</td>
<td>gustatory insular cortex</td>
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<td>ICX</td>
<td>gustatory insular cortex lesion</td>
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<td>LH</td>
<td>lateral hypothalamus</td>
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<td>LiCl</td>
<td>lithium chloride</td>
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<td>IPBN</td>
<td>lateral parabrachial nucleus</td>
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<td>LSD</td>
<td>lysergic acid diethylamide</td>
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<tr>
<td>mA</td>
<td>milliampere</td>
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<tr>
<td>MeA</td>
<td>medial nucleus of amygdala</td>
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<td>min</td>
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ml milliliter
mPBN medial parabrachial nucleus
NMDA N-methyl-D-aspartate
NST nucleus of the solitary tract
PBN parabrachial nucleus
PENT anesthesia only control animals
SHAM sham surgical control animals
TR taste reactivity
TRT taste reactivity test
V volt
µA microampere
Lesions of the gustatory thalamus (GT) completely eliminate morphine-induced conditioned taste aversions (CTAs) but have no influence on illness-induced CTAs. This lesion-based dissociation has been used to support hypotheses characterizing drug-induced taste learning as taste avoidance rather than taste aversion. However, proper comparison of lesion effects across these studies is compromised by procedural differences. The illness-induced CTA literature tends to use longer taste access periods (e.g., 15-min) whereas all investigations of morphine-induced CTA in GT-lesioned (GTX) rats have used 5-min access periods.

In order to compare lesion effects the present research used a standard 15-min taste access period and examined GTX rats in morphine-induced CTA (Experiment 1) and lithium chloride-induced CTA (Experiment 2). To examine the generality of drug effects a second set of GTX rats were tested in an amphetamine-induced CTA (Experiment 3). These rats were also tested for the occurrence and habituation of taste neophobia (Experiment 4).

The results clearly demonstrated that GT lesions do not eliminate drug-induced CTA with either morphine or amphetamine. Rather, it appeared that GTX rats acquired drug-induced CTA normally but from an elevated Trial 1 starting point. GT lesions had no influence on the acquisition of an illness-induced CTA despite elevating Trial 1 intake. Lesion-induced overconsumption of a novel taste solution was consistently found across all four experiments, a deficit that has not previously been attributed to GT lesions. The present results show that the GT has little if any role in CTA acquisition and revealed an involvement in taste neophobia that has not previously been described.
I. INTRODUCTION

Taste learning is essential for the survival of any organism that voluntarily selects its diet so that nourishing foods are consumed and toxic foods are avoided. Conditioned taste aversion (CTA) is a Pavlovian associative learning phenomenon that develops when a food becomes a conditioned stimulus (CS) associated with subsequent illness (unconditioned stimulus; US). The food is not only recognized to be poisonous resulting in a reluctance to consume that food in future encounters, but a reduction in the hedonic value (palatability) of that food also occurs (for reviews see Reilly & Schactman, 2009). CTA is a robust learning phenomenon capable of producing a conditioned response in a single trial that can guide diet selection for a lifetime (Freeman & Riley, 2009). CTA provides a valuable model in which to study learning, memory, and motivation within a laboratory setting. Determining the brain areas underlying CTA learning and the role played by each structure will allow detailed mechanistic analysis of learning, memory and motivation relevant to many areas of research beyond CTA. The current thesis concerns the function of the gustatory thalamus (GT), a central gustatory system (CGS) nucleus, whose role has been debated in the taste learning literature. Toward this end we must consider the GT within the anatomical framework of the CGS as a whole (Section A). We will then review the literature on CTA and related taste learning phenomena (Section B), which will allow a discussion of the effects of lesions of the GT (Section C) and related structures on taste learning. Finally, experiments will be proposed (Section D) to address gaps in the aforementioned literature, and to aid in determining the role of the GT in taste learning.
A. Gustatory System Anatomy and Motor Systems

Central gustatory system anatomy

To understand the role of the GT in taste learning it must be considered within the broader framework of the CGS as a whole (see Figure 1). In rodents, gustatory information from the periphery is carried to the central nervous system by three cranial nerves, facial (VII), glossopharyngeal (IX), and vagus (X), which all synapse in the rostral portion of the nucleus of the solitary tract (NST) located in the dorsomedial medulla (Barraco, El-Ridi, Ergene, Parizon, & Bradley, 1992; Halsell, Travers, & Travers, 1993; Hamilton & Norgren, 1984; Whitehead & Frank, 1983). Taste neurons from the NST then project to the medial portion of the pontine parabrachial nucleus (mPBN; Herbert, Moga, & Saper, 1990; Norgren & Leonard, 1971, 1973). Axons carrying ascending taste information from the mPBN bifurcate forming the dorsal pathway and the ventral pathway. The dorsal pathway axons synapse next in the medial portion of the ventroposterioromedial parvicellular (VPPC) area of the thalamus (Cechetto & Saper, 1987; Emmers, 1977; Halsell, 1992; Norgren, 1974; Ogawa, Hayama, & Ito, 1984), which in turn forms reciprocal connections to the gustatory portion of the insular cortex (IC; Cechetto & Saper, 1987; Kosar, Grill, & Norgren, 1986; Norgren & Wolf, 1975; Wolf, 1968). Axons of the ventral taste pathway form largely reciprocal ipsilateral connections between the mPBN and a number of structures including the amygdaloid complex (central, CNA and basolateral nucleus, BLA), bed nucleus of the stria terminalis (BNST), and lateral hypothalamus (LH; Bernard, Alden, & Besson, 1993; Fulwiler & Saper, 1984; Halsell, 1992; Krukoff, Harris, & Jhamandas, 1993; Lasiter, Glanzman, & Mensah, 1982;

The GT, the thalamic relay for taste information, occupies the medial subregion of the VPPC. The VPPC appears in coronal section as a narrow medial projection of the VPM and is wedged between the parafascicular nucleus and the medial lemniscus. Within the VPPC there is a mediolateral organization of sensory neurons showing activity in response to taste (i.e., GT), thermal, and tactile stimuli. Taste-responsive neurons are concentrated in the most medial aspect of the VPPC rapidly decreasing in frequency laterally as thermally-responsive neurons predominate (Emmers, 1977; 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. However, there are no clear anatomical demarcations of these shifts in neuronal responsivity. Thus, in the current study we define the GT as the medial half of the VPPC as defined in Paxinos and Watson (2007). For a detailed exposition of rodent CGS anatomy as well as the distribution of modality specific neurons with the VPPC see Lundy and Norgren (2004), and see Pritchard and Norgren (2004) for details concerning the CGS anatomy of human and non-human primates.

The GT is bordered dorsally by the centromedian, paracentral, and parafascicular thalamic nuclei, and ventrally by the subparafascicular nucleus, superior cerebellar peduncle, and medial lemniscus. The GT is a small nucleus measuring less than 1.0 mm in the mediolateral and anterioposterior planes, as well as, 0.5 mm in the dorsoventral plane (Paxinos & Watson, 2007). This small size has several implications for any lesion study. (1) A well-placed lesion is very likely to destroy the entire GT, but
Figure 1. Schematic diagram of the ascending central gustatory system in the rat: AMY = amygdala, BNST = bed nucleus of the stria terminalis, GT = gustatory thalamus, IC = gustatory insular cortex, IX = glossopharyngeal nerve, LH = lateral hypothalamus, mPBN = medial parabrachial nucleus, NST = nucleus of the solitary tract, VII = facial nerve, IX = glossopharyngeal nerve X = vagus nerve. Solid arrows represent ascending projections, dashed arrows represent reciprocal connections.
(2) damage is likely to encroach, at least to some extent, on surrounding structures. Also, 
(3) any deviation in placement accuracy is likely to result in a missed lesion that spares 
the GT while damaging surrounding structures.

**Ingestive motor systems**

The current investigation examines taste learning expressed during the consummatory phase of ingestive behavior. Consummatory behavior is generated through a network of orosensory and oromotor systems thought to be coordinated within the medullary reticular formation (RF). This RF system tends to generate stereotypical rhythmic behaviors (e.g., licking, mastication, swallowing) ranging from adaptively flexible (e.g., licking) to rigidly reflexive (e.g., swallowing; Travers, Dinardo, & Karimnamazi, 1997). The RF receives descending projections from many areas related to orosensory and oromotor functions including: frontal cortex, IC (gustatory and visceral), the basal ganglia, AMY, BNST, LH, and PBN as well as other brainstem structures such as the NST and spinal trigeminal nucleus (Travers et al., 1997). Motor outputs from the medullary RF project to the hypoglossal nucleus, facial nucleus, nucleus ambiguus, and the motor trigeminal nucleus, which drive orofacial musculature (Travers et al., 1997; Travers, 2004).

B. Taste Learning

*Illness-Induced CTA Learning*

CTA was first studied as an associative learning phenomenon in the laboratory by Garcia and co-workers (Garcia & Buchwald, 1963; Garcia & Kimeldorf, 1960; Garcia,
Kimeldorf, & Koelling, 1955; Hunt, Carrol, & Kimeldorf, 1965). Initially discovered in response to nausea-inducing radiation, CTAs can also be produced by other illness-inducing USs, such as vestibular perturbation (Braun & McIntosh, 1973; Cordick, Parker, & Ossenkopp, 1999; Green & Rachlin, 1973) and toxins such as lithium chloride (LiCl; Breslin, Spector, & Grill, 1992; Ossenkopp & Eckel, 1995). CTA exhibits some unique features in that a conditioned response can be established with a single trial, even with a long delay between CS and US presentations (Garcia & Ervin, 1968; Garcia, Ervin, & Koelling, 1966; McLaurin & Scarborough, 1963). These features underscore CTA learning’s role as a powerful and broadly tuned protective mechanism essential for survival. CTA has become an extensively studied phenomenon used in the laboratory to study learning, memory and motivation (for reviews see Barker, Best, & Domjan, 1977; Braveman & Bronstein, 1985; Milgram, Krames, & Alloway, 1977; Reilly & Schachtman, 2009). There are at least 5 processes involved in the formation and expression of a CTA: (1) CS detection and processing, (2) US detection and processing, (3) formation of an association between CS and US, (4) CS memory retrieval, and (5) behavioral expression. The complexity underlying this seemingly simple behavior allows the study of a variety of processes within a single model. The development of a CTA is traditionally assessed by a reduction in the volume of CS consumed subsequent to the first CS-US pairing. Such a dependent measure allows for easy assessment of CTA expression, and has served to advance CTA research for many years. However, more fine-grained analyses are useful in constructing a more complete view of ingestive behavior.

The taste reactivity test (TRT), developed by Grill and Norgen (1978a), provides one such analysis, allowing the assessment of the hedonic valence or palatability of taste
stimuli (see also Steiner, Glaser, Hawilo, & Berridge, 2001). Palatability is generally thought of as the pleasure evoking quality of foods (see Berridge, 2000; Grill & Berridge, 1985). The TRT is based on a set of stereotypical orofacial reactions to taste stimuli found to be highly conserved across mammalian species. The orofacial response of the animal to the taste stimulus is videotaped and later analyzed for the occurrence of reactions indicative of either appetitive stimulus value (e.g., mouth movements, forward tongue protrusions, lateral tongue protrusions, paw licking) or aversive stimulus value (e.g., gapes, chin rubbing, head shakes, paw flailing; for a review of TRT methodology see Berridge, 2000). These taste reactivity (TR) responses have been validated as measures of taste palatability through their relation to stimuli that humans evaluate as palatable or unpalatable. For example, as the concentration of innately preferred stimuli such as sucrose increases so too do the frequency of appetitive TR responses. Conversely, as the concentration of innately non-preferred quinine stimulus increases the occurrence of aversive TR responses increase (Berridge, 2000; Grill & Norgren, 1978a). Physiological need states, which alter human evaluations of taste palatability, also modulate TR responses. For instance, a hypertonic salt stimulus elicits aversive TR responses in salt replete rats but evokes appetitive TR responses in salt depleted rats (Berridge, Flynn, Schulkin, & Grill, 1984; Berridge & Schulkin, 1989). In the TRT, a taste solution is typically introduced directly into the oral cavity via an indwelling intraoral catheter, a procedure that affords precise control of stimulus administration by the experimenter. Brief, small volume infusions of taste stimuli limit, if not prevent, post-ingestive feedback providing a snapshot of palatability at any given point in time (see Berridge, 2000). A less common approach is to infuse a stimulus at a constant rate over
a given time interval (e.g., 1 ml/min for 5 min) while monitoring TR responses (e.g., Cubero, Thiele, & Bernstein, 1999; Lopez et al., 2010; Spray, Halsell, & Bernstein, 2000).

The TRT has been used to assess learned changes in palatability in response to pairing a taste CS with a LiCl US (Breslin et al., 1992; Ossenkopp & Eckel, 1995). These experiments show that LiCl reduces the palatability of the associated taste CS as indicated by a reduction in the occurrence of appetitive TR responses and an increase in the frequency of aversive TR responses (Berridge et al., 1981; Grill 1985; Grill & Norgren 1978b). In an illuminating series of experiments highlighting the “snapshot” nature of the TRT, Spector, Breslin, and Grill (1988) used the TRT to track the development of CTA immediately following an injection of LiCl. In their first experiment animals were injected with LiCl and then immediately placed into the TR chamber and intraorally infused with a 0.1 M sucrose solution (0.55 ml over 30 sec). The sucrose infusion was repeated once every 5 min for 30 min after the LiCl injection. Over the course of these infusions there was a significant decrease in appetitive TR responses and a concomitant increase in aversive TR responses to each sucrose infusion. Two additional experiments, employing similar designs, were conducted in this report analyzing the effects of increased delays between LiCl injection and the first sucrose infusion. Thus, the TRT was able to map the downshift in sucrose palatability at discrete time points during the development of a CTA.

An alternative methodology for assessing taste palatability, the analysis of the temporal microstructure of lick patterns (Davis, 1989, 1996, 1998; Davis & Smith, 1992; Hsiao & Fan, 1993; Spector, Klumpp, & Kaplan, 1998; Spector & St. John, 1998) provides a technique for fine-grained analysis of ingestive behavior during voluntary consumption of taste stimuli. With this approach experiments are conducted in a drinking
chamber with a conductive floor (e.g., steel bars), and a metal drinking spout. Each time a rat licks the spout it completes a very weak electrical circuit registered and time stamped by a computer. A number of dependent measures can be derived from the temporal lick data collected during a drinking session including: total number of licks, lick rate, lick cluster size, and lick efficiency. Total number of licks and lick rate are relatively self-explanatory, however, other lick analysis measures are less intuitive. Lick cluster size is typically defined as a run of licks with an interlick interval of less than a certain time interval (0.5 seconds has become standard in the literature; Arthurs, Lin, Amodeo, & Reilly, 2012; Davis, 1989, 1995, 1996, 1998; Davis & Smith, 1992; Dwyer, Boakes, & Hayward, 2008; Hsiao & Fan, 1993; Lin, Amodeo, Arthurs, & Reilly, 2012; Lin, Arthurs, Amodeo, & Reilly, 2012). Cluster size is calculated by dividing the number of licks that occurred in clusters by the total number of clusters. Various measures designed to describe lick efficiency have been employed in the literature, for example consumption per 1000 licks (Dwyer et al., 2008) and lick efficiency ratio of total intake divided by total number of licks (ml/licks; Xenakis & Sclafani, 1981). In our laboratory we calculate lick efficiency by dividing the total number of licks by the total amount consumed (licks/gram; Arthurs et al., 2012; Lin, Amodeo, Arthurs, & Reilly, 2012; Lin, Arthurs, Amodeo, & Reilly, 2012).

In experiments similar to those used to validate the TRT as a measure of palatability it has been demonstrated that both initial lick rate and lick cluster size monotonically increase with increasing concentrations of an innately preferred sucrose solution, despite an inverted U-shaped intake curve (Davis, 1989, 1995, 1996, 1998; Davis & Smith, 1992; Hsiao & Fan, 1993). Initial lick rate and cluster size also decrease
in a concentration-dependent manner in response to innately non-preferred tastes such as quinine (Davis & Perez, 1993; Davis & Smith, 1992; Hsiao & Fan, 1993). In addition, the palatability of salt solutions assessed by lick analysis is modulated by need state (Breslin, Kaplan, Spector, Zambito, & Grill, 1993). The initial rate of licking provides an assessment of taste palatability before post-ingestive consequences can feedback, while cluster size provides a more global assessment of palatability over a given stimulus presentation. Lick analysis has been used to access learned changes in palatability during voluntary consumption, and has shown that illness-induced CTAs significantly reduce cluster size of the associated taste CS (Arthurs et al., 2012; Baird, St. John, & Nguyen, 2005; Dwyer, 2009; Dwyer, Boakes, & Hayward, 2008; Kent, Cross-Mellor, Kavaliros, & Ossenkopp, 2002). This converging evidence obtained with complimentary techniques allows for strong conclusions about the downshift in palatability in response to illness-inducing USs in the CTA procedure.

**Drugs of Abuse and Taste Learning**

While LiCl has become the most commonly used illness-inducing agent employed to study CTA in the laboratory, many compounds are capable of producing a CTA (e.g., Gamzu, 1977; Gamzu, Vincent, & Boff, 1985). Indeed, the occurrence of a CTA to a given US has been used to screen and index the aversive properties of a variety of USs (Riley & Tuck, 1985). Interestingly, drugs of abuse (i.e., drugs that are readily self-administered by human and non-human animals) were discovered to cause a reduction in the volume of CS consumed just like LiCl and other toxic USs (Cappell & LeBlanc, 1971; Cappell, LeBlanc, & Endrenyi, 1973; Carey, 1973; Kumar, Pratt, & Stolerman,
1983; Nachman, Lester, & Le Magnen, 1970; Riley, Jacobs, & LoLordo, 1978). However, all these experiments used volume consumed as the dependent measure. As discussed previously, although volume consumed has proven to be a very useful dependent measure, more fine-grained approaches (i.e., TRT and lick analysis) provide a more complete evaluation of ingestive behavior.

Parker and colleagues have extensively examined drug-induced CTA with the TRT and demonstrated that CS palatability is not affected by pairings with a wide variety of drug USs including: amphetamine (Parker, 1982, 1988, 1991; Parker & Carvell, 1986; Zalaquett & Parker, 1989), cocaine (Mayer & Parker, 1993; Parker, 1993), LSD (Parker, 1996), methamphetamine (Parker, 1993), methylphenidate (Parker, 1995), morphine (Parker, 1988, 1991), low doses of nicotine (Parker & Carvel, 1986), and phencyclidine (Parker, 1993). Certain drugs have been shown to possess a bivalent effect (see Parker, Limebeer, & Rana, 2009) in that when familiar (e.g., ethanol) or at low doses (e.g., nicotine, amphetamine) they do not increase the frequency of aversive TR responses to the associated taste CS, but can cause an increase in aversive TR responses when novel (ethanol) or at higher doses (nicotine, amphetamine). This literature is a diverse, yet consistent body of work indicating that drugs of abuse do not increase the number of aversive TR responses to an associated taste CS.

This research on the palatability of drug-associated taste CSs is the basis for the widely accepted view that drugs of abuse condition an avoidance response fundamentally different from illness-induced CTAs (Parker, 1995, 2003; Parker et al., 2009). By this analysis, LiCl and other illness-inducing agents are considered to produce CTAs that lead to the avoidance of, and a concomitant reduction in the palatability of, the
associated taste CS (i.e., taste aversion). On the other hand, drugs of abuse are considered to cause avoidance of the taste CS while having no influence on CS palatability (i.e., taste avoidance). In effect, the illness-associated CS is aversive because it has become an unpleasant (even disgusting) taste, whereas the drug-associated CS is avoided because it signals (but does not acquire) some (presumably aversive) property of the drug. In fact, a single drug administration can be used to concurrently condition a taste aversion\avoidance and a place preference (Reicher & Holman, 1977; Verendeev & Riley, 2011; Wang, Huang, & Hsiao, 2010). Finding that abused drugs (in dose ranges that support self-administration and condition a place preference) also induce a CTA has been regarded as paradoxical for many years and by a number of authors (e.g., Cappell & LeBlanc, 1973; Grigson, 1997; Hunt & Amit, 1987; Parker, 1995; 2003; Vogel & Nathan, 1975). This apparent paradox provided fertile soil for the hypothesis that drugs of abuse were conditioning taste avoidance rather than taste aversion.

The differentiation of illness-induced CTA from drug-induced taste avoidance also seemed to be supported by the differential effects of brain lesions on these two forms of taste learning. This work has centered on the role of the GT, and its connections with the IC. That is, GT lesions appear to have no effect on illness-induced CTA (Flynn, 1991, Reilly & Pritchard, 1996b; Scalera, Grigson, & Norgren, 1997), but completely eliminate drug-induced taste avoidance (Grigson, Lyuboslavsky, & Tanase, 2000; Reilly & Trifunovic, 1999). Similarly, IC lesions appeared to have no effect on LiCl-induced CTA (Geddes, Han, Baldwin, Norgren, & Grigson, 2008; Mackey, Keller, & van der Kooy, 1986), but eliminate drug-induced taste avoidance (Geddes et al., 2008; Roman & Reilly, 2009). This pattern of impaired and spared behaviors seemed to indicate that the GT and
IC were serving a common purpose that was superfluous for illness-induced CTA, but essential for drug-induced taste avoidance, and was taken to support the idea that these are two separate phenomena dependent on separate brain regions (Grigson, Twining, Freet, Wheeler, & Geddes, 2009). However recent developments challenge this characterization of drug-induced taste avoidance and illness-induced CTA that has been based on different effects on palatability and dissociation by brain lesions.

Recent research from our laboratory indicates that drugs of abuse and illness-inducing agents are both producing CTAs complete with reductions in the palatability of associated taste stimuli (Arthurs et al., 2012; Lin, Arthurs et al., 2012). In these studies we used lick analysis to examine changes in the palatability of various CSs (e.g., saccharin, sodium chloride, quinine, aqueous orange odor) in response to pairings with drugs of abuse (morphine or amphetamine) or LiCl. We found that drugs of abuse and LiCl each decrease the cluster size of the associated taste CSs. These results indicate that drugs of abuse do, indeed, reduce the palatability of the associated taste CS, an effect that was not detected by research employing the TRT.

In the first set of studies, Lin, Arthurs et al. (2012) examined the effects of an amphetamine US (1 mg/kg) on a non-preferred taste CS (0.00003 M quinine), a preferred taste CS (0.1 M NaCl), and an aqueous odor CS (0.02% aqueous orange odor). Cluster size for each stimulus was significantly decreased following pairings with the amphetamine US. Thus, lick analysis was capable of detecting a downshift in palatability at a dose of 1 mg/kg that did not produce a significant shift in palatability when assessed with the TRT (Parker, 1991).

In the second set of studies, Arthurs et al. (2012) examined shifts in the
palatability of a 0.15% saccharin CS in response to various USs: LiCl (1.33 mg/kg), morphine (15 mg/kg), amphetamine (1 mg/kg), and sucrose (1.5 M). LiCl, morphine, and amphetamine served as USs in drug-induced CTA experiments, and sucrose was paired with saccharin in an anticipatory negative contrast procedure (see Flaherty, 1996) known to suppress saccharin intake, but not to condition an aversion. LiCl, morphine, and amphetamine all conditioned a CTA demonstrated as a reduction in saccharin intake and cluster size. Sucrose did suppress the intake of saccharin, but did not cause a reduction in saccharin cluster size. Importantly, a morphine dose of 15 mg/kg was found to reduce saccharin palatability, an effect not seen with the TRT even when doses as high as 80 mg/kg are used (Parker, 1991; Parker et al., 2009).

Since these results obtained with lick analysis contradict an extensive literature based on the TRT it may be useful to examine some methodological issues in the drug-induced taste avoidance literature. Many of the previously mentioned studies (e.g., Parker, 1988, 1991, 1993, 1995, 1996; Parker & Carvell, 1986; Zalequett & Parker, 1989) employed a highly palatable sucrose taste stimulus at a concentration of 0.5 M or higher. The use of such a high concentration of sucrose as a CS can not only delay learning (e.g., Grigson & Gomez, 1999), but requires a larger shift in palatability to occur for aversive TR response to be observed. A related issue is that the analysis of TR responses in these studies (notably Parker, 1995) as well as recent reviews (see Parker et al., 2009) seems to focus on aversive TR responses to the virtual exclusion of any analysis of appetitive TR responses. The reason for this focus is not entirely clear.

In addition to this evidence showing that drugs of abuse and toxins such as LiCl both decrease taste palatability, there is also recent evidence casting doubt on the
dissociation of drug-induced and illness-induced CTA based on differential effects of brain lesions. Prior evidence showing that IC lesions have no effect on CTA (Geddes et al., 2008; Mackey, Keller, & van der Kooy, 1986) has proven to be only partially correct. Using a 15-min CS presentation in experimentally naïve rats with a novel taste CS, Roman, Nebieridze, Sastre, and Reilly (2006) reported extensive Trial 1 overconsumption of a novel taste by IC-lesioned (ICX) rats. Roman and Reilly (2007) and Roman, Lin, and Reilly (2009) showed that IC lesions attenuated CTA learning as a consequence of a primary deficit in taste neophobia. That is, ICX animals misperceived the novel food as familiar and overconsumed that stimulus compared to control animals. It is well established that CTA acquisition occurs more rapidly to novel than to safe and familiar tastes, an effect called latent inhibition (Lubow, 1989; 2009). IC lesions appear to disrupt taste neophobia an effect that, in turn, results in a latent inhibition-like delay in subsequent learning.

In a 15-min procedure, ICX rats acquired a morphine-induced CTA subject to a neophobia deficit and latent inhibition-like delay of learning also seen in ICX animals during LiCl-induced CTA (Lin et al., 2011). This acquisition of drug-induced CTA had been obscured by a ceiling effect due to the use of a 5-min CS presentation in the earlier studies (Geddes et al., 2008; Roman & Reilly, 2009). Thus, IC lesions appear to disrupt taste neophobia and cause a latent inhibition-like delay in learning of both illness-induced and drug-induced CTA. As such it might be argued that the IC has no role in either drug-induced or illness-induced CTA.
C. GT Lesions and Taste Learning

As mentioned above, examination of the literature consistently demonstrates that the GT has little or no involvement in the formation of illness-induced CTAs to a novel (Flynn, Grill, Schulkin, & Norgren, 1991; Mungardee, et al., 2006; Reilly & Pritchard, 1996b; Scalera, Grigson & Norgren, 1997) or familiar taste CS (Reilly, Boronvalova, Dengler, & Trifunovic, 2003). However, a similar view that IC lesions did not affect illness-induced CTA has been recently overturned (Roman & Reilly, 2007; Roman, Lin, & Reilly, 2009). Of the prior investigations of GT lesion effects on illness-induced CTA there is a single report (Reilly et al., 2003) suggesting an overconsumption of the taste CS on Trial 1. The neuroanatomical connections for a neophobia deficit in GT-lesioned (GTX) animals exist as the GT projects strongly to the IC. Cutting off this input to the IC could affect the ability of the IC to function normally in taste neophobia even if the GT is acting as a simple relay with no role in neophobia per se. On the other hand, the Reilly et al. experiment found no indication of a delay in CTA acquisition expected if the initial overconsumption was due to an IC related deficit in taste neophobia. Overall, the cumulative literature suggests that the GT is not involved in any of the 5 stages of CTA formation and expression (outlined in Section B) for illness-induced CTA.

As previously stated it has been demonstrated that GT lesions completely eliminate drug-induced taste avoidance (Grigson et al., 2000; Reilly & Trifunovic, 1999). It should be noted, however, that these reports each used 5-min CS presentations. Such short duration CS presentations (as discussed above) do not allow confident evaluation of lesion effects. Thus we reason that the 5-min ceiling effect may be obscuring the full extent and nature of GT lesion-induced deficits in drug-induced CTA learning, just as it
was found to do with the IC lesion studies previously described. Despite the fact that recent evidence from our laboratory indicates that drugs of abuse condition a CTA fundamentally similar to illness-induced CTA it does not necessarily follow that the GT is playing an identical role in each form of taste aversion.

So, the GT could be involved in a circuit with the IC responsible for taste neophobia, and the role of the GT has been obscured in prior investigations. Or, the GT could be playing different roles in the processing of drug of abuse and illness-inducing USs. Of the five steps of CTA formation and expression (Section 2) there are only 2 that are likely to be different in drug-induced versus illness-induced CTA: US detection and processing (Step 2) and formation of an association between CS and US (Step 3). We hope that by assessing GT lesion effects in a 15-min CS procedure with different types of drugs of abuse (morphine and amphetamine) the nature of the lesion-induced deficit will be more clearly identified, thereby furthering our understanding of the role of the GT in taste learning.

D. Current Investigation

Two primary experiments (conducted in different sets of experimentally naïve animals) examined the effects of GT lesions on morphine-induced CTA (Experiment 1) and amphetamine-induced CTA (Experiment 3). Although some investigators conduct multiple experiments with a single group of animals experience indicates this is not always a good strategy. This is particularly true for experiments involving taste stimuli and distinctive contexts, which are prone to stimulus generalization and carry-over effects. However, we feel confident that secondary follow-up experiments can be of value
if they are (i) carefully designed to minimize carry-over effects and (ii) interpreted with caution. As such, we conducted follow-up experiments to clarify the role of the GT in illness-induced CTA and taste neophobia. The follow-up experiment in the first set of animals examined illness-induced CTA learning (Experiment 2); the follow-up experiment in the second set of animals (Experiment 4) examined of the role of the GT in taste neophobia.

The goal of Experiment 1 was to resolve the effect of GT lesions on morphine-induced CTA using a 15-min CS access procedure identical to that used by Lin et al. (2011). The use of a previously validated procedure and US provided an important connection to the existing literature. All previous investigations of GT function in terms of drugs of abuse have not only used a 5-min CS presentation, but have exclusively employed morphine as the US. It is possible that the observed GT lesion effects could be specific to morphine. Thus, in a second group of experimentally naïve animals we assessed GT lesion effects on amphetamine-induced CTA (Experiment 3) to address the question of the generality of drug effects. Amphetamine was selected as the second drug US because its pharmacologic effects are essentially diametrically opposed to those of morphine. For example, morphine (an opiate drug) increases appetite whereas amphetamine (a psychostimulant) has anorectic effects. All drugs of abuse including morphine and amphetamine increase dopamine release in the mesolimbic pathway (Di Chiara & Imperato, 1988; Koob, 1992). Morphine and amphetamine produce this common effect in very different ways — indirect disinhibition of dopamine release via opioid receptors by morphine and direct action on dopamine pre-synaptic terminals for amphetamine, respectively (Johnson & North, 1992; Jones, Gainetdinov, Wightman, &
Caron, 1998). If the GT has a different role in drug-induced CTA versus illness-induced CTA it seems most likely that this reflects differences in US processing or CS-US association formation (see section B). The uniformity or differences of GT lesion effects across morphine and amphetamine will provide an important interpretational tool (see Badiani, Belin, Epstein, Calu, & Shaham, 2011).

The follow-up experiments examined the role of the GT in the acquisition and expression of illness-induced CTA (Experiment 2) and taste neophobia (Experiment 4). Specifically, do GT lesions produce any of the effects of IC lesions, namely, a neophobia deficit and a subsequent latent inhibition-like delay in learning? The existing literature seems fairly conclusive on the point that the GT is not involved in illness-induced CTA learning or taste neophobia in general. However, until very recently (as previously mentioned) it was thought that the IC was also not involved in illness-induced CTA learning. This revised role of the IC was revealed by carefully designed experiments accounting for methodological shortcomings in prior investigation and prompts a similar reevaluation of the role of the GT in illness-induced CTA and taste neophobia. GT lesion effects on the response to a novel taste are no without precedent (e.g., Reilly et al., 2003), and perhaps the true magnitude and effects have been obscured in previous experimental designs, as we now know to be the case for IC lesions.

Experiment 2A employed a standard illness-induced CTA design with 15-min free access to the CS followed by an IP injection of the LiCl US. Experiment 2B was identical to Experiment 2A, except that access was limited to 5 ml on Trial 1. This experimental design allows us to determine if GT lesions influence either (1) intake on the first exposure to a taste stimulus and/or (2) the rate of CTA acquisition. The Trial 1
performance in Experiment 2A addresses this first point, while performance in Experiment 2B addresses the second point in a design that is not confounded by intake differences on Trial 1.

Although the literature is consistent in showing GT lesions do not affect illness-induced CTA learning, Reilly et al. (2003) did report a GT lesion-induced overconsumption on the initial taste exposure that did not affect acquisition of an illness-induced CTA. It would be useful to determine if this overconsumption effect was a genuine consequence of the lesion, and, if so, the nature of the deficit. Each of the previously proposed experiments provides some information about GT lesion effects on Trial 1 intake and taste neophobia. Experiment 4 focuses exclusively on the occurrence of, and recovery from, taste neophobia. This allows a more complete evaluation of the presence and magnitude of GT lesion effects on taste neophobia, prior experience in Experiment 3 notwithstanding. We also conducted this experiment in drinking chambers to allow lick analysis, which has proven to be a very sensitive technique capable of detecting differences missed by less fine-grained (i.e., volume consumed) assessments of ingestive behavior (Lin, Amodeo et al., 2012).
II. METHODS

A. Experiment 1

Subjects

Forty-one experimentally naïve, male Sprague-Dawley rats obtained from Charles River Laboratories (Wilmington, MA) served as subjects. They were individually housed in hanging stainless steel cages (Acme Metal Product, Chicago, IL) in a vivarium maintained at 21°C on a 12 hr light-dark cycle (lights on at 7:00 am). Animals were allowed to habituate to the facility for 3-5 days before surgery. All experimental treatments and procedures were conducted during the light phase of the cycle. Food and water were available at all times in the home cage except during behavioral testing as noted below. Animals (weighting 300g at the time of surgery) were treated in accordance with guidelines from the American Psychological Association (1996) and the National Institutes of Health (1996). The University of Illinois at Chicago Institutional Animal Care and Users Committee approval was obtained for all treatments.

Surgery

A total of 20 rats received bilateral GT lesions using the procedures of Sastre and Reilly (2006). These animals (Group GTX) were anesthetized with intraperitoneal (IP) injections of sodium pentobarbital (55 mg/kg) and secured in a Kopf Model 1900 stereotaxic instrument equipped with a digital readout (Kopf, La Jolla, CA) using non-traumatic earbars. Cranial sutures were exposed by a midline incision; a single trephine hole (5 mm diameter) was drilled on the skull centered over the transverse sinus at the level of the GT. Excitotoxic lesions were created with 0.15 M N-methyl-D-aspartate
(NMDA; St Louis, MO) backfilled into a glass micropipette (tip diameter ~70 µm), and infused iontophoretically into the GT with a Midgard precision current source (Stoelting, Wood Dale, IL). There was a single 6 min -10 µA current infusion per hemisphere at -3.70 mm posterior to bregma, ±0.80 mm medial/lateral to the midline, -6.30 mm ventral to dura. Body temperature was monitored throughout the surgical procedure via a rectal thermometer, and maintained at 37°C with a heating pad (Harvard Apparatus, Holliston, MA). Twenty one rats served as control subjects (Group SHAM): 10 rats received the same surgical procedures as GTX rats except no NMDA was infused and 11 rats received only pentobarbital anesthesia. All animals were monitored during the recovery from anesthesia and then returned to their home cages.

Apparatus

All testing was conducted in the home cage with fluids presented in plastic graduated cylinders fitted with silicone stoppers and stainless steel sipper tubes secured to the front of the home cage by stainless steel springs. Volumes were measured to the nearest 0.5 ml.

Procedure

Using the behavioral procedure of Lin et al. (2011), the rats were acclimated to a deprivation schedule permitting 15 min access to water each day. The experiment began when water intake stabilizes (~12 days) at which time the rats in each lesion group (SHAM; GTX) were divided into subgroups according to the drug (saline; morphine) to be administered as the US on conditioning days. Each conditioning trial consisted of 15 min
access to 0.15% saccharin followed, 5 min later, by an IP injection of either physiological saline (1ml/kg body weight) or morphine sulfate (15 mg/ml/kg). A saccharin trial occurred every third day and the rats were otherwise maintained on the water deprivation schedule as described above. Volume consumed served as the dependent measure.

**Histology**

Once the experimental procedures were completed, GTX rats were injected with sodium pentobarbital (~100 mg/kg; IP) and then transcardially perfused with physiological saline followed by 4% formalin. Brains were extracted and stored in 4% formalin for at least two days and then switched to 20% sucrose for an additional two days. Thereafter, the brains were frozen, sliced at 50 µm on a cryostat and stained with cresyl violet. Using a light microscope (Zeiss Axioskop 40), photomicrographs were taken with a Q-Imaging camera running Q-Capture software (Quantitative Imaging Corporation, Burnaby, B.C., Canada). The GT and surrounding regions were identified and labeled based on the Paxinos and Watson (2005) atlas. Animals were dropped from the study if they sustained non-bilateral GT lesions.

**Data Analysis**

Behavioral data was analyzed with repeated-measures ANOVA with Group as the between-subjects variable and Trial as the within-subjects variable. Significant main effects and interactions were followed-up by appropriate post hoc analyses, either planned comparisons (simple main effects) with the adjusted error term from the overall
ANOVA or Tukey HSD tests. All analyses were conducted using Statistica 6.0 software (StatSoft, Inc., Tulsa, OK) with alpha level set at \( p < .05 \).

B. Experiment 2

Subjects, surgery, and apparatus

The same subjects and apparatus from Experiment 1 were used in Experiment 2. They were housed, maintained and tested in the same manner as during Experiment 1 except for procedural differences discussed below.

Procedure

Following completion of Experiment 1, the rats were given 7 days free access to food and water, and were then divided into two groups of 20 (Experiment 2A) and 21 (Experiment 2B) animals, respectively, counterbalanced according to lesion and prior drug condition. Water intake was re-stabilized. Conditioning trials were conducted every third day with two water days intervening. Conditioning trials consisted of presentations of 0.0001 M quinine for 15-min (Experiment 2A) or 15-min to a maximum of 5 ml intake (Experiment 2B). Fifteen min after the quinine bottles were removed all rats received an IP injection of LiCl (13.3 ml/kg of 0.15 M LiCl). Two conditioning trials were followed by a taste only test trial. The dependent measure was volume of quinine consumed.

Histology and data analysis

Histology and data analysis procedures were identical to those used for Experiment 1.
C. Experiment 3

Subjects and Surgery

Forty experimentally naïve male Sprague-Dawley rats were acquired and housed in a manner identical to Experiment 1. Surgical procedures were also identical to those employed in Experiment 1, except 24 animals were in Group GTX.

Apparatus

Eight identical modular operant chambers (Med Associates, St. Albans, VT) were used in Experiments 3. Each chamber (30.5 cm long X 24.1 cm wide X 29.2 cm high) had a clear polycarbonate door, back wall, and ceiling, modular aluminum sidewalls, and a stainless steel bar floor. In each chamber, an oval access hole (1.3 cm wide X 2.6 cm high) was centered on the right wall panel, 6.0 cm above the bar floor. Solutions were available in retractable stimulus bottles. In the extended position, the tip of the stimulus bottle spout was centered in the access hole ~0.3 cm outside the chamber to prevent constant contact. A lickometer circuit (0.3 μA) was used to monitor licking with a temporal resolution of 10 milliseconds. A shaded light bulb (100 mA, 28 V) mounted on the sidewall opposite to the sipper tube provided illumination in the chamber. Each chamber was equipped with a white noise generator (~80 dB). Chambers were connected to a computer in an adjacent room, which controlled all events and records data using programs written in the Medstate notation language.

Procedure

After surgery animals were given 10 days to recover, and then habituated to the drinking chambers for 15 min each day over the next three days. During this habituation
phase animals were placed on a water restriction schedule consisting of 15 min water access each morning in the home cage. After the 3-day habituation phase animals received their 15-min daily water access period in the drinking chamber. Animals were divided into four experimental groups based on lesion condition (SHAM; GTX) and US condition (Saline; Amphetamine) yielding groups: SHAM-Saline, SHAM-Amphetamine, GTX-Saline, GTX-Amphetamine. Conditioning trials commenced when animals stabilized on the water access schedule as indicated by stable baselines across experimental groups on all dependent measures for three consecutive days. Conditioning trials occurred in a three-day cycle based on the nature of the US injection. On Day 1, Amphetamine rats were injected with amphetamine (1 mg/kg) whereas Saline rats were injected with an equivalent volume of physiological saline. On Day 2, Amphetamine rats received saline, and Saline rats were injected with amphetamine (this design ensures that all rats had equal exposure to saline and amphetamine). On Day 3, there were no injections. There were a total of three conditioning trials and a CS only test trial.

**Dependent Measures**

Weighing stimulus bottles before and after each session provided amount consumed. Additional dependent measures obtained from lick analysis included: total licks, cluster size, and lick efficiency (licks/g). Cluster size was calculated by dividing the total number of licks occurring in a cluster by the total number of clusters; a cluster is defined as a run of licks separated by an interlick interval of less than 500 milliseconds. Lick efficiency was calculated as the average number of licks needed to consume 1 gram of fluid.
Histology and Data Analysis

The procedures for histology and data analysis were identical to those described for Experiment 1.

D. Experiment 4

Subjects, Surgery, and Apparatus

The same subjects and apparatus from Experiment 3 were used in Experiment 4.

Procedure

After completion of Experiment 3 animals were given 14 days free access to food and water in their home cage, and then returned to the water deprivation schedule used in Experiment 3. Once water intake and lick measures restabilize in the drinking chamber taste neophobia training began with 15-min presentations of 0.0001 M quinine in place of the morning water. Each taste neophobia trial was separated by two water days. A total of four taste neophobia trials were conducted. Dependent measures were identical to those from Experiment 3: intake, total licks, cluster size, and lick efficiency.

Histology and Data Analysis

The procedures for histology and data analysis were identical to those described for Experiment 1.
III. RESULTS

A. Experiment 1

Anatomical

Only rats with complete bilateral damage to the GT were included in the statistical analyses of Group GTX. In the lesioned rats whose data were included in the behavioral analyses some minor damage extended from the GT into the centromedian, paracentral, parafascicular, and subparafascicular thalamic nuclei, as well as the VPM, but damage was unilateral and non-systematic across animals. Groups included in the primary behavioral analysis were: SHAM-Saline (n = 10), SHAM-Morphine (n = 10), GTX-Saline (n = 8), GTX-Morphine (n = 8). The discrete lesions in the current study (see Figure 2) were comparable in size and placement to prior work from our laboratory as well as other investigators using both electrolytic and excitotoxic induction techniques (e.g., Aristides & Reilly, 2006; Grigson et al., 2000; Pritchard & Reilly, 1996a; 1996b; Reilly et al., 2003; Reilly & Trifunovic, 1999; 2003), but were considerably smaller than the excitotoxic lesions reported in other studies (Scalera et al., 1997; Yamamoto, Fujimoto, Shimura, & Sakai, 1995).

Lesioned rats not included in Group GTX sustained either unilateral GT lesions or damage surrounding but not including the GT. These animals were used to form a misplaced lesion control group (Group MISS; n = 4) that was included in supplementary statistical analyses to evaluate the specificity of behavioral effects due to GT lesions versus damage to neighboring structures.
Figure 2. Experiment 1: Panel A shows a representative photomicrograph (10x) of a coronal section at the level of the GT from a SHAM rat. Panel B shows a corresponding section from a GTX animal. Panel C shows a portion of Panel B (indicated by dashed lines) at 50x magnification with the lesion outlined in red. Abbreviations: fr = fasiculus retroflexus; GT = gustatory thalamus; ml = medial lemniscus; PF = parafasicular nucleus.
Behavioral

Table 1 summarizes water data collected over the three days preceding Trial 1 for all animals included in the final analysis. Intake was analyzed with a repeated-measures ANOVA which found no significant main effects of Group ($F < 1$) or Trial, $F(2,66) = 2.48$, $p > .05$, and no significant Group x Trial interaction ($F < 1$). Thus, GT lesions did not influence water intake, which was stable across all groups for the three days prior to the first conditioning trial.

Control (SHAM) subjects and experimental (GTX) rats were given 15-min access to 0.15% saccharin followed 5 min later with an injection of either physiological saline (1 ml/kg) or morphine sulfate (15 mg/ml/kg) yielding four groups: SHAM-Saline, SHAM-Morphine, GTX-Saline, GTX-Morphine. The behavioral data for Experiment 1 are summarized in Figure 3. On Trial 1 (i.e., prior to any drug administration) animals with GT lesions showed a pronounced overconsumption of the novel saccharin tastant compared to their SHAM counterparts. An ANOVA analyzing intake across all trials revealed a significant main effect of Group, $F(3,33) = 19.80$, $p < .00001$, and of Trial, $F(4,132) = 2.82$, $p < .05$, and a significant Group x Trial interaction, $F(12,132) = 11.10$, $p < .0001$. Follow-up planned comparisons of the interaction revealed no within-groups lesion (SHAM; GTX) differences on Trial 1 intake ($ps > .25$), but a significant Trial 1 between-groups lesion difference ($p < .0001$). That is, GTX rats consumed of the novel saccharin tastant than SHAM subjects on the first exposure. On Trial 2, SHAM-Saline rats significantly increased intake thereby demonstrating some habituation of the initial taste neophobia ($p < .0001$). The GTX-Saline group also showed a significant, though numerically small, increase in intake on Trial 2 relative to Trial 1 ($p < .01$) and
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>Intake</th>
<th>Total Licks</th>
<th>Cluster Size</th>
<th>Lick Efficiency</th>
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<tbody>
<tr>
<td>1</td>
<td>SHAM-Saline</td>
<td>19.70 (0.28)</td>
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<td>16.35 (1.21)</td>
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<td>191.23 (45.19)</td>
<td>215.10 (15.33)</td>
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<td>139.76 (24.00)</td>
<td>260.29 (32.85)</td>
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<td>180.38 (31.59)</td>
<td>262.81 (34.89)</td>
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<td>135.26 (17.57)</td>
<td>210.14 (9.74)</td>
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</tr>
<tr>
<td></td>
<td>GTX-Amphetamine</td>
<td>16.85 (0.82)</td>
<td>3406.80 (176.15)</td>
<td>128.12 (17.30)</td>
<td>203.88 (9.69)</td>
</tr>
</tbody>
</table>

Table 1. Means (±SE) for water over 3 consecutive days prior to the beginning of each experiment.
maintained a significantly higher intake than the SHAM-Saline subjects on Trial 2 ($p < .05$). On Trials 3-5 saccharin intake was statistically identical in the saline-injected groups ($F_s < 1$). An inverse pattern of performance was observed in the morphine-injected groups each of which significantly reduced their saccharin consumption from Trial 1-5 ($ps < .001$). On Trial 1, SHAM-Morphine animals consumed $11.9 (±1.22)$ ml of saccharin compared to $17.5 (±1.80)$ ml in the GTX-Morphine rats, a difference of $5.6$ ml. The between-groups difference in the Morphine groups persisted across all trials ($ps < .01$). On Trial 5, SHAM-Morphine ($M = 6.7, ±1.70$) rats consumed $6.7$ ml less saccharin than GTX-Morphine ($M = 12.14, ±2.08$) rats. However, relative to initial intake, each of the Morphine groups reduced intake by a similar amount, which suggests that each of the Morphine groups acquired a CTA of comparable magnitude.

A similar pattern of results has been previously observed for morphine-induced CTA in normal rats (Lin et al., 2011). In this study normal rats acquired a morphine-induced CTA to a saccharin CS that was either novel or familiar (i.e., pre-exposed). Subjects made familiar with the CS prior to conditioning had a higher initial intake (~27 ml) than their novel counterparts (~12 ml) and, unsurprisingly, showed a latent inhibition-induced delay in CTA acquisition. Of particular interest to present purposes, the familiar CS rats in the Lin et al. experiment required 7 trials to reach asymptote versus 3 trials in the CS novel rats. Furthermore, the final asymptotic level of morphine-induced CTA was much higher in rats conditioned with a familiar saccharin CS relative to those conditioned with a novel saccharin CS. That is, after asymptotic levels of aversion were obtained rats in the CS familiar group consistently drank ~19 ml of the CS compared to
Figure 3. Experiment 1: Mean (±SE) saccharin intake for non-lesioned (SHAM) and lesioned (GTX) subjects.
~5 ml in rats conditioned with a novel CS. Thus, each group showed a similar absolute reduction of intake (novel, ~7 ml; familiar, ~8 ml) but from different starting points. As such, it would appear that the elevated asymptotic intake in GTX-Morphine rats is likely due to their higher intake on Trial 1. In Experiment 1 GTX-Morphine rats had an elevated Trial 1 intake of the saccharin CS as if it were familiar, accompanied by higher asymptotic intake, but the GTX-Morphine group did not, however, show any indication of the latent inhibition-like delay in learning that typically occurs to a familiar taste.

In the current study when CS intake was not restricted by short CS durations as used in previous work of this type, we found that GT lesions did not eliminate morphine-induced CTA. Indeed, rather than the elimination of morphine-induced CTA reported in prior studies using a 5 min procedure (Grigson et al., 2000; Reilly & Trifunovic, 1999) we found that GTX rats acquired morphine-induced CTA at about the same rate as the SHAM subjects, albeit from a different starting point on Trial 1. The higher levels of intake that occurred on the first trial suggest a lesion-induced deficit in the unconditioned response to the novel taste stimulus. It may seem that GT lesions attenuated the magnitude of morphine-induced CTA due to their high asymptotic intake relative to the SHAM-Morphine rats. However, this difference appeared to be a consequence of different starting points, and not a difference in the magnitude of the acquired aversion.

Finally, we re-analyzed the Trial 1 data of the SHAM and GTX groups along with the data from the MISS group to determine if damage to areas surrounding, but not including, the GT had any influence on saccharin intake. Inspection of Figure 4
Figure 4. Experiment 1: Mean (±SE) intake on Trial 1 for neurologically intact control (SHAM), misplaced lesion control (MISS), and lesioned (GTX) rats.
suggests that there was no difference in saccharin consumption on Trial 1 between Group SHAM and Group MISS but that intake in each of these groups was 5-6 ml lower than that of the GTX rats. A one-way ANOVA of the data summarized in Figure 4 found a significant main effect of Group, $F(2,39) = 15.43, p < .0001$. A post hoc Tukey HSD test found no difference in intake between Groups SHAM and MISS ($p > .90$), and consumption in each of these groups was significantly lower than that of Group GTX ($ps < .05$). Thus, it is concluded that bilateral damage to the GT was responsible for the Trial 1 saccharin overconsumption, and that damage to surrounding areas had no influence on this behavior.

Experiment 2

Behavioral

The water data collected over the three days preceding Trial 1 for all animals included in the behavioral analysis are summarized in Table 1. Rats from Experiment 1 were divided into new groups counterbalanced for lesion and prior drug experience (Experiment 2A, SHAM n = 10, GTX n = 8; Experiment 2B, SHAM n = 11, GTX = 8). Intake was analyzed with a repeated-measures ANOVA that found no significant main effect of Group ($p > .20$) or Trial, $F(2,66) = 2.26, p > .10$, and no significant Group x Trial interaction ($F < 1$). Thus, water intake was stable in all groups for the three days prior to the first conditioning trial.

In order to compare GTX-induced effects on morphine-induced and illness-induced CTA we used the subjects from Experiment 1 in a LiCl-induced CTA in Experiment 2. The data for Experiment 2 are shown in Figure 5. It will be recalled that in
Figure 5. Experiment 2: Mean (±SE) quinine intake in non-lesioned (SHAM) and lesion (GTX) rats over two conditioning trials and a CS only test trial. Panel A depicts quinine intake in Experiment 2A where intake was unlimited during the 15-min trials; Panel B shows consumption in Experiment 2B where intake on Trial 1 was capped to a 5 ml maximum.
Experiment 2A the rats had a 15-min quinine taste stimulus paired with LiCl on two conditioning trials and a CS only test trial. A repeated-measures ANOVA of the data summarized in Figure 5A revealed a significant main effect of Trial, $F(2,32) = 95.85, p < .0001$, as well as a Group x Trial interaction, $F(2,32) = 7.14, p < .01$, but no significant main effect of Group, $F(1,16) = 3.19, p > .05$. Post hoc planned comparisons of the interaction revealed significantly higher intake in Group GTX than in the SHAM rats on Trial 1 ($p < .05$). Each group significantly decreased intake on Trial 2 ($ps < .0001$), and there were no group differences on Trial 2 performance ($F < 1$). Each group further suppressed intake on Trial 3, again with no between-group differences ($F < 1$). To summarize, GTX animals consumed significantly more of the novel quinine solution on Trial 1 compared to their SHAM counterparts. However, after pairings with the LiCl US, the GTX group developed a CTA to the quinine CS at the same rate as SHAM subjects.

Experiment 2B was conducted in exactly the same manner as the Experiment 2A except all animals were allowed to consume a maximum of 5 ml on Trial 1. Intake was limited to prevent any between-groups differences on Trial 1 intake from confounding interpretation of subsequent performance. A repeated-measures ANOVA of the data summarized in Figure 5B revealed a significant main effect of Trial, $F(2,32) = 68.58, p < .0001$. There was, however, no significant main effect of Group ($F < 1$) and no Group x Trial interaction ($F < 1$). A Tukey HSD test showed a significant decrease from Trial 1 to Trial 2 and from Trial 2 to Trial 3 ($ps < .01$). Thus, when Trial 1 intake was equated there were no differences between SHAM and GTX animals in the rate of acquisition for a LiCl-induced CTA.

The Experiment 2 results are consistent with prior investigations of GT lesion effects finding no delay in the acquisition of a LiCl-induced CTA to a novel taste CS
(e.g., Flynn et al., 1991; Mungardee et al., 2006; Reilly et al., 2003; Reilly & Pritchard, 1996b; Scalera et al., 1997). We also found, similar to Reilly et al. (2003), a GTX-induced overconsumption of a novel tastant that did not delay subsequent CTA acquisition. Thus, the results of Experiment 1 and Experiment 2 suggest that GT lesions have a similar pattern of effects on CTAs induced with either a drug of abuse (morphine) or an illness-inducing agent (LiCl).

Experiment 3

Anatomical

In the present rats, damage to the GT and surrounding areas was comparable to that reported in the GTX rats in Experiment 1, although lesions in this group of GTX rats were on average somewhat larger (see Figure 6). In the rats with acceptable lesions, damage extended from the GT into the centromedian, paracentral, parafascicular, and subparafascicular thalamic nuclei, as well as the VPM. This damage was unilateral and non-systematic across GTX animals. A total of five animals were dropped from Group GTX due to lesions that did not damage the GT bilaterally. In the excluded animals, the same areas surrounding the GT were damaged, but to a much greater extent than in the GTX rats. As in Experiment 1, these rats were used to form a misplaced lesion control group (Group MISS; n = 5) for ancillary statistical analyses. Groups included in the primary analyses were: SHAM-Saline (n = 8), SHAM-Amphetamine (n = 8), GTX-Saline (n = 9), GTX-Amphetamine (n = 10).
Behavioral

In Experiment 3, the investigation of GT function shifted from the home cage into drinking chambers that were configured for microstructural lick analysis, allowing a more fine-grained measure of consummatory behavior. Table 1 summarizes water data collected over three days preceding Trial 1 for all animals included in the final analyses. For all four dependent measures of performance (intake, total licks, cluster size, and lick efficiency) repeated-measures ANOVAs found no significant main effect of Group ($p > .20$), Trial ($p > .20$), or any Group x Trial interactions ($p > .17$). Thus, GT lesions had no influence on performance for water, and performance of all four groups was stable over the three baseline water days.

To examine the generality of GT lesion effects on drug-induced CTA acquisition we conducted an amphetamine-induced CTA experiment in a new set of experimentally naïve rats. The behavioral data for the four treatment groups (SHAM-Saline, SHAM-Amphetamine, GTX-Saline, GTX-Amphetamine) are shown in Figure 7. On Trial 1, GTX animals appeared to have higher intake (Figure 7A), total licks (Figure 7B), and cluster size (Figure 7C) compared to the SHAM groups. Across these three measures, the SHAM-Saline group recovered from taste neophobia on Trial 2 and increased to the level of GTX-Saline counterparts on Trials 2-4. Relative to the saline groups, the SHAM-Amphetamine and GTX-Amphetamine animals showed an opposite pattern, that is, decreasing intake, total licks, and cluster size across trials. For the fourth dependent measure, lick efficiency, the two Amphetamine groups reduced lick efficiency across trials, while the Saline groups remained constant. Statistical analyses bear out these impressions.
Figure 6 Experiment 3: Panel A shows a representative photomicrograph (10x) of bilateral damage (outlined in red) in a rat from Group GTX. Panel B depicts the area of Panel A denoted by dashed lines at 50x magnification (lesion outlined in red). Abbreviations: fr = fasiculus retroflexus; GT = gustatory thalamus; ml = medial lemniscus; PF = parafasicular nucleus.
A repeated-measures ANOVA analyzing intake (Figure 7A) revealed a significant main effect of Group, $F(3,31) = 15.42, p < .00001$, and of Trial, $F(3,93) = 8.67, p < .0001$, as well as a significant Group x Trial interaction, $F(9,93) = 17.44, p < .00001$. Post hoc planned comparisons of the Group x Trial interaction showed no differences between SHAM-Saline and SHAM-Amphetamine groups or between the GTX-Saline and GTX-Amphetamine groups on Trial 1 ($Fs < 1$). There was, however, a significant elevation of intake in the GTX groups on Trial 1 compared to the SHAM groups ($p < .0001$). The SHAM-Saline group demonstrated a significant recovery from neophobia on Trial 2 relative to Trial 1 ($p < .0001$); intake in the GTX-Saline group was not significantly different from Trial 1 to Trial 2 ($p > .25$). Groups SHAM-Saline and GTX-Saline showed stable performance across Trials 2-4 ($ps > .05$). Groups SHAM-Amphetamine and GTX- Amphetamine each showed a significant reduction of intake from Trial 1-4 ($ps < .00001$). In summary, GTX rats showed higher levels of intake of Trial 1 relative to SHAM controls. This GT lesion-induced overconsumption appeared to completely disrupt taste neophobia, but did not appear to influence amphetamine-induced CTA acquisition. Curiously, the difference in Trial 1 intake did not lead to different asymptotic levels of aversion (as seen in Experiment 1). There are minimally two potential explanations for this difference across experiment: (1) the performance of the SHAM-Amphetamine rats or (2) the nature of the US (i.e., morphine or amphetamine). These alternative explanations will be considered in more detail in the Discussion section.

A repeated-measures ANOVA analyzing total licks (Figure 7B) revealed a significant main effect of Group, $F(3,31) = 9.43, p < .001$, Trial, $F(3,93) = 8.10, p < .0001$, and a significant Group x Trial interaction, $F(9,93) = 14.79, p < .0001$. Planned comparisons of the Group x Trial interaction demonstrated a pattern of significance.
Figure 7. Experiment 3: Mean (±SE) 0.15% saccharin CS directed performance for intake (A), total licks (B), cluster size (C), and lick efficiency (D) in SHAM-Saline, SHAM-Amphetamine, GTX-Saline, and GTX-Amphetamine across three conditioning trials and a CS only test trial.
identical to that described above for intake ($ps < .05$). Thus, GTX rats again demonstrated a Trial 1 overconsumption effect in terms of total licks. SHAM-Saline rats recovered from neophobia on Trial 2, while GTX-Saline rats showed consistent levels of total licks across all four trials. Each group of amphetamine-injected rats acquired a CTA as evidenced by a reduction in total licks across trials.

A repeated-measures ANOVA analyzing cluster size (Figure 7C) found a significant main effect of Group, $F(3,31) = 7.53, p < .001$, and a significant interaction of Group x Trial, $F(9,93) = 4.28, p < .001$, but no significant main effect of Trial ($p > .25$). Planned comparisons of the Group x Trial interaction revealed that on Trial 1 there were no differences between the two SHAM groups or between the two GTX groups ($Fs < 1$), but there was a significant elevation of cluster size in the GTX rats compared to SHAM subjects ($p < .001$). In the SHAM-Saline group there was a significant elevation in cluster size from Trial 1 to Trial 2 ($p < .00001$), with stable performance across Trials 2-4 ($Fs < 1$). The GTX-Saline group performance was constant across Trials 1-4 ($p > .50$). Surprisingly, in the SHAM-Amphetamine group there was no change in cluster size across Trials 1-4 ($ps > .05$). Finally, in the GTX-Amphetamine group we see a significant decrease in cluster size across Trials 1-3 ($ps < .001$) with stable performance on Trials 3 and 4 ($F < 1$). Increased cluster size across trials in the SHAM-Saline group is consistent with prior research that cluster size increases as neophobia habituates in neurologically intact (and drug naïve) rats (Lin, Amodeo et al., 2012). The lack of a significant decline in cluster size in the SHAM-Amphetamine group was surprising given consistent demonstrations of amphetamine-induced CTAs resulting in decreased cluster size in other studies (Arthurs et al., 2012; Lin, Arthurs et al., 2012), and could be due to the low initial cluster size in this group.
Lick efficiency data was analyzed with a repeated-measures ANOVA and revealed a significant Group x Trial interaction, $F(9,93) = 3.09, p < .01$. There were no main effects for Group $F(3,31) = 2.03, p = .13$, or Trial, $F(3,93) = 2.60, p = .057$. Planned comparisons of the interaction revealed no significant group differences on Trial 1 ($ps > .20$). On the final quinine trial there was not a significant difference between the two Saline groups ($p > .60$) or between the two Amphetamine groups ($p > .10$).

SHAM-Amphetamine rats had significantly lower lick efficiency compared to SHAM-Saline rats ($p < .05$). There was also a significant reduction in lick efficiency in GTX-Amphetamine rats compared to GTX-Saline subjects ($p < .05$). That is, we see a significant reduction in lick efficiency during an amphetamine-induced CTA in both Amphetamine groups; however, this effect is more pronounced in GTX rats compared to their SHAM counterparts. The results in SHAM-Amphetamine subjects are consistent with reductions in lick efficiency seen in neurologically intact animals during amphetamine-induced CTA acquisition (Arthurs et al., 2012; Lin, Arthurs, et al., 2012). The lack of a change in lick efficiency in the SHAM-Saline rats is consistent with previous lick analysis studies of CTAs induced with both drugs of abuse and LiCl (Arthurs et al., 2012; Lin, Arthurs et al., 2012). These results taken in combination (current study, Arthurs et al.; Lin, Arthurs et al.) suggest that noncontingent US injections following water access in drinking chambers have a broad impact on lick efficiency across all sessions in the drinking chamber.

The overall pattern of results in Experiment 3 demonstrates that GTX lesions do not eliminate amphetamine-induced CTA. There was a GTX-induced elevation of Trial 1 intake, total licks, and cluster size, but this elevation did not appear to influence the acquisition of an amphetamine-induced CTA. The GTX overconsumption on Trial 1
appeared to completely attenuate taste neophobia as there was no increase in intake, total licks, or cluster size across trials in Group GTX-Saline. However, in the SHAM-Saline group we found increased intake, total licks, and cluster size accompanying the attenuation of taste neophobia.

To investigate the neural specificity of the GT lesion deficits we examined the performance of the SHAM, MISS, and GTX groups on Trial 1 these data are summarized in Figure 8. It will be recalled that, subjects in Group MISS had damage to areas outside the GT, whereas the damage for Group GTX was focused in the GT with some encroachment into surrounding structures. Inspection of Figure 8 suggests that animals in groups SHAM and MISS responded in a similar fashion across all four dependent measures. In contrast, it appears that GTX rats differed from each of the other groups in terms of intake (Figure 8A), total licks (Figure 8B), and cluster size (Figure 8C). However, lick efficiency did not appear to vary across groups (Figure 8D). These impressions of the results were confirmed by statistical analyses. That is, separate one-way ANOVAs, revealed significant Group effects for intake, $F(1,37) = 6.85, p < .01$, total licks, $F(1,37) = 3.85, p < .05$, and for cluster size, $F(1,37) = 4.60, p < .05$. There was no effect of Group on lick efficiency ($F < 1$). Post hoc Tukey tests confirmed that the MISS group performance was identical to that of the SHAM group for intake ($p > .60$), total licks ($p > .98$), and cluster size ($p > .90$). In the same analyses GTX rats were significantly different from the SHAM group on intake ($p < .05$), total licks ($p < .05$), and cluster size ($p < .05$). These tests also revealed a significant difference between GTX and MISS rats for intake ($p < .05$), but not total licks ($p = .16$) or cluster size ($p = .28$).
Figure 8. Experiment 3: Mean (±SE) intake (A), total licks (B), cluster size (C), and lick efficiency (D) on Trial 1 in non-lesion (SHAM), misplaced lesion (MISS) and lesion (GTX) rats.
The lack of significant differences in these latter analyses is likely a result of low statistical power due to the small size of Group MISS. Across the three measures which showed a significant Group effect the pattern is consistent with no difference in performance between the SHAM and MISS groups and a significant elevation in GTX rats compared to SHAM subjects. Thus, we conclude that the Trial 1 overconsumption in Group GTX was due to bilateral damage to the GT, and that damage to surrounding structures had no influence on the unconditioned response to novel saccharin.

D. Experiment 4

Behavioral

Table 1 summarizes performance on all four dependent measures for the 3 days prior to the first trial. It will be noted that group names used in Experiment 3 were maintained in Experiment 4; however, all animals in Experiment 4 received identical treatment (i.e., 15 min access to a quinine solution). Each dependent measure was analyzed with repeated-measures ANOVA. The ANOVAs for all four dependent measures of performance found no significant main effect of Group (\(ps > .25\)), Trial (\(ps > .35\)) and no interaction of Group x Trial (\(ps > .30\)). Thus, neither GT lesions nor prior experience had any influence on performance and all groups were stable on all measures for the three days prior to the first conditioning trial.

Figure 9 depicts the initial occurrence, and subsequent habituation of, taste neophobia to a quinine taste stimulus in subjects previously used in the CTA procedure of Experiment 3. It would appear that prior experience differentially influenced performance in the GTX rats. That is, whereas the two groups of SHAM rats performed in a similar manner across all four dependent measures there was a notable difference.
between the two groups of GTX rats. Moreover, it would appear that prior experience in the GTX-Amphetamine group lead these rats to respond to the quinine stimulus in a manner similar to the SHAM rats. On the other hand, GTX-Saline rats showed the same pattern of higher intake, total licks, and cluster size on Trial 1 for the novel quinine as they had previously displayed for the novel saccharin solution on Trial 1 of Experiment 3.

To assess the habituation of neophobia repeated-measures ANOVAs were conducted for intake, total licks, cluster size, and lick efficiency. The ANOVA for intake (see Figure 9A) revealed a significant main effect of Trial, $F(3,31) = 56.23, p < .0001$, and a Group x Trial interaction, $F(9,93) = 2.30, p < .05$; the main effect of Group ($p > .20$) was not significant. Post hoc planned comparisons of the interaction revealed no significant difference between the SHAM rats ($F < 1$) on Trial 1, but there was a significant difference between the GTX Groups ($p < .001$). Further comparisons of Trial 1 data revealed a significant difference between the GTX-Saline group and the two SHAM groups ($p < .001$), but no difference between the SHAM groups and the GTX-Amphetamine group ($p > .30$). All Groups showed a significant attenuation of neophobia from Trial 1 to 2 ($ps < .001$), followed by stable performance on Trials 2-4 ($ps > .10$). Thus, the GTX-Saline rats overconsumed the novel quinine solution on Trial 1 relative to each of the other three groups.

A repeated-measures ANOVA of total licks (see Figure 9B) revealed a significant main effect of Trial, $F(3,93) = 56.23, p < .00001$, and a significant Group x Trial interaction, $F(9.93) = 2.30, p < .05$, but no significant main effect of Group ($p > .20$). Follow-up planned comparisons of the interaction revealed no significant
Figure 9. Experiment 4: Mean (±SE) for intake (A), total licks (B), cluster size (C), and lick efficiency (D) across the four 15-min presentations of the 0.0001 M quinine tastant. Group designations were carried over from Experiment 3 to emphasize prior experience.
difference between the two SHAM groups on Trial 1 ($p > .10$) and no difference between the GTX-Amphetamine group and the two SHAM groups ($F < 1$). However, the GTX-Saline group made significantly more licks than either the GTX-Amphetamine group or each of the two SHAM groups on Trial 1 ($p < .0001$). All groups significantly increased total licks on Trial 2 ($ps < .0001$) and were stable in performance on Trials 2-4 ($ps > .10$), except the GTX-Saline rats, which increased total licks from Trial 3-4 ($p < .05$). Thus, prior experience in GTX rats had the same differential effect on Trial 1 total licks as it did for amount consumed. That is, the GTX-Saline rats showed a significant elevation of performance relative to the other three groups.

A repeated-measures ANOVA of cluster size (see Figure 9C) showed a significant main effect of Group, $F(3,31) = 3.27$, $p < .05$, and of Trial, $F(3,31) = 20.54$, $p < .00001$, but no significant Group x Trial interaction ($F < 1$). A Tukey test was used to analyze the Group effect and revealed a significant elevation in cluster size in the GTX-Saline Group relative to the GTX-Amphetamine Group ($p < .05$); no other between-group differences were significant. A Tukey test of the main effect of Trial revealed significantly lower average cluster size on Trial 1 compared to all other Trials ($ps < .001$). There were no significant differences between Trials 2-4, ($ps > .25$). Prior experience once again differentiated the GTX groups from each other with significantly higher average cluster size in GTX-Saline than GTX-Amphetamine rats.

The repeated-measures ANOVA of lick efficiency (see Figure 9D) revealed a significant Trial effect, $F(3,93) = 38.75$, $p < .00001$. However, there was no main effect of Group ($F < 1$) and no Group x Trial interaction ($p > .40$). A Tukey test of the Trial effect revealed significantly lower lick efficiency on Trial 1 compared to Trials 2-4 ($ps < .001$). Lick efficiency was stable across Trials 2-4 ($ps > .40$). Thus, neither GT lesions
nor prior experience had any influence on lick efficiency, but lick efficiency increased as taste neophobia habituated across trials.

The overall pattern of results in the SHAM rats is consistent with previous lick analysis studies of the occurrence and habituation of neophobic reaction to quinine by neurologically intact rats (Lin, Amodeo et al., 2012). That is, the habituation of neophobia was expressed as increased intake, total licks, cluster size and lick efficiency as a novel taste becomes familiar. We see a similar pattern in GTX-Amphetamine animals, but not in the GTX-Saline rats that had initially higher levels of performance. The difference between GTX groups in initial intake, total licks, and cluster size suggests a carryover effect due to different prior experience in Experiment 3. GTX-Amphetamine rats acquired a CTA to saccharin in Experiment 3, whereas, of course, GTX-Saline rats did not. It seems likely, that these experiences in Experiment 3 may have somehow influenced behavior in Experiment 4. GTX-Saline animals showed higher intake, total licks, and cluster size for quinine in Experiment 4 as they had for saccharin in Experiment 3. GTX-Amphetamine rats, on the other hand, appeared to have generalized the CTA to saccharin acquired in Experiment 3 to the novel quinine stimulus in Experiment 4. This carryover effect could be due to the generalization across the two taste stimuli, stimuli that share a bitter component (Dess, 1993; Drewnowski, 2001).
IV. DISCUSSION

Prior to the present research GT lesions were thought to eliminate morphine-induced CTA while having no influence on LiCl-induced CTA (Grigson et al., 2000; Reilly & Trifunovic, 1999). This pattern of results was taken as evidence that morphine-induced CTA was qualitatively different from LiCl-induced CTA, and that these phenomena relied on different neural substrates (Grigson et al., 2009; Parker et al., 2009). The present results compel a reevaluation of these claims because they demonstrate that GTX rats are competent in the development of CTAs to either morphine or amphetamine. The present results also demonstrate a reliable overconsumption by GTX rats on initial exposure to a novel taste stimulus that cannot be attributed to non-specific damage to surrounding brain structures.

To determine whether proper evaluation of the role of the GT in morphine-induced CTA had been obscured by ceiling effects on intake in previous studies (due to the use of a 5-min CS access period) we conducted two drug-induced CTA experiments in GTX rats using a 15-min CS access period. In Experiment 1, GTX-Morphine rats suppressed intake across trials providing a clear demonstration that GT lesions do not eliminate morphine-induced CTA. It is worth noting that Experiment 1 would have been compromised by ceiling effects if conducted with a 5 min CS access period. This is because intake in the saline-injected rats would have been limited to a maximum of 10-12 ml on each trial thereby obscuring detection of CTA acquisition by GTX rats. In Experiment 3, the GTX-Amphetamine rats decreased intake as well as total licks and cluster size across trials. Overall, then, we conclude that GT lesions do not eliminate drug-induced CTA to either a morphine or amphetamine US.
Moving now to the follow-up procedures that were conducted after the drug-induced CTA experiments. In Experiment 2A, the GTX group consumed significantly more of the quinine CS than SHAM animals on Trial 1. Despite this lesion-induced difference in the initial starting point there was no effect of GT lesions on the acquisition of a LiCl-induced CTA. This analysis was supported in Experiment 2B, the capped procedure of the experiment, where we see SHAM and GTX rats each acquired a LiCl-induced CTA at the same rate from a common Trial 1 starting point. These results are in agreement with the existing literature showing normal LiCl-induced CTA acquisition in GTX rats (e.g., Flynn et al., 1991; Mungardee, et al., 2006; Reilly et al., 2003; Reilly & Pritchard, 1996b; Scalera et al., 1997).

In the taste neophobia procedure of Experiment 4, rats in Group GTX-Saline showed a similar pattern of Trial 1 behavior in response to a novel quinine stimulus as they did for the novel saccharin CS in Experiment 3 (i.e., elevated intake, total licks, and cluster size). Interestingly, this pattern of performance was not seen in Group GTX-Amphetamine, which responded in a manner similar to the SHAM groups on Trial 1 of Experiment 4. It seems that the prior experience of Group GTX-Amphetamine in Experiment 3 (amphetamine-induced saccharin CTA) generalized to the quinine solution in Experiment 4. This carryover effect in the GTX-Amphetamine rats could be due to shared properties of the taste stimuli used in Experiments 3 and 4 (saccharin has a bitter aftertaste that could have generalized to bitter quinine; Dess, 1993; Drewnowski, 2001).

Results in Experiment 2 are consistent with the prior literature demonstrating that GT lesions do not influence the acquisition of LiCl-induced CTA. Thus, we conclude that the GT does not have a significant role in CTA acquisition whether induced with toxins.
or drugs of abuse. Of further importance, the present work uncovered a new deficit in GTX rats: overconsumption on initial exposure to the new taste stimulus in each experiment. Interestingly, this deficit does not appear to influence the rate of subsequent taste learning. How is this new deficit to be interpreted?

The most parsimonious explanation for the pattern of trial 1 overconsumption deficits observed in GTX rats across all four experiments would be a lesion-induced deficit in taste perception. In particular, the current results suggest that GT lesions are, relative to the performance of normal subjects, causing the rats to respond as if taste stimuli were less concentrated (i.e., manifest as elevated intake, total licks and cluster size). Moreover, there is no reason that this type of lesion-induced perceptual deficit would influence subsequent CTA learning. Thus, a lesion-induced disruption of taste perception could readily explain the pattern of deficits in GTX rats of the present study.

Although a perceptual deficit might explain the performance of the GTX rats in the present study, there is very little evidence supportive of this account in the literature. Indeed, the evidence seems to refute that interpretation. A number of studies have examined GTX rats for deficits in taste perception using various tastes (e.g., sucrose, saccharin, HCl, and quinine) and intake durations (e.g., 10s, 15 min, 24 hr), finding little support for lesion effects on taste perception. Reilly and Pritchard (1996a) used both long (24 hr, two-bottle) and short (15 min, single bottle) duration tests in GTX rats, and found normal taste response curves for sucrose (0.01, 0.1, 1.0 M), NaCl (0.01, 0.1, 1.0 M), HCl (0.0003, 0.003, 0.03), and quinine (0.000003, 0.00003, 0.0003, 0.003). Flynn, Grill, Schwartz, and Norgren (1991) showed normal concentration response curves in GTX rats for sucrose (0.01, 0.1, 1.0 M), HCl (0.0003, 0.003, 0.03 M), NaCl (0.01, 0.1 M, 1.0 M), and quinine (0.000003, 0.00003, 0.003 M) during one-bottle 15-min intake tests.
Finally, Scalera et al. (1997) found normal concentration-dependent licking in GTX rats during randomly-ordered 10s presentations of six different concentrations of sucrose (0.01 to 1.0 M), NaCl (0.003 to 1.0 M), and quinine (1 x 10^{-5} to 3 x 10^{-3}; one taste type per session) when rats were either fluid replete or fluid deprived. The use of brief duration stimulus presentations increases confidence that the observed effects are attributable to taste perception and minimally influenced by post-ingestive feedback. Overall, GTX rats appear to have normal taste concentration responses to a variety of taste stimuli during long (24 hour), short (15 min), and brief (10s) duration intake tests. Thus, notwithstanding the appealing simplicity of a GT lesion-induced deficit in taste perception underlying the overconsumption of novel taste stimuli, this interpretation finds no support in the existing literature.

An alternative interpretation of the overconsumption of new taste stimuli by GTX rats concerns a disruption of taste neophobia. It will be recalled that a disruption of taste neophobia has been used to explain the pattern of effects found in rats with IC lesions. The neophobia deficit in ICX rats is characterized by the overconsumption of a novel taste stimulus and a latent inhibition-like delay in CTA acquisition to novel but not familiar taste stimuli (Lin et al., 2009; 2011). We see a similar overconsumption of a novel CS in GTX rats, but, as the present work shows, no delay in subsequent learning. How are we to interpret the pattern of deficits in GTX rats in terms of taste neophobia?

Despite the lack of a delay in learning there are several factors that suggest GT lesions do indeed cause a deficit in taste neophobia. First, support of a role for the GT in taste neophobia comes from work demonstrating c-Fos activation in the GT along with the IC, BLA, and central nucleus of the amygdala after exposure to a novel, but not a familiar, taste stimulus (Lin, Roman, Arthurs, & Reilly, 2012). This study compared c-
Fos protein levels (a marker of neuronal excitation) following volume-matched intake of a novel or familiar 0.5% saccharin taste stimulus. Second, Lin, Amodeo et al. (2012) found that taste neophobia is manifest by lower palatability (i.e., cluster size) when a taste stimulus is novel as opposed to when it is familiar. In the current study, the trial 1 overconsumption in GTX rats of Experiment 3 and the same deficit in the GTX-Saline rats of Experiment 4 was associated with a cluster size that was more representative of a familiar than a novel taste and thus supports the view that GT lesions disrupt a process necessary for the expression of taste neophobia.

Although there have been previous reports of GT lesion-induced overconsumption of novel taste stimuli these results could not be unambiguously attributed to the GT due to either misplaced (Loullis, Wayner, & Jolicoeur, 1978) or large lesions with neural damage extending to areas well outside the GT (Yamamoto et al., 1995). If GT lesions do in fact cause an overconsumption on initial exposure to taste stimuli why was it not observed in the studies cited above (Flynn et al., 1991; Reilly & Pritchard, 1996a; Scalera et al., 1997) that investigated unconditioned responsivity to various taste stimuli? A potential explanation is that none of these experiments were designed with the analysis of taste neophobia as a primary aim. In fact, many studies were designed to minimize the disrupting influence of taste neophobia on the detection of perceptual deficits. The lack of overconsumption in these studies lead to the consensus that the GT was not involved in taste neophobia. A single study employing discrete excitotoxic lesions of the GT has previously detected overconsumption of a novel taste stimulus (Reilly et al., 2003). However, this overconsumption was not interpreted as a neophobia effect due to a lack of any subsequent delay in CTA acquisition, a presumed requirement for a lesion-induced deficit in taste neophobia.
This latter point bears repeating: it has been assumed that a taste neophobia deficit is manifest as an overconsumption on initial exposure (because the taste was viewed as familiar and safe) that would impart a consequent delay in subsequent learning (i.e., a latent inhibition-like effect would also occur). However, perhaps the initial overconsumption and the subsequent delay of learning represent disruptions of two distinct processes rather than a single process as had previously been assumed. By this analysis, GT lesions disrupt the first process but not the second process whereas IC lesions disrupt both processes.

Interestingly, it has been shown that bilateral lesions of the IC, BLA, or MeA disrupt the initial expression of taste neophobia (Lin et al., 2009) just like GT lesions (current study). Furthermore, asymmetric unilateral lesions of any two of the three structures previously mentioned (IC, BLA, MeA) reveals that the IC and BLA may form a functional unit in terms of the initial expression of taste neophobia; asymmetrical lesions of the MeA-IC or MeA-BLA had no influence on taste neophobia (Lin & Reily, under review). Finally, whereas bilateral lesions of the IC (Lin et al., 2009; 2011) or BLA (St. Andre & Reilly, 2007) delay CTA acquisition to a novel taste stimulus, bilateral lesions of the GT (current study; Flynn et al., 1991; Mungardee, et al., 2006; Reilly & Pritchard, 1996b; Scalera et al., 1997; Reilly et al., 2003) or MeA (Aggleton Petrides, & Iversen, 1981; Meliza, Leung, & Rogers, 1981; Rollins, Stines, McGuire, & King, 2001; Schoenfeld & Hamilton, 1981; Yamamoto et al., 1995) have no influence on CTA learning.

To summarize, bilateral lesions of the GT or the MeA disrupt taste neophobia but have no effect on CTA learning, whereas lesions of the IC or BLA disrupt both taste neophobia and CTA acquisition. Together, these patterns of results support the view
that (1) taste neophobia may involve two processes (which are manifest as deficits in overconsumption and delayed taste learning) and that the GT (perhaps in conjunction with the MeA) is involved in the former but not the latter of these processes. However, the GT and MeA could also be acting independently to supplement the IC-BLA circuit. Clearly, the neural substrates of taste neophobia may be more complex than previously thought. Furthermore, it appears that the psychological processes underlying taste neophobia are more complicated than previously understood. Evidence of lesions that cause a deficit in initial overconsumption while not influencing subsequent learning do not fit within the current conceptual framework of taste neophobia.

Numerous studies will be needed to analyze the merits of the newly expanded neural and psychological underpinnings of taste neophobia brought to light by the current study. We will enumerate some necessary preliminary experiments. Most immediately, it will be necessary to determine the functional connectivity of the GT in taste neophobia. We propose an asymmetrical lesion study of the GT-MeA, GT-BLA, and GT-IC similar to that conducted on the IC, BLA, and MeA by Lin and Reilly (under review). It will also be necessary to examine MeA-lesioned rats in a latent inhibition CTA design similar to ones previously conducted for the IC, BLA, and GT (Lin et al., 2009; Reilly et al., 2003; St. Andre & Reilly, 2007). These lesion studies should outline the basic connectivity and function of the neural circuits involved in taste neophobia.

In addition to the above studies utilizing permanent excitotoxic lesions a variety of experiments utilizing transient pharmacological manipulations will aid in further understanding the neural substrates of taste neophobia. Temporary pharmacological inactivation of each structure (i.e., BLA, GT, IC, MeA) could be used to mimic permanent lesions while providing a level of temporal analysis that is absent with
permanent lesions. For instance, each structure could be inactivated bilaterally prior to presentations of a novel stimulus to mimic the effect of a lesion while avoiding interpretational issues that accompany permanent lesion studies, such as retrograde degeneration of related structures. Having induced a deficit in taste neophobia subsequent presentations could be made without infusions to determine if the Trial 1 deficit persists. Structures could also be inactivated following the presentation of the taste stimulus to determine potential roles in the consolidation of taste neophobia. Manipulations of specific neurotransmitter systems may also aid in elucidating the role each structure in taste neophobia. Infusions of either an NMDA receptor antagonist (MK-801) or glutamate antagonist (MPEP) before or after the presentation of taste stimuli may help to determine the function of each area in the generation of the initial neophobic response and during subsequent taste learning.

In overall summary, the research in the current thesis has in one sense succeeded in clarifying the role of the GT in taste learning. That is, we now know that lesions of the GT do not eliminate drug-induced CTA. In fact, if GT lesions have any influence on CTA acquisition in response to drugs of abuse or toxins that influence appears to be relatively minor. In another sense the current investigation has opened up a role for the GT in a previously unsuspected function: taste neophobia. The implication of a GT lesion-induced neophobia deficit that does not influence subsequent learning has enlightened our thinking about the processes involved in taste neophobia and encourages the conduct of a number of experiments that will seek to find empirical support for these processes.
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ANIMAL CARE COMMITTEE PROTOCOL APPROVAL FORM

The experiments contained in this thesis were approved and covered under ACC protocol number 11-080.
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Education

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Role of the Gustatory Thalamus in Taste Learning

B.S. 2007 University of Idaho
Major: Psychology

Academic Awards and Honors

Society for Neuroscience Annual Meeting 2010
Travel Award, UIC Department of Psychology, 2010
Travel Award, UIC Graduate College, 2010
Travel Award, UIC Graduate Student Counsel, 2010

Certificate of Achievement, Department of Psychology, 2010-2011 academic year

Society for Neuroscience Annual Meeting 2011
Travel Award, UIC Department of Psychology, 2011
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Travel Award, UIC Graduate Student Counsel, 2011

Membership in Professional Organizations

Midwestern Psychological Association
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Employment

Research Technician (Dec. 2009 to Aug. 2010) University of Illinois at Chicago
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Publications


Manuscripts in Preparation

Arthurs, J., and Reilly, S. Gustatory thalamus influence on drug-induced taste aversion learning and taste neophobia.

Arthurs, J., Lin, J. Y., and Reilly, S. Between-subject differences in morphine-induced CTA.


Lin, J. Y., Amodeo, L. R., Arthurs, J., and Reilly, S. Protein synthesis inhibition in the parabrachial nucleus and taste neophobia.
Conference Presentations


Department Colloquium Presentations


Research in Progress

Microstructural lick analysis of taste intake suppression in response to rewarding and aversive unconditioned stimuli.

Forebrain circuits involved in taste neophobia.

Central and peripheral unconditioned stimulus properties of drugs of abuse.

Laboratory Skills

Behavioral Testing:
  - Taste reactivity testing with both voluntary and involuntary intake
  - Operant chamber testing
  - Home cage consummatory intake testing with drug injections (morphine, amphetamine, lithium chloride)

Stereotaxic Microsurgery:
  - Induction of discrete excitotoxic and electrolytic brain lesions
  - Intracranial cannula implantation

Intraoral catheterization surgery

Intracranial pharmacological microinfusions

Data Analysis:
  - Statistical analysis using: STATISTICA, HLM, R, and SPSS software
  - Scoring of video taped behavioral data

Histology:
  - Transcardial tissue perfusion
  - Cryostat operation
  - Nissl staining
  - Excitotoxic lesion analysis
  - C-fos activation analysis

Teaching Experience

Teaching Assistant           Department of Psychology University of Illinois at Chicago

Class: PSCH 361 Laboratory in Learning and Conditioning
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Responsibilities: Class consists of two phases involving simulated conditioning of a virtual rat (Sniffy) in preparation for conditioning a live rat in a series of operant tasks (i.e., magazine training, shaping, discrimination, and a chain schedule), and the application of that experience to the writing of a lab report, developing writing skills as a part of the departmental writing curriculum. Also involving the preparation of class handouts, management of class blackboard page and maintenance of operant chambers.

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Responsibilities: Conduct weekly office hours to assist students in comprehension of material presented in lecture, as well as proctoring examinations and managing class blackboard page.

Mentoring of Undergraduate Volunteer Research Assistants

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