THE INFLUENCE OF THE TRIGEMINAL OROSENSORY AREA IN DRUG-INDUCED
DEVALUATION OF NATURAL REWARDS AND DEVELOPMENT OF A
CONDITIONED STATE OF WITHDRAWAL

A Dissertation in
Neuroscience

by

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ABSTRACT

Addiction is a disease of chronic relapse that costs society an estimated $484 billion per year as the addict repeatedly cycles from addiction to abstinence, withdrawal, drug seeking, and relapse. Additionally, the drug trade has far-reaching consequences that include human rights violations, increased criminal activity, community safety risks, adverse health effects, and environmental destruction. Along with society, the addict and his or her family also pay, as substance abuse, dependence, and addiction are associated with an apparent devaluation of, and inattention to, natural rewards. This consequence of addiction can be modeled using a reward comparison paradigm where rats avoid intake of a taste cue that comes to predict access to a drug of abuse. Evidence suggests rats avoid intake following such pairings, at least in part, because the taste cue pales in comparison to the highly rewarding drug expected in the near future. Along with the devaluation of natural rewards, addiction also is a disease of chronic relapse where drug seeking and drug taking are repeatedly initiated by exposure to stress, the drug itself, and drug-associated cues. Our paradigm also models this aspect of addiction. Thus, while rats may initially avoid intake of the taste cue because it pales in comparison to the drug's value, with experience, rats ultimately avoid intake because the taste cue comes to elicit the onset of a conditioned aversive state involving craving, withdrawal, and seeking.

We have made progress in delineating the underlying circuitry involved in drug-induced avoidance of a taste cue. In accordance, lesions of the gustatory thalamus or cortex eliminate avoidance of a taste cue when paired with either a drug of abuse or a rewarding sucrose solution, but not when paired with the aversive agent, LiCl. The present dissertation investigated the role of a neighboring thalamic structure, the trigeminal orosensory area (TOA), using bilateral ibotenic acid lesions. In Chapter 2, we found that the TOA lesion eliminated avoidance of a taste
cue when paired with experimenter-administered morphine or cocaine using our standard parameters. The TOA lesion, however, did not disrupt avoidance of a taste cue that predicted access to a preferred sucrose solution or the administration of the aversive agent, LiCl. This is the first lesion to selectively disrupt avoidance of an otherwise palatable taste cue when paired with a drug of abuse. In Chapter 3, we provided direct evidence that the drug-paired taste cue elicits withdrawal and that greater withdrawal elicits greater drug seeking and taking. Lesions of the TOA prevented the onset of cue-induced withdrawal. In Chapter 4, we showed that, unlike findings obtained in the home cage, the lesion failed to prevent avoidance of the drug-paired taste cue and accompanying signs of cue-induced withdrawal when tested in a runway apparatus. Factors that may have contributed to the successful performance by the lesioned rats in the runway study, such as the availability of contextual cues and a decreased inter-stimulus interval, were tested in Chapters 5 and 6. Specifically, in Chapter 5, using self-administration, we determined that contextual cues can, indeed, override the disruptive effect of the TOA lesion. In this context, TOA lesioned rats avoided the taste cue, showed signs of conditioned withdrawal, and exhibited increased instrumental responding for drug compared with intact rats. Furthermore, the TOA was essential not only for associating the explicit taste cue with the drug of abuse, but also for reactivating that memory in the absence of supporting contextual cues. Finally, in Chapter 6, we determined that the length of the interval between presentation of the taste cue and the unconditioned stimulus was pivotal for the development of drug-induced avoidance, but not sucrose-induced avoidance of a palatable cue in the lesioned rats. Therefore, in sum, we conclude that avoidance of a drug-paired taste cue develops, in part, as a result of devaluation of the taste cue, but with experience is intensified due to the onset of an aversive conditioned state of withdrawal. In the absence of other exteroceptive cues, the taste cue can support this conditioned state and lesions of the TOA block both avoidance of the taste cue and the onset of the accompanying aversive state. An intact TOA, however, is not required when the development of
the phenomenon is augmented by the addition of contextual cues and/or by the use of a very short inter-stimulus interval. Thus, while a drug of abuse can be predicted by a myriad of cues, the TOA is essential for acquisition and retention of an explicit taste-drug association. Further, the TOA is implicated in the development of the opponent process that is integral to the development of addiction and, once the association is learned, to the precipitation of cue-induced relapse.
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Chapter 1

Introduction

The Cost of Addiction

According to Results from the 2011 National Survey on Drug Use and Health (Substance Abuse and Mental Health Services Administration, 2012), in 2011, an estimated 22.5 million Americans aged 12 or older were current users of illicit drugs. This includes 18.1 million users of marijuana, 1.4 million users of cocaine, 439,000 users of methamphetamine, 620,000 users of heroin, 972,000 users of hallucinogens, and 6.1 million who used prescription-type psychotherapeutic drugs non-medically. In addition to illicit drug use, 133.4 million Americans aged 12 or older reported being current drinkers of alcohol (51.8% of the population), with 58.3 million participating in binge drinking, and 15.9 million considered heavy drinkers. Additionally, 68.2 million were current users of tobacco products. While North America represents the largest illicit drug market, drug abuse is a global epidemic with between 153 million and 300 million people aged 15-64 being current users of illicit substances in 2010, or roughly 1 out of every 20 people (UNODC, 2012). These numbers, while huge, underestimate the scope of the problem because addiction is not resolved following even extended periods of abstinence. In fact, addiction is a disease of chronic relapse (Leshner, 1999) that costs society an estimated $484 billion per year as the addict repeatedly cycles from addiction to abstinence, withdrawal, drug seeking, and relapse.

Drug use has numerous adverse health consequences. These deleterious effects on health include increased incidence of cancer, chronic respiratory disease, cardiovascular disease, liver cirrhosis, and the increased risk for communicable diseases such as tuberculosis and respiratory
infections (WHO, 2011). Furthermore, of the estimated 16 million injecting drug users worldwide, almost one in five is HIV-positive. Approximately the same proportion is infected with hepatitis B, and about half of all injecting drug users are infected with hepatitis C (Mathers et al., 2008). These health related consequences come with a cost. In the US, health related economic costs reach $143.4 billion each year (CDC, 2008; ONDCP, 2004).

Substance abuse affects all members of society. For example, in 2011, 9.4 million persons aged 12 or older reported driving under the influence of illicit drugs, as well as an additional 28.6 million who reported driving under the influence of alcohol during the past year (SAMHSA, 2012). According to reports from the National Highway Traffic Safety Administration (2012), 10,228 people were killed in alcohol-impaired-driving crashes in 2010, which accounted for 31 percent of the total motor vehicle traffic fatalities in the United States. Of the 10,228 people who died in alcohol-impaired-driving crashes, 6,627 (65%) were drivers with a blood alcohol content of .08 or higher. However, the remaining fatalities consisted of 2,872 (28%) motor vehicle occupants and 729 (7%) non-occupants. Furthermore, in 2009, there were 4.6 million drug-related emergency department visits (Owens, Mutter & Stocks, 2010) and in 2007, nearly 100 persons per day died of drug overdoses in the United States (CDC, 2011).

Every year worldwide, 245,000 deaths result from illicit drug use, 5.1 million result from tobacco use (this represents the third leading cause of premature death worldwide), and 2.3 million deaths related to alcohol abuse (WHO, 2009). Addiction, then, is not just an individual’s problem; it has far-reaching consequences that make addiction a societal concern.

The drug trade has devastating effects on the environment. Thousands of tons of chemical waste is dumped into rivers and discarded into the environment yearly in Peru and Columbia alone (UNODC, 2006; Walters, 2002). In addition to pollution, deforestation is a major environmental cost resulting from the drug trade. In Columbia, at least 60% of drug crops are grown on newly deforested land (UNODC, 2006), and according to the US Drug Tsar, 10% of
Peru’s total rainforest destruction over the past century is due to the illicit drug trade (Walters, 2002). To add insult to injury, attempts to eradicate illegal crops by fumigation result in destruction of plant and animal species in some of the most biologically diverse regions of the world (UNODC, 2006; Walsh, Sánchez-Garzoli & Abdala, 2008; Witness for Peace, 2009). Furthermore, crop fumigation leads to adverse health consequences to those inhabiting nearby areas (Richard, Moslemi, Sipahutar, Benachour & Seralini, 2005; United Nations News, 2007), and further deforestation when new plots are cleared for the continuation of drug crop farming (UNODC, 2005). Therefore, addiction to illicit substances has a global impact that cannot be ignored.

**The Devaluation of Natural Rewards**

Along with society, the addict and his or her family also pay, as substance abuse, dependence, and addiction are associated with an apparent devaluation of, and inattention to, natural rewards. Indeed, according to the Diagnostic and Statistical Manual of Mental Health Disorders, fourth edition (American Psychiatric Association [DSM-IV], 1994), substance abuse and dependence involve a failure to fulfill major obligations at work, school, or home, the giving up of important social, occupational, or recreational activities, and continued drug use in spite of recurrent physical, legal, social, or psychological problems. These assertions are supported by published data. For example, using functional magnetic resonance imaging (fMRI) of activity in the frontolimbic brain regions, Goldstein et al. (2007) found that while control subjects valued high amounts of money more than low amounts of money, cocaine addicted subjects valued all monetary amounts equally, indicating a compromised subjective sensitivity to gradients in reward value. Additionally, in an fMRI study by Wilson and colleagues (2008), smokers that were anticipating a cigarette processed monetary gains as less rewarding and monetary losses as more
punishing than those who did not anticipate a cigarette in a card-guessing task. Insidiously, addiction diminishes the reinforcing potential of natural rewards, which is problematic considering that behavioral interventions for addictive behaviors typically focus on contingency management, or working for natural rewards.

Substance abuse also involves a failure to fulfill obligations at work. According to figures from Jones et al. (1995), there is an estimated 25% reduction in work performance among heavy alcohol users in New Zealand, with a significant difference in both the number and cost of absentee and reduced efficiency days reported between the top 10% and the bottom 10% of drinkers. This equates to an estimate of alcohol-related lost productivity among the working population of New Zealand to be $57 million per year. In the US, substance abuse accounts for an astounding $128.6 billion in productivity loss per year (ONDCP, 2004).

Another example of the devaluation of natural rewards is the failure to fulfill obligations at home. In a study by Nair et al. (1997), drug use, and particularly the degree of use is predictive of disruption in primary caregiving to infants of substance abusing women. Additionally, in a study by Santolaria-Fernandez et al. (1995), drug addicts without organic pathology tend to be under-nourished. They found that 66.4% of the subjects in their study exhibited anorexia at admission, 30% of the drug addicts weighed less than 80% of the mean weight for the population, or admitted to a weight loss above 10%, and by subjective nutritional assessment 18% were deeply malnourished.

Consequently, addiction and the concurrent devaluation of natural rewards can lead to removal of children from the home, loss of home and/or job, negative health consequences, a reduced sensitivity to treatment by contingency management, and an increase in risky behaviors that affect all members of society. This emphasizes the urgency for study of drug-induced devaluation of natural rewards. While there are a number of critical animal models of craving and relapse (Grimm, Hope, Wise & Shaham, 2001; Neisewander et al., 2000; Weiss et al., 2000),
our paradigm is the first animal model for the systematic study of drug-induced devaluation of natural rewards. Support for our hypothesis has accumulated over the last decade (for a review: Grigson, Twining, Freet, Wheeler & Geddes, 2009) and the model has been expanded to include drug self-administration.

Rats will avoid intake of a palatable saccharin taste cue (referred to as the conditioned stimulus, CS) when paired with either an aversive or an appetitive unconditioned stimulus (US). In aversive conditioning, a conditioned taste aversion (CTA) occurs when rats suppress intake of the taste cue after it has been paired with an aversive, illness-inducing agent such as lithium chloride (LiCl) or x-radiation (Garcia, Kimmeldorf & Koelling, 1955). A similar result is seen in appetitive conditioning, when rats avoid intake of a palatable saccharin cue (CS) that predicts access to a more rewarding sucrose solution (US) (Flaherty & Checke, 1982). This phenomenon is referred to as an anticipatory contrast effect (ACE), and while the result may appear similar to a CTA, it is established through different means. In anticipatory contrast, reduced intake of the taste cue is thought to reflect an associative process whereby the perceived value of the saccharin CS pales in anticipation of the availability of the preferred sucrose US (Flaherty & Grigson, 1988; Flaherty & Rowan, 1985). Anticipatory contrast effects occur regardless of deprivation state (Flaherty, Grigson, Checke & Hnat, 1991) and even when using a low and high concentration of non-caloric saccharin as the first and second stimulus (Flaherty & Rowan, 1986), or low and high concentrations of corn oil (Liang, Norgren & Grigson, 2012c).

To summarize, in a CTA, rats avoid intake of a taste cue associated with an illness-inducing agent, much in the way humans avoid foods that either have caused or have been associated with illness. Furthermore, an ACE occurs when rats avoid intake of a palatable taste cue that predicts access to a preferred natural reward, similar to how humans “save room” for dessert during a meal. While both processes result in avoidance of a palatable taste cue, it is clear that they have distinct underlying mechanisms by which avoidance comes about. How then, to
explain a third phenomenon in which avoidance of a palatable taste cue develops in response to association with a drug of abuse (LeMagnen, 1969; for reviews, see Gamzu, Vincent & Boff, 1985; Goudie, 1987; Hunt & Amit, 1987). Avoidance of a drug-paired cue occurs with opiates such as morphine and heroin (Cappell, LeBlanc & Endrenyi, 1973; Grigson, Twining & Carelli, 2000; Miller, Kelly, Neisewander, McCoy & Bardo, 1990; Sherman, Pickman, Rice, Liebeskind & Holman, 1980), as well as with stimulants like cocaine and amphetamine (Cappell & LeBlanc, 1971; Glowa, Shaw & Riley, 1994). Furthermore, most abused substances support this phenomenon. For instance, ethanol, flurazepam, chlordiazepoxide, amobarbital, and phenobarbital all lead to conditioned avoidance of a palatable taste cue (Cappell et al., 1973; Lester, Nachman & LeMagnen, 1970; Vogel & Nathan, 1975). Thus, rats avoid intake of a taste cue following pairings with all drugs of abuse tested, across a range of doses (LeBlanc & Cappell, 1975; Parker, 1991), and routes of administration (Bechara & van der Kooy, 1985; Cappell & LeBlanc, 1971; Mucha & Herz, 1986; Shoaib & Stolerman, 1995; Wise, Yokel & DeWit, 1976).

This phenomenon was immediately interpreted as a CTA (Lester et al., 1970), as that was the only other phenomenon at the time that resulted in a similar reduction in CS intake (anticipatory contrast was first described over a decade later). Jumping to the conclusion that drugs of abuse are aversive, however, overlooked their well-known rewarding properties. Specifically, drugs of abuse are readily self-administered by humans and other animals (for review, see van Ree, 1979) and they sustain the development of conditioned place preferences (Bardo, Miller & Neisewander, 1984; Blander, Hunt, Blair & Amit, 1984; Katz & Gormezano, 1979; Reicher & Holman, 1977). Further, while rats avoid intake of a gustatory cue paired with either LiCl or a drug of abuse, they simultaneously decrease instrumental responding for LiCl and increase instrumental responding for morphine (White, Sklar & Amit, 1977). Therefore, avoidance of a drug-paired gustatory cue is not like that mediated by LiCl-induced malaise.
After surveying these and related data, Grigson (1997) hypothesized that drugs of abuse do not support CTA learning, but instead that the well-known rewarding properties of drugs of abuse, rather than their aversive properties, mediate the reduction in CS intake following taste-drug pairings. According to this hypothesis, rats avoid intake of a saccharin CS following taste-drug pairings because the perceived value of the saccharin cue is reduced in anticipation of the availability of the more potent rewarding properties of the impending drug. Therefore, the drug-induced avoidance of a taste cue more closely resembles that seen in anticipatory contrast (when the saccharin CS predicts access to a preferred sucrose US).

Table 1-1: Comparison of conditioned avoidance of a taste cue when paired with a natural reward, drug of abuse, or the aversive agent, LiCl.

<table>
<thead>
<tr>
<th>Manipulation</th>
<th>Sucrose US</th>
<th>Drugs of Abuse</th>
<th>LiCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deprivation State</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Strain</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Drug History</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lesions of TTA</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Manipulation has large effect on CS suppression (+), little or no effect on CS suppression (-)*

A great deal of evidence now stands in support of the reward comparison hypothesis (see Table 1-1). For example, the suppressive effects of sucrose and drugs of abuse, but to a lesser extent LiCl, are reduced when the rats are tested in a food- or water-deprived state (Flaherty et al., 1991; Gomez & Grigson, 1999; Grigson, Lyuboslavsky, Tanase & Wheeler, 1999). The suppressive effects of cocaine and sucrose, but not LiCl, are altered when tested in reward sensitive Lewis compared with less sensitive Fischer 344 rats (Grigson & Freet, 2000). The suppressive effects of sucrose and cocaine, but not LiCl, are augmented in Sprague-Dawley rats with a history of chronic morphine treatment (Grigson, Wheeler, Wheeler & Ballard, 2001).
Furthermore, lesions of the gustatory thalamus dissociate the phenomena by disrupting the suppressive effects of sucrose, morphine, and cocaine, but not those of LiCl (Grigson, Lyuboslavsky & Tanase, 2000; Schroy et al., 2005; Reilly & Pritchard, 1996a, 1996b). Finally, when the drug is self-administered, rather than passively administered, greater avoidance of CS intake is associated with increased drug self-administration (Grigson & Twining, 2002; Wise et al., 1976). Thus, while drugs of abuse may have aversive properties (Bechara & van der Kooy, 1985; Blanchard & Blanchard, 1999; Ettenberg & Geist, 1991), evidence suggests that rats suppress intake of a saccharin cue following taste-drug pairings, at least in part, because they are anticipating the well-known rewarding properties (Blander, Hunt, Blair & Amit, 1984; Reicher & Holman, 1977; Zernig et al., 2002) of the drug.

**Cue-Induced Withdrawal**

The most insidious aspect of addiction is the propensity for chronic relapse, where drug seeking and drug taking are repeatedly initiated by exposure to stress, the drug itself, and drug-associated cues. When a gustatory stimulus serves as the cue, rats will avoid intake of that taste cue following pairings with a drug of abuse such as morphine or cocaine. Initially avoidance of the cue following exposure to drugs of abuse resembles avoidance normally seen in an ACE paradigm; however, we also have uncovered evidence for aversion. Specifically, in addition to drug-induced devaluation, we also have evidence that, with experience, rats avoid the CS because it comes to elicit the onset of a conditioned aversive state involving craving and withdrawal, much like that proposed by the opponent process theory of Solomon & Corbit (1974). According to the opponent process theory (Figure 1-1), the initial response to a stimulus (State A) is automatically compensated for by an opponent response (State B) aimed at returning the overall state to neutral baseline levels. In the case of abused drugs, State A often involves feelings of
euphoria. This initial response, however, instigates an opponent response (State B), which in the case of abused drugs often presents as withdrawal. The theory suggests that, with repeated stimulation, State B gains in both intensity and duration. Furthermore, repeated stimulation leads to a decrease in State A, which represents the development of tolerance. Therefore, over time, the continued use of abused drugs results in less reward and more aversion. According to the opponent process theory, escalation in drug taking is interpreted as a means to restore the intensity of the reward while overcoming the aversive state of withdrawal.

![Diagram](image.png)

**Figure 1-1:** The opponent process theory, adapted from Solomon & Corbit (1974).

With repeated exposure, drug related cues are capable of eliciting the opponent process in the absence of drug. For example, addicted humans report aversive affect when having to wait for access to nicotine in the presence of drug-related cues (Sayette et al., 2003) and Wilson, Sayette, Delgada, and Fiez (2008) reported blunted activation of the caudate putamen following losses and gains of monetary reward. Furthermore, this negative affect is caused by physiological changes such as withdrawal and is a potent precipitator of relapse (Sinha et al., 2009). In accordance, stronger negative affect in rats, as reflected by increased aversive taste reactivity and
a shift in firing pattern in the nucleus accumbens, is associated with a shorter latency to initiate responding for cocaine, greater load up on cocaine, and faster acquisition of drug self-administration (Wheeler et al., 2008). Taken together, the data suggest that devaluation by cue-induced craving/withdrawal may occur in near parallel with devaluation by rewarding drug properties.

In support, avoidance of the CS is accompanied by elevated levels of circulating corticosterone (Gomez, Leo & Grigson, 2000), blunted accumbens dopamine (Grigson & Hajnal, 2007), and the onset of aversive taste reactivity (TR; i.e., gapes) when infused directly into the oral cavity (Wheeler et al., 2008). Withdrawal induced by an opiate antagonist also is associated with elevated corticosterone (Nunez, Foldes, Laorden, Milanes & Kovacs, 2007) and blunted dopamine in the nucleus accumbens (Shaham & Stewart, 1995). In addition, like drug-induced suppression of CS intake, rats also avoid intake of a taste cue that has been paired with naloxone-induced withdrawal and this avoidance is accompanied by aversive TR when the solution is infused directly into the oral cavity (McDonald, Parker & Siegel, 1997). This observation highlights the need to investigate the involvement of cue-induced withdrawal in the phenomenon and, therefore, the need to directly measure cue-induced withdrawal following exposure to a drug-paired taste cue.

Recent data suggest that the timing of the taste cue presentation relative to drug is also an important factor, as it can change the valence of the taste cue. Wheeler et al. (2011) found aversive responses (i.e., aversive taste reactivity, blunted accumbens dopamine, and decreased reward sensitivity) to an intraorally infused taste cue that predicted delayed cocaine self-administration, yet when the same taste cue was infused concurrently with cocaine self-administration, rats showed appetitive responses (i.e., increased accumbens dopamine) to the drug-paired cue. Therefore, the taste cue becomes aversive when it indicates that drug is coming, but the rat must wait for drug. Thus, while rats may initially avoid intake of the CS because it
pales in comparison to the drug's value, we hypothesize that they ultimately avoid intake because it comes to elicit the onset of a conditioned aversive state involving withdrawal and craving in anticipation of drug availability. Given that craving and withdrawal elicit relapse, understanding the role that drug-paired cues play in the development and maintenance of addictive behaviors is of paramount importance.

**Neural Circuitry**

In addition to behavioral evidence, progress has been made in identifying the underlying circuitry involved in drug-induced avoidance of a taste cue. Because the phenomenon relates to the avoidance of a gustatory cue, understandably, work has focused on the involvement of gustatory circuitry (see Figure 1-2). Briefly, gustatory information from the tongue and oral cavity reaches the nucleus of the solitary tract (NST) via the facial (VII), glossopharyngeal (IX), and vagus (X) nerves. The NST, in turn, projects mainly to the parabrachial nucleus (PBN) of the pons. From the PBN, the projections take two different routes to the forebrain: the thalamocortical and the ventral pathways (Norgren, 1974). The thalamocortical pathway projects from the PBN to the parvocellular region of the ventroposteromedial nucleus, or VPMpc of the thalamus. The VPMpc, in turn, projects to the agranular insular gustatory cortex and this projection is nearly 100% ipsilateral (Kosar, Grill & Norgren, 1986a, 1986b; Norgren & Wolf, 1975). The gustatory cortex also has an extensive reciprocal projection to the gustatory thalamus (Nakashima et al., 2000; Norgren, 1976; Norgren & Wolf, 1975). The ventral pathway, on the other hand, involves monosynaptic projections to limbic structures, including the central nucleus of the amygdala, the bed nucleus of the stria terminalis, the substantia innominata, and the lateral hypothalamus (Norgren, 1974, 1976).
Accordingly, lesions of nuclei along these pathways disrupt behaviors that are related to the sensory and reward processing of taste stimuli. Lesions of the PBN disrupt contrast effects for natural rewards, drugs of abuse, and the development of conditioned taste aversions (Bures, Buresova & Ivanova, 1991; Flynn, Grill, Schulkin & Norgren, 1991; Liang et al., unpublished data; Liang, Norgren & Grigson, 2012; Reilly, Grigson & Norgren, 1993; Yamamoto & Fujimoto, 1991). Lesions placed in the ventral and thalamocortical pathways projecting from the PBN, on the other hand, have effects that are more selective. When lesions are placed in the ventral pathway, in the amygdala for example, anticipatory contrast effects for natural and or drug rewards are only slightly reduced (Gilbert & Kesner, 2002; Lovaglio, Lin, Roman & Reilly,
Lesions of the thalamocortical pathway, however, differ greatly from lesions of the ventral pathway. For example, we now know that the gustatory thalamus and gustatory cortex are essential for drug-induced suppression of CS intake, but they are not necessary for the development of a LiCl-induced conditioned taste aversion (Geddes, Han, Baldwin, Norgren & Grigson, 2008; Grigson, Lyuboslavsky, & Tanase, 2000). Additionally, the thalamic lesion also prevents the development of an anticipatory contrast effect where, for example, rats avoid a lesser-valued saccharin reward in anticipation of the availability of a preferred sucrose reward (Reilly, Bornovalova & Trifunovic, 2004; Schroy et al., 2005). These findings suggest that while the ventral pathway contributes to drug-induced suppression of intake of a taste cue, the thalamocortical pathway is essential.

**Ibotenic Acid Lesions of the Thalamic Trigeminal Orosensory Area**

In this dissertation, we continue to investigate the involvement of the thalamocortical pathway; however, the focus has been shifted to a region of the thalamus 500 µm lateral to the gustatory area. This area receives trigeminal orosensory input, and is therefore referred to as the thalamic trigeminal orosensory area (TOA) by Liang and colleagues (Liang, Freet, Grigson & Norgren, 2012a; Liang, Grigson & Norgren, 2012b; Liang, Norgren & Grigson, 2012c). Briefly, the lingual branch of the mandibular nerve supplies the surface of the anterior tongue and provides intraoral somatosensory detection (Waite, 2004). The primary intraoral somatosensory axons project ipsilaterally to the dorsal and dorsomedial parts of the brainstem structures including the subnucleus caudalis, the principal trigeminal sensory nucleus (Pr5), and the interpolaris and oralis of the spinal trigeminal nucleus (Jacquin, Semba & Egger, 1983; Takemura, Sugimoto & Sakai, 1987). The secondary trigeminal neurons from these nuclei
project ipsilaterally and contralaterally to the ventroposteromedial nucleus (VPM) of the thalamus, lateral to the gustatory region (De Chazeron, Raboisson & Dallel, 2004; Guy, Chalus, Dallel & Voisin, 2005; Smith, 1973; Waite, 2004). Projections from the thalamic somatosensory area then project to the insular cortex and overlap with fibers from the gustatory thalamus (Norgren & Wolf, 1975; Yamamoto, Yuyama & Kawamura, 1981).

When ibotenic acid is injected into the TOA, the typical pattern of damage entails the middle third of the VPM as well as the majority of the taste area in the parvicellular area of the VPM (VPMpc). Some TOA lesioned subjects have extensive damage to the VPMpc with lesions crossing the midline. These particular lesions more closely resemble lesions centered exclusively on the thalamic taste area (TTA), and are therefore not included in analysis of the TOAx condition. Additionally, the lesion typically encompasses small parts of adjacent nuclei such as the paracentral nucleus (PC), centrolateral nucleus (CL), central medial nucleus (CM), parafascicular nucleus (Pf), and the posterior nucleus (Po). Variable damage to these neighboring structures, however, is described but is not an exclusion criterion.

The trigeminal orosensory pathway is of particular interest because, when damaged, it affects ingestive and motivated behavior. For example, bilateral trigeminal mandibular branch deafferentation disrupts food and water intake (Jacquin, Harris & Zeigler, 1982; Miller, 1981). Moreover, lesions of the central trigeminal nuclei such as the Pr5 and the VPM both result in an aphagia syndrome (Nadaud, Simon, Herman & Le Moal, 1984; Zeigler & Karten, 1974). Additionally, deafferentation of the mandibular nerve disrupts lever-pressing responses for food (Jacquin et al., 1982). More to the point, in a set of studies by Liang and colleagues (2012a-c), rats with lesions centered on the TOA, but also encompassing the majority of the gustatory thalamus, exhibited increased operant responding for sucrose and corn oil under sham-feeding (i.e., open gastric fistula) conditions. These TOA lesioned subjects also yielded higher break points for sucrose in a progressive ratio task compared with sham-lesioned subjects, indicating
increased motivation for sweet reward (Liang et al., 2012a). Finally, lesions centered on the gustatory thalamus prevent avoidance of a lesser-valued gustatory cue that precedes a highly rewarding sucrose solution (Reilly et al., 2004; Schroy et al., 2005), yet, rats with TOA lesions show avoidance of a lesser-valued gustatory cue when it precedes access to preferred concentrations of sham-fed sucrose or corn oil (Liang et al., 2012c). Together, these data suggest that the TOA is involved in the motivation to respond for a sweet reward. This increased motivation for a natural reward could rival the perceived rewarding value of a drug of abuse, and therefore the lesion may protect against the devaluation of such natural rewards. The effect of this particular lesion in drug-induced devaluation of natural rewards and cue-induced withdrawal is the primary focus of this dissertation.

The purpose of the first set of studies in this thesis is to follow up on the unexpected findings by Liang and colleagues (2012c) that rats with TOA lesions, often encompassing both taste and trigeminal thalamic relays, avoid intake of a taste cue that precedes access to preferred concentrations of sucrose or corn oil under sham-feeding conditions (i.e., open gastric fistula). This finding is surprising because avoidance is attenuated in rats with lesions encompassing only the gustatory thalamus (Reilly et al., 2004; Schroy et al., 2005). As such, Chapter 2 aims to test if avoidance of a sucrose-paired cue also is present under real-feeding conditions in rats with lesions of the TOA. Furthermore, previous studies indicate that drug-induced avoidance of a taste cue was eliminated in rats with bilateral lesions focused on the gustatory thalamus (Grigson, Lyuboslavsky, & Tanase, 2000). Therefore, Chapter 2 also will test the effect of the TOA lesion on drug-induced avoidance of a taste cue using experimenter-administered morphine and cocaine. Finally, the Liang et al. (2012b) study found CTA development to be intact in sham-fed TOAx rats following three pairings of either 0.3 M sucrose or 100% corn oil with LiCl. Therefore, the final experiment of Chapter 2 tests the effect of the lesion on CTA development under real-feeding conditions.
Although avoidance of the drug-paired taste cue may initially result from drug-induced devaluation of the saccharin cue, with experience, aversion develops as would be predicted by the opponent process theory of motivation (Solomon & Corbit, 1974). In Chapter 3, we test this hypothesis using the change in body weight following naloxone administration as an index of cue-induced withdrawal. In Experiment 1, we use a within-subjects design to test for indices of withdrawal in response to a taste cue paired with experimenter-administered morphine or saline. In Experiment 2, we use a between-subjects design to test this effect when the taste cue has been paired with self-administered cocaine or saline. Finally, Experiment 3 tests the effect of the TOA lesion on cue-induced withdrawal when a Polycose taste cue predicts experimenter-administered saline or morphine.

As mentioned, Liang and colleagues (2012a) found that rats with TOA lesions yielded higher break points for sucrose in a progressive ratio task compared with sham-lesioned subjects, indicating increased motivation for sweet reward. To test if the lesion affected the motivation to obtain drug, we use a runway apparatus in Chapter 4 to assess the motivation to obtain experimenter-administered morphine following access to a morphine-paired cue. Furthermore, in Chapter 5, we use cocaine self-administration to investigate the effect of the lesion on drug taking and the willingness to work for cocaine reinforcement. Additionally, Experiments 2 and 3 of Chapter 5 investigate the influence of contextual cues on the establishment of cocaine self-administration and conditioned avoidance of a drug-paired cue in intact and lesioned rats.

Finally, the interval between presentation of the taste cue and the US may serve an important role in the development of conditioned avoidance of the taste cue. For example, when the taste cue predicts delayed access to drug, the cue elicits aversive responses, yet, when the cue is delivered at the same time as the drug, the cue elicits appetitive responses (Wheeler et al., 2011). Therefore, when the cue indicates the rat must wait to receive drug, avoidance of the cue may be the result of the onset of conditioned withdrawal in anticipation of impending drug
availability. Furthermore, a lengthy interval between the cue and the US may highlight memory
deficits in lesioned subjects. Accordingly, in Chapter 6, avoidance of a cocaine-paired taste cue
is tested using a 0 sec and a 5 min interval between the cue and the drug reward. We also address
the role of a longer interval in the comparison of two disparate natural rewards in the anticipatory
contrast paradigm.
Chapter 2

Bilateral Lesions of the Thalamic Trigeminal Orosensory Area Dissociate Natural from Drug Reward in Contrast Paradigms

For decades, it has been known that rats will avoid intake of a taste cue when paired with a drug of abuse (Cappell & LeBlanc, 1971; Cappell et al., 1973; Glowa et al., 1994; Grigson, Twining, & Carelli, 2000; LeMagnen, 1969; Miller et al., 1990; Sherman et al., 1980). Because avoidance of the taste cue is also seen in the conditioned taste aversion model (CTA), findings of this nature have been interpreted as resulting from the development of a conditioned taste aversion, despite the well-known rewarding properties of these drugs (Berger, 1972; Cappell & LeBlanc, 1971; Cappell et al., 1973; Gamzu, 1977; Hunt & Amit, 1987; Lester et al., 1970; Vogel & Nathan, 1975). However, rats also suppress intake of a taste cue (e.g., saccharin) when paired with a highly rewarding sucrose solution (Flaherty & Checke, 1982; Flaherty & Grigson, 1988; Flaherty & Rowan, 1985). This observation led us to hypothesize that rats may avoid intake of a saccharin cue following pairings with a drug of abuse because they are anticipating the availability of the highly rewarding properties of the drug (Grigson, 1997). In accordance, manipulations such as deprivation state (Bell, Thiele, Seeley, Bernstein, & Woods, 1998; Gomez & Grigson, 1999; Grigson et al., 1999), drug history (Grigson, Wheeler, Wheeler, & Ballard, 2001), strain (Glowa et al., 1994; Grigson & Freet, 2000), and even bilateral lesions of the gustatory thalamus (Flynn et al., 1991; Grigson, Lyuboslavsky, & Tanase, 2000; Reilly et al., 2004; Reilly & Pritchard, 1996a; Reilly & Trifunovic, 1999; Schroy et al., 2005) and cortex (Geddes et al., 2008) can similarly affect drug and sweet-induced suppression of conditioned stimulus (CS) intake, while having relatively little impact on a LiCl-induced conditioned taste aversion (for reviews, see Grigson, 2008; Grigson et al., 2009).

Progress has been made in identifying the underlying circuitry involved in drug-induced avoidance of a taste cue. For instance, lesions of the nucleus accumbens do not disrupt
anticipatory contrast (Leszczuk & Flaherty, 2000) and 6-hydroxydopamine lesions of the ventral tegmental area have no effect on the suppression of intake of a taste cue that predicts morphine or cocaine administration (Twining et al., 2005). An intact gustatory thalamus and cortex, on the other hand, is critical for drug-induced suppression of CS intake, but these structures need not be intact for the development of a LiCl-induced CTA (Geddes et al., 2008; Grigson, Lyuboslavsky, & Tanase, 2000; Scalera, Grigson, & Norgren, 1997). As with drug-induced suppression of CS intake, both the thalamic and cortical lesions also prevent the development of an anticipatory contrast effect (ACE) where, as mentioned, rats avoid a lesser valued saccharin cue in anticipation of the availability of a preferred sucrose reward (Reilly et al., 2004; Schroy et al., 2005). Again, these data are consistent with other data suggesting that drug-induced suppression of CS intake is similar to the suppressive effects mediated by a rewarding sucrose solution, and different from a LiCl-induced CTA (Gomez & Grigson, 1999; Grigson, Cornelius, & Wheeler, 2001; Grigson & Freet, 2000; Grigson et al., 1999; Grigson, Lyuboslavsky, & Tanase, 2000; Grigson, Wheeler, Wheeler, & Ballard, 2001).

As outlined, our initial evaluations of the underlying circuitry have focused upon the gustatory pathway, in particular the region of the thalamus that is responsive to stimulation of the tongue via gustatory stimuli, hereafter referred to as the thalamic taste area (TTA). The focus here is shifted to a region of the thalamus located 500 µm lateral to the gustatory area, which receives trigeminal orosensory input. This area was referred to as the trigeminal orosensory area (TOA) of the thalamus by Liang and colleagues (2012a-c). In this set of studies (Liang, Freet, Grigson, & Norgren, 2012a; Liang, Grigson, & Norgren, 2012b; Liang, Norgren, & Grigson, 2012c), rats with lesions centered on the TOA, but also encompassing the majority of the TTA, exhibited increased operant responding for sucrose and corn oil under sham-feeding (i.e., open gastric fistula) conditions (Liang et al., 2012a). This finding is surprising, considering that lesions encompassing only the TTA do not affect operant responding for natural rewards (Reilly
& Trifunovic, 1999). Rats with TOA lesions also yielded higher break points for sucrose in a progressive ratio task compared with sham-lesioned subjects, indicating increased motivation for sweet reward (Liang et al., 2012a). This disinhibition in the lesioned subjects suggests that the TOA may exert tonic inhibition on responding for sweet reward. While this appears to be the case, lesions of the TOA have no effect on the development of CTA when sham-fed corn oil or sucrose is paired with LiCl (Liang et al., 2012b). This structure, then, is not essential for associating such natural rewards with LiCl-induced illness. Finally, while lesions of the TTA completely prevent ACE for sucrose (Reilly et al., 2004; Schroy et al., 2005), rats with TOA lesions, that often encompass both taste and trigeminal thalamic relays, are capable of developing an ACE for disparate concentrations of sham-fed sucrose or corn oil (Liang et al., 2012c).

Together, these data suggest that the TOA is involved in the motivation to respond for a sweet reward. This increased motivation for a natural reward could rival the perceived rewarding value of a drug of abuse, and therefore the lesion may protect against the devaluation of such natural rewards.

**Experiment 1: Anticipatory Contrast**

In light of the results of Liang et al. (2012c), it was necessary to investigate the influence of the TOA on sucrose reward under natural feeding conditions. While sham-feeding is essential for studying the influence of oral sensation in the absence of post-ingestive feedback, it does have potential drawbacks. For example, the gastric fistula itself as well as daily flushing of the stomach could be stressful and present discomfort, which ultimately could alter learning behaviors. Additionally, leakage into the duodenum still may occur (Sclafani & Nissenbaum, 1985). Most importantly, the procedure is by no means natural feeding, even with the fistula closed. Given the unexpected role of the TOA in sucrose reward, Experiment 1 was designed to
verify that this structure is not involved in the development of a sucrose ACE under natural feeding conditions.

Methods

Subjects

The subjects were run in two batches that totaled 64 naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise.

Surgery

Following acclimation to the colony room, the subjects (at least 300 g) underwent either bilateral ibotenic acid lesions of the thalamic trigeminal orosensory area (TOAx) or vehicle infusions (Sham). Twenty min prior to anesthesia, all rats were injected with atropine sulfate (0.1 mg/rat, ip) and Bicillin (200,000 U/0.3ml, im). They were anesthetized with sodium pentobarbital (50 mg/kg, ip) and supplemented as necessary throughout surgery. Body temperature was maintained at 37+/−1 °C. The rat’s head was mounted in a stereotaxic instrument using non-traumatic earbars. The skin over the skull was cleaned with Betadine and opened with a mid-line incision. Using a 4 mm diameter trephine, two holes were drilled in the skull centered 3.0 mm posterior to β and 1.2 mm lateral of the midline on either side. The dura mater remained
intact and moist throughout surgery with physiological saline. The skull was leveled between β and λ and a search electrode (glass-insulated tungsten microelectrode; Z = 0.5 - 1.2 Mohms at 1 kHz) was lowered into the left or right gustatory thalamus. The taste area was located by recording neural activity while stimulating the anterior tongue with 0.3 M NaCl (approximate coordinates: - 3.8 mm posterior to β, +/- 1.2 mm lateral to the midsagittal suture, and 6.0 mm below dura). Once having located the gustatory thalamus, the search electrode was removed and the lesion micropipette lowered. The lesion was made using an ibotenic acid filled glass micropipette (diameter 20 - 24 µm at the tip) that was glued directly onto the needle of a 1.0 µl Hamilton microsyringe. The presence of taste neurons was confirmed by recording background taste responses with the glass micropipette. The micropipette was then raised and lowered again 500 microns lateral to the recorded area and 0.2 µl (20 µg/µl) of ibotenic acid was infused over 10 min. The pipette remained in situ for another 10 min. This same procedure was repeated on the contralateral side. After removal of the pipette, the holes in the skull were filled with Gelfoam and the wound closed with wound clips. The animals recovered over 2-3 days and body weight returned to presurgical levels within a week.

**Apparatus**

Rats were trained in one of six 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 chambers had a clear Plexiglas top, front, and back wall. Sidewalls were made of aluminum. The grid floors consisted of nineteen 4.8 mm stainless steel rods spaced 1.6 cm apart (center to center). Each chamber was equipped with two retractable sipper tubes that were advanced into the chamber through 1.3 cm diameter holes spaced 16.4 cm apart (center to center). A lickometer circuit was used to monitor licking. A shaded bulb, which reflected light from the ceiling, was
located to the right of the cage and a white noise speaker was on the left-end wall, opposite to the sipper tubes. Each chamber was housed in a light and sound attenuated cubicle that was fitted with a ventilation fan and a white noise source that provided a background noise level of 75 dB. Control of events in the chamber and collection of the data were carried out on-line using a computer and programs written in the Medstate notation language (MED Associates, Inc., St. Albans, VT).

**Solutions**

Sodium saccharin and sucrose (Sigma Chemical, St. Louis, MO) were dissolved in distilled water (dH$_2$O) and presented at room temperature.

**Procedure**

After they recovered from surgery (no less than one week), all rats were food-deprived to 90% of their free-feeding body weight, and then maintained by a once per day feeding. Subjects were divided into saccharin-saccharin (Sham: n=12; TOAx: n=18) and saccharin-sucrose groups (Sham: n=14; TOAx: n=20). They were then habituated to the chambers for 5 min a day for 3 days with the house light and white noise on. During testing, each rat was placed in the apparatus with the house light and white noise on. The first bottle (located on the left) advanced and the rat had 3 min access to 0.15% saccharin. Thereafter, the first bottle retracted and a second bottle advanced on the right for a 3 min access period. The second bottle contained either more 0.15% saccharin (saccharin-saccharin group) or 1.0 M sucrose (saccharin-sucrose group). The bottle was then retracted and the subject was immediately removed from the chamber. Daily feeding
occurred one hour after removal from the chambers. There was one such taste-taste pairing a day for 16 days and the latency to first lick and number of licks made on each bottle was recorded.

**Histology**

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and, once deeply anesthetized, perfused transcardially for 5 min with cold physiological saline plus heparin (1.5 U/ml), immediately followed by cold 4% paraformaldehyde for 20 min. The brains were removed and stored in paraformaldehyde for 2 h, then in a mixture of 20% sucrose + 0.1 M phosphate buffer overnight. They were frozen and sliced coronally in 50 µm sections. One series of alternate sections was stained for cell bodies with cresyl echt violet and the other for Neuronal Nuclei protein (NeuN). The adequacy of the lesions was judged by comparing the acellular areas in the lesioned subjects to that of the sham-lesioned controls. The boundaries of the gustatory and oral tactile nuclei have been defined from neuroanatomical and electrophysiological data (Kosar, Grill, & Norgren, 1986a, 1986b; Norgren, 1976; for reviews, see Norgren, 1984; 1995). Here and elsewhere, data from subjects found to have misplaced or incomplete lesions were excluded from further analysis.

**Statistics**

Data were analyzed using a mixed factorial analysis of variance (ANOVA) with lesion (Sham or TOAx) and unconditioned stimulus (US; saccharin or sucrose) as the between-subjects factors and 2-day blocks (1-8) as the within-subjects factor. Post hoc Newman-Keuls tests were conducted where appropriate. Data were considered statistically significant when p < .05.
Results

Histology

Sixty-four rats were tested in Experiment 1; data from five TOAx rats were excluded from statistical analysis due to incomplete lesions, and from one control subject that failed to establish consistent licking behavior. Typical lesions damaged the middle third of the VPM as well as the majority of the taste area (VPMpc). A few subjects had extensive damage to the VPMpc with lesions crossing the midline. Additionally, the lesions encompassed small parts of adjacent nuclei such as the paracentral nucleus (PC), centrolateral nucleus (CL), central medial nucleus (CM), parafascicular nucleus (Pf), and the posterior nucleus (Po) (see Figure 2-1).

Figure 2-1: Digital photomicrographs of coronal sections of Sham (A) and TOAx (B) stained with NeuN. Abbreviations: CL, centrolateral nucleus; CM, central medial nucleus; fr, fasciculus retroflexus; mt, mammillary tract; Pf, parafascicular nucleus; Po, posterior nucleus; TOA, trigeminal orosensory area; VPM, ventral posteromedial nucleus; VPMpc, parvocellular subdivision of the VPM (thalamic taste area).
**CS Intake (Licks/3 min)**

The results showed that in real-feeding rats, as in sham-feeding (Liang et al. (2012c), TOA lesions did not prevent the development of an ACE, i.e., sucrose-induced suppression of CS intake (see Figure 2-2). This conclusion was confirmed by a significant main effect of US, $F(1,54) = 24.59, p < .001$, indicating that all rats in the saccharin-sucrose condition made fewer licks for the saccharin cue than did the saccharin-saccharin controls overall. The US x block interaction also attained statistical significance, $F(7,378) = 19.64, p < .001$, and post hoc tests revealed that anticipation of the availability of sucrose suppressed intake of the saccharin cue for all rats (Sham and TOAx combined), beginning with the 4th block ($p < .05$). There was no significant lesion x US interaction, $F(1,54) = 2.08, p = .16$. Nevertheless, while contrast was significant for both the Sham and the TOAx rats, post hoc analyses indicated higher CS intake in the TOAx saccharin-sucrose rats compared with those in the Sham saccharin-sucrose group ($p < .05$), suggesting a reduced anticipatory contrast effect for the lesioned rats. Finally, while the main effect of lesion was significant, $F(1,54) = 4.08, p < .05$ (TOAx rats consumed more of the first bottle solution overall), the lesion x US x block interaction did not attain statistical significance, $F(7,378) = 1.45, p = .21$.

In the Liang et al. (2012c) paper, the ACE for sucrose was slower to develop in the sham-feeding TOAx rats. Thus, we conducted separate follow up analyses on the Sham and the TOAx rats. Analysis of the Sham rats revealed a significant US x block interaction, $F(7,161) = 17.29, p < .001$, and post hoc tests confirmed lower CS intake in the saccharin-sucrose group relative to the saccharin-saccharin controls, beginning with the 2nd block, $p < .05$. A similar analysis for the TOAx rats also revealed a significant US x block interaction, $F(7,217) = 8.08, p < .001$. Post hoc tests, however, evidenced a significant ACE for rats in the saccharin-sucrose group only for blocks 5 through 8, $p < .05$. Rats with the TOA lesion, then, clearly can acquire the sucrose
ACE in the lick frequency measure, but with some delay. This pattern of data is unlike that obtained in rats with lesions centered on the TTA who are fully incapable of acquiring an ACE using identical testing parameters (Schroy et al., 2005).

![Graph showing CS intake (Licks/3 min) for Sham and TOAx conditions](image)

**Figure 2-2:** Mean 3-min intake (± SEM) of 0.15% saccharin in Sham (left) or TOAx (right) rats paired with 0.15% saccharin (open circles) or 1 M sucrose US (solid circles) for 16 CS-US pairings represented as 2-day blocks. *indicates a significant difference between the Sac-Sac and Sac-Sucrose conditions (p < .05). Sham: Sac-Sac N=11, Sac-Sucrose N=14; TOAx: Sac-Sac N=15, Sac-Sucrose N=18

**CS Latency**

The results of the 3-way ANOVA indicate the Sham, but not the TOAx rats, exhibited a significant contrast effect in latency by taking longer to initiate licking for the saccharin cue when it predicted access to the preferred sucrose US than when it predicted access to more saccharin (see Figure 2-3). This was evidenced by a significant lesion x US x block interaction, F(7,378) =
2.32, p < .05, as well as a significant US x block interaction, F(7,378) = 2.51, p < .05. Post hoc analyses of the significant 3-way interaction showed reliable contrast in the latency to initiate licking of the sucrose-paired saccharin CS by the sixth and eighth blocks in the Sham subjects relative to their saccharin-saccharin controls, ps < .05. No significant differences between the saccharin-saccharin and saccharin-sucrose groups were found for the TOAx rats, ps < .05.

Figure 2-3: Latency to initiate licking of 0.15% saccharin CS (± SEM) in Sham (left) or TOAx (right) rats when the CS was paired with 0.15% saccharin (open circles) or 1 M sucrose US (solid circles) for 16 CS-US pairings represented as 2-day blocks. *indicates a significant difference between the Sac-Sac and Sac-Sucrose conditions (p < .05). Sham: Sac-Sac N=11, Sac-Sucrose N=14; TOAx: Sac-Sac N=15, Sac-Sucrose N=18
**US Intake (Licks/3 min)**

Both the Sham and the TOAx rats exhibited a magnitude of reinforcement effect by making more licks for second bottle sucrose than for second bottle saccharin (see Figure 2-4). This observation was supported by the absence of a significant 3-way lesion x US x block interaction, $F < 1$, and a significant main effect of US, $F(1,54) = 190.04$, $p < .001$. The block x US interaction also was significant, $F(7,378) = 22.92$, $p < .001$. Post hoc analyses indicated lower intake of the saccharin US compared with the sucrose US for both Sham and TOAx subjects across all 2-day blocks, $p < .001$.  

![Figure 2-4](image)

**Figure 2-4:** Mean 3-min US intake (± SEM) in Sham (left) or TOAx (right) rats for 0.15% saccharin (open circles) or 1 M sucrose US (solid circles) for 16 CS-US pairings represented as 2-day blocks. *indicates a significant difference between the Sac-Sac and Sac-Sucrose conditions ($p < .05$). Sham: Sac-Sac $N=11$, Sac-Sucrose $N=14$; TOAx: Sac-Sac $N=15$, Sac-Sucrose $N=18$
**US Latency**

Likewise, all rats, regardless of lesion condition, initiated licking faster for second bottle sucrose than for second bottle saccharin (see Figure 2-5). This conclusion is supported by the absence of a significant lesion x US x block interaction, $F < 1$, and a significant main effect of US, $F(1,54) = 43.12$, $p < .001$. The main effect of block, $F(7,378) = 15.96$, $p < .001$, also was significant, showing that the latency to initiate licking decreased over successive trials, overall. Post hoc analyses indicated a significant difference between saccharin-saccharin and saccharin-sucrose groups for all rats beginning at block 2, $ps < .05$.

**Figure 2-5**: Latency to initiate licking of US ($\pm$ SEM) in Sham (left) or TOAx (right) rats for 0.15% saccharin (open circles) or 1 M sucrose US (solid circles) for 16 CS-US pairings represented as 2-day blocks. *indicates a significant difference between the Sac-Sac and Sac-Sucrose conditions ($p < .05$). Sham: Sac-Sac $N=11$, Sac-Sucrose $N=14$; TOAx: Sac-Sac $N=15$, Sac-Sucrose $N=18$
Discussion

The results indicate that all rats, Sham and TOAx, made fewer licks for the first bottle saccharin solution when it predicted subsequent access to the preferred sucrose solution than when it predicted more access to the same saccharin solution. In addition, all subjects exhibited a normal magnitude of reward effect by initiating licking for, and making more licks of, second bottle sucrose than second bottle saccharin. The failure of the TOA lesion to completely eliminate ACE in the Liang et al. (2012c) study then was not due to the use of the sham-feeding regimen. As such, the TOA is not essential for the development of an ACE (i.e., sucrose-induced suppression of CS intake). That said, the lesion was not without effect. That is, while the ACE developed in first bottle licks for the TOAx rats, it was delayed relative to the sham-lesioned subjects. Moreover, contrast also was evidenced in the Sham rats in the latency to lick the saccharin cue. Rats with lesions of the TOA, on the other hand, failed to exhibit contrast on this measure at all. TOAx rats initiated licking for first bottle saccharin very quickly, regardless of whether it predicted subsequent access to more saccharin or the preferred sucrose solution.

The delayed acquisition of ACE in the TOAx rats mirrored that seen in the Liang et al. (2012c) study. A number of hypotheses might be put forth to account for this effect. First, a reduced neophobic response by the TOAx rats may have contributed to delayed acquisition of the ACE. Neophobia is a common behavior in rats, where the animal will consume only small amounts of a novel food upon first exposure. In support, intake of the saccharin cue in the first 2-day block is higher in the lesioned group (see Figure 2-2), and while not a true test of neophobia, may suggest a reduced neophobic response in the lesioned animals. Counter to this argument, however, the same TOAx rats drank less, not more, of the 1.0 M sucrose solution upon the first exposure when presented in bottle 2. A second hypothesis is that the delay in acquisition of the ACE is the result of an increase in motivation. The TOA lesioned rat may simply be more
motivated, and therefore unwilling to wait for the preferred reward. In support, the TOAx rats not only failed to suppress intake of the taste cue, but also were quicker to initiate licking the taste cue than the sham-lesioned rats. In addition, in the Liang et al. (2012a) study, TOAx rats showed increased operant responding for sucrose when compared with Sham subjects, indicating higher motivation among the TOAx group. While possible, this account also seems unlikely because the TOAx rats were no faster (or slower) to initiate licking second bottle saccharin or sucrose relative to the sham-lesioned controls. Finally, it is possible that these lesioned subjects are slower to associate a CS with a US. In the Liang et al. (2012c) study, however, similarly lesioned rats readily learned to associate a gustatory CS with LiCl-induced malaise in a CTA paradigm. It is possible, then, that the lesion may have a small, but uniform effect on the comparison of disparate rewards.

**Experiment 2: Avoidance of a Morphine-Paired Taste Cue**

As mentioned, rats will readily learn to suppress intake of a taste cue that is paired with a drug of abuse (Cappell & LeBlanc, 1971; Cappell et al., 1973; Glowa et al., 1994; Grigson, Lyuboslavsky, & Tanase, 2000; Miller et al., 1990; Sherman et al., 1980). In previous studies, drug-induced suppression of CS intake was eliminated in rats with bilateral lesions focused on the gustatory thalamus (Grigson, Lyuboslavsky, & Tanase, 2000). Drug- and sucrose-induced suppression of CS intake are similarly affected by lesions of the gustatory thalamus (Grigson, Lyuboslavsky, & Tanase, 2000; Reilly et al., 2004; Schroy et al., 2005) or cortex (Geddes et al., 2008; Mackey, Keller, & Van der Kooy, 1986; Zito, Bechara, Greenwood, & Van der Kooy, 1988). Because drug- and sucrose-induced suppression of CS intake have been similarly affected by all previous manipulations, it was expected, based on the successful suppression of the sucrose-paired CS in Experiment 1, that the TOA lesion would have little or no effect on the
development of drug-induced suppression of intake of a gustatory cue. Experiment 2 used morphine to test this hypothesis.

**Methods**

**Subjects**

The subjects that served in Experiment 1 were used in Experiment 2 in a partial cross over design where half of each group (saccharin-saccharin or saccharin-sucrose) was assigned to one of two conditions (saline or morphine). They were returned to their free-feeding body weight, housed, and maintained as described in Experiment 1. Testing was conducted during the light phase of the cycle. Food and water were freely available, except where noted below.

**Apparatus**

The experiment was conducted in the home cages. Fluid was presented in inverted graduated Nalgene cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Intake was measured to the nearest 0.5 ml.

**Drugs and Solutions**

Morphine sulfate was provided by the National Institute on Drug Abuse (Bethesda, MD) and was prepared in sterile saline immediately before testing. Polycose (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.
Procedure

Once recovered to their free-feeding body weight, the rats were placed on a water deprivation schedule that allowed for 5 min of access to dH2O in the morning and 1 h each afternoon. Following stabilization of morning water intake (approximately 9 days), rats were matched for 5 min water intake and history. Thus, half of the rats from the saccharin-saccharin condition, and half from the saccharin-sucrose condition in Experiment 1 served in each drug treatment group in Experiment 2: Saline (Sham: n=12; TOAx: n=18) and Morphine (Sham: n=14; TOAx: n=20) groups. All rats were given 5 min access to a 0.03 M Polycose CS, and after a 5 min interval, were injected intraperitoneally with either saline or a 15 mg/kg dose of morphine. There were six such taste-drug pairings occurring at 48 h intervals. To maintain proper hydration, dH2O continued to be provided for 5 min on the days between injections and for 1 h every afternoon.

Statistics

Data were analyzed using a mixed factorial ANOVA with lesion (Sham or TOAx) and US (saline or morphine) as the between-subjects factors and trial (1-6) as the within-subjects factor. Post hoc Newman-Keuls tests were conducted where appropriate. Data were considered statistically significant when p < .05.
Results and Discussion

Histology

Sixty-four rats were tested in Experiment 2; as mentioned in Experiment 1, data from five TOAx rats were excluded from statistical analysis due to incomplete lesions. Additionally, data were excluded from five other TOAx subjects that failed to either regain free-fed body weight or had dental occlusion and could not complete the study.

CS Intake (ml/5 min)

Relative to the saline treated controls, rats with sham lesions suppressed intake of the Polycose CS following Polycose-morphine pairings. This same pattern was not evident in the lesioned group (see Figure 2-6). Polycose intake was analyzed using a mixed factorial ANOVA with lesion (Sham or TOAx) and US (saline or morphine) as the between-subjects factors and trial (1-6) as the within-subjects factor, followed by Newman-Keuls post hoc tests. There was a significant lesion x US x trial interaction, F(5,250) = 3.89, p < .01, and a significant lesion x US interaction, F(1,50) = 8.24, p < .01. Post hoc analyses indicated significant differences between the Sham saline and Sham morphine groups, beginning with trial 2, ps < .05, as well between the Sham morphine group and both the TOAx saline and TOAx morphine groups, also beginning with trial 2, ps < .05. No significant differences were found between the TOAx saline and TOAx morphine groups, ps > .05.
This is the first intervention found to differentially influence suppression of intake of a taste cue when paired with a drug of abuse versus that which occurs when a similar taste cue is paired with a naturally rewarding sucrose solution. As mentioned, using similar parameters, these two paradigms have been strikingly parallel, as they have been found to be similarly affected by strain (Glowa et al., 1994; Grigson & Freet, 2000; for a discussion, see Grigson et al., 2001; but also see Riley, 2011), deprivation state (Bell et al., 1998; Flaherty et al., 1991; Gomez & Grigson, 1999; Grigson et al., 1999), as well as lesions of the gustatory thalamus (Flynn et al., 1991; Grigson, Lyuboslavsky, & Tanase, 2000; Reilly et al., 2004; Reilly & Pritchard, 1996b; Reilly & Trifunovic, 1999; Schroy et al., 2005) or cortex (Geddes et al., 2008). It is possible that, as in the
ACE study, TOAx rats are just slower to suppress, and that an effect would have developed with more trials. While this is possible, it is unlikely as there was no evidence of suppression of CS intake by the TOAx rats following five pairings and one test. Moreover, in an unpublished study with the rats from the Liang et al. studies, TOAx rats failed to suppress intake of a Polycose CS following nine pairings with morphine. It is important to note that the number of trials typically required to attain significant suppression of a sucrose-paired taste cue in the anticipatory contrast paradigm is around eight pairings (or four 2-day blocks)(Schroy et al., 2005), whereas suppression of a drug-paired taste cue can be observed following a single taste-drug pairing (Grigson, Lyuboslavsky, & Tanase, 2000). The lack of suppression, therefore, is not likely to reflect slow learning but, rather, a complete attenuation of morphine-induced suppression of CS intake using the present parameters.

**Experiment 3: Avoidance of a Cocaine-Paired Taste Cue**

Mu opioid receptors are densely concentrated throughout the area of the thalamus targeted by the ibotenic acid lesion (Mansour, Khachaturian, Lewis, Akil, & Wartson, 1987). For this reason, it is possible that the results obtained in Experiment 2 were due to the lesion interfering with the brain’s ability to detect and process the opiate. To test the merits of this hypothesis, we employed a different class of addictive drug, namely the stimulant, cocaine. Since cocaine’s mechanism of action is modulated by, but not dependent upon, mu opioid receptor activation (Becker et al., 2002; Lesscher et al., 2005), it is possible to determine whether the effect of the TOA lesion is opiate specific or whether the lesion can interfere with a more general drug reward mechanism.
Methods

Subjects

The subjects were 36 naive, male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. The rats were housed and underwent lesion surgery as described in Experiment 1. Testing was conducted during the light phase of the cycle.

Apparatus

The apparatus was the same as in Experiment 2.

Drugs and Solutions

Cocaine hydrochloride was provided by the National Institute on Drug Abuse (Bethesda, MD). The 10 mg/kg dose of cocaine was prepared in sterile saline and administered subcutaneously as a 1.5 mg/ml stock solution, adjusted by body weight to avoid necrosis (Durazzo, Gauvin, Goulden, Briscoe, & Holloway, 1994). Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.

Procedure

The rats were placed on a water deprivation schedule that allowed for 5 min of access to dH₂O in the morning and 1 h each afternoon. Following stabilization of morning water intake (approximately 9 days), all rats were given 5 min access to a 0.15% saccharin CS, and after a 5 min interval, were injected subcutaneously with either saline or a 10 mg/kg dose of cocaine as
described. There were five such taste-drug pairings occurring at 48 h intervals. To maintain proper hydration, dH₂O was provided for 5 min each morning between conditioning trials and for 1 h every afternoon.

**Histology**

Histology was completed as described in Experiment 1.

**Statistics**

Data were analyzed using a mixed factorial ANOVA with lesion (Sham or TOAx) and US (saline or cocaine) as the between-subjects factors and trial (1-5) as the within-subjects factor. Post hoc Newman-Keuls tests were conducted where appropriate. Data were considered statistically significant when p < .05.

**Results and Discussion**

**Histology**

Of the 36 rats in Experiment 3, three died during surgery and data from an additional three subjects were removed due to incomplete lesions. The following data include 16 subjects with bilateral ibotenic acid lesions of the trigeminal thalamic orosensory area (TOAx), and 14 with vehicle infusions (Sham). As mentioned, lesions damaged the middle third of the VPM as well as the majority of the taste area in the VPMpc. Subjects that failed to meet these criteria were not included in the analyses.
Saccharin intake was analyzed using a mixed factorial ANOVA with lesion (Sham or TOAx) and US (saline or cocaine) as the between-subjects factors and trial (1-5) as the within-subjects factor, followed by Newman-Keuls post hoc tests. As was the case when the taste cue predicted an injection of morphine, the TOA lesions interfered with cocaine-induced suppression of intake of the saccharin taste cue (see Figure 2-7). This conclusion was supported by a significant lesion x US x trial interaction, $F(4,108) = 9.00$, $p < .001$, and a significant lesion x US interaction, $F(1,27) = 33.78$, $p < .001$. Post hoc tests of this significant 3-way ANOVA found that this effect was significant for the Sham group on the second through fifth trial ($ps < .001$). In the TOAx group, however, CS intake in the saline condition did not significantly differ from that in the cocaine condition on any trial ($ps > .05$), supporting the conclusion that the lesion interfered with suppression of intake of the taste cue. Furthermore, post hoc analyses indicated a significant difference in CS intake between the TOAx and Sham groups on the first trial, $p < .05$. Intake on the first trial was higher in the TOAx subjects, indicating an apparent reduced neophobic response to the CS among lesioned rats.
Figure 2-7: Mean 5-min intake (± SEM) of 0.15% saccharin in Sham (left) or TOAx (right) rats injected subcutaneously with saline (open circles) or cocaine hydrochloride (10 mg/kg; solid circles) for a total of 5 taste-drug pairings. *indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=7, Cocaine N=7; TOAx: Saline N=10, Cocaine N=6

The results show that bilateral TOA lesions interfere not only with morphine-induced suppression of CS intake, but also with cocaine-induced suppression of CS intake as well. As with the morphine experiment, the TOAx rats had an increased affinity for sweet reward, and may be unable to compare a natural gustatory reward with a drug reward. As in Experiment 1 (where saccharin served as the CS), the TOAx group appeared to lack a neophobic response to the novel saccharin taste cue and a failure to evidence neophobia has been posited as the cause for the disruptive effect of the thalamic taste area lesion (Lin, Arthurs, & Reilly, 2011). That said, reduced neophobia does not account for these results. The TOAx group showed reduced neophobia to a saccharin CS in Experiments 1 and 3, when paired with either sucrose or cocaine,
respectively. However, after several pairings, the TOAx rats suppressed intake of the saccharin CS when it predicted sucrose, but failed to avoid the CS when it predicted cocaine. Furthermore, neither the Sham nor TOAx rats exhibited neophobia in Experiment 2 when a Polycose CS predicted morphine, yet the TOAx rats failed to avoid the CS after several pairings. Therefore, while a reduced neophobic response to the CS is a notable effect of the TOA lesion, as has been found with lesions of the gustatory pathway (Lin et al., 2011); the determining factor here is not the CS, or a neophobic response to the CS, but rather the US.

**Experiment 4: Conditioned Taste Aversion**

As described above, CTA is a paradigm that also suppresses intake of a taste cue. Traditionally, a CTA occurs by pairing a taste cue with an illness-inducing agent such as LiCl or x-radiation (Garcia et al., 1955; Nachman, 1963; Smith, 1971). Unlike sweets and drugs of abuse, evidence suggests that LiCl is highly and uniformly aversive. For example, rats avoid a place associated with LiCl injections yet show preference for a place associated with a palatable sucrose solution or drugs of abuse (Bardo et al., 1984; Blander, et al, 1984; Katz & Gormezano, 1979; Reilly et al., 1993; White & Carr, 1985). Additionally, rats have increased operant responding for sucrose or drug, but not for LiCl (Guttman, 1953; Hajnal, Acharya, Grigson, Covasa, & Twining, 2007; Liang et al., 2012a; White et al., 1977; Wise et al., 1976). Even so, like ACE learning, CTA learning also depends upon identifying a CS and associating this CS with the consequences of the US. As previously mentioned, the Liang et al. (2012b) study found CTA development to be intact in sham-fed TOAx rats following three pairings of either 0.3 M sucrose or 100% corn oil with LiCl. To ensure there were no differences in real-fed rats, a subset of the subjects from Experiments 1 and 2 underwent pairings of a novel CS and the illness-inducing agent, LiCl. Because of the robust nature of the response to LiCl-induced visceral
malaise, prior experience should not affect suppression in this paradigm; however, subjects were assigned to each condition in a counterbalanced fashion to avoid generalization from previous studies. Furthermore, a novel stimulus was selected as the CS to avoid generalization between studies.

**Methods**

**Subjects**

Following Experiments 1 and 2, a subset of 32 subjects was allowed several weeks to recover before water training began. The subjects were again divided into control and experimental conditions in a counterbalanced fashion based on lesion and the previous assignments in Experiments 1 and 2. Testing was conducted during the light phase of the cycle.

**Apparatus**

The rats were housed as described in Experiment 1 and the apparatus (home cage testing) was the same as that described in Experiment 2.

**Drugs and Solutions**

Lithium chloride (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O immediately before testing. Sodium chloride (NaCl) (Sigma Chemical, St. Louis, MO) served as the gustatory CS and was prepared in dH₂O and presented at room temperature.
Procedure

The rats were placed on a water deprivation schedule that allowed for 5 min of access to dH2O in the morning and 1 h each afternoon. Once 5 min intake stabilized, subjects were divided into saline (Sham: n=8; TOAx: n=8) and LiCl (Sham: n=8; TOAx: n=8) groups. All rats were given 5 min access to 0.1 M NaCl (CS), followed 5 min later by an intraperitoneal injection of either saline or increasing concentrations of LiCl over trials. We intended to use our standard “low” concentration of 0.009 M LiCl, injected at a volume of 1.33 ml/100g body weight (5.07 mg/kg body weight) because the suppressive effects of this dose have been matched to those of a 15 mg/kg dose of morphine and a 10 mg/kg dose of cocaine (Grigson, 1997). Instead, the volume injected was 1 ml/kg body weight, which resulted in a much lower dose than anticipated. The concentration was gradually increased until an effective dose was established as follows: 0.009 M (0.38 mg/kg body weight) for the first 4 pairings, 0.018 M (0.76 mg/kg body weight) for pairings 5 and 6, 0.0375 M (1.59 mg/kg body weight) for pairings 7 and 8, and the final 4 pairings were 0.15 M (6.36 mg/kg body weight). Due to the volume of the injection, this final dose was only slightly higher than our standard low 0.009 M dose injected at a volume of 1.33 ml/100 g body weight. There was one such taste-drug pairing a day with 48 h between pairings for 11 trials, followed by one CS only test day. NaCl intake was recorded. To maintain proper hydration, dH2O was provided for 5 min each morning between conditioning trials and for 1 h each afternoon.
Statistics

Data were analyzed using a two-way ANOVA with lesion (Sham or TOAx) and US (saline or LiCl) as between-subjects factors. Data were considered statistically significant when p < .05.

Results and Discussion

CS Intake (ml/5 min)

The results verify those found in sham-fed TOAx rats in the Liang et al. (2012b) study. Thus, on the final day of testing, both the Sham and the TOAx rats suppressed intake of the LiCl-paired taste cue, indicating that the TOA lesion did not prevent a LiCl-induced conditioned taste aversion (see Figure 2-8). Support for this conclusion was provided by a non-significant lesion x US interaction, F(1,28) = 1.31, p = .26, but a significant main effect of US, F(1,28) = 31.75, p < .001. The main effect of lesion was significant on the test day, F(1,28) = 5.15, p < .05, indicating lower overall intake in the TOAx saline group compared with the Sham saline group.
The results indicate that the TOA lesions did not interfere with LiCl-induced conditioned taste aversion. As described, this also was the case in the TOAx rats in the Liang et al. (2012b) study following three pairings of 0.3 M sucrose or 100% corn oil with a potent 0.15 M (1.33ml/100g body weight, ip) LiCl. Therefore, we can assume that the TOAx rats also have a normal sensitivity to LiCl across a range of doses.

**General Discussion**

The results from Experiment 1 replicate and extend those obtained by Liang et al. (2012c) by showing that the TOA lesion did not block avoidance of a lesser-valued taste cue that predicted access to a highly palatable sucrose solution under real-feeding conditions. As such,
the lesioned subjects were able to detect the taste cue, associate the taste cue with the US, compare the available reward with the anticipated reward, and respond accordingly by suppressing intake of the lesser-valued taste cue. That said, as a group, the TOA lesioned rats did not develop an anticipatory contrast effect as fast as the sham-operated controls. In addition to contrast in lick frequency, the Sham group also exhibited contrast in latency as evidenced by an increase in the latency to lick the sucrose-paired saccharin cue. The TOAx group, however, did not develop contrast in the latency to lick the sucrose-paired cue. The lesion, then, disrupts, but does not eliminate, the development of an anticipatory contrast effect. Also consistent with the findings of Liang et al. (2012b) in sham-feeding rats, the TOA lesion had no effect on the LiCl-induced CTA when assessed in real-feeding rats in Experiment 4. Finally, in Experiments 2 and 3, the same TOA lesion fully prevented suppression of intake of a taste cue that was paired with morphine or cocaine. The TOA lesion is, then, the first manipulation in which performance in one paradigm is intact, albeit slightly delayed (anticipatory contrast), while performance in the other is eliminated (drug-mediated avoidance of the taste cue), and this is the case with both morphine and cocaine.

In previous studies, lesions focused on the VPMpc eliminated avoidance of a positive taste cue when paired with a naturally rewarding sucrose solution or a drug of abuse, but had no effect on the development of a LiCl-induced conditioned taste aversion (Flynn et al., 1991; Grigson, Lyuboslavsky, & Tanase, 2000; Reilly et al., 2004; Reilly & Pritchard, 1996b; Reilly & Trifunovic, 1999; Scalera et al., 1997; Schroy et al., 2005). Bilateral lesions of the insular cortex also disrupted the suppressive effect of a drug of abuse, but not those induced by LiCl (Geddes et al., 2008; Mackey et al., 1986). As described, lesions centered on the slightly lateral TOA, on the other hand, spared contrast effects involving the comparison of either gustatory (i.e., saccharin vs. sucrose) or trigeminal (i.e., corn oil) rewards (current data and Liang et al., 2012c), but blocked avoidance of a taste cue when paired with a drug of abuse.
This seems straightforward enough, except that the TOA lesion usually includes much or all of the VPMpc. Indeed, our histological analysis suggests that subjects with greater damage to the gustatory thalamus may, in fact, be slower to learn the ACE and have lower overall intake compared with rats with more sparing of the area. We conclude that sparing of ACE relates, at least in part, to the degree of VPMpc sparing. We also can infer that the slightly different structures in the thalamus serve discrete functions related to the comparison of, and responding to, disparate rewards over time. An intact VPMpc is required for avoidance of a lesser-valued saccharin cue when paired with sucrose or when paired with a drug of abuse such as morphine or cocaine. The TOA, on the other hand, is involved predominantly when avoiding a taste cue that predicts the availability of drug.

The most parsimonious explanation is that ACE is accomplished in TOAx rats by some sparing of cells in the VPMpc. Thus, in moving the lesion 500 microns laterally, enough cells may have been spared to enable the comparison of two disparate natural rewards (anticipatory contrast), yet still attenuate the comparison of a natural reward with a drug of abuse. In support, a few TOAx subjects had extensive damage crossing the midline. These subjects seemed to require more pairings before suppressing CS intake in the anticipatory contrast paradigm compared with other lesioned subjects. In the taste-drug pairings, however, when the taste cue predicted morphine or cocaine, lesioned rats failed to suppress intake of the drug-paired taste cue, suggesting that, unlike the ACE paradigm, the magnitude of effect is not influenced by the extent to which the lesion encroaches on the VPMpc. Of course, one might wonder how these rats with damage crossing the midline could acquire an ACE at all. Is the gustatory thalamic taste area (VPMpc) really the key thalamic nucleus for ACE? This determination will require further testing. For now, it is at least clear that ACE was spared when lesion damage was limited more to the TOA.
Understanding the deficits that result from the TOA lesion can help identify the function of this area. One possibility is that the TOA plays a specific role in developing an association between the taste cue and the motivational properties of drug. The areas damaged in the TOA lesion provide motivational input via direct projections to the caudate-putamen and anterior cingulate cortex (Iwata et al., 2011). Affective and motivational aspects are mediated by connections with the reticular formation in the midbrain as well as midline thalamic nuclei, which project to the cingulate and insular cortices (Iwata et al., 2011; Shi & Cassell, 1998). Therefore, it is possible that the lesion interferes with the association of motivational and rewarding aspects of abused drugs.

The disruptive effect of the TOA lesion also may be due to interference with descending corticothalamic input. For example, projections from the dysgranular anterior insular cortex terminate in the areas specifically damaged in the TOA lesion, including the VPM, VPMpc, CM, Po, PC, and Pf (Shi & Cassell, 1998). It is possible that the effects of the TOA lesion are a result of disconnection of this cortical circuit. Because the insular cortex has a direct contribution to morphine antinociception (Burkey, Carstens, Wenniger, Tang, & Jasmin, 1996), and is involved in cocaine-seeking behaviors (Kufahl et al., 2009), it is possible that disconnection of the corticothalamic fibers may have contributed to the failure to avoid a taste cue following pairings with a drug of abuse. Indeed, asymmetric lesions of the gustatory thalamus and insular cortex also disrupt avoidance of a taste cue when paired with either morphine or cocaine (Geddes et al., in preparation). Lesions of the gustatory cortex also disrupt the establishment of an anticipatory contrast effect following saccharin-sucrose pairings as well (Geddes et al., submitted).

Furthermore, as explored here, avoidance of the drug-paired taste cue appears to be unlike avoidance of a LiCl-paired taste cue. As with other manipulations (Bell et al., 1998; Geddes et al., 2008; Gomez & Grigson, 1999; Grigson & Freet, 2000; Grigson, Lyuboslavsky, & Tanase, 2000; Grigson et al., 1999; Grigson, Wheeler, Wheeler, & Ballard, 2001), lesions of the
TOA prevented drug- but not LiCl-induced suppression of CS intake. The suppressive effects of a drug of abuse, then, appear to have little to do with aversive properties of the drug itself (as is the case with LiCl). In this case, and as mentioned in the Introduction, we believe avoidance of the cue is due, in part, to devaluation of the taste cue in anticipation of drug availability. Unlike sucrose, however, the drug of abuse has an unnatural and potent impact on the CNS. Consequently, with experience, the cue comes to elicit the onset of a conditioned opponent process (Solomon & Corbit, 1974) and this opponent process appears highly aversive, possibly involving the onset of craving and withdrawal. In accordance, and as mentioned above, ingestion of a drug paired taste cue, like naloxone supported conditioned withdrawal (McDonald et al., 1997; Nunez et al., 2007; Shaham & Stewart, 1995), involves a conditioned elevation of circulating corticosterone (Gomez et al., 2000), reduced accumbens dopamine (Grigson & Hajnal, 2007; Wheeler et al., 2011), and, when intraorally infused, the onset of aversive taste reactivity behavior (Wheeler et al., 2008). Such an aversive conditioned state is also seen in addicted humans who are reported to show negative affect when having to wait for access to nicotine in the presence of drug-related cues (Sayette et al., 2003). Negative affect is a potent precipitator of relapse (Sinha et al., 2009). Likewise, in the rodent model, both avoidance of the taste cue and the onset of aversive taste reactivity behavior predict the latency to take drug, the length of the load up period, and the speed with which rats will acquire cocaine self-administration behavior (Wheeler et al., 2008). Therefore, unlike LiCl, cues that predict drugs of abuse can lead to a conditioned aversive state involving craving and withdrawal (Becker et al., 2010; McDonald et al., 1997, see Grigson, 2008, for a review). The TOA, then, may be involved in the development and/or expression of this more complex process. We intend to test this directly in Chapter 3.

As this is the first manipulation to yield differing results in the ACE test and suppression of CS intake when paired with a drug of abuse, parametric differences between these two paradigms may contribute to the selective effect of the TOA lesion on drug-induced suppression
of CS intake. There are a number of parallels between avoidance of a CS when paired with sucrose and when paired with a drug of abuse (Gomez & Grigson, 1999; Grigson & Freet, 2000; Grigson, Wheeler, Wheeler, & Ballard, 2001). Importantly, there also are a number of differences between these two paradigms such as the deprivation state, length of access to the CS, length of the interval between the CS and US, location of testing, number of CS-US pairings, and route of administration of the US. Understanding the influence of each of these factors in relation to the lesion is important in parsing the role of the TOA in the processing of drug-related cues.

The deprivation state of the rat differed between the anticipatory contrast and taste-drug pairings. In the anticipatory contrast paradigm, the subjects were food deprived in an effort to increase the hedonic value of the CS and US. Under these conditions, the TOA is not necessary for ACE with either corn oil (Liang et al., 2012c) or saccharin-sucrose pairings during real or sham-feeding conditions. In the studies where the CS is paired with a drug of abuse, the subjects were water-deprived to facilitate approach and consumption of the CS within the 5 min time period. While the TOA did prove essential for this paradigm, it is unlikely the result of the deprivation state, as the rats also were water deprived when the CS was paired with LiCl and the lesion was without effect on the development of the LiCl-induced CTA. Thus, it is unlikely that different deprivation states affected the behavior.

Another difference between the two paradigms is the length of CS access. Rats are allowed 3 min access to the CS in the ACE paradigm and 5 min access in the drug paradigm. In rats with lesions of the insular cortex, Lin et al. (2011) found moderate drug-induced suppression of CS intake with a 15 min compared with a 5 min access period. This suggests a ceiling effect may have prevented our ability to observe the drug-induced suppression of CS intake during the shorter access test. In the present study, however, the TOAx rats evidenced clear and reliable suppression of intake in the ACE paradigm with only 3 min access to the saccharin CS and, in the Liang et al. (2012c) study, to a corn oil CS. If the length of CS access were the mediating factor,
one would expect more suppression in a 5 min compared with a 3 min access, not less. Furthermore, a 5 min access period was also used in the CTA study and the lesion did not prevent avoidance of the LiCl-paired CS. Therefore, the length of the access period is unlikely to account for the dissociation. Nevertheless, further studies will be needed to determine whether a 15 min CS access period will reduce the disruptive effect of the TOA lesion because the information provided by the cue, and the development of the opponent process, is known to change with the access interval (Wheeler et al., 2008; Wheeler et al., 2011).

In addition to the different access times, the interval between the CS and the US differs between the two paradigms. There is no CS-US inter-stimulus interval (ISI) for the ACE paradigm, while subjects waited 5 min before receiving drug in the taste-drug pairings. This may potentially lead to behavioral changes as the 5 min wait may interfere with associating the CS and the US in the lesioned rats, or with the expression of the association in behavior (i.e., avoidance of the saccharin cue when waiting for access to drug or sweet). A simple associative deficit is unlikely, however, because there is also a 5 min ISI in the LiCl-induced CTA paradigm. Unlike LiCl, however, cues that predict drugs of abuse can lead to a conditioned aversive state involving craving and withdrawal (Becker et al., 2010; McDonald et al., 1997). The role of the ISI will be tested directly in Chapter 6. According to Wheeler et al. (2011), when an intraorally infused taste cue predicts delayed access to drug, the CS elicits aversive responses such as decreased accumbens dopamine and aversive orofacial responses. Yet when the CS is delivered at the same time as drug (Wheeler et al., 2011), there is no aversive taste reactivity and there is an increase in accumbens dopamine in response to the CS. Therefore, the avoidance of the CS when paired with drugs of abuse may very likely be the result of the onset of conditioned withdrawal in anticipation of impending drug availability. The TOA, then, may be involved in the development and/or expression of this more complex process.
Another significant difference between the ACE and drug comparison paradigms is the location in which the testing occurs. In this and the Liang et al. (2012c) study, the ACE paradigm took place in an operant chamber, which can provide many additional contextual cues compared with the home cage setting that was used for the taste-drug pairings. While the LiCl-induced CTA also was conducted in the home cage, conditioned taste aversions and drug-induced suppression of CS intake have little in common (Grigson, 1997). The test chamber may provide additional contextual cues that support ACE learning. Thus, it is possible that the lesion would effectively prevent ACE learning if tested in the home cage. The influence of the test chamber cannot be ruled out without further testing, and thus will be the topic of Chapter 5.

The number of CS-US pairings differs between the saccharin-sucrose and the taste-drug pairings. This is the case because learning typically occurs more slowly when using a natural reward as the US than when using a drug of abuse or an aversive agent. Furthermore, we began to see avoidance of the CS in the TOAx group early in the ACE paradigm, with some subjects learning faster than others. When morphine and cocaine served as the US, however, TOAx rats showed no sign of suppressing CS intake. Moreover, in an unpublished study with the rats from the Liang et al. studies, TOAx rats failed to suppress intake of a Polycose CS following nine pairings with morphine, which further suggests the deficit is not a result of delayed learning.

A final disparity between the ACE and the taste-drug experiments is the fact that the rats actively consume the sucrose US, while in the taste-drug studies described here, the drug is passively delivered by the experimenter. We plan to further evaluate this disparity in a runway apparatus in Chapter 4 with passively administered drug, and using a self-administration paradigm in Chapter 5, where the rats can actively self-administer the drug. While the drug is itself known to be rewarding, there is a body of evidence indicating that experimenter delivered drug is aversive (Chen et al., 2008; Lecca, Cacciapaglia, Valentini, Acquas, & Di Chiara, 2007; Palamarchouk, Smagin, & Goeders, 2009; Stefanski et al., 2007). We have previously
demonstrated that involuntary delivery of drug also is aversive, rendering such treated rats unwilling to work for cocaine and averse to a location associated with yoked delivery of drug (Twining, Bolan, & Grigson, 2009). When an intraorally infused CS is paired with either experimenter-delivered drug (Wheeler et al., 2011), or self-administered drug (Wheeler et al., 2008) rats emit aversive orofacial responses (i.e., gapes). In the case of self-administered drug, aversive orofacial responses develop when the signal indicates that the rat must wait 30 min for drug (Wheeler et al., 2008; Wheeler et al., 2011), not, as described, when presentation of the taste cue has been commensurate with drug delivery (Wheeler et al., 2011). The role of the TOA, then, may not have to do with the mode of administration of the US, per se, but whether the cue signals an immediate or a delayed reward. Confirmation of such a hypothesis will be addressed in Chapter 6.

In summary, the TOA appears to play a role in modulating responsiveness to a gustatory cue that predicts the availability of drug. It is not essential for avoidance of a similar taste cue when paired with a sweet in the anticipatory contrast paradigm or when paired with LiCl in the conditioned taste aversion paradigm. As such, the lesion of the TOA appears to prevent devaluation of the gustatory cue, whether mediated by comparison with the more intense drug of abuse, and/or by the onset of a conditioned aversive state, possibly involving craving and withdrawal. The selective role of the TOA in avoidance of a drug-paired taste cue appears not to depend upon sensitivity to neophobia or the length of CS access, but possibly depends upon the information provided by the taste cue – i.e., the information that drug availability, while imminent, is delayed. Future studies will test the merits of this hypothesis involving sweets and/or drugs. If confirmed, the TOA will be implicated in the development of the opponent process that may be integral to the development of addiction and, once learned, to the precipitation of cue-induced relapse.
Chapter 3

A Drug-Paired Taste Cue Elicits Withdrawal and Predicts Cocaine Self-Administration

Avoidance of a taste cue that has been paired with experimenter-administered drugs of abuse has been attributed to aversive drug properties (Berger, 1972; Cappell & LeBlanc, 1971; et al., 1973; Gamzu, 1977; Glowa et al., 1994; Grigson, Twining, & Carelli, 2000; Hunt & Amit, 1987; LeMagnen, 1969; Lester et al., 1970; Miller et al., 1990; Sherman et al., 1980; Vogel & Nathan, 1975). Here we argue that avoidance of the drug-paired taste cue, ultimately, results from the development of an aversive conditioned state of withdrawal and, as such, relates more to the development of this process than to the properties of the drug, per se. In support, and as might be predicted by the opponent process theory (Solomon & Corbit, 1974), avoidance of a drug-paired taste cue is associated with an elevation in circulating corticosterone (Gomez et al., 2000), a blunting of accumbens dopamine (Grigson & Hajnal, 2007), and frank aversive taste reactivity behavior following the intraoral infusion of the drug-paired cue (Wheeler et al., 2008). Further, as might be expected in a conditioned state of withdrawal, greater aversive taste reactivity is associated with a shorter latency to take drug (i.e., cocaine) and with greater load-up behavior (Wheeler et al., 2008). Each of these indices has been linked to conditioned withdrawal (for a review, see: Grigson, 2008). Interestingly, withdrawal induced by an opiate antagonist also is associated with elevated corticosterone (Nunez et al., 2007), blunted dopamine in the nucleus accumbens (Shaham & Stewart, 1995), and the onset of aversive taste reactivity following intraoral delivery of a naloxone-paired cue (McDonald et al., 1997). Thus, while rats may initially avoid intake of the CS because it pales in comparison to the drug's value, we hypothesize that, ultimately, avoidance is mediated by the onset of a conditioned aversive state involving craving and withdrawal in anticipation of drug availability (Grigson, 2008; Grigson et al., 2009). The present study seeks direct evidence for this effect.
Experiment 1: Morphine-Paired Cue-Induced Withdrawal

In opioid pretreated rats, the opioid antagonist naloxone can precipitate measurable indices of withdrawal of which a decrease in body weight is one of the most robust (Kanarek, D’Anci, Jurdak & Mathes, 2009; Nozaki, 1976). Accordingly, we used this measure to test if a drug-paired taste cue elicits conditioned withdrawal.

Methods

Subjects

The subjects were 15 naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise.

Apparatus

The experiment was conducted in the home cages, and solutions were presented in inverted graduated Nalgene cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Intake was measured to the nearest 0.5 ml.
Drugs and Solutions

Morphine sulfate, provided by the National Institute on Drug Abuse (Bethesda), and Naloxone (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before use. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in distilled water (dH2O) and combined with either orange or grape Kool-Aid (0.187%). These solutions were presented at room temperature.

Procedure

Before conditioning began, rats were maintained on a water deprivation schedule that allowed for 5 min access to dH2O in the morning and 1 h each afternoon. This training procedure lasted until morning water intake stabilized (9 days). During acquisition, one flavor (orange or grape) was presented each day in a 0.15% saccharin solution, alternating across days. The rats were randomly assigned to either the orange or grape CS+ group. All rats had 5 min access to the CS (either orange or grape flavored saccharin), followed 5 min later by an intraperitoneal injection of either 15 mg/kg morphine (if given access to their designated CS+ solution) or an equal volume of saline (if given access to their designated CS- solution). All subjects had one flavor paired with morphine alternated with the other paired with saline for a total of 14 conditioning days (7 grape, 7 orange). To maintain proper hydration, dH2O continued to be provided for 1 h every afternoon. Following conditioning, there were two test days, one for each flavor so every rat was tested with their designated CS+ and CS-. On each test day, rats were weighed and given 5 min access to the CS. Five minutes after CS access, each rat received a subcutaneous injection of naloxone (1 mg/kg). This sub-threshold dose of naloxone was used to increase the detectability of cue-induced withdrawal when administered in conjunction with a
drug-paired taste cue, but was insufficient to induce signs of withdrawal on its own. A second body weight was recorded 2 h after the naloxone injection. To control for the volume of CS intake (i.e., for the possibility that reduced body weight was due to low CS intake), half of the rats tested with the CS- (saline) received a volume equal to that consumed by the CS+ (morphine) group on test day. This group is hereafter referred to as the CS- Yoked group, while those with unlimited access to the CS- on test day are referred to as the CS- Ad Lib group.

Statistics

Data were analyzed using a repeated measures analysis of variance (ANOVA) with condition (CS+ or CS-) and trial (1-7) as within-subjects factors. Post hoc Newman-Keuls tests were conducted where appropriate. Data were considered statistically significant when p < .05.

Results and Discussion

CS Intake (ml/5 min)

The results showed that intake of the morphine-associated taste cue (CS+) decreased compared with intake of the saline-associated taste cue (CS-) across pairings. This conclusion was confirmed by a significant main effect of condition, F(1,14) = 16.33, p < .01, which established that all rats consumed less of the CS+ than CS- overall. The condition x trial interaction also attained statistical significance, F(6,84) = 8.29, p < .001, and post hoc tests revealed that this difference was significant for conditioning trials 3–7, ps < .05 (see Figure 3-1).
Figure 3-1: Mean 5-min intake (± SEM) of the morphine-associated taste cue (CS+; solid circles) and saline-associated taste cue (CS-; open circles) across 7 pairings of each. *indicates a significant difference between the CS+ and CS- conditions (p < .05). N=15

**Naloxone Test CS Intake (ml/5 min)**

Intake of the CS during the two test trials was analyzed using a one-way ANOVA varying group (CS- Ad Lib, CS- Yoked, and CS+). Post hoc analyses of the significant main effect of group, F(2,28) = 3.64, p < .05, demonstrated reduced intake of the CS+ compared with ad libitum intake of the CS- on test day, p < .05. Furthermore, intake of the CS+ did not differ from yoked intake of the CS-, p > .05 (see Figure 3-2a).
Naloxone Test Change in Body Weight (g/2 h)

The change in body weight following naloxone administration, which served as an index of withdrawal, was also analyzed using a one-way ANOVA. The results revealed a significant main effect of group, $F(2,28) = 13.53, p < .001$, and post hoc analyses showed that, regardless of volume consumed, naloxone administration did not induce a loss of body weight when presented following access to the saline-paired CS-. The same naloxone test, however, elicited a precipitous and significant loss in body weight when administered following presentation of the morphine-paired CS+. This conclusion was supported by a significant main effect of group, $F(2,28) = 13.53, p < .001$, and post hoc analyses showed a significant decrease in body weight
following presentation of the CS+ compared with the CS- in the Ad Lib group, p < .001, and the Yoked group, p < .001. Furthermore, no significant difference was found between the CS- Ad Lib and CS- Yoked groups, p > .05. Finally, because this was a within-subjects study, withdrawal was shown to be a conditioned response to the morphine-paired taste cue, independent of drug history (see Figure 3-2b).

Experiment 2: Cocaine-Paired Cue-Induced Withdrawal

Based on these findings, we hypothesized that if avoidance of the taste cue was due to the development of conditioned withdrawal, rather than to a specific property of the drug, this index of withdrawal should be evident with any type of abused drug. Therefore, we conducted a separate between-subjects study with naive rats and the pairing of a taste cue with cocaine. In this case, cocaine was self-administered, testing the second hypothesis that greater conditioned withdrawal, as indexed by a greater naloxone-induced loss of body weight, will be associated with greater responding for drug.

Methods

Subjects

The subjects were 17 naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light
phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise.

**Catheter Surgery**

*Catheter Construction.* The catheters were custom made in our laboratory using a modified procedure described by Koob and colleagues (1987). As described (Grigson & Twining, 2002; Twining et al., 2009), the catheter consisted of two pieces of Silastic tubing (0.03 cm id, 0.06 cm od, 14 cm long; and 0.06 cm id, 0.12 cm od, 2.5 cm long, Baxter Scientific) attached to a stainless steel guide cannula bent at one end at a 90° angle (Item #C3136, Plastics One, Roanoke, VA). The cannula and tubing assembly were molded into a permanent dental cement base by means of a custom-designed mold. A 2.5 x 2.5-cm mesh (Small Parts, Miami, FL) was permanently fixed to the base with dental cement to function as a back plate for the catheter assembly. A small silicon rubber ball was placed approximately 3.5 cm from the end of the small tubing. The entire catheter was flushed with, and then soaked in 200-proof alcohol for 24 h before implantation, then rinsed with sterile saline prior to implantation. The 3.5-cm length of tubing was placed in the jugular vein and anchored to the vein using ligating sutures, as described below.

*Catheter Implantation.* The rats were anesthetized with intramuscular administration of ketamine (70 mg/kg) and xylazine (10 mg/kg). Fur was shaved in two places: on the back between the shoulder blades and directly on top of the jugular vein on the neck, and cleaned with Betadine solution and ethanol. One incision (approximately 10.0 mm in length) was made on the neck above the jugular vein, at about a 30° angle away from midline. Another incision (approximately 25.4 mm in length) was made on the rat’s back, horizontally positioned between the shoulder blades. The skin was then separated from the muscle in both locations with
hemostats. A cannula was then pushed subcutaneously from the incision at the back, over the right foreleg, and through the incision on the ventrum of the rat. The catheter was inserted through the cannula, and the cannula was then removed. The rat was placed in a supine position, and the jugular vein exposed by gently separating the muscle surrounding the vein with blunt microforceps. Once the jugular vein was located and cleared from surrounding tissue, a stainless steel rod (3 mm in diameter) was moistened with saline and gently placed under the jugular vein. Once the rod was in place, it was used to lift the vein to enable the experimenter to make a small incision (approximately 0.5 mm) in the vein. The catheter (0.06-cm od) was then inserted into the vein through the incision. Verification that the catheter was in the jugular vein was achieved by attaching a syringe filled with saline to the other end of the catheter (coming out the back of the rat) and drawing blood back through the syringe. After verification of proper placement, the catheter was secured into position by ligating above and below the silicon ball with silk suture. The stainless steel rod was then removed and wounds were closed using suture or wound clips. General maintenance of catheter patency involved daily examination, cleaning of the coupling assembly, and flushing of the catheter with heparinized saline (0.2 ml of 13 IU/ml heparin). Patency was verified, as necessary, with Propofol (3 mg/rat iv).

**Coupling assembly.** Before the start of each self-administration session, a coupling assembly was anchored to the rat’s back to protect passage of the catheter tubing from interference by the rat. The coupling assembly (a metal spring attached to a metal spacer with Tygon tubing inserted down the center) was attached to the catheter assembly. The catheter tubing was attached to a counterbalanced swivel device (Instech, Plymouth Meeting, PA) that, in turn, was attached to a fluid injection assembly (syringe pump) in the experimental chambers. The fluid injection assembly enabled intravenous infusion of cocaine during self-administration sessions. In the rat’s home cage, the catheter was sealed with a piece of Tygon tubing, and a plastic dust cap was placed over the catheter assembly.
**Apparatus**

Rats were trained in one of twelve identical modular operant chambers (MED Associates), measuring 30.5 x 24.0 x 29.0 cm (length x width x height). All chambers had a clear Plexiglas top, front, and back wall. Sidewalls were made of aluminum. The grid floors consisted of nineteen 4.8 mm stainless steel rods spaced 1.6 cm apart (center to center). Each chamber was equipped with three retractable sipper tubes located on the left wall of the chamber with a stimulus light positioned 6 cm above each. A lickometer circuit was used to monitor licking. Drug reinforcement was controlled by an electronic circuit that operated a syringe pump (Razel Scientific Inst., Model A). A shaded bulb, which reflected light from the ceiling, was located to the right of the cage, opposite the sipper tubes. Each chamber was housed in a light and sound attenuated cubicle that was fitted with a ventilation fan and a white noise source that provided a background noise level of 75 dB. Control of events in the chamber and collection of the data were carried out on-line using a computer and programs written in the Medstate notation language (MED Associates).

**Drugs and Solutions**

Cocaine hydrochloride, provided by the National Institute on Drug Abuse (Bethesda), and Naloxone (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before use. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.
**Procedure**

Upon recovery from surgery, all rats were maintained on a water-deprivation regimen (5 min am/1 h pm), and habituated to the experimental chambers for 5 min a day for 3 days where they received 5 min access to water. Following the three habituation days, water was available ad libitum throughout the experiment in the home cages. The experimental chambers were equipped with 3 retractable spouts; the leftmost spout was used to administer the taste cue, and the remaining 2 spouts were empty and served as the active (rightmost) and inactive (center) operant spouts. During testing, the left CS spout advanced, allowing 5 min access to 0.15% saccharin. After the 5 min CS access period concluded, the CS spout retracted and the empty active and inactive spouts advanced. A stimulus light was illuminated above the active spout and the rats were placed on a fixed ratio schedule of reinforcement in which completion of 10 licks on the active spout resulted in an intravenous infusion of either saline or cocaine (0.167 mg/infusion) over a 6 sec period, depending on the group assignment. Drug or saline delivery was signaled by offset of the stimulus light and onset of a tone and house light, which remained on for a 20 sec period. Further responding during this time was not reinforced. The access period to the drug or saline lasted for 1 h, after which the active and inactive spouts retracted and the subjects were returned to their home cages. There was one taste-drug pairing per day for 7 days. One naloxone test day followed the 7 conditioning trials. Briefly, all subjects were weighed and then given 5 min access to the CS in the home cage, followed 5 min later by a subcutaneous injection of naloxone (1 mg/kg). A second body weight was recorded 2 h after the naloxone injection.
Statistics

Data were analyzed using a mixed factorial ANOVA with drug (cocaine or saline) as the between-subjects factor and trial (1-7) as the within-subjects factor. Post hoc Newman-Keuls tests were conducted where appropriate. Data were considered statistically significant when p < .05.

Results

CS Intake (Licks/5 min)

Results indicate that intake of the taste cue was significantly reduced when it predicted the opportunity to self-administer cocaine compared with saline. This was confirmed by a significant main effect of drug, F(1,15) = 8.11, p < .05, and a significant drug x trial interaction, F(6,90) = 6.74, p < .001. Post hoc analyses confirm that this effect was significant for trials 2–7, ps < .05 (see Figure 3-3a).
**Self-Administration (Infusions/60 min)**

The number of infusions per conditioning trial was also analyzed using a mixed factorial ANOVA. The results indicate that rats more readily self-administered cocaine than saline. This was demonstrated by a significant main effect of drug, $F(1,15) = 6.10$, $p < .05$, and a significant drug x trial interaction, $F(6,90) = 5.20$, $p < .001$. Post hoc analyses confirmed that the infusions were significantly higher for the cocaine group compared with the saline group for trials 2–7, $ps < .05$ (see Figure 3-3b).
Naloxone Test CS Intake (ml/5 min)

The CS intake on test day was analyzed using a one way ANOVA with drug (cocaine or saline) as the variable. Intake of the taste cue was significantly lower for the cocaine group compared with the saline group on the test day, as evidenced by a significant main effect of drug, F(1,15) = 23.23, p < .001 (see Figure 3-4a).

![Graph of Naloxone Test CS Intake](image)

Figure 3-4: (a) Mean 5-min intake (± SEM) of the 0.15% saccharin taste cue that predicts access to self-administer saline or cocaine at naloxone test. (b) Mean change in body weight (g) (± SEM) 2-h after naloxone administration. *indicates a significant difference between the saline and cocaine conditions (p < .05). Saline N=8; Cocaine N=9

Naloxone Test Change in Body Weight (g/2 h)

The change in body weight following naloxone administration was also analyzed using a one way ANOVA with drug (cocaine or saline) as the variable. As occurred with morphine, the cocaine group had a significant loss in body weight 2 h after naloxone administration compared with the saline group. This was evidenced by a significant main effect of drug, F(1,15) = 19.48, p < .001 (see Figure 3-4b).
**Correlation Analyses**

We also found that saccharin intake on the test day was significantly correlated with the change in body weight, \( R^2 = .73, F(1,13) = 35.49, p < .001 \) (Figure 3-5a), and the change in body weight on test day was significantly correlated with the number of infusions taken on the final conditioning trial, \( R^2 = .42, F(1,13) = 9.54, p < .01 \) (Figure 3-5b).

![Correlation Analyses](image)

Figure 3-5: (a) Correlation of saccharin intake and change in body weight at naloxone test. (b) Correlation of change in body weight on test day and the number of infusions taken on the final conditioning trial.

**Discussion**

In summary, the results of Experiment 2 indicate rats will avoid intake of a palatable cue when it predicts the opportunity to self-administer cocaine. Furthermore, rats will more readily self-administer cocaine than saline. When naloxone was injected following exposure to the cue,
rather than the opportunity to self-administer saline or cocaine, cocaine-treated rats displayed 
indices of withdrawal. This supports our hypothesis that avoidance of a drug-paired cue is not the 
result of aversive properties of the drug, per se, but instead the development of an aversive cue-
induced state of craving and withdrawal. In support, the change in body weight following the 
naloxone test was correlated with avoidance of the taste cue and the number of infusions taken on 
the final test day.

**Experiment 3: The Effect of TOA Lesions on Cue-Induced Withdrawal**

Failure to suppress intake of a drug-paired taste cue in previous experiments (Nyland, 
Alexander, Liang, & Grigson, 2012; Chapter 2) may be the result of the lesion interfering with 
the development of the conditioned opponent process (Solomon & Corbit, 1974). Furthermore, 
cues that predict drugs of abuse can lead to a conditioned aversive state involving craving and 
withdrawal (Becker et al., 2010; McDonald et al., 1997; see Grigson, 2008, for a review). The 
present experiment was designed to test whether the TOA may be involved in, not only avoidance 
of the drug-paired cue, but also in the development and/or expression of this complex opponent 
process. In this experiment, Sham and TOA lesioned subjects were administered naloxone 
following 5 min access to a taste cue previously associated with either saline or morphine. The 
purpose of the experiment was to determine if the TOA lesion would block not only avoidance of 
the drug-paired cue, but also the resultant loss in body weight following the naloxone test.
Methods

Subjects

The subjects included a subset of 30 male, Sprague-Dawley rats that had previously served in a saccharin-sucrose anticipatory contrast paradigm. Group assignment was established using a partial cross over design accounting for previous experience. Thus, half of the subjects that had served in each condition (saccharin-saccharin or saccharin-sucrose) were assigned to one of two drug conditions (Saline or Morphine). Testing was conducted during the light phase of the cycle. Food and water were freely available, except where noted below.

Surgery

Fourteen subjects had Sham lesions, and 16 had TOA lesions, as previously described in Chapter 2.

Apparatus

The experiment was conducted in the home cages. Fluid was presented in inverted graduated Nalgene cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Intake was measured to the nearest 0.5 ml.

Drugs and Solutions

Morphine sulfate (National Institute on Drug Abuse, Bethesda, MD) and Naloxone (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before testing.
Polycose (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.

**Procedure**

Rats were placed on a water deprivation schedule that allowed for 5 min of access to dH₂O in the morning and 1 h each afternoon. Following stabilization of morning water intake (approximately 9 days), rats were matched for 5 min water intake and history into saline and morphine drug conditions. All rats were given 5 min access to a 0.03 M Polycose CS, and after a 5 min interval, were injected intraperitoneally with either saline (Sham: n=7; TOAx: n=8) or a 15 mg/kg dose of morphine (Sham: n=7; TOAx: n=6). There were six such taste-drug pairings occurring at 48 h intervals. To maintain proper hydration, dH₂O continued to be provided for 5 min each morning on the days between injections and for 1 h every afternoon. On the final test day, all rats were weighed and then received 5 min access to the Polycose taste cue. Rather than the usual injection, all subjects received a subcutaneous injection of naloxone (1 mg/kg). Two hours after naloxone administration, a second weight was recorded. All rats had free access to water in the home cage.

**Histology**

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects with misplaced or incomplete lesions were excluded from further analysis.
Statistics

Data from the seven conditioning trials was included as part of the analysis for Chapter 2 Experiment 2, and will therefore not be analyzed further. Data from the naloxone test were analyzed using a two-way ANOVA varying lesion (Sham and TOAx) with drug condition (saline or morphine).

Results and Discussion

Histology

Data from two TOAx rats were excluded from statistical analysis due to incomplete lesions, as described in Chapter 2 (see Figure 2-1).

Naloxone Test

Intake of the taste cue and resultant change in body weight on the naloxone test day were analyzed using a two-way ANOVA varying lesion (Sham and TOAx) with drug (saline and morphine). CS Intake. Results of the two-way ANOVA indicated rats with Sham lesions drank less of the taste cue when it predicted experimenter-administered morphine compared with saline (Figure 3-6a). This was confirmed by a significant main effect of drug, F(1,24) = 8.39, p < .01, and a significant lesion x drug interaction, F(1,24) = 6.14, p < .05. Post hoc tests on the 2-way interaction indicated that intact rats avoided Polycose intake when it predicted morphine, compared with saline, p < .05. This effect was not present for the lesioned rats, p > .05. Furthermore, there was no significant main effect of lesion (F < 1), which indicates that the lesion did not interfere with consumption of the Polycose cue overall.
Change in Body Weight. The results of the two-way ANOVA on the body weight data found that Sham rats, but not TOAx rats, had a significant loss in body weight following exposure to the Polycose cue that was paired with experimenter-administered morphine but not saline (Figure 3-6b). This conclusion was supported by a significant main effect of drug, $F(1,24) = 9.24, p < .01$, and a significant lesion x drug interaction, $F(1,24) = 5.93, p < .05$. Post hoc analyses confirm that the change in body weight was lower for morphine-treated compared with saline-treated rats in the Sham, $p < .05$, but not the TOAx, $p > .05$, condition. This was not an effect of the lesion overall, as the main effect of lesion was not significant ($F < 1$).

![Figure 3-6: (a) Mean 5-min intake (± SEM) of 0.03 M Polycose at naloxone test for Sham (left) or TOAx (right) rats. (b) Mean change in body weight (g) (± SEM) 2-h following naloxone administration. *indicates a significant difference between the saline and morphine conditions (p < .05). Sham: Saline N=7, Morphine N=7; TOAx: Saline N=8, Morphine N=6](image)

General Discussion

Although avoidance of the drug-paired taste cue may initially result from drug-induced devaluation of the saccharin cue, with experience, aversion develops as would be predicted by the opponent process theory of motivation (Solomon & Corbit, 1974). The opponent process theory
proposes that the pleasurable effects of the drug are automatically opposed by central nervous system mechanisms that serve to reduce the impact of the drug. With experience, the pleasurable effects of the drug reduce in intensity due, at least in part, to tolerance. The aversive opponent process, however, gains in strength and is conditioned to drug-paired cues. As such, the opponent process is elicited in anticipation of drug-delivery and can be experienced as withdrawal in rats and man (Koob & Le Moal, 2001).

Here we provide evidence that presentation of a morphine- or cocaine-paired taste cue elicits a conditioned state of withdrawal, as indexed by a precipitous loss of body weight following injection of the opiate antagonist, naloxone. Furthermore, greater naloxone-induced loss in body weight (i.e., greater withdrawal) was correlated with greater avoidance of the drug-paired cue and, as verified with cocaine, greater cocaine self-administration behavior. These data suggest that the onset of conditioned withdrawal may precipitate a “snowball effect” involving devaluation of the natural reward cue (i.e., anhedonia), activation of the HPA axis as indicated by elevated corticosterone (Gomez et al., 2000), and blunted accumbens dopamine (Wheeler et al., 2011; Wheeler et al., 2008). Together, these consequences conspire to elicit seeking and taking in an effort to correct the aversive state as quickly as possible.

It is of note that there is a discrepancy in the magnitude of suppression in Experiments 1 and 3 (Sham rats only). This inconsistency was likely the result of differences in methodology. For example, Experiment 1 utilized a within-subjects design, and therefore each rat was subject to both the CS+ and CS- conditions. Additionally, because of the within-subjects design, the cue was adulterated with Kool-Aid, and follow up 2-bottle tests indicated that this particular batch of rats drank less of the concentration presented in the experiment (0.187%) than a saccharin solution containing half of this concentration (0.094%) of Kool-Aid. Regardless of the magnitude of the effect, it was still present in both experiments, and most importantly, it was not present in the TOA lesioned rats. In Experiment 3, the TOA lesion prevented avoidance of the
taste cue as well as cue-induced withdrawal, as indexed by a lack of a significant change in body weight following the naloxone test. These results suggest that the lesion may prevent avoidance of the drug-paired taste cue by reducing the onset of cue-induced withdrawal. Given the key role of cue-induced withdrawal in drug seeking and taking, the same lesion, then, also may prevent drug seeking and taking as well. The merits of this hypothesis will be tested directly by incorporating operant responding into the paradigm in a runway (Chapter 4) and in self-administration chambers (Chapter 5).
Chapter 4

Avoidance of a Morphine-Paired Taste Cue and Drug-Motivated Behavior in Rats with Lesions of the Trigeminal Orosensory Area

We have previously found that the TOA lesion had differing effects on avoidance of a saccharin cue when predicting access to a preferred sucrose reward versus when the cue predicts a drug of abuse (Nyland et al., 2012; Chapter 2). Specifically, we determined that TOAx rats do not avoid intake of the palatable taste cue when paired with a drug reward, yet avoidance of a sucrose-paired saccharin cue remains intact. Furthermore, TOAx rats suppress intake of a taste cue paired with an aversive stimulus in the conditioned taste aversion paradigm. This provides further evidence that, while the avoidance of a drug-paired taste cue may appear similar to the traditional conditioned taste aversion model, the meaning of the association between the taste cue and the predicted outcome is not the same. Furthermore, we discovered that, in addition to preventing suppressed intake of a drug-paired taste cue, the TOA lesion also interfered with cue-induced signs of withdrawal (Nyland & Grigson, 2013; Chapter 3). We hypothesize that this results from the lesion preventing the development of an opponent process (Solomon & Corbit, 1974) that involves cue-induced withdrawal. However, it also is possible that rats with TOA lesions fail to suppress intake of the drug-paired cue because they are unable to experience the rewarding and/or motivational properties of the drug. Therefore, it was necessary to investigate the influence of the lesion on drug motivation and reward in order to gain a full understanding the effects of the TOA lesion in this phenomenon.

Until now, suppression of a drug- or sucrose-paired taste cue has been similarly affected by numerous manipulations such as deprivation state (Gomez & Grigson, 1999), strain (Grigson & Freet, 2000), and drug history (Grigson, Wheeler, Wheeler, & Ballard, 2001). Furthermore, as
discussed, lesions of the gustatory thalamus have also yielded mostly similar effects on suppression of intake of a drug- or sucrose-paired taste cue (Grigson, Lyuboslavsky, & Tanase, 2000; Reilly et al., 2004; Schroy et al., 2005) and cortex (Geddes et al., 2008; Mackey et al., 1986; Zito et al., 1988). Therefore, the TOA lesion differs from that of cortical or thalamic taste area damage, in that it appears to interfere specifically with avoidance of a palatable taste cue that has been paired with abused drugs. One plausible explanation for these findings is disinhibition of responding for a palatable taste cue. Accordingly, rats with lesions of the TOA showed increased responding for sucrose under real and sham-feeding conditions (Liang, Freet, Grigson, & Norgren, 2012). This suggests disinhibition in responding for naturally rewarding stimuli, such as sucrose, in the TOA-lesioned rats. By this logic, in previous findings (Nyland et al., 2012; Chapter 2), the failure to avoid intake of a drug-paired cue may not have had anything to do with the drug itself, but rather with the inability to inhibit responding for a palatable taste cue. That said, TOAx rats did avoid intake of a palatable sucrose taste cue when paired with LiCl in a conditioned taste aversion experiment (Liang et al., 2012b) and they avoided saccharin when paired with a preferred sucrose solution in the ACE paradigm (Nyland et al., 2012).

On the other hand, the failure to avoid a drug-paired taste cue in our previous findings (Nyland et al., 2012; Chapter 2) may have had very much to do with the drug itself. Failure to avoid the drug-paired cue may have been due to the inability to detect experimenter-administered morphine or cocaine. Likewise, if TOAx rats are able to detect the abused drug, it is possible that the drug is not perceived as rewarding. In the following experiments, we used a runway apparatus to test these possibilities. In the first experiment, the rat was tested in an elongated runway with two distinct chambers, one at either end. One chamber served as the ‘Start Box’, where the taste cue was presented, and the other serves as the ‘Goal Box’, where experimenter-administered morphine or saline was delivered. The speed with which the rat traversed the runway from the Start Box to the Goal Box was taken as an index of the motivation to seek drug.
Previous research indicates that rats will increase running speed in the runway to reach the Goal Box paired with experimenter-administered morphine (Zernig et al., 2002) and amphetamine (Ettenberg, 1990), as well as self-administered heroin (McFarland & Ettenberg, 1995). This also is true for natural rewards such as food (Chausmer & Ettenberg, 1997; Ettenberg & Camp, 1986), water (Ettenberg & Horvitz, 1990), and sex (Lopez, Olster, & Ettenberg, 1999). Essentially, the more motivated the subject, the faster the subject traverses the runway. We hypothesize that if the TOA lesion interferes with drug-induced devaluation of a natural reward and/or cue-induced withdrawal, then it will most likely alter the motivation to seek drug. If this is the case, it will establish a new target for the study of cue-induced seeking and relapse. Therefore, the goal of this study was to test if TOAx rats were able to detect and process experimenter-administered drugs of abuse. If the lesion interferes with detection of the drug, the running speed to reach a drug-paired goal will not differ between saline-treated and morphine-treated TOAx rats. If the lesion fails to disrupt detection of the drug, however, faster or slower running speed in the runway apparatus will indicate if the TOAx rats are more or less motivated to obtain the drug, respectively.

Experiment 1: Morphine-Induced Taste Cue Avoidance in the Runway Apparatus

Whereas in the previous studies only the consummatory behavior was assessed, this paradigm allows for a clearer understanding of the subject’s instrumental responding for experimenter-administered morphine. First, avoidance of the taste cue will be assessed in the Start box. Second, the running speed from the Start box to the morphine-paired Goal box will indicate whether the rat can detect experimenter-administered drug. Third, if the drug is detected, the running speed can also determine if the rat can assign value to the drug (i.e., if the drug is
rewarding or aversive), and how motivated the subject is to seek and obtain the drug when cued by a gustatory stimulus.

Methods

Subjects

The subjects were 42 naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light phase of the cycle. The rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise.

Surgery

Following acclimation to the colony room, the subjects (at least 300 g) underwent either bilateral ibotenic acid lesions of the thalamic trigeminal orosensory area (TOA: n=24) or vehicle infusions (Sham: n=18) as described in Chapter 2.

Apparatus

All experimental manipulations were conducted in the runway. The floor, walls, and ceiling of the alleyway (7.85 ft x 7 in x 5.5 in) were made of Plexiglas. The Start box and Goal box (19.5 x 9.25 x 5.5 in) of identical dimensions were located at opposing ends of the alleyway.
The grid floors of the Start box consisted of thirty 4.8 mm stainless steel rods spaced 2 cm apart. The outer ceiling and walls of the entire runway were covered with 30% tint. The Goal box was changed as to appear different from the Start box. A tile was fitted over the grid floors in the Goal box to give it a different texture and feel then the rest of the runway. The Goal box had 1-inch thick white and black diagonal stripes covering the walls and ceiling. Computer operated sliding doors provided access from the Start box to the alleyway and from the alleyway to the Goal box. The Start box and Goal box were each equipped with one automated, retractable sipper dispenser, such that a bottle could be advanced through a 1.3 cm diameter hole in the end wall, approximately 10 cm above the grid floor. A closed circuit lick-o-meter was used to monitor licking behavior. The latency to leave the Start box and enter the Goal box was detected by interruption of six pairs of infrared photocell emitters. Output of these photocells and lick-o-meter was collected on-line by a computer. Programs were written in the Medstate notation language and the behavioral data were stored as Notepad files.

Solutions

Morphine sulfate (National Institute on Drug Abuse, Bethesda, MD) and Naloxone (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before testing. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.

Procedure

All rats were placed on a water deprivation schedule that allowed for 5 min of access to dH₂O in the morning and 1 h each afternoon. Following stabilization of morning water intake
(approximately 7 days), the rats were matched for 5 min water intake and divided into two drug treatment groups: saline and morphine.

The rats were run in the runway one at a time. They were first habituated to the runway, once a day for 2 days. During habituation, all rats were removed from their home cage, weighed and placed into the start box where they received 5 min access to water. After the 5 min period was over, the spout retracted and the door to the start box opened. Rats were then allowed to explore the runway freely (except for the goal box) for 5 min. Once the session was over, the rat was removed and placed back in the home cage. One hour after being removed from the runway, the rat was given 1 h access to water in the home cage.

Following habituation to the runway apparatus, the rats were run once a day, every other day for seven trials during the conditioning phase of the experiment. On conditioning days, the rat was removed from the home cage, weighed and placed into the start box where it received 5 min access to 0.15% saccharin. Intake of the saccharin taste cue was recorded. After the 5 min period was over, the doors to the start box and goal box opened. The rat was then given a maximum of 15 min to reach the far wall of the goal box. Once detected by the photocell at the back of the goal box, the goal box closed and the rat immediately received an intra-peritoneal injection of morphine (Sham: n=9; TOAx: n=14) or saline (Sham: n=9; TOAx: n=10), depending on the random group assignment. The time required to reach the goal box was used to determine the running speed. The rat was then placed back into the goal box for 5 min. Once the 5 min were up the rat was immediately removed from the runway and placed back in its home cage. One hour later, the rat was given 1 h access to water in the home cage. Conditioning trials occurred every other day for seven trials. On days between conditioning trials, rats received 5 min access to dH₂O in the morning and 1 h in the afternoon in the home cage.

On the final test day, the procedure was run the same as described above with the exception that all rats received subcutaneous injections of naloxone (1 mg/kg) rather than saline.
or morphine following entry into the goal box. Body weight was recorded again 2 h after the rat was injected with naloxone. Thereafter, all rats were given free access to water in the home cage.

**Histology**

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects with misplaced or incomplete lesions were excluded from further analysis.

**Results**

**Histology**

Data from six TOAx rats were excluded from statistical analysis due to incomplete lesions. Typical lesions damaged the middle third of the VPM as well as the majority of the taste area in the parvicellular area of the VPM (VPMpc). Additionally, the lesion encompassed small parts of adjacent nuclei such as the parafascicular nucleus (Pf), posterior nucleus (Po), and subparafascicular nucleus (SPF) (see Figure 4-1).
CS Intake (Licks/5 min)

Intake of the saccharin taste cue during the seven conditioning trials was analyzed using a mixed factorial ANOVA with lesion (Sham and TOAx) and drug (morphine or saline) as between-subjects factors and trial (1-7) as a within-subjects factor. The results indicate that, unlike in the home cage studies, all rats drank less of the saccharin cue when it preceded an injection of morphine compared with saline (Figure 4-2). This conclusion was supported by a significant main effect of drug, F(1,32) = 19.97, p < .001, and a significant drug x trial interaction, F(6,192) = 16.59, p < .001. Post hoc analyses of the significant interaction indicated...
that intake was lower for the morphine rats compared with saline treated rats on trials 2-7 for the Sham (ps < .05), and 3-7 for the TOAx group. Thus, in the runway apparatus, avoidance of the morphine-paired cue was observed in both the intact and the lesioned rats, as indicated by a lack of a significant main effect of lesion (F < 1), or any accompanying interactions thereof (Fs < 1). These results indicate that, all other things equal, when tested in the runway paradigm, rats with TOA lesions, like intact controls, avoid intake of a saccharin cue that predicts passively administered morphine.

Figure 4-2: Mean 5-min intake (± SEM) of 0.15% saccharin paired with experimenter-administered saline (open circles) or 15 mg/kg morphine (solid circles) across 7 trials for Sham (left) or TOAx (right) rats. * indicates a significant difference between the saline and morphine conditions (p < .05). Sham: Saline N=9, Morphine N=9; TOAx: Saline N=9, Morphine N=9


Running Speed

The running speed was determined by the time required to traverse the runway. This was calculated by subtracting the latency to leave the start box from the latency to reach the goal box. The data were analyzed using a mixed factorial ANOVA varying lesion, drug, and trial as factors. A significant main effect of drug, F(1,32) = 6.92, p < .05, indicated that drug-treated rats were faster to reach the goal than were saline-treated rats overall (Figure 4-3). The lesion, however, did not affect the running time, as there was no significant main effect of lesion (F < 1), or any accompanying interactions (Fs < 1). Furthermore, there was no significant drug x trial interaction (F < 1). These results tell us that the rats ran faster to reach the goal box when associated with passively administered morphine compared with saline, and that this effect was present in both intact and lesioned rats.

Figure 4-3: Mean running time (± SEM) to reach the goal box when it is associated with experimenter-administered saline (open circles) or 15 mg/kg morphine (solid circles) across 7 trials for Sham (left) and TOAx (right) rats. Sham: Saline N=9, Morphine N=9; TOAx: Saline N=9, Morphine N=9
**Naloxone Test**

Intake of the taste cue and the resultant change in body weight on the naloxone test day were analyzed using two-way ANOVAs varying lesion and drug. **CS Intake.** The results indicate that rats in both lesion groups drank less of the taste cue when it predicted experimenter-administered morphine compared with saline (Figure 4-4a). This was confirmed by a significant main effect of drug, $F(1,32) = 28.57, p < .001$, and lack of a significant main effect of lesion ($F < 1$) or lesion x drug interaction ($F < 1$).

**Change in Body Weight.** Furthermore, results also found both groups to have a significant loss in body weight, used here as an index of withdrawal, following exposure to the taste cue that was paired with experimenter-administered morphine but not saline (Figure 4-4b). This conclusion was supported by a significant main effect of drug, $F(1,32) = 23.10, p < .001$, and a lack of a significant main effect of lesion ($F < 1$) or lesion x drug interaction ($F < 1$). Overall, the results indicate that, when tested in the runway apparatus, intact and lesioned rats avoid intake of a morphine-paired taste cue and exhibit signs of cue-induced withdrawal.

![Figure 4-4: Mean 5-min intake (± SEM) of 0.15% saccharin at naloxone test for Sham (left) and TOAx (right) rats.](image)

*Figure 4-4: (a) Mean 5-min intake (± SEM) of 0.15% saccharin at naloxone test for Sham (left) and TOAx (right) rats. (b) Mean change in body weight (g) (± SEM) for Sham (left) and TOAx (right) rats 2-h after the naloxone test. *indicates a significant difference between the saline and morphine conditions (p < .05). Sham: Saline N=9, Morphine N=9; TOAx: Saline N=9, Morphine N=9*
**Correlation Analyses**

We also found that saccharin intake for all rats on the final trial was positively correlated to the change in body weight following naloxone administration, $R^2 = .64$, $F(1,34) = 60.13$, $p < .001$, (Figure 4-5). This correlation between intake and the change in body weight was also evident when reanalyzed separately for Sham, $R^2 = .65$, $F(1,16) = 29.12$, $p < .001$, and TOAx rats, $R^2 = .67$, $F(1,16) = 32.97$, $p < .001$. Overall, rats that drank less of the saccharin taste cue also showed a greater loss in body weight following the naloxone test.

![Graph showing correlation between saccharin intake and change in body weight](image)

Figure 4-5: Correlation of saccharin intake and the change in body weight on the final test day for Sham (red) and TOAx (blue) rats.
Discussion

When the taste-drug pairings took place in the runway apparatus, the TOA lesion failed to interfere with consummatory behavior in the way that it had when tested in the home cage. Furthermore, intact and lesioned rats ran faster to reach the goal when it was associated with morphine. This indicates that the lesion also was without effect on the instrumental performance. Finally, rats in both the TOAx and Sham groups displayed a significant loss in body weight following the naloxone test, which would be expected to precipitate drug seeking (i.e., faster running to the drug-paired goal). Overall, it appears as though the lesion was without effect on drug-induced devaluation of the saccharin taste cue when presented in the runway apparatus. This unexpected finding may have been due to the presence of abundant contextual cues in the runway apparatus. Contextual cues may have enhanced the salience of the saccharin cue, and thus enabled the saccharin-morphine association. Another, possibly simpler explanation was that the lesions were misplaced. This possibility was addressed in the following experiment using the same rats to test if avoidance of a drug-paired cue was present when tested in the home cage.

Experiment 2: Cocaine-Induced Avoidance of a Taste Cue in the Home Cage

The failure of the lesion to inhibit suppression of intake of the taste cue in the previous experiment was unexpected. To test if these results were unique to the runway paradigm, or if they were the result of misplaced lesions, the same subjects underwent a follow up study pairing a novel taste cue with experimenter-administered cocaine. The purpose of Experiment 2 was to determine if these subjects were capable of learning to avoid a taste cue paired with experimenter-delivered drug when presented in the home cage. If the lesions were well placed, the TOAx rats should fail to avoid intake of the drug-paired cue when tested in the home cage.
Methods

Subjects

The subjects that served in Experiment 1 were used in Experiment 2 in a partial cross-over design in which half of the rats from each group (saline or morphine) were reassigned to one of two new drug conditions (saline or cocaine). Testing was conducted during the light phase of the cycle. Food and water were freely available, except where noted below.

Apparatus

The experiment was conducted in the home cages. Fluid was presented in inverted graduated Nalgene cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Intake was measured to the nearest 0.5 ml.

Solutions

Cocaine hydrochloride was provided by the National Institute on Drug Abuse (Bethesda, MD). The 10 mg/kg dose of cocaine was prepared in sterile saline and administered subcutaneously as a 1.5 mg/ml stock solution, adjusted by body weight to avoid necrosis (Durazzo et al., 1994). Polycose (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.
**Procedure**

The rats were placed on a water deprivation schedule that allowed for 5 min access to dH₂O in the morning and 1 h each afternoon. Following stabilization of morning water intake (approximately 9 days), all rats were given 5 min access to a novel 0.03 M Polycose CS, and after a 5 min interval, they were injected subcutaneously with either saline or a 10 mg/kg dose of cocaine as described. There were five such taste-drug pairings occurring at 48 h intervals. To maintain proper hydration, dH₂O was provided for 5 min each morning between conditioning trials and for 1 h every afternoon.

**Histology**

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects found to have misplaced or incomplete lesions were excluded from further analysis (see Figure 4-1).

**Results**

**Histology**

As mentioned in Experiment 1, data from six TOAx rats were excluded from statistical analysis due to incomplete lesions.
CS Intake (ml/5 min)

The results of the mixed factorial ANOVA for Polycose intake over the five taste-drug pairings verified that the Sham subjects, but not the TOAx subjects, suppressed intake of the taste cue that was paired with experimenter-administered cocaine (Figure 4-6). This was evidenced by a significant lesion x drug interaction, F(1,32) = 6.78, p < .05, lesion x trial interaction, F(4,128) = 2.64, p < .05, and drug x trial interaction, F(4,128) = 7.94, p < .001. There also were significant main effects of lesion, F(1,32) = 9.96, p < .01, and drug, F(1,32) = 9.04, p < .01. Post hoc analyses revealed decreased intake for cocaine versus saline-treated rats in the Sham group on trials 3-5, ps < .05, but not on any trial for the TOAx rats, ps > .05. Intake of the taste cue also was analyzed as a function of prior drug history (from Experiment 1). The results of this analysis found no significant main effect of history, F < 1. Based on this result, we can conclude that TOAx rats failed to avoid intake of a cocaine-paired taste cue when presented in the home cage, despite the successful avoidance of a morphine-paired cue in the runway procedure. Successful performance in the runway, then, was not likely due to misplaced lesions.
Discussion

When presented in the home cage, TOAx subjects failed to suppress intake of the drug-paired taste cue. When the prior drug history from the runway was taken into account, all TOAx rats failed to suppress intake of the cocaine-paired cue – even those TOAx rats that avoided intake of the morphine-paired saccharin cue in the runway. This result confirms that the runway paradigm had a significant influence on the rats’ ability to demonstrate avoidance of a drug-paired taste cue. This also confirms that the behavioral effects of the lesion are similar to those
previously reported (Nyland et al., 2012; Chapter 2), which increases the generalizability between experiments using different batches of lesioned subjects.

**General Discussion**

The intention of this study was to investigate the effect of the TOA lesion on the motivation to obtain experimenter-administered morphine. We initially hypothesized that the lesion would alter the motivation to obtain drug; however, the original hypothesis did not take into account that the lesioned subjects would learn to suppress intake of the taste cue in this paradigm. This unexpected finding was considered while interpreting the results. The results found no difference in running speed between Sham and TOAx subjects in the morphine condition. All rats traversed the runway more quickly when the saccharin cue was paired with morphine compared with saline. Therefore, we conclude that the lesion does not interfere with motivational properties of abused drugs. That said, the TOAx rats have previously shown increased operant responding for sucrose (Liang et al., 2012a). It is possible that the TOAx rats are more responsive to rewarding stimuli in general. Therefore, instrumental performance for drug will be tested in Chapter 5 using a self-administration paradigm.

The results also unveiled the unexpected finding that, when tested in the runway, the TOAx rats were capable of detecting the taste cue and using it to predict the availability of experimenter-administered morphine. The TOAx rats also were capable of identifying the value of the drug and, under these circumstances, avoiding intake of the saccharin taste cue that preceded it. Moreover, the lesioned rats showed signs of cue-induced withdrawal when tested in the runway. These results provide evidence that the lesion does not interfere with the ability to experience and process the drug, and does not prevent the development of cue-induced
withdrawal. Why then, does the lesion inhibit drug-induced avoidance of a palatable taste cue when the experiment takes place in the home cage?

One possibility is the absence of contextual cues when tested in the home cage. Pavlovian conditioning is not as straightforward as the association of a CS and a US. The literature describes numerous factors such as phasic and contextual cues that can become associated with either the CS or the US. For example, contextual cues can become associated with the US, and can either block or enhance conditioning to a CS (Bouton & Bolles, 1979; Durlach, 1983; Grahame, Barnet, Gunther, & Miller, 1994; Randich & Ross, 1984; Tomie, 1976). Other theories suggest that the context can become associated with the CS and facilitate appropriate responses for an otherwise ambiguous CS (Bouton & Bolles, 1979).

When tested in the home cage, TOAx rats failed to avoid intake of a taste cue that was paired with experimenter-administered drugs of abuse (Chapter 2, Nyland et al., 2012). The same rats, however, do avoid intake of a LiCl-paired taste cue under identical testing procedures in the conditioned taste aversion paradigm. These findings suggest that the discrepancy lies within the ability to make the CS-US association, specifically when the US is a drug of abuse. The results from the runway study suggest that this association is possible in the presence of contextual cues, most likely because the contextual cues elicit a conditioned state of withdrawal in anticipation of drug. For example, intact rats show signs of withdrawal following exposure to a taste cue or context previously paired with an abused drug. This conditioned state of withdrawal, in turn, results in decreased CS intake. In TOAx rats, the drug-paired context, but not the CS itself, is also capable of eliciting a conditioned state of withdrawal, which may explain decreased CS intake in the runway study. Further experiments in Chapter 5 will focus on determining if context is sufficient to support taste cue avoidance in TOA lesioned rats.

Another explanation for the successful avoidance of the morphine-paired taste cue in the runway study is the length of the interval between access to the taste cue and administration of
the morphine US. For the purposes of this dissertation, we will henceforth refer to this interval as the inter-stimulus interval (ISI). In the home cage studies, there was a 5 min ISI between access to the taste cue and access to drug, while in the runway study the rat determined the length of the ISI. The ISI in the runway study was typically much less than 5 min (approximately 1.5 min). Therefore, it is possible that a short ISI also facilitates the ability of the TOA lesioned subjects to make the taste-drug association. The importance of the ISI has previously been demonstrated in rats with bilateral ibotenic acid lesions of the gustatory thalamus (TTAx). Rats with TTAx lesions do not suppress intake of a sucrose-paired cue when presented with a brief ISI; however, when presented with a 5 min ISI, the TTAx rats display a reinforcement effect where intake of the cue is increased when it predicts a more preferred sucrose solution (Reilly et al., 2004; Schroy et al., 2005). Likewise, it is possible that the extended ISI in the previous home cage studies resulted in a failure to associate the taste cue with a drug of abuse. In support, TOAx rats avoided intake of a sucrose-paired taste cue presented with a 0 sec ISI (Nyland et al., 2012). Therefore, we will further investigate the role of the ISI in Chapter 6.
Chapter 5

Self-Administration of Cocaine in Rats with Lesions of the Trigeminal Oro sensory Area

Rats avoid intake of a taste cue when paired with a drug of abuse (Cappell & LeBlanc, 1971; Cappell et al., 1973; Glowa et al., 1994; Grigson, Twining, & Carelli, 2000; LeMagnen, 1969; Miller et al., 1990; Sherman et al., 1980). While we have interpreted this as a reward comparison effect (i.e., avoidance of a less valued saccharin cue in anticipation of access to a highly rewarding drug of abuse), recent evidence shows that drug-induced avoidance of the taste cue also is accompanied by a conditioned elevation of circulating corticosterone (Gomez et al., 2000), blunted accumbens dopamine (Grigson & Hajnal, 2007), and the onset of aversive taste reactivity (i.e., gapes) (Wheeler et al., 2008). We have interpreted these data as evidence for the onset of a conditioned aversive response involving withdrawal. In accordance, and as described in Chapter 3, treatment with a low dose of naloxone elicits signs of withdrawal (i.e., significant body weight loss) when injected following ingestion of a morphine- or cocaine-paired taste cue and greater ‘withdrawal’ predicts greater cocaine self-administration (Nyland & Grigson, 2013). Further, as might be expected for a conditioned state of withdrawal, greater aversive taste reactivity is associated with a shorter latency to take drug (i.e., cocaine) and with greater load-up behavior (Wheeler et al., 2008).

We have determined that bilateral ibotenic acid lesions of the TOA interfere with avoidance of a taste cue when paired with experimenter-administered morphine or cocaine, but not when paired with either the aversive agent LiCl, or a preferred sucrose solution (Nyland et al., 2012; Chapter 2). This was not the case, however, in Chapter 4, when the taste-drug pairings took place in a runway apparatus. In this instance, the lesion failed to prevent avoidance of the drug-paired taste cue and accompanying signs of cue-induced withdrawal. Two factors may have
contributed to the successful performance by TOAx rats in the latter study: the presence of contextual cues and/or a shortened inter-stimulus interval (ISI).

Evidence suggests that drug-related contextual cues can trigger drug seeking in humans and animals (Crombag, Bossert, Koya, & Shaham, 2008; Gabbay et al., 1996; Stewart, de Wit, & Eikelboom, 1984). Accordingly, the availability of contextual cues may be sufficient to facilitate the conditioned avoidance of the drug-paired taste cue in lesioned rats. The literature suggests that contextual cues can become associated with either the conditioned stimulus (CS) or the unconditioned stimulus (US), depending on the associative strength of the context. For example, responding to a particular context lessens when the US is signaled by a more salient cue (Olding-Smee, 1975), or after repeated non-reinforced exposure to the contextual stimuli (Bouton & Bolles, 1979). Furthermore, a highly valued context can enhance conditioning to a CS (Durlach, 1983). Therefore, in Chapter 4, it is possible that the contextual cues present in the runway apparatus either increased the associative strength of the taste cue, or were themselves associated with drug, thus sidestepping the deficit caused by the TOA lesion.

Additionally, a shortened interval between the CS and US also may have contributed to the successful performance of the lesioned rats in the runway paradigm. In the runway experiment, the interval between the saccharin taste cue and drug administration was controlled by the rat. The faster the rat reached the goal box, the shorter the interval between the CS and drug. Therefore, the inter-stimulus interval (ISI) was significantly shorter (average 1.5 min) than that typically seen in home cage studies (Nyland et al., 2012; Chapter 2), where the ISI was 5 min. The length of the ISI will be addressed in Chapter 6. The present chapter will employ a short ISI, but will investigate the importance of contextual cues using a self-administration paradigm that will allow for the measurement of operant responding for cocaine.

Self-administration procedures are an integral component of addiction research. Animal models of self-administration allow for the controlled study of the reinforcing properties of a drug
and the resultant drug taking behaviors that lead to addiction. In animal models, operant conditioning is used to establish self-administration, requiring the subject to elicit a response (e.g., lever press or empty spout lick) to obtain drug (Clark, Schuster, & Brady, 1961; Weeks, 1962). In the following studies, an indwelling intrajugular catheter is used for drug delivery, and empty spout licking responses are reinforced with an intravenous cocaine or saline infusion. This method of drug delivery has become the standard for studying cocaine reward and addiction. Compared with the experimenter-administered drug models, this technique adds an additional variable to the experiment, yet more accurately models drug taking seen in humans. This is evident by the clear distinction between self-administered drug and experimenter-administered or “yoked” delivery (Dworkin, Mirkis, & Smith, 1995; Porrino, 1993; Stefanski et al., 2007; Twining et al., 2009). Furthermore, the self-administration paradigm employed here is ideally suited to study the effects of lesions of the thalamic trigeminal orosensory area (TOA) on drug reinforcement, drug-taking behaviors, and on devaluation of natural rewards.

Experiment 1 will employ both contextual cues and drug-contingent cues (i.e., lights and tones) to test if the lesion will prevent avoidance of the drug-paired cue in this paradigm. In so doing, Experiment 1 also will test the effect of the lesion on responding for drug. Additionally, the development of cue-induced withdrawal in anticipation of drug availability will be tested with, and without, the presence of contextual cues. In Experiment 2, all contextual and drug-contingent cues will be eliminated by housing the rats in the self-administration chambers and removing the drug-contingent lights and tones that signal drug delivery. The saccharin solution, then, will be the only cue for drug in Experiment 2. Finally, in Experiment 3, contextual cues will be provided by housing the rats in standard home cages and testing them in the self-administration chambers. In this case, however, drug-contingent lights and tones will not be provided. If the availability of contextual cues are sufficient to circumvent the disruptive effect of the lesion, then avoidance of the cocaine-paired cue is expected in the TOAx rats in
Experiments 1 and 3, when contextual cues are present, but not in Experiment 2 when the saccharin solution serves as the only cue. Finally, if the TOA is essential for processing the reinforcing properties of cocaine, then the lesioned subjects will fail to establish cocaine self-administration in Experiments 1-3.

**Experiment 1: Self-Administration of Cocaine in the Presence of Contextual and Drug-Contingent Cues**

The first goal of Experiment 1 was to test if the lesion will prevent avoidance of the saccharin taste cue when it predicts the opportunity to self-administer cocaine. Based on results from the runway paradigm (Chapter 4), the contextual cues present in the self-administration chambers were expected to be sufficient for the lesioned rats to establish conditioned avoidance of the taste cue in Experiment 1. Additionally, this experiment was intended to determine if the TOA lesion would interfere with the acquisition of cocaine self-administration and alter the motivation to obtain drug. Based on the findings from the runway experiment, in which the motivation to seek drug following the morphine-paired taste cue was unaltered in TOAx rats, the lesion was not anticipated to alter drug-taking or the motivation to obtain drug in Experiment 1.

**Methods**

**Subjects**

This experiment was run in three replications using a total of 96 rats. The subjects were naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights
on at 7 a.m.). Testing was conducted during the light phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise. Subjects were at least 300 g prior to surgery.

**Surgery**

Following acclimation to the colony room, the subjects underwent either bilateral ibotenic acid lesions of the thalamic trigeminal orosensory area (TOAx: n=56) or vehicle infusions (Sham: n=40) as described in Chapter 2. Following recovery from lesion surgery, all rats underwent implantation of intrajugular catheters as described in Chapter 3.

**Apparatus**

Rats were trained in one of twelve identical modular operant chambers (MED Associates), as described in Chapter 3.

**Drugs and Solutions**

Cocaine hydrochloride, provided by the National Institute on Drug Abuse (Bethesda), and Naloxone (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before use. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.
Procedure

After surgical recovery, all rats were maintained on a water-deprivation regimen (5 min am/1 h pm) and habituated to the experimental chambers for 5 min a day for 3 days. Rats received 5 min access to water from the leftmost spout on the first, the middle spout on the second, and the rightmost spout on the third habituation day. Rats were randomly assigned to either saline (Sham: n=16; TOAx: n=24) or cocaine (Sham: n=21; TOAx: n=30) conditions. Following the three habituation days, water was available ad libitum throughout the experiment in the home cages. The experimental chambers were equipped with three retractable spouts; the leftmost spout was used to present the taste cue, and the remaining two spouts were empty and served as the active (rightmost) and inactive (center) operant spouts. During testing, the left CS spout advanced, allowing 5 min access to 0.15% saccharin. After the 5 min CS access period concluded, the CS spout retracted and the empty active and inactive spouts advanced. A stimulus light was illuminated above the active spout and the rats were placed on a FR10 schedule of reinforcement where completion of 10 licks on the active spout resulted in an intravenous infusion of either saline or cocaine (0.167 mg/infusion) over a 6 sec period, depending on the group assignment. Drug or saline delivery was signaled by offset of the stimulus light and onset of a tone and house light, which remained on for a 20 sec period. Further responding during this time was not reinforced. The access period to the drug or saline lasted for 1h, after which the active and inactive spouts retracted and the subjects were returned to their home cages.

There was one taste-drug pairing per day for 7 days, followed by one progressive ratio (PR) test. During the PR test, the rats were given 5 min access to the CS. Immediately thereafter, the number of responses required for an infusion of cocaine increased by 20 for each successive infusion. The PR session ended after 30 min without an infusion.
Naloxone Test. For the last batch of rats, a naloxone test was performed to test for cue-induced withdrawal. On the naloxone test day, subjects were weighed and then given 5 min access to the taste cue in the self-administration chambers. Thereafter, the rats were given a subcutaneous injection of naloxone (1 mg/kg), rather than the opportunity to self-administer saline or cocaine. Following naloxone administration, rats were returned to the home cages. A second body weight was recorded 2 h after the naloxone injection. A second naloxone test was performed 2 d later, but this time it took place in the home cages as described in Chapter 3.

Histology

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects with misplaced or incomplete lesions were excluded from further analysis.

Statistics

Data from the conditioning trials were first analyzed using a mixed factorial ANOVA with lesion (Sham and TOAx) and drug (cocaine or saline) as between-subjects factors and trial (1-7) as the within-subjects factor. When indicated, data for the Sham and the TOAx groups were analyzed separately. Given individual differences in behavior, Sham and TOAx subjects also were divided into “low” and “high” drug-takers using a median split and those data were analyzed using a mixed factorial ANOVA varying group (Saline, Cocaine-Low, and Cocaine-High) and trial (1-7). Finally, intake of the taste cue and the resultant change in body weight on the naloxone test day were analyzed using one-way ANOVAs comparing drug condition (saline vs. cocaine) for each of the lesion groups (Sham and TOAx) separately. Post hoc Newman-Keuls
test or with unpaired t-tests (two tailed) were conducted where appropriate. Data were considered statistically significant when p < .05.

Results

Histology

Data from six TOAx rats were excluded from statistical analysis due to incomplete lesions. All other rats had substantial damage to the middle third of the VPM as well as the majority of the taste area in the parvicellular area of the VPM (see Figure 5-1) as described in Chapter 2.

Figure 5-1: Digital photomicrographs of coronal sections of Sham (A) and TOAx (B) stained with NeuN. Abbreviations: fr, fasciculus retroflexus; mt, mammillary tract; Pf, parafascicular nucleus; Po, posterior nucleus; SPF, subparafascicular nucleus; TOA, trigeminal orosensory area; VPM, ventral posteromedial nucleus; VPMpc, parvocellular subdivision of the VPM (thalamic taste area).
**CS Latency (Latency to Contact CS Spout)**

Of the 96 initial subjects, five were not run in the experiment due to problems with surgery or health concerns. Data from an additional eight Sham and nine TOAx subjects were excluded as a result of either loss of catheter patency or failure to respond to both the CS and the active spout across all seven trials. Data from these subjects were excluded from all analyses. The remaining group numbers were as follows: Sham Saline: n=12; Sham Cocaine: n=17; TOAx Saline: n=17; TOAx Cocaine n=22.

The latency to contact the CS spout was analyzed using a mixed factorial ANOVA with lesion (Sham and TOAx) and drug (cocaine or saline) as the between-subjects factors and trial (1-7) as the within-subjects factor. There was a significant main effect of drug, $F(1,64) = 12.08, p < .01$, indicating that the rats in the cocaine group were slower than rats in the saline group to initiate licking on the CS spout overall (Figure 5-2). Nevertheless, the main effect of the lesion was not significant ($F < 1$), nor were any related interactions thereof, indicating that the lesion did not affect the latency to contact the CS spout overall. There was a significant drug x trial interaction, $F(6,384) = 4.24, p < .01$, and post hoc tests confirmed that all rats were slower to make contact with the saccharin cue when it predicted the opportunity to self-administer cocaine compared with saline. This effect attained statistical significance overall on trials 6 and 7, $p < .05$. The increased latency appeared to be delayed in the TOAx rats and therefore separate ANOVAs were conducted for the Sham and TOAx rats. These analyses found a significant main effect of drug for both the Sham, $F(1,27) = 5.29, p < .05$, and the TOAx rats, $F(1,37) = 6.66, p < .05$. There was a significant drug x trial interaction for the Sham rats, $F(6,162) = 2.85, p < .05$, and post hoc analyses found a significant difference between the saline and cocaine conditions on trials 4 and 6, $p < .05$. The drug x trial interaction for the TOAx rats was marginal, $F(1,37) = 1.85, p = .09$, and unpaired t-tests indicated a significant difference between the saline and
cocaine conditions only for trial 7, p < .05. Overall, these results indicate that the rats were more hesitant to consume a saccharin taste cue that predicted the opportunity to self-administer cocaine compared with saline. Furthermore, this was true for both Sham and TOA lesioned rats overall, but the effect was delayed in rats with lesions of the TOA.

Figure 5-2: Mean latency (± SEM) to initiate licking of the 0.15% saccharin CS that predicts the opportunity to self-administer either saline (open circles) or cocaine (solid circles) for Sham (left) and TOAx (right) rats across 7 trials. *indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=12, Cocaine N=17; TOAx: Saline N=17, Cocaine N=22

CS Intake (Licks/5 min)

Intake of the saccharin taste cue during the seven conditioning trials was analyzed using a mixed factorial ANOVA varying lesion, drug, and trial as factors. A significant main effect of
drug, $F(1,64) = 27.81$, $p < .001$, indicated that the rats in the cocaine group drank less of the saccharin cue than did rats in the saline group (Figure 5-3). As was found with the latency to consume the taste cue, there was no significant main effect of lesion ($F < 1$), or any accompanying interactions for intake of the saccharin taste cue ($Fs < 1$). This means that, when the saccharin taste cue predicted the opportunity to self-administer cocaine, the lesion did not affect conditioned avoidance of the saccharin taste cue. There was a significant drug x trial interaction, $F(6,384) = 7.45$, $p < .001$, and post hoc analyses indicate that rats in the cocaine group consumed less of the saccharin taste cue compared with rats in the saline group on trials 2-7, $ps < .05$. These results indicate that rats avoid the saccharin cue when paired with the opportunity to self-administer cocaine, but not saline, irrespective of lesion. Given that the TOAx rats appeared to be delayed in their acquisition of this effect, separate ANOVAs were conducted for the Sham and TOAx groups. Results of these analyses indicated that the main effect of drug was significant for both the Sham, $F(1,27) = 15.18$, $p < .01$, and TOAx rats, $F(1,37) = 11.42$, $p < .01$. Post hoc analyses of the significant drug x trial interaction for the Sham, $F(6,162) = 2.48$, $p < .05$, and TOAx rats, $F(6,222) = 6.03$, $p < .001$, revealed significant avoidance of CS intake in the cocaine condition compared with the saline condition for the Sham rats on trials 3-7, $ps < .05$, and on trials 5-7 for the TOAx rats, $ps < .05$. These findings show that the TOAx rats acquired avoidance of the cocaine-paired taste cue, but more slowly than did rats in the Sham condition.
Figure 5-3: Mean 5-min intake (± SEM) of the 0.15% saccharin CS that predicts the opportunity to self-administer either saline (open circles) or cocaine (solid circles) for Sham (left) and TOAx (right) rats across 7 trials. * indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=12, Cocaine N=17; TOAx: Saline N=17, Cocaine N=22

Self-Administration (Infusions/60 min)

As has occurred previously (Grigson & Twining, 2002; Twining et al., 2009), individual differences were evident in cocaine self-administration for both the Sham and the TOAx subjects. Consequently, subjects were ranked on average infusions taken on terminal days 6 and 7, and divided, using a median split, into low and high drug-takers. Thereafter, the data were analyzed using mixed factorial ANOVAs varying lesion (Sham and TOAx), drug-taking group (Saline, Cocaine-Low, and Cocaine-High), and trial (1-7) as factors. The results showed that rats in the Cocaine-High group took more infusions than did the rats in both the Cocaine-Low group and the
Saline group (Figure 5-4). This conclusion was supported by a significant main effect of drug-taking group, F(2,62) = 30.74, p < .001. Furthermore, this pattern was evident for both the lesioned and the intact rats, as there was no significant main effect of lesion (F < 1) or any related interactions (Fs < 1). There was a significant drug-taking group x trial interaction, F(12,372) = 4.97, p < .001, and post hoc analyses indicated that rats in the Cocaine-High group took more infusions than rats in the Cocaine-Low group or in the Saline group on trials 2-7, ps < .05. Furthermore, there was no difference in the number of infusions between the Saline group and Cocaine-Low group on any trial overall, ps > .05. Post hoc analyses found a significant difference in the number of infusions for the Cocaine-Low condition compared with the Saline condition for TOAx rats on trials 5-7, ps < .05, but no significant differences were found for the Sham rats, ps > .05. There were significant differences for the Cocaine-High condition compared with the Saline condition for Sham rats on trials 2-7, ps < .05 and for the TOAx rats on trials 1-7, ps < .05. Post hoc tests also indicated a significant difference between the Cocaine-Low and Cocaine-High drug-takers for the Sham rats on trials 3-7, ps < .05, and for the TOAx rats on trials 1-7, ps < .05. Overall, these results confirm that individual differences within cocaine self-administering rats yields two distinct groups: “low” drug-takers and “high” drug-takers. These results also indicate that the TOA lesion did not have an effect on cocaine self-administration, nor on the division of “low” and “high” drug-takers.
Figure 5-4: Mean infusions/h (± SEM) of cocaine (0.167 mg/infusion) or saline across 7 trials for Sham (left) and TOAx (right) rats. Rats in the cocaine group were divided into “low” and “high” drug takers. Significant differences between the saline and cocaine conditions are indicated by an asterisk for the Cocaine-Low*, and Cocaine-High* groups (p < .05). # denotes a significant difference between the Cocaine-Low and Cocaine-High groups (p < .05). Sham: Saline N=12, Cocaine-Low N=8, Cocaine-High N=9; TOAx: Saline N=17, Cocaine-Low N=9, Cocaine-High N=13

Active Spout Preference (Active/Total Responses)

In an effort to assess goal-directed behavior, the preference ratio for the active spout was determined by dividing the number of active responses by the total active and inactive responses during each trial. The data were analyzed as described for the number of infusions. The results of this analysis yielded a significant main effect of drug-taking group, F(2,62) = 5.05, p < .01, and post hoc tests of this main effect showed that rats in the Cocaine-High group showed increased preference for the active spout compared with the Cocaine-Low and Saline groups overall (Figure 5-4).
This effect was seen in both intact and lesioned rats, as indicated by a lack of a significant main effect of lesion (F < 1), or any interactions thereof, p > .05. Because the effect seemed to be more prominent in the TOAx group, separate ANOVAs were conducted for the Sham and TOAx rats. The results showed that there was a significant main effect of drug-taking group for the TOAx rats, F(2,36) = 7.41, p < .01, but not for the Sham rats, F < 1. Neither Sham nor TOAx rats had a significant drug-taking group x trial interaction (F < 1); however, unpaired t-tests found greater preference for the active spout for TOAx rats in the Cocaine-High condition compared with the Saline condition on all trials except for trial 2, ps < .05. Additionally, unpaired t-tests found greater preference for TOAx rats in the Cocaine-High group compared with the Cocaine-Low group on trials 2-6, ps < .05. There were no significant differences on any trial for the Sham condition, ps > .05. These results show that rats in the Cocaine-High group displayed increased preference for the active spout compared with rats in the Saline and Cocaine-Low groups across both lesion conditions – and the effect was more marked in the TOAx rats.
Progressive Ratio-Break Point (Last Ratio Completed)

The break point was determined by the last ratio completed during the progressive ratio test. Data were analyzed using a two-way ANOVA varying lesion (Sham or TOAx) and drug-taking group (Saline, Cocaine-Low, and Cocaine-High). Post hoc tests of a significant main effect of drug taking group, F(2,62) = 23.84, p < .001, revealed that rats in the Cocaine-High group worked harder for drug than either the Cocaine-Low or Saline groups, regardless of the lesion condition, ps < .05, (Figure 5-6). Neither the main effect of lesion, F < 1, nor the lesion x
drug-taking group interaction, F < 1, was significant. Unpaired t-tests revealed significantly higher breakpoints for the Cocaine-High group compared with the Saline and Cocaine-Low groups for Sham and TOAx rats, ps < .05. Furthermore, there was also a significantly higher break point for the Cocaine-Low group compared with the Saline group in the TOAx rats, p < .05. The results indicate that the lesion did not interfere with the motivation to take drug, as measured by the progressive ratio test. Overall, high drug-takers in both lesion groups worked harder to obtain infusions of cocaine compared with low drug-takers and with saline-treated controls.

Figure 5-6: Mean break point (± SEM) for Sham (left) or TOAx (right) rats. Break point was quantified as the last completed ratio in the progressive ratio test. Data were divided into Cocaine-Low and Cocaine-High groups. Significant differences between the saline and cocaine conditions are indicated by an asterisk for the Cocaine-Low*, and Cocaine-High* groups (p < .05). # denotes a significant difference between the Cocaine-Low and Cocaine-High groups (p < .05). Sham: Saline N=12, Cocaine-Low N=8, Cocaine-High N=9; TOAx: Saline N=17, Cocaine-Low N=9, Cocaine-High N=13
**Naloxone Test (Self-Administration Chambers)**

Intake of the taste cue and resultant change in body weight for the naloxone test for the third batch of rats (Sham: Saline: n=8, Cocaine: n=9; TOAx: Saline: n=11, Cocaine: n=15) were initially analyzed using a two-way ANOVA comparing the lesion and drug-taking group. Because there was no difference in CS intake or in the 2 h change in body weight between Cocaine-Low and Cocaine-High groups, at this juncture, the data were not divided as a function of low and high drug-takers and were reanalyzed comparing lesion and drug (cocaine or saline).

**CS Intake.** The results indicate that rats drank less of the taste cue when it predicted access to self-administered cocaine compared with saline, and this effect was true for both Sham and TOAx lesioned rats (Figure 5-7a). This conclusion was confirmed by a significant main effect of drug, $F(1,39) = 86.67$, $p < .001$, and lack of a significant main effect of lesion ($F < 1$), or lesion x drug interaction ($F < 1$).

**Change in Body Weight.** The results also found a significant loss in body weight, used here as an index of withdrawal, 2 h following exposure to the taste cue that was paired with cocaine but not saline self-administration (Figure 5-7b). This was evidenced by a significant main effect of drug, $F(1,39) = 17.86$, $p < .001$. Furthermore, this was the case for both lesion conditions, as was evidenced by a non-significant main effect of lesion ($F < 1$), and a non-significant lesion x drug interaction ($F < 1$). Overall, these results confirm that the rats showed a precipitous loss in body weight following a naloxone test when presented with a taste cue that had come to predict the opportunity to self-administer cocaine. This was not the case when the taste cue predicted the opportunity to self-administer saline.
Naloxone Test (Home Cage)

The intake of the taste cue and resultant change in body weight from the naloxone test in the home cage were analyzed the same way as the first naloxone test data. Analyses compared the lesion and drug (saline or cocaine) conditions following verification that no differences existed between Cocaine-Low and Cocaine-High groups.

**CS Intake.** Post hoc analyses of a modest lesion x drug interaction, $F(1,39) = 3.18$, $p = .08$, revealed that CS intake was significantly lower for the Sham cocaine group compared with the TOAx cocaine group, $p < .05$ (Figure 5-8a). Therefore, the data were subsequently reanalyzed separately for each lesion condition. Results of these analyses found a significant main effect of drug for the Sham rats, $F(1,15) = 23.23$, $p < .001$, confirming avoidance of the taste cue in the cocaine group compared with the saline group. The main effect of drug for the TOAx rats, however, was not significant, $F(1,24) = 2.83$, $p = .11$. This indicates that, while
intake in the cocaine condition was slightly lower than that of the saline condition, avoidance of the cocaine-paired taste cue was not evident in the lesioned rats when tested subsequently in the home cage.

**Change in Body Weight.** Results of the two-way ANOVA for the change in body weight following the home cage naloxone test yielded a significant main effect of lesion, $F(1,39) = 8.01$, $p < .01$, indicating a smaller gain in body weight for the Sham rats compared with the TOAx rats overall (Figure 5-8b). The main effect of drug was significant, $F(1,39) = 8.85$, $p < .01$, as was the lesion x drug interaction, $F(1,39) = 6.53$, $p < .05$. Post hoc tests of this two-way ANOVA revealed significant loss in body weight for Sham, $p < .01$, but not TOAx, $p > .05$, rats in the cocaine group compared with their respective saline controls. Post hoc analyses also showed a significant difference between Sham and TOAx rats in the cocaine condition, $p < .001$. Taken together, these findings indicate that retention of a previously acquired saccharin-cocaine association depends upon the availability of contextual cues in TOAx rats. The drug-paired taste cue, alone, was inadequate to elicit cue-induced withdrawal in the lesioned rats.

![Figure 5-8](image)

*Figure 5-8: (a) Mean 5-min intake (± SEM) of the 0.15% saccharin CS that predicts the opportunity to self-administer either saline or cocaine for Sham (left) and TOAx (right) rats at naloxone test in the home cage. (b) Mean change in body weight (g) (± SEM) for Sham (left) or TOAx (right) rats 2-h after the naloxone test. *indicates a significant difference between the saline and cocaine conditions ($p < .05$). # denotes a significant difference between Sham and TOAx groups ($p < .05$). Sham: Saline N=8, Cocaine=9; TOAx: Saline N=11, Cocaine N=15
Discussion

As predicted, the lesion failed to inhibit avoidance of the saccharin cue when it predicted the opportunity to self-administer cocaine in the presence of contextual and drug-contingent cues. Furthermore, the lesion did not alter the motivation to obtain drug or drug-taking, as was demonstrated by increased infusions in the cocaine condition compared with the saline condition for both Sham and TOAx rats. The TOA, then, is not essential for the experience of drug reward or for the motivation to obtain drug. These findings parallel those obtained when the taste-drug pairings occurred in a runway apparatus (Chapter 4). Finally, when tested in the self-administration chamber, cue-induced withdrawal was evident in both Sham and TOAx rats. When subsequently retested in the home cage, devoid of contextual cues, only the Sham rats displayed cue-induced withdrawal in response to the cocaine-paired taste cue. These findings suggest that contextual cues present in the self-administration chambers are sufficient to support conditioned avoidance of the taste cue in intact and lesioned subjects. Therefore, in the absence of these additional cues, as was the case when retested in the home cage, the lesion prevented retention of the previously acquired taste-drug association. In Experiments 2 and 3, the involvement of context was addressed by removing all contextual and drug-contingent cues in Experiment 2, and then reintroducing only contextual cues in Experiment 3.

Experiment 2: Self-Administration of Cocaine in the Absence of Contextual and Drug-Contingent Cues

In Experiment 1, the TOA lesion failed to attenuate the avoidance of a taste cue paired with the opportunity to self-administer cocaine in our standard self-administration paradigm, but it did prevent avoidance of the taste cue and associated signs of cue-induced withdrawal when retested in the home cage. This suggests that the contextual cues present in the self-
administration chamber either increased the associative strength of the taste cue, or were themselves associated with the opportunity to self-administer drug, thus outweighing the deficit caused by the TOA lesion. The purpose of Experiment 2, then, was to determine the role of contextual cues.

Our standard self-administration procedure entails pairing 5 min access of a saccharin taste cue with 60 min to self-administer cocaine or an equal volume of saline using a fixed ratio schedule of reinforcement where 10 responses on the ‘active’ empty spout results in an intravenous infusion. A stimulus light illuminates above the active spout, and drug or saline delivery is signaled by offset of this stimulus light and onset of a tone and house light, which remain on for a 20 sec period. This procedure was modified in Experiment 2 to eliminate salient drug-contingent cues. Accordingly, no cue lights or tones were employed to signal intravenous drug or saline delivery. Furthermore, subjects resided in the self-administration chambers throughout the duration of the study to eliminate conditioning of contextual cues. We hypothesized, based on the findings from Experiment 1, that context is necessary for TOA lesioned rats to learn to avoid a drug-paired saccharin cue. If correct, the TOAx group should not avoid intake of the saccharin taste cue in the absence of contextual cues. Furthermore, if the acquisition of self-administration is dependent on these cues, neither intact nor lesioned subjects should acquire stable cocaine self-administration.

Methods

Subjects

The subjects were 24 naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed
individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise. Subjects were at least 300 g prior to surgery. During all training and testing, rats were housed in the self-administration chambers, as described below.

**Surgery**

Following acclimation to the colony room, the subjects underwent either bilateral ibotenic acid lesions of the thalamic trigeminal orosensory area (TOAx: n=14) or vehicle infusions (Sham: n=10) as described in Chapter 2. Intrajugular catheters were implanted as described in Chapter 3.

**Apparatus**

The apparatus was identical to that described in Experiment 1.

**Drugs and Solutions**

Cocaine hydrochloride, provided by the National Institute on Drug Abuse (Bethesda), and Naloxone (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before use. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.
Procedure

After recovery from surgery, all rats underwent the same procedure as in Experiment 1, with the following exceptions: (a) rather than living in the home cage and being transferred to the self-administration chambers for daily testing, subjects resided in the self-administration chambers 24 h per day. Ad libitum food and water were available in the self-administration chambers 21 h per day. Otherwise, food was removed from the chamber 1 h before daily testing and replaced 1 h after daily testing. (b) The house light was continuously illuminated and the stimulus light above the active spout was not illuminated at any time. (c) Drug or saline delivery was no longer signaled by the onset of the tone. (d) A single naloxone test took place in the self-administration chambers after the seven conditioning trials. Otherwise, all aspects of this procedure were identical to those described in Experiment 1.

Histology

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects found to have misplaced or incomplete lesions were excluded from further analysis.

Statistics

Data from the seven conditioning trials were first analyzed using a mixed factorial ANOVA with lesion (Sham and TOAx) and drug (cocaine or saline) as between-subjects factors and trial (1-7) as the within-subjects factor. When indicated, the data for the two lesion groups (Sham and TOAx) were combined and reanalyzed using a one-way ANOVA varying drug (cocaine or saline). Newman-Keuls post hoc analyses were used to further interpret the results of
significant interactions. Unpaired t-tests (two tailed) also were employed. The intake of the taste
cue and resultant change in body weight on the naloxone test day were analyzed using two-way
ANOVAs varying lesion (Sham and TOAx) and drug (cocaine or saline). Here and elsewhere,
data are considered statistically significant when p < .05.

Results

Histology

Data from one TOAx rat was excluded from statistical analysis due to an incomplete
lesion as described in Experiment 1 (see Figure 5-1).

CS Latency (Latency to Contact CS Spout)

Data from two rats (one Sham, one TOAx) were excluded due to a failure to respond to
both the CS and active spout across all seven trials. Data from these subjects was excluded from
all analyses.

The latency to make contact with the CS spout was analyzed using a mixed factorial
ANOVA with lesion (Sham and TOAx) and drug (cocaine or saline) as the between-subjects
factors and trial (1-7) as the within-subjects factor. Results of these analyses yielded no
significant main effect of drug, F(1,17) = 1.94, p = .18, or lesion (F < 1), or any accompanying
interactions thereof, Fs < 1 (Figure 5-9). This indicates that, overall, when housed in the self-
administration chambers, rats failed to show an increase in the latency to consume the saccharin
cue when it predicted the opportunity to self-administer cocaine compared with saline.
Furthermore, this was true for both lesion conditions.
**CS Intake (Licks/5 min)**

Intake of the saccharin taste cue during the seven conditioning trials was analyzed similar to the CS Latency data. Results of the mixed factorial ANOVA varying lesion, drug, and trial yielded no significant main effect of drug, $F(1,17) = 1.11$, $p = .31$, nor lesion ($F < 1$), nor any interactions thereof, $Fs < 1$ (Figure 5-10). This indicates that, overall, when housed in the self-administration chambers, rats failed to show avoidance of the saccharin cue when it predicted the opportunity to self-administer cocaine compared with saline. Furthermore, this was true for both the Sham and the TOAx rats. As a whole, these data show that, in the absence of salient
contextual cues, all rats failed to significantly avoid intake of the saccharin cue that had been paired with the opportunity to self-administer cocaine.

![Graph](image)

**Figure 5-10**: Mean 5-min intake (± SEM) of the 0.15% saccharin CS that predicts the opportunity to self-administer either saline (open circles) or cocaine (solid circles) over 7 trials for Sham (left) and TOAx (right) rats. Sham: Saline N=4, Cocaine, N=5; TOAx: Saline N=6, Cocaine, N=6

**Self-Administration (Infusions/60 min)**

The number of infusions across trials was first analyzed using a mixed factorial ANOVA varying lesion, drug, and trial. Results yielded no significant main effect of drug (F < 1) or lesion, F(1,17) = 1.87, p = .19, suggesting that, like CS Intake and Latency, the lesion, and furthermore the drug, were without effect overall. Accordingly, there were no significant accompanying interactions, Fs < 1. However, the number of infusions was low for all subjects.
until the last few trials; therefore, the data were reanalyzed for the final conditioning trial only using a two-way ANOVA varying lesion and drug. This analysis yielded a significant main effect of lesion, $F(1,17) = 5.11$, $p < .05$, which indicated higher drug taking for the TOAx rats, compared with the Sham rats overall (Figure 5-11). There also was a significant main effect of drug, $F(1,17) = 5.88$, $p < .05$, indicating a higher number of infusions of cocaine compared with saline overall. While the number of infusions was greater for cocaine than saline, and the TOAx group took more infusions than the Sham group overall, the lesion x drug interaction did not attain statistical significance, $F(1,17) = 2.05$, $p = .17$. A $t$-test revealed, however, that the TOAx rats in the cocaine condition took more infusions than rats in the TOAx saline, Sham saline, and Sham cocaine conditions, $p s < .05$. These results indicate increased drug-taking in the TOAx rats when housed in the self-administration chamber in the absence of salient cues compared with the Sham rats.

![Graph](5-11.png)

Figure 5-11: Mean infusions/h (± SEM) of saline or cocaine (0.167 mg/infusion) on the final conditioning trial for Sham (left) and TOAx (right) rats. *indicates a significant difference between the saline and cocaine conditions ($p < .05$). # denotes a significant difference between Sham and TOAx rats ($p < .05$). Sham: Saline $N=4$, Cocaine, $N=5$; TOAx: Saline $N=6$, Cocaine $N=6$
Active Spout Preference (Active/Total Responses)

The preference ratio for the active spout was determined by dividing the number of active responses by the total active and inactive responses during each trial. As before, data were analyzed using a mixed factorial ANOVA varying lesion, drug, and trial. Results yielded a significant lesion x drug interaction, F(1,17) = 4.85, p < .05. Post hoc analyses of the significant interaction indicated that the TOAx cocaine group had greater preference for the active spout compared with the TOAx saline group, p < .05, an effect that was not present in the Sham condition, p > .05 (Figure 5-12). Accordingly, there was no significant main effect of lesion (F < 1), or drug (F < 1), nor a significant lesion x drug x trial interaction (F < 1). Unpaired t-tests for the TOAx rats indicated greater preference in the cocaine condition compared with the saline condition on trials 3, 5, and 7, ps < .05. These data show that preference for the active spout was increased for the cocaine group compared with the saline group, but this was only the case for the lesioned rats. Sham rats did not show a difference in active spout preference between the saline and cocaine condition. This was unusual and likely had to do with the absence of contextual cues.
Progressive Ratio-Break Point (Last Ratio Completed)

The break point was determined by the last ratio completed during the progressive ratio test. Data were analyzed using a two-way ANOVA with lesion (Sham and TOAx) and drug (cocaine or saline) as factors. Results of these analyses yielded no significant main effect of drug, F(1,17) = 1.85, p = .19, or lesion F(1,17) = 1.65, p = .22, nor a significant lesion x drug interaction, F < 1, (Figure 5-13). Follow up analysis combining the Sham and TOAx groups also found no significant main effect of drug, F(1,19) = 2.01, p = .17. This indicates that, in the

Figure 5-12: Mean preference score (± SEM) for the active spout responses/total active and inactive responses across 7 trials for Sham (left) and TOAx (right) rats. *indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=4, Cocaine, N=5; TOAx: Saline N=6, Cocaine N=6
absence of salient contextual and drug-contingent cues, rats did not work for drug on a progressive ratio schedule of reinforcement.

![Graph](image)

**Figure 5-13:** Mean break point (± SEM) for Sham (left) and TOAx (right) rats. Break point was quantified as the last completed ratio in the progressive ratio test. Sham: Saline N=4, Cocaine, N=5; TOAx: Saline N=6, Cocaine, N=6

**Naloxone Test**

Intake of the taste cue and the resultant change in body weight on the naloxone test day were analyzed using a two-way ANOVA comparing the lesion and drug. **CS Intake.** The results found no significant main effect of drug, F(1,17) = 2.39, p = .14, or lesion, F(1,17) = 2.88, p = .11, nor a significant lesion x drug interaction, F(1,17) = 1.87, p = .19 (Figure 5-14a). The findings parallel those found for the conditioning data, indicating that, when housed in the self-administration chambers rats do not reliably avoid intake of a saccharin taste cue that predicts access to the self-administration of cocaine.
**Change in Body Weight.** Results of the two-way ANOVA for the change in body weight following the naloxone test yielded a main effect of lesion that neared significance, $F(1,17) = 4.09$, $p = .06$ (Figure 5-14b). The change in body weight appears lower for the TOAx group overall compared with the Sham group following the naloxone test, although the trend is not statistically significant. Furthermore, there was no significant main effect of drug ($F < 1$) or lesion x drug interaction ($F < 1$), indicating that the drug condition had no effect on the change in body weight following the naloxone test. Overall, these findings reveal that in the absence of salient contextual and drug-contingent cues to indicate drug availability, the rats did not avoid intake of a saccharin cue that predicted cocaine self-administration, nor did they express a resultant loss in body weight when the saccharin cue was followed by an injection of naloxone. The taste cue, then, did not support the expression of indices of conditioned withdrawal in this instance.

![Graph](image)

**Figure 5-14:** (a) Mean 5-min intake (± SEM) of the 0.15% saccharin CS that predicts the opportunity to self-administer saline or cocaine for Sham (left) and TOAx (right) rats at naloxone test. (b) Mean change in body weight (g) (± SEM) for Sham (left) or TOAx (right) rats 2-h after the naloxone test. Sham: Saline N=4, Cocaine=5; TOAx: Saline N=6, Cocaine N=6
Discussion

In the absence of contextual cues and drug-contingent cues (i.e., lights and tones), Sham and TOAx rats failed to evidence conditioned avoidance of a saccharin cue that predicted the opportunity to self-administer cocaine. This was evident by both a short latency to lick the saccharin cue and by sustained intake of the taste cue. In addition, removal of both contextual and drug-contingent cues appears to have delayed the establishment of cocaine self-administration as well. Thus, the rats consumed the sweet cue, took relatively little drug, and failed to work for the drug when challenged on a progressive ratio schedule of reinforcement. That said, the TOAx rats did show preference for the active spout when it predicted the opportunity to self-administer cocaine, and did take more infusions of cocaine compared with the Sham rats. Finally, when challenged with naloxone, intact and lesioned rats did not reliably avoid intake of the saccharin cue that predicted cocaine self-administration, nor did they express a resultant loss in body weight when the saccharin cue was followed by an injection of naloxone in this paradigm. Removal of contextual and drug-contingent cues, then, appears to have led to a failure to associate the taste cue with drug, possibly because of a failure to engage in stable drug self-administration behavior. These results emphasize the importance of contextual and drug-contingent cues in the development of operant conditioning and in the development of related Pavlovian associations.

Nevertheless, the lesion was not without effect when contextual and drug-contingent cues were removed. Results showed that preference for the active spout by the TOAx rats was increased for the cocaine group compared with the saline group. Sham rats did not show a difference in active spout preference between the saline and cocaine groups. This suggests increased goal-directed behavior and increased responsiveness to reward in general for the TOA lesioned rats. In support, the TOAx rats also exhibited a tendency to take more infusions overall.
compared with the Sham rats. Given the rather small sample size, these effects may be borne out upon a replication.

**Experiment 3: The Impact of Context in the Avoidance of a Taste Cue that Predicts Self-Administered Cocaine**

In Experiment 3, contextual cues were reintroduced by housing the rats that had served in Experiment 2 in regular home cages and exposing them to the self-administration chambers only during conditioning trials and testing. No additional drug-contingent cues (i.e., lights and tones) were present in the self-administration chamber to signal drug delivery. Therefore, the only cues that predicted the opportunity to self-administer saline or cocaine were the context of the self-administration chamber and the saccharin taste cue. In Experiment 2, the lesioned rats took more infusions overall than the Sham rats, yet failed to avoid the saccharin cue. In Experiment 3, if contextual cues are sufficient to circumvent the effects of the TOA lesion, then the lesioned rats should avoid intake of the saccharin cue when it predicts the opportunity to self-administer cocaine compared with saline. Avoidance of the saccharin cue also was not observed in the intact rats in Experiment 2; however, they took fewer infusions than the TOAx rats. Therefore, the Sham rats had little reason to suppress intake of saccharin, while the TOAx rats, who took slightly more cocaine, should have shown some avoidance of the cue. Accordingly, if contextual cues in Experiment 3 promote the development of cocaine self-administration in the intact rats, suppression of the cocaine-paired saccharin cue was expected for these rats as well.
Methods

Subjects

The subjects were 36 male, Sprague-Dawley rats. Twenty-four rats from Experiment 2 (10 Sham, 14 TOAx) were used in Experiment 3. These subjects were reassigned to cocaine and saline groups in a partial crossover design counterbalanced to control for prior experience. Furthermore, an additional 12 naive rats of the same age were included in Experiment 3. These additional rats underwent lesion and catheter surgeries at the same time as the 24 rats from Experiment 2, and were maintained under the same conditions. They all were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise.

Apparatus

The apparatus was identical to that described in Experiment 1.

Drugs and Solutions

Cocaine hydrochloride, provided by the National Institute on Drug Abuse (Bethesda), and Naloxone (Sigma Chemical, St. Louis, MO) were prepared in sterile saline immediately before use. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH2O and presented at room temperature.
Procedure

All rats underwent the same procedure as in Experiment 2, with the exception that they no longer resided in the self-administration chambers. Food and water were available Ad Libitum in the home cages and subjects were only transferred to the self-administration chambers during daily testing. All other aspects of this procedure were identical to those described in Experiment 2, i.e., drug delivery was not signaled by lights and tones.

Histology

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects found to have misplaced or incomplete lesions were excluded from further analysis.

Statistics

Data from the conditioning trials were analyzed using a mixed factorial ANOVA with lesion (Sham and TOAx) and drug (cocaine or saline) as between-subjects factors and trial (1-7) as the within-subjects factor. When indicated, the Sham and the TOAx groups were analyzed separately. Given individual differences in behavior, Sham and TOAx subjects also were divided into “low” and “high” drug-takers using a median split and those data were analyzed using a mixed factorial ANOVA varying drug-taking group (Saline, Cocaine-Low, and Cocaine-High) and trial (1-7). Finally, intake of the taste cue and resultant change in body weight on the naloxone test day were analyzed using one-way ANOVAs comparing drug (saline or cocaine) for each of the lesion groups (Sham and TOAx) separately. Post hoc Newman-Keuls tests or
unpaired t-tests (two tailed) were conducted where appropriate. Data were considered statistically significant when $p < .05$.

**Results**

**Histology**

Data from one TOAx rat were excluded from statistical analysis due to an incomplete lesion. Data from the remaining 15 Sham (Saline: $n=6$; Cocaine: $n=9$) and 20 TOAx rats (Saline: $n=6$; Cocaine: $n=14$) were included in the following analyses.

**CS Latency (Latency to Contact CS Spout)**

The latency to lick the 0.15% saccharin cue that predicted the opportunity to self-administer saline or cocaine was analyzed using a mixed factorial ANOVA varying lesion (Sham and TOAx), drug (cocaine or saline), and trial (1-7). The results indicated a significant main effect of drug, $F(1,31) = 6.76$, $p < .05$, and post hoc tests showed that the rats in the cocaine condition were slower to initiate contact with the saccharin cue compared with rats in the saline condition overall (Figure 5-15). Neither the main effect of lesion ($F < 1$), nor any interactions thereof ($Fs < .05$), were statistically significant. These data indicate that lesioned and intact rats are slower to initiate licking of a saccharin cue when it predicts the opportunity to self-administer cocaine in the presence of contextual cues. That said, and as is evident from Figure 5-15, this effect was more marked in the TOAx rats. Consequently, separate follow up drug x trial ANOVAs were conducted for the Sham and the TOAx rats. The results of these ANOVAs revealed a significant main effect of drug for the TOAx rats, $F(1,18) = 7.64$, $p < .05$, but not for
the Sham rats, F < 1. The drug x trial interaction was not significant for either Sham, F(6,78) = 1.59, p = .16, or TOAx rats, F < 1, however, unpaired t-tests revealed a significantly greater latency to contact the CS spout in the cocaine condition compared with the saline condition for the TOAx rats on trials 2, and 4-7, ps < .05. Follow up analyses for the Sham and TOAx rats varying previous experience (Saline, Cocaine, and Naive), drug, and trial indicated that this effect was carried by rats in the TOAx group with previous cocaine experience in Experiment 2. This was indicated by a significant main effect of previous experience for the TOAx rats, F(2,15) = 4.09, p < .05, but not the Sham rats, F < 1. Therefore, the increased latency to contact the CS spout was only significant for the TOAx rats. This may have been the result of the previous experience with cocaine self-administration in Experiment 2, which was slightly greater in the TOAx rats compared with the Sham rats.

Figure 5-15: Mean latency (± SEM) to initiate licking of the 0.15% saccharin cue that predicts the opportunity to self-administer saline (open circles) or cocaine (solid circles) for Sham (left) and TOAx (right) rats across 7 trials. *indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=6, Cocaine, N=9; TOAx: Saline N=6, Cocaine N=14
Results of the mixed factorial ANOVA varying lesion, drug, and trial for intake of the saccharin taste cue yielded a significant main effect of drug, F(1,31) = 21.13, p < .001. Post hoc analyses indicated that intake of the saccharin cue was decreased when it predicted the opportunity to self-administer cocaine compared with saline overall (Figure 5-16). Furthermore, there was a significant drug x trial interaction, F(6,186) = 15.86, p < .001, and post hoc analyses indicated that intake was significantly lower for the cocaine condition compared with the saline condition overall for trials 2-7, ps < .05. There was not, however, a significant main effect of lesion (F < 1), nor any related interactions, Fs < 1. Nevertheless, this effect seemed more striking in the TOAx rats, which may result from previous experience as it did for the CS latency. Therefore, the data were reanalyzed separately for Sham and TOAx rats using mixed factorial ANOVAs with drug, previous experience, and trial as factors. The results showed a significant main effect of drug for both Sham, F(1,9) = 6.39, p < .05, and TOAx rats, F(1,15) = 16.02, p < .01, and also indicated that the effect was more significant in the TOAx rats. There was no significant main effect of previous experience for either the Sham, F(2,9) = 1.92, p = .2, or the TOAx rats, F(2,15) = 2.32, p = .13. Furthermore, there were no significant interactions for either lesion group with respect to previous experience (ps > .05). This indicates that when contextual cues were available, both the Sham and TOAx rats avoided the drug-paired cue, and this was independent of previous experience. Finally, post hoc analyses of a significant drug x trial interaction for the Sham, F(6,54) = 9.19, p < .001, and TOAx rats, F(6,90) = 8.30, p < .001, indicated avoidance of the taste cue was significant on trials 2, 3, 4, and 6 for the Sham, ps < .05, and 2-7 for the TOAx rats, ps < .05. Overall, avoidance of the saccharin cue was present for both intact and lesioned rats when it predicted the opportunity to self-administer cocaine compared
with saline and, while the effect was more evident in TOAx rats, it was not a result of previous experience.

**Figure 5-16:** Mean 5-min intake (± SEM) of the 0.15% saccharin cue that predicts the opportunity to self-administer saline (open circles) or cocaine (solid circles) for Sham (left) and TOAx (right) rats across 7 trials. *indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=6, Cocaine, N=9; TOAx: Saline N=6, Cocaine N=14

**Self-Administration (Infusions/60 min)**

As found in Experiment 1, individual differences also were evident in cocaine self-administration for the Sham and the TOAx subjects in this experiment. Consequently, subjects were ranked on average infusions taken on terminal days 6 and 7, and divided, using a median split, into low and high drug-takers for each lesion. Subsequently, a mixed factorial ANOVA was
conducted varying lesion, drug-taking group (Saline, Cocaine-Low, and Cocaine-High), and trial (1-7). The results of this analysis yielded a significant main effect of lesion, $F(1,29) = 4.34, p < .05$, and post hoc analyses indicated that the TOAx rats took more infusions than the Sham rats overall, $p < .05$ (Figure 5-17). Therefore, the data were subsequently reanalyzed separately for each lesion group. The results of the separate analyses showed no significant main effect of drug-taking group for the Sham rats, $F(2,12) = 1.05, p = .38$, or a significant drug-taking group x trial interaction, $F(12,72) = 1.36, p = .21$. Furthermore, there was a significant main effect of drug-taking group for the TOAx rats, $F(2,17) = 7.86, p < .001$. Post hoc tests of this main effect showed that rats in the Cocaine-High group took more infusions than did the rats in both the Cocaine-Low group and the Saline group, $ps < .05$. There was, however, no significant drug-taking group x trial interaction, $F(12,102) = 1.19, p = .30$. Unpaired t-tests for the TOAx rats found a greater number of infusions for the Cocaine-High group compared with the Saline group on trials 3, and 5-7, $ps < .05$, and compared with the Cocaine-Low group on trials 1, 3, and 5, $ps < .05$. These results indicate that rats in the TOAx Cocaine-High group took more infusions than did rats in either the Saline or Cocaine-Low groups.
Active Spout Preference (Active/Total Responses)

The preference ratio for the active spout was determined by dividing the number of active responses by the total active and inactive responses during each trial. Data were analyzed using a mixed factorial ANOVA varying lesion, drug-taking group, and trial. These results yielded no significant main effect of lesion, $F(1,29) = 2.39$, $p = .13$, or drug-taking group, $F < 1$, nor any
significant interactions thereof, Fs < 1, (Figure 5-18). Therefore, neither the Sham nor the TOAx rats showed preference for the active spout in Experiment 3.

**Figure 5-18**: Mean preference score (± SEM) for the active spout responses/total active and inactive responses across 7 trials for Sham (left) and TOAx (right) rats. Sham: Saline N=6, Cocaine-Low N=5, Cocaine-High N=4; TOAx: Saline N=6, Cocaine-Low N=7, Cocaine-High N=7

*Progressive Ratio-Break Point (Last Ratio Completed)*

The break point was determined by the last ratio completed during the progressive ratio test. Data were analyzed using a two-way ANOVA varying lesion (Sham or TOAx) and drug-taking group (Saline, Cocaine-Low, and Cocaine-High). Results from the progressive ratio test indicated that rats in the TOAx Cocaine-High group worked harder for drug than the Cocaine-
Low or Saline groups, ps < .05, an effect that was not present in the Sham Cocaine-High group, ps > .05 (Figure 5-19). This conclusion was supported by post hoc tests of a significant lesion x drug-taking group interaction, F(2,29) = 4.47, p < .05. The main effect of drug-taking group approached significance, F(2,29) = 2.64, p = .09, which suggests that, in general, rats in the Cocaine-High group worked harder for reinforcement than did the Cocaine-Low or Saline groups. Overall, these results tell us that rats in the Cocaine-High group worked harder to obtain infusions of cocaine compared with the Cocaine-Low and Saline groups, and that this effect only reached significance for the lesioned rats.

![Sham and TOAx graphs](image)

*Figure 5-19:* Mean break point (± SEM) for Sham (left) or TOAx (right) rats. Break point was quantified as the last completed ratio in the progressive ratio test. Data were divided into Cocaine-Low and Cocaine-High groups. Significant differences between the saline and cocaine conditions are indicated by an asterisk for the Cocaine-Low*, and Cocaine-High* groups (p < .05). # denotes a significant difference between the Cocaine-Low and Cocaine-High groups (p < .05). Sham: Saline N=6, Cocaine-Low N=5, Cocaine-High N=4; TOAx: Saline N=6, Cocaine-Low N=7, Cocaine-High N=7
Naloxone Test

Intake of the taste cue and the resultant change in body weight on the naloxone test day were initially analyzed using two-way ANOVAs comparing the lesion and drug-taking group. Because there was no difference in CS intake or the change in body weight between Cocaine-Low and Cocaine-High groups, the data were reanalyzed comparing the lesion and drug (cocaine or saline).

CS Intake. The results indicated that the rats drank less of the taste cue when it predicted the opportunity to self-administer cocaine compared with saline, and this effect was true for both Sham and TOAx lesioned rats (Figure 5-20a). This was confirmed by a significant main effect of drug, $F(1,31), 12.61, p < .01$, and the lack of a significant main effect of lesion ($F < 1$), or lesion x drug interaction, $F(2,31) = 2.31, p = .14$. Post hoc analyses of the significant main effect of drug indicated decreased intake of the taste cue in the cocaine condition compared with the saline treated rats overall, $p < .001$. This effect seemed more pronounced in the TOAx rats, and unpaired t-tests confirmed that there was a significant difference in CS intake between the saline and cocaine conditions for the TOAx rats, $p < .05$, but not the Sham rats, $p > .05$.

Change in Body Weight. The results found no significant loss in body weight following exposure to the taste cue that predicted cocaine self-administration (Figure 5-20b) based on the lack of a significant main effect of drug ($F < 1$) or a lesion x drug interaction ($F < 1$). Furthermore, the lack of a significant main effect of lesion, $F(1,31) = 1.06, p = .31$ indicated that this was the case for both the lesioned and intact rats. In Chapter 3, there was a significant correlation between drug-taking and the change in body weight at test. Therefore, a similar analysis was conducted for these data. The correlational analyses found no significant relationship between drug-taking and loss in body weight for all subjects combined, $R^2 = .07$, $F(1,33) = 2.34, p = .14$, nor when analyzed separately for Sham, $F < 1$, and TOAx rats, $R^2 = .07$, $F < 1$. 

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F(1,18) = 1.44, p = .25 (data not shown). Overall, these data show that, in the presence of contextual cues, rats avoided intake of a saccharin cue that predicted the opportunity to self-administer cocaine compared with saline. Nevertheless, adding context without other contiguous cues (light and sound) was not sufficient to support the development of cue-induced withdrawal as measured by a precipitous loss in body weight when exposure to the saccharin cue was followed by a naloxone test.

Figure 5-20: (a) Mean 5-min intake (± SEM) of the 0.15% saccharin cue that predicts the opportunity to self-administer saline or cocaine for Sham (left) and TOAx (right) rats at naloxone test. (b) Mean change in body weight (g) (± SEM) for Sham (left) or TOAx (right) rats 2-h after the naloxone test. *indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=6, Cocaine=9; TOAx: Saline N=6, Cocaine N=14

Discussion

In the presence of contextual cues, both Sham and TOAx rats developed a conditioned avoidance of the saccharin cue that predicted the opportunity to self-administer cocaine. This supports the hypothesis that when context acts as a cue it can circumvent the effects of the TOA lesion. Furthermore, compared with cocaine self-administration in Experiment 2, the Sham rats showed increased drug taking and the willingness to work for drug when tested in the presence of
contextual cues. This increase in responding for drug was sufficient to support the development of conditioned avoidance of the taste cue when paired with the opportunity to self-administer cocaine and this effect was augmented in the TOAx rats. In the absence of drug-contingent cues, however, drug taking was diminished in the intact rats compared with lesioned rats. This further suggests an increased sensitivity to reward in the TOA lesioned rats.

**General Discussion**

The results of the three studies are summarized in Table 5-1. In Experiment 1, rats were housed in the home cage and tested in the drug self-administration chambers. Drug delivery was signaled by a light cue and commensurate with the presentation of a tone. Under these circumstances, the lesioned rats, like the controls, displayed conditioned avoidance of the cocaine-paired taste cue and a naloxone-induced loss in body weight. When the same subjects were retested in the home cage, however, the lesion blocked conditioned avoidance of the taste cue as well as signs of cue-induced withdrawal. This provides evidence that TOAx rats can form a taste-drug association and that they retain that association over time, but only when trained and/or tested in the presence of contextual cues. In Experiment 2, in the absence of contextual and drug-contingent cues, Sham rats failed to acquire cocaine self-administration and they failed to avoid intake of the saccharin cue. The TOAx rats, in comparison, exhibited greater drug-seeking and drug-taking behavior, but still greatly reduced suppression of intake of the saccharin cue. Finally, in Experiment 3, when contextual cues were present, but drug-contingent cues (i.e., lights and tones) were absent, the TOAx rats, like the Sham lesioned controls, learned to self-administer cocaine and significantly avoided intake of the saccharin cue. Contextual cues alone, then, are sufficient for intact and TOAx rats to establish cocaine self-administration and the associated avoidance of a cocaine-paired saccharin cue.
Based on our results, we can conclude that lesions of the TOA do not interfere with
detection and processing of the reinforcing properties of cocaine. In fact, the TOAx rats actually
increased their cocaine self-administration relative to controls. Furthermore, drug taking did not
differ from that of the Sham controls when using our standard self-administration paradigm.
Additional evidence that the lesion failed to inhibit the motivation to take drug was provided by
the use of a progressive ratio schedule of reinforcement. Elevated breakpoints confirmed that the
lesioned subjects were willing to make more responses to obtain infusions of cocaine compared
with saline, and this was similar to the Sham rats. Overall, when evaluated in our standard self-
administration paradigm, the lesion failed to attenuate drug taking and the motivation to obtain
drug reward.

Removal of the contextual and drug-contingent cues revealed a significant effect of the
lesion. Previous studies indicated that rats are less likely to self-administer cocaine when housed
in the test chambers (Caprioli et al., 2008; Caprioli et al., 2007), and that drug-contingent cues
significantly enhance the process of learning operant behaviors (Deroche-Gamonet, Piat, Le
Moal, & Piazza, 2002). Accordingly, in the absence of contextual and drug-contingent cues,
Sham rats readily consumed the saccharin cue and failed to establish steady cocaine self-
administration. Rats in the TOAx condition, however, behaved much differently. In the absence
of contextual and drug-contingent cues in Experiment 2, the TOAx rats took more infusions
overall and showed increased goal-directed behavior as indicated by preference for the active
spout. Furthermore, in the absence of drug-contingent cues in Experiment 3, the lesioned rats
exhibited escalated cocaine self-administration compared with saline self-administration while
the Sham controls did not.

It is possible that the lesion supported the development of drug taking behavior due to an
increased level of responding overall. In the absence of cues, the self-administration procedure is
signaled by the introduction of the CS spout for 5 min, followed immediately by presentation of
the active and inactive spouts. The lesioned subjects were actively engaging these spouts and exploring the chamber, behavior that is likely to trigger drug reinforcement. This increased responding could result from disconnection of thalamocortical circuits between the damaged thalamic nuclei and the prefrontal cortex (PFC) (Groenewegen, 1988). The PFC regulates motivational salience, intensity of behavioral responding (Jentsch & Taylor, 1999), and the integration of reward seeking and inhibitory control (Kalivas & Volkow, 2005). It is very possible that the lesion interferes with feedback from thalamic nuclei to the PFC. Furthermore, behavioral disinhibition resulting from the lesion may increase “sensation seeking”, as defined by the need for novel experiences and a willingness to take risks for these experiences (Zuckerman, 1979; 2001). Sensation seeking is believed to be the coalescence of reward seeking and reduced inhibitory control, both of which contribute to drug abuse vulnerability (Baler & Volkow, 2006; Bechara, 2005; Dawe & Loxton, 2004; Jentsch & Taylor, 1999). The increased goal-directed behavior and the establishment of cocaine self-administration in the absence of drug-contingent cues are consistent with a “sensation seeking” phenotype. Future studies will need to directly test whether such a phenotype will also lead to an increase in impulsivity in a delay discounting or the five-choice serial reaction time task, for example.

As mentioned, a short ISI was utilized in this model in order to specifically test if contextual cues were sufficient to circumvent the effects of the TOA lesion. In the following chapter, conditioned avoidance of a drug-paired cue with a 0 sec ISI in the home cage is tested with intact and lesioned rats. Future studies should test the influence of an increased ISI in the self-administration paradigm. The ISI could affect these results in two ways: first, the less time that passes between the CS and US, the easier it is to associate the two. Therefore, the lesion may interfere with the association over an extended length of time. This would explain why the lesion failed to inhibit avoidance of the taste cue in the runway and self-administration studies, as both paradigms have short ISIs. Secondly, forcing the rat to wait 5 min before drug administration, as
is the case in the home cage studies, may be more likely to evoke cue-induced withdrawal than if the drug is immediately available.

In summary, we have now determined that contextual and drug-contingent cues aid in the development of conditioned avoidance of a drug-paired saccharin cue. Furthermore, the availability of such cues is sufficient to circumvent the effects of the TOA lesion on drug-induced avoidance of the saccharin cue. In Experiments 1 and 3, the context was present before the taste cue and remained present throughout the self-administration period. Thus, it is hypothesized that the contextual cues draw attention to, and strengthen the associative potential of the taste cue, thereby overriding the effects of the TOA lesion. Furthermore, the drug-contingent cues, which occur only during drug delivery, served as a means to facilitate development of the operant behavior, but served no role in associating the taste cue with drug reward. These results indicate that contextual cues, which are present during presentation of the taste cue as well as cocaine self-administration are important for the development of the taste-drug association and its expression in TOA rats. In addition, we now know that the TOA lesion does not disrupt drug-taking or motivation, but rather leads to a phenotype that may be more responsive to drug reward.
Table 5-1: Summary of Findings

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Naloxone Test CS Intake | ↓ | ↓ | - | ↓* | ↓ | ↓ |
Naloxone Test Δ Body Wt | ↓ | ↓ | - | - | - | - |
Naloxone Retest CS Intake | ↓ | ↑* | NA | NA | NA | NA |
Naloxone Retest Δ Body Wt | ↓ | ↑* | NA | NA | NA | NA |

Summary of findings for Experiments 1-3. ↑ or ↓ indicates an increase or decrease for the cocaine condition with respect to the saline condition for each measure. – indicates that there was no difference between saline and cocaine conditions. NA means that this measure was not tested. *denotes differences between Sham and TOAx rats.
Chapter 6

Importance of the Inter-Stimulus Interval Length in Cocaine or Sucrose-Induced Taste Cue Avoidance

In the previous chapter, we determined that contextual cues enhance the development of drug-induced avoidance of a taste cue and override the effect of the TOA lesion. In every experiment in which the lesion was rendered ineffective, in addition to contextual cues, there was a short interval between the taste CS and the US. For example, in Chapter 4, when the rats had to traverse a straight runway to obtain experimenter-administered morphine, the running speed of the rat dictated the interval between the taste cue and drug. The average CS-US interval was less than 1.5 min and, under these conditions, the TOAx rats successfully learned to avoid intake of the drug-paired saccharin cue. Likewise, in Chapter 5, access to the saccharin cue was followed immediately by the opportunity to self-administer cocaine and, in this case, the lesion again failed to prevent drug-induced suppression of intake of the saccharin cue. In these experiments, the interval between presentation of the taste cue and access to the ensuing reward was not varied. The following tests address this variable.

The inter-stimulus interval (ISI) can potentially influence avoidance of a drug-paired cue by altering the meaning of the taste cue itself. In a study by Wheeler et al. (2011), rats displayed aversive taste reactivity and a decrease in accumbens dopamine release in response to a taste cue that predicted a delayed reward, but ingestive behavior and an increase in accumbens dopamine in response to the same taste cue when it predicted immediate reward. These data suggest that the two manipulations support different phenomena and reveal the development of an aversive state when the cue signals that the rat must wait for drug. In the first instance, the taste cue was intraorally infused with no CS-US interval. Thus, a short ISI can make the cue appetitive but a long ISI leads to a conditioned aversive state triggered by the cue. Why then, did the intact rats avoid intake of the taste cue with a long ISI in Chapter 2 (Nyland et al., 2012), and with a short
ISI in Chapters 4 and 5? One possibility is that, in the absence of a conditioned aversive state triggered by the cue (short ISI), avoidance of the drug-paired taste cue stems from the comparison of the two disparate rewards. Not surprisingly, the reward value of the drug outweighs that of the taste cue. When the presentation of the cue signals that the rat must wait for drug, however, avoidance of the taste cue is driven by a conditioned aversive state of withdrawal in response to the cue itself. Experiment 1 tested if TOA lesioned rats would avoid intake of a cocaine-paired saccharin cue when presented with a 0 sec or 5 min ISI. Published data show such rats fail to avoid intake of a cocaine or morphine-paired taste cue when 5 min elapse between access to the taste cue and the administration of drug. As in our published findings (Nyland et al., 2012; Chapter 2), the procedure took place in the home cage to eliminate a contribution from contextual cues. We hypothesized that if the lesioned rats failed to avoid intake of the cue with a 0 sec ISI, this would indicate that the findings from the runway and self-administration studies resulted from recruitment of contextual cues. Contrarily, successful avoidance of the drug-paired cue when presented with a 0 sec ISI, however, would indicate that the lesion interferes with the association or behavioral expression of the association specifically in response to the taste CS.

Importantly, the ability of the lesioned rats to successfully avoid intake of a drug-paired cue may be the result of a memory deficit. It is possible that the 5 min interval between the CS and the US prevents the lesioned rats from comparing the CS and US. As such, the possibility must be considered that TOAx rats successfully exhibited an anticipatory contrast effect, i.e., avoidance of a sucrose-paired saccharin cue, because access to saccharin was followed immediately by access to sucrose. Thus, if the sucrose data parallel the drug data, the lesioned rats also would fail to avoid intake of a sucrose-paired cue in the anticipatory contrast paradigm when 5 min elapse between access to saccharin and sucrose. Experiment 2 tested this hypothesis.
**Experiment 1: Avoidance of a Cocaine-Paired Taste Cue with 0 sec or 5 min ISI**

We have previously shown that rats with lesions of the TOA failed to avoid a taste cue when it predicted experimenter-administered morphine or cocaine after a 5 min ISI (Nyland et al., 2012; Chapter 2). In Chapter 4, however, when the taste cue was presented in a runway apparatus, the lesioned rats avoided intake of a saccharin cue that predicted experimenter-administered morphine. Likewise, when the saccharin cue was presented in the self-administration chambers in Chapter 5, lesioned rats avoided intake of the cue when it predicted the opportunity to self-administer cocaine. While the role of context was deemed sufficient to enhance the development of drug-induced avoidance of the saccharin cue in Chapter 5, the interval between the saccharin cue and experimenter-administered morphine or self-administered cocaine was significantly reduced compared with the home cage studies (Nyland et al., 2012; Chapter 2). Therefore, the aim of this experiment was to test if rats with TOA lesions would avoid intake of a taste cue (as they do in the standard ACE paradigm when using a zero sec ISI) when paired with experimenter-administered cocaine in a similar home cage study using a zero sec inter-stimulus interval.

**Methods**

**Subjects**

The subjects were 30 naive male, Sprague-Dawley rats delivered from Charles River at approximately 50 days of age. Following one week of quarantine, the rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light
phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise.

**Surgery**

Following acclimation to the colony room, the subjects (at least 300 g) underwent either bilateral ibotenic acid lesions of the thalamic trigeminal orosensory area (TOAx: n=18) or vehicle infusions (Sham: n=12) as described in Chapter 2.

**Apparatus**

The experiment was conducted in the home cages. Fluid was presented in inverted graduated Nalgene cylinders with silicone stoppers and stainless steel spouts affixed to the front of each home cage with springs. Intake was measured to the nearest 0.5 ml.

**Solutions**

Cocaine hydrochloride was provided by the National Institute on Drug Abuse (Bethesda, MD). The 10 mg/kg dose of cocaine was prepared in sterile saline and administered subcutaneously as a 1.5 mg/ml stock solution, adjusted by body weight to avoid necrosis (Durazzo et al., 1994). Naloxone (Sigma Chemical, St. Louis, MO) was prepared in sterile saline immediately before use. Sodium saccharin (Sigma Chemical, St. Louis, MO) was dissolved in dH₂O and presented at room temperature.
Procedure

Before conditioning began, rats were maintained on a water deprivation schedule that allowed for 5 min access to dH2O in the morning and 1 h each afternoon. This training procedure lasted until morning water intake stabilized (9 days). **Phase I: Zero sec ISI.** During acquisition, all rats were given 5 min access to a 0.15% saccharin CS. Immediately following removal of the CS bottle, the rats were injected subcutaneously with either 10 mg/kg cocaine (Sham: n=6; TOAx: n=8) or an equal volume of saline (Sham: n=6; TOAx: n=10). There were five such taste-drug pairings occurring at 48-hour intervals. To maintain proper hydration, dH2O continued to be provided for 5 min on the days between injections and for 1 h every afternoon. **Phase I: Naloxone Test.** A naloxone test was performed 48 h following the last cocaine injection. On the test day, all rats were weighed before receiving 5 min access to the saccharin CS. Immediately following removal of the CS bottle, all rats received a subcutaneous injection of naloxone (1 mg/kg). Two hours after naloxone administration, a second body weight was recorded. Following the naloxone test, rats were maintained on the water-deprivation schedule for 2 days. **Phase II: 5 min ISI.** Phase II began 48 h following the Phase I naloxone test. Subjects remained in the same drug condition and underwent the same procedure as described in Phase I, with the exception that there was a 5 min interval between CS access and the subcutaneous injections of cocaine (10 mg/kg) or saline. As in Phase I, there were five taste-drug pairings occurring at 48-hour intervals, and dH2O continued to be provided for 5 min on the days between injections and for 1 h every afternoon. **Phase II: Naloxone Test.** A second naloxone test was performed 48 h following the last cocaine injection. The procedure was identical to the first naloxone test with the exception of a 5 min interval between CS access and naloxone administration.
**Histology**

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects found to have misplaced or incomplete lesions were excluded from further analysis.

**Statistics**

Data from the conditioning trials were analyzed using a mixed factorial ANOVA varying lesion (Sham and TOAx), drug (cocaine or saline), and trial (1-5) as factors. Intake of the taste cue and resultant change in body weight on the naloxone test days were analyzed using two-way ANOVAs comparing lesion and drug. Post hoc Newman-Keuls tests or unpaired t-tests (two tailed) were conducted where appropriate. Data were considered statistically significant when \( p < .05 \).

**Results**

**Histology**

Data from five TOAx rats were excluded from statistical analysis due to incomplete lesions. Typical lesions damaged the middle third of the VPM as well as the majority of the taste area (VPMpc). Additionally, the lesion encompassed small parts of adjacent nuclei such as the parafascicular nucleus (Pf), posterior nucleus (Po), and subparafascicular nucleus (SPF) (see Figure 6-1).
**CS Intake (ml/5 min)**

Intake of the saccharin taste cue was analyzed separately for each phase (i.e., 0 sec or 5 min ISI). When the saccharin CS was immediately followed by an injection of saline or cocaine (Phase I: 0 sec ISI), the rats in the cocaine condition avoided intake of the CS compared with saline treated rats (Figure 6-2, left panels of Sham and TOAx graphs). This conclusion was supported by a significant main effect of drug, $F(1,21) = 64.59, p < .001$, and drug x trial interaction, $F(4,84) = 24.74, p < .001$. When the saccharin cue and experimenter-administered cocaine were presented with a 0 sec ISI, the TOA lesion did not interfere with conditioned
avoidance of the saccharin cue. This conclusion was supported by the lack of a significant main effect of lesion, $F(1,21) = 1.59, p = .22$, and lesion x drug x trial interaction, $F(4,84) = 1.14, p = .34$. There was, however, a significant lesion x drug interaction, $F(1,21) = 4.35, p < .05$, and post hoc analyses indicated that the intake was greater for the saline-treated compared with the cocaine-treated rats on trials 2-5 for the Sham group, $ps < .01$, and trials 3-5 for the TOAx rats, $ps < .01$.

In Phase II, the rats were forced to wait 5 min after the saccharin CS access before receiving an injection of saline or cocaine. Under these conditions, rats in the Sham, but not the TOAx group, avoided intake of the CS when it was paired with cocaine compared with saline treated rats (Figure 6-2, right panels of Sham and TOAx graphs). This was supported by significant main effects of lesion, $F(1,21) = 9.84, p < .01$, and drug, $F(1,21) = 23.18, p < .001$, and a significant lesion x drug interaction, $F(1,21) = 12.78, p < .01$. Post hoc analyses of this significant interaction indicated that the Sham rats in the cocaine condition avoided intake of the saccharin CS compared with saline treated rats, $ps < .05$, while TOAx rats showed no difference in intake between saline or cocaine treated rats, $ps > .05$. The lack of a significant lesion x drug x trial interaction ($F < 1$) indicates that this effect did not change over trials. When each lesion group was analyzed separately, unpaired t-tests indicated that the intake was greater for the saline- compared with the cocaine-treated rats on trials 1-5 for the Sham group, $ps < .01$, and was not significant on any trial for the TOAx rats, $ps > .05$.

Overall, these data indicate that rats in both lesion conditions suppressed intake of the cocaine-paired saccharin cue when there is a 0 sec ISI. When the ISI was extended to 5 min, the lesioned rats no longer suppressed intake of the cocaine-paired saccharin cue.
**Phase I: 0 sec ISI Naloxone Test**

Intake of the taste cue and the resultant change in body weight on the naloxone test day were analyzed using two-way ANOVAs varying lesion and drug as factors.

**CS Intake.** The results indicated that, when there was a 0 sec ISI, rats in both lesion groups drank less of the taste cue when it predicted experimenter-administered cocaine compared with saline (Figure 6-3a). This was confirmed by a significant main effect of drug, $F(1,21) = 141.60, p < .001$, and a lack of a significant main effect of lesion ($F < 1$). The lesion x drug interaction approached significance, $F(1,21) = 3.50, p = .08$, which suggested this effect was more prominent in the Sham condition. Post hoc analyses indicated that the intake was greater for the saline- compared with the cocaine-treated rats for both lesion groups, $ps < .05$.  

Figure 6-2: Mean 5-min intake (± SEM) of 0.15% saccharin in Sham (left) or TOAx (right) rats paired with saline (open circles) or 10 mg/kg cocaine (solid circles). The first 5 trials had a 0-sec ISI between the CS and US, and the last 5 trials had a 5-min ISI. The two phases were divided by the first naloxone test (indicated as NC on graph). *indicates a significant difference between the saline and cocaine conditions ($p < .05$). Sham: Saline N=6, Cocaine N=6; TOAx: Saline N=7, Cocaine N=6
Change in Body Weight. Results found that the change in body weight following naloxone administration for both lesion conditions was decreased for cocaine-, but not saline-treated rats (Figure 6-3b). These conclusions were supported by a significant main effect of drug, F(1,21) = 43.96, p < .001, and lack of a significant main effect of lesion (F < 1). Furthermore, post hoc analyses of a significant lesion x drug interaction, F(1,21) = 7.60, p < .05, indicated that this effect was significant for both lesion groups, p < .05, but it was more pronounced in the Sham, p < .001, than the TOAx rats, p < .05. Furthermore, post hoc analyses indicated greater weight loss for the Sham cocaine rats compared with the TOAx cocaine rats, p < .01. Overall, these results indicate that when the saccharin cue was immediately followed by experimenter-administered cocaine, the lesion was without effect. Both intact and lesioned rats avoided intake of the saccharin cue and showed signs of cue-induced withdrawal – even when tested in the home cage.

Figure 6-3: (a) Mean 5-min intake (± SEM) of the 0.15% saccharin taste cue that predicts experimenter-administered saline or cocaine for Sham (left) and TOAx (right) rats at naloxone test following 5 taste-drug pairings with a 0-sec ISI. (b) Mean change in body weight (g) (± SEM) for Sham (left) or TOAx (right) rats 2-h after the naloxone test. *indicates a significant difference between the saline and cocaine conditions (p < .05). # denotes a significant difference between Sham and TOAx groups (p < .05). Sham: Saline N=6, Cocaine N=6; TOAx: Saline N=7, Cocaine N=6
**Phase II: 5 min ISI Naloxone Test**

The intake of the taste cue and resultant change in body weight on the naloxone test day were analyzed as described above.

**CS Intake.** The results indicated that when there was a 5 min ISI, only rats in the Sham condition drank less of the taste cue when it predicted experimenter-administered cocaine compared with saline (Figure 6-4a). This was confirmed by significant main effects of lesion, $F(1,21) = 6.62, p < .05$, and drug, $F(1,21) = 34.74, p < .001$. Furthermore, post hoc analyses of a significant lesion x drug interaction, $F(1,21) = 22.03, p < .001$, indicated decreased intake of the cocaine-paired saccharin cue for rats in the Sham group, $p < .001$, but not in the TOAx group, $p > .05$. Moreover, the post hoc analyses also indicated that the intake for the Sham cocaine group was significantly lower than that of the TOAx cocaine group, $p < .001$.

**Change in Body Weight.** The results found a significant decrease in body weight for cocaine-treated, compared with saline-treated, rats in the Sham group and no difference in body weight for cocaine- and saline-treated rats in the TOAx group (Figure 6-4b). These conclusions were supported by significant main effects of lesion, $F(1,21) = 14.12, p < .001$, and drug, $F(1,21) = 39.21, p < .001$. Post hoc analyses of the significant lesion x drug interaction, $F(1,21) = 16.74, p < .001$, indicated weight loss for the cocaine-treated rats in the Sham, $p < .001$, but not the TOAx group, $p > .05$, compared with saline-treated rats. Additionally, weight loss in the Sham cocaine group was significantly lower than the TOAx cocaine group, $p < .001$. Essentially, these results indicate that when the interval between the saccharin cue and experimenter-administered cocaine was increased to 5 min, earlier findings were replicated such that intact rats avoided intake of the cocaine-paired cue and showed indices of cue-induced withdrawal, while the lesioned rats did not.
Discussion

Results from Experiment 1 confirmed the significance of the ISI in avoidance of a cocaine-paired saccharin cue in rats with lesions of the TOA. Increasing the interval between the saccharin cue and experimenter-administered cocaine had no effect on saccharin avoidance for the Sham rats. On the other hand, the TOAx rats learned to avoid intake of the cocaine-paired saccharin cue when experimenter-administered cocaine immediately followed access to the saccharin cue; however, cocaine-induced avoidance of the saccharin cue was delayed in the lesioned rats. Furthermore, in the Phase I naloxone test, both intact and lesioned rats showed indices of withdrawal when tested with a 0 sec ISI. The magnitude of this effect was somewhat
smaller for the TOAx rats compared with the Sham rats. While the lesioned rats were capable of avoiding intake of the cocaine-paired cue, these findings suggest that this developed much more slow than in intact rats. This could result from a decreased ability to associate the taste cue with experimenter-administered cocaine. In support, a weak association would explain the decreased magnitude of cue-induced withdrawal in the lesioned rats. Alternatively, Liang et al., (2012a) found increased responding for natural reward amongst TOAx rats; therefore, it could also be the case that the lesioned rats are simply unwilling to suppress intake of the taste cue. While possible, this does not account for the decreased magnitude of cue-induced withdrawal observed in the Phase I naloxone test. Accordingly, it is possible that the lesion interferes with the development of an aversive state of withdrawal in response to the drug-paired taste cue.

In Phase II, when the interval between the taste cue and experimenter-administered cocaine was increased to 5 min, the TOAx rats no longer avoided intake of the saccharin cue. This supports the conclusion that the TOA lesion weakens the associative strength of the taste cue. In the final naloxone test, the lesioned rats failed to display signs of withdrawal in response to the drug-paired taste cue. These findings advocate an associative deficit in the lesioned rats. While the associative strength of the taste cue is a possible mediator of this deficit, it is also possible that the results are due to memory failure. This seems unlikely, given that the TOAx rats were capable of avoiding intake of a LiCl-paired cue when a similar 5 min ISI was utilized in Chapter 2 (Nyland et al., 2012). Therefore, it is likely that the lesion specifically interferes with the association of a taste cue and a drug of abuse. To test this, the final experiment in this chapter will test the effects of an increased ISI on the avoidance of a sucrose-paired taste cue in Sham and TOAx rats.
Experiment 2: Anticipatory Contrast with 5 min ISI

Previously, lesions of the TOA delayed, but did not prevent the development of an anticipatory contrast effect when a saccharin cue predicted immediate access to a more rewarding sucrose solution (Nyland et al., 2012; Chapter 2). Several factors in this paradigm may have led to the success of the TOAx rats in developing an anticipatory contrast effect, such as contextual cues and a short ISI. In Experiment 2, the significance of the ISI was tested to determine if the lesion would inhibit comparison of two disparate natural rewards when there was a 5 min interval between presentations of the lesser- and higher-valued solutions.

Methods

Subjects

The subjects from Experiment 1 were used in Experiment 2, and were allowed 9 weeks off in between experiments. The rats were housed individually in stainless steel hanging cages in a temperature-controlled (21 °C) animal care facility with a 12-h light-dark cycle (lights on at 7 a.m.). Testing was conducted during the light phase of the cycle. Rats were maintained on dry Harlan Teklad rodent diet (Madison, WI) and water ad libitum, except where noted otherwise.

Apparatus

The experiment was conducted in 12 identical modular operant chambers as described in Chapter 2.
**Solutions**

Sodium saccharin and sucrose (Sigma Chemical, St. Louis, MO) were dissolved in dH2O and presented at room temperature.

**Procedure**

Following a 9-week interval, all rats were food-deprived to 90% of their free-feeding body weight, maintained by a once per day feeding. Rats were divided into saccharin-saccharin and saccharin-sucrose groups in a counterbalanced fashion with respect to their previous experience. They were then habituated to the chambers for 5 min a day for 3 days with the house light and white noise on. During testing, each rat was placed in the apparatus with the house light and white noise on. The first bottle (located on the left) advanced and the rat had 3 min access to 0.15% saccharin. Thereafter, the first bottle retracted and 5 min later, a second bottle advanced on the right for a 3 min access period. The second bottle contained either more 0.15% saccharin (saccharin-saccharin group; Sham: n=6; TOAx: n=8) or 1.0 M sucrose (saccharin-sucrose group; Sham: n=6; TOAx: n=10). The bottle was then retracted and the rat was immediately removed from the chamber. Daily feeding occurred one hour after removal from the chambers. There was one such taste-taste pairing a day for 16 days and the latency to first lick and number of licks made on each bottle was recorded.

**Histology**

At the end of all behavioral tests, the rats were given an overdose of Pentobarbital Sodium (100 mg/kg, ip) and perfused transcardially as described in Chapter 2. Data from subjects found to have misplaced or incomplete lesions were excluded from further analysis.
Statistics

Data were analyzed using a mixed factorial ANOVA varying lesion (Sham and TOAx), US (saccharin or sucrose), and block (1-8) as factors. Post hoc Newman-Keuls or unpaired t-tests (two tailed) were conducted where appropriate. Data were considered statistically significant when p < .05.

Results

Histology

Data from five TOAx rats were excluded from statistical analysis due to incomplete lesions, as described in Experiment 1 (see Figure 6-1).

CS Intake (Licks/3 min)

Data from two Shams and one TOAx rat were removed from analysis due to failure to establish licking on either spout across the eight 2-day blocks. Intake of the saccharin taste cue was analyzed using a mixed factorial ANOVA varying lesion, US, and block. Results indicate that rats in both the Sham and TOAx lesion condition avoided the saccharin cue when it preceded access to a highly preferred sucrose solution (Figure 6-5). This conclusion was supported by a significant main effect of US, F(1,18) = 23.27, p < .001, and lack of a significant main effect of lesion (F < 1), or any accompanying interactions (Fs < 1). There was a significant US x block interaction, F(7,127) = 8.19, p < .001, and post hoc analyses indicate that this effect was significant for blocks 2-8, ps < .05. Unpaired t-tests for each lesion group indicated greater saccharin CS intake in the saccharin-saccharin condition compared with the saccharin-sucrose
condition on blocks 2-8 for the Sham group, ps < .05, and blocks 3-8 for the TOAx group, ps < .05.

**Figure 6-5:** Mean 3-min intake (± SEM) of 0.15% saccharin in Sham (left) or TOAx (right) rats paired with more 0.15% saccharin (open circles) or 1 M sucrose (solid circles) across 16 CS-US pairings represented as 2-day blocks. The interval between CS and US presentation was 5-min. *indicates a significant difference between the Sac-Sac and Sac-Sucrose conditions (p < .05). Sham: Saccharin N=4, Sucrose N=6; TOAx: Saccharin N=5, Sucrose N=7

**CS Latency (Latency to Contact CS Spout)**

Results indicate that rats in both the Sham and TOAx lesion condition failed to display contrast in the latency to contact the CS spout (Figure 6-6). This conclusion was supported by lack of significant main effects of lesion, F(1,18) = 1.27, p = .28, and US, F(1,18) = 1.38, p = .26, or any accompanying interactions, Fs < 1. The lesion x US interaction was nearing significance,
F(1, 18) = 3.21, p = .09, which suggested, from an observation of the data, that the Sham group was trending towards developing contrast (i.e., a longer latency to lick first bottle saccharin when paired with sucrose than when paired with more saccharin).

Figure 6-6: Mean latency (± SEM) to make contact with the CS spout that precedes access to 0.15% saccharin (open circles) or 1 M sucrose (solid circles) in Sham (left) and TOAx (right) rats across 16 CS-US pairings represented as 2-day blocks. The interval between CS and US presentation was 5-min. Sham: Saccharin N=4, Sucrose N=6; TOAx: Saccharin N=5, Sucrose N=7

**US Intake (Licks/3 min)**

Results of the mixed factorial ANOVA indicate that rats in both the Sham and TOAx lesion condition drank more of the sucrose than saccharin US, demonstrating a magnitude of reward effect (Figure 6-7). This conclusion was supported by a significant main effect of US,
F(1,18) = 12.62, p < .01, and lack of a significant main effect of lesion F(1,18) = 2.20, p = .16, or any accompanying interactions, Fs < 1. The US x block interaction approached significance, F(7,127) = 1.96, p = .07. Separate t-tests for each lesion group indicated greater intake of second bottle sucrose compared with second bottle saccharin on blocks 4, and 6-8 for the Sham group; ps < .05, and blocks 2-8 for the TOAx group, ps < .05.

Figure 6-7: Mean 3-min US intake (± SEM) of 0.15% saccharin (open circles) or 1 M sucrose (solid circles) in Sham (left) or TOAx (right) rats across 16 CS-US pairings represented as 2-day blocks. The interval between CS and US presentation was 5-min. *indicates a significant difference between the Sac-Sac and Sac-Sucrose conditions (p < .05). Sham: Saccharin N=4, Sucrose N=6; TOAx: Saccharin N=5, Sucrose N=7
**US Latency (Latency to Contact US Spout)**

Results indicate that rats in both the Sham and TOAx lesion conditions failed to display a magnitude of reward effect in the latency to contact the US spout (Figure 6-8). This conclusion was supported by lack of significant main effects of lesion, F < 1, and US, F < 1, or any accompanying interactions, Fs < 1. These results indicate that all rats were quick to initiate licking the US, whether saccharin or sucrose.

![Graph showing US Latency](image)

**Figure 6-8**: Mean latency (± SEM) to make contact with the US spout containing either 0.15% saccharin (open circles) or 1 M sucrose (solid circles) in Sham (left) or TOAx (right) rats across 16 CS-US pairings represented as 2-day blocks. The interval between CS and US presentation was 5-min. Sham: Saccharin N=4, Sucrose N=6; TOAx: Saccharin N=5, Sucrose N=7
Discussion

The results show that the lesion does not prevent the development of an anticipatory contrast effect when there is a 5 min interval between presentation of the CS and US. Thus, in a similar manner as was seen in the previously published study (Nyland et al., 2012; Chapter 2), rats in the lesion condition avoided intake of the saccharin cue when it predicted access to a preferred sucrose solution, though the effect was somewhat delayed in both cases. The Sham rats developed contrast in the latency to contact the CS spout when the ISI was short in the published data (Nyland et al., 2012), but this was not observed when the ISI was 5 min. Rats with lesions of the TOA, on the other hand, did not develop this contrast effect in latency when CS and US presentation was separated by either a 0 sec ISI (Nyland et al., 2012; Chapter 2) or a 5 min ISI. When tested with a 5 min ISI, like the 0 sec ISI, both intact and lesioned animals evidenced a magnitude of reward effect as indicated by greater consumption of second bottle sucrose compared with second bottle saccharin. The only difference observed between the results from testing with a 0 sec ISI in Chapter 2, and a 5 min ISI in the present chapter was the latency to contact the US spout. In the published data (Nyland et al., 2012; Chapter 2), both intact and lesioned rats were faster to contact the US spout when it contained sucrose compared with saccharin. When the ISI was increased to 5 min, however, there was no difference in the latency to contact the spout containing saccharin compared with sucrose—all rats were quick. Taken together, these results show that ACE remains intact for Sham and TOAx rats when 5 min elapse between access to the saccharin cue and access to the preferred sucrose reward. That said, the effect is somewhat diminished in the TOAx rats.
**General Discussion**

In Experiment 1, when the saccharin cue was immediately followed by experimenter-administered cocaine, intact and lesioned rats avoided intake of the taste cue. These results suggest that, in the absence of contextual cues, the lesion is without effect when a brief ISI is employed. Accordingly, the brief ISI enabled rats with lesions of the TOA to compare the value of the taste cue with that of the drug of abuse, or at the very least, to associate the two. Furthermore, following five taste-drug pairings, intact and lesioned rats exhibited signs of cue-induced withdrawal; however, this effect was less significant in the TOAx rats. This confirms that the TOA lesion does not eliminate, but perhaps, attenuates development of a conditioned aversive state in anticipation of drug when using a brief ISI.

After the first naloxone test, taste-drug pairings were tested with a 5 min interval between the taste cue and drug. In this case, the lesioned rats no longer avoided intake of the saccharin cue, nor did they express signs of cue-induced withdrawal on the final naloxone test. The reversal of saccharin avoidance in the lesioned group following the shift to a 5 min ISI was indicative of a deficit in retention of the previously learned association of the drug-paired cue. This reversal may result from an insufficient, or weak, association of the saccharin cue and experimenter-administered cocaine, which was suggested by the delay in avoidance of the saccharin cue in Phase I (0 sec ISI).

Accordingly, a weak association would make it easier to forget the association after just one unreinforced presentation of the saccharin cue (i.e., the first naloxone test). Most likely, the temporal contiguity in Phase I (0 sec ISI) increased the salience, or significance, of the saccharin taste cue, thus enabling the association between the saccharin cue and drug in the lesioned rats to persist. When the ISI was extended to 5 min, this was no longer the case. In Phase II (5 min ISI), the lack of temporal proximity between the cue presentation and cocaine delivery was no longer
sufficient to draw attention to the saccharin cue, which on its own, lacked salience for the lesioned rats.

It should be noted that immediately following the first naloxone test (i.e., during the first acquisition trial of Phase II testing; Figure 6-3), intake of the saccharin cue immediately increased in the TOAx cocaine group before a single pairing with the increased ISI. According to Bouton (1993), it is possible that the single non-reinforced presentation of the taste cue that took place during the first naloxone test could have been sufficient to reverse the weak association between the taste cue and experimenter-administered cocaine. Additionally, temporal contiguity facilitated the development of indices of cue-induced withdrawal, an effect that dissipated once retention of the taste-drug association was lost. These findings suggest that temporal contiguity acts similar to context by facilitating this association in rats with TOA lesions.

Based on the results from Experiment 2, the length of the ISI does not affect avoidance of a sucrose-paired taste cue in rats with TOA lesions. These data imply that avoidance of a taste cue that predicts a natural reward (i.e., sucrose) is processed by different means than avoidance of a taste cue that predicts a drug of abuse. Further, the effect of the TOA lesion is specific to the association of a gustatory cue paired with a drug of abuse. As such, avoidance of the taste cue when paired with experimenter-administered cocaine was established with a 0 sec ISI, but was not retained when the ISI was extended to 5 min, while the interval between the presentation of two natural rewards (i.e., saccharin and sucrose) reduced, but did not prevent, avoidance of the taste cue in TOA lesioned rats.
Chapter 7

Conclusions

Summary of Findings

The results from Chapter 2 replicate and extend those obtained by Liang et al. (2012c) by showing that the TOA lesion does not block avoidance of a lesser-valued taste cue that predicts access to a highly palatable sucrose solution under real-feeding conditions. As such, the lesioned subjects were able to detect the taste cue, associate the taste cue with the US, compare the available reward with the anticipated reward, and respond accordingly by suppressing intake of the lesser-valued taste cue. That said, as a group, the TOA lesioned rats did not develop an anticipatory contrast effect as fast as the sham-operated controls and did not develop contrast in the latency to lick the sucrose-paired cue. The lesion disrupts, but does not eliminate, the development of an anticipatory contrast effect. Surprisingly, the same TOA lesion fully prevented avoidance of a taste cue when paired with experimenter-administered morphine or cocaine. The TOA lesion is, then, the first manipulation in which performance in one paradigm is intact, albeit slightly delayed (anticipatory contrast), while performance in the other is eliminated (drug-mediated avoidance of the taste cue), and this is the case with both morphine and cocaine. Finally, and consistent with the findings of Liang et al. (2012b) in sham-fed rats, the TOA lesion also had no effect on the LiCl-induced CTA when assessed in real-fed rats using parameters identical to those employed when a drug of abuse served as the US.

Chapter 3 (Nyland & Grigson, 2013), provides evidence that presentation of a morphine- or cocaine-paired taste cue elicits a conditioned state of withdrawal, as indexed by a loss of body weight following injection of the opiate antagonist, naloxone. Furthermore, greater naloxone-induced loss in body weight (i.e., greater withdrawal) was correlated with greater avoidance of
the drug-paired cue and, as verified with cocaine, greater cocaine self-administration behavior. Significantly, the TOA lesion prevented avoidance of the taste cue as well as cue-induced withdrawal when training and testing occurred in the home cage. These results suggest that the lesion may prevent avoidance of the drug-paired taste cue by reducing the onset of cue-induced withdrawal. This is particularly important, given that cue-induced withdrawal is a major precipitating factor of relapse.

In Chapter 4, we used a runway apparatus to test if the TOA lesion interfered with detection of the drug, and if not, to investigate the effect of the TOA lesion on the motivation to obtain experimenter-administered morphine. We found no difference in instrumental behavior (i.e., running speed) between intact and lesioned rats, such that all rats ran more quickly when the saccharin cue presented in the start box predicted experimenter-administered morphine in the goal box, compared with saline. In this experiment, however, the lesioned rats also avoided intake of the taste cue that predicted experimenter-administered morphine. This was unexpected, as the lesion fully prevented avoidance of a morphine-paired taste cue when presented in the home cage (Nyland et al., 2012; Chapter 2). A follow up study indicated that these same rats failed to avoid intake of a cocaine-paired taste cue when presented subsequently in the home cage using the same procedure as described in Chapter 2. Why then, did the lesion prevent avoidance of a drug-paired cue when presented in the home cage, but not in the runway apparatus? While both procedures paired a palatable taste cue with experimenter-administered drug, the runway experiment provided contextual cues that may have aided the development of the taste-drug association in lesioned rats. Further, results from the runway experiment indicated that the lesion also did not prevent the development of cue-induced withdrawal in this apparatus.

Using a self-administration paradigm in Chapter 5, we tested the effect of the TOA lesion on drug-taking and the willingness to work for drug reinforcement. Again, the lesioned rats avoided intake of the saccharin cue that predicted the opportunity to self-administer cocaine and
they displayed indices of cue-induced withdrawal when tested in the self-administration chambers. When retested in the home cage, however, the lesioned rats no longer avoided intake of the saccharin cue, nor exhibited signs of cue-induced withdrawal. This suggests that contextual cues in the self-administration chamber, and in the runway apparatus in Chapter 4, may act as occasion setters that facilitate the development of the taste-drug association. To test this hypothesis, the self-administration experiment was repeated in the absence of contextual and drug-contingent cues. In this case, all rats failed to avoid the saccharin taste cue. There were very low levels of cocaine self-administration, and this may account for the failure to avoid intake of the taste cue. The TOAx rats did display a significant preference for the active spout, which is indicative of greater goal-directed behavior. When the contextual cues were reintroduced in the final experiment of Chapter 5, both intact and lesioned rats avoided intake of the saccharin taste cue; however, only the lesioned rats exhibited an escalation in cocaine self-administration and an increased willingness to work for drug. The progressive ratio findings parallel those found by Liang et al. (2012a), indicating increased responding for sham-fed sucrose. Thus, the lesion does not interfere with detection of rewarding stimuli, but instead increases responding for a rewarding stimuli (US), be it natural or drug.

Finally, in Chapter 6, we determined that a brief inter-stimulus interval (ISI) between the presentation of the taste cue and experimenter-administered cocaine was sufficient to facilitate avoidance of the drug-paired cue in lesioned rats when presented in the home cage. Thus, the TOAx rats learned to avoid intake of the cocaine-paired saccharin cue when experimenter-administered cocaine immediately followed access to the saccharin cue. Furthermore, following five taste-drug pairings, both intact and lesioned rats showed signs of cue-induced withdrawal in response to the saccharin cue. Nevertheless, following the first naloxone test, when the ISI was increased to 5 min, the lesioned rats no longer avoided intake of the saccharin cue or showed signs of cue-induced withdrawal upon a second naloxone test. These results imply that the
association between the saccharin cue and experimenter-administered cocaine may be facilitated by temporal proximity in addition to contextual cues. The association facilitated by temporal proximity was relatively weak, however, as evidence of this association was absent following a single non-reinforced presentation of the saccharin cue (during the first naloxone test) and this disruption was maintained when the ISI was subsequently increased to 5 min.

Despite the effects of a 5-min ISI on cocaine-induced avoidance of a taste cue, using the same interval, the TOA lesion slowed, but did not prevent, the development of an anticipatory contrast effect, i.e. pairing saccharin with sucrose. Taken together, these results indicate that increasing the interval between a saccharin cue and experimenter-administered cocaine prevents avoidance of the taste cue in TOA lesioned rats; but not avoidance of the saccharin cue that precedes similarly delayed access to a preferred natural reward (i.e., 1.0 M sucrose).

Conclusions

**Conditioned Taste Aversion**

In the CTA paradigm, robust avoidance of a taste cue occurs when it is associated with an aversive stimulus such as LiCl (Nachman, 1963). The data have confirmed the findings of Liang et al. (2012b), that rats with lesions of the TOA develop a conditioned taste aversion to a LiCl-paired taste cue. These results are not surprising, given that conditioned taste aversions are present in rats with lesions of the gustatory thalamus (Flynn et al., 1991; Grigson, Lyuboslavsky, & Tanase, 2000) and cortex (Geddes et al., 2008).
Anticipatory Contrast

Anticipatory contrast occurs when rats learn to avoid a taste cue when it comes to predict a preferred natural reward (Flaherty & Checke, 1982). In this dissertation, the ability of TOAx rats to avoid a palatable saccharin cue when paired with a more preferred sucrose solution was tested under real-feeding conditions. In addition, lesions of the TOA have previously been tested using disparate concentrations of sham-fed sucrose or corn oil by Liang and colleagues (2012c). Both the current findings and those of Liang et al. (2012c), demonstrate that the lesion does not eliminate avoidance of a palatable taste cue that predicts access to a more preferred natural reward. That said, the lesion did delay this effect significantly, whether the saccharin cue predicted immediate (see Figure 2-2) or delayed (see Figure 6-5) access to a more rewarding sucrose solution, or when using two disparate sucrose concentrations (Liang et al., 2012c). Possible explanations for the delay are addressed in detail below.

Avoidance of a Drug-Paired Taste Cue

When a palatable taste cue is paired with a drug of abuse, intake of the taste cue is suppressed. The reward comparison hypothesis (Grigson, 1997) suggests that this avoidance is the result of devaluation of the naturally rewarding cue, as its perceived value pales in comparison to that of the ensuing drug of abuse. The comparison of a natural reward to that of impending drug, however, is not the only factor at play. With repeated trials, the taste cue also produces evidence of conditioned aversion such as an elevation of corticosterone (Gomez et al., 2000), blunted accumbens dopamine (Grigson & Hajnal, 2007), and the onset of aversive taste reactivity (Wheeler et al., 2008). Lesions of the TOA eliminated avoidance of a drug-paired taste cue as well as indices of cue-induced withdrawal when presented in a familiar setting (i.e., when
presented in the home cage), but not when presented in the presence of contextual cues or with a 0 sec ISI. As in anticipatory contrast, development of taste cue avoidance was clearly evident, but was delayed under these circumstances. Therefore, the TOA lesion did not eliminate the avoidance of a drug-paired taste cue in all circumstances. The means by which the lesion can cause such results are discussed in the following section.

**Overall Effects of the Trigeminal Orosensory Area Lesion on Taste Cue Avoidance**

There are several steps in the development of conditioned avoidance of a palatable taste cue. First, the rat must be able to detect both the taste cue (CS) and the unconditioned stimulus (US). Failure to detect either would result in failure to associate one with the other. Second, the rat must then associate and compare the perceived value of the taste cue with that of the US. Failure at this stage could be due to an inability to make the association, or discrepancies in the perceived value of the stimuli. In the case of drugs of abuse, there also is an element of conditioned aversion that supports drug-induced avoidance of the taste cue. Accordingly, presentation of the taste cue should evoke an aversive conditioned state in anticipation of ensuing drug reward. Unsuccessful development of this conditioned aversive state could also lead to failure to avoid the drug-paired taste cue. Finally, the rat must use this information to suppress, or avoid, intake of the taste cue. Failure at this stage is related to performance, and could result from deficient memory or increased impulsivity. I will address each of these steps in the development of appetitive conditioning to gain a better understanding of why, in certain instances, avoidance of the taste cue does not occur.

First, I will address what the lesioned rats *can* do, before discussing what they *cannot* do. I have demonstrated that TOAx rats are capable of avoiding intake of a taste cue that predicted an aversive stimulus (LiCl), a preferred sucrose solution under real-feeding conditions (Nyland et
al., 2012), and drugs of abuse when presented with contextual or temporal cues (Chapters 4-6). Furthermore, consumption of the saccharin and Polycose taste cue in the home cage taste-drug pairings was greater than that seen for water intake on the alternating water days (data not shown). This is not surprising, given that rats with lesions encompassing the gustatory thalamus respond in a manner similar to sham-operated controls when presented with varied concentrations of sucrose, NaCl, hydrochloric acid, and quinine hydrochloride in 24-hour two bottle tests (Reilly & Pritchard, 1996a). Therefore, I conclude that the deficit does not lie in the ability to detect either the taste cue or the unconditioned stimulus.

The TOAx rats avoid intake of a sweet- or drug-paired taste cue only under particular circumstances. Therefore, the main effect of the lesion was not a disruption in performance, per se. Liang et al. (2012a) found increased responding for real and sham-fed sucrose in TOAx rats compared with sham-lesioned controls. In the anticipatory contrast experiment (Chapter 2: Nyland et al., 2012), intake appeared higher overall for the TOAx rats, which supports the observation by Liang and colleagues (2012a) that the TOAx rats showed increased responsiveness to natural rewards. Additionally, TOAx rats in the saccharin-sucrose condition did not avoid intake of the saccharin cue to the degree seen in the Sham rats. Increased responding for natural rewards, then, is a notable effect of the lesion. Furthermore, in the taste-drug studies, failure to avoid intake of the drug-paired taste cue could have resulted from this increased responsiveness for natural rewards in the lesioned rats. Thus, it is possible that the lesioned rats were, in fact, capable of detecting and comparing the taste cue with experimenter-administered drug, but preferred the taste cue to the drug.

While possible, this scenario is not likely, as the lesioned rats also tended to show increased cocaine self-administration compared with the intact rats in Chapter 5. Based on these findings, when compared with controls, the lesioned rats may have a higher level of responding overall, be it for sweet or drug. Nevertheless, lesioned rats showed lower baseline intake of a
neutral NaCl solution in the conditioned taste aversion experiment (Nyland et al., 2012; Chapter 2). Thus, it appears that the lesioned rats did not express increased responsiveness overall, the increased responsiveness was specific to rewarding stimuli. Accordingly, rats with lesions of the TOA quickly recognized rewarding stimuli and increased responding. This may account for their lack of neophobia for sweet rewards and in the immediate elevation in cocaine self-administration. Therefore, based on our current understanding of the TOA lesion, we can conclude that rats with this lesion exhibited increased responding for rewarding stimuli.

The delayed or absent avoidance of the taste cue by the TOAx rats also could be interpreted as impulsivity. While it is possible that failure to avoid intake of the taste cue was a result of a decreased response inhibition, impulsivity also includes persistent and apparently aimless responding (Chudasama et al., 2003). The finding that the TOAx rats showed an increased active spout preference compared with the Sham rats in the self-administration paradigms does not support an increased impulsivity interpretation. If there were an increase in impulsivity, increased responding would occur on both the active and the inactive spouts. Nevertheless, increased impulsivity cannot be completely ruled out without further testing. Future studies using the 5-choice serial reaction time task would provide more specific analysis of compulsive (perseveration) and impulsive (premature) responses in lesioned rats (Dalley et al., 2007).

Rats with TOA lesions were capable of detecting the stimuli and avoiding intake of the taste cue. Why then, did these animals show a delay in acquisition of sucrose-induced avoidance of the taste cue? Further, why did the lesion prevent drug-induced avoidance of the cue under some, but not all circumstances? The main effect of the lesion appears to be an inability to associate the palatable taste cue with a drug of abuse. Previous findings with lesions of the gustatory thalamus eliminate (or in the case of a long ISI, reverse) anticipatory contrast effects (Reilly & Pritchard, 1996b), and prevent drug-induced avoidance of a palatable taste cue.
(Grigson, Lyuboslavsky, & Tanase, 2000). Perhaps the less complete loss sustained by the
gustatory thalamus in the TOA lesion was sufficient to reduce, but not eliminate, the associative
strength of the taste cue. Here, the physiological relevance of the CS-US pairing may have
significance. Accordingly, TOAx rats showed no deficit in avoidance of a taste cue paired with
visceral malaise, an association that has physiological relevance and is significant for survival.
Furthermore, the TOAx rats were delayed in avoiding a taste cue that predicted a preferred
sucrose reward. While still physiologically relevant, this association is less significant for
survival. Finally, avoidance of a drug-paired taste cue holds the least physiological relevance,
and was only seen in the TOAx rats in the presence of contextual cues or a short ISI. According
to Rescorla and Wagner (1972), the associative value of the taste cue gains strength when
concurrently presented with other stimuli. For example, context can act as an occasion-setter that
may strengthen the association of the taste cue and the US (Bouton & Bolles, 1979).
Alternatively, the salient context and the weak cue can form one combined stimulus, that, when
presented together, can form a single association with the unconditioned stimulus (Darby &
Pearce, 1995). As such, if the associative value of the taste cue is only effective after
enhancement by the contextual stimuli, it may take longer to develop avoidance of the taste cue.
This would explain the delayed acquisition of avoidance of a drug-paired taste cue in the TOAx
rats.

Effects of Trigeminal Orosensory Area Lesions on Avoidance of a Sucrose-Paired Taste Cue

The TOAx rats were capable of suppressing intake of a less valued taste cue when it
predicted access to a more preferred sucrose solution. The delay in acquisition, however,
suggests that the mechanism by which this occurs was different for TOAx rats than for Sham rats.
In support, when the saccharin taste cue was immediately followed by a preferred sucrose
solution rather than more saccharin, intact and lesioned rats were quicker to contact the US spout (see Figure 2-5). This indicates that they were anticipating the US. Whether this was in response to the saccharin taste cue is unclear. For both intact and lesioned rats, the latency to contact the US spout was significantly reduced starting with the second 2-day block. At the same time, while the intact rats also were avoiding intake of the saccharin CS starting with the second 2-day block, the lesioned rats did not evidence CS avoidance until the fifth 2-day block (see Figure 2-2). The delayed suppression of intake suggests that the lesioned rats were anticipating the US before developing avoidance of the taste cue that predicted it. Therefore, it is possible that the sucrose US was being associated either with the contextual cues present in the operant chamber or a combination of the taste cue and context. This also may explain the absence of a contrast effect in the latency to contact the CS spout (see Figure 2-3). Unlike the controls that were slower to contact the CS spout when it predicted a preferred sucrose solution, the lesioned rats were quick to contact the CS spout. This suggests increased responding for sweet reward in general, and further, that the TOAx rats were associating the context, rather than the saccharin cue, with the preferred sucrose solution. To determine the facilitative effects of context, the anticipatory contrast paradigm should be retested in a familiar setting by housing the rats in the operant chambers or testing in the home cages. This will allow an assessment of the lesion’s effect on avoidance of a sucrose-paired taste cue without the confounding effects of contextual cues.

**Effects of Trigeminal Orosensory Area Lesions on Avoidance of a Drug-Paired Taste Cue**

The hypothesis that the lesion weakens the associative strength of the taste CS also explains the results found in the taste-drug studies. For example, the lesioned rats failed to avoid the taste cue when it was paired with experimenter-administered morphine in the home cage, but they avoided it when presented in the runway apparatus. In this case, the main difference
between the two experimental procedures was the presence of contextual cues. While the literature is teeming with theories on the role of context in associative learning, most agree that the presence of contextual cues alters the development of the CS-US association. Furthermore, a weak cue is not likely to be associated with an unconditioned stimulus (in this case, experimenter-administered morphine) when the interval between the offset of the cue and onset of the US is increased (Balsam, 1984; Broadbent & Cunningham, 1996). This is particularly true when there are no additional cues present during this interval (Balsam, 1984). Therefore, when presented in the home cage, the 5 min interval, in which there were no cues, may not have been conducive to the association of a weakened cue and the drug of abuse in the TOA lesioned rats (Figure 7-1a). An association did occur, however, when the weak cue was presented in a salient context (Figure 7-1b). This raises the question: are rats with lesions of the TOA associating the taste cue with drug, or are they associating the context with drug?

Figure 7-1: Intake of a taste cue when paired with experimenter-administered morphine or saline in Sham and TOAx rats when the taste-drug pairings take place in (a) the home cage, and (b) a runway apparatus. In the home cage, the TOAx rats did not avoid intake of the morphine-paired taste cue. However, when the taste cue was presented in one end of a runway apparatus, and the experimenter-administered morphine delivered on the other, the lesioned rats avoided intake of the morphine-paired taste cue. The schematics above each graph show how context can bridge the interval between the taste cue and drug to make this association possible. These figures are reproduced from Figures 2-6, and 4-2, respectively.
Context alone may not account for the successful avoidance of the taste cue in lesioned rats. Evidence suggests that the association of the taste cue and ensuing drug is not just a matter of what is coming, but also, when (Miller & Barnet, 1993). In addition to contextual cues, there also was a shortened interval between the presentation of the taste cue and drug in both the runway and self-administration studies. Therefore, in Chapter 6, the influence of temporal contiguity was tested in an experiment identical to the previous home cage study (Nyland et al., 2012; Chapter 2), with the exception that the length of the inter-stimulus interval (ISI) was decreased during taste-drug testing. In the previously published data, rats with TOA lesions did not avoid intake of a saccharin cue that predicted experimenter-administered cocaine in the home cage following a 5 min delay (Figure 7-2a). In Chapter 6, however, when the saccharin cue was immediately followed by experimenter-administered cocaine in the home cage, both intact and lesioned rats avoided intake of the saccharin cue (Figure 7-2b). In this instance, there were no contextual cues. Here the association was strengthened only by altering the temporal contiguity, which has been shown to increase the associative strength of a weak stimulus (Balsam, 1984; Broadbent & Cunningham, 1996). Nevertheless, as in the previous findings, avoidance of the cocaine-paired taste cue was delayed.
**Effects of Trigeminal Orosensory Area Lesions on Development of Cue-Induced Withdrawal**

In Chapter 5, when a saccharin taste cue predicted the opportunity to self-administer cocaine, both intact and lesioned rats avoided intake of the CS and showed signs of cue-induced withdrawal in the presence of contextual cues (see Figure 5-7). When retested in the home cage, the saccharin cue alone was not sufficient to induce withdrawal in the lesioned rats (see Figure 5-8). This suggests that the lesioned rats associated the contextual cues with the opportunity to self-administer cocaine. Furthermore, as the opponent process theory suggests, the development of a conditioned state of withdrawal in anticipation of drug also was associated with the context, rather than the taste cue. This would explain why the lesioned rats neither avoided saccharin intake nor showed signs of cue-induced withdrawal when retested in the absence of contextual cues. The establishment of a conditioned aversive state may be the reason that the effects of the

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Figure 7-2: Intake of a taste cue when paired with experimenter-administered cocaine or saline in Sham and TOAx rats when the taste-drug pairings take place in the home cage with either (a) a 5-min ISI or (b) a 0-sec ISI. With a 5-min ISI, the TOAx rats did not avoid intake of the cocaine-paired taste cue. However, when the interval was reduced to a 0-sec ISI, the TOAx rats avoided intake of the cocaine-paired taste cue. The schematics above each graph show how decreasing the interval between the taste cue and drug make this association possible. These figures are reproduced from Figures 2-7, and 6-2, respectively.
TOA lesion are more prominent in the avoidance of a taste cue paired with drug compared with sucrose. As the development of an aversive state in anticipation of drug is unique to the taste-drug association, this provides evidence that the avoidance of a drug-paired cue occurs by a different mechanism than sweet-induced avoidance.

In previous lesion studies, Geddes et al. (2008) found that avoidance of a drug-paired taste cue was eliminated in rats with lesions of the gustatory region of the insular cortex. In these experiments, our standard taste-drug paradigm was used. Specifically, rats were given 5 min access to a taste cue in the home cage and, 5 min after CS access, the rats received experimenter-administered drug or vehicle. In similarly lesioned rats, Lin et al. (2011) found moderate drug-induced avoidance of a taste cue intake when given a longer 15 min CS access period followed by the standard 5 min ISI. I found similar results with TOAx rats in a pilot study that entailed 5 taste-drug pairings with our standard paradigm (5 min CS access, 5 min ISI, and experimenter-administered cocaine or vehicle), followed by 3 taste-drug pairings with an extended taste cue access period of 15 min (Figure 7-3). Specifically, in the pilot study, avoidance of the cocaine-paired cue was observed in Sham, but not TOAx rats with a 5 min CS access period. When the CS access period was extended to 15 min, cocaine-treated Sham rats maintained a similar level of intake, while the saline-treated Sham rats increased intake, as would be expected with a longer access period. The lesioned rats in the cocaine condition, however, showed only a moderate escalation of intake when compared with the saline-treated rats. Therefore, with a 15 min CS access period, the TOAx rats showed moderate avoidance of the cocaine-paired taste cue. As discussed previously, extending the testing period should lead to a failure to evidence drug-induced suppression of CS intake by the TOAx rats. Why then, was there facilitation (i.e., significant avoidance) when the CS access period was increased from 5 to 15 min?
Figure 7-3: Mean intake (± SEM) of 0.03 M Polycose in Sham (left) or TOAx (right) rats paired with experimenter-administered saline (open circles) or 10 mg/kg cocaine (solid circles) across 5 taste-drug pairings with 5-min CS access followed by 3 taste-drug pairings with 15-min CS access. *indicates a significant difference between the saline and cocaine conditions (p < .05). Sham: Saline N=2, Cocaine N=6; TOAx: Saline N=4, Cocaine N=4

As mentioned, when the interval between the offset of CS access and the onset of drug administration (ISI) was short, the lesioned rats avoided intake of a drug-paired taste cue, but when the ISI was increased to 5 min, this was no longer the case (see Figure 7-2). Note that the same 5 min ISI was present throughout the pilot study, regardless of the CS access duration (see Figure 7-4). Additionally, testing occurred in the home cage, so there were no contextual cues to facilitate drug-induced avoidance of the palatable taste cue in the TOAx rats (see Figure 7-1). So why, then, if the ISI remains the same, and the context remains the same, do TOAx rats exhibit moderate avoidance of the cocaine-paired taste cue when the CS access period was increased to 15 min?
Figure 7-4: Diagram depicting the series of events over time when (a) 5-min or (b) 15-min access to a Polycose taste cue was followed by experimenter-administered cocaine with a 5-min interval between the offset of the taste cue and onset of cocaine stimulus (i.e., 5-min ISI). Note: in the absence of contextual cues, a 5-min ISI was sufficient to prevent avoidance of a drug-paired cue in TOAx rats.

Much in the way contextual cues facilitate avoidance of a drug-paired taste cue, interoceptive contexts, such as craving and withdrawal in anticipation of drug, also can enhance this effect in TOAx rats (see Figure 7-5). Nevertheless, one could argue that this interoceptive context would also have facilitated drug-induced avoidance of the taste cue regardless of the length of the ISI. While this is a valid point, the interoceptive context, involving cue-induced craving and withdrawal, differs from external contextual cues in that the interoceptive context is multidimensional and dynamic. Thus, the presence of an external contextual cue remains constant, while the aversive interoceptive state intensifies when there is a longer wait for drug. Furthermore, the onset of this interoceptive context starts when the taste cue is initially presented. Therefore, increasing the interval between the initial onset of the taste cue and drug delivery (referred to here as ‘wait time’) leads to a more intense interoceptive context, thus facilitating the association of the taste cue and drug. The increased intensity, then, may explain moderate taste
cue avoidance in the lesioned rats when there was a longer wait time. This provides evidence that the avoidance of a drug-paired taste cue may very likely be the result of the onset of conditioned withdrawal in anticipation of impending drug availability. Furthermore, these results support the hypothesis that the TOA lesion weakens the associative strength of a palatable taste cue, an effect that may be overridden by increasing the intensity of the aversive state.

![Diagram](image)

Figure 7-5: Diagram depicting the involvement of cue-induced withdrawal in anticipation of cocaine when (a) 5-min or (b) 15-min access to a Polycose taste cue was followed by experimenter-administered cocaine with a 5-min interval between the offset of the taste cue and onset of cocaine stimulus (i.e., 5-min ISI).

In conclusion, because avoidance of the taste cue that predicted either a natural reward or drug of abuse either was absent or delayed in all cases for rats with TOA lesions, I question if the contextual or temporal cues were facilitating the CS-US association, or instead establishing their own associations with the US. Because the lesioned rats expressed a conditioned taste aversion to a LiCl-paired taste cue with a 5 min ISI, and because this occurred at the same rate as in intact controls, there does not seem to be a general delay in learning overall. Most likely, then, the absence or delay in learning resulted from the compensation that was necessary to overcome the weakened associative strength of gustatory cue that resulted from the TOA lesion. Understanding
the relative associations between drug use and external and/or internal cues is a necessary first step in preventing or weakening these associations. Because these associations trigger an aversive state that can lead to relapse even after months of abstinence, eliminating this threat is of utmost importance for successful rehabilitation of addicted individuals. The TOA lesion has provided a means by which to understand how specific drug-related cues can evoke this powerful phenomenon, and opens the door to a more detailed analysis of the factors that perpetuate the cycle of addiction.

**Future Directions**

While not all who suffer from addiction are ready or willing to break the cycle of addiction, those who do are constantly at risk of cue-induced relapse (Everitt & Robbins, 2005; Field & Duka, 2002; Heinz et al., 2004; Reid, Flammino, Starosta, Palamar, & Franck, 2006). Of the 21.6 million persons in the US who needed treatment for a drug or alcohol problem in 2011, only 2.3 million (10.8%) actually received specialized treatment (SAMHSA, 2012). Therefore, improvements in the treatment of addiction, as well as increased availability of treatment options are sorely needed. Furthermore, understanding the mechanisms by which drug or withdrawal related cues devastate attempts at recovery is essential in combating the cycle of relapse in addiction.

Lesion studies, such as those presented in this dissertation, aid in deciphering the circuitry that is and is not important for the association of cues with a drug of abuse. As such, lesion studies can help guide researchers and clinicians towards relevant targets for therapy. For example, locating areas involved in drug-cue associations could be the target for direct current stimulation or fMRI feedback training. Direct current stimulation has proven a useful tool in altering cocaine craving. For example, high frequency transcortical magnetic stimulation of the
skull overlying the PFC decreases craving for cocaine by increasing the excitability of critical inhibitory areas (Camprodon, Martínez-Raga, Alonso-Alonso, Shih, & Pascual-Leone, 2007). On the other hand, low frequency applied to the same area increased risk-taking behaviors (Knoch et al., 2006). Furthermore, fMRI feedback training has proven useful in reducing pain in chronic pain patients by training them to alter the activity in the right anterior cingulate cortex (deCharms et al., 2005). Likewise, similar techniques could be used to train individuals to alter activity in areas deemed relevant for the association of drug related cues. Therefore, further investigation should focus on identifying, and exploiting neural structures essential for the association of drug related cues in hopes of giving addicts a fighting chance against cue-induced craving, withdrawal, and the propensity to relapse.
References


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Ruth L. Kirschstein NRSA (F31) DA029369, May 3, 2011 – May 3, 2014
National Research Council Research Associate Postdoctoral Award, Jan 2013

MEMBERSHIPS IN PROFESSIONAL SOCIETIES

The Society for Neuroscience
Society for the Study of Ingestive Behaviors
Psi Chi Psychological Honors Society

PUBLICATIONS


