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THE GUSTATORY INSULAR THALAMOCORTICAL TRACT IS NECESSARY FOR ACQUISITION AND RETENTION OF DRUG-INDUCED REWARD COMPARISONS, BUT NOT LICL-INDUCED TASTE AVERSION

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ABSTRACT

This dissertation systematically tested the conditioned avoidance of a (taste) stimulus when repeatedly paired with cocaine, morphine, sucrose, or LiCl in male Sprague-Dawley rats with discrete bilateral lesions of the insular gustatory cortex (GCTx). To address this problem, we utilized stereotaxic-guided, ibotenic-acid lesions of the taste cortex, in order to determine the role of the insular gustatory cortex in conditioned suppression of intake of a gustatory CS when contingently paired with a drug of abuse (specifically morphine and cocaine), the aversive agent LiCl, and a known reinforce, such as sucrose (Chapters 2 and 3). At the end of Chapter 3, unilateral lesions of the insular gustatory cortex were combined either an ipsilateral (Ip-CNTL) or a contralateral (THCx) electrophysiological-guided excitotoxic disruption of the taste thalamus, and tested under the consummatory successive negative contrast paradigm. In Chapter 4, rats with asymmetric THCx lesions were tested under drug contrast acquisition, retention, and LiCl-induced conditioned taste aversion (CTA). Next, in order to test the effect of asymmetric THCx lesions on retrieval or expression of taste memory, the rats in Chapter 5 were first exposed to taste-morphine pairings, then lesioned and retested for retention of acquiring a conditioned taste avoidance response. Taken together, the results of our behavioral experiments suggest that the taste thalamocortical loop is required for particular types of reward- and some forms of drug-induced suppression of CS intake, but not essential for innate taste preference/aversion, eliciting taste stimulated orofacial responses, producing LiCl-CTA, or an association between the taste CS and drug reward (i.e., cue-induced craving), *per se*. In Chapter 6, the interpretations of our findings are summarized and discussed in terms of addiction in general and the importance of learning, memory, reward signaling and comparison.

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-Rastafa I. Geddes, M.S.

Chapter 1

Introduction

1.1 Drugs of abuse, addiction, and the "War on Drugs"

While the interplay between human kind and numerous types of "mind-altering" or psychoactive compounds could be traced back to the earliest known human recordings (Brecher and Reports. 1972; Caldwell 1978), the drug war in America began almost a century ago (Susman 1975). In 1914 a series of drug laws were enacted under the Harrison Narcotics Act. Similar to the liquor and cigarette taxes of today, the Marijuana Tax act of 1937 attempted to regulate the use of cannabis, opium, and heroin (Brecher and Reports. 1972). Between 1909 and 1968 the United States Government Printing Office grew to consist of at least 54 different federal drug laws , over which time, the leading approach to eradicating drug abuse in America was varying levels of prohibition and targeting the sale and drug trafficking (Susman 1975).

Possibly to no avail, since first, the consumption of beer/wine (82%), cigarettes (72%) or hard liquor (65%) an even illicit drug (35%) were surprisingly high among adolescent in attending New York State public schools in 1971 (Kandel, Single et al. 1976). Second, drug use in America is believed to have peaked at 14% of the population in 1979 according the National Survey on Drug Use and Health (NSDUH, 2007). Third, in New York City (and other major cities across the nation) a new era of pandemic drug use was ushered in by the availability and low cost of crack cocaine, heroin, and prescription drugs during the 1980s, 1990s, and this century (Hamid, Curtis et al. 1997; Manchikanti 2006). Fourth, NSDUH has estimates that the use of marijuana (14.8 million), prescription drugs (7 million), and cocaine (2.4 million) are high pervasive in America today *(NSDUH, 2006).* Fifth,

although 8.3% lower than ever observed, more than the 19.9 million Americans are estimated to have reportedly habitually engaged in illegal drug use during 2007, according the NSDUH statistics (2007).

While the 8.3% decrease in drug use from 1979 may be attributed to the legal "War on Drugs" two facts say otherwise. First, we know that hardened incarceration among drug and non-violent offenders has lead to an initiation or increase in drug use, recidivism, sexually transmitted diseases, and a decrease in successful rehabilitation, prosperity, and the life itself (Grobsmith and Dam 1990; Gross, DeJong et al. 1994; Dawson 1997; Barrow, Herman et al. 1999; Calzavara, Burchell et al. 2003; Blankenship, Smoyer et al. 2005; Davis, Johnson et al. 2006; Narevic, Garrity et al. 2006; Cuellar, Kelleher et al. 2008; Moore and Elkavich 2008; Krinsky, Lathrop et al. 2009). Second, in addition to the legal war, the administration has also furiously funded basic and clinical research on addiction and to date the data suggest that addiction in general (i.e., food, shopping, gambling and drugs) develops as the brain goes a rye due to effects of initial or repeated stimuli-consequence experience on sensory and interoceptive perception as well as learning and memory processing (Davis and Smith 1976; Wise 1984; Lett 1989; Koob and Weiss 1992; Nestler, Hope et al. 1993; White 1996; Dudish-Poulsen and Hatsukami 1997; Smith, Jones et al. 2001).

Unfortunately, despite increasingly expanding our knowledge about the phenomenon of addiction, the disease is still a pervasive. To combat this trend, more recently, according to the National Drug Control Strategy FY 2009 Budget Summary, released by the White House in February 2008, the US Presidential administration requested 1.5 billion for drug use prevention, 3.4 billion for treatment programs, and 9.2 billion to combat drug trafficking *(NDCS, 2009 FY Budget Summary, 2008).* Therefore, while the use of illicit drugs is a personal one, the public's concern is not unwarranted and this is mainly because addiction impacts family and co-workers, destroys communities and drains

national funds (Hammersley, Forsyth et al. 1989; French, Mauskopf et al. 1996; Hamid, Curtis et al. 1997; Potenza, Steinberg et al. 2000).

1.2 Drugs of abuse and the devaluation of non-drug rewards

So, why do so many individuals misuse or abuse drugs? Human kind have reported creative and robust motives for initially engaging in drug use and some includes; autonomy, curiosity, bored dumb, social acceptance, recreation, athletic achievement, spirituality (religion), pain relief, and emotion or cognitive modification (Grilly 1992; Vetulani 2001). Compulsivity is a key feature of drug addiction and refers to an animal's or human's propensity for uncontrolled drug-seeking, drug-taking, and relapse. Compulsion arises, in part, from positively reinforced learning and negative drug-induced withdrawal effects (Gardner and Lowinson 1993; Wolffgramm and Heyne 1995; Berlin, Rolls et al. 2004; Davis, Strachan et al. 2004; Kelley 2004; Baler and Volkow 2006). The current thesis focuses of another key component of drug addiction, the lack of interest or devaluation of non-drug rewards. In this case, the non-drug reward is a palatable taste cue which, through conditioning, comes to predict the availability of drug.

In "Über Coca" (published in 1884) Sigmund Freud wrote, "The psychic effect of cocaïnum muriaticum…consists of exhilaration and lasting euphoria, which does not differ in any way from the normal euphoria of a healthy person…" Über Coca', Centralblatt für die ges. Therapie, 2, pp. 289–314, 1884. This implies, the cognitive effects of engaging in naturally motivating behaviors (i.e., resolution of caloric, hydration, and copulation needs) parallel the effects of taking psychoactive drugs (i.e., the "High"). Interestingly, animals who also engage in naturally motivated behaviors as noted by William James, "…have been observed to seek out substances with mind-altering properties" (Kenny 2007). In fact, intoxication has been proposed as a universal "fourth drive," (following hunger, thirst, and sex)

(Kenny 2007). These ‘drives’, however, are not always on equal footing. Some final common substrate allows for competition among alternative rewards for the time and the investment of the organism and drugs of abuse often win out (Di Chiara, Acquas et al. 1993; Schroeder, Binzak et al. 2001; Kelley, Schiltz et al. 2005).

Drug dependent humans, for example, readily forgo natural and secondary rewards (e.g., money) for drug access and prefer viewing drug-conditioned (non-pharmacological) stimuli (e.g., pictures), over unpleasant images, while non-drug experienced controls avoided cocaine images more than the unpleasant ones (Hamilton, Voris et al. 1998; Heishman, Schuh et al. 2000; Goldstein, Alia-Klein et al. 2007; Goldstein, Parvaz et al. 2008; Moeller, Maloney et al. 2009). Under forced-choice conditions, primates choose cocaine over food almost exclusively (Aigner and Balster 1978). When rats were given long-term access to cocaine, after showing deteriorations in body weight, eating, and general health, 90% of the rats overdosed within 30 days (Bozarth and Wise 1985). Finally, in a more regimented paradigm, rats avoid intake of an otherwise palatable taste cue, referred to as the conditioned stimulus (CS), when it comes through repeated pairings, to predict subsequent access to a powerful drug of abuse, referred to here as the unconditioned stimulus (US). This phenomenon has been studied for decades (see, LeMagnen, 1969) and allows for the investigation of underlying mechanisms and circuitry (Grigson 1997).

1.3 Taste-guided learning: Anticipatory avoidance and CTA

Despite years of study, it is not immediately clear why rats avoid intake of a taste cue when paired with a drug of abuse (Cappell and LeBlanc 1971; Nachman and Hartley 1975; Sklar and Amit 1977). Suppression of CS intake may relate to the drugs rewarding properties (Grigson 1997), aversive

properties (Nachman, Lester et al. 1970; van der Kooy, O'Shaughnessy et al. 1983; Ettenberg and Geist 1991; Geist and Ettenberg 1997), and/or to addictive properties (Grigson, Twining et al. 2008; Wheeler, Twining et al. 2008; Liu, Showalter et al. 2009). Consummatory behaviors are highly susceptible to post-ingestive consequences (Garcia, Hankins et al. 1974; Le Magnen 1984). In support, when a food source provides vital nutrients the preference for that food is increased (Pfaffmann, Norgren et al. 1977). We know that food-deprived rats devalue or avoid intake of a lesser valued gustatory CS that predicts brief access to a more rewarding calorie-dense sucrose US solution(Flaherty and Checke 1982). This phenomenon, referred to as an Anticipatory Contrast Effect (ACE), depends on the relative value of the first reward cue (i.e., the CS) vs. the second reward (i.e., US). Of course, this effect can be obtained even when rats are not food-deprived and even when non-caloric saccharin solutions serve as the lesser valued cue and the more valued consequence (Flaherty and Rowan 1986; Flaherty and Grigson 1989; Warwick, Bowen et al. 1997). Together, these data demonstrate that conditioned taste avoidance can be mediated by positive consequences.

Finally, if flavor perception or food consumption results in sickness, the food source and similar flavored foods are actively rejected and treated as disgusting even during moderate deprivation (Pfaffmann 1964). In the laboratory, this phenomenon is studied by pairing a novel gustatory stimulus with illness induced by either lithium chloride (LiCl) or x-radiation, for example (Garcia, Kimeldorf et al. 1955; Smith, Morris et al. 1964; Barker and Smith 1974). LiCl-induced conditioned taste aversion (CTA*)* is distinct from the avoidance of the taste cue induced by anticipatory contrast. In this case, devaluation and avoidance of the taste cue arises via an association of the novel taste cue with the negative US consequence of visceral malaise due to poisoning or visceral malaise, for example(Nachman and Ashe 1973; Coil, Hankins et al. 1978; Pelchat, Grill et al. 1983; Rabin and Hunt 1983; Spector, Smith et al. 1986; Spector, Breslin et al. 1988) . Given these different taste-guided

phenomenon, and the evidence that avoidance of an otherwise palatable taste cue can occur when the taste cue predicts a highly rewarding outcome (i.e., ACE) or a highly aversive outcome (i.e., CTA), the initial aim of this thesis work was to use lesions of the bilateral insular gustatory cortex (GCTx) to determine whether the circuit mediating drug-induced suppression of CS intake matched that involved in other instances of taste-guided reward or aversion learning.

1.4 Central gustatory processing: The dorsal vs. ventral tract

Taste detection occurs via taste specific receptors (Zhang, Hoon et al. 2003; Kim, Breslin et al. 2004) which are restricted to the anterior 2/3 and posterior 1/3 of the tongue, palate, and upper esophagus (Mistretta 1972). Taste buds and surrounding epithelium are innervated by fibers from several cranial nerves (CN V, VII, IX and X) (Frank and Pfaffmann 1969; Contreras, Beckstead et al. 1982; Miller and Spangler 1982). These fibers project to the nucleus of the solitary tract, NST (Norgren and Leonard 1971; Travers, Pfaffmann et al. 1986). In Sprague Dawley rats, the rostral NST (rNST) mainly sends fibers to the ipsilateral medial pontine parabrachial nuclei, mPBN (Norgren and Pfaffmann 1975). Use of that information in learning, however, does. To this end, the mPBN send two sets of projections: A ventral pathway diffuses unilaterally to at least 3 major limbic regions including the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST); The dorsal pathway projects ipsilaterally and contralaterally from the mPBN relays to the parvicellular part of the ventral posteromedial nucleus of the thalamus (VPMpc). From there taste information is transferred by an ipsilateral projection to the insular gustatory cortex (GC) (Benjamin and Akert 1959; Wolf 1968; Norgren and Wolf 1975; Kosar, Grill et al. 1986).

1.5 The effect of central gustatory lesions on taste-guided learning

Table 1-1 summarizes the disruptive effects of bilateral lesions of the gustatory pons (PBNx), taste thalamus (THLx) or insular taste cortex (GCTx) on distinct devaluation learning induced by sucrose-induced ACE, consummatory successive negative contrast (SNC), drug-induced taste avoidance, and LiCl-induced CTA (see rows 1-4, respectively). A SNC effect is another form of contrast where rats, with a history of brief daily access to a very concentrated sucrose reward, for example, are unwilling to consume a lesser valued sucrose solution when unexpectedly downshifted from the high to the low reward (see Flaherty, 1996, for a review). This form of contrast is included in this summary because it involves processes and circuits that tend to overlap with ACE. The impact of these lesions is designated as **No effect, BLOCKED, or untested (?)** in Table 1-1.

Lesions BLOCKED avoidance of CS **NO EFFECT** of lesion on CS Intake	Bilateral PBN Lesions (PBNx)	Bilateral taste thalamus Lesions (THLx)	Lesions of taste Insular Cortex (GCTx)
sucrose-induced anticipatory contrast effect (ACE)	**BLOCKED**	**BLOCKED**	**?**
consummatory successive negative contrast (SNC)	**BLOCKED**	**BLOCKED**	**?**
Addictive substance-induced CS devaluation	**BLOCKED**	**BLOCKED**	**BLOCKED** **Mackey et al., 1986**
LiCl--induced CTA	**BLOCKED**	**No effect**	**No effect** **Mackey et al., 1986**

Table 1-1: Summary of bilateral lesions of PBNx, THLx GCTx on conditioned taste devaluation.

First, as shown in Table 1-1, column 1, the bilateral lesions of the gustatory PBNx (PBNx) blocked all means of suppression of intake (ACE, SNC, drug-induced suppression, and LiCl-induced CTA. Bilateral lesions of the gustatory thalamus (THLx) and cortex (GCTx), on the other hand, had no

effect on LiCl-induced CTA, but blocked suppression of intake induced by ACE, SNC, and by pairings with morphine (Mackey, Keller et al. 1986; Flynn, Grill et al. 1991; Spector, Norgren et al. 1992; Bechara, Martin et al. 1993; Grigson, Spector et al. 1994; Reilly and Trifunovic 1999; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005; Liang and Norgren 2009).

From these data, we drew two conclusions: (1) Drug-induced suppression more closely parallels sucrose-induced suppression of CS intake than LiCl-induced CTA. (2) Drug-induced suppression of CS intake depends upon either (a) the gustatory cortex or (b) upon communication between the gustatory thalamus and gustatory cortex. Many of the chapters to follow tested this latter hypothesis in rats with asymmetric lesions of the gustatory thalamus and cortex (e.g., left thalamus and right cortex). Given the ipsilateral and reciprocal anatomy of the gustatory thalamus and the cortex, such a lesion serves to functionally disconnect this gustatory thalamocoritical loop. Specifically, we predict that consummatory avoidance requires an intact gustatory thalamococrtical loop when consumption of the tastant is reduced by (a) anticipation of drug delivery or access, (b) the memory of a previously received preferred reward (SNC) or (c) anticipation of access to a highly preferred sucrose reward (ACE). This same lesion, however, was predicted to have no impact on CTA learning. In some cases, the conclusions were strengthened through the use of within subjects studies; where the same lesioned rats might failed at one task, while being successful at another). Since all of the CS's engage the ascending gustatory pathway the effectiveness of the same lesion could be tested in different paradigm. Chapter 2 of this thesis describes the effect of bilateral lesions of the insular gustatory cortex (GCTx) on acquisition of morphine-, cocaine-, and LiCl-induced suppression of CS intake (see Geddes, 2008). The effect of the same bilateral cortical lesion on sucrose-sucrose and saccharin-sucrose ACE is described in Chapter 3. Chapter 4 introduces the asymmetric gustatory THCx lesion and, thereby, describes the role of the gustatory thalamocoritical loop in the expression of a SNC effect (i.e., in

avoidance of a lesser valued reward following the unexpected downshift from a preferred reward). Chapter 5 tests whether gustatory THCx lesions prevented a preoperatively acquired taste-drug avoidance response and, thereafter, whether the same THCx rats would also avoid a novel taste cue paired with cocaine, or between a novel taste cue and LiCl. Chapter 6 is the Thesis General Discussion.

Bilbliography

Aigner, T. G. and R. L. Balster (1978). "Choice Behavior in Rhesus Monkeys: Cocaine Versus Food." Science **201**(4355): 534-535.

Baler, R. D. and N. D. Volkow (2006). "Drug addiction: the neurobiology of disrupted self-control." Trends Mol Med **12**(12): 559-66.

Barker, L. M. and J. C. Smith (1974). "A comparison of taste aversions induced by radiation and lithium chloride in CS-US and US-CS paradigms." J Comp Physiol Psychol **87**(4): 644-54.

Barrow, S. M., D. B. Herman, et al. (1999). "Mortality among homeless shelter residents in New York City." Am J Public Health **89**(4): 529-34.

Bechara, A., G. M. Martin, et al. (1993). "The parabrachial nucleus: a brain stem substrate critical for mediating the aversive motivational effects of morphine." Behav Neurosci **107**(1): 147-60.

Benjamin, R. M. and K. Akert (1959). "Cortical and thalamic areas involved in taste discrimination in the albino rat." J Comp Neurol **111**: 231-59.

Berlin, H. A., E. T. Rolls, et al. (2004). "Impulsivity, time perception, emotion and reinforcement sensitivity in patients with orbitofrontal cortex lesions." Brain **127**(Pt 5): 1108-26.

Blankenship, K. M., A. B. Smoyer, et al. (2005). "Black-white disparities in HIV/AIDS: the role of drug policy and the corrections system." J Health Care Poor Underserved **16**(4 Suppl B): 140-56.

Bozarth, M. A. and R. A. Wise (1985). "**Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat**." Jama **254**(1): 81-3.

Brecher, E. M. and t. e. o. C. Reports. (1972). "Licit and Illicit Drugs. ." The Consumers Union Report on Narcotics, Stimulants, Depressants, Inhalants, Halluncinogens, and Marijuana--including Caffeine, Nicotine, and Alcohol. **Boston: Little, Brown and Company. .**

Caldwell, A. (1978). History of Psychopharmacology. . New York, New York: Academic Press

Calzavara, L. M., A. N. Burchell, et al. (2003). "Prior opiate injection and incarceration history predict injection drug use among inmates." Addiction **98**(9): 1257-65.

Cappell, H. and A. E. LeBlanc (1971). "Conditioned aversion to saccharin by single administrations of mescaline and d-amphetamine." Psychopharmacology **22**(4): 352-356.

Coil, J. D., W. G. Hankins, et al. (1978). "The attenuation of a specific cue-to-consequence association by antiemetic agents." Psychopharmacology (Berl) **56**(1): 21-5.

Contreras, R. J., R. M. Beckstead, et al. (1982). "The central projections of the trigeminal, facial, glossopharyngeal and vagus nerves: an autoradiographic study in the rat." J Auton Nerv Syst **6**(3): 303-22.

Cuellar, A. E., K. J. Kelleher, et al. (2008). "Incarceration and psychotropic drug use by youth." Arch Pediatr Adolesc Med **162**(3): 219-24.

Davis, C., S. Strachan, et al. (2004). "Sensitivity to reward: implications for overeating and overweight." Appetite **42**(2): 131-8.

Davis, W. M. and S. G. Smith (1976). "Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior." Pavlov J Biol Sci **11**(4): 222-36.

Davis, W. R., B. D. Johnson, et al. (2006). "Risks for HIV infection among users and sellers of crack, powder cocaine and heroin in central Harlem: implications for interventions." AIDS Care **18**(2): 158-65.

Dawson, G. (1997). "The African-American male: brief insights on health, drugs, and incarceration--Part 1." J Natl Med Assoc **89**(9): 580-4.

Di Chiara, G., E. Acquas, et al. (1993). "Drugs of abuse: biochemical surrogates of specific aspects of natural reward?" Biochem Soc Symp **59**: 65-81.

Dudish-Poulsen, S. A. and D. K. Hatsukami (1997). "Dissociation between subjective and behavioral responses after cocaine stimuli presentations." Drug Alcohol Depend **47**(1): 1-9.

Ettenberg, A. and T. D. Geist (1991). "Animal model for investigating the anxiogenic effects of self-administered cocaine." Psychopharmacology (Berl) **103**(4): 455-61.

Flaherty, C. F. and S. Checke (1982). ""anticipation of incentive gain."" Animal learning and Behavior(10): 171-182.

Flaherty, C. F. and P. S. Grigson (1989). "Effect of clonidine on sucrose intake and water intake varies as a function of dose, deprivation state, and duration of exposure." Pharmacol Biochem Behav **32**(2): 383-9.

Flaherty, C. F. and G. A. Rowan (1986). "Successive, simultaneous, and anticipatory contrast in the consumption of saccharin solutions." J Exp Psychol Anim Behav Process **12**(4): 381-93.

Flynn, F. W., H. J. Grill, et al. (1991). "Central gustatory lesions: II. Effects on sodium appetite, taste aversion learning, and feeding behaviors." Behav Neurosci **105**(6): 944-54.

Frank, M. and C. Pfaffmann (1969). "Taste nerve fibers: a random distribution of sensitivities to four tastes." Science **164**(884): 1183-5.

French, M. T., J. A. Mauskopf, et al. (1996). "Estimating the dollar value of health outcomes from drug-abuse interventions." Med Care **34**(9): 890-910.

Garcia, J., W. G. Hankins, et al. (1974). "Behavioral regulation of the milieu interne in man and rat." Science **185**(4154): 824-31.

Garcia, J., D. J. Kimeldorf, et al. (1955). "Conditioned aversion to saccharin resulting from exposure to gamma radiation." Science **122**(3160): 157-8.

Gardner, E. L. and J. H. Lowinson (1993). "Drug craving and positive/negative hedonic brain substrates activated by addicting drugs." Seminars in Neuroscience

The Neurobiology of Drug Addiction/Dependency **5**(5): 359-368.

Geist, T. D. and A. Ettenberg (1997). "Concurrent positive and negative goalbox events produce runway behaviors comparable to those of cocaine-reinforced rats." Pharmacol Biochem Behav **57**(1-2): 145-50.

Goldstein, R. Z., N. Alia-Klein, et al. (2007). "Is decreased prefrontal cortical sensitivity to monetary reward associated with impaired motivation and self-control in cocaine addiction?" Am J Psychiatry **164**(1): 43-51.

Goldstein, R. Z., M. A. Parvaz, et al. (2008). "Compromised sensitivity to monetary reward in current cocaine users: an ERP study." Psychophysiology **45**(5): 705-13.

Grigson, P. S. (1997). "Conditioned taste aversions and drugs of abuse: a reinterpretation." Behav Neurosci **111**(1): 129-36.

Grigson, P. S., P. Lyuboslavsky, et al. (2000). "Bilateral lesions of the gustatory thalamus disrupt morphine- but not LiCl-induced intake suppression in rats: evidence against the conditioned taste aversion hypothesis." Brain Res **858**(2): 327-37.

Grigson, P. S., A. C. Spector, et al. (1994). "Lesions of the pontine parabrachial nuclei eliminate successive negative contrast effects in rats." Behav Neurosci **108**(4): 714-23.

Grigson, P. S., R. C. Twining, et al. (2008). Drug-induced suppression of CS intake: Reward, aversion, and addiction. Conditioned Taste Aversion: Behavioral and Neural Processes,. NY, NY., Oxford University Press.

Grilly, D. M. (1992). Drugs and Human Behavior, Allyn & Bacon.

Grobsmith, E. S. and J. Dam (1990). "The revolving door: substance abuse treatment and criminal sanctions for Native American offenders." J Subst Abuse **2**(4): 405-25.

Gross, M., W. DeJong, et al. (1994). ""Drugs and AIDS--reaching for help": a videotape on AIDS and drug abuse prevention for criminal justice populations." J Drug Educ **24**(1): 1-20.

Hamid, A., R. Curtis, et al. (1997). "The heroin epidemic in New York City: current status and prognoses." J Psychoactive Drugs **29**(4): 375-91.

Hamilton, M. E., J. C. Voris, et al. (1998). "Money as a tool to extinguish conditioned responses to cocaine in addicts." J Clin Psychol **54**(2): 211-8.

Hammersley, R., A. Forsyth, et al. (1989). "The relationship between crime and opioid use." Br J Addict **84**(9): 1029-43.

Heishman, S. J., K. J. Schuh, et al. (2000). "Reinforcing and subjective effects of morphine in human opioid abusers: effect of dose and alternative reinforcer." Psychopharmacology **148**(3): 272-80.

Kandel, D., E. Single, et al. (1976). "The epidemiology of drug use among New York State high school students: Distribution, trends, and change in rates of use." Am J Public Health **66**(1): 43-53.

Kelley, A. E. (2004). "Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning." Neurosci Biobehav Rev **27**(8): 765-76.

Kelley, A. E., C. A. Schiltz, et al. (2005). "Neural systems recruited by drug- and food-related cues: Studies of gene activation in corticolimbic regions." Physiology & Behavior **86**(1-2): 11-14.

Kenny, P. J. (2007). "Brain reward systems and compulsive drug use." Trends Pharmacol Sci **28**(3): 135-41.

Kim, U. K., P. A. Breslin, et al. (2004). "Genetics of human taste perception." J Dent Res **83**(6): 448-53.

Koob, G. F. and F. Weiss (1992). "Neuropharmacology of cocaine and ethanol dependence." Recent Dev Alcohol **10**: 201-33.

Kosar, E., H. J. Grill, et al. (1986). "Gustatory cortex in the rat. II. Thalamocortical projections." Brain Res **379**(2): 342-52.

Krinsky, C. S., S. L. Lathrop, et al. (2009). "Drugs, detention, and death: a study of the mortality of recently released prisoners." Am J Forensic Med Pathol **30**(1): 6-9.

Le Magnen, J. (1984). "Ingestive behaviour in the homeostatic control of internal environment." Appetite **5**(2): 159-67.

Lett, B. T. (1989). "Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine." Psychopharmacology (Berl) **98**(3): 357-62.

Liang, N.-C. and R. Norgren. (2009). "Pontine and thalamic influence on oral sucrose and oil reward." from http://etda.libraries.psu.edu/theses/approved/WorldWideIndex/ETD-4257/index.html

Liu, C., J. Showalter, et al. (2009). "Ethanol-induced conditioned taste avoidance: reward or aversion?" Alcohol Clin Exp Res **33**(3): 522-30.

Mackey, W. B., J. Keller, et al. (1986). "Visceral cortex lesions block conditioned taste aversions induced by morphine." Pharmacol Biochem Behav **24**(1): 71-8.

Manchikanti, L. (2006). "Prescription drug abuse: what is being done to address this new drug epidemic? Testimony before the Subcommittee on Criminal Justice, Drug Policy and Human Resources." Pain Physician **9**(4): 287-321.

Miller, I. J. and K. Spangler (1982). "Taste bud distribution and innervation on the palate of the rat." Chemical Senses **7**: 99 - 108.

Mistretta, C. M. (1972). "Topographical and histological study of the developing rat tongue, palate and taste buds." Third Symposium on Oral Sensation and Perception: The Mouth of the Infant: 163 - 187.

Moeller, S. J., T. Maloney, et al. (2009). "Enhanced choice for viewing cocaine pictures in cocaine addiction." Biol Psychiatry **66**(2): 169-76.

Moore, L. D. and A. Elkavich (2008). "Who's using and who's doing time: incarceration, the war on drugs, and public health." Am J Public Health **98**(5): 782-6.

Nachman, M. and J. H. Ashe (1973). "Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl." Physiol Behav **10**(1): 73-8.

Nachman, M. and P. L. Hartley (1975). "Role of illness in producing learned taste aversions in rats: A comparison of several rodenticides." Journal of Comparative and Physiological Psychology **89**(9): 1010-1018.

Nachman, M., D. Lester, et al. (1970). "Alcohol aversion in the rat: behavioral assessment of noxious drug effects." Science **168**(936): 1244-6.

Narevic, E., T. F. Garrity, et al. (2006). "Factors predicting unmet health services needs among incarcerated substance users." Subst Use Misuse **41**(8): 1077-94.

Nestler, E. J., B. T. Hope, et al. (1993). "Drug addiction: A model for the molecular basis of neural plasticity." Neuron **11**(6): 995-1006.

Norgren, R. and C. M. Leonard (1971). "Taste pathways in rat brainstem." Science **173**(2): 1136-9.

Norgren, R. and C. Pfaffmann (1975). "The pontine taste area in the rat." Brain Res **91**(1): 99-117.

Norgren, R. and G. Wolf (1975). "Projections of thalamic gustatory and lingual areas in the rat." Brain Res **92**(1): 123-9.

Pelchat, M. L., H. J. Grill, et al. (1983). "Quality of acquired responses to tastes by Rattus norvegicus depends on type of associated discomfort." Journal of Comparative Psychology **97**(2): 140-153.

Pfaffmann, C. (1964). "Taste, its sensory and motivating properties." Am Sci **52**: 187 - 206.

Pfaffmann, C., R. Norgren, et al. (1977). "Sensory affect and motivation." Ann N Y Acad Sci **290**: 18-34.

Potenza, M. N., M. A. Steinberg, et al. (2000). "Illegal behaviors in problem gambling: analysis of data from a gambling helpline." J Am Acad Psychiatry Law **28**(4): 389-403.

Rabin, B. M. and W. A. Hunt (1983). "Effects of antiemetics on the acquisition and recall of radiation- and lithium chloride-induced conditioned taste aversions." Pharmacology Biochemistry and Behavior **18**(4): 629-635.

Reilly, S. and R. Trifunovic (1999). "Gustatory thalamus lesions eliminate successive negative contrast in rats." Behav Neurosci **113**(6): 1242-8.

Schroeder, B. E., J. M. Binzak, et al. (2001). "A common profile of prefrontal cortical activation following exposure to nicotine- or chocolate-associated contextual cues." Neuroscience **105**(3): 535-45.

Schroy, P. L., R. A. Wheeler, et al. (2005). "Role of gustatory thalamus in anticipation and comparison of rewards over time in rats." Am J Physiol Regul Integr Comp Physiol **288**(4): R966-80.

Sklar, L. S. and Z. Amit (1977). "Manipulations of catecholamine systems block the conditioned taste aversion induced by self-administered drugs." Neuropharmacology **16**(10): 649-55.

Smith, B., H. Jones, et al. (2001). "Physiological, subjective and reinforcing effects of oral and intravenous cocaine in humans." Psychopharmacology **156**(4): 435-444.

Smith, J. C., D. D. Morris, et al. (1964). "Conditioned Aversion to Saccharin Solution with High Dose Rates of X-Rays as the Unconditioned Stimulus." Radiat Res **22**: 507-10.

Spector, A. C., P. Breslin, et al. (1988). "Taste reactivity as a dependent measure of the rapid formation of conditioned taste aversion: a tool for the neural analysis of taste-visceral associations." Behav Neurosci **102**(6): 942-52.

Spector, A. C., R. Norgren, et al. (1992). "Parabrachial gustatory lesions impair taste aversion learning in rats." Behav Neurosci **106**(1): 147-61.

Spector, A. C., J. C. Smith, et al. (1986). "Radiation-induced taste aversion: effects of radiation exposure level and the exposure-taste interval." Radiat Res **106**(2): 271-7.

Susman, R. M. (1975). "Drug Abuse, Congress and the Fact-Finding Process." The ANNALS of the American Academy of Political and Social Science, **417** (1): 16-26.

Travers, S. P., C. Pfaffmann, et al. (1986). "Convergence of lingual and palatal gustatory neural activity in the nucleus of the solitary tract." Brain Research **365**(2): 305-20.

van der Kooy, D., M. O'Shaughnessy, et al. (1983). "Motivational properties of ethanol in naive rats as studied by place conditioning." Pharmacol Biochem Behav **19**(3): 441-5.

Vetulani, J. (2001). "Drug addiction. Part I. Psychoactive substances in the past and presence." Pol J Pharmacol **53**(3): 201-14.

Warwick, Z. S., K. J. Bowen, et al. (1997). "Learned suppression of intake based on anticipated calories: cross-nutrient comparisons." Physiol Behav **62**(6): 1319-24.

Wheeler, R. A., R. C. Twining, et al. (2008). "Behavioral and electrophysiological indices of negative affect predict cocaine self-administration." Neuron **57**(5): 774-85.

White, N. M. (1996). "Addictive drugs as reinforcers: multiple partial actions on memory systems." Addiction **91**(7): 921-49; discussion 951-65.

Wise, R. A. (1984). "Neural mechanisms of the reinforcing action of cocaine." NIDA Res Monogr **50**: 15-33.

Wolf, G. (1968). "Projections of thalamic and cortical gustatory areas in the rat." J Comp Neurol **132**(4): 519-30.

Wolffgramm, J. and A. Heyne (1995). "From controlled drug intake to loss of control: the irreversible development of drug addiction in the rat." Behavioural Brain Research **70**(1): 77-94.

Zhang, Y., M. A. Hoon, et al. (2003). "Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways." Cell **112**(3): 293-301.

Gustatory Insular Cortex Lesions Disrupt Drug-Induced, but not Lithium Chloride-Induced, Suppression of Conditioned Stimulus Intake

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ABSTRACT

Rats suppress intake of a normally preferred 0.15% saccharin conditioned stimulus (CS) when it is paired with an aversive agent like lithium chloride (LiCl) or a preferred substances such as sucrose or a drug of abuse. The reward comparison hypothesis suggests that rats avoid intake of a saccharin cue following pairings with a drug of abuse because the rats are anticipating the availability of the rewarding properties of the drug. The present study used bilateral ibotenic acid lesions to examine the role of the gustatory cortex in the suppression of CS intake induced by cocaine, morphine, and LiCl. The results show that bilateral lesions of the insular gustatory cortex (1) fully prevent the suppressive effects of both a 15 and a 30 mg/kg dose of morphine, (2) attenuate the suppressive effect of a 10 mg/kg dose of cocaine, but (3) are overridden by a 20 mg/kg dose of the drug. Finally, these same cortical lesions had no impact on LiCl-induced conditioned taste aversion. The current data show that the insular taste cortex plays an integral role in drug-induced avoidance of a gustatory CS.

Keywords: reward comparison, drugs of abuse, anticipatory contrast, avoidance, CTA

Introduction

Rats avoid intake of a saccharin cue when paired with an aversive, illness-inducing agent, such as lithium chloride (LiCl) or x-radiation (Barker & Smith, 1974; Nachman & Ashe, 1973). They also avoid intake of the same saccharin conditioned stimulus (CS) when paired with a drug of abuse (Goudie, Dickins, & Thornton, 1978; Kulkosky, Sickel, & Riley, 1980; Kumar, Pratt, & Stolerman, 1983; LeBlanc & Cappell, 1975; Wise, Yokel, & DeWit, 1976). With further testing, however, the parallels between LiCl and drugs of abuse break down. Although LiCl and drugs of abuse both suppress CS intake, LiCl promotes conditioned place aversion and suppression of operant responding for the emetic agent (Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982; Paredes-Olay & Lopez, 2002; Schalomon, Robertson, & Laferriere, 1994; Turenne, Miles, Parker, & Siegel, 1996). Drugs of abuse, on the other hand, produce conditioned place preferences and increased operant responding for the abused substance (D'Mello, Goldberg, Goldberg, & Stolerman, 1981; Gomez, 2001; LeBlanc & Cappell, 1975; Mackey, Keller, & van der Kooy, 1986; Schenk & Partridge, 1999; Wakonigg, Sturm, Saria, & Zernig, 2003; Wise et al., 1976; Zito, Bechara, Greenwood, & van der Kooy, 1988). In fact, the simultaneous display of a "positive" instrumental response (i.e., increased running speed, place preference, and drug-seeking) and a "negative" consummatory response (i.e., avoidance of the associated gustatory CS) was thought to be provoked by simultaneous opposing positive and negative properties of abused drugs, thereby creating a theoretical paradox (Corrigall, Linseman, D'Onofrio, & Lei, 1986; Hunt & Amit, 1987; Parker, 1995; White, Sklar, & Amit, 1977).

The reward comparison hypothesis attempts to resolve this paradox by postulating that the same rewarding properties that drive increased operant responding for drugs of abuse also drive the decrease in intake of an associated gustatory cue (Grigson, 1997). This hypothesis is based on a phenomenon, referred to as anticipatory contrast, in which rats reduce intake of a normally preferred saccharin CS

when it comes to predict access to more preferred 32% sucrose solution (Flaherty & Checke, 1982). In support, the suppressive effect of a rewarding sucrose unconditioned stimulus (US) and a drug of abuse, but not LiCl, are similarly affected by the deprivation state of the rat, the strain of the rat, and by a history of chronic exposure to morphine (Glowa, Shaw, & Riley, 1994; Grigson & Freet, 2000; Grigson, Lyuboslavsky, Tanase, & Wheeler, 1999; Grigson, Twining, & Carelli, 2000; Grigson, Wheeler, Wheeler, & Ballard, 2001). Data from lesion experiments also support the reward comparison hypothesis by demonstrating that different central gustatory pathways mediate reward contrast effects and learned aversions. For example, bilateral lesions of the gustatory thalamus (THLx) have no impact on the development of a LiCl-induced conditioned taste aversion (CTA), but fully prevent morphine- and sucrose-induced suppression of CS intake (Flynn, Grill, Schulkin, & Norgren, 1991; Grigson, Lyuboslavsky, & Tanase, 2000; Mungarndee, Lundy, & Norgren, 2006; Reilly & Pritchard, 1996b, 1997; Scalera, Grigson, & Norgren, 1997; Schroy et al., 2005). Given the strong projections from taste thalamus to the insular cortex, however, it is not clear whether an intact taste thalamus is critical for the phenomenon, or whether the taste thalamus is involved simply because it serves as a conduit to the taste cortex (Benjamin & Pfaffmann, 1955; Norgren & Leonard, 1971; Norgren & Wolf, 1975; Wolf, 1968).

Evidence in support of the latter conclusion is provided by reports showing that bilateral lesions of the taste cortex, like lesions of the taste thalamus, selectively disrupt sucrose- and morphine-, but not LiCl-induced avoidance of the CS (Kiefer & Orr, 1992; Mackey et al., 1986; Zito et al., 1988). Despite these data, a firm conclusion regarding the function of the gustatory cortex in these phenomena is hampered by methodological constraints, a paucity of data, and mixed results. In fact, other data suggest that the insular gustatory cortex is required for normal acquisition and retention of a CTA (Dunn & Everitt, 1988; Escobar, Fernandez, Guevara-Aguilar, & Bermudez-Rattoni, 1989; Schafe & Bernstein, 1996; Yamamoto, Yuyama, Kato, & Kawamura, 1984).

Regarding the apparent disruptive effect of lesions of the taste cortex on drug-induced suppression of CS intake, only morphine has been investigated and, only at a single dose of the drug (Mackey et al., 1986; Zito et al., 1988). In the single known study that specifically examined the role of the taste cortex in sucrose-induced suppression of CS intake (i.e., anticipatory contrast), Kesner and Gilbert (2007) failed to include an "un-shifted" control group, thus complicating the interpretation of the data. Finally, the disruptive effect of lesions of the gustatory insular cortex on CTA learning depends on the training procedure (i.e., intraoral infusion vs. bottle licking) and the type of CS (e.g., saccharin or sucrose) employed (Fresquet, Angst, & Sandner, 2004; Koh & Bernstein, 2005; Wilkins & Bernstein, 2006; Yamamoto, Sako, Sakai, & Iwafune, 1997). The current experiments sought to resolve these conflicting data. Thus, Experiment 1 tested whether lesions of the taste cortex would disrupt the suppressive effect of cocaine, another drug of abuse. Experiment 2 tested whether the same cortex lesions would disrupt drug-induced suppression of CS intake when a high dose of morphine, served as the US. Finally, Experiment 3 tested whether these lesions would disrupt CTA learning when using a relatively low or a standard dose of LiCl.

Experiment 1: Bilateral GCTx and taste-cocaine pairings

The objective of Experiment 1 was to test whether the gustatory cortex, the main projection target of the taste thalamus (Krettek & Price, 1977; Norgren & Wolf, 1975), is essential for the conditioned suppression of CS intake that follows taste-drug pairings. As mentioned, both bilateral gustatory thalamic (electrophysiologically guided) and insular gustatory cortical (stereotaxically placed) excitotoxic lesions fully prevent morphine- but not LiCl-induced suppression of CS intake (Grigson, Lyuboslavsky, et al., 2000; Mackey et al., 1986; Zito et al., 1988). Furthermore, drug-induced suppression of CS intake, but not LiCl-induced CTA, was blocked by (1) pretreatment with centrally acting cholinergic antagonist atropine or (2) by injecting a dopamine antagonist directly in

the insular cortex (Hunt, Segal, & Amit, 1987; Hunt, Switzman, & Amit, 1985). Unpublished data from our laboratory suggest that lesions of the taste thalamus also disrupt cocaine-induced suppression of intake of a taste cue. The disruptive effect of the lesion, however, was overridden when using a higher 20 mg/kg dose of cocaine (Twining, Baldwin, Palomo, Han, Horvath, & Grigson, 2008). The present study, then, was designed to test whether bilateral ibotenic acid lesions of the gustatory cortex also will disrupt the suppressive effect of a standard dose of cocaine (i.e., 10 mg/kg), but not that induced by a higher dose of the drug (i.e., 20 mg/kg).

Methods:

Subjects

The subjects were 70 naı¨ve male Sprague–Dawley rats (Charles River) weighing 250 to 350 g at the beginning of the experiment. Experiment 1 was run in two complete replications consisting of 33 and 37 rats, respectively. The rats were individually housed in standard suspended stainless steel wire mesh cages in a temperature- and humidity-controlled colony room maintained on a 12-hr light–dark cycle. Food (Teklad #6068) and distilled water (dH2O) were available ad libitum, unless otherwise noted. During all experiments, acquisition trials were conducted in the home cages using inverted Nalgene graduated cylinders with silicone stoppers and stainless steel spouts attached to the front of the cage with springs. Intake was measured to the nearest 0.5 ml.

Drugs and solutions

The CS was a 0.03 M Polycose solution (Sigma Chemical Company, St. Louis, MO) prepared 24 h in advance and presented at room temperature. This concentration of Polycose is highly preferred by rats and readily supports CTA learning (Sako et al., 1994; Smith, Norgren, & Grigson, 2004). The US, cocaine hydrochloride, was generously provided by the National Institute on Drug Abuse (NIDA) and mixed in 0.9% saline every morning, approximately 1 hr prior to the CS–US pairings. The cocaine

was prepared as a stock solution (1.5 mg/ml) in saline and the dose [injected subcutaneously (s.c.)] was adjusted for bodyweight. This regimen diluted the solution to avoid necrosis at the injection site (Durazzo, Gauvin, Goulden, Briscoe, & Holloway, 1994; Mayer & Parker, 1993).

Surgery

Thirty-six rats received bilateral injections of 0.2 _l of ibotenic acid over 10 min in the gustatory cortical region of each cortex (Group GCTx), 16 received similar infusions of sodium phosphate buffer (PBS), and 18 served as nonsurgical controls (NSC). The coordinates for the stereotaxically placed lesions are modified from those of Mackey and colleagues (Mackey et al., 1986). Prior to surgery, each rat was injected with atropine sulfate (0.25 mg/ i.p.) and Gentamicin (6 mg/kg, i.p.). Twenty minutes later, each rat was injected with pentobarbital sodium (50 mg/kg, i.p.), which was supplemented throughout surgery as necessary. Body temperature was maintained at 37 ^{o} 1 °C. After the rat reached a surgical level of anesthesia, the head was shaved, secured in a stereotaxic instrument, and the skull leveled between lamda and bregma and then the nose bar was set at -3.3 mm. The skin was cleaned with Betadine and a midline incision was made. Then a hole, centered at 0.5 mm anterior to bregma, was drilled in the skull on either side of the midline, using a ball tip burr. The dura mater was exposed and kept moist with physiological saline as needed. Lesions were placed at the following coordinates: anterior/posterior + 0.5 mm from bregma, medial/lateral +/-4.8 mm, and dorsal/ventral -5.5 mm from the dura mater using a 1-μl Hamilton microsyringe, with a glass pipette (tip: 5–10 μm) glued to its tip. The pipette microsyringe assembly was attached to a carrier and lowered into the target area. Immediately thereafter, 0.2 μl (20 μg/μl) of ibotenic acid was infused over 10-min. After the infusion, the pipette remained in place for 10 more minutes. This procedure was then repeated in the contralateral hemisphere. Controls were treated identically to the GCTx rats

except, instead of ibotenic acid, rats were bilaterally infused with 0.2 μl of PBS (pH = 7.4). After surgery, the holes in the skull were filled with Gelfoam and the incision closed with wound clips.

Procedure

Following at least 5 days recovery from surgery, water was restricted to 5 min in the a.m. and 1 h in the p.m. Daily dH2O intake stabilized within a week. Morning water intake was used to match and assign rats, in a counterbalanced manner, to one of two US conditions: saline or 10 mg/kg cocaine (s.c.). Thus, 16 control lesion rats (8 PBS and 8 NSC) and 18 GCTx rats received saline injections, while the remaining 18 control lesion rats (8 PBS and 10 NSC) and 18 GCTx rats received cocaine as the US. During conditioning, all rats were given 5-min access to 0.03 M Polycose and, after a 5-min interval, were injected s.c. with either saline or cocaine. There was a total of 9 CS–US pairings, with one dH2O day elapsing between each, at which time rats received 5 min access to water in the morning and 1 h each afternoon. The concentration of cocaine was increased from 10 mg/kg to 20 mg/kg on Trials 8 and 9. All rats were given 1 h access to water each afternoon to maintain hydration.

Histology

After completion of all behavioral experiments, the rats were deeply anesthetized with pentobarbital sodium [100 mg/kg (i.p.)] and perfused transcardially for 5 to 10 min with physiological saline, followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer for 15 to 20 min. The brains were stored overnight in 20% sucrose in phosphate buffer (20 g/100 ml) at 4 °C. The next day, the brains were cut into 50-micron thick coronal sections using a freezing microtome. This tissue was immunostained for neuron-specific nuclear protein (NeuN) (Jongen-Relo & Feldon, 2002). This staining procedure permits assessment of possible retrograde damage in the taste thalamus due to the cortical lesion. The sections were collected in phosphate buffer (PB) overnight. Sections were transferred to a PBS solution, incubated for 30 min in 0.5% H2O2, 1 h in blocking solution, and then

24 h in primary antibody. The secondary antibody incubation, avidinbiotin complex (ABC Elite kit) step, and 3,3'−diamino-benzidine (DAB) incubation were each preceded and followed by 3 rinses in PBS for 5 min. Finally, the sections were mounted on gelatincoated slides, allowed to dry overnight, and then cover-slipped using Permount. When processing could not occur immediately, the sections were collected in 30% ethylene glycol glycerol in 50 mM phosphate buffer and stored at -20 °C for approximately 1 week before being thawed and placed in PBS overnight.

Results and Discussion:

Histology

Histological analysis of the 36 rat brains from the GCTx group revealed 28 well-placed bilateral lesions of the gustatory cortex. The remaining eight rats were eliminated from the experiment, five due to misplaced lesions and three because extensive cortical lesions led to marked retrograde degeneration of the corresponding thalamic taste relay. Thus, for Experiment 1, the behavioral data from 34 control lesion rats and 28 GCTx rats contributed to the current statistical analyses. In Figure 1, panels Athrough C depict photomicrographs of NeuN stained coronal sections (2x) of an intact rat brain at 3 of the 5 levels analyzed during cortical lesion assessment. Levels 1 through 5 roughly correspond with Plates 26, 28, 30, 32, and 34 (Paxinos & Watson, 2005). In the labeled photomicrograph shown in panel B of Figure 1, the region identified as agranular/dysgranular insular (AI/DI) represents a section of the insular cortex where gustatory neurons were previously found to have maximal responsiveness to taste stimuli applied to the anterior tongue in rats (Benjamin & Pfaffmann, 1955; Kosar, Grill, & Norgren, 1986a; Yamamoto et al., 1984; Yamamoto, Yuyama, & Kawamura, 1981). On the surface of the brain, this region lies just dorsal to where the middle cerebral artery traverses the rhinal sulcus. In Figure 1, Levels 1 (panel A) and 5 (panel C) are approximately 1 mm anterior and posterior to Level 3 (panel B), respectively.

Panel D of Figure 1 depicts a representative section of an ideal gustatory cortex lesion with damage limited primarily to the gustatory cortex and little to no retrograde degeneration of the corresponding taste thalamus (thalamus not shown). As mentioned earlier, data from three GCTx rats were discarded due to large cortical cysts (Figure 1, panel E) accompanied by extensive cell loss in the thalamic nuclei (i.e., ventroposteromedial pars compacta [VPMpc], ventroposteromedial/ ventroposterolateral [VPM/ VPL], central medial [CM], and posterior [Po] nuclei of the thalamus, see Figure 2, panel D). In three rats the cortical lesions were too small or asymmetric (sparing cortical taste nuclei in either hemisphere) and, therefore, incomplete (Figure 1, panel F). The data from two GCTx rats also were omitted when the histology revealed a complete lack of cortical injury. An intact brain is shown in Figure 2A and 2B under low and higher magnification, respectively), NeuN stained cell bodies can readily be seen throughout the taste thalamus (also referred to as VPMpc) and other structures of the thalamus (i.e., VPM and Po). Figure 2, panel C is a representative section from a GCTx rat that incurred minimal retrograde neuronal cell loss in the taste thalamus. Similar to the control nuclei operated rat brain, the thalamic taste control nuclei of these GCTx rats were evenly stained with the NeuN marker indicating that the vast majority of neurons survived. Finally, Figure 2 panel D depicts a representative brain section with extensive retrograde gustatory thalamic neuronal degeneration. The data from this rat was, of course, eliminated from the analysis.

Figure 2-1

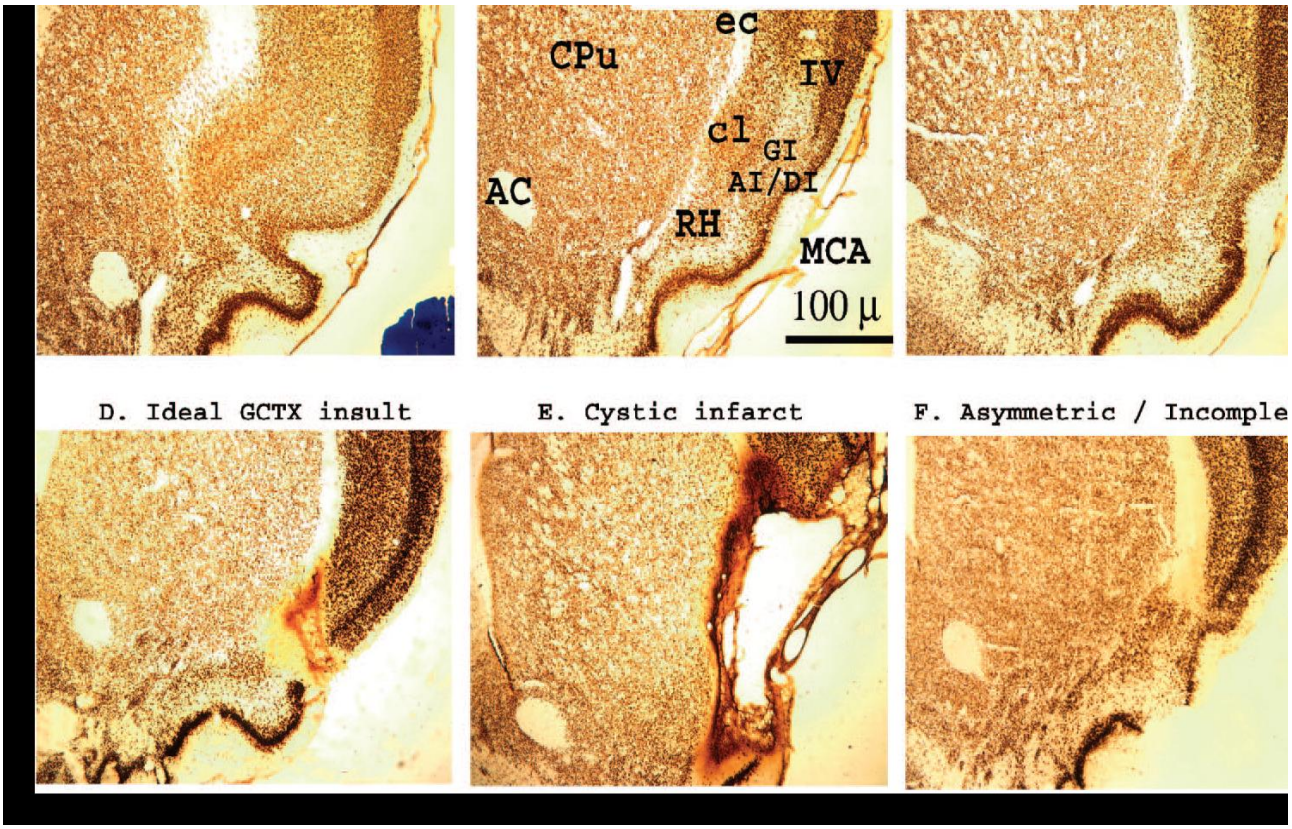

Figure **2-1**: Photomicrographs of NeuN-stained coronal brain sections (2x) through the insular gustatory cortex in Sprague–Dawley rats. As a histological control, the cytoarchitectonic organization of the gustatory and surrounding nuclei was assessed at 5 levels throughout the insular cortex. Sections in A through C represent the rostral (Level 1), middle (Level 3), and caudal (Level 5) limits of the gustatory cortical zone in an intact control lesion Sprague–Dawley rat, respectively (Levels 2 and 4 not shown). Bilateral ibotenic acid lesions of the gustatory cortex (GCTx) were systematically rated from anterior to posterior in five brain sections corresponding with the Levels 1 through 5 (data shown from Levels 1, 3, and 5 in panels D–F). While most ibotenic acid lesions were (D), ideal (i.e., limited to the gustatory cortical nuclei), some were (E), overwhelmingly large resulting in cystic infarction, and others (F), were misplaced or asymmetrical (unilateral), thus sparing some gustatory cortical neurons and rated as misplaced. Of the 19 GCTx rats only the behavioral data for 11 GCTx rats, with complete gustatory lesions, were retained for statistical analysis. In total, the data for 8 GCTx rats were discarded due to incomplete lesions (n = 3), extensive retrograde damage (see Figure 2 panel D) to the taste thalamus (n = 3), or both (n = 2). AC = anterior commissure; AI/DI/GI = agranular/dysgranular/granular insular cortex; cl = claustrum; Cpu = caudate putamen; ec = external capsule; MCA = middle cerebral artery (anterior branch); RH = rhinal horn; IV = cortical layer 4.

Figure 2-2

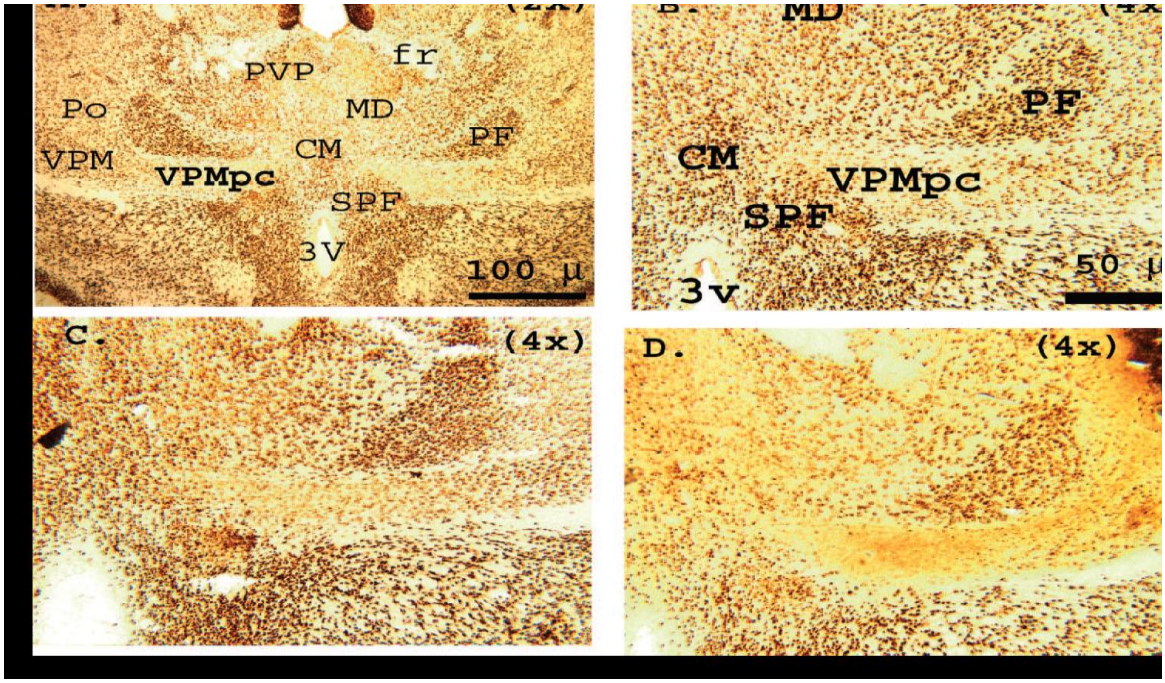

Figure **2-2**: Photomicrographs of NeuN-stained coronal brain sections at the level of the ventral posteromedial (gustatory) thalamus (VPMpc) in Sprague–Dawley rats. Histological analysis of the gustatory thalamus was conducted at four levels, roughly corresponding to Figures 31–34 in the standard laboratory rat stereotaxic atlas (Paxinos & Watson, 2005a). A. Intact rat brain (2x). Note the cytoarchitectonic organization of the gustatory thalamus and surrounding nuclei, the bilateral symmetry of the region (between hemispheres), and the distinct distribution of NeuN stained cell bodies B. Higher (4x) magnification of the gustatory thalamus in the right hemisphere shown in panel A. Despite the dense appearance of other thalamic nuclei (i.e., CM, MD, PF, SPF) NeuN staining within the gustatory thalamic nuclei, was relatively abundant, organized, and uniform. C. Similar NeuN staining was observed in the thalamus of GCTx rats having ideal lesions of the gustatory cortex (4x). These GCTx rat brains appeared anatomically identical to intact rat brains at the level of the gustatory thalamus. In some cases, upon closer inspection, minor cell loss was observed in the gustatory thalamus of a couple of GCTx rat brains, which were otherwise indistinguishable from control lesion rat brains and thus were retained for analysis. In contrast, D depicts a representative section of GCTx rat brains with extensive retrograde degeneration (4_). The total absence of cell bodies indicates extensive neuronal degradation throughout the gustatory thalamus. This damage extends laterally into the VPM/VPL thalamic nucleus. Note the loss of cytoarchitectal organization overall. As previously mentioned the behavioral data for these GCTx rats were omitted from statistical analysis. CM = centromedial thalamus; fr = fronix; MD = mediodorsal thalamus; PF = parafasiculus thalamus; Po = posterior thalamus; PVP = paraventricular posterior thalamus; SPF = subparafasiculus thalamus; VPM/VPL = ventroposteromedial / ventroposterolateral thalamus; VPMpc = pericellular region of the ventroposteromedial (gustatory) thalamus; 3v = third ventricle.

Panel D of Figure 1 depicts a representative section of an ideal gustatory cortex lesion with damage limited primarily to the gustatory cortex and little to no retrograde degeneration of the corresponding taste thalamus (thalamus not shown). As mentioned earlier, data from three GCTx rats were discarded due to large cortical cysts (Figure 1, panel E) accompanied by extensive cell loss in the thalamic nuclei (i.e., ventroposteromedial pars compacta [VPMpc], ventroposteromedial/ ventroposterolateral [VPM/ VPL], central medial [CM], and posterior [Po] nuclei of the thalamus, see Figure 2, panel D). In three rats the cortical lesions were too small or asymmetric (sparing cortical taste nuclei in either hemisphere) and, therefore, incomplete (Figure 1, panel F). The data from two GCTx rats also were omitted when the histology revealed a complete lack of cortical injury. An intact brain is shown in Figure 2A and 2B under low and higher magnification, respectively), NeuN stained cell bodies can readily be seen throughout the taste thalamus (also referred to as VPMpc) and other structures of the thalamus (i.e., VPM and Po). Figure 2, panel C is a representative section from a GCTx rat that incurred minimal retrograde neuronal cell loss in the taste thalamus. Similar to the control nuclei operated rat brain, the thalamic taste control nuclei of these GCTx rats were evenly stained with the NeuN marker indicating that the vast majority of neurons survived. Finally, Figure 2 panel D depicts a representative brain section with extensive retrograde gustatory thalamic neuronal degeneration. The data from this rat was, of course, eliminated from the analysis.

Figure 2-3

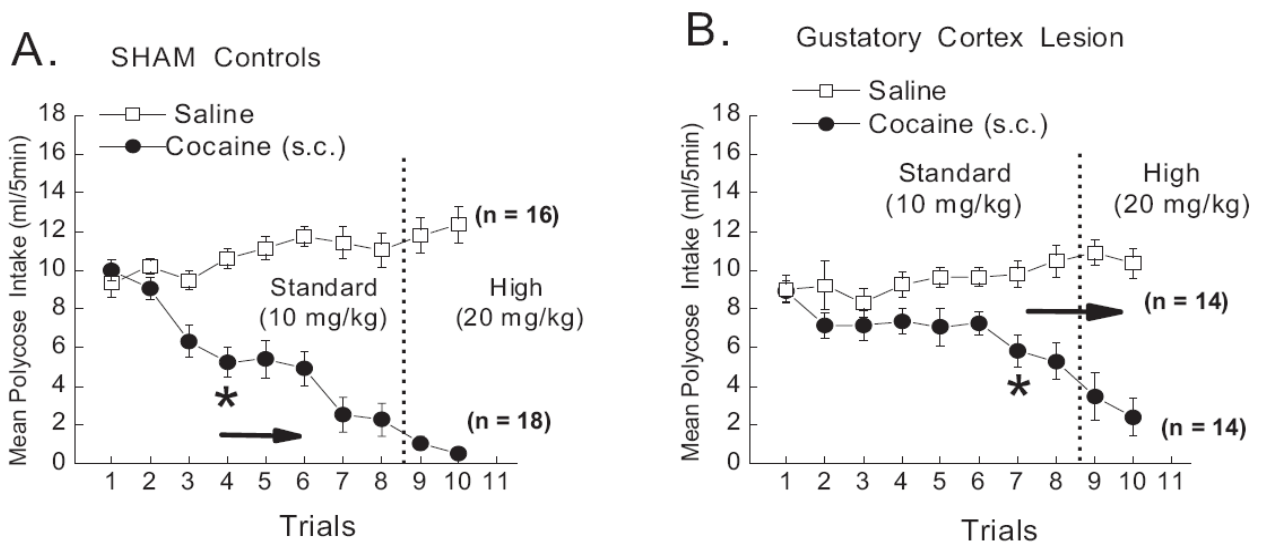

Figure **2-3**: Mean conditioned stimulus (CS) intake (ml/5 min) of the 0.1 M sodium chloride (NaCl) CS in Sham and gustatory cortex lesioned (GCTx) rats following pairings with saline (open squares) or 0.009 M or 0.15 M lithium chloride (LiCl; closed circles). A three-way analysis of variance (ANOVA) varying US (saline or LiCl) x Lesion (Sham or GCTx) x Trials (1–9) revealed that GCTx failed to disrupt LiCl-induced CTA such that both the Sham (panel A) and GCTx rats (panel B) learned to suppress intake of the CS when repeatedly paired with either dose of LiCl, $F(8, 200) = .669$, $p > .05$. Post hoc tests on a significant US x Trials interaction, $F(8, 200) = 18.75$, $p < .05$, showed that intake of the CS cue was suppressed by the fourth trial in both Sham and GCTx rats alike, ps < .05. * indicates a significant difference between US (saline vs. LiCl) groups.

CS intake

Intake of the gustatory cue serves as the dependent measure. The intake data from PBS and NSC rats did not significantly differ, $F < 1$. Consequently, the data from these two groups were collapsed and will hereafter be referred to as Group Sham. The results of a 2 x 2 x 2 x 10 mixed factorial analysis of variance (ANOVA) varying replication (1 or 2), lesion (GCTx or Sham), US (saline vs. cocaine), and trials (1–10) also failed to demonstrate a significant effect of replication, $F < 1$. Thus, the behavioral data from the two replications were combined and analyzed using a 2 x 2 x 10 mixed factorial ANOVA. The results revealed a significant Lesion x Drug x Trials interaction, F (9,

522) = 2.77, $p < .05$, see Figure 3. Following the significant 3-way ANOVA, post hoc comparisons revealed that, while Sham rats in the Polycose-cocaine condition suppressed intake of the taste cue by the fourth trial, $p < .05$ (see Figure 3, left panel), the GCTx rats did not show a significant reduction in CS intake, relative to their saline controls, until the seventh taste-drug pairing (see Figure 3, right panel). This finding suggests that bilateral lesions of the gustatory cortex disrupt the suppressive effects of a 10, but not a 20, mg/kg dose of cocaine. Post hoc analysis also revealed that gustatory cue intake did not significantly differ between the Sham and GCTx saline-treated groups across Trials 1 to 6, p .05. As such, the cortical lesion-induced deficit in cocaine-induced suppression of CS intake could not simply be attributed to the reduction in overall fluid intake by the saline treated rats. Of course, it is possible that the 10 mg/kg dose would have exerted a greater reduction in CS intake in the GCTx rats if training had continued with the lower dose of the drug. Although it may be important to address this possibility in a future study, here we opted to raise the dose on the eighth pairing because we thought it most critical to fully challenge the effectiveness of these gustatory cortical lesions. Regardless (i.e., whether mediated by more trials or new trials with the higher dose), the insular cortex lesion delays, but does not eliminate the suppressive effects of cocaine on intake of the drug-associated taste cue. Therefore, it appears that, as with bilateral lesions of the taste thalamus (Baldwin et al., 2002; Liu, Showalter, & Grigson, 2008), lesions of the gustatory cortex also reduce the suppressive effects of a lower, but not a higher dose of cocaine.

Experiment 2: Bilateral GCTx and taste-morphine pairings

Although the effects of the cortex lesion closely parallel those associated with damage to the taste thalamus (Mackey et al., 1986; Zito et al., 1988), the gustatory cortex lesion appears less disruptive than bilateral lesions of the taste thalamus (Baldwin et al., 2002; Grigson, Lyuboslavsky et al., 2000; Reilly & Trifunovic, 1999). This suggests that the suppressive effects of cocaine may differ

from those of morphine. Alternatively, the same data could reflect insufficient lesions of the gustatory cortex, leaving cocaine-induced suppression of CS intake partially intact. Mackey et al. (1986) demonstrated that, in adult male Wistar rats, bilateral ibotenic acid lesions of the gustatory cortex fully disrupted the suppressive effect of a 15 mg/kg dose of morphine on intake of a saccharin CS, after 5 CS–US pairings. Unlike morphine, when paired with an injection of a 15 or 75 mg/kg dose of LiCl (i.p.), these same rats acquired an aversion to a similar saccharin cue (Mackey et al., 1986). In our Exp. # # 2, the rats from Exp. # 1 were used to replicate the finding of Mackey and colleagues on the highly disruptive effect of bilateral cortical lesions on morphine-induced suppression of CS intake. Moreover, since the high dose of cocaine in Exp. # 1 appeared to override the disruptive effect of the cortical lesion, the dose of morphine was increased after six trials from 15 to 30 mg/kg. The results of this study speak to the adequacy of the lesion and also test whether the suppressive effects of cocaine and morphine might be mediated, at least in part, by different neural substrates.

Methods:

Subjects, surgery, and apparatus.

Thirty-seven rats (18 Sham and 19 GCTx) from the second replication of Exp. # 1 were counterbalanced as a function of drug-history and used to examine morphine-induced avoidance of CS intake in Exp. #2. The rats were housed and maintained as described in Exp. # 1.

Drugs and solutions:

The CS, sodium saccharin was purchased from Sigma Chemical Co., St. Louis, MO, mixed in dH2O and presented at room temp. A 0.15% saccharin solution is highly preferred and readily supports drug-induced contrast, anticipatory contrast, and CTA (Bardo & Valone, 1994; Flaherty & Grigson, 1988; Flaherty & Mitchell, 1999; Grigson, 2000; Grigson & Hajnal, 2007; Nachman & Ashe, 1973; Reilly & Pritchard, 1997; Schroy et al., 2005; Yamamoto & Fujimoto, 1991). The US

morphine sulfate was generously provided by NIDA and mixed in 0.9% saline, approximately 1 hr prior to the CS–US pairings (LeBlanc & Cappell, 1975; Wise et al., 1976).

Procedure:

Approximately 2 weeks following the end of Experiment 1, the rats were returned to the water deprivation regiment (5 min a.m., 1 h p.m.). Based on their drug experience in Experiment 1, a mixed cross over design was used to match rats into 2 US conditions: saline or 15 mg/kg morphine. Treatment groups were balanced by placing half the Sham and GCTx rats with previous cocaine history in the saline group and the other half in the morphine group for the present experiment. Thus, of the 18 control lesion (Sham) rats, eight received saline and 10 now received morphine as the US. Out of the 19 GCTx rats, 9 GCTx rats served in the saline group and 10 GCTx rats were placed in the morphine group. During testing, all rats were allowed 5 min access to 0.15% saccharin, and after a 5-min interval, were injected i.p. with the appropriate US. There was a total of 8 CS–US pairings with one dH2O day (5 min a.m., 1 h p.m.) elapsing between each. The concentration of morphine was increased from 15 mg/kg to 30 mg/kg on Trials 7 through 8, followed by one CS only test day. All of the rats were given 1 h access to water each afternoon to maintain hydration.

Results and Discussion:

Histology

As previously mentioned, the data for 8 of the 19 GCTx rats were eliminated from the statistical analysis. Thus, only 11 GCTx rats (5 saline, 6 drug) contributed data to Experiment 2 (see the previous discussion of the Histological results).

Figure 2-4

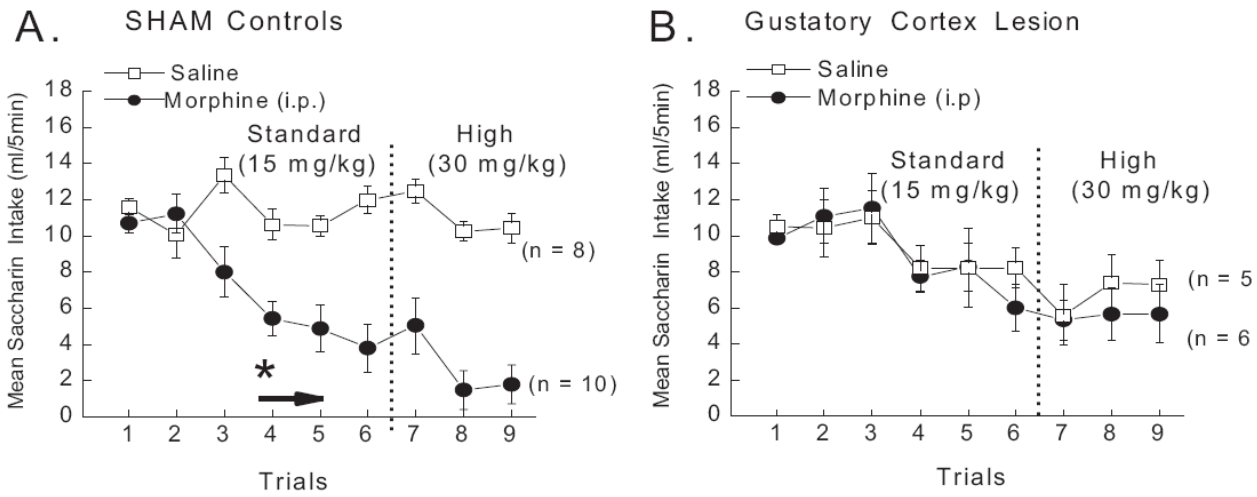

Figure **2-4**: Mean conditioned stimulus (CS) intake (ml/5 min) of the 0.15% saccharin CS in Sham and gustatory cortex lesioned (GCTx) rats following pairings with saline (open squares) or 15 and then 30 mg/kg morphine (closed circles) across trials. A three-way analysis of variance (ANOVA) varying Drug (saline or morphine) x Lesion (Sham or GCTx) x Trials (1–9) revealed that bilateral lesions of the gustatory cortex disrupted morphine-induced contrast such that Sham (A), but not GCTx rats (B), suppressed intake of the 0.15% saccharin cue when paired with the passive administration of the low or high dose of morphine, $F(8, 200) = 2.37$, $p < .05$. The Sham rats suppressed intake of the 0.15% saccharin CS cue by the fourth CS-morphine pairing, $p < .05$ (indicated by an *), while the GCTx rats never significantly reduced intake of the saccharin CS relative to the saline treated GCTx controls, *p*s >.05.

CS intake

Mean intake of the 0.15% saccharin solution (ml/5 min) served as the dependent measure. Intake by the PBS and NSC rats did not significantly differ, p .05. Consequently, these groups were collapsed. The resultant data were reanalyzed using a 2 x 2 x 9 repeated measures ANOVA varying lesion (GCTx or Sham), US (saline or morphine), and trials (1–9). Post hoc analyses were conducted on significant ANOVAs using the Newman–Keuls test. Figure 4 shows the mean +/- SEM intake of the saccharin CS for all groups across the nine trials. The Sham but not the GCTx rats suppressed intake of the saccharin cue following saccharin-morphine pairings. This observation was confirmed

by a significant Lesion x US x Trials interaction, F (8, 200) = 2.37, $p < .05$. Post hoc analysis confirmed that Sham rats, but not GCTx rats, suppressed intakeof the CS cue beginning with the fourth saccharin-morphine pairing, ps <.05. The GCTx rats treated with morphine, on the other hand, never significantly reduced intake relative to their saline treated controls. It should also be noted that post hoc analysis comparing the CS intake of vehicle-treated Sham versus vehicle-treated GCTx rats revealed no significant differences on Trials 1–6, $p >.05$. These data confirm that the lesion-induced deficit in morphine-induced suppression of CS intake cannot be simply attributed to the slightly lower intake by vehicle treated GCTx. Moreover, the GCTx rats also tended to drink less water on the days between conditioning trials (data not shown), but this effect also was not statistically significant, and essentially identical for both the morphine and saline treated subgroups. A similar decrement in intake has been seen in rats with bilateral thalamic lesions (Grigson, Lyuboslavsky, et al., 2000). Therefore, it appears that fluid consumption by fluid deprived GCTx rats may, on occasion, be lower than that of Sham rats. In sum, despite minor differences in general fluid intake, the results show that bilateral lesions of the insular cortex fully abolish morphine-induced suppression of a saccharin cue.

The outcome of the current experiment is critical on a number of levels. First, in Sprague–Dawley rats, we replicated the finding that bilateral lesions of the taste cortex disrupt morphine-induced contrast with a standard dose of the drug (Mackey et al., 1986). Second, we demonstrated that the lesion also fully disrupted the suppressive effects of even a relatively high 30 mg/kg dose of morphine. This finding differs from the dose dependent effect obtained with cocaine in Experiment 1. Thus, the neural mechanisms mediating the suppressive effects of morphine and high doses of cocaine may differ. Potential experimental approaches to address this possibility are discussed below (see General Discussion). An alternative consideration, however, is that prior experience with either Polycose-saline or Polycose-cocaine in Experiment 1 may have retarded the development of a subsequent saccharin-

morphine association in Experiment 2 and that the magnitude of this effect may have been augmented in rats with lesions of the gustatory cortex. Bilateral lesions of the ventral tegmental area (VTA), that do not affect drug-induced suppression of CS intake in naive rats, nevertheless retard the development of this effect in rats with a history of either saccharin-morphine or saccharin-saline pairings (Twining, Hajnal, Bruno, Hess, & Grigson, 2005). Bilateral lesions of the insular taste cortex, then, also may facilitate this latent inhibition-like effect (Lubow & De la Casa, 2005; Lubow, Markman, & Allen, 1968). If so, these taste-drug experienced rats with cortical lesions also should demonstrate deficits in CTA learning when a novel cue is paired with the illness-inducing agent, LiCl (as addressed in Exp. #3).

Experiment 3: Bilateral GCTx and taste-LiCl pairings

Bilateral lesions of the parabrachial nucleus (PBN) have been shown to fully prevent CTA learning (Grigson, Reilly, Shimura, & Norgren, 1998; Grigson, Shimura, & Norgren, 1997; Spector, Norgren, & Grill, 1992; Spector, Scalera, Grill, & Norgren, 1995). Gustatory information from the medial PBN is transmitted to cells in the taste thalamus, which in turn send projections to the gustatory insular cortex (Kosar, Grill, & Norgren, 1986b; Norgren & Leonard, 1971; Norgren & Pfaffmann, 1975; Shi & Cassell, 1998). Given the gustatory PBN connections to the gustatory thalamocortical pathway the possibility remains, that PBN lesions block CTA learning by preventing pertinent information about the CS cue from reaching the insular gustatory cortex (Allen, Saper, Hurley, & Cechetto, 1991). Thus, Experiment 3 tested whether these same lesions of the gustatory cortex also disrupt acquisition of a LiClinduced conditioned taste aversion. As described, there are data in support of this possibility (Dunn & Everitt, 1988; Koh & Bernstein, 2005; Naor & Dudai, 1996; Ramirez-Amaya et al., 1996), and data that, either directly or indirectly argue against it (Flynn et al., 1991; Geddes et al., 2004, 2003; Grigson, Lyuboslavsky, et al., 2000; Lasiter & Glanzman, 1982; Mackey et al., 1986; Reilly & Pritchard, 1996a; Scalera et al., 1997; Zito et al., 1988). In the

present study, we used a low (0.009 M) and standard (0.15 M) dose of LiCl because, when passively administered to intact rats, these doses suppress CS intake in a manner similar to the standard and higher doses of cocaine and morphine (Grigson, 1997).

Methods: Subjects, surgery, and apparatus.

All 37 rats from Experiment 2 served as subjects (*n* = 18 Sham and *n* = 19 GCTx).

Drugs and solutions:

The CS, 0.1 M sodium chloride (NaCl) (Sigma Chemical Co., St. Louis, MO), was mixed in water and presented at room temperature. As a gustatory cue, NaCl has been shown to be fairly preferred in rats and to readily support CTA learning (Baird, St John, & Nguyen, 2005; Grill & Norgren, 1978; Spector, Grill, & Norgren, 1993; Yamamoto, 1984; Yamamoto et al., 1984). The US, LiCl, was purchased from Sigma Chemical Company, St. Louis, MO, prepared in a stock solution, and maintained at 4 °C between trials. Either the 0.009 M or 0.15 M doses were injected i.p.

Procedure

Approximately 2 weeks following the end of Exp. # 2, the rats were returned to the water deprivation regimen (5 min a.m., 1 h p.m.). Based on their drug experience from Exp. # 1 and 2, we used a mixed cross over design to match rats into 2 US conditions: saline or LiCl. Treatment groups were balanced by placing half the Sham and GCTx rats with previous cocaine and morphine history in the saline group and the other half in the LiCl group for the present experiment. Thus, of the 18 Sham rats, 9 received saline and 9 now received LiCl as the US. Out of the 19 GCTx rats, 9 GCTx rats served in the saline group and 10 GCTx rats were placed in the LiCl group. As previously mentioned, the data for 8 of the 19 GCTx rats were discarded based on histological analysis of lesion placement and retrograde thalamic degeneration (see the Results section in Exp. # 1 above). Thus, 6 GCTx rats contributed data to the saline condition and the remaining 5 GCTx rats to the LiCl

condition. During testing, all rats were allowed 5 min access to 0.1 M NaCl and, after a 5 min interval, were injected i.p. with the appropriate US. There was a total of 8 CS–US pairings with one dH2O day between each pairing. The concentration of LiCl was increased from 0.009 M to 0.15 M on Trials 7 and 8, followed by one CS only test. The rats were given 1 h p.m. access to rehydrate.

Results and Discussion:

CS intake

Intake of the 0.1 M NaCl CS (ml/5 min) served as the dependent measure. Intake by the PBS and NSC groups did not significantly differ, p .05, so the data were collapsed and reanalyzed using a 2 x 2 x 9 mixed factorial ANOVA varying lesion (GCTx or Sham), US (saline or LiCl), and trials (1–9). Post hoc analyses were conducted on significant interactions using the Newman–Keuls test. The results of the CTA test with Sham and GCTx rats are shown in Figure 5, panels A and B, respectively. The 3 way ANOVA varying Lesion x Drug x Trials was not significant, $F > 1$, nor were any analyses involving lesion as a factor, ps .05. The US x Trials interaction, however, did attain statistical significance, $F(8, 200) = 18.75$, $p > .05$, and post hoc tests revealed that all rats (Sham and GCTx combined) suppressed intake of the NaCl taste cue following four taste-drug pairings, ps <.05. These findings confirm that rats with bilateral ibotenic acid lesions of the gustatory cortex retain the ability to demonstrate a LiCl-induced CTA. Suppression of CS intake in the CTA paradigm by GCTx rats is apparent over several acquisition trials and is marked, as in the Sham rats, on the final two conditioning trials. Since the rate of acquisition between the two lesion groups is the same, this demonstrates that lesions of the gustatory cortex not only have no effect on the LiCl-induced CTA, but also that they have no effect on the potential disruptive effects of prior experience with other CSs and USs, on latent inhibition like effect (De la Casa & Lubow, 1995).

Figure 2-5

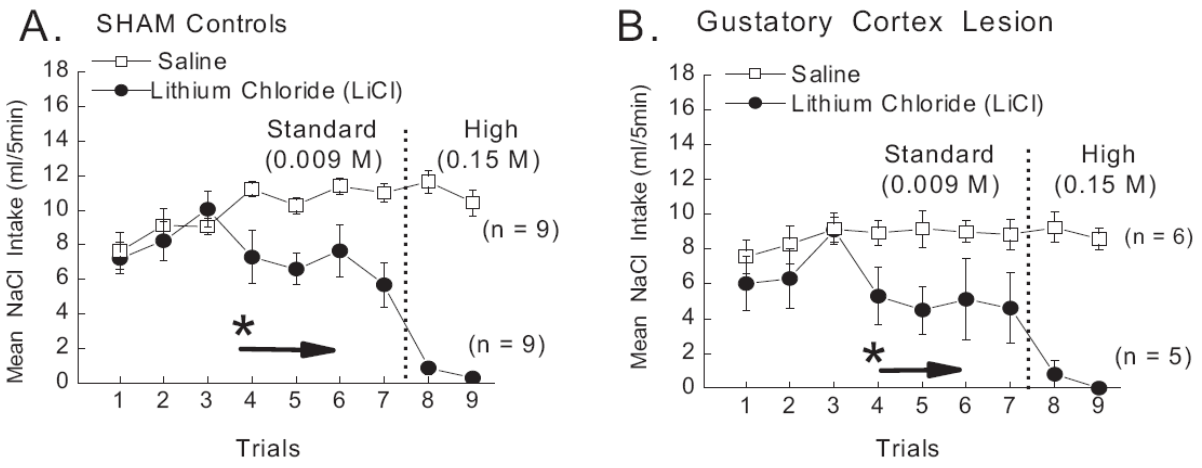

Figure **2-5**: Mean conditioned stimulus (CS) intake (ml/5 min) of the 0.15% saccharin CS in Sham and gustatory cortex lesioned (GCTx) rats following pairings with saline (open squares) or 15 and then 30 mg/kg morphine (closed circles) across trials. A three-way analysis of variance (ANOVA) varying Drug (saline or morphine) x Lesion (Sham or GCTx) x Trials (1–9) revealed that bilateral lesions of the gustatory cortex disrupted morphine-induced contrast such that Sham (A), but not GCTx rats (B), suppressed intake of the 0.15% saccharin cue when paired with the passive administration of the low or high dose of morphine, $F(8, 200) = 2.37$, $p < .05$. The Sham rats suppressed intake of the 0.15% saccharin CS cue by the fourth CS-morphine pairing, $p < .05$ (indicated by an *), while the GCTx rats never significantly reduced intake of the saccharin CS relative to the saline treated GCTx controls, ps > .05.

General Discussion

The results confirm that Sprague–Dawley rats with bilateral ibotenic acid lesions of the gustatory cortex can learn a LiCl-induced CTA, even at very low US doses. The same lesions, however, attenuated the development of cocaine-induced suppression of CS intake and prevented the suppressive effects of the standard 15 mg/kg and higher 30 mg/kg dose of morphine. These findings suggest that the thalamocortical gustatory system is not necessary for rats to experience the LiCl US exposure as an aversive event. Nor is the pathway necessary to learn an association between the taste cue and the unconditioned stimulus in a CTA paradigm. As summarized in Table 1, this pattern of

data mirrors that obtained from rats with bilateral lesions of the taste thalamus. Specifically, we have shown that THLx rats suppress intake of a gustatory CS when paired with a low or standard dose of LiCl but, fail to suppress intake of the same gustatory cue when paired with the standard doses of morphine or cocaine. In THLx rats, the higher dose of morphine remains untested, while unpublished data suggest that the higher dose of cocaine overrides the disruptive effective of these lesions. Similarly, in the present experiment, the higher dose of cocaine also was sufficient to override the disruptive effect of bilateral lesions of the taste cortex, but the higher dose of morphine was not. Finally, as discussed earlier, rats with lesions of the taste thalamus also fail to suppress intake of the 0.15% saccharin solution when paired with a normally preferred sucrose solution (Reilly & Pritchard, 1997; Schroy et al., 2005). Other researchers have reported that in Long-Evans rats, sucrose anticipatory contrast is also disrupted by bilateral lesions of the gustatory insular cortex (Kesner & Gilbert, 2007; Ragozzino & Kesner, 1999). Similar to Kesner and Gilbert (2007), but with the appropriate unshifted control group, we also have found that bilateral lesions of the gustatory insular cortex block the suppressive effects of a high concentration of sucrose on intake of a lesser saccharin or sucrose cue (Geddes, Han, & Grigson, 2005). Thus, taken together, the data show that both the taste thalamus and the taste cortex need to be intact for drug- and sucrose-induced suppression of CS intake, but not for LiCl-induced "CTA."

Table 2-1

Table **2-1**

Table 1
Comparison of Bilateral Gustatory Thalamus and Gustatory Cortex Lesion-Induced Deficits

	Thalamic lesions (THLX)	Gustatory cortex (GCTX)
Low dose LiCl-induced CTA	+	+
High dose LiCl-induced CTA	+	+
Standard morphine (15 mg/kg)	−	−
Standard cocaine (10 mg/kg)	−	−
High morphine (30 mg/kg)	untested	−
High cocaine (20 mg/kg)	+ (in preparation)	+
Sucrose-induced ACE	−	− (in preparation)

Note. LiCl = lithium chloride; CTA = conditioned taste aversion; ACE = anticipatory contrast effect; + = can learn to suppress intake of conditioned stimulus cue; − = fail to suppress taste cue.

What is the role of the gustatory cortex in the comparison of a natural reward cue with a drug of abuse? Evidence suggests that the gustatory cortex plays a role in responding to both the absolute and the relative properties of reward. Regarding absolute properties, similar insular cortex lesions disrupt short-term working memory for the magnitude of a food reward, but not its spatial location (DeCoteau, Kesner, & Williams, 1997; Ragozzino & Kesner, 1999). Utilizing neuroimaging techniques, human data suggest that the temporal- insular cortex is involved in the processing of naturally (appetitive) rewarding taste stimuli (Kobayakawa et al., 1996; O'Doherty, Dayan, Friston,

Critchley, & Dolan, 2003; Small, 2006). Human studies also have shown that neuronal activity in this region is preferentially affected by changes in reward value (O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001; Yamamoto et al., 2006).

If the reward comparison hypothesis is correct and avoidance of the taste cue is due to the relative rewarding properties of the drug US, then an intact gustatory cortex also must be essential for the comparison of the relative value of the taste cue with not only sweets, but also with the drug US. Specifically, the rat must (1) appropriately detect the taste cue and experience the drug consequence, (2) associate the taste cue with the effects of the drug, (3) remember the value of the drug experience upon presentation of the taste cue, (4) compare the value of the available gustatory CS with the memory of the value of the subsequent drug US, and finally (5) reduce intake of the lesser valued CS. Conditioned taste aversion follows a similar paradigm, but CTA learning does not involve comparing the *relative* reward values of the CS cue and US experience, nor does it require the thalamocortical gustatory pathway. As such, the taste cortex appears critical for making the explicit comparison between a less rewarding taste cue and a more rewarding drug of abuse that is anticipated in the near future and expressing the consequence of these comparisons in ingestive behavior.

While an intact taste cortex was essential for avoidance of the taste cue when paired with a low and a high dose of morphine and the low dose of cocaine, the disruptive effect of the lesion was overridden by the use of a relatively high 20 mg/kg dose of cocaine. Twining et al. (2008) have obtained a similar pattern in rats with bilateral lesions of the taste thalamus (see Table 1, column 2, row 5). The suppressive effect of the higher dose of cocaine may represent a quantitative (i.e., moderate to highly rewarding) and/or a qualitative (i.e., reward vs. aversion) change in the suppression of CS intake. For instance, the reinforcing properties of 20 mg/kg cocaine, but not 10 mg/kg, were sufficiently rewarding for rats with THLx or GCTx lesions to suppress intake of the CS

cue. Such an increase in the magnitude of the US reward value may increase the number of neuronal systems recruited during the US exposure, thus rendering the THLx and cortex less critical.

As for changes is the quality of the drug US, it is possible that at the higher dose of the cocaine, the suppression of CS intake may reflect an aversion, similar to a LiCl-CTA. This could occur if this dose of drug is toxic, thus creating an aversive US consequence. The 20 mg/kg (i.p.) dose of cocaine, however, has been shown to be sufficient to decrease the time taken to traverse a runway and to support the development of a conditioned place preference (Ettenberg & Geist, 1993; Hansen-Trench, Segar, & Barron, 1996; Knackstedt & Ettenberg, 2005; Lett, 1989; Mucha et al., 1982; Wakonigg et al., 2003; Zernig et al., 2002). Furthermore, 0.33 mg/infusion [the intraventricular (i.v.) equivalent of the 20 mg/kg (i.p.) dose of cocaine], not only caused suppression of intake of the 0.3M Polycose cue in both groups, but this dose was also readily self-administered by Sham and THLx rats, alike (Baldwin et al., 2002). More recently, we have attempted to address this question in rats with asymmetric lesions of the THLx and gustatory cortex using cocaine self-administration in operant chambers. We have found that in adult male Sprague–Dawley rats, disconnecting the taste thalamus from the gustatory insular cortex specifically blocks avoidance of the taste cue, but not the ability of the gustatory cues to induced drug seeking or instrumental responding for 0.33 mg i.v. infusions of cocaine (Geddes, Han, & Grigson, 2007). These data suggest that cocaine retains its positive attributes in rats with lesions of the thalamocortical gustatory system.

A final possibility is that, while the drug is not aversive, per se, the state elicited by the drug-associated cue is. In this case, the drug-associated taste cue may elicit cue-induced withdrawal, for example, which is an aversive state known to support taste aversion (Frumkin, 1976; McDonald & Hong, 2004; McDonald, Parker, & Siegel, 1997; Siegel, 1975, 1999; Weise-Kelly & Siegel, 2001; Wheeler & Miller, 2007; Wheeler et al., 2008). Taken together, the development of an aversive state

(perhaps, the most likely interpretation) also would be expected to recruit other neuronal circuits. Future studies will test the merits of these hypotheses.

In sum, the gustatory cortex appears to play a major role in comparing the relative value of an available taste reward with the memory of the alternative reward that is anticipated in the near future. Bilateral lesions of the gustatory cortex parallel those of the taste thalamus (see Table 1). In addition to the thalamic afferent axons, insular gustatory cortical cells have been shown to project back to the taste thalamus, forming a gustatory thalamocortical loop (Allen et al., 1991; Kosar et al., 1986a, 1986b; Norgren & Wolf, 1975; Shi & Cassell, 1998; Wolf, 1968). These observations support our recent hypothesis that drug-induced suppression of CS intake depends upon communication between the two nuclei in this thalamocortical loop. Current experiments are testing this hypothesis (Geddes, Han, Baldwin, & Grigson, 2006; Geddes, Han, & Grigson, 2005). Reciprocal connections between the cerebral cortex and the thalamus have been studied, in other modalities including vision, odor, touch and pain (de Carvalho, 1994; Ghazanfar & Nicolelis, 1997; Kuroda, Murakami, Oda, Shinkai, & Kishi, 1993). Understanding the relationship between the thalamus and cortex during higher-order behaviors, such as attention and sleep, may give some insight to the role and manner by which the gustatory thalamocortical loop contributes to relative reward learning over time (Mayer, Schuster, & Claussen, 2006; Smythies, 1997).

List of Figures and Tables

Acknowledgements

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Bibliography

Allen, G. V., Saper, C. B., Hurley, K. M., & Cechetto, D. F. (1991). Organization of visceral and limbic connections in the insular cortex of the rat. *Journal of Comparative Neurology, 311,* 1–16.

Baird, J. P., St John, S. J., & Nguyen, E. A. (2005). Temporal and qualitative dynamics of conditioned taste aversion processing: Combined generalization testing and licking microstructure analysis. *Behavioral Neuroscience, 119,* 983–1003.

Bardo, M. T., & Valone, J. M. (1994). Morphine-conditioned analgesia using a taste cue: Dissociation of taste aversion and analgesia. *Psychopharmacology (Berlin), 114*, 269–274.

Barker, L. M., & Smith, J. C. (1974). A comparison of taste aversions induced by radiation and lithium chloride in CS-US and US-CS paradigms. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology Psychology, 87*, 644–654.

Benjamin, R. M., & Pfaffmann, C. (1955). Cortical localization of taste in albino rat. *Journal of Neurophysiology, 18,* 56–64.

Corrigall, W. A., Linseman, M. A., D'Onofrio, R. M., & Lei, H. (1986). An analysis of the paradoxical effect of morphine on runway speed and food consumption. *Psychopharmacology (Berlin), 89,* 327–333.

de Carvalho, L. A. (1994). Modeling the thalamocortical loop. *International Journal of Bio-Medical Computing, 35,* 267–296.

De la Casa, G., & Lubow, R. E. (1995). Latent inhibition in conditioned taste aversion: The roles of stimulus frequency and duration and the amount of fluid ingested during preexposure. *Neurobiology of Learning and Memory, 64,* 125–132.

D'Mello, G. D., Goldberg, D. M., Goldberg, S. R., & Stolerman, I. P. (1981). Conditioned taste aversion and operant behavior in rats: Effects of cocaine, apomorphine and some long-acting derivatives. *Journal of Pharmacology and Experimental Therapeutics, 219,* 60–68.

DeCoteau, W. E., Kesner, R. P., & Williams, J. M. (1997). Short-term memory for food reward magnitude: The role of the prefrontal cortex. *Behavioural Brain Research, 88,* 239–249.

Dunn, L. T., & Everitt, B. J. (1988). Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. *Behavioral Neuroscience, 102,* 3–23.

Durazzo, T. C., Gauvin, D. V., Goulden, K. L., Briscoe, R. J., & Holloway, F. A. (1994). Cocaine-induced conditioned place approach in rats: The role of dose and route of administration. *Pharmacology, Biochemistry and Behavior, 49,* 1001–1005.

Escobar, M., Fernandez, J., Guevara-Aguilar, R., & Bermudez-Rattoni, F. (1989). Fetal brain grafts induce recovery of learning deficits and connectivity in rats with gustatory neocortex lesion. *Brain Research, 478,* 368–374.

Ettenberg, A., & Geist, T. D. (1993). Qualitative and quantitative differences in the operant runway behavior of rats working for cocaine and heroin reinforcement. *Pharmacology, Biochemistry and Behavior, 44,* 191–198.

Flaherty, C. F., & Checke, S. (1982). "Anticipation of incentive gain." *Animal Learning and Behavior, 10,* 171–182.

Flaherty, C. F., & Grigson, P. S. (1988). From contrast to reinforcement: Role of response contingency in anticipatory contrast. *Journal of Experimental Psychology: Animal Behavior Processes, 14,* 165–176.

Flaherty, C. F., & Mitchell, C. (1999). Absolute and relative rewarding properties of fructose, glucose, and saccharin mixtures as reflected in anticipatory contrast. *Physiology and Behavior, 66,* 841–853.

Flynn, F. W., Grill, H. J., Schulkin, J., & Norgren, R. (1991). Central gustatory lesions: II. Effects on sodium appetite, taste aversion learning, and feeding behaviors. *Behavioral Neuroscience, 105,* 944–954.

Fresquet, N., Angst, M-J., & Sandner, G. (2004). Insular cortex lesions alter conditioned taste avoidance in rats differentially when using two methods of sucrose delivery. *Behavioural Brain Research, 153,* 357–365.

Frumkin, K. (1976). Differential potency of taste and audiovisual stimuli in the conditioning of morphine withdrawal in rats. *Psychopharmacologia, 46,* 245–248.

Geddes, R. I., Han, L., Baldwin, A., & Grigson, P. S. (2006). Asymmetric lesions of the gustatory thalamocortical loop selectively disrupt morphine-induced contrast, while sparing LiCl-induced conditioned taste aversion (CTA) learning. *Appetite, 46,* 354.

Geddes, R. I., Han, L., & Grigson, P. S. (2005). *Morphine-induced suppression of intake of a taste cue depends upon an intact thalamocortical loop in the dorsal taste pathway*. Program No. 801.15. 2005 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience.

Geddes, R. I., Han, L., & Grigson, P. S. (2007). Lesions of the gustatory thalamocortical loop block drug-induced devaluation of a natural saccharin reward cue, while leaving instrumental responding for the drug intact. *Appetite, 49,* 292–311. Ghazanfar, A. A., & Nicolelis, M. A. (1997). Nonlinear processing of tactile information in the thalamocortical loop. *Journal of Neurophysiology,78,* 506–510.

Glowa, J. R., Shaw, A. E., & Riley, A. L. (1994). Cocaine-induced conditioned taste aversions: Comparisons between effects in LEW/N and F344/N rat strains. *Psychopharmacology (Berl), 114,* 229–232.

Gomez, F. (2001). Induction of conditioned taste aversion with a selfadministered substance in rats. *Brain Research. Brain Research Protocols, 8,* 137–142.

Goudie, A. J., Dickins, D. W., & Thornton, E. W. (1978). Cocaine-induced conditioned taste aversions in rats. *Pharmacology, Biochemistry and Behavior, 8,* 757–761.

Grigson, P. S. (1997). Conditioned taste aversions and drugs of abuse: A reinterpretation. *Behavioral Neuroscience, 111,* 129–136.

Grigson, P. S. (2000). Drugs of abuse and reward comparison: A brief review. *Appetite, 35,* 89–91.

Grigson, P. S., & Freet, C. S. (2000). The suppressive effects of sucrose and cocaine, but not lithium chloride, are greater in Lewis than in Fischer rats: Evidence for the reward comparison hypothesis. *Behavioral Neuroscience, 114,* 353–363.

Grigson, P. S., & Hajnal, A. (2007). Once is too much: Conditioned changes in accumbens dopamine following a single saccharin-morphine pairing. *Behavioral Neuroscience, 121,* 1234–1242.

Grigson, P. S., Lyuboslavsky, P., & Tanase, D. (2000). Bilateral lesions of the gustatory thalamus disrupt morphine- but not LiCl-induced intake suppression in rats: Evidence against the conditioned taste aversion hypothesis. *Brain Research, 858,* 327–337.

Grigson, P. S., Lyuboslavsky, P. N., Tanase, D., & Wheeler, R. A. (1999). Water-deprivation prevents morphine-, but not LiCl-induced, suppression of sucrose intake. *Physiology and Behavior, 67,* 277–286.

Grigson, P. S., Reilly, S., Shimura, T., & Norgren, R. (1998). Ibotenic acid lesions of the parabrachial nucleus and conditioned taste aversion: Further evidence for an associative deficit in rats. *Behavioral Neuroscience, 112,* 160–171.

Grigson, P. S., Shimura, T., & Norgren, R. (1997). Brainstem lesions and gustatory function: III. The role of the nucleus of the solitary tract and the parabrachial nucleus in retention of a conditioned taste aversion in rats. *Behavioral Neuroscience, 111,* 180–187.

Grigson, P. S., Twining, R. C., & Carelli, R. M. (2000). Heroin-induced suppression of saccharin intake in water-deprived and water-replete rats. *Pharmacology, Biochemistry and Behavior, 66,* 603–608.

Grigson, P. S., Wheeler, R. A., Wheeler, D. S., & Ballard, S. M. (2001). Chronic morphine treatment exaggerates the suppressive effects of sucrose and cocaine, but not lithium chloride, on saccharin intake in Sprague-Dawley rats. *Behavioral Neuroscience, 115,* 403–416.

Grill, H. J., & Norgren, R. (1978). The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Research, 143,* 263–279.

Hansen-Trench, L. S., Segar, T. M., & Barron, S. (1996). Neonatal cocaine and/or ethanol exposure: Effects on a runway task with suckling reward.*Neurotoxicology and Teratology, 18,* 651–657.

Hunt, T., & Amit, Z. (1987). Conditioned taste aversion induced by self-administered drugs: Paradox revisited. *Neuroscience and Biobehavioral Reviews, 11,* 107–130.

Hunt, T., Segal, R., & Amit, Z. (1987). Differential involvement of central cholinergic mechanisms in the aversive stimulus properties of morphine and amphetamine. *Pharmacology, Biochemistry and Behavior, 28,* 335–339.

Hunt, T., Switzman, L., & Amit, Z. (1985). Involvement of dopamine in the aversive stimulus properties of cocaine in rats. *Pharmacology Biochemistry and Behavior, 22,* 945–948.

Jongen-Relo, A. L., & Feldon, J. (2002). Specific neuronal protein: A new tool for histological evaluation of excitotoxic lesions. *Physiology and Behavior, 76,* 449–456.

Kesner, R. P., & Gilbert, P. E. (2007). The role of the agranular insular cortex in anticipation of reward contrast. *Neurobiology of Learning and Memory, 88,* 82–86.

Kiefer, S. W., & Orr, M. R. (1992). Taste avoidance, but not aversion, learning in rats lacking gustatory cortex. *Behavioral Neuroscience, 106,* 140–146.

Knackstedt, L. A., & Ettenberg, A. (2005). Ethanol consumption reduces the adverse consequences of self-administered intravenous cocaine in rats. *Psychopharmacology (Berlin), 178,* 143–150.

Kobayakawa, T., Endo, H., Ayabe-Kanamura, S., Kumagai, T., Yamaguchi, Y., Kikuchi, Y., et al. (1996). The primary gustatory area in human cerebral cortex studied by magnetoencephalography. *Neuroscience Letters, 212,* 155–158.

Koh, M. T., & Bernstein, I. L. (2005). Mapping conditioned taste aversion associations using c-Fos reveals a dynamic role for insular cortex. *Behavioral Neuroscience, 119,* 388–398.

Kosar, E., Grill, H. J., & Norgren, R. (1986a). Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture. *Brain Research, 379,* 329–341.

Kosar, E., Grill, H. J., & Norgren, R. (1986b). Gustatory cortex in the rat. II. Thalamocortical projections. *Brain Research, 379,* 342–352.

Krettek, J. E., & Price, J. L. (1977). The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. *Journal of Comparative Neurology, 171,* 157–191.

Kulkosky, P. J., Sickel, J. L., & Riley, A. L. (1980). Total avoidance of saccharin consumption by rats after repeatedly paired injections of ethanol or LiCl. *Pharmacology Biochemistry and Behavior, 13,* 77–80.

Kumar, R., Pratt, J. A., & Stolerman, I. P. (1983). Characteristics of conditioned taste aversion produced by nicotine in rats. *British Journal of Pharmacology, 79,* 245–253.

Kuroda, M., Murakami, K., Oda, S., Shinkai, M., & Kishi, K. (1993). Direct synaptic connections between thalamocortical axon terminals from the mediodorsal thalamic nucleus (MD) and corticothalamic neurons to MD in the prefrontal cortex. *Brain Research, 612,* 339–344.

Lasiter, P. S., & Glanzman, D. L. (1982). Cortical substrates of taste aversion learning: Dorsal prepiriform (insular) lesions disrupt taste aversion learning. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology Psychol, 96,* 376–392.

LeBlanc, A. E., & Cappell, H. (1975). Antagonism of morphine-induced aversive conditioning by naloxone. *Pharmacology, Biochemistry and Behavior, 3,* 185–188.

Lett, B. T. (1989). Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology (Berlin), 98,* 357–362.

Liu, C., Showalter, J., & Grigson, P. S. (2008). *Ethanol-induced conditioned taste aversion: Reward or aversion?* Manuscript Submitted for publication.

Lubow, R. E., & De la Casa, L. G. (2005). There is a time and a place for everything: Bidirectional modulations of latent inhibition by timeinduced context differentiation. *Psychonomic Bulletin & Review, 12,* 806–821.

Lubow, R. E., Markman, R. E., & Allen, J. (1968). Latent inhibition and classical conditioning of the rabbit pinna response. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology Psychol, 66,* 688–694.

Mackey, W. B., Keller, J., & van der Kooy, D. (1986). Visceral cortex lesions block conditioned taste aversions induced by morphine. *Pharmacology, Biochemistry and Behavior, 24,* 71–78.

Mayer, J., Schuster, H. G., & Claussen, J. C. (2006). Role of inhibitory feedback for information processing in thalamocortical circuits. *Phys Rev E Stat Nonlin Soft Matter Phys, 73*(3 Pt 1), 031908.

Mayer, L. A., & Parker, L. A. (1993). Rewarding and aversive properties of IP and SC cocaine: Assessment by place and taste conditioning. *Psychopharmacology (Berl), 112,* 189–194.

McDonald, R. J., & Hong, N. S. (2004). A dissociation of dorso-lateral striatum and amygdala function on the same stimulus-response habit task. *Neuroscience, 124,* 507–513.

McDonald, R. V., Parker, L. A., & Siegel, S. (1997). Conditioned sucrose aversions produced by naloxone-precipitated withdrawal from acutely administered morphine. *Pharmacology, Biochemistry and Behavior, 58,* 1003–1008.

Mucha, R. F., van der Kooy, D., O'Shaughnessy, M., & Bucenieks, P. (1982). Drug reinforcement studied by the use of place conditioning in rat. *Brain Research, 243,* 91–105.

Mungarndee, S. S., Lundy, R. F., Jr., & Norgren, R. (2006). Central gustatory lesions and learned taste aversions: Unconditioned stimuli. *Physiology and Behavior, 87,* 542–551.

Nachman, M., & Ashe, J. H. (1973). Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl. *Physiology and Behavior, 10,* 73–78.

Naor, C., & Dudai, Y. (1996). Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. *Behavioural Brain Research, 79,* 61–67.

Norgren, R., & Leonard, C. M. (1971). Taste pathways in rat brainstem. *Science, 173,* 1136–1139.

Norgren, R., & Pfaffmann, C. (1975). The pontine taste area in the rat. *Brain Research, 91,* 99–117.

Norgren, R., & Wolf, G. (1975). Projections of thalamic gustatory and lingual areas in the rat. *Brain Research, 92,* 123–129.

O'Doherty, J. P., Dayan, P., Friston, K., Critchley, H., & Dolan, R. J. (2003). Temporal difference models and reward-related learning in the human brain. *Neuron, 38,* 329–337.

O'Doherty, J., Rolls, E. T., Francis, S., Bowtell, R., & McGlone, F. (2001). Representation of pleasant and aversive taste in the human brain. *Journal of Neurophysiology, 85,* 1315–1321.

Paredes-Olay, C., & Lopez, M. (2002). Lithium-induced outcome devaluation in instrumental conditioning: Dose-effect analysis. *Physiology and Behavior, 75,* 603–609.

Parker, L. A. (1995). Rewarding drugs produce taste avoidance, but not taste aversion. *Neuroscience and Biobehavioral Reviews, 19,* 143–157.

Paxinos, G., & Watson, C. R. (2005a). Rat brain in stereotaxic coordinates [CD-ROM]. *Journal of Neuroscience Methods, 3,* 129–149.

Ragozzino, M. E., & Kesner, R. P. (1999). The role of the agranular insular cortex in working memory for food reward value and allocentric space in rats. *Behavioural Brain Research, 98,* 103–112.

Ramirez-Amaya, V., Alvarez-Borda, B., Ormsby, C. E., Martinez, R. D., Perez-Montfort, R., & Bermudez-Rattoni, F. (1996). Insular cortex lesions impair the acquisition of conditioned immunosuppression. *Brain, Behavior, and Immunity, 10,* 103–114.

Reilly, S., & Pritchard, T. C. (1996a). Gustatory thalamus lesions in the rat: I. Innate taste preferences and aversions. *Behavioral Neuroscience, 110,* 737–745.

Reilly, S., & Pritchard, T. C. (1996b). Gustatory thalamus lesions in the rat II. Aversive and appetitive taste conditioning. *Behavioral Neuroscience, 110,* 746–759.

Reilly, S., & Pritchard, T. C. (1997). Gustatory thalamus lesions in the rat: III. Simultaneous contrast and autoshaping. *Physiology and Behavior, 62,* 1355–1363.

Reilly, S., & Trifunovic, R. (1999). Progressive ratio performance in rats with gustatory thalamus lesions. *Behavioral Neuroscience, 113,* 1008–1019.

Sako, N., Shimura, T., Komure, M., Mochizuki, R., Matsuo, R., & Yamamoto, T. (1994). Differences in taste responses to polycose and common sugars in the rat as revealed by behavioral and electrophysiological studies. *Physiology and Behavior, 56,* 741–745.

Scalera, G., Grigson, P. S., & Norgren, R. (1997). Gustatory functions, sodium appetite, and conditioned taste aversion survive excitotoxic lesions of the thalamic taste area. *Behavioral Neuroscience, 111,* 633– 645.

Schafe, G. E., & Bernstein, I. L. (1996). Forebrain contribution to the induction of a brainstem correlate of conditioned taste aversion: I. The amygdala. *Brain Research, 741,* 109–116.

Schalomon, P. M., Robertson, A. M., & Laferriere, A. (1994). Prefrontal cortex and the relative associability of taste and place cues in rats. *Behavioural Brain Research, 65,* 57–65.

Schenk, S., & Partridge, B. (1999). Cocaine-seeking produced by experimenter-administered drug injections: Dose-effect relationships in rats. *Psychopharmacology (Berlin), 147,* 285–290.

Schroy, P. L., Wheeler, R. A., Davidson, C., Scalera, G., Twining, R. C., & Grigson, P. S. (2005). Role of gustatory thalamus in anticipation and comparison of rewards over time in rats. *American Journal of Physiology Regul Integr Comp Physiol, 288,* R966–R980.

Shi, C. J., & Cassell, M. D. (1998). Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *Journal of Comparative Neurology, 399,* 440–468.

Siegel, S. (1975). Evidence from rats that morphine tolerance is a learned response. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology Psychol, 89,* 498–506.

Siegel, S. (1999). Drug anticipation and drug addiction. The 1998 H. David Archibald lecture. *Addiction, 94,* 1113–1124.

Small, D. M. (2006). Central gustatory processing in humans. *Advances in Oto-Rhino-Laryngology, 63,* 191–220.

Small, D. M., Zatorre, R. J., Dagher, A., Evans, A. C., & Jones-Gotman, M. (2001). Changes in brain activity related to eating chocolate: From pleasure to aversion. *Brain, 124*(Pt 9), 1720–1733.

Smith, M. E., Norgren, R., & Grigson, P. S. (2004). A mixed design reveals that glucose moieties facilitate extinction of a conditioned taste aversion in rats. *Learn Behav, 32,* 454–462.

Smythies, J. (1997). The functional neuroanatomy of awareness: With a focus on the role of various anatomical systems in the control of intermodal attention. *Consciousness and Cognition, 6,* 455–481.

Spector, A. C., Grill, H. J., & Norgren, R. (1993). Concentration-dependent licking of sucrose and sodium chloride in rats with parabrachial gustatory lesions. *Physiology and Behavior, 53,* 277–283.

Spector, A. C., Norgren, R., & Grill, H. J. (1992). Parabrachial gustatory lesions impair taste aversion learning in rats. *Behavioral Neuroscience, 106,* 147–161.

Spector, A. C., Scalera, G., Grill, H. J., & Norgren, R. (1995). Gustatory detection thresholds after parabrachial nuclei lesions in rats. *Behavioral Neuroscience, 109,* 939–954.

Turenne, S. D., Miles, C., Parker, L. A., & Siegel, S. (1996). Individual differences in reactivity to the rewarding/aversive properties of drugs: Assessment by taste and place conditioning. *Pharmacology, Biochemistry and Behavior, 53,* 511–516.

Twining, R. C., Baldwin, A. E., Palomo, A., Han, L., Horvath, N. & Grigson, P. S. (2008). *Gustatory thalamus lesions block the suppressive effects of low, but not high, doses of cocaine following passive or self-administration* (Manuscript in preparation).

Twining, R. C., Hajnal, A., Bruno, K., Hess, E. J., & Grigson, P. S. (2005). Lesions of the ventral tegmental area disrupt drug-induced appetite stimulating effects but spare reward comparison. *International journal of Comparative Psychology, 18,* 372–396.

Wakonigg, G., Sturm, K., Saria, A., & Zernig, G. (2003). Opioids, cocaine, and food change runtime distribution in a rat runway procedure. *Psychopharmacology (Berlin), 169,* 52–59.

Weise-Kelly, L., & Siegel, S. (2001). Self-administration cues as signals: Drug self-administration and tolerance. *Journal of Experimental Psychology: Animal Behavior Processes, 27,* 125–136.

Wheeler, D. S., & Miller, R. R. (2007). Interactions between retroactive interference and context-mediated treatments that impair Pavlovian conditioned responding. *Learning & Behavior, 35,* 27–35.

Wheeler, R. A., Twining, R. C., Jones, J. L., Slater, J. M., Grigson, P. S., & Carelli, R. M. (2008). Behavioral and electrophysiological indices of negative affect predict cocaine self-administration. *Neuron, 57,* 774 –785.

White, N., Sklar, L., & Amit, Z. (1977). The reinforcing action of morphine and its paradoxical side effect. *Psychopharmacology (Berlin), 52,* 63–66.

Wilkins, E. E., & Bernstein, I. L. (2006). Conditioning method determines patterns of c-fos expression following novel taste-illness pairing. *Behavioural Brain Research, 169,* 93–97.

Wise, R. A., Yokel, R. A., & DeWit, H. (1976). Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. *Science, 191,* 1273–1275.

Wolf, G. (1968). Projections of thalamic and cortical gustatory areas in the rat. *Journal of Comparative Neurology, 132,* 519–530.

Yamamoto, C., Nagai, H., Takahashi, K., Nakagawa, S., Yamaguchi, M., Tonoike, M. (2006). Cortical representation of taste-modifying action of miracle fruit in humans. *Neuroimage, 33,* 1145–1151.

Yamamoto, T. (1984). Taste responses of cortical neurons. *Progress in Neurobiology, 23,* 273–315.

Yamamoto, T., & Fujimoto, Y. (1991). Brain mechanisms of taste aversion learning in the rat. *Brain Research Bulletin, 27,* 403–406.

Yamamoto, T., Sako, N., Sakai, N., & Iwafune, A. (1997). Gustatory and visceral inputs to the amygdala of the rat: Conditioned taste aversion and induction of c-fos-like immunoreactivity. *Neuroscience Letters, 226,* 127–130.

Yamamoto, T., Yuyama, N., Kato, T., & Kawamura, Y. (1984). Gustatory responses of cortical neurons in rats. I. Response characteristics. *Journal of Neurophysiology, 51,* 616–635.

Yamamoto, T., Yuyama, N., & Kawamura, Y. (1981). Cortical neurons responding to tactile, thermal and taste stimulations of the rat's tongue. *Brain Research, 221,* 202–206.

Zernig, G., Harbig, P., Weiskirchner, I., Auffinger, M., Wakonigg, G., & Saria, A. (2002). Reinforcing effect of subcutaneous morphine in a modified Ettenberg runway. *Journal of Molecular Neuroscience, 18,* 135–142.

Zito, K. A., Bechara, A., Greenwood, C., & van der Kooy, D. (1988). The dopamine innervation of the visceral cortex mediates the aversive effects of opiates. *Pharmacology, Biochemistry and Behavior, 30,* 693–699.

Bilateral Lesions of the Insular Gustatory Cortex Block Anticipatory Contrast

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ABSTRACT

Rats suppress intake of a lesser valued taste cue, such as saccharin, when it predicts future access to a highly preferred 1.0 M sucrose solution. This phenomenon, referred to as an anticipatory contrast effect, is thought to occur because the lesser valued cue pales in anticipation of the highly rewarding sucrose solution that is anticipated in the very near future (Flaherty and Checke 1982). This phenomenon is eliminated by bilateral, taste-guided, electrolytic or ibotenic acid lesions of the gustatory thalamus (Reilly and Pritchard 1996; Schroy, Wheeler et al. 2005). The present set of experiments examined the development of ACE in rats with similar bilateral ibotenic acid-induced lesions aimed at the insular gustatory cortex (GC), the major target site of efferent axons projecting from the taste thalamus (for a recent review of the pathway see (Pritchard and Norgren 2004)). The results of the current experiments showed that bilateral lesions of the gustatory insular cortex fully prevented the development of anticipatory contrast in rats whether access to a highly preferred 1.0 M sucrose US was predicted by access to a less palatable 0.05 M sucrose (Experiment 1) or 0.15% saccharin CS (Experiment 2). These data are considered in light of data showing that the same lesion disrupts avoidance of a taste cue when paired with morphine and low doses of cocaine, but not when paired with low or high doses of LiCl (Geddes, Han et al. 2008).

Key words: CTA, avoidance, devaluation, incentive, thalamocortical loop, ibotenic acid.

Introduction

Rats will avoid intake of an otherwise palatable taste cue when the gustatory solution is paired with either a highly rewarding or an aversive outcome. An **a**nticipatory contrast effect (ACE) occurs when rats avoid intake of a lesser valued gustatory conditioned stimulus (CS), such as 0.15% saccharin, when it comes through once daily pairings to predict the availability of a highly preferred unconditioned stimulus (US), such as 1.0 M sucrose (Flaherty and Checke 1982). In this case, the rats are thought to avoid the lesser valued saccharin cue in anticipation of the impending access to the more preferred sucrose reward (Flaherty, Grigson et al. 1996). A conditioned taste aversion (CTA) occurs when a similar gustatory CS is followed by a bout of illness induced by irradiation or lithium chloride (LiCl) injections (Garcia, Kimeldorf et al. 1955; Smith, Morris et al. 1964; Nachman and Ashe 1973; Barker and Smith 1974). In CTA the effects of the aversive US must be associated with and then attributed to the gustatory CS solution. Such a phenomenon is thought to mediate the reduction in intake found in cancer patients undergoing treatment with chemotherapy and tumor-bearing rats given sweets (but not salt) (Smith and Blumsack 1981; Carrell, Cannon et al. 1986; Smith, Barker et al. 1994). Conditioned taste aversion is a robust phenomenon that is observed with sweets and salts, may last for years in rodents and man, occurs following a single taste-illness episode, and can be acquired *in utero* and expressed postnatally (Carroll and Smith 1974; Garb and Stunkard 1974; Mickley, Remmers-Roeber et al. 2000; Gruest, Richer et al. 2004). Like aversive and rewarding USs, rats also will avoid intake of a gustatory CS when paired with a psychoactive drug such as ethanol, amphetamine, cannabinoids, cocaine, morphine, and nicotine (Le Magnen 1969; Cappell and LeBlanc 1971; Corcoran, Bolotow et al. 1974; Sklar and Amit 1977; Switzman, Hunt et al. 1981; Kumar, Pratt et al. 1983).

Perhaps influenced by the time at which it was discovered, this drug-induced taste avoidance phenomenon was first interpreted as a CTA and taken as evidence that drugs of abuse must have not only rewarding, but also aversive, properties (Nachman, Lester et al. 1970; Wise, Yokel et al. 1976; White, Sklar et al. 1977; Hunt and Amit 1987). While it is the case that drugs of abuse have some aversive properties (Burgdorf, Knutson et al. 2001; Knackstedt, Samimi et al. 2002; Stalnaker, Roesch et al. 2006; Goldstein, Alia-Klein et al. 2007; Tomasi, Goldstein et al. 2007; Calu and Schoenbaum 2008), and we now have evidence that these taste-drug pairings support the onset of a conditioned aversive state (Wheeler, Twining et al. 2008; Liu, Showalter et al. 2009; Twining, Bolan et al. 2009), we have hypothesized that rats avoid intake of a taste CS when paired with a drug of abuse because the rats are anticipating access to the highly rewarding drug of abuse (Grigson 1997). According to this hypothesis, the suppressive effects of drugs of abuse are more akin to sucrose-induced contrast than they are to LiCl-induced CTA learning In support, relative to a LiCl-induced CTA, sucrose- and drug-induced taste avoidance are similarly affected by manipulations of CS intensity and, in some cases, taste quality, deprivation state, strain differences, and a history of chronic morphine exposure (Flaherty and Geary 1993; Glowa, Shaw et al. 1994 Flaherty, 1994 #237; Giorgi, Corda et al. 1997; Grigson 1997; Gomez and Grigson 1999; Grigson, Lyuboslavsky et al. 1999; Grigson and Freet 2000; Grigson, Wheeler et al. 2001; Risinger and Boyce 2002). Furthermore, after learning a CTA, animals also will display a conditioned place aversion to the environment in which the taste-LiCl pairings took place (Barker and Smith 1974), while they will exhibit an increase in instrumental responding and a conditioned place preference for a drug or sweet associated context (Mucha, van der Kooy et al. 1982; White and Carr 1985; Carroll, Lac et al. 1989; Bickel, DeGrandpre et al. 1995; Agmo and Marroquin 1997).

Along with other manipulations, lesions of the gustatory pathway also have dissociated the suppressive effects of drugs of abuse from those of LiCl. In rodents, primary taste quality (salty, sour,

bitter, sweet, umami) and non-gustatory sensations are carried by the facial nerve, glossopharyngeal nerve, and the vagus nerve from the periphery to the spinal trigeminal nuclei and, for taste, to the nucleus of the solitary tract (NST) (Travers, Pfaffmann et al. 1986). The rostral NST in rats sends taste projections to the medial ipsilateral parabrachial nucleus (mPBN) in the pons. The mPBN cells send efferent projections via a ventral pathway to the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), substantia innominata, and bed nucleus of the stria terminalis (BNST) (Norgren 1978). Other mPBN fibers project via a dorsal pathway to the VPMpc (parvicellular region of the ventral posteromedial nucleus of the thalamus). These thalamic cells then project to the ipsilateral insular cortex (Benjamin and Akert 1959; Wolf 1968; Norgren and Wolf 1975; Augustine 1985).

Bilateral lesions of the gustatory mPBN neurons disrupt conditioned suppression produced by all three US categories (i.e., aversive, rewarding, and addictive substances) (Spector, Norgren et al. 1992; Bechara, Martin et al. 1993; Reilly, Grigson et al. 1993; Scalera, Spector et al. 1995; Liang and Norgren 2009). Bilateral lesions of the VMPpc spares CTA but Blocks avoidance of the taste cue induced by cocaine, morphine, and sucrose (Flynn, Grill et al. 1991; Scalera, Grigson et al. 1997; Reilly and Trifunovic 1999; Grigson, Lyuboslavsky et al. 2000; Reilly, Bornovalova et al. 2004; Schroy, Wheeler et al. 2005). Given the projection from the gustatory thalamus to the cortex, the goal of the present set of experiments was to determine whether the gustatory cortex, like the thalamus, need be intact for the development of a sucrose-induced ACE.

Experiment 1: Bilateral GCTx and 0.5 M sucrose – 1.0M sucrose pairings

Bilateral agranular insular cortex lesions block the reduction in CS intake induced by morphine, but not by LiCl (Mackey, Keller et al. 1986; Zito, Bechara et al. 1988; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005). We recently expanded upon these findings by showing that bilateral

lesions of the gustatory cortex (GCTx) fully prevented conditioned avoidance of the taste cue induced by both a 15 and a 30 mg/kg dose of morphine and greatly diminished that induced by a 10 mg/kg dose of cocaine (Geddes, Han et al. 2008). The same bilateral lesions of the insular gustatory cortex, however, had no impact on a LiCl-induced CTA or on the suppressive effects of a higher 20 mg/kg dose of cocaine (Geddes, Han et al. 2008). We predict, then, that an intact gustatory insular cortex also will be essential for sucrose-induced suppression of CS intake. In partial support, Kesner and Gilbert (2007) reported that rats failed to suppress intake of a sucrose-associated saccharin CS. In this case, however, no saccharin-saccharin unshifted controls were included, by definition, preventing conclusions related to contrast (Kesner and Gilbert 2007). Thus, in the following experiments we will test whether GCTx lesions that disrupted drug-induced avoidance also would block ACE when using disparate concentrations of sucrose in Experiment 1 or saccharin-sucrose pairs in Experiment 2. In each case, contrast in the shifted condition will be assessed relative to intake exhibited by appropriate unshifted controls.

Methods:

Subjects

The subjects were 38 naive male Sprague-Dawley rats (Charles River) weighing 300-500 grams at the start of this experiment. After the current study these rats were exposed to taste-drug (morphine and cocaine) pairings followed by taste-LiCl pairings and the data from the three subsequent studies has been published separately (see (Geddes, Han et al. 2008)). For the current study, all rats were individually housed in standard suspended stainless steel wire-mesh cages in a temperature and humidity controlled colony room maintained on a 12/12 hour light/dark cycle. Water was available ad libitum in the back of the home cage and access to dry chow (Teklad #6068) was restricted as described in the **Procedure** section.

Surgery

Nineteen rats received bilateral ibotenic acid lesions of the gustatory cortex (group GCTx), 10 rats were infused into the insular GC with sodium phosphate buffer (PBS), and 9 rats served as non-surgical controls (NSC). The coordinates for the stereotaxically-placed lesions were modified from (Mackey, Keller et al. 1986). Prior to surgery, each rat was injected with atropine sulfate (0.25 mg/ i.p.) and Gentamicin (6 mg/kg, i.p.). Thereafter, each rat was anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and supplemented throughout surgery. Body temperature was maintained at 37 $\pm 1^0$ C. Lesions were placed at the following coordinates: A/P +0.5 mm from bregma, M/L +/- 4.8, and D/V -5.5 from the dura mater. A single 1 μl Hamilton microsyringe attached to a carrier was lowered into the target area and 0.2 μl (20 μg / ml) of ibotenic acid was infused over a 10 min interval. The needle then remained in place for an additional 10 min. This procedure was repeated in the contralateral hemisphere. Surgical controls were treated identically to the GCTx rats, except that instead of ibotenic acid, rats were infused with 0.2 μl of PBS (pH = 7.4). After surgery, holes in the skull were filled with Gelfoam and the wound was closed with wound clips.

CS and US solutions

*S*ucrose powder was obtained from Fischer Chemical (Pittsburg, PA) and the solutions (presented at room temperature) were prepared 24 h prior to CS-US pairings.

Apparatus

The sapid stimuli were presented in one of four identical modular operant chambers (MED Associates, Inc., St. Albans, VT) measuring 30.5 x 24.0 x 29.0 cm (length x width x height). All testing chambers have clear Plexiglas on the front and back walls, while the end and top are made of aluminum and stainless steel, respectively. The grid floors consist of nineteen 4.8 mm stainless steel rods spaced

1.6 cm apart (center to center). Each chamber is housed in a sound- and light-attenuated cubicle, fitted with a ventilation fan, and a white noise generator that provides a background noise of 75 dB. The inside of each chamber is illuminated by a shaded bulb reflecting off the ceiling. The bulb is located on the left end of the wall, to the right of the cage speaker. Each chamber is equipped with two retractable sipper tubes that enter and exit the left wall of the chamber through 1.3 cm diameter holes, spaced 16.4 cm apart (center to center), located approximately 10 cm above the grid floor. When advanced the tip of the sipper tube was aligned in the center of the hole, flush with the right-end wall. A lickometer circuit (0.3μA) was used to monitor licking. Control of events in the chambers and data collection was carried out on-line using a 33-MHz computer. Programs are written in the Medstate notation language.

Procedure

Food restriction and habituation. Following at least 5 d recovery from surgery, all rats were restricted to once-a-day feeding until each rat reached 85% of its free-feeding body weight. The rats were then habituated to the operant conditioning chambers for 5 min/day for 3 days. During which the white noise and house light remained on with the empty sipper tubes retracted.

Experimental design and testing. The 19 GCTx and 19 Sham (10 PBS and 9 non-surgical controls) rats were then assigned to one of 2 US conditions: 0.05 M – 0.05 M sucrose or 0.05 M – 1.0 M sucrose. One Sham rat died during acquisition and its data consequently were excluded from analysis. Thus, 9 GCTx rats and 8 Sham rats (4 PBS and 4 NSC) received 0.05 M sucrose solution in both bottle 1 and 2, while the remaining 10 GCTx rats and 10 Sham rats (6 PBS and 4 NSC) received 0.05 M in bottle 1 and 1.0 M sucrose in bottle 2. During testing, each rat was taken from its home cage, weighed, and placed in the test chamber. Once in the chamber, the signal light over bottle 1 turned on, and bottle 1, containing 0.05 M sucrose, advanced for a 3 min access period. Immediately thereafter, bottle 1

retracted and the cue light turned off. Simultaneously the cue light over bottle 2 turned on and the bottle advanced. Rats were then given 3 min access to either 0.5 M sucrose or 1.0 M sucrose. At the end of the session, bottle 2 retracted and the house light was turned off, signaling the end of the session. The rat was removed from the test chamber and returned to its home cage. One such CS-US pairing occurred daily for 12 days in succession. The rats were given their daily food ration no sooner than 45 min after being returned to their home cage.

Histology

After completing 3 additional behavioral studies (see (Geddes, Han et al. 2008)), the rats were deeply anesthetized with an injection of Pentobarbital Sodium (100 mg/kg i.p.) and perfused transcardially for 5-10 min with physiological saline, followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4) for 15-20 min. The brains were stored overnight in 20% sucrose in phosphate buffer (20 g/100 ml) at 4 ^{o}C. The next day the brains were cut into 2 alternating series of 50-μ-thick coronal sections using a freezing microtome. The first series was collected in 30% ethylene glycol glycerol in 50 mM phosphate buffer (PB) and stored at –20 ^{o}C for at least 1 week before being thawed and placed in PBS overnight. The next day the tissue was immunostained for neuron-specific nuclear protein (NeuN) (Jongen-Relo and Feldon 2002). The second series of sections was immediately mounted on gelatin-coated slides, dried overnight, and then stained alternately for cell bodies with cresyl Lecht violet stain. These staining procedures were used to qualitatively determine if the insular taste cortex was the only region completely damaged by the bilateral ibotenic acid-induced lesion.

----------Insert Figure 3-1 about here ----------

Results and Discussion:

Histological lesion analysis

Again, although the rats from Experiment 1 were naïve at the start of sucrose-sucrose testing reported here, these rats were subsequently tested in saccharin-cocaine, then Polycose-morphine, and finally NaCl-LiCl pairings. The drug and LiCl data for these rats were published (Geddes, Han et al. 2008). Figure 1 presented here is a modified photomicrograph from Geddes and colleagues (2008) of a representative coronal section of an intact brain (A), an ideal Lesion (B), and an extensive infarct (C) in the right hemisphere at the level of the insular taste cortex.

Below these NeuN stained sections in A-C are more posterior sections from the same rats at the level of the ipsilateral medial VPMpc in the thalamus (D - F). While the VMPpc is intact in the rat with the control Lesion (panels A and D), and in the rat that sustained an ideal Lesion (panels B and E), clear retrograde degeneration occurred in the VPMpc of the rat that sustained a large infarct (panels C and F). The NeuN stain shown for this rat in panels C and F can be compared to the same tissue stained with cresyl Lecht violet shown in the cortex in panel C2 and in the VPMpc in the thalamus in panel F2. Histological analysis of the insular GC was conducted at 4-5 levels corresponding to Figures 15-19 in the standard rat stereotaxic atlas (Paxinos and Watson 2005). Level I correspond to the prefrontal/insular cortex and cells here are reportedly multi-sensory. Level II is considered anterior insular cortex and contains taste specific cells (see Fig. 1, A). Dramatic changes in the rhinal sulcus and the location of the anterior commissure, corpus collosum, and a diminishing cortical layer IV are commonly used for rostral to caudal orientation between levels. Level III (moving rostral to caudal) resides two-thirds of the way through the taste responsive insula. Level IV is considered posterior dorsal insular and marks the caudal border of oral sensation in the insula. Finally, Level V is thought to receive visceral instead of orosensory input from the thalamus.

In Experiment 1, one control rat died and the data from eight of the nineteen GCTx rats were eliminated because lesions were misplaced (n=3), incomplete (n=2), or too large (n=3). Thus, in Experiment 1, the data from 29 rats total contributed to the current statistical analyses, 11 rats in the GCTx group and 18 in the Sham group (9 PBS and 9 NSC). All eleven GCTx rats that contributed data in Experiment 1 had complete bilateral damage to the insular GC at levels I-V. While all lesions extended beyond the boundaries of the agranular and dysgranular layers of the insular cortex, the insular GC in these rats appears to be the only region to sustain complete damage from neurotoxin application (see Fig. 1, B). In stark contrast to these smaller lesions, as described, ibotenic acid infusions also resulted in extremely large cortical damage in three rodents (see Fig. 1, C), and the data from these rats were excluded. Cystic infarct damage spread laterally to the pia mater, medially to the claustrum into and beyond the external capsule reaching the putamen. This damage also incorporated extensive somatosensory (dorsal), rhinal and piriform (ventral) cortices, see Fig. 1, C.

As mentioned, cystic infarct also led to retrograde damage in the medial VPMpc, which was assessed at four levels which correspond to Figures **31–34,** see (Paxinos and Watson 2005). The total absence of cell bodies indicates extensive neuronal degeneration throughout the medial VPMpc of the thalamus. Level I rest anterior to the taste thalamus. Level II marks the beginning of the taste thalamus. Level III correspond to the midway point through the taste thalamus on a rostral-caudal plane. Level IV is the most posterior region of the medial VPMpc (where cells respond to taste specifically). An example of the brain of a control lesion rat at Level III of the medial VPMpc of the thalamus is depicted in Fig. 1, D.

----------Insert Figure 3-2 about here ----------

CS intake: 0.05 M sucrose in Bottle 1 (taste cue)

Bottle 1 intake data was collapsed into 2-day Blocks. Intake of the 0.5 M sucrose solution did not differ between the PBS and the NSC rats, $F < 1$, and thus the data were collapsed and are referred as the Sham group. All data were then analyzed using a 2 x 2 x 6 mixed factorial ANOVA varying Lesion (GCTx or Sham), US (0.05 M sucrose vs. 1.0 M sucrose), and Blocks (1 – 6). Although the 3 way analysis revealed a significant Lesion x US x Blocks interaction, $F (5, 170) = 3.11$, $p < .05$, the main effect of Lesion just missed significance, $F (1, 34) = 3.87$, $p = .057$ and unfortunately there was no significant main effect of US, $F (1, 34) = 0.20$, $p > .05$. An independent t-test of intake within trial showed that the Sham rats in the 0.05 M – 1.0 M sucrose condition suppressed intake of the 0.05 M sucrose cue relative to first bottle intake by the 0.05 M – 0.05 M sucrose controls beginning with the 4th two-day block, $p < .05$, (see Fig. 2, panel A). The GCTx rats, on the other hand, did not show a significant difference in Bottle 1 intake as a function of US condition (0.05 M or 1.0 M sucrose in Bottle 2), $ps > .05$, see Fig. 2, panel B.

US intake: 0.05 M sucrose vs. 1.0 M sucrose in Bottle 2

As with Bottle 1 intake, the Bottle 2 intake data were analyzed using a 2 x 2 x 6 mixed factorial ANOVA varying Lesion (GCTx or Sham), US (0.05M sucrose vs. 1.0M sucrose), and Blocks (1 – 6). The results of this analysis revealed a significant main effect of US, $F (1, 25) = 72.77$, $p < .05$, and as expected no 3-way interaction $F (5, 125) = 0.37$, $p > .05$. The subsequent 2-way ANOVA varying US and Blocks, however, was significant, $F (5, 125) = 15.89$, $p < .05$. Post hoc tests showed that by the 3rd block all rats, made more licks of second bottle 1.0 M sucrose than 2nd bottle 0.05 M sucrose, $ps < .05$ (see the panels C and D in Fig. 2). Consistent with our expectation, the insular GC was found to be essential for the comparison of disparate natural rewards and for the resultant reduction in CS intake.

The strength of the current investigation, however, comes from the fact that the same GCTx rats that failed to exhibit the anticipatory contrast effect in the present Experiment 1 were subsequently tested with morphine, cocaine, and LiCl (see, (Geddes, Han et al. 2008)).

Experiment 2: Bilateral GCTx and saccharin-sucrose pairings

In Experiment 2, a naïve set of rats was used to test whether bilateral ibotenic acid lesions of the insular GC are sufficient to eliminate the suppressive effects of 1.0 M sucrose on the intake of a 0.15% saccharin CS, the more traditional anticipatory contrast design. This modification (i.e., saccharin-sucrose) is expected to yield larger contrast effects than those observed in Experiment 1. If the saccharin-sucrose ACE is blocked by these lesions, these data will verify a mediating role for the insular GC in the comparison of disparate concentrations of a natural sapid reward over time.

Methods:

Subjects and apparatus

The subjects were 53 (31 Sham and 22 GCTx) naïve male Sprague-Dawley rats (Charles River) weighing 350-550 g at the beginning of the experiment. The rats were obtained, maintained, and housed as described in Exp. # 1. Testing was conducted in the four modular operant chambers described above.

CS and US solutions

A .15% saccharin (Sigma Chemical Company, St. Louis, MO) solution and sucrose (Fischer Chemical, Pittsburg, PA) served as the CS and/or the US. Both were prepared 24 h in advance and presented at room temperature.

Surgery

Twenty-two rats received bilateral GCTx lesions, nine rats served as PBS infused controls, and 22 rats served as NSC. For Experiment 2, the lesion coordinates were adjusted to yield a higher number of successful lesions. The modified coordinates were adjusted according to body weight: rats weighing 350 grams or less received infusions at: A/P +0.8 mm anterior to bregma, M/L +/- 4.0 mm away the midline at 10^{o} lateral and D/V -6.7 mm from the skull surface; rats weighing greater than 350 grams received infusions at: A/P +1.0 mm, M/L +/- 4.0 mm from midline at 10^{o} lateral, and D/V -7.7 mm from the skull surface. These adjustments were based on axon tracing and multi-unit recording data showing that the location of the insular taste receptive field changes as a function of body size (Kosar, Grill et al. 1986; Kosar, Grill et al. 1986).

Procedure

Following at least 5 d recovery, rats were deprived to 85% of their free-feeding body weight as described in Experiment 1. *Experiment 2* was run in two replications consisting of 34 and 19 rats, respectively. Rats (31 Sham and 22 GCTx) were then assigned, in a counterbalanced manner, to one of two US conditions: 0.15% saccharin in bottle 2 or 1.0 M sucrose in bottle 2. Thus, during testing, all rats were given 3 min access to 0.15% saccharin and immediately thereafter, given either 3 min additional access to the same 0.15% saccharin solution (n= 8 GCTx rats and 12 Sham (4 PBS and 8 NSC)) or 3 min access to a highly preferred 1.0 M sucrose solution (n=12 GCTx rats and 21 Sham rats (5 PBS and 16 NSC).

Histology

The histology was conducted as described in Experiment 1.

Results and Discussion:

Histological lesion analysis

Five GCTx rats (3 in the saccharin US group and 2 in the sucrose US group) died during the acquisition trials, their data were excluded from further analysis. Eleven of the seventeen remaining rats in the GCTx group had complete, well-placed bilateral damage to the insular taste cortex that were consistent in placement and extent with those described in Experiment 1 (data not shown). The remaining six rats were eliminated from the experiment due to either misplaced (n=2), incomplete (n=2), or extensive cortical lesions accompanied by marked retrograde degeneration of the corresponding thalamic taste relay (n=2).

CS intake: 0.15% saccharin in Bottle 1

The data from two additional Sham rats in the saccharin-saccharin group were lost as the rats stopped licking after their tails were inadvertently pinched in the operant door. Thus, for Experiment 2, the behavioral data from 29 Sham rats (n = 10 saccharin - saccharin; n = 19 saccharin - sucrose) and 11 GCTx rats (n = 7 saccharin - saccharin; n = 4 saccharin - sucrose) contributed data to the current statistical analyses. Intake (licks / 5-min) of first bottle 0.15% saccharin served as the dependent measure and the data were averaged into seven 2-day Blocks. Bottle 1 intake data were then analyzed using a 2 x 2 x 2 x 7 mixed factorial ANOVA varying Replication (1 vs. 2), Lesion (GCTx or Sham), US (0.15% saccharin vs. 1.0M sucrose), and Blocks (1 – 7). The results of this analysis showed no effect of Replication, all Fs < 1. Consequently, the data from the two replications were combined and the data were reanalyzed using the 2 x 2 x 7 mixed factorial ANOVA. The results of this analysis revealed a significant 3-way interaction, $F(6, 216) = 3.74$, $p<.05$, see Fig. 3.

----------Insert Figure 3-3 about here ----------

Post-hoc Newman-Keuls tests showed that Sham rats in the saccharin-sucrose condition suppressed intake of first bottle saccharin relative to their saccharin-saccharin controls beginning with the 2nd block, $p < .05$, (see Fig. 3, panel A). In contrast, rats with bilateral GCTx lesions in the same saccharin-sucrose condition failed to show any evidence of contrast relative to their saccharin-saccharin treated counterparts, $ps > .05$ (see Fig. 3, panel B). Post hoc assessment also reveal that Bottle 1 saccharin intake differed between Sham and GCTx rats in the saccharin-saccharin condition only transiently (i.e., only on Blocks 5 and 6). Bottle 1 saccharin intake between the Sham and GCTx rats in the saccharin-sucrose condition, on the other hand, differed on Blocks 2-7, with the Sham rats in the saccharin-sucrose condition making fewer licks of the saccharin cue than similarly treated GCTx rats.

US intake: saccharin vs. 1.0 M sucrose in Bottle 2

Intake (licks/3 min) of second bottle saccharin or sucrose served as the dependent measure (see Figure 3, panels C and D). As with bottle 1 intake, statistical tests confirmed that second bottle intake could be collapsed across replication, $Fs < 1$. The final intake data were then analyzed using a 2 x 2 x 7 mixed factorial ANOVA varying Lesion (GCTx or Sham), US (0.15% saccharin vs. 1.0 M sucrose), and Blocks (1 – 7). The 3-way analysis on the bottle 2 intake was not significant, $F< 1$. The main effect of Lesion condition (on Bottle 2 intake) was also not of significance, $F < 1$, $p > .05$ meanwhile the main effect of US condition was $F (1, 36) = 85.07$, $p< .05$. A 2-way AVOVA varying US x Blocks was also significant, $F (6, 216) = 9.99$, $p<.05$ meanwhile post hoc tests revealed that all rats, regardless of lesion condition, made more licks for bottle 2 sucrose than saccharin and intake by the Sham and GCTx rats did not differ for either 2nd bottle saccharin or sucrose, $ps > .05$.

Experiment 2 here was designed to challenge this latter finding by testing whether bilateral GCTx lesions would be disruptive when using parameters that support the development of a more robust

ACE, as compared to Exp. # 1. The rats in Exp. # 1 were vital for a different reason. Not only did they provide evidence that bilateral insular taste lesions disrupt sucrose contrast, but also by serving in subsequent taste CS-US paradigms they served as a within subjects comparison of lesion deficit. That is to say, the rats in Exp. # 1 of the current sucrose anticipatory contrast studies were subsequently ran in three follow-up CS-US paring experiments (which was, saccharin-morphine, followed by polycose-cocaine and ending with pairing a palatable sodium chloride solution with LiCl injections).Table 1 (below) summarizes the effect of these bilateral insular gustatory cortex lesions (GCTx) on the acquisition of conditioned taste avoidance. As shown, the same bilateral GCTx lesions that prevented avoidance of a lesser taste cue in anticipation of a highly rewarding 1.0 M sucrose solution in the near future also, fully disrupt avoidance of a taste cue paired with a 15 or 30 mg/kg dose of morphine, and attenuate the gustatory CS avoidance produce with passive administration of a 10 mg/kg dose of cocaine. The same rats successfully, however, acquired LiCl-induced CTA, using either a low or a high dose of the drug. Finally, these cortical lesion data parallel the data our lab has also collected with the effects under the same taste conditioning paradigms when rats are given bilateral lesions of the gustatory thalamus (THLx), which is the major source of projections to insular taste cortex.

----------Insert Table 3-1 about here ----------

General Discussion

The results of these two studies demonstrate, unequivocally, that an intact gustatory cortex is essential for the development of an ACE, i.e., for the suppression of intake of a lesser valued taste cue when paired with a highly rewarding sucrose US. The lesion fully prevented sucrose-induced suppression of intake of a lesser valued sucrose cue in Experiment 1 and of a lesser valued saccharin cue in Experiment 2. Bottle 2 data made it clear that this lesion-induced deficit was not due to a failure to

detect either the CS or the US or to an inability to appropriately respond to different levels of gustatory reward. All rats, Sham and GCTx alike, made more licks on bottle 2 for the stronger than for the weaker rewards. Despite the absence of un-shifted controls, these data are consistent with the conclusion drawn by Kesner and Gilber (2007) using a sucrose-sucrose pair in Long Evans rats. They also are consistent with the supposition of our reward comparison hypothesis that the suppressive effects of drugs of abuse, at least in part, parallel those induced by a rewarding sucrose solution (Grigson 1997). Thus, lesions of the insular GC disrupt avoidance of a taste cue when paired with sucrose, morphine, or a lower dose of cocaine, but not when paired with LiCl (Mackey, Keller et al. 1986; Geddes, Han et al. 2008).

As shown in Table 1, a very similar behavioral profile is obtained following bilateral lesions of the gustatory thalamus. For two decades, it has been known that bilateral lesions of the gustatory thalamus exert no impact, whatsoever, on the establishment of a LiCl-induced CTA (Flynn, Grill et al. 1991). This initial finding has since been replicated many times (Reilly and Pritchard 1996; Scalera, Grigson et al. 1997; Grigson, Lyuboslavsky et al. 2000; Mungarndee, Lundy et al. 2006). The same lesion, however, greatly disrupts suppression of CS intake by sucrose and morphine (Reilly and Pritchard 1996; Reilly and Trifunovic 1999; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005) as well as cocaine (Twining, in prep). Together, the data suggest the possibility that the cortex, rather than the thalamus, is critical for avoidance of a taste cue when paired with a rewarding sucrose solution or a drug of abuse. An alternative interpretation is that the phenomena depend not upon the thalamus or the cortex, per se, but upon communication between the two structures. Studies are underway to test the involvement of the gustatory thalamocortical loop in these and related phenomena.

Whether dependent upon the thalamus, cortex, or the thalamocortical loop, consideration must be given to the role of these nuclei in this phenomenon. Why does this lesion so disrupt the development of a sucrose-sucrose or saccharin-sucrose ACE? To address this question, we must rely upon the

confluence of data across lesion studies. As mentioned above, it is unlikely that rats with bilateral lesions of the gustatory cortex failed to suppress intake of the gustatory cue because they failed to detect the CS or the US or because they did not recognize one as more palatable than the other. All rats readily licked the 2nd bottle solutions and clearly made more licks for the stronger of the two solutions. In all cases, 2nd bottle intake increased across trials in a normal acquisition function, suggesting that all rats remembered the taste of the solution from day to day. In this study, there was a slight tendency for the lesioned rats in the saccharin-sucrose condition in Experiment 2 to make more licks for first bottle saccharin than the lesioned saccharin-saccharin controls. This pattern of behavior is robust in rats with bilateral lesions of the gustatory thalamus, particularly when a 5 min interstimulus interval was imposed between CS and US access (Schroy, Wheeler et al. 2005). This finding (perhaps occluded by a ceiling effect here) is reminiscent of a reinforcement effect and, as such, suggests that the failure to exhibit an ACE by the lesioned rats is likely not due to a failure to associate the CS with the US.

In this case, the rats do not only have to associate the CS with the US, they need to compare their relative value. Further, unlike simultaneous contrast where the rats compare disparate concentrations of sucrose, for example, in an alternating fashion over very short periods of time (Flaherty and Largen 1975; Flaherty and Rowan 1986; Grigson, Kaplan et al. 1997), in the case of anticipatory contrast, the rats need to compare an available reward level with the memory of a different reward that is anticipated in the near future. This distinction suggests that the gustatory cortex may be essential for just such a comparison. Support for this suggestion comes from data related to a third form of consummatory contrast – successive negative contrast. In the consummatory successive negative contrast paradigm, rats with a history of daily access to a very high concentration of sucrose, for example, avoid intake of a lesser concentration when unexpectedly downshifted (Flaherty 1996). Data suggest that, while somewhat different, this phenomenon also depends upon comparison of the available reward with the

memory of the previously received reward (see Flaherty, 1996 for a review). Consistent with this interpretation, bilateral lesions of the gustatory thalamus (Reilly and Trifunovic 1999; Reilly and Trifunovic 2003; Sastre and Reilly 2006), insular gustatory cortex (Lin, Roman et al. 2009), and asymmetric lesions of the gustatory thalamocortical loop (see (Geddes, Li et al. in prep (2), Thesis Chapter 4)) all fully disrupt the occurrence of a successive negative contrast effect following reward downshift. Our current hypothesis, then, is that the gustatory cortex and/or the thalamocortical loop, is essential for comparing an available reward with the memory of an alternative reward and for expression this comparison process in changes in consummatory behavior.

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Table 3-1. Comparison of the effects of bilateral lesions of the gustatory cortex (GCTx) and the gustatory thalamus (THLx). Table 1 depicts the disruptive effects of bilateral insular GCTx and bilateral taste thalamic lesions on LiCl-induced sucrose-induced anticipatory contrast effects (ACE), the suppressive effects of various drugs of abuse (i.e., morphine or cocaine), and LiCl-induced conditioned taste aversion (CTA). Both lesion types disrupt the suppressive effects of a highly reinforcing sucrose solution (ACE) which is a rewarding US and blocked or attenuated taste avoidance produced by a lower dose morphine and cocaine, respectively. The GCTx lesions also render the higher dose of morphine ineffective meanwhile neither type of bilateral thalamic (THLx) or cortical (GCTx) lesion was sufficient to block LiCl-induced CTA learning.

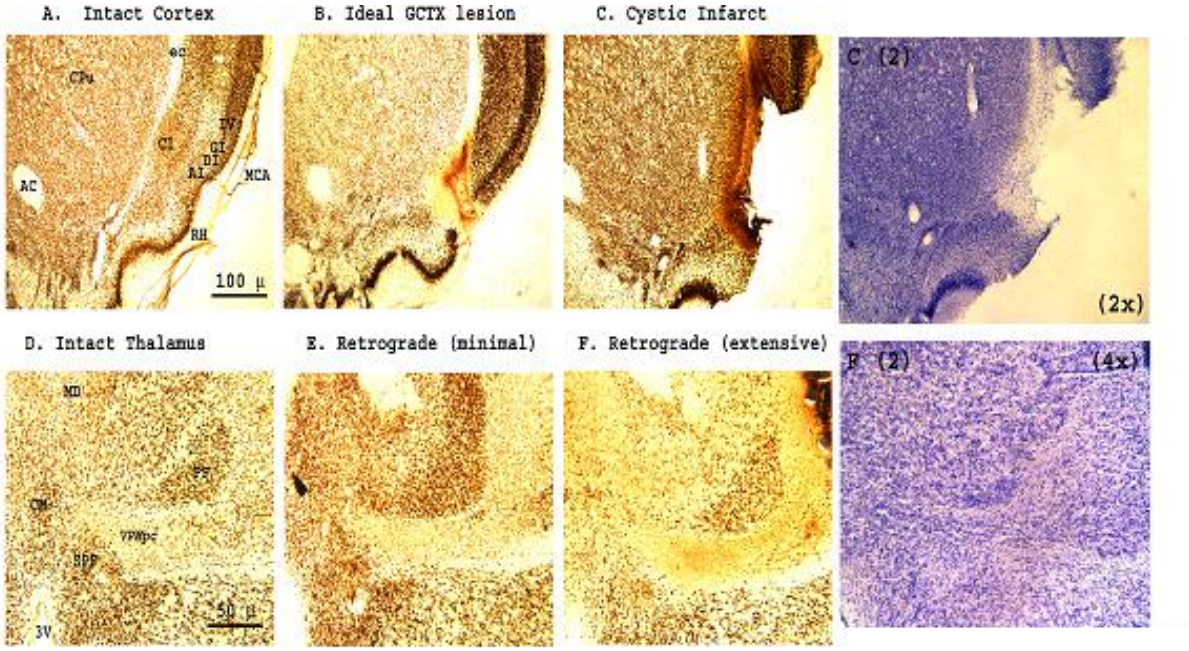
Figure captions

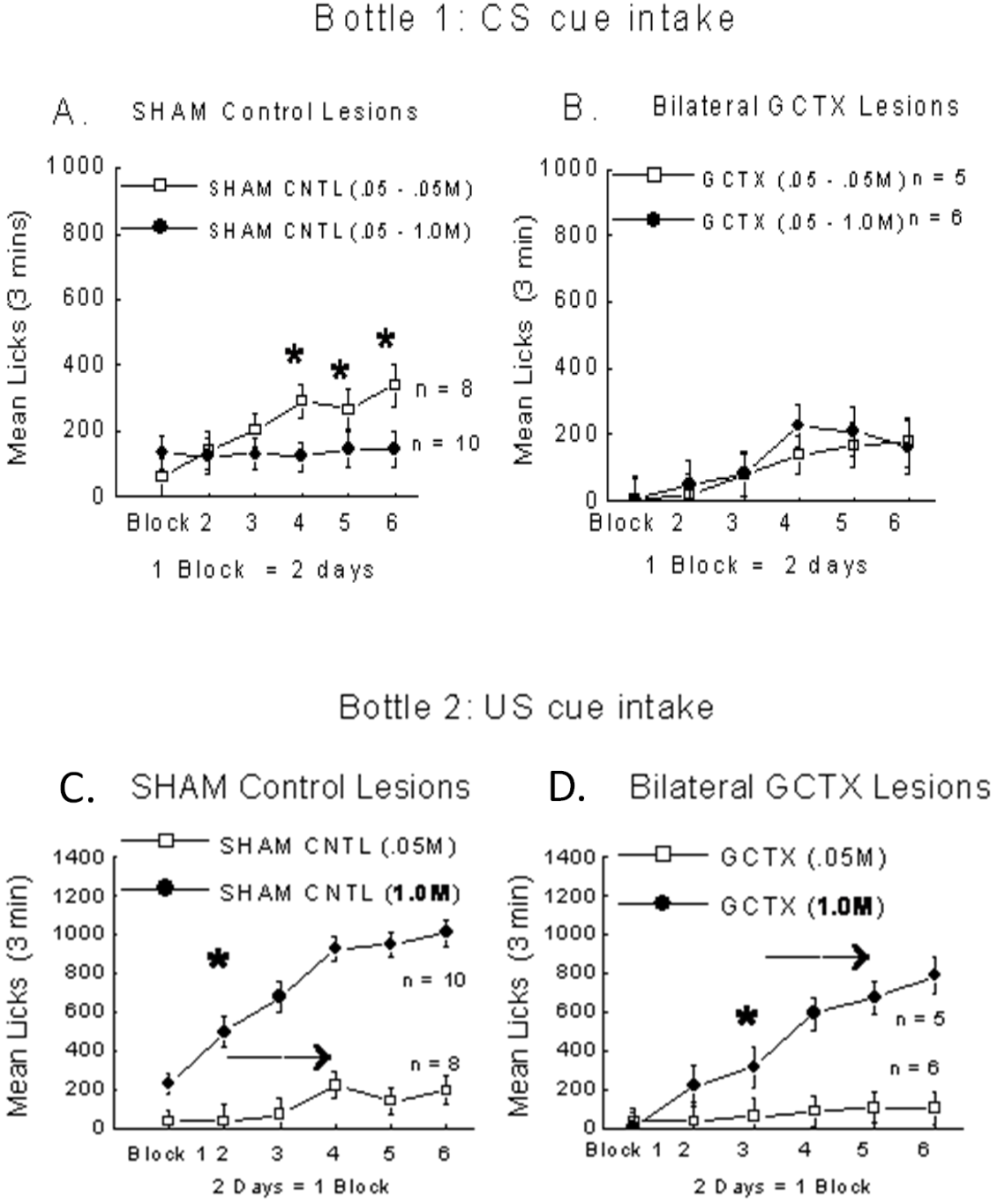
Figure 3-1. Photomicrographs of NeuN- and cresyl violet- stained coronal brain sections at the level of the gustatory cortex and the taste thalamus. Top panel are coronal sections at the level of the taste cortex from three rats. (A) An intact insular gustatory cortex (at Level II) in a Sham-operated rat (2x). (B) An ideal GCTx lesion (Level III). (C) A rat brain with cystic infarct of cortical lesion site. (D to F) are the corresponding thalamic nuclei from the rats in A-C (4x). Adjacent section from C and F which were stained with cresyl violet are depicted in C2 and F2.

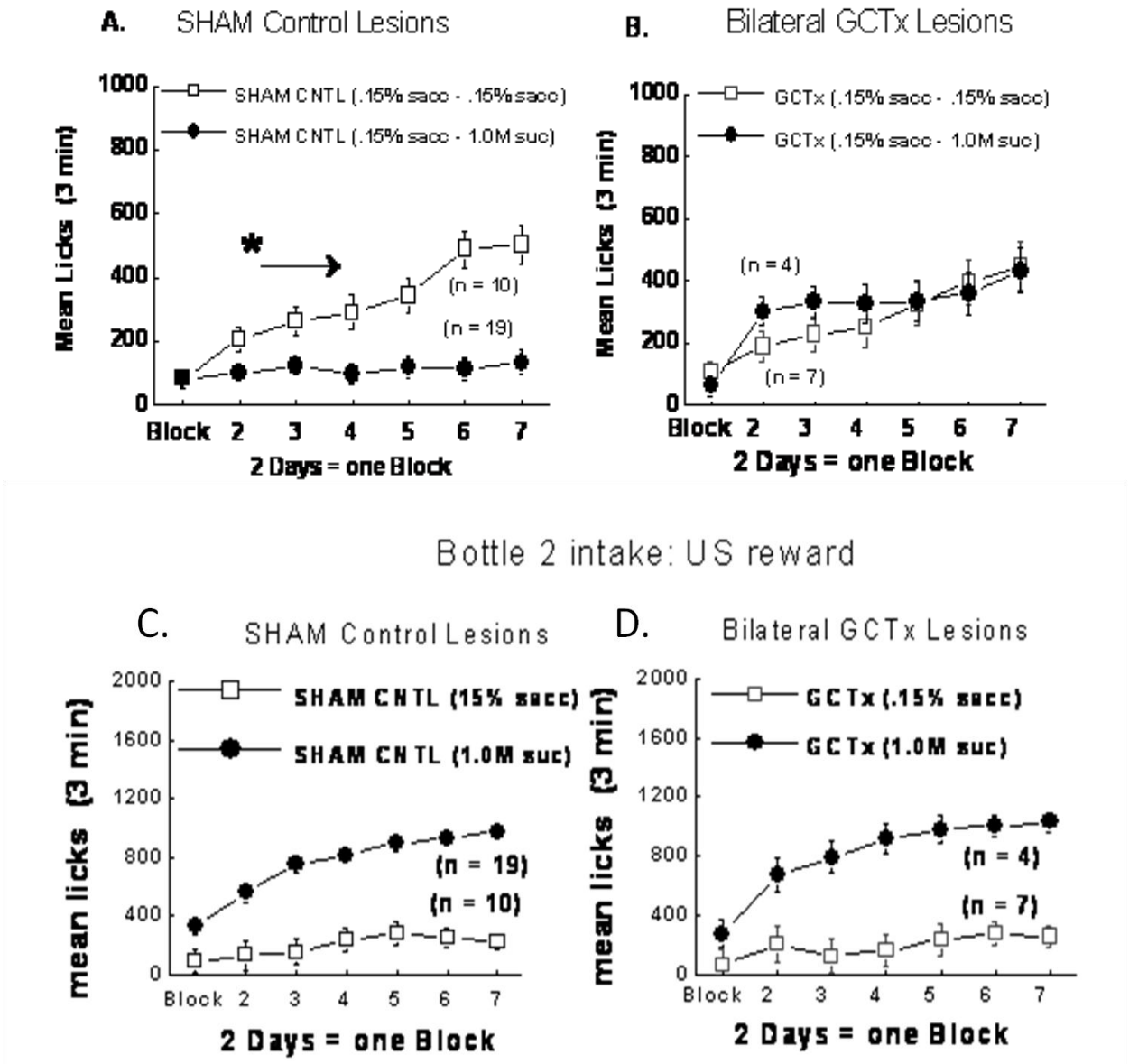
Figure 3-2. Mean number of licks (+/- SEM) for the CS and US in Experiment 1. Top panel, mean number of licks (+/- SEM) for the CS (0.05 M sucrose) in Bottle 1 by rats with control (Sham) lesions (panel A) and by rats with bilateral ibotenic acid lesions of the gustatory cortex (GCTx) (panel B) for rats in the unshifted 0.05 M – 0.05 M condition vs. rats in the shifted 0.05 M – 1.0 M sucrose condition. Bottom panel, mean number of licks (+/- SEM) for the US (0.05 M or 1.0 M sucrose) in Bottle 2 by rats with Sham (panel C) or GCTx (panel D) lesion. * indicates points of significant difference.

Figure 3-3. Mean number of licks (+/- SEM) for the CS and US in Experiment 1. Top panel, mean number of licks (+/- SEM) for the CS (0.15% saccharin) in Bottle 1 by rats with control (Sham) lesions (panel A) and by rats with bilateral ibotenic acid lesions of the gustatory cortex (GCTx) (panel B) for rats in the unshifted (saccharin-saccharin) condition vs. rats in the shifted saccharin-sucrose condition. Bottom panel, mean number of licks (+/- SEM) for the US (0.15% saccharin or 1.0 M sucrose) in Bottle 2 by rats with Sham (panel C) or GCTx (panel D) lesion. * indicates points of significant difference.

Lesions BLOCKED avoidance of CS **NO EFFECT** of lesion on CS Intake	Bilateral Insular GC Lesions (GCTx)	Bilateral Taste Thalamic Lesions (THLx)
sucrose-induced anticipatory contrast effect (ACE)	**BLOCKED** (Geddes, in prep 1)	**BLOCKED** **(Schroy et al., 2005)**
RELATED BEHAVIORAL DATA		
15 mg/kg dose morphine-induced CS devaluation	**BLOCKED** **(Geddes et al., 2008)**	**BLOCKED** **(Grigson et al., 2000)**
30 mg/kg dose morphine-induced CS devaluation	**BLOCKED** **(Geddes et al., 2008)**	**BLOCKED** (Twining, in prep)
10 mg/kg dose cocaine-induced CS devaluation	**Attenuated** **(Geddes et al., 2008)**	**BLOCKED** **(Grigson et al., 2000)**
20 mg/kg dose cocaine-induced CS devaluation	**No effect** **(Geddes et al., 2008)**	**No effect** (Twining, in prep)
.009 M LiCl--induced CTA	**No effect** **(Geddes et al., 2008)**	**No effect** **(Grigson et al., 2000)**
.015 M LiCl--induced CTA	**No effect** **(Geddes et al., 2008)**	**No effect** **(Grigson et al., 2000)**


A. Intact Cortex
B. Ideal GCTX lesion
C. Cystic Infarct
CPu
ec
LV
CI
GI
DI
AI
MCA
AC
RH
100 µ
C (2)
(2x)
D. Intact Thalamus
E. Retrograde (minimal)
F. Retrograde (extensive)
MD
PF
CM
VPMpc
BPF
3V
50 µ
F (2)
(4x)


Bottle 1: CS cue intake
A. SHAM Control Lesions
SHAM CNTL (.05 - .05M)
SHAM CNTL (.05 - 1.0M)
Mean Licks (3 mins)
1000
800
600
400
200
0
n = 8
n = 10
Block 2 3 4 5 6
1 Block = 2 days
B. Bilateral GCTX Lesions
GCTX (.05 - .05M) n = 5
GCTX (.05 - 1.0M) n = 6
Mean Licks (3 min)
Block 2 3 4 5 6
1 Block = 2 days
Bottle 2: US cue intake
C. SHAM Control Lesions
SHAM CNTL (.05M)
SHAM CNTL (1.0M)
Mean Licks (3 min)
1400
1200
1000
800
600
400
200
0
n = 10
n = 8
Block 1 2 3 4 5 6
2 Days = 1 Block
D. Bilateral GCTX Lesions
GCTX (.05M)
GCTX (1.0M)
Mean Licks (3 min)
n = 5
n = 6
Block 1 2 3 4 5 6
2 Days = 1 Block


Bottle 1 intake: CS cue
A.
SHAM Control Lesions
SHAM CNTL (.15% sacc - .15% sacc)
SHAM CNTL (.15% sacc - 1.0M suc)
Mean Licks (3 min)
1000
800
600
400
200
0
*
(n = 10)
(n = 19)
Block 2 3 4 5 6 7
2 Days = one Block
B.
Bilateral GCTx Lesions
GCTx (.15% sacc - .15% sacc)
GCTx (.15% sacc - 1.0M suc)
Mean Licks (3 min)
(n = 4)
(n = 7)
Block 2 3 4 5 6 7
2 Days = one Block
Bottle 2 intake: US reward
C.
SHAM Control Lesions
SHAM CNTL (15% sacc)
SHAM CNTL (1.0M suc)
mean licks (3 min)
2000
1600
1200
800
400
0
(n = 19)
(n = 10)
Block 2 3 4 5 6 7
2 Days = one Block
D.
Bilateral GCTx Lesions
GCTx (.15% sacc)
GCTx (1.0M suc)
mean licks (3 min)
(n = 4)
(n = 7)
Block 2 3 4 5 6 7
2 Days = one Block

Bilbliography

Agmo, A. and E. Marroquin (1997). "Role of gustatory and postingestive actions of sweeteners in the generation of positive affect as evaluated by place preference conditioning." Appetite **29**(3): 269-89.

Augustine, J. R. (1985). "The insular lobe in primates including humans." Neurol Res **7**(1): 2-10.

Barker, L. M. and J. C. Smith (1974). "A comparison of taste aversions induced by radiation and lithium chloride in CS-US and US-CS paradigms." J Comp Physiol Psychol **87**(4): 644-54.

Bechara, A., G. M. Martin, et al. (1993). "The parabrachial nucleus: a brain stem substrate critical for mediating the aversive motivational effects of morphine." Behav Neurosci **107**(1): 147-60.

Benjamin, R. M. and K. Akert (1959). "Cortical and thalamic areas involved in taste discrimination in the albino rat." J Comp Neurol **111**: 231-59.

Bickel, W. K., R. J. DeGrandpre, et al. (1995). "The behavioral economics of concurrent drug reinforcers: a review and reanalysis of drug self-administration research." Psychopharmacology (Berl) **118**(3): 250-9.

Burgdorf, J., B. Knutson, et al. (2001). "Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs." Psychopharmacology (Berl) **155**(1): 35-42.

Calu, D. J. and G. Schoenbaum (2008). "Cocaine-Paired Cues Activate Aversive Representations in Accumbens Neurons." Neuron **57**(5): 633-633.

Cappell, H. and A. E. LeBlanc (1971). "Conditioned aversion to saccharin by single administrations of mescaline and d-amphetamine." Psychopharmacology **22**(4): 352-356.

Carrell, L. E., D. S. Cannon, et al. (1986). "Nausea and radiation-induced taste aversions in cancer patients." Appetite **7**(3): 203-8.

Carroll, M. E., S. T. Lac, et al. (1989). "A **concurrently available nondrug reinforcer prevents the acquisition or decreases the maintenance of cocaine-reinforced behavior**." Psychopharmacology (Berl) **97**(1): 23-9.

Carroll, M. E. and J. C. Smith (1974). "Tme course of radiation-induced taste aversion conditioning." Physiol Behav **13**(6): 809-12.

Corcoran, M. E., I. Bolotow, et al. (1974). "Conditioned taste aversions produced by active and inactive cannabinoids." Pharmacology Biochemistry and Behavior **2**(6): 725-728.

Flaherty, C. and N. Geary (1993). "Contrast in consummatory behavior." Appetite **21**(1): 81.

Flaherty, C., P. S. Grigson, et al. (1996). "Anticipatory contrast as a function of access time and spatial location." Anim. Learn. Behav **24**: 68-81.

Flaherty, C. F. (1996). Incentive relativity. Cambridge [England] ; New York, Cambridge University Press.

Flaherty, C. F. and S. Checke (1982). ""anticipation of incentive gain."" Animal learning and Behavior(10): 171-182.

Flaherty, C. F. and J. Largen (1975). "Within-subjects positive and negative contrast effects in rats." J Comp Physiol Psychol **88**(2): 653-64.

Flaherty, C. F. and G. A. Rowan (1986). "Successive, simultaneous, and anticipatory contrast in the consumption of saccharin solutions." J Exp Psychol Anim Behav Process **12**(4): 381-93.

Flynn, F. W., H. J. Grill, et al. (1991). "Central gustatory lesions: II. Effects on sodium appetite, taste aversion learning, and feeding behaviors." Behav Neurosci **105**(6): 944-54.

Garb, J. L. and A. J. Stunkard (1974). "Taste Aversions in Man." Am J Psychiatry **131**(11): 1204-1207.

Garcia, J., D. J. Kimeldorf, et al. (1955). "Conditioned aversion to saccharin resulting from exposure to gamma radiation." Science **122**(3160): 157-8.

Geddes, R. I., L. Han, et al. (2008). "Gustatory insular cortex lesions disrupt drug-induced, but not lithium chloride-induced, suppression of conditioned stimulus intake." Behav Neurosci **122**(5): 1038-50.

Geddes, R. I., H. Li, et al. (in prep (2), Thesis Chapter 4). "An Intact Taste Thalamocortical Loop is Required for Consummatory Successive Negative Contrast." in PSU THESIS

Giorgi, O., M. G. Corda, et al. (1997). "Effects of cocaine and morphine in rats from two psychogenetically selected lines: a behavioral and brain dialysis study." Behav Genet **27**(6): 537-46.

Glowa, J. R., A. E. Shaw, et al. (1994). "Cocaine-induced conditioned taste aversions: comparisons between effects in LEW/N and F344/N rat strains." Psychopharmacology (Berl) **114**(2): 229-32.

Goldstein, R. Z., N. Alia-Klein, et al. (2007). "Is decreased prefrontal cortical sensitivity to monetary reward associated with impaired motivation and self-control in cocaine addiction?" Am J Psychiatry **164**(1): 43-51.

Gomez, F. and P. S. Grigson (1999). "The suppressive effects of LiCl, sucrose, and drugs of abuse are modulated by sucrose concentration in food-deprived rats." Physiol Behav **67**(3): 351-7.

Grigson, P. S. (1997). "Conditioned taste aversions and drugs of abuse: a reinterpretation." Behav Neurosci **111**(1): 129-36.

Grigson, P. S. and C. S. Freet (2000). "The suppressive effects of sucrose and cocaine, but not lithium chloride, are greater in Lewis than in Fischer rats: evidence for the reward comparison hypothesis." Behav Neurosci **114**(2): 353-63.

Grigson, P. S., J. M. Kaplan, et al. (1997). "Reward comparison in chronic decerebrate rats." Am J Physiol **273**(2 Pt 2): R479-86.

Grigson, P. S., P. Lyuboslavsky, et al. (2000). "Bilateral lesions of the gustatory thalamus disrupt morphine- but not LiCl-induced intake suppression in rats: evidence against the conditioned taste aversion hypothesis." Brain Res **858**(2): 327-37.

Grigson, P. S., P. N. Lyuboslavsky, et al. (1999). "Water-deprivation prevents morphine-, but not LiCl-induced, suppression of sucrose intake." Physiol Behav **67**(2): 277-86.

Grigson, P. S., R. A. Wheeler, et al. (2001). "Chronic morphine treatment exaggerates the suppressive effects of sucrose and cocaine, but not lithium chloride, on saccharin intake in Sprague-Dawley rats." Behav Neurosci **115**(2): 403-16.

Gruest, N., P. Richer, et al. (2004). "Emergence of long-term memory for conditioned aversion in the rat fetus." Dev Psychobiol **44**(3): 189-98.

Hunt, T. and Z. Amit (1987). "Conditioned taste aversion induced by self-administered drugs: paradox revisited." Neurosci Biobehav Rev **11**(1): 107-30.

Jongen-Relo, A. L. and J. Feldon (2002). "Specific neuronal protein: a new tool for histological evaluation of excitotoxic lesions." Physiol Behav **76**(4-5): 449-56.

Kesner, R. P. and P. E. Gilbert (2007). "The role of the agranular insular cortex in anticipation of reward contrast." Neurobiol Learn Mem **88**(1): 82-6.

Knackstedt, L. A., M. M. Samimi, et al. (2002). "Evidence for opponent-process actions of intravenous cocaine and cocaethylene." Pharmacol Biochem Behav **72**(4): 931-6.

Kosar, E., H. J. Grill, et al. (1986). "Gustatory cortex in the rat. I. Physiological properties and cytoarchitecture." Brain Res **379**(2): 329-41.

Kosar, E., H. J. Grill, et al. (1986). "Gustatory cortex in the rat. II. Thalamocortical projections." Brain Res **379**(2): 342-52.

Kumar, R., J. A. Pratt, et al. (1983). "Characteristics of conditioned taste aversion produced by nicotine in rats." Br J Pharmacol **79**(1): 245-53.

Le Magnen, J. (1969). "Peripheral and systemic actions of food in the caloric regulation of intake." Ann N Y Acad Sci **157**(2): 1126-57.

Liang, N.-C. and R. Norgren. (2009). "Pontine and thalamic influence on oral sucrose and oil reward." from http://etda.libraries.psu.edu/theses/approved/WorldWideIndex/ETD-4257/index.html

Lin, J.-Y., C. Roman, et al. (2009). "Insular cortex and consummatory successive negative contrast in the rat." Behavioral Neuroscience **123**(4): 810-814.

Liu, C., J. Showalter, et al. (2009). "Ethanol-induced conditioned taste avoidance: reward or aversion?" Alcohol Clin Exp Res **33**(3): 522-30.

Mackey, W. B., J. Keller, et al. (1986). "Visceral cortex lesions block conditioned taste aversions induced by morphine." Pharmacol Biochem Behav **24**(1): 71-8.

Mickley, G. A., D. R. Remmers-Roeber, et al. (2000). "Ketamine Blocks a Taste-Mediated Conditioned Motor Response in Perinatal Rats." Pharmacology Biochemistry and Behavior **66**(3): 547-552.

Mucha, R. F., D. van der Kooy, et al. (1982). "Drug reinforcement studied by the use of place conditioning in rat." Brain Res **243**(1): 91-105.

Mungarndee, S. S., J. Lundy, Robert F., et al. (2006). "Central gustatory lesions and learned taste aversions: Unconditioned stimuli." Physiology & Behavior **87**(3): 542-551.

Nachman, M. and J. H. Ashe (1973). "Learned taste aversions in rats as a function of dosage, concentration, and route of administration of LiCl." Physiol Behav **10**(1): 73-8.

Nachman, M., D. Lester, et al. (1970). "Alcohol aversion in the rat: behavioral assessment of noxious drug effects." Science **168**(936): 1244-6.

Norgren, R. (1978). "Projections from the nucleus of the solitary tract in the rat." Neuroscience **3**(2): 207-18.

Norgren, R. and G. Wolf (1975). "Projections of thalamic gustatory and lingual areas in the rat." Brain Res **92**(1): 123-9.

Paxinos, G. and C. R. Watson (2005). "Rat brain in stereotaxic coordinates (CD-ROM)." J Neurosci Methods **3**(2): 129-49.

Pritchard, T. C. and R. Norgren (2004). Gustatory System

The Human Nervous System (Second Edition). G. Paxinos and J. K. Mai. San Diego, Academic Press**:** 1171-1196.

Reilly, S., M. Bornovalova, et al. (2004). "Excitotoxic lesions of the gustatory thalamus spare simultaneous contrast effects but eliminate anticipatory negative contrast: evidence against a memory deficit." Behav Neurosci **118**(2): 365-76.

Reilly, S., P. S. Grigson, et al. (1993). "Parabrachial nucleus lesions and conditioned taste aversion: evidence supporting an associative deficit." Behav Neurosci **107**(6): 1005-17.

Reilly, S. and T. C. Pritchard (1996). "Gustatory thalamus lesions in the rat: II. Aversive and appetitive taste conditioning." Behav Neurosci **110**(4): 746-59.

Reilly, S. and R. Trifunovic (1999). "Gustatory thalamus lesions eliminate successive negative contrast in rats." Behav Neurosci **113**(6): 1242-8.

Reilly, S. and R. Trifunovic (1999). "Progressive ratio performance in rats with gustatory thalamus lesions." Behav Neurosci **113**(5): 1008-19.

Reilly, S. and R. Trifunovic (2003). "Gustatory thalamus lesions eliminate successive negative contrast in rats: evidence against a memory deficit." Behav Neurosci **117**(3): 606-15.

Risinger, F. O. and J. M. Boyce (2002). "Conditioning tastant and the acquisition of conditioned taste avoidance to drugs of abuse in DBA/2J mice." Psychopharmacology (Berl) **160**(3): 225-32.

Sastre, A. and S. Reilly (2006). "Excitotoxic lesions of the gustatory thalamus eliminate consummatory but not instrumental successive negative contrast in rats." Behav Brain Res **170**(1): 34-40.

Scalera, G., P. S. Grigson, et al. (1997). "Gustatory functions, sodium appetite, and conditioned taste aversion survive excitotoxic lesions of the thalamic taste area." Behav Neurosci **111**(3): 633-45.

Scalera, G., A. C. Spector, et al. (1995). "Excitotoxic lesions of the parabrachial nuclei prevent conditioned taste aversions and sodium appetite in rats." Behav Neurosci **109**(5): 997-1008.

Schroy, P. L., R. A. Wheeler, et al. (2005). "Role of gustatory thalamus in anticipation and comparison of rewards over time in rats." Am J Physiol Regul Integr Comp Physiol **288**(4): R966-80.

Sklar, L. S. and Z. Amit (1977). "Manipulations of catecholamine systems block the conditioned taste aversion induced by self-administered drugs." Neuropharmacology **16**(10): 649-655.

Smith, B. K., K. Barker, et al. (1994). "Development of altered taste preferences in tumor-bearing rats." Appetite **23**(3): 219-30.

Smith, J. C. and J. T. Blumsack (1981). "Learned taste aversion as a factor in cancer therapy." Cancer Treat Rep **65 Suppl 5**: 37-42.

Smith, J. C., D. D. Morris, et al. (1964). "Conditioned Aversion to Saccharin Solution with High Dose Rates of X-Rays as the Unconditioned Stimulus." Radiat Res **22**: 507-10.

Spector, A. C., R. Norgren, et al. (1992). "Parabrachial gustatory lesions impair taste aversion learning in rats." Behav Neurosci **106**(1): 147-61.

Stalnaker, T. A., M. R. Roesch, et al. (2006). "Abnormal associative encoding in orbitofrontal neurons in cocaine-experienced rats during decision-making." Eur J Neurosci **24**(9): 2643-53.

Switzman, L., T. Hunt, et al. (1981). "Heroin and morphine: aversive and analgesic effects in rats." Pharmacol Biochem Behav **15**(5): 755-9.

Tomasi, D., R. Z. Goldstein, et al. (2007). "Thalamo-cortical dysfunction in cocaine abusers: Implications in attention and perception." Psychiatry Research: Neuroimaging **155**(3): 189-201.

Travers, S. P., C. Pfaffmann, et al. (1986). "Convergence of lingual and palatal gustatory neural activity in the nucleus of the solitary tract." Brain Research **365**(2): 305-20.

Twining, R. C., M. Bolan, et al. (2009). "Yoked delivery of cocaine is aversive and protects against the motivation for drug in rats." Behav Neurosci **123**(4): 913-25.

Wheeler, R. A., R. C. Twining, et al. (2008). "Behavioral and electrophysiological indices of negative affect predict cocaine self-administration." Neuron **57**(5): 774-85.

White, N., L. Sklar, et al. (1977). "The reinforcing action of morphine and its paradoxical side effect." Psychopharmacology (Berl) **52**(1): 63-6.

White, N. M. and G. D. Carr (1985). "The conditioned place preference is affected by two independent reinforcement processes." Pharmacology Biochemistry and Behavior **23**(1): 37-42.

Wise, R. A., R. A. Yokel, et al. (1976). "Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats." Science **191**(4233): 1273-5.

Wolf, G. (1968). "Projections of thalamic and cortical gustatory areas in the rat." J Comp Neurol **132**(4): 519-30.

Zito, K. A., A. Bechara, et al. (1988). "The dopamine innervation of the visceral cortex mediates the aversive effects of opiates." Pharmacol Biochem Behav **30**(3): 693-9.

An Intact Taste Thalamocortical Loop is Required for Consummatory Successive Negative Contrast

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ABSTRACT

Consummatory successive negative contrast (SNC) occurs when animals that are unexpectedly downshifted from a strong to a weak concentration of sucrose consume far less of the weaker reward, relative to animals that only experienced the lesser reward (Flaherty and Largen 1975; Grigson, Spector et al. 1993; Flaherty 1996). Similar contrast effects have been reported in humans and in primates (Weinstein 1972; Specht and Twining 1999). In rats, the central taste pathway is predominately unilateral (Norgren and Leonard 1971; Pfaffmann, Norgren et al. 1977) and bilateral lesions of the gustatory region of the pontine parabrachial nucleus (Grigson, Spector et al. 1994), the thalamus (Reilly and Trifunovic 1999) or the insular cortex (Lin, Roman et al. 2009) prevent SNC in consummatory behavior. These data implicate a mediating role for the taste cortex or, alternatively, for communication between the cortex and the thalamus. The present study used asymmetric lesions of the reciprocally connected taste thalamus and insular gustatory cortex (GC) to test whether SNC depended upon an intact gustatory thalamocortical loop. We found that asymmetric lesions (i.e., right thalamus and left cortex, THCx), but not ipsilateral control lesions (i.e., right thalamus and cortex, Ip-CNTL), prevented SNC. Rats, then, require an intact gustatory thalamocortical loop to evidence a SNC effect in consummatory behavior following an unexpected downshift in reward.

Key words: gustatory, reward, taste avoidance, devaluation, expectation.

Introduction

The perception of the value of a given stimulus by rats, monkey, and man depends upon the perceived relative value of alternative rewards presented closely in time. In humans, a 7% sucrose solution was reportedly devalued when it was preceded by experience with a more preferred 28% sucrose solution (Specht and Twining 1999). In monkeys, Tinklepaugh (1928) showed that when grapes or a piece of banana was replaced with an otherwise acceptable piece of lettuce, monkeys completely avoided the lettuce (Williams 1997). Similarly, rats unexpectedly shifted from 16% to 4% sucrose responded less for the 4% sucrose solution relative to unshifted rats that only experienced the lower 4% sucrose reward (Weinstein 1972). Avoidance of the otherwise acceptable solution following reward downshift is referred to as a consummatory successive negative contrast (SNC) effect. Finally, while differences in caloric load may contribute to the phenomenon, negative contrast effects also occur following a shift from a high to a low concentration of saccharin and they occur whether rats are sated or hungry (Weinstein 1978; Riley and Dunlap 1979; Flaherty and Rowan 1986; Grigson, Spector et al. 1993; Mitchell and Flaherty 2005). Contrast, then, can be mediated by taste factors alone.

Avoidance of the lesser reward as a result of SNC requires comparison between the currently available, lesser valued, solution and the memory of the more preferred, but missing reward (Flaherty and Checke 1982; Flaherty, Rowan et al. 1989). According to Flaherty (1996), Stage 1 of SNC occurs when the less preferred solution is first encountered (post-shift day 1) and involves detection, rejection, and searching for the missing reward (Flaherty 1996). Stage 2 occurs on the 2nd post-shift day and involves approach/avoidance 'conflict' as the hungry rat approaches a reward that is, from an absolute perspective, perfectly acceptable, but from a relative perspective, sub par. Contrast, at this stage, can be attenuated by selective pretreatment with a benzodiazepine (Flaherty and Rowan 1986) and it is

associated with elevated levels of circulating corticosterone (Flaherty, Rowan et al. 1986). Stage 3 occurs on the third or fourth postshift day and involves acceptance of the less preferred reward and, thus, increasing intake to the level of the unshifted controls (Flaherty 1996). Moving on, although SNC has been considered an animal model of emotion (e.g., anxiety and/or disappointment), lesion data implicate an important mediating role for the central gustatory pathway (see Figure 1).

----------Insert Figure 4-1 (Horizontal view of rat taste pathway) about here ----------

Despite slight differences between the central taste projections in rats and primates (Pfaffmann, Norgren et al. 1977; Beckstead, Morse et al. 1980; Norgren 1983) the innate orofacial responses to specific taste qualities are highly conserved in mammals (Grill and Norgren 1978; Claude Marcel and Bruno 1996). Taste transduction occurs in the oral cavity via taste specific receptors (Zhang, Hoon et al. 2003; Kim, Breslin et al. 2004), which are restricted to the anterior 2/3 and posterior 1/3 of the tongue, palate, and upper esophagus (Mistretta 1972). Taste buds and surrounding epithelium are innervated by fibers from several cranial nerves (CN V, VII, IX and X) (Frank and Pfaffmann 1969; Contreras, Beckstead et al. 1982; Miller and Spangler 1982). These fibers project to the nucleus of the solitary tract, NST (Norgren and Leonard 1971; Travers, Pfaffmann et al. 1986). In Sprague Dawley rats, the rostral NST (rNST) mainly sends fibers to the ipsilateral medial pontine parabrachial nuclei, mPBN, (Norgren and Pfaffmann 1975). The mPBN send two sets of projections: A ventral pathway diffuses unilaterally to at least 3 major limbic regions including the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST), (see gray tract in Fig. 1). The dorsal pathway, on the other hand, projects ipsilaterally and contralaterally from the mPBN relays to the parvicellular part of the ventral posteromedial nucleus of the thalamus (VPMpc). From there taste

information is transferred by an ipsilateral projection to the agranular insular cortices, see black tract in Fig. 1 (Benjamin and Akert 1959; Wolf 1968; Norgren and Wolf 1975; Augustine 1985; Kosar, Grill et al. 1986; Halsell 1992).

Orosensory processing in the brain is important for taste-guided learning (Kawamura 1975). Unilateral damage to the insular gustatory cortex (GC) in humans may result in ipsi- and contralateral deficits in taste perception (Bornstein 1940b; Pritchard, Macaluso et al. 1999; Mathy, Dupuis et al. 2003). In rats, bilateral lesions of the mPBN, VPMpc, or insular GC block acquisition of SNC following an unexpected downshift in reward (Grigson, Spector et al. 1994; Reilly and Trifunovic 1999; Lin, Roman et al. 2009). These findings support one of two conclusions: (1) The mPBN and the VPMpc are important only because they serve as conduits to the gustatory cortex; (2) The phenomenon depends not upon the gustatory thalamus or cortex, per se, but upon communication between the two structures. The present study tested the latter hypothesis by examining whether SNC would be prevented following asymmetric (THCx), but not ipsilateral control lesions (Ip-CNTL), of the gustatory thalamocortical loop.

Methods:

Subjects and apparatus

The subjects were 34 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 500-650 g at the beginning of the experiment. Rats were individually housed in standard suspended stainless steel wire-mesh cages in a temperature and humidity controlled environment.

Pre- and post-shift solutions

Sucrose powder obtained from Fischer Chemical (Pittsburg, PA) was used to mix two concentrations of sucrose solution: 0.1 M and 1.0 M. These solutions were prepared 24 h prior to testing and presented at room temperature.

Surgery: Asymmetric gustatory thalamocortical lesions (THCx)

Unilateral gustatory thalamic lesion (Stage 1): For surgery, each rat was injected with atropine sulfate (.25 mg/ i.p.), Gentamicin (6 mg/kg, i.p.), and 20-min later, .5 cc of pentobarbital sodium (50 mg/kg, i.p.) with .1 cc supplemented as necessary. Body temperature was maintained at 37+1 ^{0}C. Once under surgical anesthesia, taste neurons in the thalamus were located by applying 0.3 M NaCl to the anterior tongue while recording multiunit activity through a glass-insulated tungsten microelectrode (Z = 1.0-1.5 MΩ at 1 kHz). Taste receptive cells in the taste thalamus (VPMpc) were located by stimulating the anterior part of the tongue with 0.3 M NaCl while recording multiunit activity through a glass-insulated tungsten microelectrode [impedance (Z) = 1.0-1.5 MΩ at 1 kHz].The coordinates for electrode penetrations ranged from -3.5 to -4.1 mm posterior to bregma, -1.1 to -1.4 mm lateral to the midsagittal suture, and -5.5 to -6.8 mm below the skull surface. Once the thalamic taste area had been located, the search microelectrode was replaced with a double-barreled micropipette-electrode (M/E; OD 50–60 mm; Z = 0.5–1 MΩ at 1 kHz), one lumen of which was glued directly onto the needle of a 1.0-μl Hamilton microsyringe. Thereafter, the microsyringe/electrode assembly and 0.2 μl (20 μg / μl) of ibotenic acid was infused over 10 mins. The electrode remained in place for an additional 5-10 mins. After removal of the microsyringe/electrode assembly, the skull hole was filled with gelfoam. The rat was then transported to a similar surgery room next door for the stereotaxically-guided insular gustatory cortex lesions.

Unilateral insular gustatory cortex lesion (Stage 2): The coordinates for the stereotaxically placed cortical lesions are modified from those of Mackey and colleagues (Mackey, Keller et al. 1986), and are the same as recently published (Geddes, Han et al. 2008), except the cortical lesion, in this case, was placed in only one hemisphere (which was ipsilateral to thalamic lesion in the control lesion group

(Ip-CNTL), and contralateral to thalamic lesion in the asymmetric lesion group, THCx). The range of coordinates is as follows: A/P +0.7 to +1.0 mm anterior to β, M/L +/- 4.0 lateral to midline with the tip oriented 10^{o} lateral, and D/V -6.7 to -7.7 from the skull. Once again, 0.2 μl (20 μg/μl) of ibotenic acid was infused over a 10 min period and the needle of the Hamilton microsyringe remained in place for an additional 10 mins. Once the ibotenic acid filled Hamilton microsyringe was removed, the hole in the skull was filled with gelfoam and the incision closed with wound clips.

Procedure:

Recovery from surgery took 5-7 days. Thereafter, rats were handled and weighed for another 5 days. Access to chow was then restricted by a once/day feeding until each rat reached 85% of their free-feeding bodyweight. During testing, 16 rats (8 Ip-CNTL and 8 THCx) served in the shifted experimental group. These rats received 5 min access to 1.0 M sucrose on trials 1-10 and were then downshifted to 0.1 M sucrose on trials 11-14. Eighteen rats (6 Ip-CNTL and 12 THCx) served in the unshifted control group and, as such, received 5 min daily access to 0.1 M sucrose on all 14 days of testing. All rats were given their daily food ration no sooner than 45 min after the daily test session.

----------Insert Figure 4-2 (Levels I-VI) about here ----------

Results

Histological lesion analysis

The brains were removed and sectioned using the NeuN stain as initially described by Jongen-Relo and Feldon (Jongen-Relo and Feldon 2002). The data from 5 rats (3 unshifted THCx and 2 shifted THCx) were eliminated due to misplaced lesions (which spared the intended taste specific target site) or

oversized lesions (which resulted in cystic infarct and significant damage to adjacent, non-taste nuclei). Thus, 15 rats (6 Ip-CNTL and 9 THCx) from the unshifted group and 14 rats (8 Ip-CNTL and 6 THCx) rats from the shifted group contributed data for analysis. As depicted in Figure 2, cortical and thalamic damage were assessed at six levels (Level I-VI), spanning the rostral-caudal extent of the respective taste areas. Figure 3 (below) shows photomicrographs of rat brains with representative Ip-CNTL and asymmetric THCx lesions. The insular cortex (GC) and VPMpc of the thalamus are clearly labeled and the core of the damaged area has been outlined. The damage to non -taste nuclei in these rats was partial at best, otherwise the data was dropped from analysis.

----------Insert Figure 4-3 (NeuN Histology) about here ----------

Sucrose intake

Pre-shift: Sucrose intake for Ip-CNTL (left panel) and THCx (right panel) rats in the unshifted and shifted groups are shown in Figure 3. A terminal pre-shift score was calculated for each group by averaging total licks across pre-shift days 9 and 10. These intake data were then analyzed, along with the post-shift data, using a 2 x 2 x 5 mixed factorial ANOVA varying lesion (THCx or Ip-CNTL), post-shift condition (unshifted or shifted) , and trials (1-5). The results of this analysis revealed a significant main effect of Lesion and Shift conditions, $Fs > 1$, $ps < .05$. In addition, the Lesion x Shift condition x Trial interaction also was significant, $F(4,100) = 2.59$, $p < .05$. Post hoc tests of this significant three-way ANOVA revealed that during the pre-shift phase, both the Ip-CNTL and THCx rats made more licks for the high than for the low concentration of sucrose, $ps < .05$ (see Figure 3, panels A and B).

----------Insert Figure 4-4 (behavioral SNC data) about here ----------

Post-shift: Once downshifted, the Ip-CNTL rats, but not the asymmetric THCx rats, exhibited a significant successive negative contrast effect. This conclusion was supported by post hoc Newman-Keuls tests of the significant 2 x 2 x 5 interaction. Thus, on post-shift day 1 (i.e., day 11), the CNTL rats shifted from 1.0 – 0.1 M sucrose consumed significantly less 0.1 M sucrose than did their unshifted controls, $p < .05$. Rats with asymmetric THCx lesions also reduced intake following the unexpected decrease in reward, but intake did not fall below that of their unshifted asymmetric THCx controls, $p > .05$. Over the 3-day recovery period or postshift day 3, intake by the shifted Ip-CNTL rats recovered to the level of the unshifted controls, $ps > .05$, meanwhile intake by the shifted THCx rats actually exceeded that of their unshifted controls on both the 2nd and the 3rd post-shift day, $ps < .05$. The asymmetric gustatory THCx lesion, then, fully prevented the occurrence of successive negative contrast in consummatory behavior following the unexpected downshift from 1.0 to 0.1 M sucrose.

Discussion

In rats, the mPBN send ascending gustatory fibers to the taste thalamus, bilaterally. Each taste thalamic nuclei, in turn, is reciprocally connected to the ipsilateral insular dysgranular/agranular cortex. Bilateral lesions of the mPBN, taste thalamus or insular gustatory cortex completely block consummatory SNC (Grigson, Spector et al. 1994; Reilly and Trifunovic 2003; Lin, Roman et al. 2009). The present study is the first to demonstrate that contralateral lesions of the taste thalamocortical axis, which disconnect the taste thalamus from the insular taste cortex in both hemispheres, also block consummatory SNC in rats. Ipsilateral control lesions (which anatomically spare the gustatory thalamocortical loop in at least one hemisphere), on the other hand, do not prevent the expression of a consummatory contrast. These data show that successive negative contrast effects in consummatory behavior depend upon communication between the taste thalamus and cortex.

During the pre-shift phase of consummatory SNC, regardless of lesion type, rats receiving 1.0 M sucrose drink more than rats receiving 0.1 M sucrose. An intact gustatory thalamocortical loop, then, is not required to respond appropriately to the absolute reward properties of the gustatory stimuli. This finding is consistent with other published data. For instance, sucrose licking results in dopamine overflow in the NAc in intact rats, and this effect is completely blocked by bilateral lesions of the mPBN, but not by bilateral lesions of the gustatory thalamus (Hajnal and Norgren 2005). Rats with bilateral lesions of the gustatory thalamus also respond normally for sucrose, salt, and acid when tested using short or long-term intake tests (Reilly and Pritchard 1996; Scalera, Grigson et al. 1997). Finally, as mentioned, rats with bilateral lesions of the gustatory thalamus or insular gustatory cortex have no difficulty learning to avoid intake of a gustatory cue when paired with LiCl-induced illness (Braun, Slick et al. 1972; Lorden 1976; Mackey, Keller et al. 1986; Flynn, Grill et al. 1991; Scalera, Grigson et al. 1997; Grigson, Lyuboslavsky et al. 2000; Mungarndee, Lundy et al. 2006), also see Chapter 2 of this Thesis or (Geddes, Han et al. 2008).

Unlike the clear absence of involvement in the processing of absolute reward properties for sweets, a great deal of evidence implicates a mediating role for the gustatory thalamus and cortex in responding to relative reward properties. Thus, as alluded to above, bilateral lesions of the gustatory thalamus or cortex block the avoidance of a saccharin or weak sucrose cue when paired with a high concentration of sucrose in the anticipatory contrast paradigm (Geddes and Grigson in prep (1), Thesis Chapter 3); Reilly, 1996 #675; Schroy, 2005 #756}. Bilateral lesions of the gustatory thalamus or cortex also block avoidance of a gustatory cue when paired with a low or a high dose of morphine or when paired with a 10 mg/kg dose of cocaine (Mackey, Keller et al. 1986; Reilly and Trifunovic 1999; Grigson, Lyuboslavsky et al. 2000; Geddes, Han et al. 2008). In each case, as with consummatory SNC, avoidance of the lesser reward involves the comparison of the available tastant with the ‘memory’ of the

preferred reward that is anticipated in the near future. This finding contrast with other data showing that the same nucleus is not involved in the short-term memory dependent comparison of disparate concentrations of a sweet when the two solutions are presented closely in time in the simultaneous contrast paradigm (Reilly and Pritchard 1997; Reilly, Bornovalova et al. 2004).

Although strong evidence suggests that thalamocortical nuclei are involved in the comparison of an available reward with the memory of a preferred reward, additional evidence suggests that the thalamic lesion may not disrupt this comparison process, per se, but the expression of the comparison process in consummatory behavior. Thus, bilateral lesions of the taste thalamus completely block SNC in consummatory behavior while sparing SNC in the same rats when provided with contextual cues in a runway (Sastre and Reilly 2006). Opposite dissociations have been obtained following lesions of other structures. For example, bilateral lesions of the NAc or the hippocampus spare consummatory SNC, but block SNC in instrumental performance in the runway (Flaherty, Rowan et al. 1989; Flaherty, Coppotelli et al. 1998; Leszczuk and Flaherty 2000). Together, these lesion data suggest that rats with lesions of the gustatory thalamus can compare disparate rewards over time, but this devaluation effect is not reflected by suppression of intake of the lesser reward. Future studies will test whether asymmetric lesions of the gustatory thalamocortical loop induce a parallel effect or whether, alternatively, disconnection of the gustatory thalamocortical loop disrupts SNC in both consummatory and instrumental responding.

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Figure captions

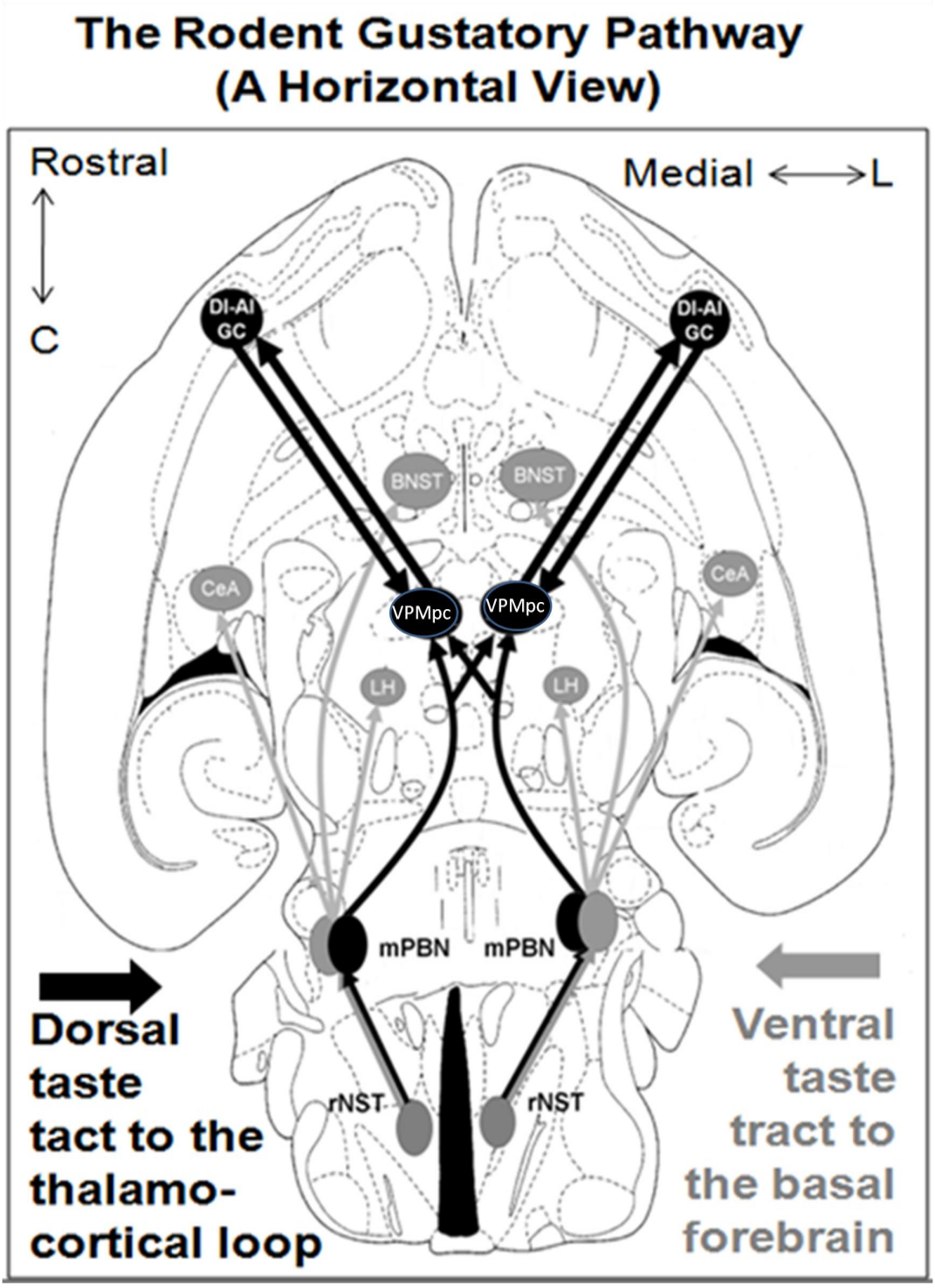
Figure 4-1. The Central Gustatory Pathway in the Rodent brain (A Horizontal View). Depicts a simplified version of the major ascending predominately ipsilateral projections of the three cranial nerves (e.g., the facial, CN V; glossopharengal, CN IX; and the vagus, CN X), to the nucleus of the solitary tract (NST) in the brainstem. The chorda tympani (CT), greater superficial petrosal (GSP), lingual-tonsilar branch of IX (LT- CN IX), and superior laryngeal branch of CN X (SL) relay taste input to the rostral NST (rNST), meanwhile non-taste input from the SL were intermingled with LT- CN IX terminals and visceral (e.g., post-ingestive and cardio-respiratory) afferents in the caudal medulla**.** These cranial nerves have fibers also omitted here, synapse on the spinal trigeminal nucleus, the dorsal motor nucleus (of the vagus), and the reticular formation in the medualla, which are all involved in innate orosensory and oromotor reflexes. The taste sensitive rNST rely taste sensation to the medial parabrachial nucleus (mPBN) meanwhile the visceral or cadual (cNST) medulla projects to the lateral PBN (not shown). The mPBN orosensory relays target (similar forebrain sites as the visceral neurons in the cNST and LPBN) the lateral hypothalamus (LH), and extended amygdala, more specifically the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST), all shown in with grey circles. A second set of mPBN taste fibers relay sensory information to the dorsal forebrain via projecting to the ipsilateral (50%) and (50%) contralateral to the most medial portion of the parvicellular region of the thalamus (VPMpc), which in turn is reciprocally connected to the ipsilateral insular gustatory cortex (GC), as indicated with black double arrows for the thalamocortical loop fibers and circles representing the thalamic and cortical nuclei. While this illustration is not totally inclusive of central taste-sensitive connections, it does representation connections verified by published (1) electrophysiological stimulation, (2) neuroantomical tracing, and (3) behavioral lesion studies. Also see references in text for more through discussion of connectivity.

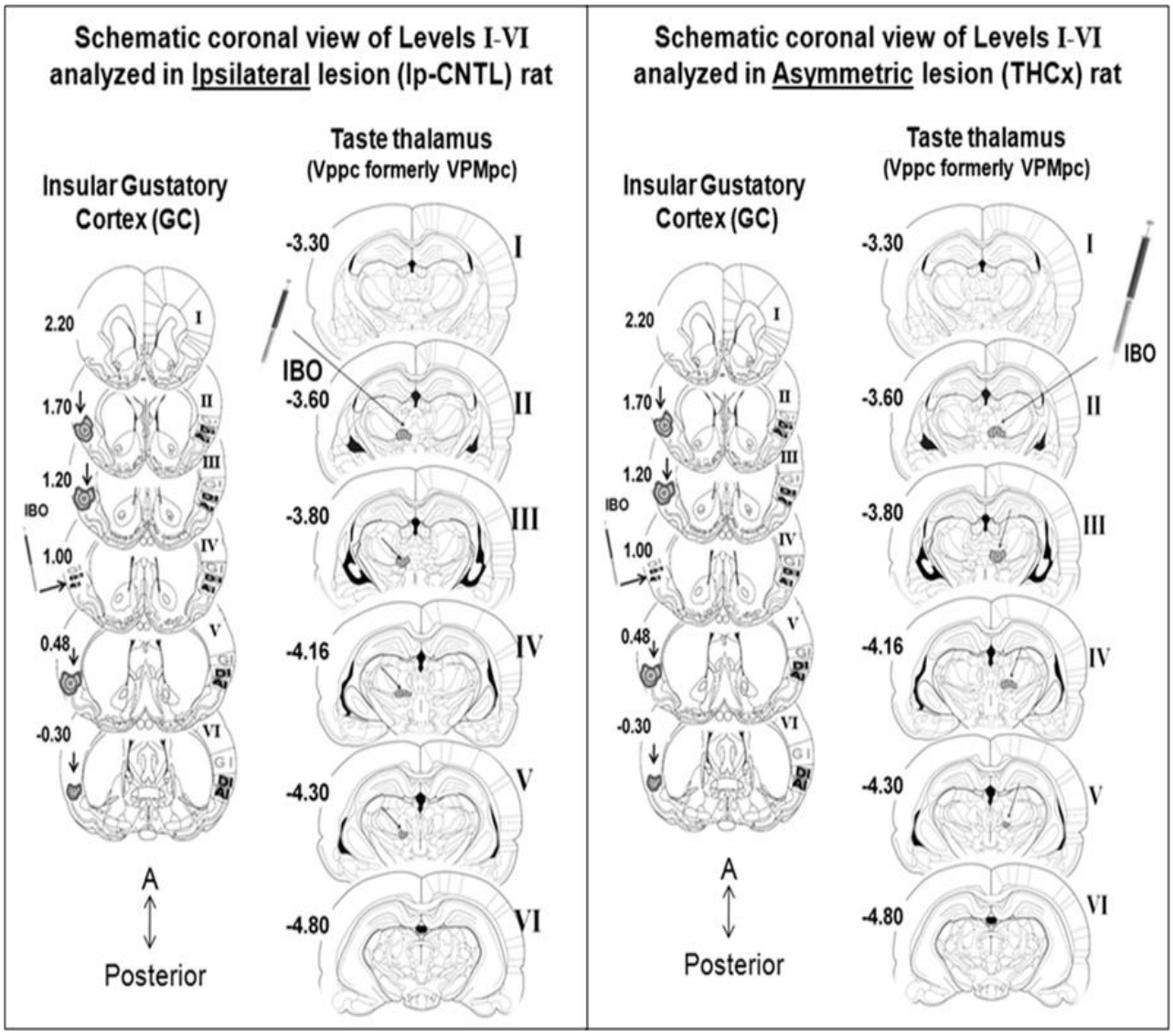
Figure 4-2. Superimposed schematic section at level of taste thalamus and insular gustatory cortex. Analysis of brain sections at Levels I to VI (in both the thalamus and insular taste cortex), were carried out to determine if any non-taste nuclei adjacent to our target site was damage from lesion surgery (see text). The behavioral data of a rat was omitted from analysis if damage to the areas surrounding the taste thalamus or cortex was considerable (i.e., cystic infarct, retrograde or anterograde degeneration).

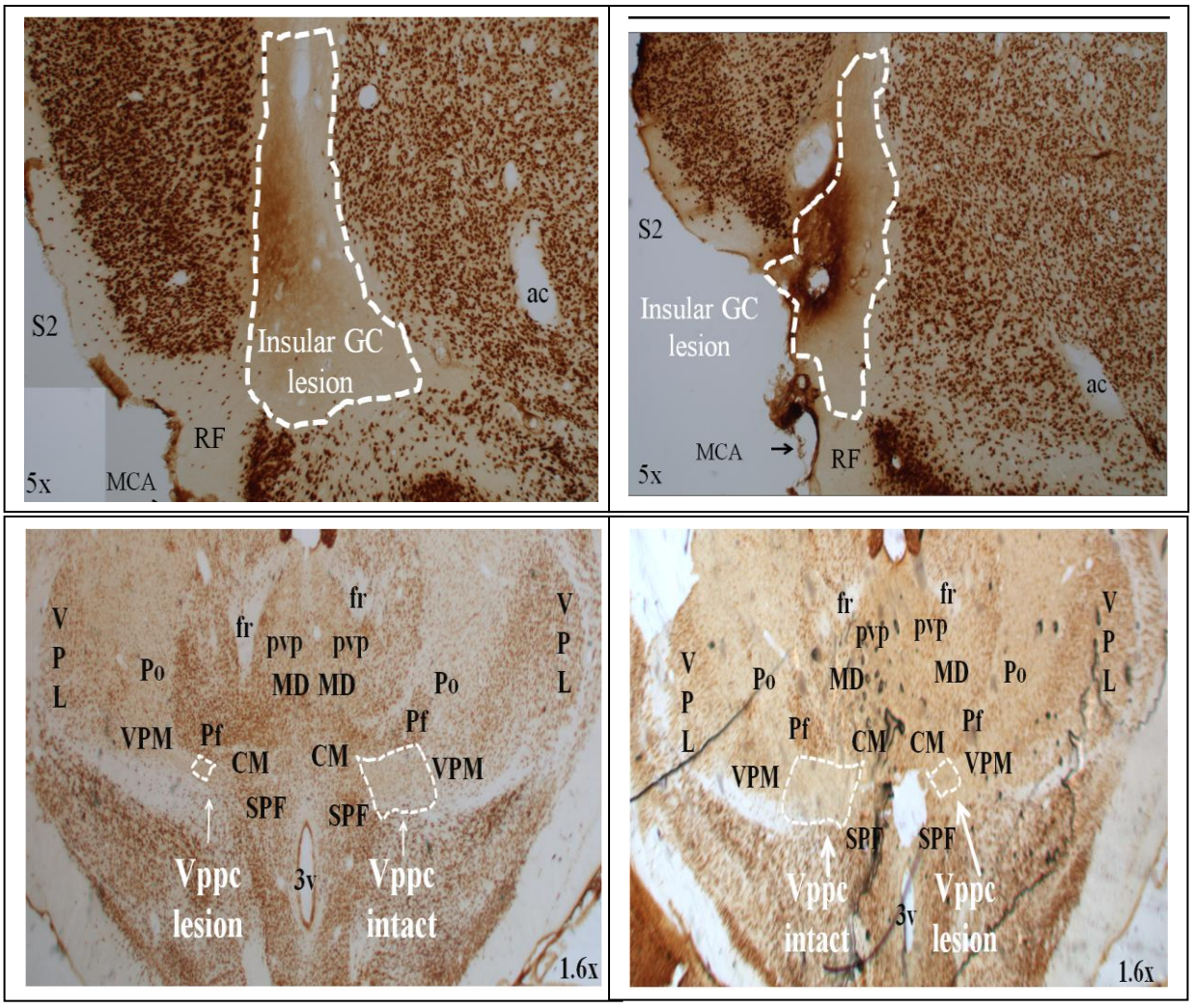
Figure 4-3. Representative insular and thalamic taste areas, damage, and the Levels (I-VI) analyzed. Coronal sections at the level of the insular cortex are displayed to the left and the corresponding thalamus to the right. The gray ring around the taste region indicates the focus of the lesion damage and analysis. Below each set of schematic diagrams are representative photomicrographs of lesion injury at the level insular gustatory cortex and thalamus of the same right with control (A-B) or asymmetric lesion (C-D). In each image the dotted outline encapsulates the damage taste area. Note the sparing of the non-taste nuclei and cortical tissue, respectively. As best as we can determine, total destruction of surrounding (non-gustatory) nuclei occurred in 2 of the 5 rats that were discarded for further analysis. The remaining 3 had insufficient lesion damage or sparing of the target either cortical (2) or the VPMpc (1) of the thalamus. A more detailed but early version of similar histological analysis has been previously published and used to analyze bilateral ibotenic acid induced lesions of the VPMpc (Schroy, Wheeler et al. 2005) and the insular gustatory cortex (Geddes, Han et al. 2008).

Figure 4-4. Pre- and post-shift solution intake data Intake (ml/5 min) of the 0.1 M sucrose in 85% food restricted rats with ipsilateral control lesions (Ip-CNTL) and rats with asymmetric thalamocortical loop lesions (THCx) in the shifted (1.0 M to .1 M) and unshifted (.1 M to .1 M) groups, are shown in panel A (left) and B (right) respectively. The pre-shift data is expressed here as an average of intake

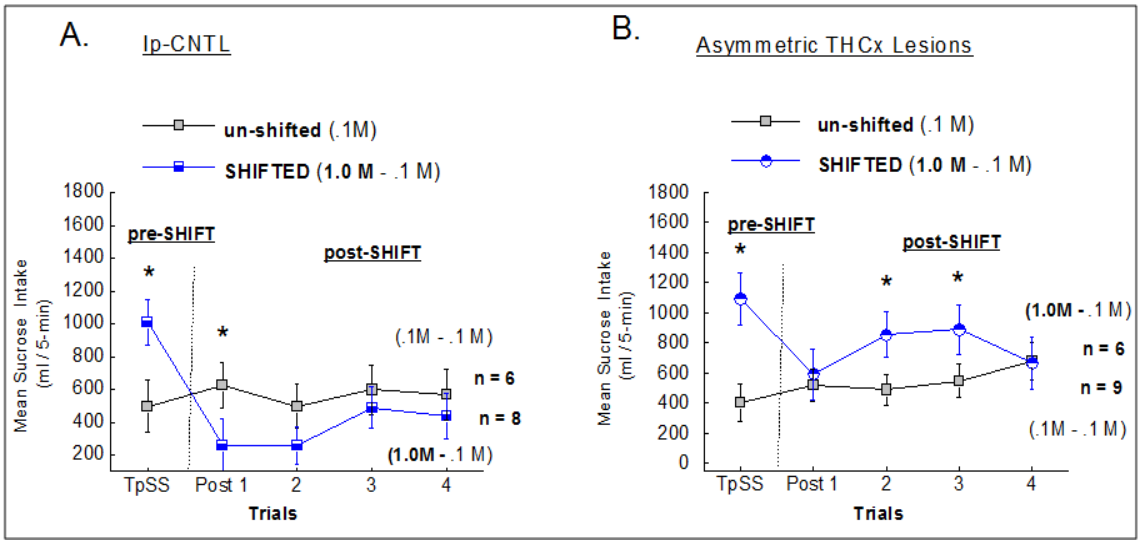
over the last two (9th and 10th) pre-shift day and the four days post-shift (days 11-14) are displayed individually. The Ip-CNTL rats suppressed intake of the .1 M sucrose well beyond their Ip-CNTL counterparts that only received the .1 M sucrose, $p < .05$ (indicated by an *), while the asymmetric THCx rats remarkable reduced their intake exactly to the level of their asymmetric THCx unshifted counterparts, $ps > .05$ ($n = 6 - 9$ per group). While the asymmetric THCx lesions were effective in disrupting the magnitude of gustatory avoidance due to SNC, based on intake THCx rat appear to retain the same absolute value for the lesser .1 M sucrose as do Ip-CNTL rats, see intake on the 4th post-shift day, $ps > .05$.


The Rodent Gustatory Pathway
(A Horizontal View)
Rostral
C
Medial ⟷ L
DI-AI GC
DI-AI GC
BNST
BNST
CeA
CeA
VPMpc
VPMpc
LH
LH
mPBN
mPBN
rNST
rNST
Dorsal taste tact to the thalamo-cortical loop
Ventral taste tract to the basal forebrain


Schematic coronal view of Levels I-VI analyzed in Ipsilateral lesion (Ip-CNTL) rat
Insular Gustatory Cortex (GC)
Taste thalamus (Vppc formerly VPMpc)
2.20
1.70
1.20
IBO
1.00
0.48
-0.30
-3.30
IBO
-3.60
-3.80
-4.16
-4.30
-4.80
I
II
III
IV
V
VI
A
Posterior
Schematic coronal view of Levels I-VI analyzed in Asymmetric lesion (THCx) rat
Insular Gustatory Cortex (GC)
Taste thalamus (Vppc formerly VPMpc)
2.20
1.70
1.20
IBO
1.00
0.48
-0.30
-3.30
IBO
-3.60
-3.80
-4.16
-4.30
-4.80
I
II
III
IV
V
VI
A
Posterior


S2
Insular GC lesion
ac
RF
5x
MCA
S2
Insular GC lesion
ac
MCA
RF
5x
V P L
fr
fr
pvp
pvp
Po
MD MD
Po
Pf
Pf
VPM
CM
CM
VPM
SPF
SPF
Vppc lesion
3v
Vppc intact
1.6x
V P L
fr
fr
pvp
pvp
V P L
Po
MD
MD
Po
Pf
Pf
CM
CM
VPM
VPM
SPF
SPF
Vppc intact
3v
Vppc lesion
1.6x

Ipsilaterl control (Ip-CNTL) lesion		Experimental Design	Asymmetric lesion (THCx) lesion	
Un-shifted	**Shifted**	groups	Un-shifted	**Shifted**
0.1 M sucrose	**1.0 M sucrose**	Preshift 5 min access per day for 10 days	0.1 M sucrose	**1.0 M sucrose**
0.1 M sucrose	**0.1 M sucrose**	**Postshift 5 min access per day for 4 days**	0.1 M sucrose	**0.1 M sucrose**
6 **(6)**	8 **(8)**	n = rats / group **(post-histology)**	12 **(9)**	8 **(6)**



Bilbliography

Augustine, J. R. (1985). "The insular lobe in primates including humans." Neurol Res **7**(1): 2-10.

Beckstead, R. M., J. R. Morse, et al. (1980). "The nucleus of the solitary tract in the monkey: projections to the thalamus and brain stem nuclei." J Comp Neurol **190**(2): 259-82.

Benjamin, R. M. and K. Akert (1959). "Cortical and thalamic areas involved in taste discrimination in the albino rat." J Comp Neurol **111**: 231-59.

Bornstein, W. S. (1940b). "Cortical representation of taste in man and monkey: II. The localization of the cortical taste area in man, a method of measuring impairment of taste in man." Journal of Biology and Medicine **13**: 133-156.

Braun, J. J., T. B. Slick, et al. (1972). "Involvement of gustatory neocortex in the learning of taste aversions." Physiol Behav **9**(4): 637-41.

Claude Marcel, H. and S. Bruno (1996). "Taste perception and feeding behavior in nonhuman primates and human populations." Evolutionary Anthropology: Issues, News, and Reviews **5**(2): 58-71.

Contreras, R. J., R. M. Beckstead, et al. (1982). "The central projections of the trigeminal, facial, glossopharyngeal and vagus nerves: an autoradiographic study in the rat." J Auton Nerv Syst **6**(3): 303-22.

Flaherty, C. F. (1996). Incentive relativity. Cambridge [England] ; New York, Cambridge University Press.

Flaherty, C. F. and S. Checke (1982). ""anticipation of incentive gain."" Animal learning and Behavior(10): 171-182.

Flaherty, C. F., C. Coppotelli, et al. (1998). "Excitotoxic lesions of the hippocampus disrupt runway but not consummatory contrast." Behav Brain Res **93**(1-2): 1-9.

Flaherty, C. F. and J. Largen (1975). "Within-subjects positive and negative contrast effects in rats." J Comp Physiol Psychol **88**(2): 653-64.

Flaherty, C. F. and G. A. Rowan (1986). "Successive, simultaneous, and anticipatory contrast in the consumption of saccharin solutions." J Exp Psychol Anim Behav Process **12**(4): 381-93.

Flaherty, C. F., G. A. Rowan, et al. (1989). "Effects of intrahippocampal administration of colchicine on incentive contrast and on radial maze performance." Behav Neurosci **103**(2): 319-28.

Flaherty, C. F., G. A. Rowan, et al. (1986). "Corticosterone, novelty-induced hyperglycemia, and chlordiazepoxide." Physiol Behav **37**(3): 393-6.

Flynn, F. W., H. J. Grill, et al. (1991). "Central gustatory lesions: I. Preference and taste reactivity tests." Behav Neurosci **105**(6): 933-43.

Frank, M. and C. Pfaffmann (1969). "Taste nerve fibers: a random distribution of sensitivities to four tastes." Science **164**(884): 1183-5.

Geddes, R. I. and P. S. Grigson (in prep (1), Thesis Chapter 3). "Bilateral Lesions of the Insular Gustatory Cortex Block Anticipatory Contrast." In PSU THESIS.

Geddes, R. I., L. Han, et al. (2008). "Gustatory insular cortex lesions disrupt drug-induced, but not lithium chloride-induced, suppression of conditioned stimulus intake." Behav Neurosci **122**(5): 1038-50.

Grigson, P. S., P. Lyuboslavsky, et al. (2000). "Bilateral lesions of the gustatory thalamus disrupt morphine- but not LiCl-induced intake suppression in rats: evidence against the conditioned taste aversion hypothesis." Brain Res **858**(2): 327-37.

Grigson, P. S., A. C. Spector, et al. (1993). "Microstructural analysis of successive negative contrast in free-feeding and deprived rats." Physiol Behav **54**(5): 909-16.

Grigson, P. S., A. C. Spector, et al. (1994). "Lesions of the pontine parabrachial nuclei eliminate successive negative contrast effects in rats." Behav Neurosci **108**(4): 714-23.

Grill, H. J. and R. Norgren (1978). "The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats." Brain Res **143**(2): 263-79.

Hajnal, A. and R. Norgren (2005). "Taste pathways that mediate accumbens dopamine release by sapid sucrose." Physiol Behav **84**(3): 363-9.

Halsell, C. B. (1992). "Organization of parabrachial nucleus efferents to the thalamus and amygdala in the golden hamster." J Comp Neurol **317**(1): 57-78.

Jongen-Relo, A. L. and J. Feldon (2002). "Specific neuronal protein: a new tool for histological evaluation of excitotoxic lesions." Physiol Behav **76**(4-5): 449-56.

Kawamura, Y. (1975). "Role of sensory factors in chewing and feeding behavior." Pharmacol Biochem Behav **3**(1 Suppl): 163-73.

Kim, U. K., P. A. Breslin, et al. (2004). "Genetics of human taste perception." J Dent Res **83**(6): 448-53.

Kosar, E., H. J. Grill, et al. (1986). "Gustatory cortex in the rat. II. Thalamocortical projections." Brain Res **379**(2): 342-52.

Leszczuk, M. H. and C. F. Flaherty (2000). "Lesions of nucleus accumbens reduce instrumental but not consummatory negative contrast in rats." Behav Brain Res **116**(1): 61-79.

Lin, J.-Y., C. Roman, et al. (2009). "Insular cortex and consummatory successive negative contrast in the rat." Behavioral Neuroscience **123**(4): 810-814.

Lorden, J. F. (1976). "Effects of lesions of the gustatory necortex on taste aversion learning in the rat." J Comp Physiol Psychol **90**(7): 665-79.

Mackey, W. B., J. Keller, et al. (1986). "Visceral cortex lesions block conditioned taste aversions induced by morphine." Pharmacol Biochem Behav **24**(1): 71-8.

Mathy, I., M. J. Dupuis, et al. (2003). "[Bilateral ageusia after left insular and opercular ischemic stroke]." Rev Neurol (Paris) **159**(5 Pt 1): 563-7.

Miller, I. J. and K. Spangler (1982). "Taste bud distribution and innervation on the palate of the rat." Chemical Senses **7**: 99 - 108.

Mistretta, C. M. (1972). "Topographical and histological study of the developing rat tongue, palate and taste buds." Third Symposium on Oral Sensation and Perception: The Mouth of the Infant: 163 - 187.

Mitchell, C. P. and C. F. Flaherty (2005). "Differential effects of removing the glucose or saccharin components of a glucose-saccharin mixture in a successive negative contrast paradigm." Physiol Behav **84**(4): 579-83.

Mungarndee, S. S., J. Lundy, Robert F., et al. (2006). "Central gustatory lesions and learned taste aversions: Unconditioned stimuli." Physiology & Behavior **87**(3): 542-551.

Norgren, R. (1983). "The gustatory system in mammals." Am J Otolaryngol **4**(4): 234-7.

Norgren, R. and C. M. Leonard (1971). "Taste pathways in rat brainstem." Science **173**(2): 1136-9.

Norgren, R. and C. Pfaffmann (1975). "The pontine taste area in the rat." Brain Res **91**(1): 99-117.

Norgren, R. and G. Wolf (1975). "Projections of thalamic gustatory and lingual areas in the rat." Brain Res **92**(1): 123-9.

Pfaffmann, C., R. Norgren, et al. (1977). "Sensory affect and motivation." Ann N Y Acad Sci **290**: 18-34.

Pritchard, T. C., D. A. Macaluso, et al. (1999). "Taste perception in patients with insular cortex lesions." Behav Neurosci **113**(4): 663-71.

Reilly, S., M. Bornovalova, et al. (2004). "Excitotoxic lesions of the gustatory thalamus spare simultaneous contrast effects but eliminate anticipatory negative contrast: evidence against a memory deficit." Behav Neurosci **118**(2): 365-76.

Reilly, S. and T. C. Pritchard (1996). "Gustatory thalamus lesions in the rat: I. Innate taste preferences and aversions." Behav Neurosci **110**(4): 737-45.

Reilly, S. and T. C. Pritchard (1997). "Gustatory thalamus lesions in the rat: III. Simultaneous contrast and autoshaping." Physiol Behav **62**(6): 1355-63.

Reilly, S. and R. Trifunovic (1999). "Gustatory thalamus lesions eliminate successive negative contrast in rats." Behav Neurosci **113**(6): 1242-8.

Reilly, S. and R. Trifunovic (1999). "Progressive ratio performance in rats with gustatory thalamus lesions." Behav Neurosci **113**(5): 1008-19.

Reilly, S. and R. Trifunovic (2003). "Gustatory thalamus lesions eliminate successive negative contrast in rats: evidence against a memory deficit." Behav Neurosci **117**(3): 606-15.

Riley, E. P. and W. P. Dunlap (1979). "Successive Negative Contrast as a Function of Deprivation Condition Following Shifts in Sucrose Concentration." The American Journal of Psychology **92**(1): 12.

Sastre, A. and S. Reilly (2006). "Excitotoxic lesions of the gustatory thalamus eliminate consummatory but not instrumental successive negative contrast in rats." Behav Brain Res **170**(1): 34-40.

Scalera, G., P. S. Grigson, et al. (1997). "Gustatory functions, sodium appetite, and conditioned taste aversion survive excitotoxic lesions of the thalamic taste area." Behav Neurosci **111**(3): 633-45.

Schroy, P. L., R. A. Wheeler, et al. (2005). "Role of gustatory thalamus in anticipation and comparison of rewards over time in rats." Am J Physiol Regul Integr Comp Physiol **288**(4): R966-80.

Specht, S. M. and R. C. Twining (1999). "Human taste contrast and self-reported measures of anxiety." Percept Mot Skills **88**(2): 384-6.

Travers, S. P., C. Pfaffmann, et al. (1986). "Convergence of lingual and palatal gustatory neural activity in the nucleus of the solitary tract." Brain Research **365**(2): 305-20.

Weinstein, L. (1972). "Contrast effects in animal and human learning: recent results and interpretations." J Psychol **81**(2d Half): 235-47.

Weinstein, L. (1978). "Negative and positive incentive contrast effects with saccharine versus sucrose." J Gen Psychol **98**(2d Half): 225-40.

Williams, B. (1997). "Varieties Of Contrast: A Review Of Incentive Relativity By Charles F. Flaherty." J Exp Anal Behav **68**(1): 133-141.

Wolf, G. (1968). "Projections of thalamic and cortical gustatory areas in the rat." J Comp Neurol **132**(4): 519-30.

Zhang, Y., M. A. Hoon, et al. (2003). "Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways." Cell **112**(3): 293-301.

An Intact Taste Thalamocortical Loop is Necessary for Acquisition and Retention of Morphine-Induced Avoidance of a Taste Cue in Rats

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ABSTRACT

Rats with bilateral lesions of the gustatory thalamus fail to reduce intake of a gustatory conditioned stimulus (CS) when paired with morphine, but not when paired with the illness-inducing agent, lithium chloride (LiCl) (Grigson, Lyuboslavsky et al. 2000). A similar pattern of results occurs following bilateral lesions of the gustatory insular cortex (Mackey, Keller et al. 1986; Geddes, Han et al. 2008). These data led us to hypothesize that it was not the thalamus or the cortex, per se, but the communication between the two structures that is essential for drug-induced suppression of CS intake. To test this hypothesis, Sprague-Dawley rats were given either ipsilateral ibotenic acid lesions of the gustatory thalamus and gustatory cortex (Ip-CNTL) or contralateral (asymmetric) lesions of these same structures (THCx). In Experiment 1 and 2, respectively, we tested whether these asymmetric THCx lesions would disrupt acquisition of morphine- or LiCl-induced suppression of CS intake. In Experiment 3, we tested whether rats with similar asymmetric gustatory THCx lesion could retain a preoperatively acquired morphine-induced suppression of CS intake and, in Experiment 4, whether these same rats (from the morphine retention study) could acquire suppression of CS intake using another drug of abuse, cocaine. The results showed that, while the LiCl-induced conditioned taste aversion was robust for all rats, the asymmetric THCx lesion fully disrupted acquisition and retention of morphine- and cocaine-induced suppression of CS intake.

Key words: gustatory cortex, unilateral damage, devaluation, learning and memory, LiCl, CTA.

Introduction

Given the variable dangers and toxins in any environment, adaptive learning is an important survival skill for all living animals. Thus, while timely procurement of nutrients is necessary for survival, the ingestion of toxins (e.g., poison or spoiled foods) could be deadly (Garcia, Hankins et al. 1974). Humans, primates, and rats decrease intake of a gustatory conditioned stimulus (CS) when repeatedly paired with an unconditioned stimulus (US) that is aversive, rewarding, or addictive (Barker and Smith 1974; Booth, Pilcher et al. 1977; Flaherty and Checke 1982). Ample data from clinical diagnosis as well as data from lesion studies in non-human primates and rodents suggest that the insular gustatory cortex (GC) plays a vital role in the perception of taste quality and in the discrimination of taste intensity (Bornstein 1940a; Bornstein 1940b; Benjamin 1955; Bradley 1963; Rolls 1989; Ogawa, Hasegawa et al. 1992; Pritchard, Macaluso et al. 1999; Mathy, Dupuis et al. 2003).

Bilateral lesions of the gustatory parvicellular region of the ventroposteromedial (VPMpc) nucleus of the thalamus and bilateral lesions of the gustatory insular cortex have been used to determine whether these dorsal taste nuclei contribute to avoidance of a taste cue when paired with aversive, rewarding, or addictive USs . For instance, bilateral lesions aimed at the gustatory thalamus or insular cortex disrupts avoidance of a lesser sucrose reward cue when paired with a highly preferred 32% sucrose solution. This phenomenon, referred to as an anticipatory contrast effect, is thought to be mediated by anticipation of the highly preferred sucrose reward expected in the very near future (Flaherty and Checke 1982; Reilly and Pritchard 1996; Reilly, Bornovalova et al. 2004; Kesner and Gilbert 2007; Roman and Reilly 2009; Geddes and Grigson in prep (1), Thesis Chapter 3). Similar thalamic or insular lesions also have been found to disrupt avoidance of a taste cue when paired with morphine or cocaine (Mackey, Keller et al. 1986; Reilly and Trifunovic 1999; Grigson, Lyuboslavsky et al. 2000; Geddes, Han et al. 2008; Roman and Reilly 2009), but not when paired with LiCl (Braun, Slick

et al. 1972; Lorden 1976; Lasiter and Glanzman 1982; Flynn, Grill et al. 1991; Scalera, Grigson et al. 1997; Mungarndee, Lundy et al. 2006). Together, these data show that the gustatory thalamus and/or the insular cortex are essential for avoidance of a taste cue when paired with a highly preferred sweet or a drug of abuse, but not when paired with LiCl-induced malaise.

-------*Insert Figure 5-1 (Horizontal view of rat gustatory pathway) about here* ------

As shown in gray in Figure 1 (top panel) the taste cells in the medial pons (mPBN) project to the ventral, or as is coded in black, dorsally to the gustatory thalamus and then insular cortex. Note that the projection from the gustatory thalamus to the cortex is fully ipsilateral and that it is reciprocal. Given the anatomy, two conclusions can be drawn from the from the data: (1) The insular cortex, as the ultimate projection region, is the key structure involved in sucrose and drug-induced suppression of CS intake; or (2) These phenomena depend upon not the thalamus or the cortex, per se, but upon communication between the two structures. In support, we recently disconnected the gustatory thalamus from the insular gustatory cortex via an asymmetric lesion of the gustatory thalamus in one hemisphere and the gustatory cortex. Thereafter, we tested whether these lesioned rats could demonstrate a consummatory successive negative contrast effect (SNC) when unexpectedly downshifted from a high to a low concentration of sucrose (Geddes, Li et al. in prep (2), Thesis Chapter 4). The results showed that SNC was fully disrupted by the lesion. Interestingly, while anticipatory contrast (ACE) and SNC differ in several ways (see Flaherty, 1996), both are prevented by bilateral lesions of the gustatory thalamus or cortex (Reilly and Pritchard 1996; Reilly and Trifunovic 1999; Schroy, Wheeler et al. 2005; Lin, Roman et al. 2009; Geddes and Grigson in prep (1), Thesis Chapter 3).

This parallel led us to speculate that the suppressive effects of drugs of abuse, which to date depend upon similar circuits as ACE, also would depend upon communication between the gustatory thalamus and cortex, i.e., via the gustatory thalamocoritcal loop. To test this hypothesis, we used 36 rats with either asymmetric THCx or Ip-CNTL lesions to determine if direct ipsilateral communication between the taste thalamus and insular gustatory cortex is necessary for acquisition of morphine-induced suppression of CS intake (Exp. # 1) or for LiCl-induced CTA (Exp. # 2). Using a naïve set of rats (n = 36), we then tested the effects of the asymmetric THCx lesion on retention of a preoperatively acquired morphine-induced suppression of CS intake (Exp. # 3). These same rats were then used to test the effects of the asymmetric THCx lesion on acquisition of drug-induced suppression using another drug of abuse, cocaine, as the US (Exp. # 4).

Experiment 1 and 2: Asymmetric THCx, taste-morphine and taste-LiCl pairings

Morphine-induced suppression of CS intake. Experiment 1 tested the hypothesis that asymmetric gustatory THCx lesions are similar to bilateral taste thalamus and insular GC lesions (Mackey, Keller et al. 1986; Reilly and Trifunovic 1999; Grigson, Lyuboslavsky et al. 2000; Geddes, Han et al. 2008; Roman and Reilly 2009) and, as such, that they would prevent avoidance of a palatable 0.15% saccharin CS following repeated pairings with a 15 or a 30 mg/kg dose of morphine administered ip. If confirmed, this approach will provide novel evidence that drug-induced suppression of CS intake depends upon communication between the gustatory thalamus and gustatory insular cortex.

LiCl-induced CTA: Experiment 2 used the same rats from Exp. # 1 to test the hypothesis that asymmetric THCx lesions, akin to bilateral lesions of the taste thalamus and bilateral insular gustatory cortex lesions (Mackey, Keller et al. 1986; Flynn, Grill et al. 1991; Reilly and Pritchard 1996; Reilly and Pritchard 1996; Scalera, Grigson et al. 1997; Grigson, Lyuboslavsky et al. 2000; Geddes, Han et al.

2008), would have no effect on acquisition of a LiCl-induced CTA (see Figure 2, below). If confirmed, the current data will show a clear distinction in the central taste nuclei required for learning to avoid a CS paired with an abused drug vs. LiCl.

Methods

Subjects and apparatus:

The subjects were 36 naïve male Sprague-Dawley rats (Charles River) weighing 350-500 g at the beginning of the experiment. They were individually housed in standard suspended stainless steel wire-mesh cages in a temperature and humidity controlled colony room maintained on a 12/12 hour light/dark cycle. Food (Teklad #6068) and distilled water (dH_2O) were available ad lib, unless otherwise noted. All CS-US trials were conducted in the home cages using inverted Nalgene graduated cylinders affixed to the front of the cage with a spring with silicone stoppers and stainless steel spouts. Fluid intake was measured to the nearest 0.5 ml.

CS and US Solutions:

In Experiment 1, saccharin (Sigma Chemical Company, St. Louis, MO) served as the CS and was dissolved in distilled water in a concentration of 0.15% at least 24 h in advance and was presented at room temperature. Morphine sulfate (generously provided by the National Institute on Drug Abuse) was prepared in saline 1 h prior to testing and was delivered at a 15 mg/kg dose injected intraperitoneally (i.p.). For Exp. # 2, sodium chloride (Sigma Chemical Co., St. Louis, MO) served as the CS solution, was dissolved in dH_2O, and was presented at room temperature. A 0.009 M or 0.15 M dose of LiCl (Sigma Chemical Company, St. Louis, MO) was prepared every 3 days and maintained in the refrigerator between trials.

Surgery

Unilateral gustatory thalamic lesion. On the day of surgery rats were fist injected (ip) with atropine sulfate (.25 mg/rat) and gentamicin (6 mg/rat). Twenty minutes later they were then anesthetized with pentobarbital sodium (50 mg/kg ip) and supplemented as necessary. Body temperature was maintained at 37 $\pm$ 1°C. The rat's head was then mounted in a stereotaxic with non-traumatic ear bars, with the skull level between bregma (β) and lambda (λ). The skin over the skull was cleaned with Betadine and opened with a midline incision. Using a 4-mm-diameter trephine at 3.0 mm posterior to bregma a hole was drilled in the skull on one side of the midline. During surgery, the dura matter was left intact and kept moist with saline. Taste receptive cells in the taste thalamus (VPMpc) were located by stimulating the anterior part of the tongue with 0.3 M NaCl while recording multiunit activity through a glass-insulated tungsten microelectrode [impedance (Z) = 1.0-1.5 MΩ at 1 kHz].The coordinates for electrode penetrations ranged from -3.5 to -4.1 mm posterior to bregma, -1.1 to -1.4 mm lateral to the midsagittal suture, and -5.5 to -6.8 mm below the skull surface. Once the thalamic taste area had been located, the search microelectrode was replaced with a double-barreled micropipette-electrode (M/E; OD 50–60 mm; Z = 0.5–1 MΩ at 1 kHz), one lumen of which was glued directly onto the needle of a 1.0-μl Hamilton microsyringe. The microsyringe and its attached micropipette were filled with mineral oil. The other lumen was filled with an etched tungsten wire similar to the search electrode. The ibotenic acid was drawn in through the tip of the M/E immediately before the injections were made. It was then lowered directly into the hole in the dura that was left by the penetration producing the best response to NaCl oral application. The thalamic taste area was relocated electrophysiologically, and 0.2 μl (20 μg / μl) of ibotenic acid was infused over 10 min. The M/E remained in place for another 10 mins, after which the hole in the skull was filled with gelfoam.

Unilateral insular gustatory cortex lesion. The coordinates for the stereotaxically placed cortical lesions are modified from those of Mackey (1986), except that the cortical lesion was performed in only one hemisphere. The range of coordinates is as follows: A/P +0.7 to +1.0 mm anterior to β, M/L +/- 4.0 lateral to midline with the tip oriented 10^{o} lateral, and D/V -6.7 to -7.7 from the skull. A 4-mm-diameter trephine was used to drill a 2^{nd} hole in the skull, ~1 mm anterior to bregma. Ibotenic acid, 0.2 μl (20 μg / μl), was infused over a 10 min period using a 1 μl Hamilton syringe which remained in place for another 10 mins. Once the ibotenic acid filled syringe was removed, the incision was rinsed and closed with wound clips. For Experiments 1 and 2, twenty rats with received asymmetric lesions (Left Thalamus and Right Cortex, n= 12; Right Thalamus and Left Cortex, n = 8). The remaining 16 rats were given ipsilateral lesions (Left Thalamus and Cortex, n= 6; Right Thalamus and Cortex, n = 10).

Procedure

Experiment 1: Morphine-induced suppression of CS intake. The rats recovered within 3-5 days post surgery and body weight returned to presurgical levels within a week. All rats were then placed on the restricted water regimen where they received 5 min access to dH_2O in the morning and 1 h in the afternoon. Daily dH_2O intake stabilized within a week. Five minute morning water intake was used to match and assign rats to one of two US conditions: saline or 15 mg/kg morphine (i.p.). Thus, 7 Ip-CNTL rats and 8 THCx rats received saline injections, while the remaining 9 Ip-CNTL rats and 12 THCx rats were administered morphine. During conditioning, all rats were weighed, returned to the home cage, and then given 5 min to consume the saccharin CS. After a 5 min interval, the rats were injected (i.p.) with either saline or morphine. There were a total of 7 CS-US pairings and one CS only test. Finally, one dH_2O day elapsed between trials, and the morphine concentration was increased to 30 mg/kg on trial 6.

--Insert Figure 5-2 (Experimental design for taste-morphine and LiCl studies) about here --

Experiment 2: LiCl-induced CTA. As shown in Fig. 2, three weeks following the end of Exp. # 1, the rats were again water restricted as described, and matched into two new US conditions (saline or LiCl). Approximately half the Ip-CNTL and THCx rats with previous morphine history were placed in the LiCl group and the other half in the saline group. A similar approach was taken with the rats' previously injected ip with saline. Thus, of the 16 Ip-CNTL rats, 8 now received saline and 8 received LiCl as the US. Out of the 20 THCx rats, 8 THCx rats served in the saline group and 12 THCx rats were placed in the LiCl group. During testing, all rats were allowed 5 min access to 0.1 M NaCl and, after a 5 min interval, were injected ip with the appropriate US. There was a total of 5 CS-US pairings with one dH_2O day elapsing between each pairing. The concentration of LiCl was increased from 0.009 M to 0.15 M on the 5^{th} trial, followed by one CS only test. In both Exp. # 1-2, rats were allowed 1 h access to dH_2O each afternoon to hydrate.

Histology. After completion of Experiment 2 (LiCl-induced CTA), *the* rats were deeply anesthetized with a 100 mg/kg (i.p.) injection of Pentobarbital Sodium and perfused transcardially for 5-10 min with physiological saline, followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer for 15-20 min. Brains were stored overnight in 20% sucrose mixed in phosphate buffer (20 g/100 ml) in the refrigerator. The next day, the brains were cut into 50-μ-thick coronal sections using a freezing microtome, placed in phosphate buffer (PB) in preparation for the neuron-specific nuclear protein (NeuN) immunostaining procedure (Jongen-Relo, 2002). This staining procedure permits easy assessment of missing or damaged cell bodies in cortical and subcortical tissue. The sections were collected in PB overnight. Sections were transferred to a PBS solution, incubated for 30 min in 0.5% H_2O_2, 1 h in blocking solution, and then 24 h in primary antibody. The secondary antibody incubation,

avidin-biotin complex (ABC Elite kit) step, and DAB incubation were each preceded and followed by 3 rinses in PBS for 5 min. Finally, the sections were mounted on gelatin-coated slides, dry overnight, and then cover-slipped. We recently described this staining procedure elsewhere (Geddes, Han et al. 2008).

----------*Insert Figure 5-3 (super-imposed Paxinos & Watson sections) about here* ----------

Results:

Histology.

As depicted in Fig. 3, analysis of the taste thalamus and insular GC was conducted at 6 different levels (I-VI), in which ibotenic acid (IBO) was infused at Level IV in the insular cortex and Level II in the thalamus. The taste regions (Levels II-V) of the thalamus correspond to stereotaxic Plates 65-68, respectively, meanwhile the taste receptive insular cortex (Levels II to V) corresponds to Plates 27, 29, 31, and 33, respectively (Paxinos and Watson 2005). NeuN- stained sections from a rat with asymmetric THCx and ipsilateral control lesions are shown below in the top and bottom panel of Figure 4, respectively. Assessment of lesion damage revealed that a majority of the thalamic lesions extended beyond the boundaries of the VPMpc and included portions of the centromedial (CM), ventromedial, paracentral, centrolateral, VPM, posterior (Po), parafascicular (PF), subparafascicular, and mediodorsal (MD) thalamic nuclei. Damage to these structures, however, was at best incomplete. Otherwise, neighboring nuclei were largely spared.

----------*Insert Figure 5-4 (Photomicrograph from rats in Exp. # 1-2) about here* ----------

Moreover, since the thalamic taste nuclei and insular gustatory cortex lesion was unilateral, the remaining (intact) thalamic nucleus and insular Cortex in the same rat (whether ipsilateral or

contralateral to each other) served as the control when assessing cell loss due to lesion surgery, for comparison at the level of the taste thalamus see Fig. 4 A-B (top). In fact, Fig. 4 A-D (top) is a set of photomicrographs from a rat with an asymmetric THCx lesion representing the intact left taste thalamus (A), the damaged right taste thalamus (see dotted white lines) (B), damaged left insular taste cortex (C) and the intact right insular taste cortex (D). Importantly, assessment of the taste regions in Fig. 4 B and D failed to reveal any significant retrograde or anterograde damage commensurate with the thalamic or cortical lesion respectively. An ipsilateral control lesion is shown in Figure 4 A-D (bottom panel). The nuclei are not labeled, but are set up similar to Figure 4 A-D in the top panel.

In general, these rats incurred the same amount of thalamocortical insult, but the damage was limited to the same (in this case right) hemisphere (see dotted white line in B and D, bottom). Upon inspection, the brains from 18 out of 20 rats in the THCx group revealed well-placed asymmetric lesion aimed at the taste thalamus and cortex. Two asymmetric THCx rats were eliminated from the study due to misplaced lesions. Moreover, one Ip-CNTL rat failed to acquire normal water or CS intake and was therefore removed from the analysis. Thus, for Exp. # 1 and 2, the behavioral data from 15 Ip-CNTL rats and 18 asymmetric THCx rats contributed to the current statistical analyses (n = 33).

---------*Insert Figure 5-5 (behavioral data from the morphine and LiCl studies) about here* --------

CS intake and morphine. Mean intake of the 0.15% saccharin solution (ml/5min) served as the dependent measure. The data were analyzed using a 2 x 2 x 8 mixed factorial ANOVA varying lesion (THCx or Ip-CNTL), US (saline or morphine), and trials (1-8). Post hoc analyses were conducted on significant interactions using the Newman-Kuels test with alpha set at .05. The results of pairing a taste CS with morphine US injections in rats with Ip-CNTL and THCx lesions are shown in Fig. 5 A and B

(top panel), respectively. The Ip-CNTL rats, but not THCx rats, readily expressed avoidance of the taste CS that was paired with the morphine US. This observation was confirmed by post hoc tests of a significant Lesion x US x Trials interaction, $F(7,203) = 13.52$, $p<.05$. Post hoc analysis confirmed that Ip-CNTL rats, but not THCx rats, avoided intake of the CS cue by the 4th saccharin-morphine pairing relative to the saline treated controls, ps <.05. Intake of the saccharin CS by the asymmetric THCx group was not reduced by morphine pairings, at the 15 or the 30 mg/kg dose of the drug, ps > .05.

CS intake and LiCl-induced CTA. Mean intake of the 0.1 M NaCl solution (ml/5min) served as the dependent measure. The data were analyzed using a 2 x 2 x 6 mixed factorial ANOVA varying lesion (THCx or Ip-CNTL), US (saline or LiCl), and trials (1-6). Post hoc analyses were conducted on significant interactions using the Newman-Keuls test. The results of the CTA test with Ip-CNTL and THCx rats are shown in Fig. 5 C and D (bottom panel), respectively. The 3 way ANOVA varying Lesion x US x Trials was not significant, $F<1$, nor were any analyses involving lesion as a factor, ps >.05. The US x Trials interaction, however, did attain statistical significance, $F(8,200) = 18.75$, $p<.05$, and post hoc tests revealed that, relative to the saline-treated controls, both the Ip-CNTL and THCx rats suppressed intake of the NaCl CS following pairings with LiCl, ps <.05 (see Figure 5).

Discussion

The results from Experiments 1 and 2 revealed that asymmetric THCx lesions prevent CS avoidance due to taste-morphine pairings but spare CTA acquisition. These results verify that it is not the taste thalamus or insular gustatory cortex, *per se,* but communication between these two structures that is essential for drug-induced suppression of CS intake. Since rats with asymmetric lesions of the gustatory thalamocortical loop (which disconnect the thalamus form the insular cortex in both hemispheres) acquired a CTA, we can conclude that the taste processing required for the suppressive

effects of morphine, but not those associated with a CTA, require a functional gustatory thalamocortical loop. This conclusion is consistent with published data showing that bilateral lesions of the taste thalamus or insular taste cortex eliminate drug- and sucrose-, but not LiCl-induced CS suppression (Reilly and Pritchard 1996; Scalera, Grigson et al. 1997; Reilly and Trifunovic 1999; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005; Kesner and Gilbert 2007; Geddes, Han et al. 2008; Lin, Roman et al. 2009; Roman and Reilly 2009; Geddes and Grigson in prep (1), Thesis Chapter 3).

Experiment 3: Asymmetric THCx and retention of taste-morphine pairings

Retention of morphine-induced CS avoidance. It is unknown whether the insular GC is required for retention of a preoperatively acquired taste-morphine association. The results from Exp. # 1 and 2 demonstrate that an intact gustatory thalamacortical loop is necessary for acquisition of drug-induced CS devaluation but not for LiCL-induced CTA while previous studies demonstrate that and the insular cortex is required for retention of CTA. We hypothesize that the ability to recall or retrieve a previously acquired drug-induced CS devaluation may depend upon communication between the VPMpc of the thalamus and the insular GC, rather than upon the activity of either structure alone; and therefore, asymmetric THCx lesions will block retention of a preoperatively acquired taste-morphine association. To test this possibility, the current investigation (Exp. # 3) used asymmetric THCx lesions to determine the role of ipsilateral communication between the two taste nuclei on a previously acquired taste-drug association. A naïve set (n = 36) of rats was given taste-morphine parings followed by Ip-CNTL or THCx surgeries. The rats were re-exposed to the CS only in a one-bottle test followed by a water day and then a two bottle (choice) retention test with the saccharin CS and water. After the completion of Exp. # 3 the same rats were later tested in taste-cocaine pairings in Exp. # 4.

--Insert Figure 5-6 (Experimental design for morphine retention and cocaine studies) about here--

Methods.

Subjects.

Thirty-six naïve male Sprague-Dawley rats (Charles River) weighing 350-500 g at the beginning of the experiment. They were individually housed and maintained as described above.

Solutions, apparatus, procedure (pre-lesion), and histology

Acquisition. During acquisition, there were a total of 8 saccharin CS – morphine US pairings. The (pre-lesion) taste-morphine pairings were conducted as described in Exp. # 1, but they occurred prior to lesion surgery. Thereafter, the Ip-CNTL lesions and the THCx lesions were completed as described for Exp. # 1 and 2. **Retention tests.** Following at least 5 d recovery for lesion surgery, water access was restricted as described in Exp. # 1, for 3 days, which was followed up the next day with a 15 min, one bottle saccharin test. After another water day, the rats were given a 2 bottle choice test, where they could consume either saccharin or water. All rats were given 1-h access to water each afternoon. Thereafter, the rats were anesthetized, perfused, and the brains were removed, prepared, sectioned, and stained as described above.

Results and Discussion.

Histological analysis.

Prior to histological analysis, one Ip-CNTL rat suffered clear motor deficits and thus its data were dropped. The data for two saline treated rats (n = 1 Ip-CNTL, n = 1 THCx) that died relatively early in the study also were eliminated. Histological analysis of the 33 remaining rats revealed that, out of the 20 rats with asymmetric THCx lesions (6 saline, 14 morphine), five (2 saline, 3 morphine) rats had

either misplaced or oversized injury to the targeted lesion site. Thus, the data for nine (5 Ip-CNTL, 4 THCx) rats receiving taste-saline pairings and nineteen (8 Ip-CNTL, 11 THCx) rats receiving taste-morphine pairings contributed to the statistical analysis (histological data in keeping with that shown in Figure 3 and, thus, is not shown).

----------*Insert Figure 5-7 (behavioral data from morphine retention study) about here* ----------

CS intake (Pre-lesion).

Mean intake of the 0.15% saccharin solution (ml/5min) prior to gustatory thalamocortical lesion surgery served as the dependent measure. Statistical analysis of the data using a 2 x 8 mixed factorial ANOVA varying US (saline or morphine), and trials (1-8), revealed a significant US x trial interaction, $F(7, 182) = 11.52$, $p < .05$. Post hoc analyses were conducted on significant interactions using the Newman-Kuels test with alpha set at .05. These data showed that intake of the saccharin CS was significantly reduced by the 2nd trial, see Fig. 7 A.

CS intake (post-lesion): 5-minute One-Bottle Retention Test.

During one-bottle testing, intake of the saccharin CS served as the dependent measure. A US x lesion interaction resulting from a 2-way ANOVA varying US (saline or morphine) and lesion (Ip-CNTL or THCx) narrowly missed significance, $F(1, 24) = 4.08$, $p = .055$. Even so, group differences on these planned comparisons were evidenced by an analysis using a Student's t-test. Specifically, as indicated by the asterisk and dotted line, this latter analysis revealed that the Ip-CNTL rats treated with morphine drank significantly less saccharin than Ip-CNTL rats treated with saline (see Figure 7, panel B). In fact, the morphine-treated rats with Ip-CNTL lesions drank significantly less saccharin (3.6 ml)

than any of the other 3 groups (8.45 – 10.6 ml), ps < .05. The asymmetric THCx lesions menwhile prevented retention of the preoperatively acquired avoidance of the morphine-paired taste CS, p > .05.

CS intake (Post-lesion): 5-minute Two-Bottle Retention Test.

During two-bottle testing, the consumption of water and the saccharin CS served as the dependent measures. Figure 7 C (bottom panel) shows mean intake of water (open bars) and saccharin CS (solid bars) by rats with Ip-CNTL (left) and asymmetric THCx (rigjht) lesions as a function of US condition (saline or morphine) prior to lesion surgery. A 2-way ANOVA varying US (saline or morphine) and lesion (Ip-CNTL or THCx), unfortunately, failed to reach significance, $F(1, 24) = 1.3$, $p > .05$. We assume this occurred because in 3 of the 4 groups, saccharin intake was greater than water intake. Independent t-test support this assumption and further revealed that the saccharin intake by the Ip-CNTL lesion group treated with morphine (2.38 ml) was significantly lower (†) than intake of the saccharin CS intake in all other groups and the exact oppose was true of this groups water intake (#), see Fig. 7 C. That is to say, while all rats given taste-morphine pairings learned (prior to receiving lesion surgery) to avoid the drug-paired CS, only preventing the taste thalamus from communicating with the taste cortex in both hemisphere prevented the expression of prior taste-drug association (as measured by CS intake). The consumption of similar amounts of saccharin in the asymmetric group, regardless of lesion suggest that the asymmetric lesions did appear to interfere with the absolute reinforcing value of either gustatory solution or the preference for saccharin over water. Taken together, these data show that an intact thalamocortical loop is required not only for acquisition of the taste-morphine association, but for retention of that conditioned taste avoidance when acquired preoperatively.

Experiment 4: Asymmetric THCx and taste-cocaine pairings

Cocaine-induced suppression of CS intake. Given that the asymmetric lesion of the gustatory thalamocoritcal loop prevented acquisition of CS devaluation produced by the passive administration of morphine, we hypothesize that the same lesion also would prevent the suppression of CS intake induced by a 10 mg/kg dose of cocaine. The rats from Exp. # 3 were used to test this hypothesis, see Figure for an illustration of this experimental design.

Methods

Subjects and apparatus.

The subjects and apparatus were the same as those described in Experiment 3.

Drugs and solutions.

The CS was a 0.03 M Polycose solution (Sigma Chemical Company, St. Louis, MO) prepared 24 h in advance and presented at room temperature. The US, cocaine hydrochloride, was generously provided by the National Institute on Drug Abuse and was mixed in 0.9% saline every morning, 1 h prior to the taste-cocaine pairings. Cocaine was prepared as a stock solution (1.5 mg/ml) in saline. The volume was increased to administer a 10 mg/kg dose subcutaneously (s.c.). The solution was diluted to avoided necrosis at the injection site (Mayer and Parker 1993; Durazzo, Gauvin et al. 1994).

Procedure

At the completion of Experiment 3, the rats were returned to a period of ad lib access to food and water. Then previous experience with passive injections of morphine or saline was used to match and assign rats, in a counterbalanced manner, to one of two US conditions: saline or the standard 10 mg/kg dose of cocaine (s.c.), see experimental design in Fig. 6, above. As shown, eight (8) Ip-CNTL rats and

eight (8) THCx rats received saline injections, while the remaining six (6) Ip-CNTL rats and seven (7) THCx rats received cocaine as the US. During conditioning, all rats were given 5 min access to 0.03 M Polycose and, after a 5 min interval, were injected s.c. with either saline or 10 mg/kg cocaine. There were a total of 6 CS-US pairings and one test. All rats were given 1 h access to water every afternoon. One dH_2O day (5 min a.m., 1 h p.m.) elapsed between each trial.

-----Insert Figure 5-8 (behavioral data from taste-cocaine acquisition study) about here ----

Results and Discussion

CS intake:

Intake of the CS served as the dependent measure. The results of a 2 x 2 x 7 mixed factorial ANOVA varying lesion type (THCx or Ip-CNTL), US (saline vs. cocaine), and trials (1 – 7) found a significant lesion x US x trials interaction, $F(6, 150) = 2.8$, $p < .01$. Post-hoc comparisons of intake revealed that relative to their saline treated controls, Ip-CNTL rats in the Polycose-cocaine condition suppressed intake of the CS on trials 3-7, $p < .01$ (see Fig. 8 A). The suppression of CS intake in the THCx rats occurred on the trials 3 and 4, but this effect was only transient, see Figure 8 B (right). Additional post-hoc tests indicated that intake of the CS did not differ between the Ip-CNTL and THCx rats treated with saline across trials 1 – 7, $p > .05$. Using asymmetric THCx lesions, the data from Experiment 4 shows that bilaterally disconnecting the taste thalamus from the insular gustatory cortex severely attenuated the suppressive effect of a 10 mg/kg dose of cocaine on CS intake. Thus, the gustatory thalamocortical loop is, as with taste-morphine conditioning, vital for avoidance of a taste cue following pairing with cocaine.

General Discussion

Using bilateral lesions of the insular GC or taste thalamus, we previously demonstrated that the gustatory thalamocortical axis is essential for gustatory reward comparisons and subsequent devaluation of a taste CS when the CS is repeatedly paired with passive injections of an addictive drug or access to a more palatable sucrose solution (Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005; Geddes, Han et al. 2008). Using asymmetric gustatory THCx lesions, here, we demonstrate that the same gustatory thalamocortical axis (which is not required for taste detection, concentration discrimination, or innate orofacial taste reactive responses to the different primary taste qualities, such as sweet, sour, salty, bitter and umami) is vital for acquisition (Exp. # 1 and 4) and retention (Exp. # 3) of taste-drug association, but nnot for acquisition of a LiCl-induced CTA (Exp. # 2), see Table 1 for a summary of these and related lesion data. As summarized in Table 1, the effects of asymmetric gustatory THCx lesions (column 1) on taste-guided learning, parallel those obtained with bilateral insular GC (column 2) and taste thalamic (column 3) lesions. While all three types of gustatory lesion functionally prevent communication between the taste thalamus and insular gustatory cortex in both hemispheres, only the asymmetric THCx lesions spare one taste thalamic nuclei and one taste cortex region (in opposing hemispheres). In this manner, it is possible to specifically test whether it is communication between the two structures, rather than the structure(s) themselves, is vital for avoidance of a taste cue following pairings with a drug of abuse.

----------*Insert Table 5-1 about here* ----------

As indicated in Table 1, while the effect of asymmetric THCx lesions on ACE remains untested (column 1, row 1), bilateral lesions of the insular gustatory cortex or taste thalamus have been shown to prevent acquisition of ACE. The related form of contrast, successive negative contrast has been tested in

each lesion condition and is known to be blocked following bilateral or asymmetric lesions of this dorsal taste pathway (see Row 2). Experiment 1 and 2 from the current analysis revealed that asymmetric gustatory THCx lesions also disrupt the suppressive effects of passively administered morphine at a low and a higher dose of the addictive substance (column 1, row 3-4), but the same lesion does not impair the ability to acquire a LiCl-induced CTA (column 1, row 5-6). As revealed in Experiment 3, retention of a preoperatively acquired taste-morphine pairing (column 1, row 7) was blocked by asymmetric gustatory THCx lesions. The same asymmetric THCx lesions also prevented drug-induced suppression of CS intake following taste-cocaine pairings with a novel CS, see Experiment 4 (column 1, row 8). Given these data, it is clear that an intact gustatory thalamocortical loop is essential for avoidance of an otherwise palatable taste cue when anticipating access to a preferred sucrose solution, morphine, or cocaine, but not when paired with the aversive agent, LiCl.

In theory, sucrose-induced ACE requires comparing the rewarding value of consuming two distinct, concurrently available gustatory solutions meanwhile drug-induced conditioned taste avoidance requires comparing the rewarding value of consuming a gustatory solution that predicts passive injections of an addictive substance with the reinforcing drug effects on the body and central nervous system, once the addictive substances is administered. In contrast to these behaviors, LiCl-induced CTA does not require the same taste reward comparison process and is actually thought to arise from associating the gustatory CS and the aversive US, followed by attributing the negative effects of the US to the gustatory CS. These latter events, together, result in subsequent CS devaluation and modification of innate orofacial responses when re-exposed to taste CS a.k.a. conditioned taste aversion learning. The reward comparison hypothesis states that drug-induced conditioned taste avoidance of a predictive gustatory cue is qualitatively similar to sucrose-induced anticipatory avoidance of a less preferred gustatory saccharin CS (i.e., sucrose-induced ACE). Assuming the latter hypothesis is correct, the acquisition of morphine-

or cocaine-induced taste CS devaluation must also require distinguishing between the effects of consuming the CS from the physiologically effects of US and the establishment of an association between the two. . In the case of drugs and sucrose, then, the reinforcing consequence of consuming the predictive CS, is thought to pale in comparison to the reinforcing consequences of the more preferred US. It is possible that acquiring SNC, which is an exaggerated avoidance of a less concentrated, unexpected, novel sweet, may share some similarities with LiCl-induced CTA. For instance, LiCl-induced CTA and SNC may be accompanied by a negative affective state and both responses require retrieval or activation of long-term taste memory. Similar to ACE, however, SNC involves comparing the rewarding effects associated with consuming the two disparate solutions (one available, one remembered or anticipated), which may be a key feature that links these two forms of contrast.

From the current data it is clear that the gustatory thalamocortical loop is needed for acquisition of taste reward comparison-dependent consummatory learning and taste memory-dependent consummatory responding. In support of a qualitative distinction between reward comparisons dependent gustatory avoidance (due to taste-drug or saccharin-sucrose pairings) and the reward comparison independent LiCl-induced CTA, the gustatory thalamocortical loop plays a role in retention of preoperatively acquired consummatroy responses regardless of its dependency on reward comparison. That is to say, unlike acquisition of LiCl-induced CTA, remembering a LiCl-induced CTA, acquiring or retaining drug-induced CS avoidance, sucrose-induced ACE, and consummatory SNC all require ‘on-site’ retrieval of the information-laden gustatory stimulus and all depend upon that remembered orosensory information reaching the insular gustatory cortex via the ipsilateral taste thalamus.

While it is the case that all of these phenomena depend upon an intact gustatory thalamocortical loop, there is an exception. That is, although the asymmetric lesion blocks the suppressive effects of low and high doses of morphine and lower (i.e., 10 mg/kg) doses of cocaine, the disruptive effect of bilateral

lesions of the insular gustatory cortex are overridden by the use of a higher 20 mg/kg dose of the drug (Geddes, Han et al. 2008). A similar pattern has been obtained with ethanol (Liu, Showalter et al. 2009). Some cortical involvement in high dose ethanol and CTA cannot be disputed. For instance, chronic decerebrate rats, which display innate taste reactivity to different taste qualities, failed to acquire LiCl-induced CTA in response to taste-LiCl pairings and decortices rats are highly deficient in extinction and retention of CS devaluation following taste-ethanol pairings (Kiefer, Metzler et al. 1985). The CS devaluation produced by the higher doses of cocaine or ethanol thus may be more akin to CTA.

In support, cocaine and ethanol at the higher doses and LiCl at the low or high dose consistently produces conditioned place and taste aversion (i.e. consummatory avoidance plus increase negative orofacial responses to drug paired taste CS) (Mucha, van der Kooy et al. 1982). In addition, evidence suggests that experimenter delivered cocaine (or yoked delivery) may be aversive (Goudie, Dickins et al. 1978; Parker 1995; Grigson 1997; Grigson, Twining et al. 2008; Twining, Bolan et al. 2009). Specifically, brief CS access paired with yoked (passive) iv administration of cocaine caused greater avoidance of the taste CS, resulted in a decreases in responding for cocaine when then placed on a progressive-ratio schedule, and greater avoidance of a location associated with the yoked delivery of drug (Twining, Bolan et al. 2009). We suspect, however, that avoidance of a drug-associated taste cue differs from a LiCl-induced CTA. Specifically, while we now know that intraoral infusion of a cocaine-associated taste cue elicits aversive taste reactivity behavior like a LiCl-induced CTA, we also have determined that greater avoidance of the taste cue (and, importantly, greater aversive taste reactivity behavior) predicts a shorter latency to take the first cocaine infusion, greater initial load-up on the drug, and faster acquisition of drug-taking over trials. Thus, while a LiCl-induced CTA develops because of the aversive properties of the drug, avoidance of a cocaine-associated taste cue occurs, we believe, because of the development of a conditioned aversive state – including withdrawal.

Acknowledgements

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Figure captions

Figure 5-1. The Central Gustatory Pathway in the Rodent brain (A Horizontal View).

Depicts a simplified version of the major ascending predominately ipsilateral projections of the three cranial nerves (e.g., the facial, CN V; glossopharengal, CN IX; and the vagus, CN X), to the nucleus of the solitary tract (NST) in the brainstem. The chorda tympani (CT), greater superficial petrosal (GSP), lingual-tonsilar branch of IX (LT- CN IX), and superior laryngeal branch of CN X (SL) relay taste input to the rostral NST (rNST), meanwhile non-taste input from the SL were intermingled with LT- CN IX terminals and visceral (e.g., post-ingestive and cardio-respiratory) afferents in the caudal medulla**.** These cranial nerves have fibers also omitted here, synapse on the spinal trigeminal nucleus, the dorsal motor nucleus (of the vagus), and the reticular formation in the medualla. The taste sensitive rNST rely taste sensation to the medial parabrachial nucleus (mPBN) meanwhile the visceral or cadual (cNST) medulla projects to the lateral PBN (not shown). The mPBN orosensory relays target (similar forebrain sites as the visceral neurons in the cNST and LPBN) the lateral hypothalamus (LH), and extended amygdala, more specifically the central amygdala (CeA) and the bed nucleus of the stria terminalis (BNST), all shown in with grey circles. A second set of mPBN taste fibers relay sensory information to the dorsal forebrain via projecting to the ipsilateral (50%) and (50%) contralateral to the most medial portion of the parvicellular region of the thalamus (VPMpc), which in turn is reciprocally connected to the ipsilateral insular gustatory cortex (GC), as indicated with black double arrows for the thalamocortical loop fibers and circles representing the thalamic and cortical nuclei. See text for references.

Figure 5-2. Unconditioned Stimulus History. The sample size, lesion type, and unconditioned stimulus treatment groups. This diagram also illustrates the manner in which Ip-CNTL and THCx rats (n = 33, post-histology) from the morphine study in Exp. # 3 were crossed-over based on their prior morphine experience in order to subsequently test the effects of gustatory asymmetric thalamocortical (THCx) lesions on LiCl-induced conditioned taste aversion in Exp. # 4.

Figure 5-3. Representative insular and thalamic taste areas, damage, and the Levels (I-VI) analyzed. Schematic sections from Paxinos and Watson were superimposed from rostal to caudal for the insular and vice versa for the taste thalamus.

Figure 5-4 A-D (Top panel). A series of photomicrographs of a representative coronal brain section of with an ideally placed asymmetric Thalamocortical (THCx) lesion. In this figure, the intact gustatory thalamus (top left, panel A) and intact cortex (bottom right, panel D) is outlined with dotted circles and labeled VPMpc with arrow (panel A) and GI, AI, & DI (panel D), respectively. Ibotenic acid induced neuroanatomical changes in the cytoarchitecture of the gustatory lesion target sites can be viewed in both the right thalamus (top right, panel B) and left cortex (bottom left, panel C).

Figure 5-4 A-D (Bottom Panel). A series of photomicrographs of a representative coronal brain section of with an ideally placed ipsilateral control (Ip-CNTL) lesion. These images (not labeled) are set up similar to Figure 4A-D in the top panel, however in this figure the same degree of thalamocortical lesion insult was within the same hemisphere. This particular Ip-CNTL rat sustained a lesion of the right thalamus and right cortex, panels B (top, right) and D (bottom, right). Since, the ibotenic acid induced damage of the gustatory lesion target sites are limited to one hemisphere, rats with these type of lesion still have at least one intact thalamocortical loop, see right thalamus (top right, panel B) and right cortex (bottom right, panel C). AC: anterior commissure, AI/DI/GI: agranular/ dysgranular/ granular insular

cortex, cl: claustrum, Cpu: caudate putamen, ec: external capsule, MCA: (anterior branch of) middle cerebral artery, RH: rhinal horn, IV: cortical layer 4. CM: centromedial thal., fr: fronix, MD: mediodorsal thal., PF: parafasciulus thal., Po: posterior thal., PVP: paraventricular posterior thal, SPF: subparafasciulus thal., VPM/VPL: ventro-posteriomedial/ventro-posteriolateral thal., VPMpc: ventro-posteriomedial (gustatory) thal., 3v:3rd ventricle.

Figure 5-5 (A-B). Mean CS intake (ml/5 min) of the 0.15% saccharin CS in Ip-CNTL and THCx lesion rats following pairings with saline (open squares) or 15 and then 30 mg/kg morphine (closed circles) across trials. A 3-way ANOVA varying Drug (saline or morphine) x Lesion (Ip-CNTL or THCx) x Trials (1-9) revealed that asymmetric Thalamocortical THCx lesions disrupted morphine-induced contrast such that Ip-CNTL (Left panel), but not THCx rats (Right panel), suppressed intake of the 0.15% saccharin cue when paired with the passive administration of the low or high dose of morphine, $F(8,232) = 13.637$, $p < .05$. The Ip-CNTL rats suppressed intake of the 0.15% saccharin CS cue by the 2nd CS-morphine pairing, $p < .05$ (indicated by an *), while the THCx rats never significantly reduced intake of the saccharin CS relative to the saline treated THCx rats, ps > .05 (n = 7-10 per group). **(C-D) Mean CS intake (ml/5 min) of the 0.1 M NaCl CS in Ip-CNTL and THCx rats following pairings with saline (open squares) or 0.009 M or 0.15 M LiCl (closed circles) across trials.** A 3-way ANOVA varying Drug (saline or LiCl) x Lesion (Ip-CNTL or THCx) x Trials (1-6) revealed that THCx failed to disrupt LiCl-induced CTA such that both the Ip-CNTL (Left panel) and THCx rats (Right panel) learned to suppress intake of the CS when repeatedly paired with either dose of LiCl, $F(5,145) = 1.075$, $p > .05$. Post hoc tests on a significant US x Trials interaction, showed that intake of the CS cue was definitely suppressed by the 3rd trial in both Ip-CNTL and THCx rats alike, ps < .05 (n = 6-12 per group).

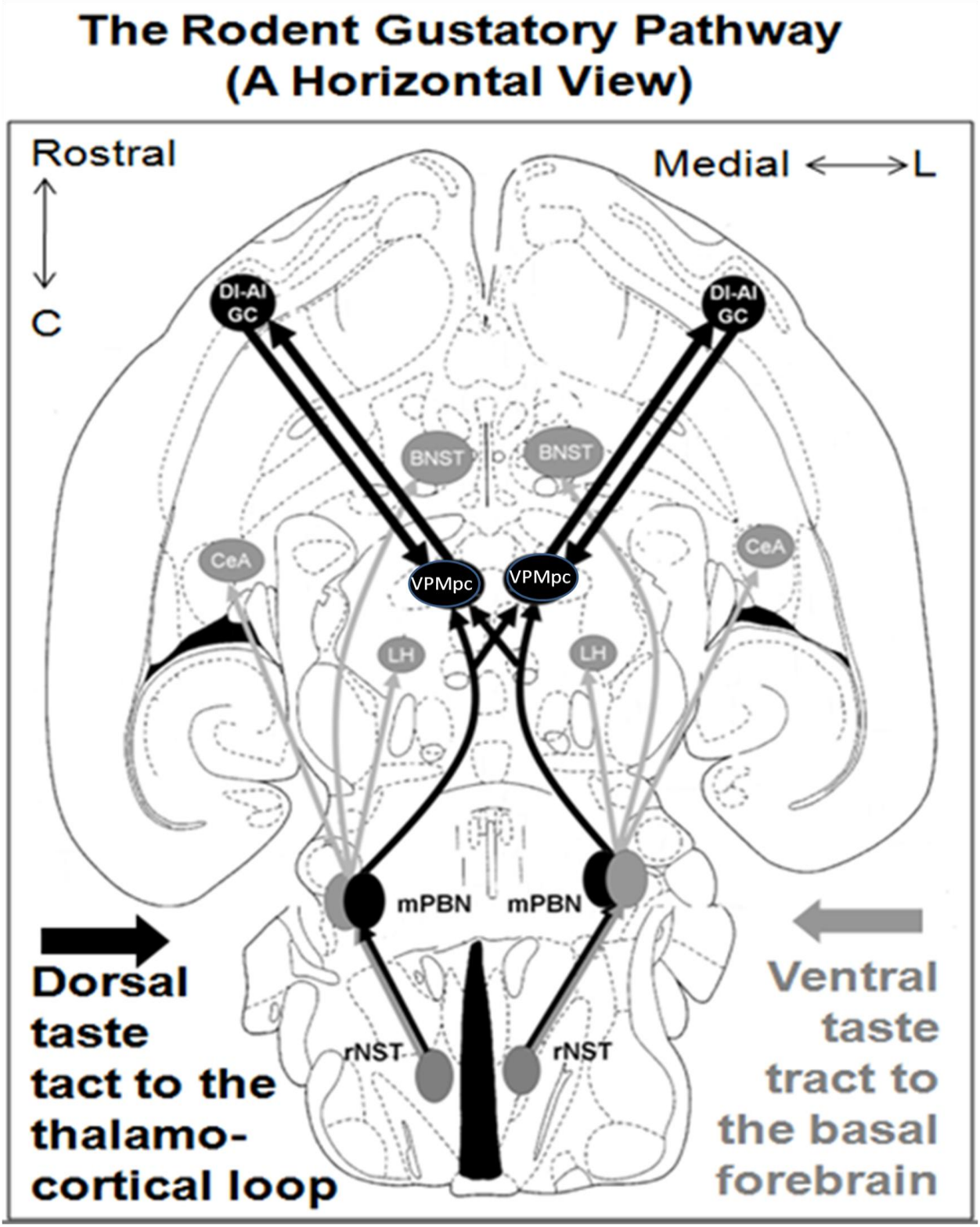
Figure 5-6: Experimental Design: This diagram shows the sample size, lesion type, & unconditioned stimulus (US) groups. Upon histological analysis th data for 5 of the 20 asymmetric THCx lesion rats were discarded. Of the 15 remaining asymmetric THCx lesion rats 8 received Polycose-saline and 7 received Polycose-cocaine pairings. The missing Ip-CNTL rat from the morphine retention study (Exp. # 1) was returned to the saline treated group for the current cocaine study. Thus, the post-histology sample size varied between the morphine retention (n=28) study and cocaine contrast (n=29) stud by one rat.

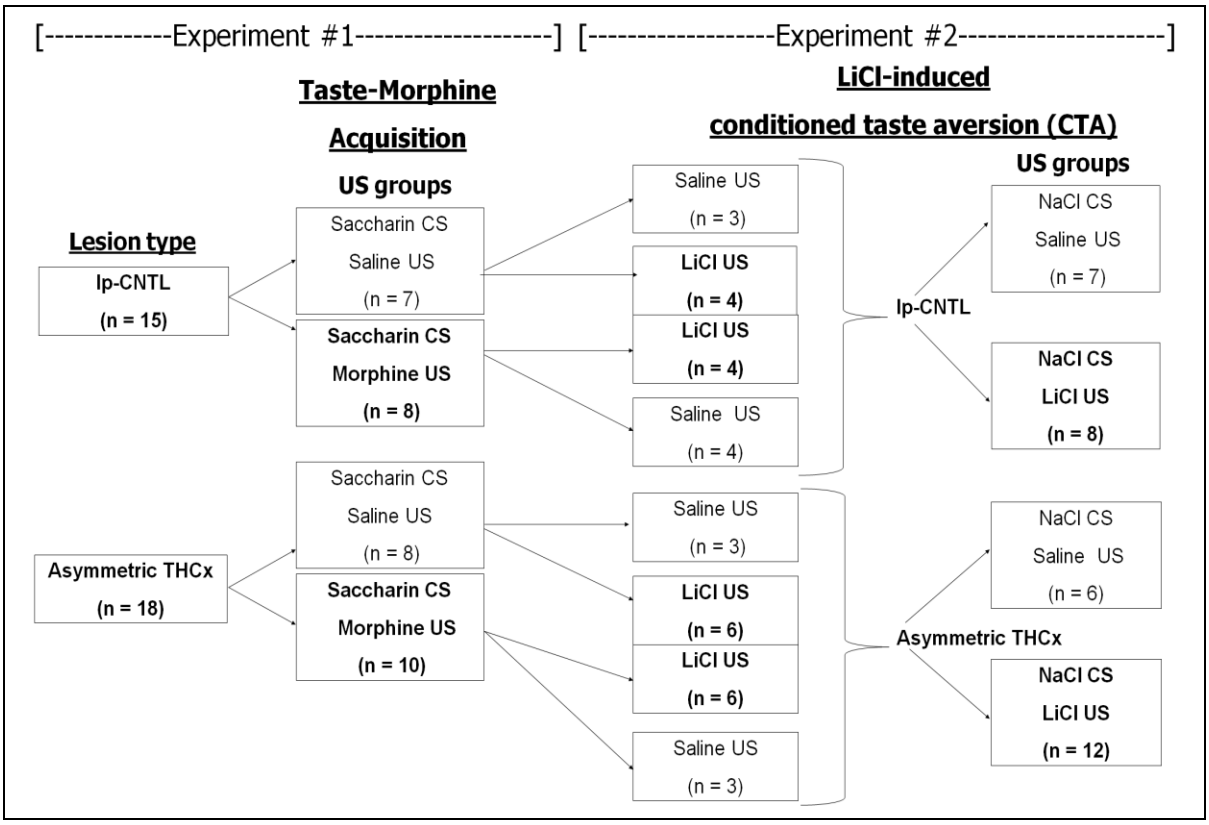
Figure 5-7: Mean pre-lesion and post-lesion saccharin CS intake. (A) Mean saccharin CS intake during acquisition training (**Pre-lesion**) between saline (black squares) and morphine (gray circles) treated rats. **(B)** Mean saccharin cue intake (Post-lesion) during one-bottle test. **(C)** Mean water and saccharin cue intake (Post-lesion) during two-bottle test. The asterisks and arrow represents significant differences ($p < .05$) between saline and morphine treated rats. $n = 5 – 11$/ group.

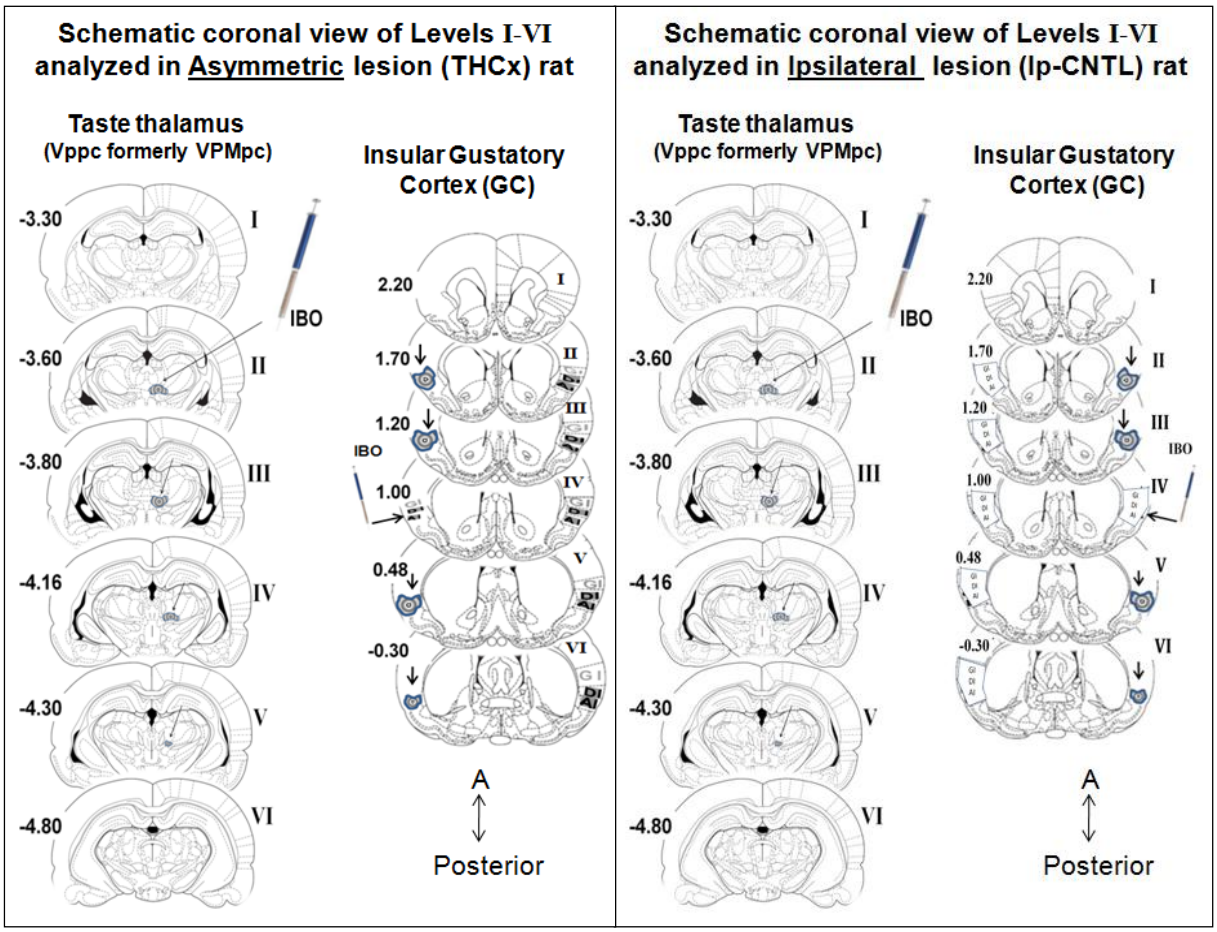
Figure 5-8 (A-B). Mean CS intake (ml/5 min) of the 0.03M Polycose CS cue. This graph shows mean intake of the Polycose CS cue following pairings with saline (black squares) or 10 mg/kg cocaine (green circles) by rats with Ip-CNTL (panel A, left) and gustatory thalamocortical lesions (THCx), shown on the right in panel B. n = 6-8 / group. * indicate significant ($p < .05$) difference between saline and cocaine treatment.

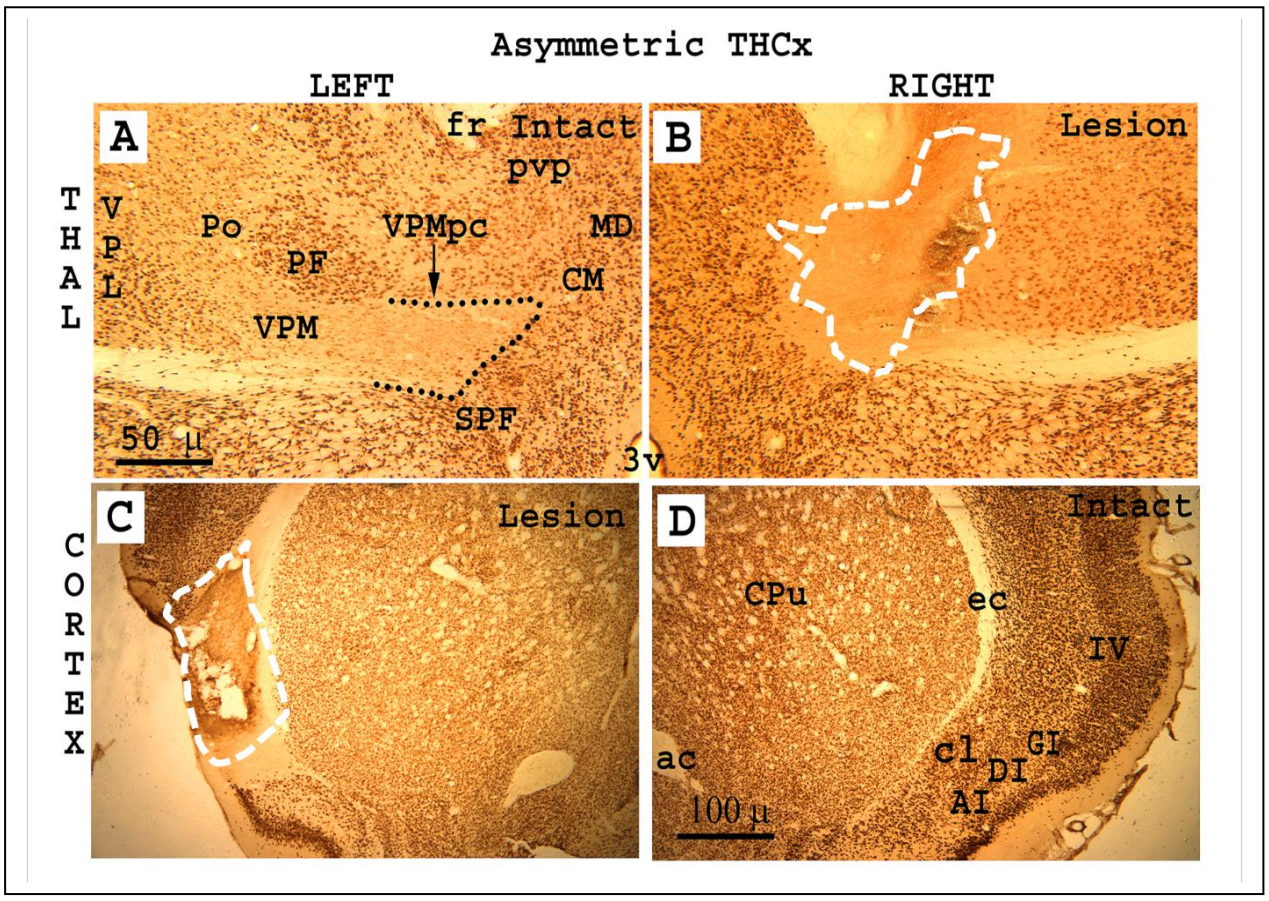
Table 5-1. Comparison of the effect of asymmetric THCx lesions and bilateral lesions of the gustatory thalamus and bilateral insular gustatory cortex lesions on three types of taste-guided responses. Both type of bilateral lesion (thalamic or cortical) lesions were effective in blocking the suppressive effects of the standard dose of morphine, cocaine or a sucrose reinforcer, none of these lesions were sufficient to disrupt LiCl-induced CTA. The present data also shows that asymmetric THCx lesions selectively disrupted morphine and cocaine contrast with the lower doses and the retention of a preoperatively acquire taste-morphine association, but not LiCl-induced CTA. Taken together, these data further illustrates the fact that blocking communication between the gustatory thalamus and cortex selectively disrupts drug-induced devaluation of a natural gustatory reward, possible through learning or memory processes.

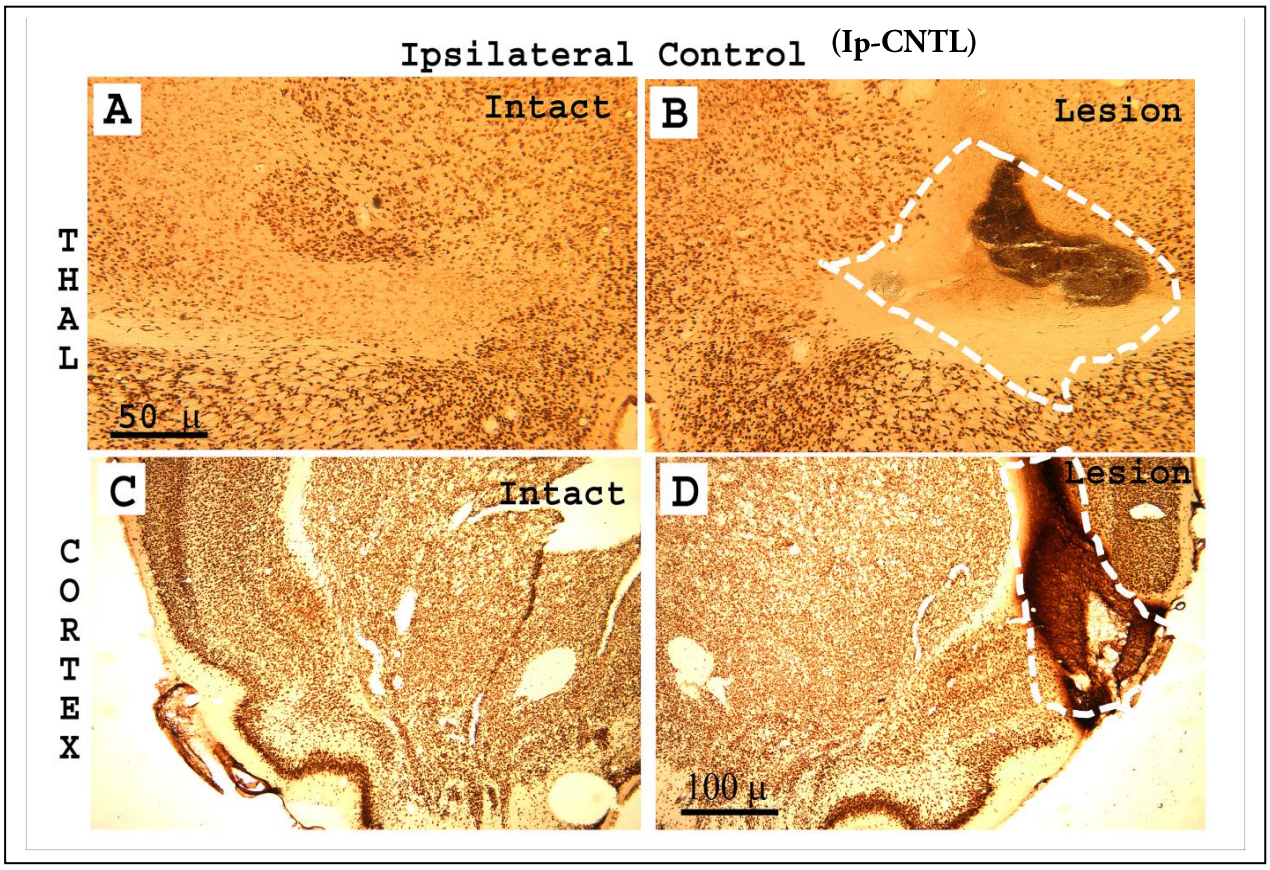
Figures

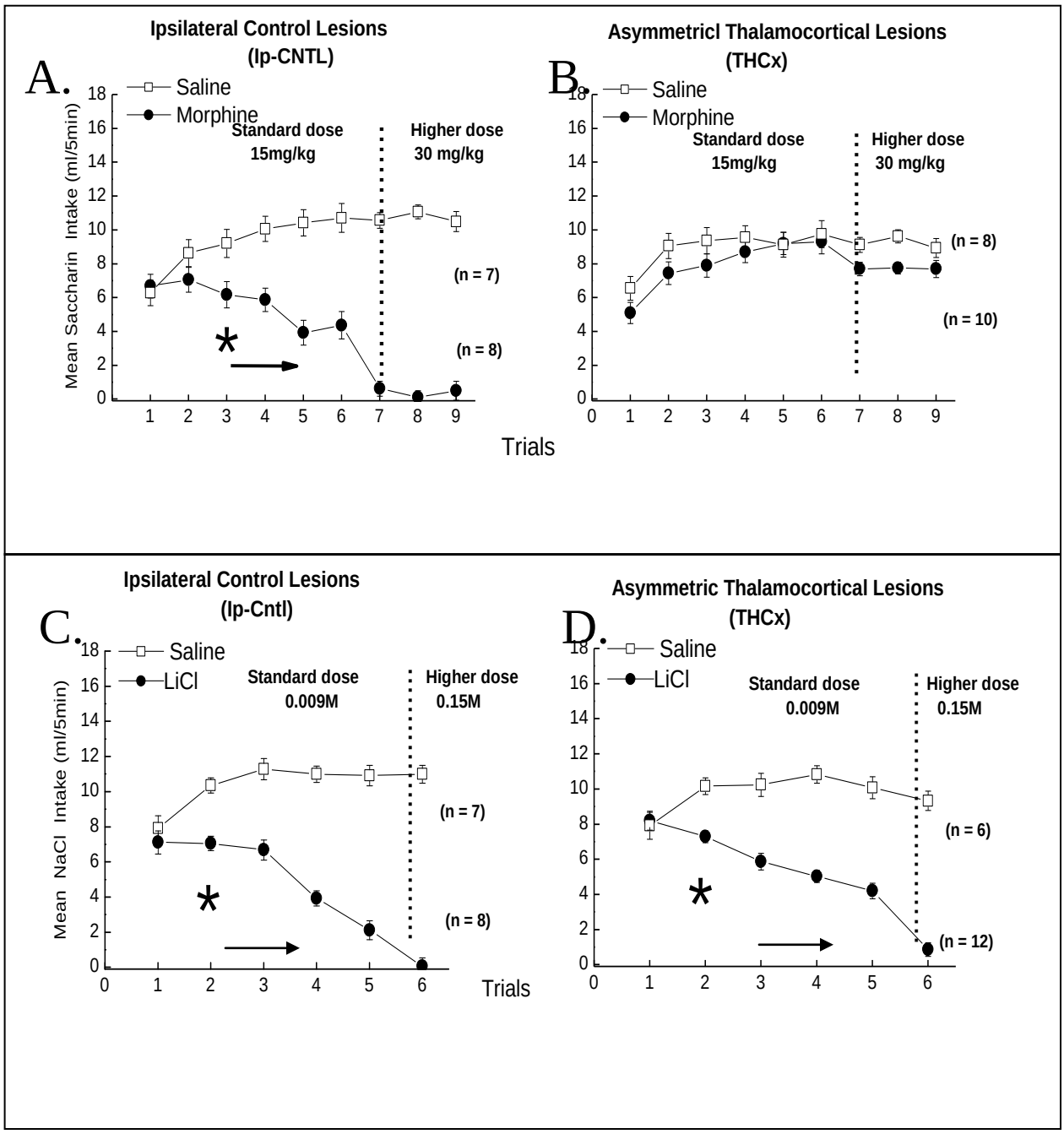

The Rodent Gustatory Pathway
(A Horizontal View)
Rostral
C
Medial ⟷ L
DI-AI GC
DI-AI GC
BNST
BNST
CeA
CeA
VPMpc
VPMpc
LH
LH
mPBN
mPBN
rNST
rNST
Dorsal taste tact to the thalamo-cortical loop
Ventral taste tract to the basal forebrain

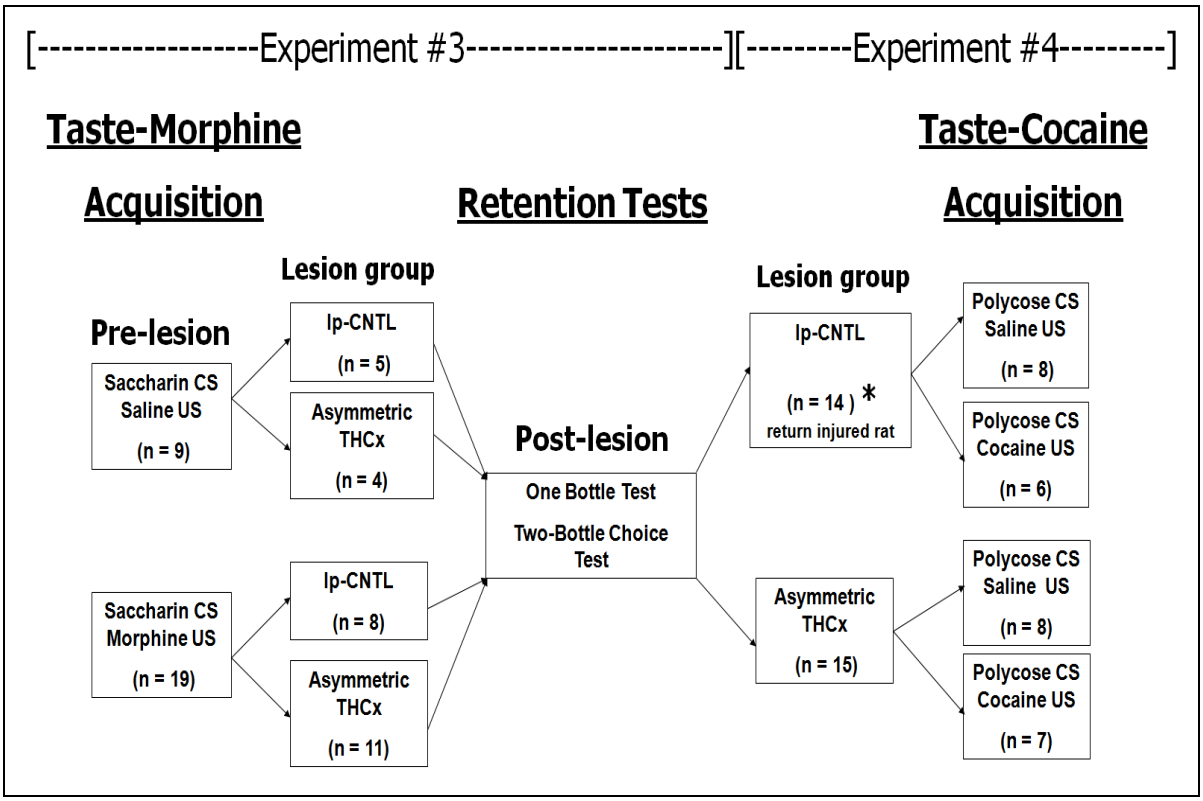

[----------Experiment #1----------] [----------Experiment #2----------]
Taste-Morphine Acquisition
LiCl-induced conditioned taste aversion (CTA)
Lesion type
Ip-CNTL (n = 15)
Asymmetric THCx (n = 18)
US groups
Saccharin CS Saline US (n = 7)
Saccharin CS Morphine US (n = 8)
Saccharin CS Saline US (n = 8)
Saccharin CS Morphine US (n = 10)
Saline US (n = 3)
LiCl US (n = 4)
LiCl US (n = 4)
Saline US (n = 4)
Saline US (n = 3)
LiCl US (n = 6)
LiCl US (n = 6)
Saline US (n = 3)
US groups
Ip-CNTL
NaCl CS Saline US (n = 7)
NaCl CS LiCl US (n = 8)
Asymmetric THCx
NaCl CS Saline US (n = 6)
NaCl CS LiCl US (n = 12)

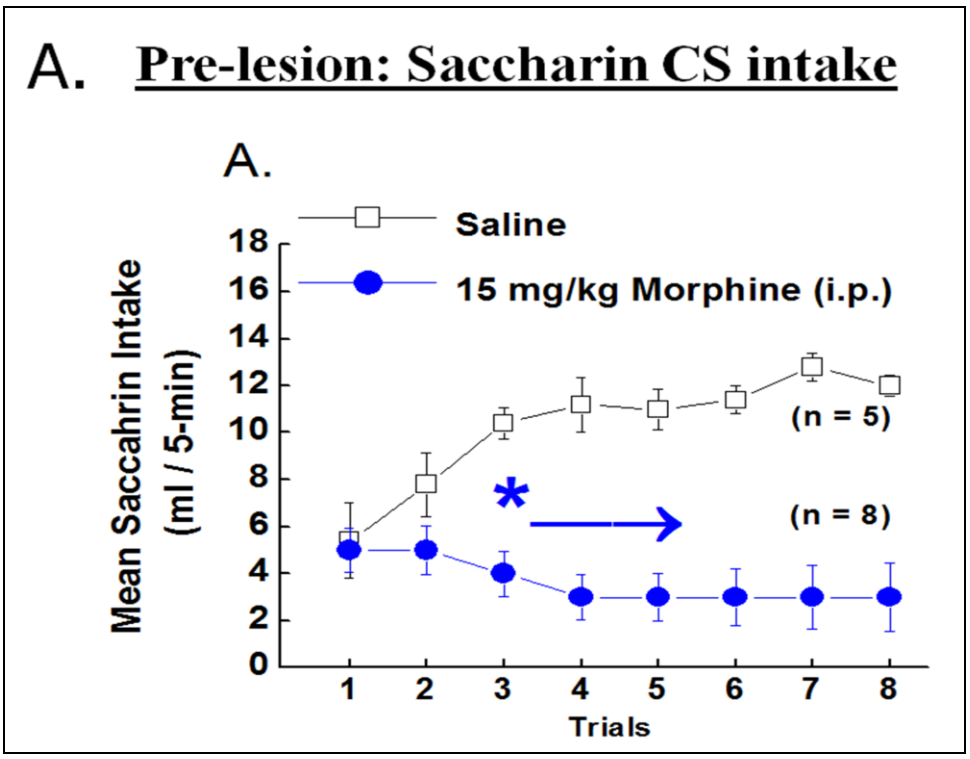

Schematic coronal view of Levels I-VI analyzed in Asymmetric lesion (THCx) rat
Taste thalamus (Vppc formerly VPMpc)
Insular Gustatory Cortex (GC)
-3.30
-3.60
-3.80
-4.16
-4.30
-4.80
I
II
III
IV
V
VI
IBO
2.20
1.70
1.20
1.00
0.48
-0.30
A
Posterior
Schematic coronal view of Levels I-VI analyzed in Ipsilateral lesion (Ip-CNTL) rat
Taste thalamus (Vppc formerly VPMpc)
Insular Gustatory Cortex (GC)
-3.30
-3.60
-3.80
-4.16
-4.30
-4.80
I
II
III
IV
V
VI
IBO
2.20
1.70
1.20
1.00
0.48
-0.30
A
Posterior

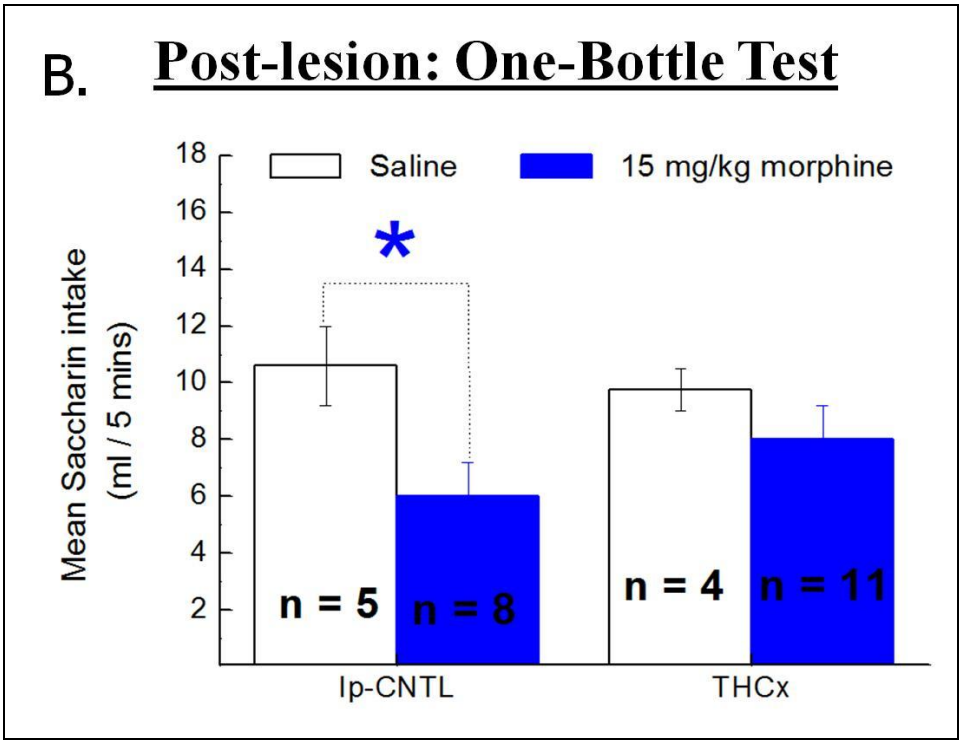

Asymmetric THCx
LEFT
RIGHT
A
fr
Intact
pvp
THAL
VPL
Po
VPMpc
MD
PF
CM
VPM
SPF
50 µ
3v
B
Lesion
C
Lesion
CORTEX
D
Intact
CPu
ec
IV
ac
cl
DI
GI
AI
100 µ

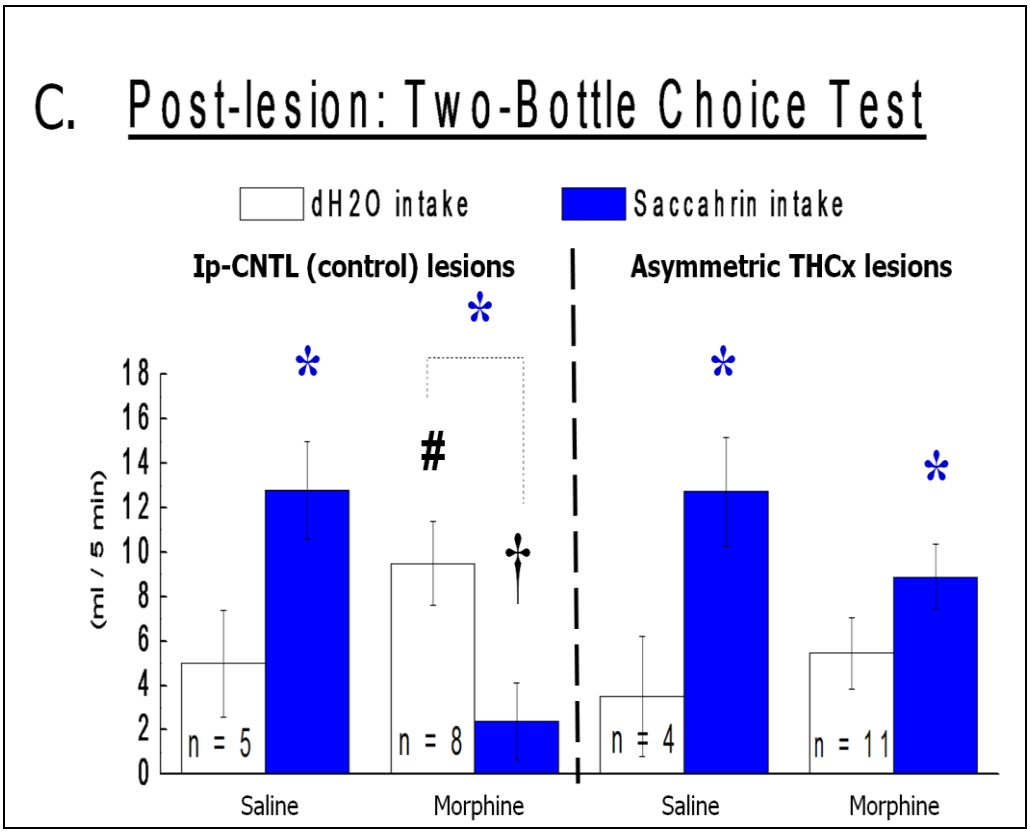

Ipsilateral Control (Ip-CNTL)
A
Intact
THAL
50 µ
B
Lesion
C
Intact
CORTEX
D
Lesion
100 µ

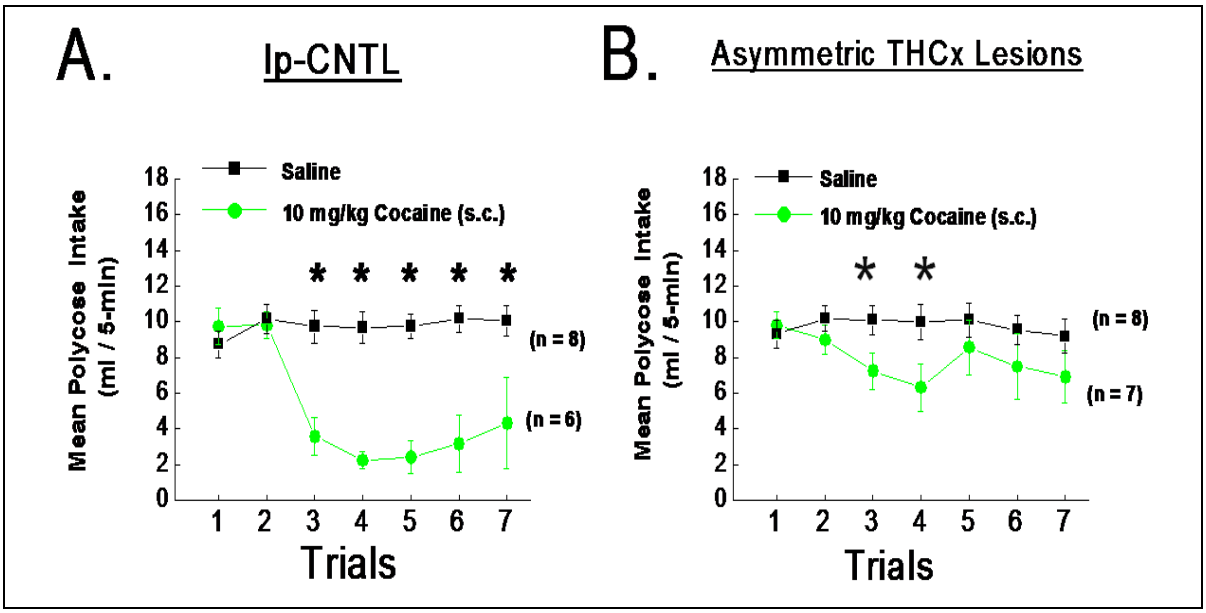

A.
Ipsilateral Control Lesions
(Ip-CNTL)
Saline
Morphine
Standard dose
15mg/kg
Higher dose
30 mg/kg
Mean Saccharin Intake (ml/5min)
(n = 7)
(n = 8)
B.
Asymmetricl Thalamocortical Lesions
(THCx)
Saline
Morphine
Standard dose
15mg/kg
Higher dose
30 mg/kg
(n = 8)
(n = 10)
Trials
C.
Ipsilateral Control Lesions
(Ip-Cntl)
Saline
LiCl
Standard dose
0.009M
Higher dose
0.15M
Mean NaCl Intake (ml/5min)
(n = 7)
(n = 8)
Trials
D.
Asymmetric Thalamocortical Lesions
(THCx)
Saline
LiCl
Standard dose
0.009M
Higher dose
0.15M
(n = 6)
(n = 12)


[----------Experiment #3----------][---------Experiment #4---------]
Taste-Morphine
Taste-Cocaine
Acquisition
Retention Tests
Acquisition
Lesion group
Pre-lesion
Saccharin CS
Saline US
(n = 9)
Ip-CNTL
(n = 5)
Asymmetric
THCx
(n = 4)
Saccharin CS
Morphine US
(n = 19)
Ip-CNTL
(n = 8)
Asymmetric
THCx
(n = 11)
Post-lesion
One Bottle Test
Two-Bottle Choice
Test
Lesion group
Ip-CNTL
(n = 14) *
return injured rat
Asymmetric
THCx
(n = 15)
Polycose CS
Saline US
(n = 8)
Polycose CS
Cocaine US
(n = 6)
Polycose CS
Saline US
(n = 8)
Polycose CS
Cocaine US
(n = 7)


A. Pre-lesion: Saccharin CS intake
A.
Saline
15 mg/kg Morphine (i.p.)
Mean Saccharin Intake
(ml / 5-min)
18
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
8
Trials
(n = 5)
(n = 8)
*


B. Post-lesion: One-Bottle Test
Saline
15 mg/kg morphine
Mean Saccharin intake
(ml / 5 mins)
18
16
14
12
10
8
6
4
2
*
n = 5
n = 8
n = 4
n = 11
Ip-CNTL
THCx


C. Post-lesion: Two-Bottle Choice Test
dH2O intake
Saccahrin intake
Ip-CNTL (control) lesions
Asymmetric THCx lesions
(ml / 5 min)
18
16
14
12
10
8
6
4
2
0
n = 5
n = 8
n = 4
n = 11
Saline
Morphine
Saline
Morphine


A.
Ip-CNTL
B.
Asymmetric THCx Lesions
Saline
10 mg/kg Cocaine (s.c.)
Mean Polycose Intake
(ml / 5-min)
Trials
(n = 8)
(n = 6)
(n = 7)

Lesions BLOCKED avoidance of CS **NO EFFECT** of lesion on CS Intake	Asymmetric Taste Thalamocortical Lesions (THCx)	Bilateral Insular GC Lesions (GCTx)	Bilateral Taste Thalamic Lesions (THLX)
RELATED BEHAVIORAL DATA			
sucrose-induced anticipatory contrast effect (ACE)	**(Untested)**	**BLOCKED** **(Geddes, in prep 1)**	**BLOCKED**
consummatory successive negative contrast (cSNC)	**BLOCKED** **(Geddes, in prep 2)**	**BLOCKED**	**BLOCKED**
	DATA SET ONE (N = 33)		
15 mg/kg dose morphine-induced CS devaluation	**BLOCKED** **(Exp. # 1)**	**BLOCKED**	**BLOCKED**
30 mg/kg dose morphine-induced CS devaluation	**BLOCKED** **(Exp. # 1)**	**BLOCKED**	**BLOCKED** (Twining, in prep)
.009 M LiCl--induced CTA	**No effect** **(Exp. # 2)**	**No effect**	**No effect**
.015 M LiCl--induced CTA	**No effect** **(Exp. # 2)**	**No effect**	**No effect**
	DATA SET TWO (N = 29)		
RETENTION of morphine-induced CS devaluation	**BLOCKED** **(Exp. # 3)**	**BLOCKED** **(Apo and CTA)**	**?**
10 mg/kg dose cocaine-induced CS devaluation	**Attenuated** **(Exp. # 4)**	**Attenuated**	**BLOCKED**
COCAINE AT HIGHER DRUG DOSE			
20 mg/kg dose cocaine-induced CS devaluation	**(Untested)**	**No effect**	**No effect** (Twining, in prep)

Bilbliography

Barker, L. M. and J. C. Smith (1974). "A comparison of taste aversions induced by radiation and lithium chloride in CS-US and US-CS paradigms." J Comp Physiol Psychol **87**(4): 644-54.

Benjamin, R. M. (1955). "Cortical taste mechanisms studied by two different test procedures." J Comp Physiol Psychol **48**(2): 119-22.

Booth, D. A., C. W. Pilcher, et al. (1977). "Comparative potencies of amphetamine, fenfluramine and related compounds in taste aversion experiments in rats." Br J Pharmacol **61**(4): 669-77.

Bornstein, W. S. (1940a). "Cortical representation of taste in man and monkey: I. The localization of the cortical taste area in man, a method of measuring impairment of taste in man." Journal of Biology and Medicine **12**: 719-736.

Bornstein, W. S. (1940b). "Cortical representation of taste in man and monkey: II. The localization of the cortical taste area in man, a method of measuring impairment of taste in man." Journal of Biology and Medicine **13**: 133-156.

Bradley, W. H. (1963). "Central Localization of Gustatory Perception: an Experimental Study." J Comp Neurol **121**: 417-23.

Braun, J. J., T. B. Slick, et al. (1972). "Involvement of gustatory neocortex in the learning of taste aversions." Physiol Behav **9**(4): 637-41.

Durazzo, T. C., D. V. Gauvin, et al. (1994). "Technical report: the subcutaneous administration of cocaine in the rat." Pharmacol Biochem Behav **49**(4): 1007-10.

Flaherty, C. F. and S. Checke (1982). ""anticipation of incentive gain."" Animal learning and Behavior(10): 171-182.

Flynn, F. W., H. J. Grill, et al. (1991). "Central gustatory lesions: II. Effects on sodium appetite, taste aversion learning, and feeding behaviors." Behav Neurosci **105**(6): 944-54.

Flynn, F. W., H. J. Grill, et al. (1991). "Central gustatory lesions: I. Preference and taste reactivity tests." Behav Neurosci **105**(6): 933-43.

Garcia, J., W. G. Hankins, et al. (1974). "Behavioral regulation of the milieu interne in man and rat." Science **185**(4154): 824-31.

Geddes, R. I. and P. S. Grigson (in prep (1), Thesis Chapter 3). "Bilateral Lesions of the Insular Gustatory Cortex Block Anticipatory Contrast." In PSU THESIS.

Geddes, R. I., L. Han, et al. (2008). "Gustatory insular cortex lesions disrupt drug-induced, but not lithium chloride-induced, suppression of conditioned stimulus intake." Behav Neurosci **122**(5): 1038-50.

Geddes, R. I., H. Li, et al. (in prep (2), Thesis Chapter 4). "An Intact Taste Thalamocortical Loop is Required for Consummatory Successive Negative Contrast." in PSU THESIS

Goudie, A. J., D. W. Dickins, et al. (1978). "Cocaine-induced conditioned taste aversions in rats." Pharmacol Biochem Behav **8**(6): 757-61.

Grigson, P. S. (1997). "Conditioned taste aversions and drugs of abuse: a reinterpretation." Behav Neurosci **111**(1): 129-36.

Grigson, P. S., P. Lyuboslavsky, et al. (2000). "Bilateral lesions of the gustatory thalamus disrupt morphine- but not LiCl-induced intake suppression in rats: evidence against the conditioned taste aversion hypothesis." Brain Res **858**(2): 327-37.

Grigson, P. S., R. C. Twining, et al. (2008). Drug-induced suppression of CS intake: Reward, aversion, and addiction. Conditioned Taste Aversion: Behavioral and Neural Processes,. NY, NY., Oxford University Press.

Kesner, R. P. and P. E. Gilbert (2007). "The role of the agranular insular cortex in anticipation of reward contrast." Neurobiol Learn Mem **88**(1): 82-6.

Kiefer, S. W., C. W. Metzler, et al. (1985). "Neocortical involvement in the acquisition and retention of learned alcohol aversions in rats." Alcohol **2**(4): 597-601.

Lasiter, P. S. and D. L. Glanzman (1982). "Cortical substrates of taste aversion learning: dorsal prepiriform (insular) lesions disrupt taste aversion learning." J Comp Physiol Psychol **96**(3): 376-92.

Lin, J.-Y., C. Roman, et al. (2009). "Insular cortex and consummatory successive negative contrast in the rat." Behavioral Neuroscience **123**(4): 810-814.

Liu, C., J. Showalter, et al. (2009). "Ethanol-induced conditioned taste avoidance: reward or aversion?" Alcohol Clin Exp Res **33**(3): 522-30.

Lorden, J. F. (1976). "Effects of lesions of the gustatory necortex on taste aversion learning in the rat." J Comp Physiol Psychol **90**(7): 665-79.

Mackey, W. B., J. Keller, et al. (1986). "Visceral cortex lesions block conditioned taste aversions induced by morphine." Pharmacol Biochem Behav **24**(1): 71-8.

Mathy, I., M. J. Dupuis, et al. (2003). "[Bilateral ageusia after left insular and opercular ischemic stroke]." Rev Neurol (Paris) **159**(5 Pt 1): 563-7.

Mayer, L. A. and L. A. Parker (1993). "Rewarding and aversive properties of IP and SC cocaine: assessment by place and taste conditioning." Psychopharmacology (Berl) **112**(2-3): 189-94.

Mucha, R. F., D. van der Kooy, et al. (1982). "Drug reinforcement studied by the use of place conditioning in rat." Brain Res **243**(1): 91-105.

Mungarndee, S. S., J. Lundy, Robert F., et al. (2006). "Central gustatory lesions and learned taste aversions: Unconditioned stimuli." Physiology & Behavior **87**(3): 542-551.

Ogawa, H., K. Hasegawa, et al. (1992). "Difference in taste quality coding between two cortical taste areas, granular and dysgranular insular areas, in rats." Exp Brain Res **91**(3): 415-24.

Parker, L. A. (1995). "Rewarding drugs produce taste avoidance, but not taste aversion." Neurosci Biobehav Rev **19**(1): 143-57.

Paxinos, G. and C. R. Watson (2005). "Rat brain in stereotaxic coordinates (CD-ROM)." J Neurosci Methods **3**(2): 129-49.

Pritchard, T. C., D. A. Macaluso, et al. (1999). "Taste perception in patients with insular cortex lesions." Behav Neurosci **113**(4): 663-71.

Reilly, S., M. Bornovalova, et al. (2004). "Excitotoxic lesions of the gustatory thalamus spare simultaneous contrast effects but eliminate anticipatory negative contrast: evidence against a memory deficit." Behav Neurosci **118**(2): 365-76.

Reilly, S. and T. C. Pritchard (1996). "Gustatory thalamus lesions in the rat: I. Innate taste preferences and aversions." Behav Neurosci **110**(4): 737-45.

Reilly, S. and T. C. Pritchard (1996). "Gustatory thalamus lesions in the rat: II. Aversive and appetitive taste conditioning." Behav Neurosci **110**(4): 746-59.

Reilly, S. and R. Trifunovic (1999). "Gustatory thalamus lesions eliminate successive negative contrast in rats." Behav Neurosci **113**(6): 1242-8.

Reilly, S. and R. Trifunovic (1999). "Progressive ratio performance in rats with gustatory thalamus lesions." Behav Neurosci **113**(5): 1008-19.

Rolls, E. T. (1989). "Information processing in the taste system of primates." J Exp Biol **146**: 141-64.

Roman, C. and S. Reilly (2009). "Insular cortex lesions and morphine-induced suppression of conditioned stimulus intake in the rat." Behavioral Neuroscience **123**(1): 206-211.

Scalera, G., P. S. Grigson, et al. (1997). "Gustatory functions, sodium appetite, and conditioned taste aversion survive excitotoxic lesions of the thalamic taste area." Behav Neurosci **111**(3): 633-45.

Schroy, P. L., R. A. Wheeler, et al. (2005). "Role of gustatory thalamus in anticipation and comparison of rewards over time in rats." Am J Physiol Regul Integr Comp Physiol **288**(4): R966-80.

Twining, R. C., M. Bolan, et al. (2009). "Yoked delivery of cocaine is aversive and protects against the motivation for drug in rats." Behav Neurosci **123**(4): 913-25.

General Discussion

6.1 Summary of Thesis Behavioral Data

The data presented in this thesis used bilateral lesions of the insular gustatory cortex (GCTx) in data Chapter 2 and asymmetric lesions of the gustatory thalamocortical loop (THCx) in data Chapter 5. Together these lesion studies demonstrated that the communication between the insular taste cortex and taste thalamus (which receives its primary projections from the medial parabrachial nucleus bilaterally) is necessary for acquisition (and retention in case of the asymmetric THCx rats) of morphine US-induced conditioned gustatory CS avoidance following repeated taste CS - drug US pairings. Whether paired with active cocaine US self-administration (which was tested but omitted from current thesis data chapters) or paired with passive morphine US injections, access to the gustatory CS also serves as a predictive signal for the physiological effects of the more preferred addictive drug US, to be experienced in the near future.

Anticipatory contrast effect (ACE) arises from contingently pairing brief access to two distinct (in this case edible) CS-US stimuli. This occurs, for example, allowing 3 min access to a palatable saccharin or low concentration sucrose solution followed by 3 min access to a more concentrated sucrose solution in the near future. In data Chapter 3 bilateral GCTx lesions were shown to block the acquisition of ACE, which suggesting a commonality in the gustatory processing required for both forms of conditioned gustatory CS avoidance responses. A third phenomenon, consummatory successive negative contrast (SNC) requires the comparison of two gustatory rewards (one present and one missing) and, is therefore also dependent on the retrieval of taste encoded memory. As shown in data Chapter 4, asymmetric gustatory THCx lesions blocked consummatory SNC in rats. Finally, by exposing a subset of rats with bilateral insular GCTx or asymmetric THCx lesions that served in the taste-morphine experiments to LiCl-

induced CTA we were able separate (as did Mackey, 1986) the need for an intact taste thalamocortical loop in conditioned taste avoidance vs. taste aversion.

6.2 Summary of Thesis Histological Data

For histological analysis of central brain lesions our lab has traditionally used nissl-stained sections (Grigson, Reilly et al. 1998; Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005; Twining, Hajnal et al. 2005). Instead of using a Nissl-stain, the neuron nuclei (NueN) specific immunohistochemical marker was use to determine the boundaries of the bilateral insular GC lesions and asymmetric lesions of the taste thalamocortical axis (Jongen-Relo and Feldon 2002). Thalamic retrograde and/or anterograde insular cortex injury was separately reviewed and found to be minimal in both bilateral insular and asymmetrical thalamocortical loop lesions. In a majority of the lesions analysis of NeuN immunostained sections revealed, little to no loss in cell bodies or alterations in the morphology of the non-gustatory thalamic nuclei (i.e., adjacent VPM, VPL, Pf, Po and centromedian) and the dorsally and ventrally located somatosensory and piriform/rhinal cortices. Rats with large cystic infracts (i.e., damage to non- gustatory nuclei), retrograde or, anterograde degeneration from the lesion site were omitted for further analysis.

The experiments using asymmetric lesions of the thalamocortical loop are critical in understanding the neuroanatomical mechanisms behind the comparison of disparate rewards over time. These contralaterally placed lesions extent the findings with bilateral lesions of either nuclei because they demonstrate that, it is not the gustatory thalamus or gustatory cortex, per se, but communication between these two structures that is essential for conditioned avoidance of a sweet taste cue following pairings with a drug of abuse and sucrose anticipation. This means, one

intact gustatory thalamic and one intact gustatory cortical nucleus is sufficient to acquire sucrose- and morphine-induced devaluation of an otherwise palatable saccharin CS, but only if those intact nuclei are in the same hemisphere (i.e., only if the circuitry is intact to allow for communication between the thalamus and cortex). The same restrict is not placed on CTA learning, presumably because it involves but does not require the taste thalamocortical loop.

The behavioral results found following asymmetric THCx lesions mimic those obtained following bilateral lesions of the taste thalamus or bilateral lesions of the insular gustatory cortex, which has revealed the importance facts about the neural circuitry involved in complex learning responses. For instance, bilateral lesions of the taste thalamus (Grigson, Lyuboslavsky et al. 2000; Schroy, Wheeler et al. 2005) or insular gustatory cortex (Kesner and Gilbert 2007; Roman and Reilly 2009) prevents acquisition of gustatory CS avoidance produced by a drug of abuse or sucrose anticipation, as did the asymmetric THCx lesions. The largely ipsilateral reciprocal projection between the taste (VMPpc) thalamus and cortex (see schematic diagram in data Chapters 3-5), allows only minimal communication between the structures when the lesions are place contralateral, as do bilateral lesions at either level. Moreover, similar to the disruptive effect of asymmetric THCx lesions on the acquisition of consummatory successive negative contrast (see data Chapter 3), bilateral lesions at either level (e.g., the insular gustatory cortex or taste thalamus) were previously shown to also prevent consummatory SNC in rats (Reilly and Trifunovic 1999; Lin, Roman et al. 2009). In summary, communication between the taste thalamus and insular taste cortex, then, appears critical for making the explicit comparison between two disparate rewards and expressing the consequence of this comparison in ingestion. LCl-induced CTA, on the other hand, develops in rats with bilateral damage to the taste thalamocortical loop (as shown here), as well as independent of nutrient demands, and while the

animal is unconscious (Provenza, Lynch et al. 1994). Finally, more than 20 years ago, chronic thalamic and chronic decerebrate rats both have been shown to appropriately detection and discrimination different tasting solutions as well as distinct concentrations of the same taste (Grill and Norgren 1978; Grill and Norgren 1978). These data provide strong evidence that the gustatory thalamocortical loop is only required fro specific forms of conditioned taste learning responses.

6.3 Reward Comparisons Hypothesis: Implication of Thesis Data

The presumed sequences of events necessary for conditioned taste suppression arising from taste CS-US reward comparison process, and the positively-reinforced ACE are (1) appropriate detection of the taste CS and experience the drug or sucrose consequence, (2) associate the taste CS with the effects of the drug (or sucrose) US, (3) remember the value of the US experience upon future presentation of the taste CS, (4) distinguish the drug-induced effects from those of the CS, which is followed by (5) comparing current CS with the US value in memory and (6) suppressing intake of the less valued CS upon repeated re-exposure. The events involved in the negatively-reinforced LiCl-induced CTA overlap but differ from the other two behaviors. Learning a CTA requires (1) appropriate detection of the taste CS which is temporally linked to an [aversive] US consequence or experience, (2) associate the taste CS with internal malaise or the effects of the aversive (LiCl or x-ray) US, (3) recall the negative event produced by the US experience upon future presentation of the taste CS. At this point the paradigms differ, since CTA learning does not involve comparing [the relative reward values of] the CS cue and the US experience. Instead, (4) the internal malaise that is produced by the aversive US is treated as a by-

product of CS intake and thus is attributed to the CS. Finally, (5) intake of the taste CS is drastically suppressed upon re-exposure.

While an intact taste cortex was essential for reward comparison and the consequential suppression of a taste cue when paired with a low and a high dose of morphine and the low dose of cocaine, the disruptive effect of the lesion was overridden by the use of a relatively higher 20 and 40 mg/kg dose of cocaine. Twining et al., (in preparation) have obtained a similar pattern in rats with bilateral lesions of the taste thalamus. The suppressive effect of the higher dose of cocaine may represent a quantitative (i.e., moderate to highly rewarding) and/or a qualitative (i.e., reward vs. aversion) change in the type of conditioned taste suppression being expressed. For instance, the reinforcing properties of 20 mg/kg cocaine, but not 10 mg/kg, may be sufficiently rewarding for rats with THLX or GCTx lesions to suppress intake of the CS cue. Such an increase in the magnitude of the US reward value may increase the number of neuronal systems recruited during the US exposure while rendering the taste thalamocortical loop less critical.

As for changes is the quality of the drug US, it is possible that at the higher dose of the cocaine, the devaluation of the drug signal may reflect a shift from reward (pCTS) to aversion (nCTS) learning, similar to the traditional LiCl-induced "CTA". This could occur if this dose of drug is toxic and results in a negative emotional state, thus creating an aversive US consequence. The 20 mg/kg (i.p.) dose of cocaine, however, has been shown to be sufficient to increase run speed in the runway and to support CPP acquisition (Mucha, van der Kooy et al. 1982; Ettenberg and Geist 1993). Thus a third possibility is that, while the drug is not aversive in-of-itself, the state elicited by the drug-associated cue is. In this case, the drug-associated taste cue may elicit cue-induced withdrawal, for example, which is an aversive state known to support taste aversion

(Siegel 1975; Frumkin 1976; McDonald, Parker et al. 1997; Weise-Kelly and Siegel 2001; McDonald and Hong 2004; Wheeler and Miller 2007). This most likely interpretation, the development of an aversive state, also would be expected to recruit if not depend on other neuronal circuits.

6.4 Future directions

In addition to the work laid out in this thesis, we have also collected data demonstrating that despite a failure to suppress the taste CS solution during the CS access period, asymmetric THCx rats worked just as hard as rats with control lesions for cocaine-self infusions during the US consumption phase. When tested in the runway, however, the asymmetric THCx rats ran slower in the saccharin-morphine vs. saccharin-saline condition. This latter finding we believe suggest the taste cue may be induced a negative emotion state and must be studied further.

Bilbliography

Ettenberg, A. and T. D. Geist (1993). "Qualitative and quantitative differences in the operant runway behavior of rats working for cocaine and heroin reinforcement." Pharmacol Biochem Behav **44**(1): 191-8.

Frumkin, K. (1976). "Differential potency of taste and audiovisual stimuli in the conditioning of morphine withdrawal in rats." Psychopharmacologia **46**(3): 245-8.

Grigson, P. S., P. Lyuboslavsky, et al. (2000). "Bilateral lesions of the gustatory thalamus disrupt morphine- but not LiCl-induced intake suppression in rats: evidence against the conditioned taste aversion hypothesis." Brain Res **858**(2): 327-37.

Grigson, P. S., S. Reilly, et al. (1998). "Ibotenic acid lesions of the parabrachial nucleus and conditioned taste aversion: further evidence for an associative deficit in rats." Behav Neurosci **112**(1): 160-71.

Grill, H. J. and R. Norgren (1978). "Neurological tests and behavioral deficits in chronic thalamic and chronic decerebrate rats." Brain Res **143**(2): 299-312.

Grill, H. J. and R. Norgren (1978). "The taste reactivity test. II. Mimetic responses to gustatory stimuli in chronic thalamic and chronic decerebrate rats." Brain Res **143**(2): 281-97.

Jongen-Relo, A. L. and J. Feldon (2002). "Specific neuronal protein: a new tool for histological evaluation of excitotoxic lesions." Physiol Behav **76**(4-5): 449-56.

Kesner, R. P. and P. E. Gilbert (2007). "The role of the agranular insular cortex in anticipation of reward contrast." Neurobiol Learn Mem **88**(1): 82-6.

Lin, J.-Y., C. Roman, et al. (2009). "Insular cortex and consummatory successive negative contrast in the rat." Behavioral Neuroscience **123**(4): 810-814.

McDonald, R. J. and N. S. Hong (2004). "A dissociation of dorso-lateral striatum and amygdala function on the same stimulus-response habit task." Neuroscience **124**(3): 507-13.

McDonald, R. V., L. A. Parker, et al. (1997). "Conditioned sucrose aversions produced by naloxone-precipitated withdrawal from acutely administered morphine." Pharmacol Biochem Behav **58**(4): 1003-8.

Mucha, R. F., D. van der Kooy, et al. (1982). "Drug reinforcement studied by the use of place conditioning in rat." Brain Res **243**(1): 91-105.

Provenza, F. D., J. J. Lynch, et al. (1994). "Food aversion conditioned in anesthetized sheep." Physiol Behav **55**(3): 429-32.

Reilly, S. and R. Trifunovic (1999). "Gustatory thalamus lesions eliminate successive negative contrast in rats." Behav Neurosci **113**(6): 1242-8.

Roman, C. and S. Reilly (2009). "Insular cortex lesions and morphine-induced suppression of conditioned stimulus intake in the rat." Behavioral Neuroscience **123**(1): 206-211.

Schroy, P. L., R. A. Wheeler, et al. (2005). "Role of gustatory thalamus in anticipation and comparison of rewards over time in rats." Am J Physiol Regul Integr Comp Physiol **288**(4): R966-80.

Siegel, S. (1975). "Evidence from rats that morphine tolerance is a learned response." J Comp Physiol Psychol 89(5): 498-506.

Twining, R. C., A. Hajnal, et al. (2005). "Lesions of the Ventral Tegmental Area disrupt drug-induced appetite stimulating effects but spare reward comparison." International journal of Comparative Psychology 18: 372-396.

Weise-Kelly, L. and S. Siegel (2001). "Self-administration cues as signals: drug self-administration and tolerance." J Exp Psychol Anim Behav Process 27(2): 125-36.

Wheeler, D. S. and R. R. Miller (2007). "Interactions between retroactive-interference and context-mediated treatments that impair Pavlovian conditioned responding." Learn Behav 35(1): 27-35.

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PUBLICATIONS

Geddes,R., R.C. Vannucci, & S.J. Vannucci. 2001. *Delayed Cerebral Atrophy following Moderate Perinatal Hypoxic-Ischemia in the Immature Rat. Dev Neurosci, 23:180-185.*

Geddes, R.I., Han, L., Norgren, R., A.E. Baldwin and P.S. Grigson (2008). *"Gustatory insular cortex lesions disrupt drug-induced, but not lithium chloride-induced, suppression of conditioned stimulus intake." Behav Neurosci **122**(5): 1038-50.*

Grigson, P. S., Twining, R. C., Freet, C. S., Wheeler, R. A., & Geddes, R. I. (2008). *Drug-induced suppression of CS intake: Reward, aversion, and addiction. Conditioned Taste Aversion: Behavioral and Neural Processes,. NY, NY, Oxford University Press.*

MANUSCRIPTS IN PREPARATION

Geddes, R. I. and P. S. Grigson (in prep (4), ommitted from Thesis). "Lesions of the gustatory thalamocortical loop block drug-induced devaluation of a natural saccharin reward cue, while leaving instrumental responding for the drug intact." NOT in PSU THESIS.

PROFESSIONAL MEETINGS (Abstracts)

Geddes, R.I., L. Han & P.S. Grigson. (2008) *Disconnection of the gustatory thalamocortical loop prevents both learning and memory of the devaluation of a sweet cue by a drug of abuse. (abstract in Appetite, data presented in Paris France July 2008)*

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