PONTINE AND THALAMIC INFLUENCE ON ORAL SUCROSE
AND OIL REWARD

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Abstract

Brain reward systems are fundamental for survival, reproduction, and fitness. Investigating the neural circuits of reward is critical for understanding how the brain organizes behavior as a function of experience. Oral food stimuli such as sugars and fats provide good probes to study reward because they are basic constituents of food that are rewarding to both humans and rodents. Although both orosensory stimuli are highly preferred, much less is known about how those rewarding effects are elaborated in the brain. Sensory detection of sucrose depends on the gustatory system. The sensory systems needed for detecting oil in the mouth have not been determined, but probably involve the olfactory, gustatory, and trigeminal somatosensory systems. The assumption in this dissertation is that coding of sucrose and corn oil depends on the gustatory and the intraoral trigeminal system, respectively. Previous dialysis studies demonstrated that dopamine (DA) overflow in the nucleus accumbens (NAc) increases during sham intake of sucrose solutions. The first objective was to examine whether sham feeding corn oil produces similar DA efflux in the NAc. It does and thus supports the hypothesis that the sensory activities from different orosensory stimuli are transmitted to the forebrain structures linked to reward. Based on the anatomical differences between the central gustatory and the intraoral trigeminal systems, it was hypothesized that the gustatory parabrachial nucleus (PBN) is important in transmitting sucrose, but not corn oil reward. Conversely, the thalamic orosensory area (TOA) was involved in the processing of corn oil, but not sucrose reward. This hypothesis was tested in rats with PBN or TOA lesions using three reward-related behavioral tasks. Because orosensory reward was of interest, all the studies were designed using sham feeding. The first study tested whether rats can switch the hedonic value of an oral stimulus from positive to negative after conditioned taste aversion learning. The second study determined whether rats could measure the apparent reward strength of sucrose and corn oil after PBN or TOA lesions. Finally, an anticipatory contrast paradigm was used to assess whether lesions of the PBN and the TOA eliminate reward comparison for sucrose and corn oil, respectively. The results of these studies reveal that the PBN is important for processing not only sucrose but also corn oil rewards. On the other hand, an intact TOA is not necessary for either sucrose or corn oil rewards.
rewards. Therefore, the original hypothesis that sucrose reward depends on the PBN and oil reward on the TOA requires revision. Given that brainstem gustatory relays receive projections from the intraoral trigeminal system, one alternative neural mechanism for corn oil reward is that the PBN processes both gustatory and trigeminal orosensory activity. Furthermore, this research has eliminated the classical thalamocortical sensory system as a likely candidate for transmitting the sensory neural activity generated by oral food stimuli to the central reward systems. This then points toward more direct projections from brainstem sensory relays to the limbic forebrain as exemplified by parabrachial nuclei.
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List of Abbreviations

ACE          anticipatory contrast effect
AI           angranular insular cortex
B1           bottle 1
B2           bottle 2
BNST         bed nucleus of stria terminalis
BP           break point
CeA          central nucleus of amygdala
CFP          conditioned flavor preference
CS           conditioned stimulus
CTA          conditioned taste aversion
DA           dopamine
DC           dorsal cochlear nucleus
DI           dysgranular insular cortex
FR           fixed ratio
GI           granular insular cortex
GF           gastric fistula
HPLC         high-performance liquid chromatography
LC           locus coeruleus
LETO         Long-Evans Tokushima Otsuka
LH           lateral hypothalamus
L-H          low-high condition in anticipatory contrast effect
LiCl         lithium chloride
L-L          low-low condition in anticipatory contrast effect
MD           mediodorsal nucleus
Me5          mesencephalic trigeminal nucleus
Mo5          motor trigeminal nucleus
NAc          nucleus accumbens
NPY          neuropeptide Y
NST          nucleus of the solitary tract
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<th>Full Form</th>
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<tr>
<td>OLEF</td>
<td>Otsuka Long-Evans Tokushima Fatty</td>
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<tr>
<td>PBN</td>
<td>parabrachial nucleus</td>
</tr>
<tr>
<td>PBNx</td>
<td>parabrachial nucleus lesions</td>
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<tr>
<td>PR</td>
<td>progressive ratio</td>
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<td>Pr5</td>
<td>principal sensory trigeminal nucleus</td>
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<td>PRP</td>
<td>postreinforcement pause</td>
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<td>SNC</td>
<td>successive negative contrast effect</td>
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<tr>
<td>Sp5</td>
<td>spinal trigeminal nucleus</td>
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<tr>
<td>TSNC</td>
<td>trigeminal sensory nuclear complex</td>
</tr>
<tr>
<td>TOA</td>
<td>thalamic orosensory area</td>
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<tr>
<td>TOA</td>
<td>thalamic orosensory area lesions</td>
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<tr>
<td>TR</td>
<td>taste reactivity</td>
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<tr>
<td>TTA</td>
<td>thalamic taste area</td>
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<tr>
<td>US</td>
<td>unconditioned stimulus</td>
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<tr>
<td>VPL</td>
<td>ventral posterolateral nucleus</td>
</tr>
<tr>
<td>VPM</td>
<td>ventral posteromedial nucleus</td>
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<tr>
<td>VPPC</td>
<td>the ventral posterior thalamus nucleus, parvicellular part</td>
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<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
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<tr>
<td>ZI</td>
<td>zona incerta</td>
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Chapter 1

Introduction

Brain reward systems are fundamental for survival, reproduction, and fitness. Investigating the neural circuits of rewards is critical for understanding how the brain organizes behavior as a function of experience. The pioneering brain stimulation research by Olds and Milner (Olds and Milner, 1954) initiated the concept that a neural circuit in the brain modulates behaviors activated by rewarding stimuli. Later studies show that the lowest thresholds and response rates for intracranial electrical self-stimulation are associated with areas of the densest packing of dopaminergic neurons in the ventral forebrain (Wise and Rompre, 1989). Therefore, it is believed that central reward circuits are located in these regions. This reward system known as the mesolimbic dopamine (DA) system is involved in processing of both nonnatural and natural rewards. It comprises DA neurons in the ventral tegmental area (VTA) and their projections to the ventral striatum, predominantly to the nucleus accumbens (NAc), and also to the amygdala, prefrontal cortex, and other forebrain regions (Oades and Halliday, 1987; Kelley and Berridge, 2002; Geisler and Zahm, 2005). Supportive evidence comes from the finding that brain stimulation can activate DA neuronal activities in the VTA, and the administration of addictive drugs such as cocaine, opiates and others (Wise, 1989; Wise et al., 1995; Ranaldi et al., 1999; Cadoni and Di Chiara, 2007) can increase release of NAc DA. Furthermore, the administration of DA agonist and antagonist in the NAc can facilitate and attenuate the rewarding effects of additive drugs and brain stimulation, respectively (Wise and Rompre, 1989). For natural rewards, studies also show that food (Wilson et al., 1995; Bassareo and Di Chiara, 1999a, b) or sexual contact (Pfaus et al., 1990) elevates NAc DA levels. The presentation of food reward also can trigger bursting of DA neurons in the VTA (Ljungberg et al., 1992; Schultz et al., 1993; Schultz, 1998). Finally, in operant studies, where animals make lever presses to receive food reward, DA antagonists decrease performance for food reward without inducing motor deficits (Smith, 1995). There is, then, a great deal of evidence for the role of DA in mediating the rewarding properties of reward. Even so, little is known about where and how rewarding properties of oral food stimuli are extracted from their sensory properties. This dissertation will investigate this problem.
Orosensory stimulation and reward

Sugars and fats provide a good model for investigating the neural basis of the hedonic effects of food rewards. The concept of reward seems straightforward. A rewarding stimulus is a stimulus that, when ‘consumed’ “feels good” to humans and presumably to animals as well. Hence, it is difficult to measure reward because “feels good” is a subjective state and is difficult to define operationally. A perceived rewarding value of a stimulus can be affected by motivation (or drive). Orosensory stimuli such as sucrose and oil are basic food elements, and can be easily specified and applied. They reflect the circularity conundrum in definition of reward because they are rewarding, and those rewarding effects can induce motivation or can be altered by motivation. Nevertheless, the sense of oral stimuli serves several functions that can be used as probes to understand the brain mechanisms for reward. Those functions can be measured, and such measurement can effectively break the circularity conundrum. That is, oral stimuli induce innate concentration dependent intake as the intake is increased for a preferred taste and decreased for an aversive taste. The preferences or aversions can be altered by the motivational state i.e. satiety and food or water restriction; they can also be altered by experience i.e. association learning (Spector, 2000; Norgren et al., 2003). For example, rats normally avoid drinking 1.0M NaCl, but will drink 1.0M avidly after being salt depleted. Another example is that palatable sucrose or corn oil becomes aversive after being associated with malaise induced by lithium chloride (LiCl). In both cases, the sensory characteristic of the oral stimuli do not change, but the hedonic value of the sensory message is increased or diminished. This is the best evidence to show that the rewarding effects of a stimulus are processed in the brain. It is also best explanation of why orosensory stimulation serves as a good model for investigating the central pathways for reward.

Sugars and fats share similar rewarding effects with or without postingestive feedback. It is well known that sucrose is innately rewarding. When preweanling rats are tested only once postnatally, intake of sucrose increases with increasing concentrations and this relationship is greater as the rats grow older (Ackerman et al., 1992). This kind of preference is shown in taste reactivity (TR) test. The TR test was originally developed as a method to assess responses to sapid stimuli (Grill and Norgren, 1978b). The stimuli are infused into the oral cavity through an intraoral cannula, and the immediate orofacial responses are videotaped for
analysis. Animals generate stereotyped responses to different sapid stimuli. For example, sucrose elicits an ingestion response sequence beginning with “low amplitude, rhythmic mouth movements, followed by tongue protrusions, and then lateral tongue movements”. In contrast, quinine elicits a rejection response beginning with gaping and then body movements including chin rubbing, head shaking, face washing, forelimb flailing, and paw pushing (Grill and Norgren, 1978b). Later, Berridge applied this TR test as a method to measure the hedonic aspect of reward in rodent, primates, and human infants (Berridge, 2000; Steiner et al., 2001). The results indicate that the intraoral infusion of sucrose initiates an inherent positive hedonic response across species. Furthermore, adult rats ingest sucrose as a function of concentration during real and sham feeding. In the sham feeding model, a gastric cannula is opened to prevent food from entering the small intestine and so to minimize or exclude postingestive effects (Sclafani and Nissenbaum, 1985a; Smith, 1999). As the metabolic effects are excluded in sham feeding, the response curve for sucrose during sham feeding differs from that demonstrated during real feeding. The curve is an inverted-U function with the highest intake at around 0.3M during real feeding (Nissenbaum and Sclafani, 1987; Smith and Sclafani, 2002). For sham feeding, the curve is a monotonic function (Weingarten and Watson, 1982; Nissenbaum and Sclafani, 1987). The increasing intake function obtained during sham feeding is similar to that obtained when rats are allowed only very brief access to sucrose solutions (Shimura et al., 1997). Rats also drink non-nutritive saccharin solutions in a concentration dependent manner (Smith and Sclafani, 2002). However, the response curves during real and sham feeding are both inverted-U function, presumably reflecting a shift from reward to aversion with higher concentrations of saccharin (Sclafani and Nissenbaum, 1985b). Given that animals show concentration dependent preferences to sweet solutions when the metabolic effect is minimized, orosensory stimulation of sucrose alone can produce rewarding effects and these effects are not learned (i.e., they are innate) Along with innate reward properties, oral stimuli also can become rewarding via learning. For example, rats will come to prefer a flavor that has been paired with the ingestion of a sweet solution to another flavor that has been paired with the ingestion of water. This phenomenon is referred to as a conditioned flavor preference (CFP). The CFP learning, however, not only occurs with real feeding (Messier and White, 1984; Sclafani, 2002) but also when rats are sham feeding as well
(Sclafani et al., 1994; Yu et al., 1999). Oral stimulation alone, then, can support both innate and learned preferences.

All the rewarding effects mentioned above for sucrose are shown for corn oil except for the inherent TR responses. By 14 days of age, rat pups prefer corn oil (Ackroff et al., 1990; Smith and Greenberg, 1991; Ackerman et al., 1992) or nonnutritive mineral oil (Ackroff et al., 1990) emulsions to water, and show concentration dependent intake of corn oil (Ackerman et al., 1992). Previous studies have shown that animals increase food intake when it is mixed with different dietary fat such as lard (Hamilton, 1964), corn oil (Carlisle and Stellar, 1969; Lucas et al., 1989; Smith, 2004), and vegetable shorting (Lucas et al., 1989). Rats also prefer chow mixed with nonnutritive fat substitutes, petrolatum and mineral oil (Hamilton, 1964; Carlisle and Stellar, 1969; Lucas et al., 1989). Compared with sucrose, however, less research has been done in terms of understanding the preference-aversion response of adult animals to different oil concentrations. Unlike sweet sapid stimuli, which have been evaluated using the TR test, there has been no report regarding to animals’ oro-facial response to oil stimuli. One study was conducted for humans and this study demonstrated that infants less than 4 days old showed no specific response to corn oil (Graillon et al., 1997). One study using mice showed that the licking rate during the first 60 s of a 30-min intake session increased with increasing concentrations of corn oil (Saitou et al., 2009). Although lacking data from real feeding, the sham intake of corn oil in adult rats is an inverted-U function of concentration with the maximal intake produced by 12.5%, 25% and 50% corn oil emulsions (Mindell et al., 1990). Finally, as described for sweet oral stimuli, corn oil and mineral oil also support the development of CFP (Elizalde and Sclafani, 1990). Thus, oil also activates the reward circuit via innate and learned processes.

In summary, sucrose and corn oil both have rewarding value and this value drives behavior in an orderly fashion. The rewarding effects of each can be produced on the basis of orosensory stimulation alone (e.g., in rats with an open gastric fistula) and these effects can be innate or they can be learned. Given that both sucrose and corn oil are sensory stimuli, the pathways for their detection and coding must be examined to understand how the sensory signal for sucrose and oil is translated to reward in the brain.
Sensory detection of fats

Sucrose and corn oil have different sensory properties and sensory coding of the two stimuli very likely depends on different central neural systems. Sucrose has a sweet taste quality and so sensory coding of sucrose apparently depends on the gustatory system. The central neural system responsible for sensory coding of corn oil, however, is still inconclusive. Gustatory, olfactory, and trigeminal systems are possible candidates for sensory coding of oils. There are three pieces of evidence that support the involvement of the gustatory system in detecting fat and oil. First, recordings from taste receptors demonstrate that free fatty acids stimulate the gustatory system through the delayed rectifying potassium channels in the taste receptor cells (Gilbertson et al., 1997). Second, it has been shown in rats that lingual lipase can hydrolyze triglycerides to free fatty acids within 1-5 seconds (Kawai and Fushiki, 2003). Finally, a fatty acid transporter, CD36, has been discovered on the taste cells (Laugerette et al., 2005). These data suggest that fats or oils are digested into free fatty acids, and those free fatty acids activate the gustatory signal through taste receptors. Despite these data, other biological and behavioral data are at odds with this theory. Lingual lipase is secreted from the von Ebner’s gland in different species, but there is not necessary lipase activity in the oral cavity in each species (Gilbertson et al., 1997; Schiffman et al., 1998). Although chorda tympani nerve transection impairs discrimination of free fatty acids (Stratford et al., 2006; Pittman et al., 2007), rats with transaction of this important nerve are still able to acquire a conditioned aversion to corn oil when paired with LiCl (Pittman et al., 2007). Finally, adding a lipase inhibitor to a corn oil emulsion does not change the preference for the emulsion (Kawai and Fushiki, 2003). Therefore, the detection of or preference for oils does not necessarily require the gustatory system.

Free fatty acids solutions and corn oil emulsions do not provide the same orosensory cue for learning. For the investigators who suggest that sensory detection of fats depends on taste, the detection of fatty acids in the oral cavity is often considered a pivotal cue to the recognition of dietary fats (Saitou et al., 2009). One study, however, showed that fatty acids and oil emulsions are distinguishable when using them as the CS in conditioned taste aversion (CTA). In this study, rats were first trained to associate a 16% corn oil and 0.25M sucrose mixture
with LiCl. They were then tested for their preference to the oil/sucrose mixture, 0.25M sucrose solution, or 16% corn oil emulsion over water. In this case, the rats showed similar aversion to the oil/sucrose mixture and 16% corn oil emulsion, but they did not avoid 0.25M sucrose. The rats generalized their CTA to the oil/sucrose mixture more to the corn oil than to the sucrose. Thus, the salient orosensory cue of the oil/sucrose mixture was corn oil. The investigators then trained another group of rats to associate a linoleic acid and sucrose mixture with LiCl, and then tested their preference to the mixture, linoleic acid, or sucrose over water. These rats showed strongest aversion to sucrose, then to linoleic/sucrose mixture, and least aversion to linoleic acid. These results indicate that the salient orosensory cue in the linoleic/sucrose mixture was sucrose (Smith et al., 2000). The combination of the two experiments suggests that corn oil emulsions and fatty acid solutions do not have similar orosensory feature. Likely, other features of corn oil such as texture and olfaction make oil emulsions different from fatty acid solutions.

Although oils can contain olfactory features for sensory detection, the olfactory cue does not seem necessary for sensory processing for fats. For example, the preference for chows mixed with corn oil to mineral oil is only temporarily impaired after olfactory nerve damage (Kinney and Antill, 1996). One could argue that olfactory function recovers due to regeneration of olfactory neurons. Other studies, however, used zinc sulfate (ZnSO₄), and showed that anosmic rats were able to 1) show preference for higher concentrations of corn oil emulsions (Takeda et al., 2001), 2) perform conditioned place preference with corn oil (Takeda et al., 2001), and 3) form conditioned aversion to lard and generalize the aversion to butter, margarine, and vaseline (Larue, 1978). Furthermore, studies with human subjects indicate that the detection of odor is not required for the detection of fats. Fluid dairy products contain 0.5 to 36% fat. When the subjects are tested with nose clipped or without visual cues, the detection threshold for those dietary fats does not vary (Mela, 1988; Schiffman et al., 1998). The sensation of fat in the oral cavity is often described as a texture that is creamy, greasy, oily or buttery. The sensation of texture can be mediated by viscosity and/or oiliness. Although viscosity is thought to be the most important component of the texture, a study using the conditioned aversion paradigm has suggested that rats use lubricating properties to discriminate triglyceride oil, silicone oil, and mineral oil (Ramirez, 1994). These data suggest
that the trigeminal system, rather than the gustatory system, is the most important sensory coding system for the detection of oil. Here we will focus upon corn oil. Thereby, the general hypothesis in this thesis project is that sensory coding of corn oil depends on the tactile receptors in the intraoral trigeminal system.

Although the assumption of this thesis is that sucrose and corn oil are detected through the gustatory and trigeminal pathway separately, it should be noted that a firm conclusion regarding the role of gustation, olfaction, and somatosensory in the perception of fat is premature. It is expected that the studies in this thesis will provide further understanding of how the sensory and reward properties of fats are processed in the brain. If the general hypothesis is true, lesions of gustatory relays will disrupt reward-related behavior to sucrose and lesions of trigeminal relays will disrupt reward-related behavior to corn oil. The following section will describe the gustatory and intraoral trigeminal somatosensory pathways, and select central nuclei along the pathways.

**Gustatory pathway and function**

In rodents, the taste signals are detected from the oral cavity via the chorda tympani (CT) and the greater superficial petrosal (GSP) branches of the facial (VII), the linguotonsilar branch of the glossopharyngeal (GL, IX), and the superior laryngeal branch of the vagus (X) cranial nerves (Lundy and Norgren, 2004). These first-order taste neuron projects to the medial and intermediate nucleus of the solitary tract (NST) (Hamilton and Norgren, 1984), then the second-order taste neurons originate from the NST and terminate in the medial PBN (MPBN)(Norgren and Leonard, 1971, 1973; Norgren, 1978). Axons originating from the PBN bifurcate to two pathways. One is the classical thalamocortical pathway that sends taste information to the gustatory insular cortex (Kosar et al., 1986) via the gustatory thalamus, the parvicellular part of the ventral postero medial thalamic nucleus (VPMpc) (Norgren, 1974; Saper and Loewy, 1980; Bester et al., 1999). The other projections involve monosynaptic connections from the PBN to the ventral limbic structures including the central nucleus of amygdala (CeA) (Bernard et al., 1993), the substantia innominata (Norgren, 1974), the lateral hypothalamus (LH) (Bester et al., 1997), and the bed nucleus of stria terminalis (BNST) (Norgren, 1976; Saper and Loewy, 1980; Alden et al., 1994). In addition to the ascending
projections, there are reciprocal connections between the taste relays. The NST receives descending input from the PBN and other forebrain gustatory structures. Similarly, the PBN receives input from the LH, CeA, and the gustatory cortex; and the gustatory cortex also sends axons back to its thalamic relay (Lundy and Norgren, 2004). Except for the connections between the PBN and VPMpc, the ascending and descending projections in the gustatory system are primarily ipsilateral (Lundy and Norgren, 2004). Illustration of the gustatory pathway is shown in Fig 1.1.

At the peripheral level, bilateral single gustatory nerve transection such as chorda tympani or glossopharyngeal nerve section increases but not eliminates the detection threshold and ingestive and rejection responses (Travers et al., 1987; Grill et al., 1992). Those gustatory functions are blunted after a combined transection of the gustatory nerves. For example, it takes a combined transection of CT and GL to eliminate the concentration-dependent increases in sucrose elicited TR responses (Grill and Schwartz, 1992), and a combined transection of the CT, GL and GSP to flatten the sucrose concentration-response function (Spector et al., 1996). In addition, the detection threshold for taste and unconditioned preference-aversion is also impaired following damage to brainstem taste relays. Lesions of the gustatory NST and PBN, respectively, flattened and reduced the concentration-response function of sucrose (Flynn et al., 1991b; Spector et al., 1993; Shimura et al., 1997). Rats with lesions of one of the brainstem nuclei also are unresponsive to increasing sucrose concentration in a TR test (Flynn et al., 1991b). In contrast, lesions of the gustatory thalamus, VPMpc, have relatively minor effects on the unconditioned intake of rewarding or aversive sapid stimuli (Flynn et al., 1991b; Reilly and Pritchard, 1996b).

Lesions of the PBN compromise reward related responses of taste stimuli. The inherent rewarding effects of oral stimuli can be altered by experience in a CTA task. Animals acquire a CTA when a novel taste, the conditioned stimulus (CS), is followed by an intraperitoneal injection (i.p.) of LiCl, the unconditioned stimulus (US), that induces visceral illness. After one such pairing of the CS and the US, the inherently rewarding sucrose CS can become aversive, i.e. rejected or avoided. It has been demonstrated in our laboratory that lesions of the PBN, but not those of the NST or VPMpc prevent rats from
Fig. 1.1. Gustatory (blue) and oral-trigeminal (red) pathway. Abbreviations: AI, angranular insular cortex; Amyg, amygdala; CeA, central nucleus of amygdala; CI, claustrum; cp, cerebral peduncle; DC, dorsal cochlear nucleus; DI, dysgranular insular cortex; GI, dysgranular insular cortex; LC, locus coerules; LH, lateral hypothalamus; MD, mediadorsal thalamic nucleus; Me5, mesencephalic trigeminal nucleus; Mo5, motor trigeminal nucleus; NST, nucleus of the solitary tract; PBN, parabrachial nuclei; Pr5, principal sensory trigeminal nucleus; Sp5, spinal trigeminal nucleus; TTA, thalamus taste area; VPM, ventral posteromedial thalamic nucleus; ZI, zona incerta.
learning a CTA (Grill and Norgren, 1978a; Flynn et al., 1991a; Spector et al., 1992; Reilly et al., 1993; Scalera et al., 1995; Reilly and Pritchard, 1996a; Grigson et al., 1997a, b; Scalera et al., 1997; Grigson et al., 1998). As with bilateral lesions of the pontine PBN, rats also cannot learn a CTA following decerebration (Grill and Norgren, 1978a). Because decerebration functionally disconnects the pons from the forebrain, these observations imply that the PBN is necessary but not sufficient for learning a CTA [for review see (Reilly, 1999)]. Similar to CTA learning, lesions of the PBN but not the NST or the VPMpc disrupt a salt appetite that is induced by injections of furosemide (Scalera et al., 1995; Grigson et al., 1997b; Scalera et al., 1997). Those findings lead to the hypothesis that the gustatory PBN is required for transmitting the rewarding effects of taste to forebrain. This hypothesis is further supported by neurochemical and Fos studies with rats sham drinking sucrose. In one study, the DA level in the NAc was measured using microdialysis and reverse phase HPLC; and in the other study, neuronal activation in the brain in response to sham intake of sucrose was measured by immunohistological labeling of Fos. For normal rats, sham drinking of sucrose increased DA overflow in the NAc (Hajnal et al., 2004), and increased Fos expression in the brainstem and forebrain gustatory relays as well as in the NAc (Mungarndee et al., 2008). Lesions of the PBN, but not the VPMpc, blunted the DA increase in the NAc during sucrose intake (Hajnal and Norgren, 2005), and eliminated the sucrose-induced Fos increase in not only the forebrain gustatory relays but also in the NAc (Mungarndee et al., 2008). Taken together, these results suggest that the PBN is essential for transmitting the rewarding effects of oral sucrose to the forebrain reward circuits.

**Intraoral trigeminal pathway and function**

Beside jaw muscles and skin over the mandible and lower lips, the mandibular branch of the trigeminal nerve innervates the intraoral lower jaw mucosa, teeth and the anterior tongue. Particularly, 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, interpolaris and oralis of spinal trigeminal nucleus (Sp5), and the principal trigeminal sensory nucleus (P5) (Jacquin et al., 1983; Takemura et al., 1987). The secondary trigeminal neurons from these nuclei then project to
the ventroposteromedial nucleus (VPM) of the thalamus (Waite, 2004). Those projections include ipsilateral and contralateral projections (Smith, 1973; De Chazeron et al., 2004; Waite, 2004; Guy et al., 2005). Unlike the gustatory system, the thalamocortical projections are the only connecting pathway between the brainstem trigeminal nuclei and the forebrain. The projections from the thalamic somatosensory areas to the cortex terminate parallel to overlap with the gustatory cortex (Norgren and Wolf, 1975; Yamamoto et al., 1981). Illustration of the oral-trigeminal pathway is shown in Fig 1.1.

Damage to the trigeminal orosensory system affects ingestive and motivated behavior. There has been no analysis of the effects of trigeminal orosensory lesions on the response to intraoral sapid stimuli. The effects of trigeminal damage are best documented for food and water intake. Bilateral trigeminal mandibular branch deafferentation disrupts food and water intake. The more extensive the deafferentation, the more severe and longer lasting the impairment of food and water intake (Miller, 1981; Jacquin and Zeigler, 1983). Rats with mandibular nerve damage could not initiate meals at normal rate, and thereby increased meal duration to compensate for the consummatory inefficiency (Miller, 1981). Moreover, lesions of the central trigeminal nuclei such as the Pr5 and the VPM both results in an aphagia syndrome (Zeigler and Karten, 1974; Nadaud et al., 1984). Thus, while a little is known about the role of the trigeminal orosensory system in feeding, the knowledge is sparse regarding the means by which damage to the intraoral trigeminal system may affects reward learning behaviors. The only record in the literature is that sensory deafferentation of the mandibular nerve disrupts lever pressing responses for food (Jacquin et al., 1982).

**Specific studies**

The general hypotheses are that similar forebrain structures, ultimately, are involved in the orosensory reward of sucrose and corn oil. The rewarding effects of sucrose, however, reach the reward circuit via the gustatory pathway, while the rewarding effects of oil reach the reward circuit via the trigeminal sensory pathway. Using DA level in the NAc as an index of reward, it has been demonstrated that orosensory stimulation with sucrose increases DA in the NAc (Hajnal et al., 2004) as mentioned above. The first study in this thesis project was to determine whether sham drinking 100% corn oil also will produce an increase of DA in the NAc (Chapter 2). If true, then lesions of a nucleus of the central gustatory pathway should
 disrupt reward-related behaviors associated with the orosensory stimulation of sucrose and
lesions of a nucleus of the trigeminal sensory pathway should disrupt reward-related
behaviors associated with the orosensory stimulation of oil. Based on previous studies of
gustatory function, it is hypothesized that the PBN is the required gustatory relay for
translating the sucrose signal to the forebrain reward circuits. Given that less is known about
the functions of the oral trigeminal system, the nucleus for testing the trigeminal part of the
hypothesis has been chosen based on comparisons of the anatomical characteristic of the two
pathways. For the gustatory pathway, there are two nuclei that connect to the forebrain
structures, the PBN and the VPMpc. The oral trigeminal pathway, in contrast, has only the
thalamic trigeminal somatosensory area, the VPM, that connects to the forebrain structures.
Thus, it is hypothesized that the thalamic oral trigeminal nucleus is important in transmitting
orosensory stimulation of corn oil reward to the forebrain structures.

There are three studies involved in testing different aspects of reward using three behavioral
tasks. Study 1 tests rats’ ability to switch the hedonic value of an oral stimulus from preferred
to avoided using the CTA paradigm (Chapter 3). If the hypotheses are true, lesions of the
PBN and the VPM will disrupt the acquisition of conditioned aversion to sucrose and corn oil,
respectively. Study 2 measures the relative reward strength of sucrose and corn oil via operant
conditioning (Chapter 4). If the hypotheses are true, lesions of the parabrachial nuclei and the
VPM will decrease the innate reward strength of sapid sucrose and corn oil, respectively.
Finally, study 3 (Chapter 5) tests whether rats with central lesions are able to compare
different concentrations of sucrose and corn oil in a Pavlovian conditioning paradigm referred
to as an anticipatory contrast effect (ACE). An ACE occurs when rats avoid intake of a weak
sapid stimulus in anticipation of the immanent availability of a strong (preferred) sapid
stimulus in the very near future (Flaherty and Checke, 1982). If the hypotheses are true,
lesions of the parabrachial nuclei will disrupt the ACE for sucrose concentration pairs, while
lesions of the VPM will disrupt the ACE for corn oil emulation pairs. In the literature, these
behavioral tasks have always been tested with normal feeding. Since this dissertation is
focused on the orosensory effects of sucrose and corn oil, each paradigm used with animals
that were sham feeding to minimize postingestive feedback. Therefore, in Chapter 4 and 5,
sham feeding were tested with operant tasks and the reward comparison paradigm to obtain
baseline data for comparison performance after central gustatory and trigeminal lesions. A
general discussion for the results of all experiments is presented in Chapter 6.
Chapter 2

Sham feeding corn oil increases accumbens dopamine in the rat

Although the exact role of mesolimbic dopamine (DA) in reward remains controversial, considerable evidence demonstrates that both natural (Smith, 1995; Wilson et al., 1995; Smith, 2004) and non natural (Wise and Rompre, 1989) rewards release DA in this system. Dopamine neurons in the ventral tegmental area (VTA) project predominantly to the nucleus accumbens (NAc) (Oades and Halliday, 1987; Geisler and Zahm, 2005). Intake of palatable foods such as chocolate (Wilson et al., 1995) and shortcake (Martel and Fantino, 1996a, b) results in an increase of extracellular DA in the NAc. Among the constituents of these foods, sucrose has been tested most because it has inherent rewarding properties. In our laboratory, we have demonstrated that both real (Hajnal and Norgren, 2001, 2002) and sham (Hajnal et al., 2004) feeding of sucrose increase DA overflow in the NAc. During sham feeding, sucrose solution is drained out from the stomach by a gastric fistula and so the postingestive effects of sucrose are excluded. Thus, the result of the sucrose sham feeding experiment indicates that the orosensory effects of sucrose alone are sufficient to increase extracellular levels of NAc DA.

Fat is another macronutrient that appears to be inherently preferred by both humans and rodents. Rats prefer 25%, 50% and 100% corn oil emulsions to water. Using corn oil emulsions, Smith and colleagues demonstrated that sham intake of corn oil is an inverted-U function of concentration in both preweanling (Smith and Greenberg, 1991; Ackerman et al., 1992) and adult (Mindell et al., 1990; Smith and Greenberg, 1991) rats. Furthermore, systemic application of the DA receptor antagonists SCH23390 and raclopride dose dependently decrease the intake of corn oil emulsions without affecting the latency to sham feed or producing obvious motor impairment (Weatherford et al., 1988; Weatherford et al., 1990). These results support the hypothesis that the rewarding effects of oral corn oil are mediated by central dopaminergic activity, but do not specify the site of the effect. A more direct support for this hypothesis requires measuring mesolimbic DA levels during orosensory stimulation with corn oil. Therefore, in the present study, we used microdialysis in combination with reverse-phase high-performance liquid chromatography (HPLC) to
investigate DA levels in the medial shell of NAc during sham feeding of 100% corn oil emulsion. Some of these data were presented at the annual meeting of the Society for Neuroscience in Washington D.C. in 2005 (Liang et al., 2005). The results of this chapter are published as short communication in American Journal of Physiology in 2006 (Liang et al., 2006).

Materials and Methods

Subject

A total of 43 male Sprague-Dawley rats (275-325 g, Charles River, Wilmington, MA) were used in five iterations of this study. They were individually housed on a 12:12-h light-dark schedule with ad libitum tap water and standard laboratory diet [Rodent diet (W) 8604; Harlan Teklad, Madison, WI].

Surgery

For the implantation of gastric fistulas and microdialysis cannulas, the subjects were food deprived overnight, then treated with atropine sulfate (0.15mg/kg, ip) and, 20 min later, anesthetized with pentobarbital sodium (50mg/kg, ip). Each rat was fitted with a stainless steel gastric fistula and bilateral, 21-guage stainless steel guide cannulas aimed above the posterior medial NAc [A 1.0 mm, L 1.0 mm from the bregma, and V 4.0 mm from the skull; Ref.(Paxinos and Watson, 2005)]. The design and implantation of the gastric cannulas are described elsewhere (Smith, 1999). The guide cannulas were fixed to the skull using stainless steel screws (Fillister head 1-72 × 1/8", Small Parts, Inc, FL) and dental acrylic. All the procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine, and comply with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

Training Procedure

After 7-10 days recovery, the rats were transferred to individual hanging cages that had a longitudinal slot in the floor, and placed on an 18-h food and water deprivation regimen. One hour before the sham licking training session (7:30 AM), the stomach was flushed with
lukewarm water. A flexible tube was screwed in the gastric fistula and passed through the slot to drain solutions. On alternative days, the subjects received dH₂O or 100% corn oil emulsion [100 ml corn oil blended with 0.75 ml tween-80 (Sigma-Aldrich Inc, St Louis, MO)] for 20 min (9:00 – 9:20 AM). They were then allowed 2-3 hr (12:00 – 15:00) real intake of normal powder chow and dH₂O. Each rat had 6 to 10 training trials with dH₂O and 100% corn oil emulsion. They were then transferred to one of the six microdialysis chambers and received the same training regimen for 4-7 more days. On the last day of training in the chamber, the concentric microdialysis probes with 2-mm active membrane were implanted bilaterally in the medial shell of the NAc through the guide cannulas. The active membrane of the probes consisted of cellulose tubing (20-kDa cutoff, 0.2-mm OD × 2-mm length; Spectrum, Ranch Dominguez, CA; see ref. 5 for details). The probes were perfused with artificial cerebrospinal fluid [aCSF; in mM: 145 NaCl, 2.7 KCl, 1.2 CaCl₂, 1.0 MgCl₂, and 2.0 Na₂HPO₄ in HPLC-grade water (Fisher Scientific, Pittsburgh) adjusted to pH 7.4] through a microdialysis swivel (375/D/22QE; Instech Laboratories, Plymouth Meeting, PA ) at a rate of 1.0 μl/min using microsyringe pumps (Model A99; Razel Scientific Instruments, Stamford, CT). On the test days, 20-min dialysis samples were taken before, during, and after sham licking. Because of the limits of the microdialysis probes, each subject had at most three test days.

Histology
At the end of the experiment, the rats were sacrificed with an overdose of pentobarbital sodium (150 mg/kg ip), then perfused transcardially with 0.9% saline solution followed by 10% formalin. The brains were frozen, serially sectioned at 50 μm, and stained with cresyl violet to verify placement of the microdialysis probes.

HPLC
Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) from microdialytic samples (20 μl) were analyzed by reverse-phase HPLC with coulometric detection (ESA CoulArray system, ESA, Inc., Chelmsford, MA, Analytic cell: Model 5014B, electrode 1: -175 mV; electrode 2: +175 mV; guard cell: Model 5020: +300 mV). The chromatograms were recorded and analyzed off line by ESA data system on a PC.

Statistical Analysis
The data includes intake of dH2O and 100% corn oil. For neurochemistry data analysis, the raw results from the chromatograms were converted to a percentage of the mean value of the 3 baseline samples taken before the sham licking sessions. These normalized data for DA, DOPAC and HVA were analyzed by separate two-way ANOVAs (stimulus × sample/time), followed by post hoc Newman-Kuels tests when justified.

Results
Probe Placement
After exclusions for poor placement (n = 4), malfunctioning probes (n = 20), and inadequate fistula drainage (n = 7), data from 12 rats with a total of 15 probes were included in the results. The probes excluded for location were in the rostral shell of NAc (β+2.50 mm, n=2), lateral to the NAc core (β+1.20 mm, n=1), and dorsal to the core (β+1.70 mm, n=1). During sham licking of dH2O and corn oil, dopamine overflow at these sites was unchanged or increased somewhat (data not shown). Because the subject numbers were small, the results were not analyzed further. The successful probes were located between 1.00 mm to 1.40 mm anterior to β in the medial shell of NAc (Fig. 2.1).

Fig. 2.1. **Localizations of the microdialysis probes.** Microdialysis sites in the NAc are drawn in the sections of the rat brains’s left hemisphere. The active membranes of the probes (0.2 mm × 2 mm), depicted with gray bars, were located between 1.40 - 1.00 mm rostral to the bregma in the medial shell of the NAc based on the atlas of Paxions and Watson (Paxinos and Watson, 2005).
Intake
Sham intake of both dH2O and corn oil emulsion increased during training. There was a significant trial effect and a stimulus×trial interaction [F(5, 110)=13.64, p<0.001; F(5, 110)=7.29, P<0.001]. The dH2O intake reached peak on the third training trial (20.08 ± 3.18 ml / 20 min) and then decreased in the following trials. In contrast, corn oil emulsion intake increased continuously with mean intake reaching 22.33 ± 1.89 ml / 20min by the sixth trial. On the first trial in the microdialysis chamber, both dH2O and corn oil emulsion intakes decreased significantly compared with the prior training trial (dH2O: 16.92 ± 3.41 ml vs. 7 ± 1.11 ml, t-test, p<0.02; corn oil emulsion: 22.33 ± 1.89 ml vs. 12.47 ± 2.43 ml, t-test, p<0.005). Although the dH2O intake was smaller than the corn oil emulsion intake on the first trial in the chamber, the difference was not quite significant (t-test, p=0.053). During the dialysis tests, however, sham intakes of corn oil and dH2O were statistically identical (corn oil vs. dH2O: 13.97 ± 2.08 ml vs. 11.73 ± 1.39 ml; t-test, p=0.37).

Monoamine Level
The results showed that sham licking corn oil stimulated accumbens DA flux, while licking dH2O did not. The percent increase in DA overflow, however, was not correlated with the volume of oil consumed (r=−0.17). Two-way ANOVAs (stimulus × sample) revealed that there were stimulus [F(1, 25)=10.17, p<0.004], sample [F(8, 200)=2.85, p<0.006], and stimulus×sample [F(8, 200)=2.52, p<0.02] effects. After 20-min of sham corn oil intake, DA levels were significantly higher than baseline and higher than DA levels after dH2O intake (corn oil vs. dH2O: 157.5 ± 18.8% vs. 93.0 ± 4.0%, p<0.001; Fig. 2). Dopamine levels continued to be significantly higher for 20 minutes after oil intake ceased (corn oil vs. dH2O sample 5: 141.9 ± 21.7% vs. 96.0 ± 5.4%, p<0.05). Two-way ANOVAs demonstrated that DOPAC and HVA levels also were higher than baseline after sham licking of corn oil. There were stimulus [DOPAC: F(1, 25)=8.98, p<0.007; HVA: F(1, 25)=8.53, p<0.008] and sample [DOPAC: F(8, 200)=2.67, p<0.009; HVA: F(8, 200)=5.89, p<0.001] effects in both cases, but no interaction between stimulus and sample [DOPAC: F(8, 200)=1.17, p=0.32; HVA: F(8, 200)=1.08, p=0.38].
Discussion

This experiment has demonstrated that sham licking 100% corn oil increases DA and its metabolites in the NAc. The design controlled for licking behavior because the rats received dH₂O and corn oil on alternative days and ingested similar amounts. The DA activation during licking corn oil, therefore, was unlikely to result from differential oromotor activity. Because the rats were sham feeding to minimize gastrointestinal feedback, the nutritive component in the corn oil should not contribute to the increased DA overflow in the NAc. The results support the hypothesis that the oral sensory properties of corn oil drive accumbens dopaminergic activity.

Figure 2.2. Extracellular levels of DA (top), DOPAC (middle), and HVA (bottom) in the NAc before, during, and after sham licking of dH₂O and corn oil. Licking corn oil stimulated DA flux in the NAc. This effect lasted at least 20 min after the end of the corn oil bout. Sham licking of corn oil also increased DOPAC and HVA overall, but none of the post hoc comparisons was significant. *; significant post hoc tests for differences from baseline samples and from sham water intake, p < 0.05. #; significant difference in sample 5 when the rats ingested water in sample 4 compared with corn oil in the same period, p < 0.05)
Sham licking of a gustatory stimulus, sucrose, stimulates accumbens DA overflow as a function of concentration (Hajnal et al., 2004). The effects of 0.3M sucrose and 100% corn oil on DA overflow in the NAc did not differ -- 156.05 ± 11.78% and 157.5 ± 18.8%, respectively, ref. 6. Behaviorally, rats prefer 100% corn oil to 10% sucrose [≈ 0.24 M, Ref.(Weatherford et al., 1990)]. Theoretically, if both the behavioral and neurochemical indexes reflected the same underlying reward mechanisms, the measures would match. In fact, it is unlikely that either NAc DA overflow or preference reflect reward similarly because the construct cannot be defined precisely, especially in neural terms (Norgren et al., 2006). Nevertheless, the results from the present study and the sucrose studies (Hajnal and Norgren, 2001, 2002; Hajnal et al., 2004) support the hypothesis that the reward produced via different sensory modalities are mediated by the same or closely related substrates in the forebrain.

Anatomical studies have demonstrated direct and indirect connections between the forebrain gustatory relays and the mesolimbic DA areas, including the NAc and the ventral tegmental area (VTA) (Oades and Halliday, 1987; Norgren et al., 2003; Lundy and Norgren, 2004; Geisler and Zahm, 2005). Those forebrain and hindbrain connections provide possible substrates for DA activation in the accumbens, and may also be involved in the hedonic effects of taste (Norgren et al., 2003). Hajnal and Norgren (2005) demonstrated that lesions in the secondary taste relay, the parabrachial nuclei (PBN), but not in the gustatory thalamus blunt the DA overflow during sucrose licking (Hajnal and Norgren, 2005). This result implies that the hedonic information of the sucrose taste reaches the NAc via the PBN and the limbic forebrain circuits.

Dopamine flux occurs in other areas during tasks involving ingestive behavior but not always directly in register with oral stimulation. Dopamine release in the striatum occurred during operant learning for food reward but peaked around the lever press rather than reward consumption (Nakazato, 2005). Extracellular DA in the medial prefrontal cortex (MPC) was increased in response to presentation of a neutral stimulus, a plastic box, that had been associated with a palatable food. The same neutral stimulus, however, did not modify
extracellular DA in the medial NAc (Bassareo and Di Chiara, 1997). In the MPC, dopamine appears to be essential for attention and working memory related learning. In an eight arm radial maze task, MPC DA efflux increased in the absence of food reward (Phillips et al., 2004). These and other studies indicate that increased accumbens DA release during sham intake is not just a general response to food, but one facet of the complex, highly orchestrated neural activity that accompanies rewarded behavior.

The sensory mechanisms by which corn oil is detected are not known. The best candidates are olfaction, taste, and the oral somatosensory system. Rats made anosmic by nasal instillation of ZnSO$_4$ still discriminated fats such as margarine and lard mixed with food (Mindell et al., 1990). After olfactory bulbectomy, rats still preferentially ingest 0.5% and 1% corn oil (Ramirez, 1993). Anosmic mice can show conditioned place preference to 100% corn oil (Takeda et al., 2001). Although preference for 1 and 3% corn oil is decreased in anosmic mice, their preference for higher concentrations 5 and 10% is not affected (Takeda et al., 2001). These results suggest that an olfactory mechanism is not necessary for processing oral oil stimulation. Recent studies suggest that fatty acids are important for the gustatory recognition of fats. Rats can detect free fatty acids and acquire a conditioned aversion to them (McCormack et al., 2006). In addition, a fatty acid transporter, CD36, is located on the taste cells (Laugerette et al., 2005). Although provocative, this evidence does not prove that taste is responsible for detecting oils or dietary fats. The main reason is that the dietary fats consist mainly of triglycerides, and triglycerides need to be digested first to become fatty acids. Although lingual lipase can hydrolyze triglycerides to free fatty acids (Kawai and Fushiki, 2003), how effective this mechanism is for the gustatory recognition of dietary lipids remains unknown. In rats, addition of a potent lipase inhibitor diminished preference for a triacylglyceride solution. This effect, however, did not occur when the lipase inhibitor was added to a corn oil emulsion (Kawai and Fushiki, 2003). Furthermore, rats with lesions in the secondary gustatory nucleus, the PBN, fail to learn aversions to taste stimuli but do learn to avoid 100% corn oil (Norgren et al., 2001). Thus it is possible that the olfactory or the gustatory systems are not essential for processing the sensory and hedonic aspects of corn oil. This would leave the trigeminal system as the best candidate.
The intraoral trigeminal system, however, does not project directly to the limbic or DA systems. The mandibular branch of the trigeminal nerve innervates the anterior tongue, lower teeth, and much of the intraoral mucosa (Jacquin et al., 1983; Takemura et al., 1987; Waite, 2004). The maxillary branch distributes to the hard and soft palate. The axons of these nerves project to the mediodorsal principal and spinal trigeminal sensory nuclei as well as the nucleus of the solitary tract (NST) (Jacquin et al., 1983; Takemura et al., 1987; Waite, 2004).

In contrast to the anterior oral cavity, somatosensory information from the posterior oral cavity reaches the brain through the glossopharyngeal nerve. Tactile information detected by the glossopharyngeal nerve is also carried to the NST (Hamilton and Norgren, 1984). There is little if any evidence of direct projections to the mesolimbic areas from the trigeminal system. The NST and the spinal trigeminal nuclei project strongly to the parabrachial nuclei. Thus it is possible that intraoral somatosensory activity reaches the ventral forebrain via the PBN (Yoshida et al., 1997; Lundy and Norgren, 2004; Waite, 2004). Electrophysiological confirmation of this possibility is lacking and, as mentioned above, behavioral evidence suggests that, at minimum, additional routes exist (Norgren et al., 2001). The current experiment demonstrates that sham licking of corn oil releases accumbens DA much the same way as does sucrose ingestion. The central pathways that are critical for this effect can be determined using experiments parallel to those used to narrow the sucrose hedonic response down to the parabrachial ventral pathway (Hajnal and Norgren, 2005).

Using DA in the NAc to track the reward mechanism in the forebrain, this study has shown that the rewarding effects of sucrose and corn oil involve similar forebrain structures. Three behavioral tasks are used to test whether the same or different orosensory pathways are involved in transmitting sucrose and corn oil rewards in the next three chapters.

* This chapter was published as a short report in,
Chapter 3

Differential effects of lesions of the gustatory parabrachial nucleus and the thalamic orosensory area on conditioned taste aversion for sucrose and corn oil in sham feeding rats.

Studies with microdialysis and reverse-phase HPLC provide neurochemical evidence that orosensory stimulation of sucrose and corn oil produces similar rewarding effects. As discussed in chapter 2, animals respond to oral sucrose and corn oil similarly (Ackerman et al., 1992; Hajnal et al., 2004; Liang et al., 2006). The neurochemical study along with previous pharmacological studies suggest that the two oral stimuli produce reward using the same or closely related neural substrates in the forebrain (Weatherford et al., 1988; Weatherford et al., 1990; Hajnal and Norgren, 2001). The next question then is to understand whether the two oral rewards are transmitted through the same or different neural pathways to the forebrain reward circuits. Substantial studies have demonstrated that the sensory detection and reward processing of oral sucrose is through the gustatory pathway. The central neural system responsible for sensory detection of corn oil, however, is far from clear. In chapter 1, the possible role of the olfactory, gustatory, and trigeminal system was discussed. The evidence suggests that the sensory detection of corn oil depends on the trigeminal system. The anatomical significant differences between the gustatory and the trigeminal system lead to the hypothesis that the gustatory parabrachial nucleus (PBN) and the thalamic trigeminal nucleus, ventral posteromedial nucleus (VPM), are important in processing oral sucrose and corn oil reward, respectively. This hypothesis was tested with three different behavioral tasks that examine different aspects of reward. The purpose of each test was to understand whether lesions of the PBN disrupt processing the reward related information of sucrose but not of corn oil; conversely, whether lesions of the thalamic orosensory area (TOA) disrupt processing reward related information of corn oil but not of sucrose. The first study tested whether lesions of the PBN eliminate conditioned aversion learning to sucrose but not to corn oil, and whether the reverse is true for TOA lesions.
The secondary gustatory relay, the PBN, is required for the acquisition of a CTA (Reilly et al., 1993; Grigson et al., 1997a, b). The experimental procedures conducted in the laboratory for the conditioned aversion learning include the presentation of a novel oral stimulus such as a taste solution followed, after a 15-min interstimulus interval, by an intraperitoneal (ip) injection of LiCl. After one such pairing of the oral stimulus with the injection, animals learn to avoid the gustatory stimulus upon its next presentation. In this classical conditioning paradigm (Domjan, 2005), the novel oral stimulus is the conditioned stimulus (CS) and the LiCl is the unconditioned stimulus (US). Conditioned taste aversion is a robust phenomenon because the aversion can be acquired after one single pairing of the CS and the US, and the learning does not require close temporal contiguity between the CS and US (Garcia et al., 1966; Bernstein, 1999). The conditioned aversion paradigm provides a good model for the investigation of the reward pathways activated by orosensory stimuli. That is, following acquisition of a CTA, the inherent hedonic properties of a stimulus can switch from positive to negative (Norgren et al., 2003). We predict that orosensory stimulation with sucrose or oil will be sufficient to support the development of a CTA following pairings with the illness-inducing agent LiCl. Given that lesions of the PBN block CTA learning in real feeding rats (Spector et al., 1992; Sakai and Yamamoto, 1998), we predict that these same lesions will block the acquisition of the CTA to sucrose in sham feeding rats. The PBN lesion should have no effect on the CTA to the corn oil CS because previous experiments in our laboratory indicate that PBN lesioned rats can learn a conditioned aversion to a 100% corn oil CS when paired with LiCl (Norgren et al., 2001). Oil, then, has properties that circumvent the gustatory pathway. Bilateral lesions of the TOA, on the other hand, are expected to have no effect on the development of the CTA to sucrose, but should block the development of the CTA to the corn oil CS. In the present study, lesions in the thalamus were centered in the trigeminal area, which was 500μm lateral to the gustatory area.

Materials and Methods

Subjects
The subjects were 30 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 275-300g at the beginning of this study. They were individually housed in hanging wire mesh cages on a 12:12-h light-dark schedule with ad libitum tap water and standard laboratory media.
pellets [Rodent diet (W) 2018; Harlan Teklad, Madison, WI]. Once the experiment began, the rats were maintained on an 18 hr food and water deprivation regimen with distilled water (dH2O) and the same chow powdered [Rodent diet (W) 2018C; Harlan Teklad, Madison, WI] available from noon to 4PM daily.

Surgery
The rats were divided into PBN lesions (PBNx, n=10), PBN surgical control (Con PBN, n=5), TOA lesions (TOAx, n=10), and TOA surgical control (Con TOA, n=5). For each surgery, the rats were treated with atropine sulfate (0.15 mg/kg ip; Gensia Sicor Pharmaceuticals, Irvine, CA) and, 20 min later, anesthetized with pentobarbital sodium (50 mg/kg ip; Nembutal; Abbott Laboratories, North Chicago, IL). Once anesthetized, the rat was mounted in a stereotaxic instrument and a midline incision is made to expose the dorsal surface of the skull for the brain surgery. Using electrophysiological guidance, the taste PBN was localized for rats in the PBNx group. For the rats in the TOAx condition, the thalamic taste areas were localized first, and then moved the electrode 500 μm lateral to the thalamic taste areas. The electrode was oriented 20° off vertical pointing anterior for the recording in the PBN and 90° vertical for recording in the thalamus. Taking bregma as reference, the coordinates for penetrations in the PBN were AP: -12.0 ± 1.0 mm, ML: ±1.8 ± 0.4 mm, DV: -7.5 ± 0.5 mm; and in the thalamus AP: -3.6 ± 0.6 mm, ML: ±1.0 to ±1.4 mm, DV: -7.0 ± 0.5 mm. A Hamilton 1.0μl syringe fitted with a glass pipette on the tip was filled with ibotenic acid (20 μg/μl; Sigma, St Louis, MO) and then the pipette tip was lowered into the brain at the appropriate coordinates. For PBNx rats, taste activity was recorded through the Hamilton injector to confirm the location. When the pipette tip reached the desired depth, the ibotenic acid (0.1 – 0.15μl /side for PBN; 0.17 – 0.2μl /side for TOA) was delivered by pressure over 15 min. Afterward, the syringe remained in place for an additional 10 to 15 min to allow for dispersal. The same procedure was repeated on the other side of the brain. For the surgical controls, the skull was opened and the electrode was lowered several times to just above the PBN or the TOA. At the end of each cranial surgery, the skull openings were filled with Gelfoam (Upjohn, Kalamazoo, MI) and the skin was closed with wound clips. The rat was then allowed to recover until its body weight reached pre-surgical level, usually more than one week. Subsequently the rats were anesthetized again for the gastric fistula surgery for
sham feeding. Details for the design and implantation of the gastric fistulas (Plouse Machine Shop, Harrisburg, PA) are described elsewhere (Smith, 1999; Mungarndee et al., 2008). At the end of each surgery, the rats were given gentamicin (4mg ip; Abbott Laboratories, North Chicago, IL), and an anti-inflammatory (Meloxicam, 1 mg/kg sc; Metacam, Boehringer Ingelheim Vetmedica, St. Joseph, MO) every 12 hrs for as long as needed. In all, rats had at least one month of recovery until the experimental procedure began.

Training Protocol

Four days before baseline water training, the rats were transferred to training cages. These stainless steel wire cages (11” long × 9” wide × 7” high) have a slot down the center of the floor to accommodate the gastric cannula drain tube. During this period, the rats were acclimated to consume powdered chow and lick dH2O through a water spout in front of the hanging cage. Water and food were removed in the afternoon the day before the training began, and from then on, the rats were maintained at the 18 hr food and water restriction schedule. Every morning they received fluid for 15 min with the gastric fistula opened and dH2O and powdered food for 4 hr in the afternoon with the gastric fistula closed. The CTA training included baseline water intake, a series of conditioning trials, and one-bottle and two-bottle tests. The rats were first trained and tested for CTA using 100% corn oil (Mazola, ACH Food Companies, INC., Memphis, TN) as the CS, then returned to free drinking and feeding for two weeks. Thereafter, the food and water restriction regimen was re-imposed for a second LiCl-induced CTA using 0.3M sucrose (Fisher Chemicals, Fairlawn, NJ) as the CS. Every 100 ml corn oil contained 0.75 ml Tween-80 (Sigma-Aldrich, St. Louis, MO) so that it would remain fluid in the stomach.

Each morning before the 15 min fluid intake access period, the screw of the gastric fistula was removed for each rat and the stomach was flushed with lukewarm water until came out clear. A flexible tube was then screwed into the gastric fistula and passed through the slot to drain the solutions. The fluid (dH2O or the CS) was presented in an inverted Nalgene graduated cylinder with a silicone stopper and a stainless steel spout affixed to the front of the hanging cage. Once the rat finished its daily session, its stomach was flushed again with lukewarm
water, and the gastric fistula closed with its screw. The recovery of solutions from the gut was measured to ensure the success of sham feeding. Baseline water intake normally stabilized within 7 days. Once water intake stabilized, the CTA acquisition cycle began. On the first day of the cycle, the rats were presented with the CS for 15 min, and after a 15 min interval, the rats were given an intraperitoneal injection of 0.15M lithium chloride (US, 1.33ml/100g body weight). The following two days were regular water days without injections. There were a total of three such CS-US acquisition cycles. Afterward, there was a one-bottle test, presentation of the CS without the injection of US, and a two-bottle test, presentation of the CS and dH2O. There were two regular water days in between these tests as well. To maintain the accuracy of the CS-US contingency, the presentations of the CS or the injections of the US were staggered 30 seconds apart for each rat. For this reason, the daily training of 30 rats were divided into two squads.

Histology
At the end of the study, the rats were euthanized with an overdose of pentobarbital sodium (150mg/kg ip), then perfused transcardially with cold heparinized 0.9% saline solution followed by 4% buffered paraformaldehyde at 4°C. The brains were removed and placed in paraformaldehyde. A few hours later, the brains were cryoprotected in 30% sucrose in 0.1M phosphate buffer (PB, pH 7.4) overnight also at 4°C. They were then blocked, frozen, and sectioned coronally at 50 μm. The sections were kept in PB, then mounted and stained with cresyl violet to verify the area of lesions. Included in the analyses were data from rats that showed bilateral damage of the PBN or TOA, characterized by loss of neuronal cells and increased gliosis.

Statistical analysis
The data consist of the means of the AM 15 min sham fluid intake, and the means of the PM 4 hrs food and water intake. These data were analyzed by repeated measures ANOVA followed by post hoc Newman-Keuls tests when appropriate.
Results

Lesions

Five PBNx rats did not have adequate bilateral damage of the gustatory PBN, and their data were excluded. The remaining 5 PBNx rats had lesions centered in the medial PBN. Besides the medial and lateral PBN, two of the five rats had lesions extended to the supratrigeminal areas and part of the locus ceruleus. The lesions of the TOA were centered in the ventral posteromedial nucleus of the thalamus (VPM). Besides the VPM, the lesions also included most of the parvicellular part of the VPM (the taste areas, VPMpc). The lesions of the PBN and the TOA are illustrated in Fig. 3.1. A and B, respectively.

Body weight and food and water intake

Before surgeries, rats were assigned to one of the 4 lesion conditions based on their body weight, and the average body weight for each group ranged from 325g to 333g. Differences in body weight occurred during the two surgeries (i.e., lesion surgeries and gastric fistula surgeries) and recovery periods. There were two CTA training experiments, 100% corn oil as the CS was conducted before 0.3M sucrose as the CS. Body weights across three days before the beginning of each CTA training were analyzed comparing the lesion group and its surgical controls. It was at least one month after the lesion surgery that the first CTA training began. Rats with damages in the PBN did not gain as much weight as the surgical controls across days. Before the beginning of oil CTA, the PBNx rats weighed significantly less than the control rats [n=5 each group, Con PBN, 442.1±7.9 g vs. PBNx, 405.3±8.6 g; F(1, 8)=9.86, p<0.02]. The differences in body weight for the two groups were found also before the beginning of the sucrose CTA [Con PBN, 547.5±20.9 g vs. PBNx, 471.3±7.6 g; F(1, 8)=11.70, p<0.01]. These kinds of differences were not shown in comparisons between the Con TOA (n=5) and TOAx (n=10) [before oil CTA, Con TOA, 438.5±11.2 g vs. TOAx, 411.4±10.8 g; F (1, 13)=0.87, p=0.37. before sucrose CTA, 519.3±20.6 g vs. 514.7±15.9 g; F(1,13)=0.03, p=0.88]. That is, lesions of the TOA did not affect body weight change over time.
Fig. 3.1. Photomicrograph of coronal sessions stained with cresyl violet, (A) surgical control for PBN (B) PBN lesions (C) surgical control for TOA (D) TOA lesions. The images for the PBN are presented at a 4× while for the TOA are presented at 2× magnitudes. The line bar in (A) or (C) presents 1mm. The arrows indicate gliosis formed after lesions. (BC: brachium; CL: central lateral nucleus; CM: central medial nucleus; MD: medial dorsal nucleus; Me5: mesencephalic trigeminal nucleus; VPM: ventral posteromedial nucleus; VPMpc: the parvicellular part of VPM)
During the CTA experiments, the rats were placed on a food and water deprivation schedule. In the morning of each CTA experiment, the rats were sham fed with dH2O during baseline training and the two days between each LiCl injection and bottle-test day. They were provided access to sham lick a CS solution on the injection days and the one-bottle test day, and dH2O and a CS solution on the two-bottle test day. Each afternoon, the rats were allowed to real feed food and dH2O for four hours. The PBN groups and the TOA groups consistently showed difference in body weight throughout the experimental period. For example, the PBNx rats weighed significantly less than the Con PBN rats across the 9 days of 3 injection cycles during sucrose CTA but not during oil CTA [oil CTA, Con PBN, 399.3±9.5 g vs. PBNx, 373.2±10.0 g; F(1, 8)=3.62, p=0.09; sucrose CTA, 504.9±24.2 g vs. 428.9±9.5 g; F(1, 8)=8.55, p<0.02]. Despite the body weight differences, the afternoon food intakes during these days were similar in both PBN groups [oil CTA, Con PBN, 18.5±0.6 g vs. PBNx, 22.3±3.4 g; F(1, 8)=1.15, p=0.32; sucrose CTA, 18.3±1.2 g vs. 20.7±3.4 g; F(1, 8)=0.44, p=0.52]. However, the PBNx rats always drank less water in the afternoon than the Con PBN rats did during the injection cycles [oil CTA, Con PBN, 23.1±0.2 ml vs. PBNx, 15.0±3.1 ml; F(1, 8)=6.85, p<0.04; sucrose CTA, 24.5±1.5 ml vs. 16.9±2.4 ml; F(1, 8)=7.49, p<0.03]. In contrast to the PBN groups, there were no significant differences in body weight between the Con TOA and TOAx rats during either injection cycles for oil or sucrose CTA [oil CTA, Con TOA, 398.0±11.5 g vs. TOAx, 386.1±12.2 g; F(1, 13)=0.38, p=0.55; sucrose CTA, 478±18.9 g vs. 477.6±15.4 g; F(1, 13)=0.00, p=0.99]. During these injection cycles, there were no significant differences in the afternoon intake of either food [oil CTA, Con TOA, 18.5±0.9 g vs. TOAx, 19.0±0.7 g; F(1, 13)=0.18, p=0.68; sucrose CTA, 16.6±0.8 g vs. 18.0±0.8 g; F(1, 13)=1.11, p=0.31] or water [oil CTA, Con TOA, 22.0±1.6 ml vs. TOAx, 22.2±1.1 ml; F(1, 13)=0.01, p=0.94; sucrose CTA, 22.1±1.2 ml vs. 22.2±1.4 ml; F(1, 13)=0.00, p=0.98] between the TOA groups.

Conditioned aversion for 100% corn oil

Over the last three days of baseline morning dH2O training, the sham intakes did not differ between the Con PBN and PBNx rats [F(1, 8)=1.20, p=0.30]. The same comparison was done between the Con TOA and TOAx rats, and no differences in intakes were found (F<1, p=0.73). The intakes of the oil CS during the 3 conditioning trials and 1 one-bottle test are
shown in the left most panels of Fig. 3.2 A, PBNx, and B, TOAx. Repeated measures ANOVA varying group (lesions vs. its controls) and trials (4 CS presentations), revealed that there was a lesion \([F(1, 8)=5.60, p<0.05]\), a trial \([F(3, 24)=11.23, p<0.0001]\), and a lesion \(\times\) trial interaction effect \([F(3, 24)=3.84, p<0.03]\). The lesion and interaction effects indicated that on the first and second trial the sham intake of the PBNx rats was significantly higher than the controls (first, 8.8±2.0 ml vs. 2.5±0.5 ml, post hoc p<0.002; second, 4.6±3.0 ml vs. 0.06±0.04 ml, post hoc p<0.04). The trial effect revealed that both the PBNx and the control rats learned to avoid 100% corn oil after it had been pairing with injections of LiCl.

Comparisons of the TOAx and its surgical control, on the other hand, showed only an effect of trial \([F(3, 39)=50.47, p<0.0001]\) without an effect of lesion \([F(1, 13)=1.81, p=0.20]\) or lesion \(\times\) trial interaction \([F(3, 39)=1.50, p=0.22]\). These results indicate that there was no difference between the TOAx and the control rats in corn oil sham intake and that all rats learned to avoid the corn oil CS.

The results of the two-bottle tests are shown in the left most panels of Fig. 3.3 A, PBNx, and B, TOAx. When the oil CS was presented along with a bottle of dH2O, both control and lesion groups avoided the CS. Student’s paired t-tests comparing the dH2O and corn oil intakes revealed that the Con PBN and TOAx rats prefer dH2O, p<0.05. Although the p values for the PBNx and con TOA rats were not to a significant level, both had p=0.055. The non-significant results can be attributed to the low subject numbers, both had n=5. Further, the results shown at the lefts of Fig. 3.3 might seem to indicate that sham intakes of dH2O were different between the controls and lesions [Con PBN, 17.8±3.7 ml vs. PBNx, 10.2±3.9 ml; Con TOA, 8.6±3.2 ml vs. TOAx, 12.4±2.3 ml]. The t-tests, however, showed no significant effects. Therefore, data from the CS and dH2O intakes during acquisition and bottle tests were consistent- rats with PBN or TOA lesions spared conditioned aversion to 100% corn oil.
Fig. 3.2. The acquisition of CTA for left, 100% corn oil and right: 0.3M sucrose. (A) lesions of the PBN disrupted conditioned aversion to 0.3M sucrose but not to 100% corn oil. (B) lesions of the TOA spared conditioned aversion to both corn oil and sucrose. The data presented include the sham intake of the last day of baseline dH2O, the CS during three acquisition trials and one-bottle test.
Conditioned aversion for 0.3M sucrose
The results of CTA learning for 0.3M sucrose distinguished the PBN lesions from the TOA lesions. Over the last three days of baseline morning dH2O training, PBNx rats sham licked significantly more than Con PBN rats [F(1, 8)=7.34, p<0.03]. The same comparison was done between the Con TOA and TOAx rats, and no differences in intakes were found (F<1, p=0.87). The intakes of the oil CS during the 3 conditioning trials and 1 one-bottle test are shown in the right most panels of Fig. 3.2.A, PBNx, and B, TOAx. Rats with PBN lesions sham licked significantly more sucrose CS over trials than did their control rats [F(1, 8)=13.14, p<0.007]. The PBNx rats appeared to decrease their sham intakes of the CS as did the control rats [trial effect, F(3, 24)=6.70, p<0.002]. However, the intakes in PBNx rats did not fall under their baseline water intake while the intakes in the control rats were reduced to zero [interaction, F<1, p=0.44]. In contrast to the PBN rats, all the TOA rats completely avoided the sucrose CS by the trial of one-bottle test [trial effect, F(3, 39)=11.16, p<0.0001]. No effect of group (F<1, p=0.35) or interaction (F<1, p=0.79) was found when comparing the CS intake of the Con TOA and the TOAx.

The results of the two-bottle tests are shown in the right most panels of Fig. 3.3 A, PBNx, and B, TOAx. The Con PBN rats drank significantly more dH2O than sucrose (dH2O, 15.8±4.3 ml vs. sucrose, 1.4±1.2 ml; Student’s t-test, p<0.05) while the PBNx rats drank more sucrose and showed no preference to either dH2O or sucrose (dH2O, 2.9±2.0 ml vs. sucrose, 9.3±4.2 ml; Student’s t-test, p=0.26). Both TOA groups, on the other hand, showed preference to dH2O during the two-bottle test (Student’s t-tests, both p<0.05). Therefore, data from the CS and dH2O intakes during acquisition and bottle tests were consistent. Lesions of the PBN disrupted while lesions of the TOA spared a CTA to 0.3M sucrose.

Discussion
The results of this study support the hypothesis that the PBN is required for conditioned aversion learning to a taste stimulus, sucrose, but not to a non-taste, 100% corn oil. In contrast, lesions including thalamic taste and trigeminal areas failed to block conditioned aversion learning to either a sucrose or a 100% corn oil cue. The results are consistent with previous observations that lesions of the PBN disrupt conditioned aversion learning
Fig. 3.3. The sham intake during 15 min two-bottle test for left: 100% corn oil and right: 0.3M sucrose. (A) The PBNx rats avoided 100% corn oil but not 0.3M sucrose while the Con PBN rats avoided both stimuli. (B) The Con TOA and TOAx rats all avoided 100% corn oil and 0.3M sucrose. (* p<0.05)
particularly when the CS is a taste cue, but not when the CS is a trigeminal cue: e.g. capsaicin (Grigson et al., 1998) and 100% corn oil (Norgren et al., 2001). Although the lesions of the thalamus included not only the trigeminal orosensory but also the taste area, conditioned aversion learning was not disrupted to either the taste or the presumed trigeminal CS (i.e., sucrose or corn oil). This result is consistent with previous studies showing that acquisition of a CTA to a taste cue is intact following lesions of the thalamic taste area. However, it does not support our original hypothesis that large TOA lesions (lesions centered on the trigeminal component) would interfere with conditioned aversion to 100% corn oil.

Despite the lack of saline injection and unpaired controls, the results of this study are clear. Experimentally, there are different control procedures for the establishment of a CTA. These control procedures focus on the relationships between the CS and the US. One control is that animals receive saline injections after the consumption of the CS (Spector et al., 1992; Grigson et al., 1997a). Other controls focus on the temporal contiguity of the CS and US. The LiCl injection can be administered before, during or after the presentation of the CS. When the LiCl injection precedes the US, it is the unpaired condition or so called pseudoconditioning. In one case the US injection is applied hours before the CS (Yamamoto et al., 1994; Tokita et al., 2007), and in another procedure, the injection is administered the day previous to the CS-US acquisition day (Lundy et al., 2004). In this study, those kinds of controls were omitted for sham feeding. Performing the conditioned aversion experiment with sham feeding adds more time requirement, and limits the number of subjects that can be complete within daily trails. Conditioned aversion is a well-established phenomenon, and the procedures are standardized in our laboratory. Furthermore, previous studies with gustatory lesions included the saline injection controls, and such control does not alter the results of the lesion effects. Therefore, comparisons of the surgical controls and lesion animals are sufficient to demonstrate the lesion effects on conditioned aversion learning.

Lesions of the PBN increased while lesions of the TOA did not change the intake of the CS upon the first exposure. It may seem that the PBNx rats were retarded in the acquisition of the conditioned aversion to corn oil, as they did not completely avoid corn oil until after two
acquisition trials. Such an effect can be attributed to the fact that the initial intake of the corn oil was more than three times higher in the PBNx rats than in the control rats. The slopes of decreasing intake in the two groups were parallel indicating that the learning speed was the same. When the CS was 0.3M sucrose, rats with PBN lesions again sham drank more of the solution upon the first exposure though intake was not significantly different from that of control rats. After three acquisition trials, PBNx rats still did not avoid sucrose. Although the intake of sucrose did decrease from its maximum, intake was never significantly lower than baseline water. During the two-bottle intake test, PBNx rats still showed a tendency to prefer to sucrose. After excluding unsuccessful lesions, only 5 PBNx rats were included in the results. The small subject number probably contributes to the non-significant difference between the water and sucrose intake during two-bottle test. One of the 5 PBNx rats had a relatively small lesion with some taste neurons spared within the rostral gustatory PBN bilaterally. This rat subsequently reduced its intake of sucrose, but still drank about 10 ml of sucrose during tests without US. Therefore, it is quite clear that rats with bilateral damage in the gustatory PBN can learn a conditioned aversion to 100% corn oil solution, but not to sucrose. The case with the TOAx rats also is clear. The TOAx rats consumed equivalent amounts of both water and the CS relative to intake by their surgical controls. As such, there is no doubt that conditioned aversion learning to sucrose and corn oil survive excitotoxic lesions of the TOA.

The calorie effect of the CS is not a necessary component of conditioned aversion learning. In this sham feeding procedure, rats did not receive metabolic feedback but still acquired conditioned aversion indicating that the orosensory cue alone can support the conditioned aversion learning. This is consistent with the fact that conditioned aversions can be acquired using a non-nutritional oral stimulus such as saccharin (Sakai and Yamamoto, 1998) and capsaicin (Grigson et al., 1998; Reilly and Trifunovic, 2000) as CS or brief intraoral infusion of the CS (Spector et al., 1992).

The absence of neophobia did not contribute to the failure of PBNx rats to acquire CTA. Neophobia is defined by animals consuming small amounts on first exposure to a novel food. Rats with PBN lesions often do not show neophobia as their initial intakes of CS are more
than the intact rats (Reilly et al., 1993; Grigson et al., 1998). The PBNx rats in this study showed no neophobia to either sucrose or corn oil, but they learned conditioned aversion to one but not the other stimulus. Furthermore, when there was no difference between the PBNx and control rats in the initial intake of a CS, the control but not the PBNx rats acquired an aversion to the CS (Di Lorenzo, 1988; Grigson et al., 1997a). Thus, the PBNx rats did not fail to learn aversion to sucrose as a result of lack of neophobia. It should be noted that it has been suggested that the absence of neophobia in PBNx rats is due to inadvertent damage of the lateral, visceral PBN (LPBN). Indeed, rats with LPBN lesions did not show neophobia to novel sapid stimuli (Reilly and Trifunovic, 2001). In this study, the phenomenon occurred while rats were sham feeding. As the visceral feedback factor was excluded in both lesions and control subjects, the previous assumption is not likely to be true. Rats with gustatory PBN or LPBN lesions both fail to express neophobia to novel sapid stimuli. The mechanisms for this lesion effect are not clear. However, it is believed that the mechanisms for the neophobia and conditioned aversion deficits in gustatory and visceral PBN lesions are independent (Aguero et al., 1993; Reilly et al., 1993; Sakai and Yamamoto, 1998; Reilly and Trifunovic, 2000, 2001; Wang and Chambers, 2002).

Substantial evidence suggests that an associative deficit mediates the disruptive effect of PBN lesions on CTA learning. Successful learning of conditioned aversion requires rats to detect, process, integrate, and associate the CS and US, and express the learned association in behavior. Previous experiments have demonstrated that the deficit in CTA learning by the PBN lesioned rats is not due to a failure to detect of the CS (Spector et al., 1995) or the US (Reilly et al., 1993). The failure to acquire the sucrose CTA also is not likely due to a simple failure to associate a gustatory CS with a US as PBNx rats can acquire a conditioned flavor preference and can express that new learning in behavior (Reilly et al., 1993; Grigson et al., 1998; Sclafani et al., 2001). Moreover, rats with excitotoxic PBN lesions also can express a CTA to a CS that was acquired prior to the PBN lesions (Grigson et al., 1997a). They can also learn a conditioned aversion to a trigeminal stimulus such as 100% corn oil, as shown in the presented study, and capsaicin (Grigson et al., 1998). Taken together, the data suggest that PBN lesion disrupt acquisition of a CTA because they block formation of a specific taste-illness association.
Mechanisms for the disruption of CTA differ in rats with gustatory PBN and LPBN lesions. As described, an association deficit is thought to mediate the disruptive effect of the gustatory PBN lesion. Lateral PBN lesions also disrupt CTA learning, but in this case it is believed that these lesions disrupt visceral input and thereby block the effectiveness of the US (Reilly, 1999). That is, LPBNx animals cannot detect the illness induced by LiCl and so cannot learn a conditioned aversion regardless the sensory modality of the CS i.e. taste or trigeminal stimuli (Reilly, 1999; Reilly and Trifunovic, 2000). Furthermore, the disruption occurs after LPBN lesions even using USs other than LiCl such as methylscopolamine, ethanol (Cubero et al., 2001), and cyclophosphamide (Mungarndee et al., 2006). It is shown in one study that gustatory PBN lesions also block a CTA when the US is cyclophosphamide (Mungarndee et al., 2006), but it is unclear whether gustatory PBN lesions will also block a CTA induced by other drugs mentioned above. Finally, rats with excitotoxic (Grigson et al., 1997a) but not electrolytic (Sakai and Yamamoto, 1998) gustatory PBN lesions are able to show a CTA to a CS that was acquired prior to the lesion. In contrast, rats with electrolytic LPBN lesions can show such retention of a preoperatively acquired CTA (Sakai and Yamamoto, 1998). Since the electrolytic lesions damage not only the neuronal cells located in the nucleus but also the passing fibers, those results suggest that gustatory PBN connections with forebrain structures are important for the retention of preoperatively acquired CTA. Along with retention, as described, it also appears that forebrain gustatory and visceral relays are involved in the acquisition of a CTA as well. This conclusion is supported by the fact that CTA learning is eliminated in decerebrate rats whose connections between the brainstem and the forebrain are disrupted (Grill and Norgren, 1978; Tokita et al., 2007). Researches with forebrain and PBN-forebrain asymmetric lesions, as well as c-Fos induction in response to CTA, also support this conclusion (Schafe and Bernstein, 1996, 1998; Sakai and Yamamoto, 1999; Navarro et al., 2000; Clark and Bernstein, 2009).

It would be important to investigate whether the hedonic value of the CS is altered in PBNx rats after the conditioned aversion procedures. As mentioned above, it is known in the literature that the disruption of conditioned aversion in PBNx rats is due to a taste-illness association deficit. Nevertheless, it is not clear whether rats with PBN lesions cannot switch
the hedonic value of the CS as a result of the association deficit. It has been demonstrated that DA overflow in the NAc is switched from significantly above to significantly below baseline after intake of saccharin has been followed by LiCl injection (Mark et al., 1991). Furthermore, the efflux of acetylcholine (ACh) in the NAc is increased during the intake of saccharin that has become aversive after CTA learning (Mark et al., 1995). As such, DA and ACh levels in the NAc can be used as an index of positive and negative hedonic value, respectively. In the future, it will be worthwhile to evaluate the fluctuations of DA and ACh levels in the NAc during intake of oral stimuli such as sucrose and corn oil that have been paired with LiCl in PBNx rats. The neurochemical study from our laboratory has shown that lesions of the PBN blunt the DA overflow in the NAc during sucrose intake (Hajnal and Norgren, 2005). Based on the neurochemical and conditioned aversion results, one would predict that DA and ACh levels in the NAc during sucrose or corn oil intake would be different in rats with PBN lesions. Since PBNx rats did not acquired a CTA to sucrose, the hedonic value of sucrose would not be altered and so their DA and ACh levels in the NAc should show no indication of switching hedonic value. In contrast, the PBNx rats were able to acquired aversion to 100% corn oil and so their DA and ACh levels in the NAc should be significantly below and above baseline, respectively. Future studies will test the merits of these hypotheses.

The TOA is not necessary for conditioned aversion learning regardless of the sensory properties of the CS. Unlike the PBN lesions, the TOA lesions had no effect on the acquisition of conditioned aversion to either sucrose or corn oil. Although the lesions included not only the trigeminal area but also most of the taste area of the thalamus, they failed to disrupt conditioned aversion learning. This result is consistent with previous studies showing that conditioned aversion learning is not impaired in rats with lesions centered on the gustatory thalamus (Lasiter, 1985; Scalera et al., 1997; Reilly, 1998; Grigson et al., 2000; Mungarande et al., 2006). In the literature, some studies have reported that lesions of the gustatory thalamus impair a CTA (Yamamoto, 1993; Yamamoto et al., 1995). The CTA protocol in this laboratory requires three CS-US pairing trails. The reports that showed impairment of conditioned aversion learning in thalamic lesioned rats typically included only one pairing. Therefore, it seems obvious that the discrepancy is attributed to the procedural differences. In support, studies show that lesions of the gustatory thalamus have no effect on
CTA learning when tested across 3 or more CS-US pairings (Lasiter, 1985; Grigson et al., 2000). The present lesion, however, included the taste area, but was focused on the trigeminal area. As such, the results in this study do not support the hypothesis that the thalamic trigeminal area is necessary for conditioned aversion learning to a corn oil CS. This could indicate that we were incorrect in our assumption that the trigeminal system mediates the sensory detection of corn oil. The results for the PBN lesions, though, indicate that sensory processing pathways for sucrose and corn oil are different. The alternative explanations, then, include: 1) trigeminal nuclei other than the thalamic trigeminal area is necessary for the conditioned aversion learning to a corn oil CS, 2) the trigeminal detection of corn oil maybe concentration dependent. It would be worthwhile to test the two possibilities for future studies using the conditioned aversion paradigm. Before that, however, we need to have some understanding of the role of the PBN and TOA in responding to the innate rewarding properties of a range of concentrations of sucrose and corn oil. The next chapter will address this question using operant tasks.
Chapter 4

Lesions of the gustatory parabrachial nucleus but not the thalamic orosensory area disrupt operant response to sucrose and corn oil reward in rats.

In this chapter, the reward strength of orosensory sucrose and corn oil is measured by operant tasks using fixed ratio schedule (FR) and progressive ratio (PR) schedules. If the gustatory and trigeminal systems are important in transmitting sucrose and corn oil reward respectively, lesions of the PBN will decrease the reward strength of sucrose but not corn oil solutions and the reverse effects will occur in rats with TOA lesions. In the literature, the relative reward strength of foods is usually measured during real feeding model. In order to measure the reward strength of orosensory stimuli, I first demonstrate that normal rats will work for sucrose rewards when sham feeding in Experiment 1, and then test the hypothesis with lesion animals included in Experiment 2.

Experiment 1

The intake function of sucrose solutions varies in testing methods for rodents. In 30-min, real feeding tests, sucrose intake was an invert-U function of concentration (Nissenbaum and Sclafani, 1987; Kawai and Fushiki, 2003). The highest intake occurred at around 0.3M. In brief access (Davis, 1973; Cagan and Maller, 1974; Shimura et al., 1997) or sham feeding tests (Weingarten and Watson, 1982; Nissenbaum and Sclafani, 1987), however, sucrose intake increased as the concentration increased. The major factor that accounted for differences in the intake function was the opportunity for post-ingestive negative feedback. Brief access and sham feeding both prevent accumulation in the gastrointestinal tract and the subsequent induction of satiety. In a sham feeding model, a gastric fistula (GF) is implanted, and so excludes post-ingestive feedback by allowing ingested fluid to drain out the stomach. Moreover, sham feeding rats maintained a significantly higher rate of licking than real feeding animals throughout the testing period (Davis and Smith, 1990). The fact that sham feeding rats have a concentration dependent intake of sucrose implies reward without metabolic effects. Thus sham feeding provides a good model for studying the rewarding properties of orosensory stimulation.
Sham feeding not only increases the intake of sucrose, but also increases dopamine overflow in the shell of nucleus accumbens (NAc). Previous studies from our laboratory (Hajnal et al., 2004) and another group (Avena et al., 2006) have shown that orosensory stimulation of sucrose increases the release of DA in the NAc as does real feeding (Hajnal and Norgren, 2001, 2005). Since accumbens DA is consistently related to rewards (Smith, 2004), these results further support that orosensory stimulation without metabolic outcome can produce rewarding effects. In the present study, the reward strength of sucrose was measured with operant tasks, FR and PR schedules of reinforcement. Among other uses, the FR task is used to screen the reinforcement properties of addictive drugs (Winger and Woods, 1985; Arnold and Roberts, 1997). Hodos (1961) first introduced the PR schedule as a method to measure reward strength (Hodos, 1961). In a PR schedule, the work requirement needed to receive a fixed reward increased progressively throughout testing. Reward strength is often identified by the break point (BP), the highest ratio completed within a given test session. Since this time, many studies have applied these operant tasks to measure the strength of natural (Hodos and Kalman, 1963; Reilly, 1999; Brennan et al., 2001; Sclafani and Ackroff, 2003; Hajnal et al., 2007) and non-natural rewards (Roberts et al., 1977; Caine and Koob, 1994; Richardson and Roberts, 1996). It has been shown that BP increases as the concentration (Reilly, 1999; Brennan et al., 2001; Sclafani and Ackroff, 2003) or volume (Hodos and Kalman, 1963; SKjoldager et al., 1993) of a reward increases. Studies testing the reward strength of sucrose with real feeding have shown similar results (Reilly, 1999; Brennan et al., 2001; Sclafani and Ackroff, 2003). Based on the sham-feeding effects mentioned above, we hypothesized that the absence of satiety signals not only maintains, but actually increases the reward value of sucrose. The effects of sham feeding on the reward strength of sucrose were tested with a FR10 and then two PR5 schedules. The FR10 schedule compared the feeding effects with a between group design while the PR5 schedule compared the effects with both a between and a within group design. A preliminary report of this study was presented as an abstract at the annual meeting of the Society for the Study of Ingestive behavior in 2008 (Liang et al., 2008).

Materials and Methods
The subjects were 16 male Sprague-Dawley rats (Charles River, Wilmington, MA) from a previous microdialysis experiment with a GF implanted in the stomach. The rats weighed 350-600g at the beginning of this experiment. They also had bilateral guide cannulas affixed to the skull. They were individually housed on a 12:12-h light-dark cycle with ad libitum access to tap water and standard laboratory diet [Rodent diet (W) 8604; Harlan Teklad, Madison, WI]. Once the experiment began, the rats were maintained on a 15 hr food deprivation regimen. Water was removed one hour before and returned one hour after the experiment daily. The operant sessions occurred between 8:00 to 10:30 AM daily. Normal powdered chow was available between 12:00 to 5:00 PM. Body weight was monitored daily to ensure it did not fall below 85% of their free-feeding weight.

Apparatus
The rats were tested in 12 identical modular operant chambers measuring L30.5 × W24.0 × H29.0 cm. Details of this chamber have been described elsewhere (Schroy et al., 2005). In brief, each chamber was equipped with a house light, a white noise generator, and 3 sipper tubes that could be programmed to advance and retract depending on the testing schedule. These sipper tubes entered the chamber through 1.3-cm holes spaced 16.4-cm apart from left to right of a Plexiglas wall. The house light and white noise generator (75 dB) were located opposite the sipper tubes. Licking was monitored using a triple lickometer circuit. Each test chamber was located in a sound attenuating cubicle that was fitted with a ventilation fan. This set up for operant tasks and on-line data collection was operated by a PC computer and an interface (MedPC; Medassociates Inc, St. Albans, VT).

Experimental Design
The rats were divided into two groups. Their responses to 5 different concentrations of sapid sucrose (0.03M, 0.1M, 0.3M, 1.0M, and 2.0M) were measured with a once daily FR10 or PR5 session. Eight rats (G1, sham-real) were tested while sham feeding on an FR10 and the first PR5 schedule, and then when real feeding on a second PR5 schedule. Another 8 rats (G2, real-sham) had the order reversed, real feeding during the FR10 and the first PR5, then sham feeding on the second PR5. The three retractable sipper tubes entering from left, center, to right were the reward (e.g., sucrose), empty spout operant, and inactive empty spout,
respectively. Contacting the empty inactive spout had no programmed consequences. The FR10 schedule required the rat to touch the center dry active spout 10 times to obtain 10-sec access to the left most reward spout that contained one of five concentrations of sucrose. These sessions were 30 min long. The PR 5 schedule was similar except that the requirement increased by 5 contacts for each 10-sec trial starting at 10 licks. The PR5 sessions ended when 10 min elapsed without achieving a PR requirement.

Procedure
Before each rat was placed in an operant chamber, its stomach was flushed with lukewarm water. For sham feeding, a flexible tube was screwed into the gastric fistula and passed through a slot in the wire mesh floor to drain solutions to a waste pan. For real feeding, a flexible tube glued with silicone rubber adhesive sealant (white RTV 12, GE Silicone) was screwed into the fistula to prevent drainage. Once the rat finished its daily session, the recovery of solutions was measured and, for sham feeding, the rat’s stomach was flushed again with lukewarm water. The gastric fistula was closed with its screw for all rats before they were returned to their home cages. During habituation, the rats were placed in the chamber with house light and white noise on for 15 minutes. After 3 days of habituation, the operant responding for 5 concentrations of sucrose then began. For both the real and sham conditions, the FR10 schedule was repeated twice and the PR5 schedule three times at each concentration in ascending order. All the procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine, and comply with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

Statistical Analysis
One rat in G1 had inappropriate drainage and one rat in G2 died during the experiment. Thus, data from these two rats was excluded. The data from both groups (each n=7) consist of the means of intake, spout contacts, and break points during 2 FR10 or 3 PR5 sessions for each stimulus. These data were analyzed using a $5 \times 2$ ANOVA varying sucrose concentration (0.03M – 2.0M) and feeding condition (real vs. sham). Post hoc Newman-Keuls tests were conducted when appropriate. Separate ANOVAs were conducted during FR and PR testing.
Results

Sham Feeding increased FR10 responses to sucrose at 1.0M and 2.0M. As predicted, the sham feeding group (G1) made more operant spout contacts than the real feeding group (G2) to receive reward of high concentrations. A two-way ANOVA (stimulus × group) showed there was a group [F(1, 12)=5.27, p< 0.05], concentration [F(4, 48)=16.41, p<0.0001], and group × concentration interaction effect [F(4, 48)=6.08, p<0.003]. Fig. 4.1A demonstrates these effects. The response curve for G1 increased as a function of sucrose concentration, and showed maximal responses to 1.0M and 2.0M. In contrast, the operant contacts in G2 rats reached maximal at 0.3M and showed no significant differences from concentrations 0.1M to 2.0M. The intake curves for both groups were essentially identical to their operant response curves, as shown in Fig. 4.1B. The sham feeding intakes were significantly higher than the real feeding intakes at 1.0M and 2.0M. There was again a group [F(1, 12)=5.94, P<0.04], concentration [F(4, 48)=17.30, P<0.0001], and group × concentration interaction effect [F(4, 48)=6.33, P<0.0004]. The results of reward spout licks showed the same effects as the results of operant spout contacts and intakes. Thus, the lick data are omitted in this report.

Sham feeding increased PR5 responses at high concentrations. The design of two PR5 tests was to measure and compare how hard the two groups of rats would work for sucrose during real and sham feeding. Group 1 responded for sucrose with sham feeding in the first PR5 test and with real feeding in the second PR5 test, and G2 had the feeding order reversed. The conclusions from operant spout contact, break point, intake, and reward spout lick are primarily the same. Therefore, the data presented here include only the results of operant spout contact (Fig. 4.2A) and break point (Fig. 4.2B). The results of operant spout contacts are presented for within group analysis comparing real and sham feeding. For both G1 (Fig. 4.2A, left) and G2 (Fig. 4.2A, right), the response was a monotonic function of sucrose concentration during sham feeding and an inverted-U function of sucrose concentration during real feeding. A Two-way ANOVA (feeding × stimulus) revealed that there was a main effect of concentration [G1, F(4, 48)=16.28, P<0.0001 vs. G2, F(4, 48)=3.97, P<0.008] and feeding × concentration interaction [G1, F(4, 48)=9.59, P<0.0001 vs.
Fig. 4.1. Sham feeding increased FR10 responses to high concentrations of sucrose. (A) Post hoc Newman–Keuls test showed that the operant spout contacts during sham feeding were significantly higher at 1.0M (p<0.0003) and 2.0M (p<0.0002) during than during real feeding. (B) The same effect was shown in intake. The sham intakes during sham feeding at 1.0M (p<0.0002) and 2.0M (p<0.0002) were significantly higher than during real feeding. (*: significant difference between feeding conditions)
G2, \( F(4, 48)=8.16, P<0.0001 \) for both groups. The main effect of feeding, however, was shown in G1 \( [F(1, 12)=11.31, P<0.006] \) but not in G2 \( [F(1, 12)=1.19, P=0.30] \). Although the response curves were similar for the two groups, more significant differences between real and sham feeding were shown in G1 than in G2. Operant spout contacts were significantly higher at 1.0M and 2.0M during sham feeding than during real feeding in G1, but similar significance was shown only at 2.0M in G2. These data is consistent with the FR10 data that rats responded more for high concentration of sucrose solution during sham feeding than during real feeding.

Break point is the highest operant spout contacts that a rat accomplished to gain a 10s sucrose reward. Thus, the response function of the break points was similar to that of the operant spout contacts. The design of the PR testing allows to compare the effects of feeding by within group analysis, and to compare the effects of feeding experience by between group analysis. The effects of feeding on operant responses were presented in the previous paragraph. Here, shown in Fig. 4.2B is the result of between group analysis comparing the effects of feeding experience on break point. Group 1 had only sham feeding experience while G2 had only real feeding experience when they were tested with the first PR5 schedule. When they were tested with the second PR5 schedule, G1 had the first real while G2 had the first sham feeding experience. During sham feeding (Fig. 4.2B, left), the slope of the response curve for G1 (sham-real) is steeper than that for G2 (real-sham). A two-way ANOVA (group \( \times \) stimulus) demonstrated a main effect of concentration \( [F(1, 48)=39.08, P<0.0001] \) and group \( \times \) concentration interaction \( [F(4,48)=8.35, P<0.0001] \) effect, but not of group \( [F(1,12)=1.02, P=0.33] \). Break points for G1 were higher than for G2 at 1.0M and 2.0M, but lower than for G2 at 0.03M. The two groups of rats also showed differences in break point during real feeding (Fig. 4.2B, right). There was a main effect of group \( [F(1,12)=5.34, P<0.04], \) concentration \( [F(4,48)=14.10, P<0.0001], \) and group \( \times \) concentration interaction \( [F(4,48)=4.26, P<0.005] \). Rats with previous sham feeding experience (G1) had lower break points than had G2 at low concentrations of sucrose (0.03M to 0.3M).

Overall, rats responded more to receive high concentrations of sucrose during sham feeding than during real feeding. The difference was more significant when the rats were tested with sham feeding and then with real feeding, than when the rats were tested the other way around.
Fig. 4.2. Regardless of testing order, on a PR5 schedule operant responses increased at high concentrations during sham feeding. (A) Post hoc Newman–Keuls test showed that the operant spout contacts during sham feeding were significantly higher at 1.0M (p<0.0002) and 2.0M (p<0.0002) than during real feeding in G1, left. The same significant difference was only shown at 2.0M (p<0.0002) in G2, right. (B) Previous real feeding experience increased the break point for 0.03M (p<0.03), but decreased break points for 1.0 (p<0.002) and 2.0M (p<0.0002) during sham feeding, left. Previous sham feeding experience, on the other hand, decreased break points for 0.03, 0.1 and 0.3M sucrose during real feeding. The decrease of break points was significant at 0.1M (p<0.02), and close to significant at 0.03M (p=0.051). (*: significant difference between feeding conditions)
That is, the real feeding experience decreased the within group differences on break points between sham and real feeding conditions. During sham intake, the break points for G1 rats were 53 and 100 licks higher for 1.0 and 2.0 M sucrose, respectively, than when they real fed. For G2 rats the same differences were 20 and 56 licks, respectively [Wilcoxon matched pairs test: 1.0M (G1 vs. G2): 53.1±19.5 vs. 20.2±9.9, z = 3.12, p<0.002; 2.0M (G1 vs. G2): 100.7 ± 15.5 vs. 56.2 ± 13.6, z= 1.98, p<0.05].

Discussion
This is the first report to show that an orosensory cue without metabolic feedback can support an operant behavior. Further, the intake and reward value of sucrose increases monotonically with concentration in a sham feeding condition. Those results support our hypothesis that the absence of satiety signals not only maintains, but actually increases the reward value of sucrose. Moreover, the hypothesis was supported by using both between and within group designs. Postingestive negative feedback was minimized by draining the ingested fluid through a gastric fistula. During FR10, sham feeding rats responded more for 1.0 and 2.0M sucrose solutions than did real feeding rats. The PR5 experiments demonstrated that the break points for concentrated sucrose were significantly higher during sham feeding than real feeding. In general, the results of the FR10 with real feeding were also consistent with previous studies using operant responses as a measure of the reward strength of sucrose (Reilly, 1999; Brennan et al., 2001; Sclafani and Ackroff, 2003). On a FR schedule, the concentration-response/intake function is an inverted-U (Sclafani and Ackroff, 2003). The purpose of imposing a PR schedule is to acquire a break point for different magnitudes of reinforcer. Thus, it is expected that the break point will be positively related to the magnitude of reward. The volume of a fixed solution, the concentrations of the reward stimulus, and the duration of reward access are different ways of varying the magnitude of reward. The results of this study, consistent with previous studies (SKjoldager et al., 1993; Reilly, 1999; Brennan et al., 2001; Sclafani and Ackroff, 2003), revealed a positive relationship between break point and reward magnitude.
The reward strength of a stimulus, however, is not an absolute value. It varies depending on several other factors. These factors include session length, criteria for end of a session, the ratio of the PR, deprivation state etc. All factors contribute to how, and how soon animals become satiated. For example, studies from Reilly (Reilly, 1999) as well as Experiment 1 of Sclafani and Ackroff (Sclafani and Ackroff, 2003) showed that the break points of sucrose solutions increased as the concentration increased and reached plateau at 1 and 2 M (or 32 % and 64 %). Hodos and Kalman (Hodos and Kalman, 1963) as well as Brennan et al (Brennan et al., 2001), in contrast, demonstrated an inverted-U function of break points under real feeding condition as shown in this study. The major differences among those and the current studies are the session length and the criteria to end a session. In the first two studies, the criterion to end a session was when no response occurred for 3 minutes. In Experiment 1 of the Sclafani and Ackroff’s report, the session ended after 30 minutes. In contrast, the session length was 60 min or 10 min elapsed without a response in Brennan et al (Brennan et al., 2001). There was no limited session length as long as the animal made a response within 15 min in the Hodos and Kalman study or reached a ratio requirement within 10 min in the present study. The designs of the first two studies shortened the session length whereas the designs of the later studies lengthened them. The shorter length limited consumption, and consequently reduced the effects of postingestive feedback. Thus, the break points of sucrose in those studies are similar to the break points with sham feeding condition in the current study.

Satiation effects can also be prevented by increasing the PR requirement. In the same study, Hodos and Kalman (Hodos and Kalman, 1963) were able to switch the function of break points from an inverted-U to a monotonic function by increasing the PR requirement from 5 to 40. Increasing the PR ratio kept the total consumption of the reinforcer relatively low, and so decreased the effects of satiety. Another way of limiting the influence of the satiety signals is using long-term sessions. In Experiment 2 of the Sclafani and Ackroff’s paper, the authors allowed rats to perform a PR1 schedule for sucrose solutions daily for 23 hr with food and water available ad libitum. The break points across sucrose concentrations were similar to their results of 30-min session, the break point increased as the concentration increased. Besides the long session length, this experiment was done without food deprivation. The PR
responding reflects the reward value of sucrose while food was also freely available. The reward strength of sucrose, therefore, is probably most close to its absolute value and, thus, supports a monotonic function. Finally, when the rats were water but not food deprived (Reilly, 1999; Hajnal et al., 2007), they showed no concentration-dependent increase in break point for sucrose. In this case, the satiation does not seem to play a role probably because the animals were motivated for hydration but not for energy.

Satiety signals play an important role in the reward strength of a natural reward. During sham feeding, the ingested fluid is prevented from accumulating in the gastrointestinal tract and so excluded the postingestive feedback. When the satiation does not occur, the rewarding effects of a palatable food do not decline. The operant tasks in the current study were conducted when the rats were food deprived. The sham feeding procedure not only prevented satiety from emerging, but also maintained the deprivation. Thus, over time the reward value of sucrose was higher in rats with sham feeding than with real feeding. Consistent with this study, other data have shown that operand responding for sucrose was increased or decreased depending on the pre-administration of orexigenic or anorexigenic peptides in the brain. Injections of neuropeptide Y (NPY) in the hypothalamus increased break points for sucrose pellets (Brown et al., 1998) whereas intraventricular administration of insulin and leptin both decreased bar presses for 5% sucrose (Figlewicz et al., 2006). Similar effects were seen in the cholecystokinin-1 receptor deficient, Otsuka Long-Evans Tokushima fatty (OLEF) rats. Compared with Long-Evans Tokushima Otsuka (LETO) age-matched lean controls, OLEF rats showed higher break points to 0.3M and 1.0M sucrose (Hajnal et al., 2007). Moreover, lesions of the area postrema increased the break point for 15% sucrose solutions (Miller et al., 2005). This lesion also produced overexpression of NPY mRNA in the arcuate nucleus of the hypothalamus, amygdala, and hippocampus (Edwards et al., 1997; Miller et al., 2002). These results suggest that there is cross talk between reward circuits and the satiety/energy regulatory signals. This possibility was further supported by the demonstration that injections of ghrelin, a powerful orexigenic peptide, into the ventral tegmental area and the NAc dose dependently increased food intake (Naleid et al., 2005).
The effects of sham feeding appeared to be dependent on the stimulus concentration. During both FR and PR testing, operant responding was only significantly greater for high concentrations of sucrose in sham feeding than in real feeding. It has been shown that there are conditioned and unconditioned associations between the ingested stimulus and its postingestive feedback (Davis and Smith, 1990). The conditioned feedback occurred during the early while the unconditioned feedback during the later period of a feeding session. These associations were best demonstrated when animals ingested a small amount of concentrated carbohydrate solution in their first experience with sham feeding. The sham intake as well as the rate of licking then gradually increased until they reached plateau. Previous sham feeding studies have shown that four to five daily, consecutive, sham feeding experiences were required to diminish previous conditioned postingestive negative feedback (Mook et al., 1983; Weingarten and Kulikovsky, 1989; Davis and Smith, 1990). Nevertheless, it has been shown that the initial intake was significantly higher during sham feeding than during real feeding when presented with a less concentrated solution such as 0.25M glucose (Mook et al., 1983). In the current study, sham feeding rats did not show any difference in responding for the low concentrations relative to real feeding rats. Since the FR10 was repeated only twice for each concentration, there were not enough trials for the conditioned postingestive effects to extinguish. The feeding condition was probably not the only factor that influenced the operant responses. Because the concentrations were presented in an ascending order, all rats were still in the acquisition process during the first few FR sessions. Therefore, the operant responses and intakes for 0.03 and 0.1M did not differ between real and sham feeding. As the trial went on and rats learned to perform the operant tasks, the responses to 0.3M were higher in the sham than the real feeding condition, although not significantly so. The responses to 1.0M and 2.0M were significantly higher during sham feeding than real feeding. Within the sham feeding group, the responses to 1.0M and 2.0M were essentially equal. A ceiling effect during the short 30-min test session could account for this finding. When the same rats were performing PR5, the session length was not limited by time but by their responses. Their responses to 2.0M were higher than to 1.0M (left of Fig. 4.2A, ANOVA: p=0.11).

During the first PR5 test, the sham feeding rats already had had 10 trials of consecutive FR10 experience. Their PR responses to low concentrations, nonetheless, were still not significantly
higher than the real feeding rats. When the schedule switched from FR10 to PR5, the reward stimulus was switched from 2.0M to 0.03M. A strong negative contrast effect may have occurred after this concentration change. The rats made operant responses but did not lick vigorously for the reward. Whenever the reward switched from 2.0M to 0.03M their contacts on the operant spout decreased but increased on the inactive spout. This contrast effect could work against the sham feeding effect. Therefore, the operant responses for low concentration of sucrose were not higher in sham than real feeding after the rats had had large amount of sham feeding experience. Furthermore, the rats were switched from sham to real feeding, or vice versa, during the second PR5 schedule. At this time, they not only were under the influence of a contrast effect, but also the change of feeding condition. Rats switching from real to sham feeding (real-sham, G2) responded more to 1.0M and 2.0M sucrose compared with their PR5 performance during real feeding, but less when compared with G1 (sham-real) during sham feeding. These results were consistent with the factor mentioned above. The learned inhibitory control over ingestion does not extinguish until several trials of sham feeding experiences, particularly when concentrated solutions are presented. There were different effects when rats switched from sham feeding to real feeding. Sham-real rats showed less response to 0.03M, 0.1 and 0.3M during real feeding than during sham feeding. By rationale, it is not unusual that rats responded less to low concentrations of sucrose when they were real fed than sham fed. A between group comparison, however, showed those responses were lower than real-sham rats performing under the same feeding condition. It has been suggested that once rats had learned sham feeding, they relearn the postingestive feedback rapidly within one trial (Mook et al., 1983). Moreover, the volume of their meals was expected to be the same as rats under normal feeding conditions (Mook et al., 1983). It was not the case for the current study. The results of current study suggested that different processes occurred while switching from real to sham feeding or vice versa. Further studies are required to elucidate the mechanism.

The results of the 30-min FR and the PR schedule were parallel. They both showed that sham feeding increased the apparent reward value of concentrated sucrose solutions. It has been a controversial issue of whether FR schedule itself is enough to measure the reward strength of a reinforcer, particularly when the reinforcer is an addictive drug (Arnold and Roberts, 1997).
This study demonstrated that the FR and PR schedules provided similar information. The major difference between the two schedules is in comparing the reinforcing efficacy. Fixed ratio schedule is a rate dependent measure while PR schedule is not. The reinforcing efficacy is important to identify for drugs of abuse because their rewarding/reinforcing effects are strongly related to their pharmacological effects. Even with PR schedules, the experimental procedures and interpretation of the results require extra caution because drug effects may alter behavioral performance (Richardson and Roberts, 1996). Those issues are less likely to occur when using food as the reinforcer. Despite the fact that the FR schedule does not measure the break point of a reward, with appropriate experimental design, a FR task can provide accurate information about the reinforcing effects of a natural reward.

In summary, this study supports our hypothesis that minimizing postingestive negative feedback not only maintains, but also increases the reward strength of sucrose. The results also suggested that processes involved in switching from real to sham feeding or vice versa might be different. Many studies have investigated the process of learning to sham feed. Whether a relearning process is necessary for an animal with enormous amount of sham feeding experience to start real feeding, however, requires further studies.

**Experiment 2**

As mentioned above, the general hypothesis in this study is that the rewarding and sensory properties of sucrose and corn oil are processed through the gustatory and trigeminal pathways, respectively. If they are processed through different pathways, lesions of nuclei along one of the two pathways will disrupt behaviors that are related to the sensory and reward processing of one but not the other stimulus. Accordingly, we tested the hypothesis that the PBN is important for taste reward while the TOA is important for trigeminal somatosensory reward by measuring the reward strength of sucrose and corn oil on intact and lesioned rats using operant tasks, FR and PR schedules (Hodos, 1961; Liang et al., 2006). This study is the first to investigate and compare the effects of PBN and TOA lesions on FR
and PR schedules using both real and sham feeding paradigm. Part of the results was presented as an abstract for the annual meeting of the Society for Neuroscience in Washington D.C. in 2008.

Materials and Methods
Subjects
The subjects were 30 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 295-335g at the beginning of this study. They were individually housed on a 12:12-h light-dark cycle with ad libitum access to tap water and standard laboratory diet [Rodent diet (W) 8604; Harlan Teklad, Madison, WI]. Once the experiment began, the rats were maintained on a 15 hr food deprivation regimen. Water was removed one hour before and returned one hour after the experiment every day. Normal powder chow was available for 4 hours at least one hour after the daily session, normally between 12:00 to 4:00 PM. Body weight was monitored daily to ensure it did not fall below 85% of their free-feeding weight.

Surgeries
The rats were divided into PBN lesions (PBNx, n=10), PBN surgical controls (Con PBN, n=5), TOA lesions (TOAx, n=10), and TOA surgical controls (Con TOA, n=5). All surgeries were conducted as described above. Rats had at least one month to recover prior to the start of experimental testing.

Apparatus
The apparatus was the same as that used in Experiment 1.

Experimental Design
The rats were divided into four groups as described above. Their responses to 3 different concentrations of sapid sucrose, 0.03M, 0.3M and 2.0M, and 3 different concentrations of corn oil emulsions, 2.5%, 25% and 100% were measured with a once daily FR10 or PR5 session. All sucrose solutions were made with distilled water. Each corn oil emulsion was blended with distilled water and Tween 80 for 8 minutes (Sigma-Aldrich, St. Louis, MO; 0.75ml Tween 80 was added to every 100ml of corn oil and water mixture). All rats were
tested while sham feeding on an FR10 and then on the first PR5 schedule, and finally when real feeding on a second PR5 schedule for each reward solution.

Procedure
Before each rat was placed in an operant chamber, its stomach was flushed with lukewarm water as described above. Before operant tasks for sucrose or corn oil began, rats were provided 15 minutes free access with sham feeding to a sucrose or corn oil solution. There were 3 days of free access (15 min) to sucrose (in the order of 0.3M, 0.3M, 0.03M) and 2 days of 15 min access to corn oil (both 25%). During habituation, the house light and white noise was on, and the reward and active spouts were both advanced into the chamber. The rats received sucrose or corn oil from the reward or the active spout on alternative habituation days. The responses for the sucrose solutions were tested before the responses for corn oil emulsions, both in ascending order. During testing, the FR10 and PR5 schedule was repeated twice and three times, respectively, for each concentration under both sham and real feeding conditions (details of the daily schedule is shown in Table 4.1). After completing the last PR5 for corn oil with real feeding, all rats were tested again with 2 sessions of FR10 using 0.3M sucrose while sham feeding. All of the procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine, and comply with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

Table 4.1. Testing schedule.

<table>
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<th>DAY</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4-9</th>
<th>10-18</th>
<th>19-27</th>
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<td>0.03</td>
<td>FR10</td>
<td>PR5</td>
<td>PR5</td>
<td></td>
</tr>
<tr>
<td>DAY</td>
<td>28</td>
<td>29</td>
<td>30-35</td>
<td>36-44</td>
<td>45-53</td>
<td></td>
</tr>
<tr>
<td>Corn Oil(%)</td>
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<td>25</td>
<td>FR10</td>
<td>PR5</td>
<td>PR5</td>
<td></td>
</tr>
<tr>
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<td>Free access</td>
<td>Sham Feeding</td>
<td>Real Feeding</td>
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Histology
At the end of the experiment, the rats were sacrificed with an overdose of pentobarbital sodium (150 mg/kg ip), then perfused transcardially with cold heparinized 0.9% saline solution followed by 4% buffered paraformaldehyde at 4°C. The brains were removed to paraformaldehyde and, a few hours later, cryoprotected in 30% sucrose in 0.1M phosphate buffer (PB, pH 7.4) overnight, also at 4°C. Then they are blocked, frozen, sectioned coronally at 50 µm. The tissues were kept in PB, then mounted and stained with cresyl violet to verify the area of lesions. Only rats showing a bilateral damage of the PBN or TOA, characterized by loss of neuronal cells and intense gliosis leading to shrinkage of those areas, were included in the data analysis.

Statistical Analysis
Total number of PBNx rats involved in this study was 9 because one rat died during recovery of surgeries. Two PBNx rats died during the corn oil operant tasks, and so their data was excluded from the corn oil results. After histology, one TOAx rat did not show lesions on one side of the brain, thus its data was excluded. Three TOAx rats did not perform during the sucrose operant sessions, and thus data from these rats was not included for the sucrose results. The gastric fistula of one TOAx rat failed during corn oil experiments, and so its data was not included in the corn oil results. The data consist of the means of intake, spout contacts, and break points during 2 FR10 or 3 PR5 sessions for each stimulus. These data were analyzed by two-way ANOVA with repeated measures followed by post hoc Newman-Keuls tests when justified.

Results
Lesions
The cresyl violet staining for the PBN and the TOA is shown in Fig. 4.3. The gustatory PBN is located in the areas dorsal and ventral of the brachium, and lateral to the mesencephalic trigeminal nucleus (Fig. 4.3A). After lesions, gliosis occurred in the damage areas (Fig. 4.3B). Among the 9 PBNx rats, two rats had large lesions, and the lesions included the entire gustatory PBN, the locus coeruleus, the supratrigeminal nucleus, and extended to the edge of the motor trigeminal nucleus. The rest 7 rats had damages centered in the PBN, but the bilateral lesions were not quite symmetrical. Five rats had lesions extended to the locus
Fig. 4.3. Photomicrograph of coronal sessions stained with cresyl violet, (A) surgical control for PBN (B) PBN lesions (C) surgical control for TOA (D) TOA lesions. The images for the PBN are presented at a 4× while for the TOA are presented at 2× magnitudes. The line bar in (A) or (C) presents 1mm. The arrows indicate gliosis formed after lesions. (BC: brachium; CL: central lateral nucleus; CM: central medial nucleus; MD: medial dorsal nucleus; Me5: mesencephalic trigeminal nucleus; VPM: ventral posteromedial nucleus; VPMpc: the parvicellular part of VPM)
coeruleus on one side, and 3 of these rats had little taste spared on the other side of the brain. The TOA includes the thalamic taste area (VPMpc) and the medial part of the VPM (Fig. 4.3C). The TOA lesions included entire VPMpc and the medial half of the VPM in all 9 rats. Four of the 9 rats also had damage in the midline, and so the lesions of the left and the right sides of the brain are continuous (Fig. 4.3D).

Free access licks
Before an operant was imposed for sucrose or corn oil reinforcement, sucrose and corn oil baseline licks were measured. There were three 15-min free access sessions of sucrose and two such sessions of corn oil. Both 0.3M sucrose and 25% corn oil were presented in two 15-min free access sessions (see table 4.1). The results of the free access licks in PBN (A) and TOA (B) groups are shown in Fig. 4.4. The data included in the figure were licks of 0.03M sucrose, and licks of 0.3M sucrose and of 25% corn oil during their second free access session. Rats with bilateral lesions of the PBN licked less sucrose than did surgical control rats \[ F(1, 12)=15.70, p<0.002 \]. The differences in number of licks were significant when 0.3M sucrose was presented \[ group \times concentration, F(2, 24)=9.01, p<0.002; 2nd 0.3M, Con PBN, 3934.8\pm481.0 \text{ vs. PBNx, } 937.4\pm299.9, \text{ post hoc, } p<0.0002 \]. Although the PBNx rats licked less sucrose than the did Con PBN, the number of licks were concentration dependent in both groups \[ F(2, 24)=22.49, P<0.0001 \]. When 25% corn oil emulsion was the stimulus during the 15-min free access, there was no significant difference in licking between the PBNx and Con PBN rats \[ group, F(1, 10)=0.86, p=0.38; \text{ interaction, } F<1, p=0.99 \]. Although 25% oil emulsion was presented in the two sessions of oil free access, both groups increased licks of 25% corn oil in the 2nd session \[ F(1, 10)=14.06, p<0.004; (\text{Con PBN vs. PBNx}), 1st session, 993.6 \pm422.8 \text{ vs. } 1496.1\pm232.8; 2nd session, 2165.4 \pm637.9 \text{ vs. } 2657\pm450.1 \]. Only for the first exposure of 25% corn oil was the solution stirred and not emulsified using a blender. This was a mistake due to the inexperience of the experimenter. The difference in preparation resulted in a difference in the actual concentrations because the oil floated to the top in the 1st 25% oil solution \[ \text{This effect on oil free access was also seen in the TOA groups (see bellow).} \]. Overall, these results indicated that lesions of the PBN decreased baseline intake of sucrose but spared normal baseline intake of corn oil.
Fig. 4.4. Effects of bilateral PBN or TOA lesions on 15 min free access to sucrose or corn oil. (A) PBNx rats licked significantly less 0.3M sucrose than the control rats, left. Their licks at 0.03M sucrose were close to significantly less than the controls (post hoc, p=0.07). They licked the same amount of 25% corn oil as the control rats, right. (B) The free access lick did not differ between TOAx rats and their controls for sucrose and corn oil (25% corn oil, p=0.07). (*, significant differences between the lesion and the control groups)

In contrast to the comparisons of the PBN groups, there were no significant differences in baseline licks of either sucrose or corn oil between the TOA groups. The TOAx rats showed normal licks of sucrose and corn oil as comparing to the surgical control rats [(sucrose vs.
corn oil): group, F<1, P=0.07 vs. F(1, 11)=3.0, p=0.11; interaction, F<1, p=0.46 vs. F<1, p=0.35]. Both TOAx and Con TOA rats showed concentration dependent intake of sucrose [F(2, 18)=6.4, p<0.008]. For the same reason mentioned above, those rats also increased licking in the 2nd session of free access to 25% oil emulsion [F(1, 11)=27.6, p<0.0001; (Con TOA vs. TOAx), 1st session, 619.4 ±158.9 vs. 1410.4±202.9; 2nd session, 1006.9 ±282.7 vs. 2160.8±260.9]. These results demonstrated that lesions of the TOA had no significant influence on baseline licks of either sucrose or corn oil.

FR10 responses
When the operant FR10 schedule was imposed, PBNx rats showed severe performance deficits (Figure 4.5.A, left). Compared to the surgical controls, the PBNx rats not only did not work for sucrose rewards but also had no concentration dependent responses of the operant spout contacts [group, F(1, 12)=15.65, p<0.002; concentration, F(2, 24)=19.97, p<0.0001; interaction, F(2, 24)=19.58, p<0.0001]. The PBNx rats made close to zero response regardless of the concentration of sucrose rewards. When the reward was corn oil, the PBNx rats still responded significantly less then the Con PBN rats did [Figure 4.5.A, right; group, F(1, 10)=37.66, P<0.0002]. However, the PBNx rats showed concentration dependent responses for corn oil as did the control rats [concentration, F(2, 20)=20.35, p<0.0001; interaction, F(2, 20)=5.05, p<0.02]. The responses of the PBNx rats for 100% corn oil were almost significantly more than for 2.5% corn oil [2.5%, 126±40.2 vs. 100%, 274.7±90; post hoc, p=0.06]. Thus, the hypothesis that lesions of the PBN would disrupt operant responses for sucrose was supported, but that same lesions would spare operant responses for corn oil was not supported by these results.

Consistent with the results of baseline licks, lesions of the TOA produced no deficits on operant responses for either sucrose or corn oil (Figure 4.5.B). Although the TOAx rats tended to respond more for both sucrose and corn oil rewards than did the Con TOA rats, the differences were not significant [(sucrose vs. corn oil), group: F(1, 9)=1.12, p=0.32 vs. F(1, 11)=1.81, p=0.21; interaction: F(2, 18)=1.51, p=0.25 vs. F<1, p=0.47]. Both TOA groups responded for sucrose and corn oil as a function of reward concentration [(sucrose, F(2, 18)=10.47, p<0.001 vs. corn oil, F(2, 22)=28.37, p<0.0001]. These results did not support the
Fig. 4.5. Effects of bilateral PBN or TOA lesions on FR10 operant responses to sucrose or corn oil. (A) Compared to the surgical controls, damages in the PBN disrupted operant responding for sucrose, and greatly reduced responding for corn oil. (B) TOAx and Con TOA rats showed no significant difference in operant responding for sucrose and corn oil.
hypothesis that lesions of the TOA would disrupt operant responses for corn oil but not for sucrose.

PR5 responses
The results for PR5 were similar to those for FR10. The PBNx rat showed severe performance deficit while performing PR5 under both sham (A) and real (B) feeding conditions (Figure 4.6). They did not respond for either sucrose or corn oil. The Con PBN group had significantly higher break points for both sucrose [F(1, 12)=48.73, p<0.0001] and corn oil [F(1, 10)=24.12, p<0.0007] than did the PBNx group during sham feeding. Consistent with Experiment 1, the break points for both the Con PBN rats were a monotonic function of concentration during [F(2, 24)=38.71, p<0.0001]. The PBNx rats, on the other hand, responded equally to different concentrations of sucrose [interaction, F(2,24)=36.94, p<0.0001; post hoc, Con PBN, p<0.0002 vs. PBNx, p>0.8]. When the reward was corn oil, the results of break points during sham feeding were similar. For the Con PBN rats, the break points for corn oil were not a linear function of concentration as were for sucrose, but the break points increased as the corn oil concentration increased [F(2, 20)=29.40, p<0.0002]. Although the break points for corn oil were higher in the PBNx rats than were for sucrose, the break points for different concentrations of corn oil did not differ from each other [interaction, F(2, 20)=11.46, p<0.0005; post hoc, p>0.4]. Compared the break points for sucrose during real feeding, the Con PBN group had an inverted-U function of concentration when the PBNx group still showed no concentration dependent responses [group, F(1. 12)=65.24, p<0.0001; concentration, F(2, 24)=22.74, p<0.0001; interaction, F(2, 24)=115.28, p<0.0001]. The break points for sucrose concentrations were significantly different from each other in the Con PBN rats but not in the PBNx rats [post hoc, Con PBN, p<0.007 vs. PBNx, p>0.3]. The PBNx rats again had significantly lower break points for corn oil during real feeding than did the Con PBN rats [group, F(1, 10)=17.17, p<0.003; concentration, F(2, 20)=9.84, p<0.002; interaction, F(2, 20)=5.30, p<0.02]. There were no significant differences in the break points for different concentrations of corn oil in the PBNx rats [post hoc, p>0.4]. These data indicates that when the work requirement to receive a reward increases after each reward trial, lesions of the PBN severely impaired the operant responses for both sucrose and corn oil.
Fig. 4.6. Effects of bilateral PBN lesions on break points of PR5 for sucrose and corn oil during (A) sham and (B) real feeding. Except for during sham feeding 0.03M sucrose (post hoc, p=0.08), the break points of the PBNx rats were significantly less than of the control rats for sucrose and corn oil during both sham and real feeding (post hoc, p<0.03).
There were also differences between the Con PBN and PBNx rats when comparing the break points during real and sham feeding. For the Con PBN rats, the break points for high concentrations of either sucrose or corn oil differed between feeding conditions. Consistent with the results in Experiment 1, the break point for 2.0M sucrose (post hoc, $p<0.0002$) was significantly higher during sham feeding than was during real feeding [interaction of feeding×concentration, $F(2, 20)=5.30$, $p<0.02$]. Similarly, the break points for corn oil in the Con PBN rats were higher during sham feeding than were during real feeding [$F(1, 8)=6.24$, $p<0.04$]. In contrast to the control rats, the PBNx rats showed no differences in the break points during real and sham feeding for either sucrose [$F(1, 16)=0.15$, $p=0.70$] or corn oil [$F(1, 12)=0.43$, $p=0.52$]. The major difference between real and sham feeding was that the rats received calorie during real feeding but not during sham feeding. Thus, these results further demonstrated that lesions of the PBN disrupted operant responses in a PR5 schedule regardless of the nutritional effect of the reward.

During the PR5 testing, the Con TOA and TOAx rats both showed concentration dependent responses for both sucrose and corn oil during sham (A) and real (B) feeding (Figure 4.7). During sham feeding, the break points for sucrose were significantly higher in the TOAx rats than in the Con TOA rats [$F(1, 9)=11.75$, $p<0.008$]. Particularly, the break points for 0.3M (post hoc, $p<0.002$) and 2.0M (post hoc, $p<0.0004$) sucrose were significantly higher in the TOAx than in the control rats [concentration, $F(2, 18)=56.95$, $p<0.0001$; interaction, $F(2, 18)=4.19$, $p<0.04$]. These effects did not occur in the break points for corn oil because there were no significant differences between the two groups [group, $F(1, 11)=1.41$, $p=0.26$; concentration, $F(2, 22)=23.21$, $p<0.0001$; interaction, $F<1$, $p=0.68$]. During real feeding, the TOAx rats still showed concentration dependent of break points for sucrose [$F(2, 18)=26.30$, $p<0.0001$], and their break points were significantly different from those of the Con TOA rats [group, $F(1, 9)=5.31$, $p<0.05$; interaction, $F(2, 18)=1.11$, $p=0.35$]. The two groups of rats responded similarly for corn oil and the break points did not differ during real feeding [group, $F(1, 11)=1.12$, $p=0.31$; concentration, $F(2, 22)=20.84$, $p<0.0001$; interaction, $F(2, 22)=1.59$, $p=0.23$]. These results did not support the hypothesis that the TOA is necessary for corn oil but not sucrose reward. Lesions of the TOA did not decrease operant responses for either
Fig. 4.7. Effects of bilateral TOA lesions on break points of PR5 for sucrose and corn oil during sham (A) and real (B) feeding. The TOAx rats performed significantly higher break points for sucrose at 0.3M and 2.0M than the control rats did during sham feeding. They did not differ while responding for corn oil. During real feeding, there was no difference in break points between the TOAx and control rats for both sucrose and corn oil.
sucrose or corn oil. In fact, the lesions enhanced operant responses for sucrose but not for corn oil in a PR5 testing.

When comparing the break points during real and sham feeding, the Con TOA rats had significantly higher break point for 2.0M sucrose (post hoc, p<0.007) during sham feeding than real feeding [interaction, F(2, 16)=5.79, p<0.02]. This result was consistent with that of the Con PBN rats. However, unlike the Con PBN rats, the Con TOA rats showed no differences in break points for corn oil during real and sham feeding [F(1, 8)=2.08, p=0.19]. Interestingly, the TOAx group had significantly higher break points for sucrose during sham feeding than during real feeding. The effect was more persuasive than the same effect in the TOA controls because there were significant differences in every main effect of statistics [feeding, F(1, 10)=19.96, p<0.002; concentration, F(2, 20)=60.42, p<0.0001; interaction, sucrose [F(2, 20)=13.69, p<0.0002; post hoc at 2.0M, p<0.0002]. When responding for corn oil, the TOAx rats again had significantly higher break points during sham feeding than during real feeding [F(1, 14)=15.79, p<0.002]. These results indicate that lesions of the TOA facilitate the differences in operant responses during real and sham feeding compared to their controls.

Discussion
This is the first study to demonstrate that lesions of the PBN and TOA have different effects on the reward strength of orosensory stimulation. On the one hand, the results support our hypothesis that the PBN is important for processing sucrose reward and PBNx rats responded differently to sucrose and corn oil. Although showing concentration dependent responses, the PBNx rats licked significantly less sucrose while ingesting normal amounts of corn oil during the 15-min free access session. They did not respond for sucrose while showing steady responses for corn oil. The impaired performance during corn oil operant tasks, on the other hand, failed to support our hypothesis that lesions of the PBN would have no effect on the processing of oil reward. The hypothesis that the TOA is important for oil but not sucrose reward was not supported. Nonetheless, the responses to the two stimuli were not identical. The TOAx rats tended to over-respond for both sucrose and corn oil, and they responded
significantly more for sucrose than did the surgical control rats during the PR5 testing. Although some TOAs rats failed to respond during the sucrose sessions, they responded as well as the other TOA lesioned rats once they started responding. This initial disruption of performance was probably due to a problem of recovery of function.

The progressive ratio schedule was first designed to measure the relative strength of a reward using the break point instead of the rate of responding (Hodos, 1961; Hodos and Kalman, 1963). The rate of responding, an index of reinforcing efficacy of a stimulus for FR schedule, can reflect both the motor and motivational functions, which confound the interpretation of results. Animals can show impairment of motor function but still be able to change the break point for a reward according to manipulations of motivational variables (Eagle et al., 1999). This advantage was the reason why the PR schedule was chosen to test the lesion effects on the reward strength of sucrose and corn oil. Nevertheless, the PBN lesioned rats in this study showed severe performance deficits with both sucrose and corn oil, and thus a microanalysis of their responses for the cause of deficit is necessary. Dependent variables in the operant tasks included: break point, postreinforcement pause (PRP, the time interval between the end of a reward trial and the first contact of the operant spout), licks per reward trial (number of licks during each 10 sec reward trial), operant duration (the time spend to accomplish a FR10 requirement to receive reward), and the latency to consume gained reward. The first two variables are considered as the motivational aspects. The second two variables represent the motor aspects, and the last variable could indicate the associative learning aspect of performance. The following discussions will include comparisons of the performance differences between the two lesions as well as the two stimuli according to the analysis of these variables.

Despite the fact that PBNx rats licked normal amounts of 25% corn oil emulsion during 15-min free access, their operant responses for corn oil were impaired. That PBNx rats showed significantly decreased licking during the free access sessions to sucrose but not corn oil suggesting that the lesions influenced their responses to sucrose and corn oil differently. At first glance, it seems that the PBNx rats failed to respond for sucrose but performed stably, though significantly less than normal, for corn oil. During the sucrose sessions, responses
were so limited that appropriate microanalysis could not be done. The microanalysis thus included only data from corn oil and the last two FR10 sessions of 0.3M sucrose. Compared with the surgical controls, the PBNx rats made the same number of licks per reward trials (average licks 60-80/10sec), too similar times to complete an FR10 requirement, and showed similar latencies to consume the reward. That gustatory PBNx rats could make the same amount of licks as the control rats during a short 10s period indicates that there was no apparent tongue movement impairment after lesions. Since the operant duration for each 10 contacts on the operant spout did not significantly differ from control animals, the gustatory PBN lesions did not seem to damage other functions of movement such as nose poking and paw pressing, which may account for some operant responses. Although not analyzed, the latency to consume the reward varied greatly during sucrose sessions. Once oil FR10 tasks had begun, this latency stabilized after two sessions and eventually did not differ from the control rats. The latency to lick the reward could be an indication of whether the animals acquired the association between the operant response and the consequent reward delivery. The results indicated that the PBNx rats learned the contingency between the response and reward during oil operant tasks. Moreover, they responded to 0.3M sucrose with similar latency to lick gained reward during the last two FR10 testing sessions. These two sessions of 0.3M sucrose were designed to determine whether the responses performed by the PBNx rats during corn oil operant tasks were stimulus specific. Data from the last two sessions revealed that the learned responses were not specific to corn oil. Considering that the training was conducted post-surgery, there are two possible explanations for this result. One states that the PBNx rats had not yet completely recovered from the lesions while they were first performing the operant task. They did not have the ability to learn the task while they were first exposed to the sucrose operant tasks. The function recovered by the time the stimulus was switched to oil emulsions. However, there were differences in lesion size as well as in recovery rate among the PBNx rats. Under such condition, the points where animals started performing would vary in each animal, which was the case for the TOAx rats. The observation that all PBNx rats started responding after the free access to corn oil makes it unlikely that the recovery of function was the cause of deficit. Accordingly, it is more likely that the sensory and rewarding processing of corn oil for the PBN lesions was damaged not as severely as the processing of sucrose. The oil stimulus served as a stronger salient stimulus to drive the
operant behavior of the PBNx rats. Therefore, those rats were able to learn the task while corn oil was the reward, and they could perform in sucrose tasks once they had learned a response-reward association during the corn oil operant sessions.

The deficits of the PBNx rats were shown primarily in the motivational aspects of performance. Different concentrations of sucrose and corn oil were used as the salient stimulus to assess manipulations of altered reward magnitude on the operant performance in this study. Overall, the PBNx rats showed no concentration dependent response for sucrose, but a weak one for corn oil. As mentioned in the result section, the break points for sucrose were close to zero, and those for corn oil were low in the PBNx rats. Same deficit was shown in the postreinforcement pause (PRP) data. The analysis of the PRP for the FR10 and PR5 sessions revealed that the PBNx rats had very long PRP as comparing to the control rats (ANOVA: group × concentration, p<0.05). The PBNx rats also did not show a tendency of increasing PRP as normal rats did while the ratio requirement increased. Furthermore, the PBNx rats not only responded poorly to the operant spout but also responded little to the inactive spout. These results indicated that lesions centered in the gustatory PBN produced a general impaired motivation for sucrose and corn oil reward.

This impairment could be due to the disability of sensory detection or the decreased perception of the hedonic value of the stimuli. The PBN is the second central gustatory nucleus that receives projections from the rostral end of the nucleus of the solitary tract. This nucleus is unique in the way that it is the location where the gustatory pathways split into a ventral and a dorsal pathway. The dorsal projections are classical thalamocortical pathway. The ventral projections reach the central reward circuits (Norgren et al., 2003; Geisler and Zahm, 2005) by way of lateral hypothalamus, bed nucleus of stria terminalis and the central nucleus of amygdala (Lundy and Norgren, 2004). It has been shown that animals with PBN lesions could neither learn a LiCl-induced CTA nor express a furosemide elicited salt appetite (Flynn et al., 1991a; Spector et al., 1992; Scalera et al., 1995). Although these animals changed the detection threshold for sapid stimuli variously under different physiological conditions, their ability to perform on a taste-guided shock avoidance task was spared (Spector et al., 1993). The decrease in the perceived sensory intensity of the stimulus could
retard acquisitions of taste-related learning tasks (Shaw, 1983). This alone, however, cannot account for the deficit shown in previous CTA and salt appetite studies as well this study because in all cases the concentrations used were above the detection threshold. Moreover, several learning trials were conducted in this and other studies, but the animals still showed poor performance and no sign of learning (Spector et al., 1995). Therefore, the elimination of CTA and operant performance following PBN lesions is not necessary due to the disability of sensory detection. Instead, considerable studies have demonstrated that the deficits of the PBNx animals in a CTA learning was due to an associative deficit (Reilly et al., 1993; Grigson et al., 1998). Although the latency to lick gained reward showed that the PBNx rats seemed to acquire the contingency between the response and reward, the poor performance leaves the strength of the associative learning questionable.

The decreased perception of the hedonic value of the oral stimuli is a better candidate for the cause of impairment in the operant performance. One piece of supporting evidence comes from the study that demonstrated PBNx rats were unresponsive to increasing sucrose concentration during a taste reactivity (TR) test even though they showed concentration dependent intake of sucrose solutions (Flynn et al., 1991b). Normal rats showed stereotypic orofacial and body movements in responding to sapid stimulation (Grill and Norgren, 1978b). The TR test has been used as an index of animals’ perception of the hedonic effects of oral stimuli (Pecina and Berridge, 2000). Accordingly, PBN lesions damaged the function for perceiving the rewarding effects of sucrose and so disrupted the operant performance for oral rewards. Consistent with this assumption, lesions of the PBN blunt DA increases in the NAc during sucrose intake (Hajnal and Norgren, 2005). Does this mean that the gustatory PBN plays an important role in the motivational and rewarding aspects of oral sucrose stimulation? To further elucidate this issue, investigations using operant tasks can focus on the following projects. If the associative learning deficit were the cause of poor performance, would the use of a stronger and easier response-reward contingency task have improved the performance? The FR10 and PR5 or higher ratio of operant requirement has been used in previous studies (Sclafani and Ackroff, 2003; Hajnal et al., 2007; Liang et al., 2008). In these studies, animals without neurological damages could acquire and perform the task within a few sessions. Nevertheless, using a lower ratio for FR and PR tasks such as FR1 and PR 1 might allow
animals with lesions to learn and perform the task better. This assignment would avoid the
floor effect that could confound the interpretation of the results, which occurred in this study
when the PBNx rats did not responded for sucrose. Secondly, previous studies with CTA and
salt appetite have shown that the PBN lesions block the acquisition but not retention of the
tasks (Scalera and Norgren, 1993; Grigson et al., 1997b). Could it be the same case in the
operant task? Because the operant training in this study was conducted post-operatively, the
deficits could be purely on the acquisitive aspect of the task. The necessity of the PBN in
processing the rewarding effects of orosensory stimulation would be further demonstrated in
animals trained with operant tasks before the PBN lesion surgery and then tested the retention
of operant responses post-operatively.

In contrast to the results of PBN lesions, lesions of the TOA produced significantly higher
break points of sucrose and minor over-responding for corn oil. The thalamic lesions in this
study included not only the nucleus of taste, the ventral posterior thalamus nucleus,
parvicellular part (VPPC), but also the nucleus of trigeminal, the ventral posteromedial
nucleus (VPM). The results are similar to Reilly and Trifunovic (1999b) in the direction that
thalamic lesions including thalamic taste areas do not disrupt operant responding for sucrose
reward. In that study, the lesions were centered in the gustatory zone of the thalamus and such
lesions did not increase or decrease operant responding for sucrose solutions compared with
controls (Reilly and Trifunovic, 1999b). With lesions including larger thalamic areas in this
study, the break points for sucrose actually increased. The microanalysis showed that during
both sucrose and corn oil sessions, the TOAx rats required less time to complete an FR10 and
shorter PRP compared with control. However, the only significance was shown in the shorter
PRP specifically during sucrose sessions (last two FR10 of 0.3M sucrose, Student’s t test;
PR5 of sucrose, ANOVA: group × concentration, p<0.05). The over-responding effect did not
appear while the lesions were confined to the gustatory thalamus (Reilly and Trifunovic,
1999b). Moreover, the over-responding did not occur at the inactive spout demonstrating a
specific behavioral disinhibition. The TOA lesions selectively enhanced responding on the
conditioned reinforcement spout for sucrose but not for corn oil. It seems most parsimonious
to conclude that the sucrose is a more salient stimulus in the operant task under the TOA
lesioned condition. Therefore, the effects of TOA lesions on the processing of sucrose and
corn oil reward differ. Similarly, selective disinhibition effect on operant responding was seen in lesions of the subthalamic nucleus (Baunez et al., 2002). This is consistent with the knowledge that the thalamic circuits, particularly the ventrobasal part including the VPM and ventral posterolateral portions (VPL), are primarily inhibitory (Roberts et al., 1992; Salt and Turner, 1998). The lesions in this study included the caudal and ventralmost part of VPM and to some extent the VPL. The results that taste and trigeminal combined lesions but not the one centered in the VPPC (taste) resulted in over-responding for sucrose are intriguing. Moreover, the differential disinhibition effects of TOA lesions on the operant responding to sucrose and corn oil suggested that different neural mechanisms are involved in the sensory and hedonic processing of the two stimuli.

The results of this study did not support the general hypothesis that the rewarding effects of sucrose and corn oil are processed through gustatory and trigeminal pathways, respectively. This hypothesis was supported by the demonstration that lesions in the gustatory PBN disrupted CTA learning to taste stimuli but not 100% corn oil (Norgren et al., 2001). In this study, lesions of the PBN but not TOA impaired operant responding to both sucrose and corn oil. Several interpretations could be drawn from this result regarding the hypothesis. The first would be that the processing of sucrose and corn oil both rely on the gustatory PBN. This statement, however, does not mean that sensory detection and reward processing of both stimuli depends on the gustatory system. The spinal and brainstem trigeminal nuclei project strongly to the parabrachial nuclei (Dallel et al., 2004; Waite, 2004). It is possible that the thalamic trigeminal areas are not the critical substrates involved in the processing of oral trigeminal reward. Lesions of the trigeminal sensory nucleus in the pons induced aphagia in rats (Nadaud et al., 1984). Thus, the principal trigeminal sensory nucleus (Pr5) is a strong candidate for investigation of the processing of orosensory corn oil. That said, our hypothesis could still be supported by showing that lesions of the Pr5 block behavioral performance to corn oil but not sucrose reward. Finally, the testing sequence of the stimulus could affect the results. Should the testing order of the sucrose and corn oil be reversed, would the PBNx and TOAx rats perform differently? In summary, future studies addressing issues mentioned above are necessary to determine the neural mechanisms involved in processing orosensory stimulation of corn oil.
Using operant tasks to measure the strength of reward, this study demonstrated that lesions of the PBN severely disrupted operant responding for sucrose, and impaired responding for corn oil. Lesions of the TOA, in contrast, had subtle effects on responding for corn oil. Thus, the hypothesis that the PBN and TOA are important for processing sucrose and corn oil reward respectively was not supported. The results, nevertheless, suggested that different neural mechanisms are responsible for the behavioral performance to sucrose and corn oil reward. As the operant responding was trained post-operatively, whether the deficits reflect impairment in acquisition or performance requires further investigation.
Chapter 5
Lesions of the gustatory parabrachial nucleus but not the thalamic orosensory area eliminate anticipatory contrast for sucrose and corn oil in sham feeding rats.

In previous two chapters, I presented the effects of PBN and TOA lesions on an animal’s ability to switch the hedonic value of sucrose or corn oil from positive to negative in a conditioned aversion paradigm, and on their ability to estimate the reward value of sucrose and corn oil using FR and PR schedules. From the two aspects of orosensory reward, the results did not fully support the hypothesis that the gustatory PBN is important for orosensory sucrose but not corn oil, and conversely, that the thalamic orosensory area (TOA) is necessary for oil but not sucrose reward. This chapter will present the last task, anticipatory contrast, which was used to test the same hypothesis, but in this case having to do with an estimation of relative, rather than absolute, reward value. Again, previous experiments with anticipatory contrast were done with real feeding. I first demonstrate that normal rats can show an anticipatory contrast effect (ACE) for sucrose and corn oil (which has never been tested) in Experiment 1. Then in Experiment 2, I tested whether this behavioral effect for sucrose is blocked by lesions of the PBN and the effect for oil is blocked by lesion of the TOA.

Experiment 1
Ingestion of one palatable sapid stimulus is affected by the relative value of other sapid stimuli presented closely in time. This change in responding as a function of experience is referred to as a contrast effect (Flaherty, 1996). An anticipatory contrast effect (ACE) develops when rats suppress intake of a weak stimulus (e.g., 0.15% saccharin or 0.06 M sucrose) when it comes to predict the future availability of a stronger, more preferred, stimulus (e.g., 1.0 M sucrose) compared to rats that only experience the weak solution. Over daily trials, the ACE develops and the first bottle intake of the weaker solution in the experimental condition (i.e., weak-strong concentration pair) is suppressed while first bottle intake by rats in the control condition (i.e., weak-weak concentration pair) is increased (Flaherty and Cheke, 1982; Lucas et al., 1988; Lucas and Timberlake, 1992; Flaherty et al.,
1994). Previous studies have shown that this contrast effect is due to the anticipation of the more rewarding solution, not to the memory of having received the preferred 1.0M sucrose solution on the previous day [(Flaherty and Rowan, 1985; Flaherty et al., 1995), but see ref. (Timberlake and Engle, 1995)]. Thus, the first solution has been considered as a conditioned stimulus (CS) and the second, preferred sucrose solution as an unconditioned stimulus (US), as in a classical conditioning context (Flaherty and Rowan, 1985; Flaherty et al., 1991). This contrast effect occurs when the CS and US are at either the same or different locations (Lucas et al., 1988; Flaherty et al., 1996). Moreover, it has been demonstrate that the caloric value of the CS plays an important role in developing an ACE when the intersolution interval (ISI) varies. When the ISI is within seconds, similar ACEs are shown in both sucrose-sucrose and saccharin-sucrose parings. As the ISI increases from seconds to minutes (e.g., 5 or 10 minutes), the ACE diminished in sucrose-sucrose pairings. This difference between the sucrose-sucrose and saccharin-sucrose paring is not due to differences in taste complexity but to the motivational process based on the load of the weak, but available CS. Food deprived rats are unwilling to forgo the available caloric reward when they have to wait for access to the preferred reward. When not deprived, however, anticipatory contrast is evident in behavior (i.e., rats avoid intake of a lesser sucrose cue even when having to wait for access to the preferred and highly caloric second solution (Flaherty et al., 1991).

The ACE also occurs when neither the CS nor the US contains a caloric load. Using saccharin-saccharin pairings, Flaherty and Rowan (1986) showed a statistically reliable ACE which using highly disparate concentrations of saccharin as the first and second solution (e.g., 0.05% - 0.15%). The magnitude of the ACE did not differ when the duration of access to the second solution was 3 or 5 min (Flaherty and Rowan, 1986). These results suggest that the ACE could be based solely upon relative taste cues via an associative process. Accordingly, this study was designed to determine whether an ACE can be obtained when the CS and US provides limited or no metabolic consequence by using a sham feeding model. As described, in this model, a fistula is implanted in the stomach of a rodent so that, when opened, the ingested fluid drains out before producing a postingestive effect (Smith, 1999). The sham feeding paradigm not only limits the caloric load of an oral stimulus but also excludes the intestinal distension that may play a role in the postingestive feedback.
process (Sclafani and Nissenbaum, 1985). Furthermore, previous studies have always used a sweet stimulus for the ACE. This study, therefore, investigates whether different orosensory stimuli, sucrose and corn oil, with similar rewarding effects (Weatherford et al., 1990; Hajnal et al., 2004; Liang et al., 2006) can support an ACE. The contrast paradigm used included one 3 min access period to a low concentration, followed immediately by another 3 min access period to either the same low concentration in unshifted controls or to a preferred, higher concentration in the experimental subjects. In Experiment 1A, rats were first trained with 0.06M vs. 1.0M sucrose and then with 1% and 2.5% vs. 25% corn oil. Since an ACE with oil was not obtained, in Experiment 1B, naïve rats were trained with 1.5, 2.5 and 5% corn oil in bottle 1 vs. 25% corn oil in bottle 2 to determine an appropriate concentration for the CS.

Materials and Methods
The subjects were 36 naïve male Sprague-Dawley rats (Charles River, Wilmington, MA), 18 for each experiment, weighing 275-300g at the beginning of the study. They were individually housed on a 12:12-h light-dark schedule with ad libitum access to tap water and standard laboratory diet [Rodent diet (W) 2018; Harlan Teklad, Madison, WI]. Once the experiment began, the rats were maintained on a food deprivation regime with distilled water available at all times except when the rats were in the chamber. Normal pelleted chow was weighed and provided at least one hour after the daily session.

Surgery
For experiment 1A and 1B, the rats were divided into low-low (L-L) and low-high (L-H) groups, n=9 each. The rats were treated with atropine sulfate (0.15 mg/kg ip) and, 20 min later, anesthetized with pentobarbital sodium (50 mg/kg ip) for the gastric fistula surgery. Details for the design and implantation of the gastric fistulas are described elsewhere (Smith, 1999). Rats had at least two weeks to recover until the experimental procedure began.

Apparatus
The rats were tested in 6 identical modular operant chambers measuring 30.5 cm x 24.1 cm x 29.2 cm. Each chamber was equipped with a house light, a white noise generator, and 3 sipper tubes that could be programmed to advance and retract depending on the testing schedule (only 2 sipper tubes were used for this experiment). These sipper tubes could enter the chamber through 1.3-cm holes spaced 16.4-cm apart from left to right of an aluminum wall. The house light and white noise generator was located opposite to the sipper tubes. The white noise generator provided a background noise level of 75 dB. Three chambers served as L-L chambers where only low concentration pairs were presented. The other three served as L-H chambers where both the low and the high concentration pairs were presented. Spout licking was recorded using a triple lickometer circuit. Each test chamber was located in a sound attenuating cubicle that was fitted with a ventilation fan. This set up for operant tasks and on-line data collection was operated by a PC computer and an interface (MedPC; Medassociates Inc. St. Albans, VT).

Procedure
Rats were run in squads of 6, with 3 rats placed in the L-L chambers and the other three placed in the L-H chambers. Before each rat was placed in a chamber, its stomach was flushed with lukewarm water as described in Chapter 4. Testing was preceded by one 5-min habituation trial, where rats were placed in the chamber with house light and white noise on. Food was removed from the home cage the day before the habituation trial. Thereafter, normal pelleted chow was weighed (20-25 g) and given to the rats in their home cage at least one hour after they finished their daily trial. The body weight was maintained at 90% of free feeding. During testing, the rats were given 3 min access to 0.06M sucrose. Bottle 1 (B1) retracted and bottle 2 (B2) advanced. Rats in the L-L condition were then given 3 min access to the same 0.06M sucrose solution in B2. Rats in the L-H condition, on the other hand, were given 3 min access to 1.0M sucrose. There was one such pairing a day for 14 days in succession. After a week off, the L-L and L-H groups were reversed and tested for ACE using corn oil concentration pairs. During the first 7 trials, 1% corn oil served as the Low concentration and 25% corn oil served as the H concentration. Thereafter, the Low concentration was increased to 2.5% corn oil for another 8 trials. This design failed to support the development of an ACE in rats with open fistulas and a history of experience.
with sucrose. Experiment 1B, then, addressed the same question, but in rats that were naïve to sucrose.

In Experiment 1B, the rats were first trained through 14 days, using 1.5% followed by a second 1.5% corn oil as the L-L condition and 1.5% followed by 25% corn oil as the L-H condition. After 14 trials, it became clear that even sucrose naïve rats did not lick 1.5% corn oil emulsions consistently when tested at 90% of free feeding body weight. Given the low intake of the L concentration by rats in the L-L control group, it was not possible to assess contrast (i.e., suppressed intake of the L solution when paired with the future availability of the H concentration). Consequently, the rats were placed back in a free feeding condition for two weeks and then began training with the 2.5% vs. 25% condition using the same L-L and L-H groups. The rats licked 2.5% corn oil consistently after 10 trials. After the 10th trial, more normal pellet chow (3-5g) were given to the rats for the rest of 8 trials in order to bring up the body weight from 90% to 95% of free feeding. The rats developed ACE with corn oil using 2.5% as the L stimulus and 25% as the high stimulus. The rats were then given two weeks of free feeding without training, and then placed back to food deprivation regimen with a target of 95% of the body weight. The day before testing, the L-L and L-H groups were reversed and the L concentration was increased to 5% corn oil. There were 8 such trials.

The sucrose solutions were made with distilled water and the corn oil emulsions were blended with distilled water and Tween-80 [100 ml corn oil-water mixture blended with 0.75 ml Tween-80 (Sigma-Aldrich, St. Louis, MO)]. All the procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine, and comply with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

Statistical Analysis
It took the rats 6-8 days to begin licking consistently. Only rats that performed consistent licking there after contributed data to the analysis. In Experiment 1A, all rats contributed data to the analyses. In experiment 1B, data from three rats were omitted. One rat died after surgery, one rat from each L-L and L-H group did not lick throughout the training days. The
data included daily 3-min sham licks of B1 and B2, and the latency to start licking each bottle. The lick and latency data were averaged into 2-day blocks and were analyzed by repeated-measures ANOVAs varying solutions and blocks. Post hoc Newman-Keuls tests were conducted where appropriate.

Results
Experiment 1A. Sucrose ACE
After 14 trials, rats in the L-L (0.06M-0.06M) and L-H (0.06M-1.0M) condition showed different intake of the B1 solution (Fig. 5.1). Comparisons of B1 licks (Fig. 5.1A) between groups revealed that the L-H group developed a clear ACE. Specifically, the average B1 licks of L-H during the 3 min presentation were significantly lower than the B1 licks made by rats in the L-L group, beginning with block 4[group: F(1, 16)=8.87, p<0.009; block: F(6, 96)=14.02, p<0.0001; group \times block: F(6, 96)=12.45, p<0.0001]. The number of licks at each bottle did not differ for the L-L groups, but the licks increased across block, F(6, 96)=19.66, p<0.0001. The L-H group, in contrast, licked significantly more at B2 than at B1 from the very first block until the last block [bottle: F(1, 16)=313.16; block: F(6, 96)=61.76; bottle \times block: F(6, 96)=52.72; p<0.0001 for all effects]. The results of B2 licks in the L-L and L-H groups are shown in Fig. 5.1B. In addition to the contrast effect shown in B1 licks, a decrease of latency was shown in licking the B2 solution (Fig. 5.2). The latency to lick B2 1.0M sucrose decreased across blocks, relative to the latency to lick B2 0.06M sucrose beginning with block 5 [group: F(1, 16)=25.61, p<0.0002; block: F(6, 96)=4.41, p<0.0006; group \times block: F(6, 96)=2.91, p<0.02]. The latency to make the first lick on B1, however, did not differ between rats in the L-L vs. L-H condition so the data are not presented.
Fig. 5.1. The anticipatory contrast effect for 0.06M vs. 1.0M sucrose comparison. (A) An ACE for sucrose was shown in bottle 1 licks. (B) Comparisons of bottle 2 licks between the L-L and L-H groups. Mean (± SEM) number of 3 min licks (sham intake) made for the bottle 1 (B1: 0.06M sucrose) or bottle 2 (B2: 0.06M or 1.0 M sucrose) over 7 2-day blocks for rats in the Low-Low (L-L, n=9) or Low-High (L-H, n=9) condition.

At the end of the sucrose ACE training, rats in L-L and L-H were switched for the corn oil ACE training. The data from this part of experiment are not presented, but the results can be summarized as follows. First, rats at 90% of free feeding body weight did not approach 1% corn oil emulsion. When the concentration was increased to 2.5%, they made 473 licks at highest average within 3 minutes. However, at this level of deprivation, the L-L and L-H groups showed no difference of B1 licks. Thus, Experiment 1B was conducted in sucrose naïve rats to determine the parameters required to obtain an ACE with disparate concentrations of corn oil in sham feeding rats.
Fig. 5.2. The latency to lick for B2 sucrose. Mean (± SEM) second of latency to lick for the B2 over 7 2-day blocks for rats in the L-L (0.06M) and L-H (1.0M) groups.

Experiment 1B. Corn oil ACE

Three low concentrations, 1.5%, 2.5% and 5%, were used to compare with 25% corn oil emulsions. Rats with body weight of 90% free feeding did not lick 1.5% corn oil voluntarily. They made less than 50 licks within 3 min at highest average. When the concentration was increased to 2.5%, they started licking, and the licking patterns of L-L (2.5%-2.5% and 5%-5%) and L-H (2.5%-25% and 5%-25%) across trials are shown in Fig. 5.3 and Fig. 5.4 (A, B1 licks and B, B2 licks). The statistical analysis includes comparisons of B1 licks between the L-L and L-H groups (for ACE), and comparisons of B1 and B2 lick within the L-L or the L-H group. When the L concentration was 2.5% oil, the contrast effect was seen in block 8 [Fig. 5.3A; group × block interaction, F(7, 91)=4.27, p<0.0005; post hoc p<0.008]. The licks of block 7 did not differ using post hoc Newman-Keuls tests, but a Student t-test comparing
Fig. 5.3. The anticipatory contrast effect for 2.5% vs. 25% corn oil comparison. (A) An ACE for corn oil was shown in B1 licks. (B) Bottle 2 licks in the L-L (n=7) and L-H (n=8) groups. Mean (± SEM) number of 3 min licks (sham intake) made for the B1 (2.5% corn oil) or B2 (2.5% or 25% corn oil) over 7 2-day blocks for rats in the L-L or L-H condition. The arrows indicate the point when the rats began to receive more food post training. (Note: the vertical scales in A and B are different.)

only block 7 showed that the licks in the L-L was significantly higher than in the L-H (p=0.044). Thus, after the food provided post training was increased, it took more than two trials to develop a contrast effect. In the L-L condition, the average licks of B1 and B2 (both 2.5% oil) increased across blocks [F(7, 84)=6.92, p<0.0001], but were not significantly different from each other. In the L-H group, the average licks of B2 25% oil were significantly higher than of B1 2.5% oil across blocks [bottle: F(1, 14)=29.39, p<0.0001; block: F(7, 98)=6.16, p<0.0001; bottle × block: F(7, 98)=3.2 p<0.005].

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Fig. 5.4. The anticipatory contrast effect for 5% vs. 25% corn oil comparison. (A) An ACE for corn oil was shown in B1 licks. (B) Bottle 2 licks in the L-L (n=8) and L-H (n=7) groups. Mean (± SEM) number of 3 min licks (sham intake) made for the B1 (5% corn oil) or B2 (5% or 25% corn oil) over 4 2-day blocks for rats in the L-L or L-H condition.

After the rats returned to free feeding for two weeks and reversed their group assignment for the 5% vs. 25% experiment, they quickly showed a contrast effect at mild food deprivation (body weight of 95% of free feeding, Fig. 5.4). When the L concentration was 5%, the B1 licks in the L-L group were significantly higher than in the L-H group from block 2 to 4 [group, F(1, 13)=7.32, p<0.02; group × block, F(3, 39)=3.81, p<0.02]. Thus, an ACE was shown in 5% corn oil. There was no significant difference in B1 and B2 licks in the L-L condition [bottle, F<1, p=0.57; block, F(3, 42)=2.31, p=0.09; bottle × block, F(3, 42)=2.78, p=0.053]. The L-H group licked significantly more B2 25% oil than B1 5% oil [F(1,
but there was no significant main effect of either block \([F(3, 36)=1.09, P=0.36]\) or bottle by block interaction \([F<1, p=0.88]\). Overall, the results of the 2.5% vs. 25% and 5% vs. 25% indicate that rats can show an ACE for oil when they are mildly food deprived. When the H concentration is 25% corn oil, an ACE for 5% corn oil can be formed within 4 blocks (8 days).

Unlike the sucrose comparisons in Experiment 1A, there was not a dominant effect as far as the latency to lick for each bottle was concerned. During the 2.5% vs. 25% experiment, there was no significant difference between the L-L and L-H groups in latency to lick for B1 [group, \(F<1, p=0.51\)], but the L-L rats began to lick for B1 sooner than did the L-H rats at block 8 \([F(7, 91)=4.19, p<0.0005; \text{post hoc}, p<0.05]\). When comparing the latency to lick for B2, the L-H approached B2 sooner than the L-L group did [group, \(F(1, 13)=6.49, p<0.03\); block, \(F(7, 91)=3.9, p<0.001\); interaction, \(F(7, 91)=1.41, p=0.21\)]. During the 5% vs. 25% experiment, there was no significant difference between the L-L and L-H groups in latency to lick for either B1 or B2.

Discussion
This is the first study to demonstrate that an ACE can be developed using different orosensory stimuli, sucrose or corn oil, under sham feeding conditions. It is determined that using sucrose-sucrose pairings, a statistically reliable ACE could be obtained. The concentrations of sucrose were chosen based on previous studies of ACE with normal feeding condition, 0.06M is close to 2% sucrose and 1.0M is close to 32% sucrose (Flaherty et al., 1991). These concentrations turned out to be appropriate for the ACE under sham feeding condition. The results of this study agree with previous study using saccharin-saccharin pairings that differences in the concentration of taste cues alone are sufficient to suppress intake of the initial solution (Flaherty and Rowan, 1986). When the stimuli were oil-oil pairings, the same reliable contrast effects were obtained with 2.5 or 5% vs. 25% emulsions. Because this was the first study to test whether animals can develop an ACE for corn oil emulsions, the concentrations tested were chosen based on the data collected from our laboratory. In a sham feeding experiment, rats with overnight food and water deprivation
were provided daily 30-min free access to a corn oil emulsions. This experiment demonstrated that the sham intake of 2.5% corn oil (8.9 ml) was about a third of the 25% corn oil emulsion (28.7 ml). Thus, the first few concentrations chosen for the contrast paradigm were lower than 2.5%. It turned out that rats with food restriction alone did not consume the 1 and 1.5% corn oil emulsions voluntarily and constantly.

Whether an ACE will develop depends on the reward value rather than the caloric value of the low concentration of the two stimuli in the anticipatory contrast paradigm. It has been suggested that animals can associate the orosensory properties of oral stimuli with their nutritional values according to previous feeding experience (Booth, 1972). It has been shown that although some fluid leakage into the duodenum was possible even when the recovery of drained fluid was more than ingested during sham feeding (Sclafani and Nissenbaum, 1985). In this study, rats had never consumed either sucrose or corn oil before sham feeding. The stimuli were all novel, and the rats never associated the orosensory cue and the caloric value of them. One or 1.5% corn oil contains no less caloric value than 0.06M sucrose. If somehow a leakage to the duodenum were sufficient for the rats to form an association between the orosensory cue and the caloric value of the stimulus, 1 or 1.5% corn oil and 0.06M sucrose would have been equal salient oral stimuli. This experiment showed that rats developed an ACE for sucrose using 0.06M sucrose but not one for corn oil using 1 or 1.5% oil as the CS. These suggest that the caloric value of the stimulus is not the important factor for developing an ACE. This notion is supported by the results that 2.5% corn oil contained more caloric value than 0.06M sucrose, but using 2.5% oil as the CS formed a smaller degree of ACE than using 0.06M sucrose. One possibility to explain the results mentioned in this paragraph is that the reward value of the 0.06M sucrose is higher than of the 1% to 2.5% corn oil solutions. Thus, 0.06M sucrose can drive the licking behavior while the oil solutions cannot. This notion is supported by previous experiments showing that the reward value of the CS cue modulates the degree of an ACE (Flaherty et al., 1994). When the CS cue contains little or no reward value, animals do not lick the CS consistently. A reliable and significant ACE is developed when the reward value of the CS is high enough to initiate consistent licking behavior (Flaherty et al., 1994). This possibility can be examined by two-bottle tests with 0.06M sucrose and a corn oil emulsion presented
at the same time while the animals are food and water deprived or food deprived alone. These two-bottle tests have not yet been conducted.

The deprivation state plays an important role for forming an ACE for corn oil under sham feeding condition. In Experiment 1A, rats did not develop a contrast effect for 2.5% vs. 25% comparisons with the deprivation to 90% of free feeding weight. When the same comparison was tested again in Experiment 1B, The rats did not develop an ACE until the deprivation level was brought down from 90 to 95% of free feeding weight. Anticipatory contrast experiments with sweet tasting stimuli demonstrated that the deprivation state does not alter the contrast effects that are produced with saccharin as the first solution. The deprivation state only diminished an ACE when the ISI prolonged with sucrose-sucrose pairings. That is, when the ISI was 5 min, a contrast effect for 2 or 4% vs. 32% was obtained with free feeding rats but not when they were food deprived (Flaherty et al., 1991). In other words, deprivation state is not a factor for developing a contrast effect when the first solution, the CS, carries only an orosensory cue. In this experiment, it was expected that the metabolic effects of corn oil were excluded through sham feeding. If that were the case, one would expect that the deprivation state did not change the ACE as in the case in saccharin-sucrose pairings. That a contrast effect for 2.5% vs. 25% corn oil varies depending on the deprivation level suggests that the rats in the corn oil experiment somehow perceived the nutritive aspect of the stimulus. This is not impossible although the recovery of the drained solutions were measured regularly and only data with the recovery that was the same or more than the ingested volume were included in the analysis. Measuring the recovery of drained fluid cannot completely assure that gastric sham feeding prevents all of the postingestive effects of food (Sclafani and Nissenbaum, 1985). Besides using a pyloric cuff to prevent fluid from entering the duodenum during sham feeding (Sclafani and Nissenbaum, 1985), another way to test whether a caloric factor was involved in the contrast effect for corn oil is to test the same 2.5% vs. 25% comparisons with various ISIs. The same degree of contrast effects should be obtained as the saccharin-sucrose pairings did if the caloric factor of the CS is not involved in the developing of an ACE for corn oil under sham feeding condition. The caloric factor can also be excluded by using nonnutritive mineral oil as the CS or as the CS and US both for the contrast paradigm. Mineral oil has similar
rewarding properties to corn oil (Mindell et al., 1990). Comparisons of ACEs for mineral oil and corn oil varying real or sham feeding condition, the deprivation state, ISI or the concentrations of the stimuli will provide more understanding of whether caloric information is necessary for the occurrence of anticipatory contrast of oil. Finally, it could also be that the animals could detect the caloric value of a nutritive stimulus from the oral cavity. Previous studies suggest that there is a direct pathway from the oral cavity to the brain for a glucose taste (Maller et al., 1967; Kare, 1969; Kare et al., 1969; Stephan et al., 1999). It is possible that such direct pathway exists and provides the information of the caloric value of corn oil emulsions when some free fatty acid are produced from the digestion of corn oil by some lipid lipase resides in the oral cavity (Kawai and Fushiki, 2003).

Latency measures are not as accurate as consummatory measures for identifying ACE. In this study, contrast effects in the latency to lick B1 did not occur regularly. For the sucrose comparisons, the L-H started licking B2 sooner than did the L-L group. Their latency to initiate licking on B1, however, did not differ. For the corn oil comparisons, the L-H group approached more slowly to B1 but sooner to B2 during 2.5% vs. 25% comparisons than did the L-L group. No significant effect on latency to lick B1 or B2 was shown during 5% vs. 25% comparisons. The latency to lick B1 can occur as a contrast or a reinforcement effect. When a contrast effect occurs, the latency to lick B1 for the L-H condition increases and becomes significantly longer than in the L-L condition over trials. A reinforcement effect occurs when the latency to lick B1 for the L-H condition decreases and becomes significantly shorter than for the L-L condition (Flaherty and Grigson, 1988). The experimental design in this study was an autoshaping procedure. The rats did not need to make a required number of licks on the B1 CS to receive the B2 US. With this procedure, previous experiments have demonstrated that a contrast effect in latency to B1 occurs and does not vary as a function of ISI within 15 seconds range. That is, the L-H group was slower to make the first lick to B1 than was the L-L group (Flaherty and Grigson, 1988). Other experiments using the same procedure, however, have shown inconsistent results. In a sucrose-sucrose pairing experiment, it was shown that latency to lick B1 was shorter when B2 contained a higher concentration (Flaherty et al., 1991). It was in saccharin-sucrose
pairings that a longer latency to B1 in the L-H group, a contrast effect, appeared (Flaherty and Grigson, 1988; Flaherty et al., 1996). When a contingent-lick requirement on the B1 CS is imposed, a reinforcement effect on the latency to lick B1 occurs. The latency to the first lick on B1 was shorter in the L-H condition than in the L-L condition. This reinforcement effect has been demonstrated with saccharin-sucrose pairings (Flaherty and Grigson, 1988). The latency to the first lick of B1 also varies depending on the access time and spatial location of B1 and B2 (Flaherty et al., 1996). If an effect on latency to lick B2 occurs, it was always a shorter latency in the L-H than in the L-L group for both sucrose-sucrose and saccharin-sucrose pairings (Flaherty and Grigson, 1988; Flaherty et al., 1991). In our experimental design, the latency to lick B2 cannot present either a contrast or a reinforcement effect because a H-H condition is not included in the experiment. Despite these data, most previous experiments showed no latency effect to either B1 or B2 (Flaherty et al., 1995). It has been suggested that animals in an anticipatory contrast paradigm acquired the association between the orosensory cues of the B1 and B2 before they learned the contextual cues for the prediction of the B2 or for the orosensory associations. It would take extended training for the contrast in latency to develop, and the latency measure is not as precise as the consummatory measure of contrast. (Flaherty and Grigson, 1988). Based on the discussion above, it is now uncommon for our experiments to show no consistent contrast effect in latency for either sucrose or corn oil.

This study is the first to demonstrate an ACE for corn oil, and ACEs for either corn oil or sucrose obtained during sham feeding. In our laboratory, we have shown that rodents can learn conditioned taste aversions, operant tasks (Liang et al., 2008c) and anticipatory contrast effects when postingestive feedback was excluded or limited by sham feeding. The results of those studies suggest that sucrose and corn oil are different orosensory stimulation; they drive behavioral differently i.e. the deprivation level influences the degree of contrast effects for corn oil but not for sucrose. Since this was not a parametric study, future studies varying the concentrations of the stimuli, the deprivation state, the ISI, the access time, the spatial location of the solutions and the nutritive value of the stimuli are required to compare the ACEs for sucrose and corn oil.
**Experiment 2**

The major hypothesis in this dissertation is that the sensory detection of corn oil requires the intraoral trigeminal somatosensory system, which by pass the PBN and project directly to the thalamocortical pathway (Waite, 2004). The hypothesis has been tested by investigating the effects of lesions of the PBN and the TOA on reward related behaviors including CTA and operant tasks using sucrose and corn oil as orosensory stimuli. If the hypothesis were true, lesions of a gustatory relay would disrupt CTA learning or decrease operant responding to sucrose but not to corn oil; the converse effect would occur in lesions of a trigeminal relay. The results of these studies support the hypothesis as the PBN lesions eliminate learning a conditioned aversion to sucrose but not to 100% corn oil and disrupt operant responding for sucrose but only depress it for corn oil. Lesions of the TOA, however, have no effect on CTA learning, and if anything, disinhibit operant responses for sucrose and corn oil. These results are inconclusive about whether the PBN is required for processing corn oil reward. The next experiment investigated the effects of the PBN and TOA lesions on reward comparisons of sucrose or corn oil solutions using the anticipatory contrast paradigm. If the orosensory reward of sucrose and corn oil are processed respectively through the gustatory PBN and the oral trigeminal thalamic, lesions of the PBN and the TOA will prevent ACE for sucrose and corn oil, respectively.

As demonstrated above, rats are able to develop an anticipatory contrast effect (ACE) when two rewarding solutions of either sucrose or corn oil are presented sequentially and daily over several days. The anticipatory contrast paradigm tests a different aspect of reward, but shares the components with the CTA and operant tasks. All include a conditioned stimulus (CS) and an unconditioned stimulus (US). In this contrast paradigm, the CS is the first solution presented and has a low concentration. The US has a higher concentration and follows the weak one (Flaherty and Rowan, 1985; Flaherty and Grigson, 1988; Flaherty et al., 1991). To test the hypothesis mentioned above, the stimuli used were 0.06M vs. 1.0M sucrose and 1.5% vs. 25% corn oil emulsions for the reward comparison of orosensory sucrose and corn oil, respectively.

**Materials and Methods**
Subjects
The subjects were 72 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 275-300g at the beginning of this study. They were individually housed on a 12:12-h light-dark schedule with ad libitum tap water and standard laboratory diet [Rodent diet (W) 2018; Harlan Teklad, Madison, WI]. Once the experiment began, the rats were maintained on a food deprivation regime with distilled water available at all times except when the rats were in the chamber. Food was weighed and provided at least one hour after the daily session.

Surgery
The rats were divided into PBN lesions (PBNx, n=28), PBN surgical controls (n=6), TOA lesions (TOAx, n=20), TOA surgical controls (n=6), and naïve controls (n=12). All surgeries were conducted as described in chapter 3. After surgeries, 5 PBNx, 1 TOAx, and 2 control rats died during recovery. In all, rats had at least one month to recover before the experimental procedure began.

Apparatus
The apparatus used were the same as in Experiment 1.

Procedure
Due to the limited number of operant chambers, the rats were run in two squads. The first iteration included 27 rats, 15 PBNx and 12 controls; the second iteration included 37 rats, 19 TOAx, 8 PBNx and 10 controls. The rats in each surgical group were divided into an L-L and L-H training for ACE. The rats were run in batches of 6, 3 in L-L chambers, and 3 in L-H chambers. Before each rat was placed in a chamber, its stomach was flushed with lukewarm water as described in Chapter 4. The anticipatory contrast paradigm included a daily trial of 3-min each to B1 and B2 solutions for 14 days. Food was removed from the home cage the day before the habituation trial. Since then, normal pelleted chow was weighed and given to the rats in the home cage at least one hour after they finished their daily trial. Body weight was maintained at 90% of their free feeding weight for sucrose ACE, and 95% of their free feeding weight for corn oil ACE. The 5-min habituation occurred the day before the actual ACE training began, rats were placed in the chamber with
the house light and white noise on. The rats were first trained for 14 days of sucrose ACE, using 0.06M followed by a second 0.06M sucrose as the L-L and 0.06M followed by 1.0M sucrose as the L-H condition. After a week long break with free feeding, the L-L and L-H groups were reversed and trained for 14 more days of corn oil ACE, using 1.5% followed by a second 1.5% corn oil emulsion as the L-L and 1.5% followed by 25% corn oil emulsion as the L-H condition. The sucrose solutions were made with distilled water and the corn oil emulsions were blended with distilled water and Tween-80 [100 ml corn oil-water mixture blended with 0.75 ml Tween-80 (Sigma-Aldrich, St. Louis, MO)]. All the procedures in this experiment were approved by the Institutional Animal Care and Use Committee of the Pennsylvania State University College of Medicine, and comply with the American Physiological Society’s “Guiding Principles for Research Involving Animals and Human Beings.”

Histology
At the end of the experiment, the rats were sacrificed with an overdose of pentobarbital sodium (150 mg/kg ip), then perfused transcardially with 0.9% saline solution followed by 4% buffered paraformaldehyde at 4°C. The brains were removed to paraformaldehyde and a few hours later cryoprotected in 30% sucrose in paraformaldehyde overnight, also at 4°C. Then they were blocked, and frozen sectioned coronally (50 µm) in three series. One series is mounted and stained with the cresyl violet to verify the lesion areas. Another series was processed for immunohistochemical staining of NeuN, a neuron specific protein, using standard procedures, i.e. ABC-Elite, Vector Labs. The lesions are charted onto standardized sections taken from normal animals.

Statistical analysis
Only rats that licked consistently licking in each session were included in the data analysis, i.e. 12 PBNx, 18 TOAs, and 20 controls for sucrose ACE; 15 PBNx, 12 TOAx and 16 controls for corn oil ACE. There were no significant differences between naïve and surgical controls so their data were combined. The data included daily 3-min sham licks of B1 and B2 and the latency to begin licking each bottle. The lick and latency data were averaged into
2-day blocks and were analyzed by repeated-measures ANOVAs varying lesions, solutions, and blocks. Post hoc Newman-Keuls tests were conducted where appropriate.

Results
Lesion size
Twenty-three PBNx rats were trained for the ACE experiments, data from 8 of them were excluded from the statistical analysis. These 8 rats did not perform consistently, and their licking were on and off across days. They had substantial large lesions extended beyond the gustatory PBN rostral-caudally. Besides the gustatory PBN, their lesions also included the supratrigeminal nucleus, the locus coeruleus, and in some rats, the motor trigeminal nucleus. Therefore, it appeared that the large lesions prevented the rats from licking consistently. The 15 PBNx rats included in the data analysis had lesions centered in the gustatory PBN (Fig. 5.5B). The lesions included both medial and lateral PBN and, in some cases, extended to the lateral part of mesencephalic trigeminal nucleus and the supratrigeminal area. The lesions of the TOA also were quite substantial. In 6 rats, the lesions extended across the midline. These lesions damaged the entire orosensory area of the thalamus (Fig. 5.5D). In the other 13 rats, the lesions included the trigeminal nucleus, ventral posteromedial nucleus (VPM), nucleus, ventral posteromedial nucleus (VPM), and the taste areas, the parvicellular part of VPM (VPMpc). As the lesions extended to the fasciculous retroflexus, the midline was spared. At this level, the lesions included not only the trigeminal and taste areas, but also the parafascicular thalamic nucleus and the rostral end of the posterior thalamic nuclear group.

Sucrose ACE
Control. The control rats showed a contrast effect in B1 licks, and the L-H group initiated licking at B2 sooner than the L-L group. Consistent with the previous sham feeding study, the control rats developed an ACE while using 0.06M sucrose as low and 1.0M sucrose as high concentration in the paradigm (Fig. 5.6A). The average licks of B1 0.06M increased across blocks for the L-L group, and became significantly more than the L-H group from block 4 to 7 [group, F(1, 18)=9.53, p<0.007; block, F(6, 108)=15.14, p<0.0001, interaction, F(6, 108)=6.75, p<0.0001]. The results of B2 licks in the L-L and L-H condition are shown
Fig. 5.5. Photomicrograph of coronal sections stained with NeuN. (A) surgical control for PBN (B) PBN lesions (C) surgical control for TOA (D) TOA lesions. The images for the PBN are presented at a 4× while for the TOA are presented at 2× magnitudes. The line bar in (A) or (C) presents 1mm. (BC: brachium; CL: central lateral nucleus; CM: central medial nucleus; MD: medial dorsal nucleus; Me5: mesencephalic trigeminal nucleus; VPM: ventral posteromedial nucleus; VPMpc: the parvicellular part of VPM)

in Fig. 5.6B. When comparing the average licks of B1 and B2, the L-L group increased licking across trials but the number of licks did not differ between bottles [block, F(6, 108)=26.73, p<0.0001; bottle, F(1, 18)=1.43, p=0.25]. The L-H group licked more B2 than
B1, and the differences were significant from block 2 to 7 [bottle, F(1, 18)=100.58; block, F(6, 108)=32.04; bottle × block interaction, F(6, 108)=20.55; all p<0.0001]. Moreover, the latency to the first lick of B2 was significantly shorter in the L-H group than the L-L group [F(1, 18)=7.65, p<0.02; Fig. 5.9, control] while the latency to first lick of B1 did not differ between groups (F<1, p=0.89). The lick and latency data in this experiment was consistent with those shown above (Experiment 1A).

Fig. 5.6 Control. The anticipatory contrast effect for 0.06M vs. 1.0M sucrose comparison. (A) An ACE for sucrose was shown in bottle 1 licks. (B) Comparisons of bottle 2 licks between the L-L (n=10) and L-H groups (n=10). Mean (± SEM) number of 3 min licks (sham intake) made for the bottle 1 (B1: 0.06M sucrose) or bottle 2 (B2: 0.06M or 1.0 M sucrose) over 7 2-day blocks for rats in the L-L or L-H condition.

Parabrachial nucleus lesions. In contrast to the control groups, the PBNx rats did not show any contrast effect when comparing either the average B1 licks or latency to first lick of B1. The comparisons of B1 licks between L-L and L-H showed that the PBNx rats performed an
Fig. 5.7. **PBNx.** Anticipatory contrast effect for 0.06M and 1.0M sucrose comparisons. (A) The PBNx rats failed to show an ACE for sucrose in bottle 1 licks. (B) Comparisons of bottle 2 licks between the L-L (n=7) and L-H (n=5) groups. Mean (± SEM) number of 3 min licks (sham intake) made for the bottle 1 (B1: 0.06M sucrose) or bottle 2 (B2: 0.06M or 1.0 M sucrose) over 7 2-day blocks for rats in the L-L or L-H condition.

induction instead of suppression effect, particularly in block 6 and 7 (Fig. 5.7A). Two-way ANOVA with group and block showed a block effect [F(6, 60)=18.28, p<0.001] but no group × block interaction. The group effect was marginal but not significant [F(1, 10)=3.82, p=0.08]. The L-H group licked significantly more than the L-L group at block 6 (post hoc, p<0.03), and close to significantly more at block 7 (post hoc, p=0.06). In both L-L and L-H groups, the licks to both bottles increased across trials [L-L vs. L-H: F(6, 72)=13.68 vs. F(6, 48)=15.67, both p<0.0001] but the average licks did not differ between bottles (Fig. 5.7A and B). Although the L-H group licked more at the higher concentration B2 1.0M than the
B1 0.06M, the difference was not significant [F(1, 8)=1.99, p=0.2]. When comparing licks of the high concentration B2 to the control and TOAx groups, PBNx rats consumed significantly less than did the others [F(2, 22)=12.45, p<0.0003]. Consistent with the licking data, both PBNx groups did not show any effect on the latency to lick B1 (data not shown) or B2 (Fig. 5.9, PBNx). As trials went on, they approached B1 sooner in both conditions, and the L-H group also approached B2 sooner across trials though never significantly faster than the L-L group (F<1, p=0.87). These data demonstrate that the PBNx rats were unable to form an ACE for sucrose when using either number of licks or the latency to lick to identify the effect.

Thalamic orosenaory area lesions. The licking patterns and latency data of TOAx rats were essentially identical to the control rats. As shown in Fig. 5.8A, the B1 licks were significantly higher in the L-L than in the L-H group from block 5 to 7 [group, F(1, 16)=4.04, p=0.06; block, F(6, 96)=10.60, p<0.0001; group × block interaction, F(6, 96)=8.59, p<0.0001; post hoc, p<0.02]. Thus, the TOAx rats showed an ACE for sucrose as the controls did. Unlike the control and PBNx groups, comparison of B1 and B2 licks (Fig. 5.8A and B) in the L-L group showed that their licks increased across blocks [F(6, 84)=11.76, p<0.0001]. There was an interaction between bottle and block [F(6, 84)=3.48, p<0.005] and the rats licked more on B1 than on B2 at block 5 and 7 (post hoc, p<0.04). For the L-H group, the licks of B2 were significantly higher than of B1 from block 3 to 7 [bottle, F(1, 18)=218.84; block, F(6, 108)=38.08; interaction, F(6, 108)=27.09, p<0.0002 in all cases]. Furthermore, compared with the L-L group, the L-H group suppressed their B1 licks and started to lick B2 sooner. The latency to lick B2 was also consistent with the control groups. The L-H group started to lick B2 significantly sooner than the L-L at the first block, but the latency reduced greatly at block 2 and stayed shorter than the L-L group (Fig. 5.9, TOAx). The ANOVA revealed a main effect of group [F(1, 16)=14.9, p<0.002], block [F(6, 96)=7.1, p<0.0001] and interaction [F(6, 96)=4.13, p<0.001]. In both groups, the latency to lick B1 became sooner as trials went on (data not shown). The L-H group again began to approach the B1 slower than the L-L group at the first two blocks, but eventually there was no difference between groups.
Fig. 5.8. **TOAx.** Anticipatory contrast effect for 0.06M and 1.0M sucrose comparisons. (A) The TOAx rats showed an ACE for sucrose in bottle 1 licks. (B) Comparisons of bottle 2 licks between the L-L (n=8) and L-H (n=10) groups. Mean (± SEM) number of 3 min licks (sham intake) made for the bottle 1 (B1: 0.06M sucrose) or bottle 2 (B2: 0.06M or 1.0 M sucrose) over 7 2-day blocks for rats in the L-L or L-H condition.

In terms of B1 licks across control and lesion groups, comparisons of the L-L groups showed an effect of block [F(6, 132)=29.44, p<0.0001] and lesion × block interaction [F(12, 132)=1.94, p<0.04] without an effect of lesion. The control and TOAx group did not differ from each other, but their B1 licks were both significantly higher than the PBNx rats at the last two blocks (post hoc, p<0.02). Comparisons of the L-H groups showed an effect of lesion [F(2, 22)=6.62, p<0.006], block [F(6, 132)=10.62, p<0.0001], and lesion × block interaction [F(12, 132)=2.81, p<0.002]. The B1 licks of the L-H condition for the control and TOAx were not significantly different. The B1 licks of the L-H condition for the PBNx,
however, were significantly higher than those for the control in the last 4 blocks (post hoc, p<0.02) and those for the TOAx rats in the last 3 blocks (post hoc, p<0.03). Thus, lesions of the PBN but not TOA prevent the ACE for sucrose.

![Graph showing latency to first lick for B2 for the control, PBNx, and TOAx conditions over 7 2-day blocks for rats in the L-L (0.06M sucrose) and L-H (1.0M sucrose) groups.](image)

**Fig. 5.9.** Sucrose. Latency to the first lick on B2 for the control, PBNx, and TOAx. Mean (± SEM) second of latency to lick for the B2 over 7 2-day blocks for rats in the L-L (0.06M sucrose) and L-H (1.0M sucrose) groups. Except for the PBNx groups, the L-H group approached B2 sooner than the L-L group.

The latency to B1 of the L-L condition for the control and lesion groups was not different from each other, and neither was that of the L-H condition. In contrast, the L-H group of the PBNx had longer latency to B2 than of the control and the TOAx [lesion, F(2, 22)=4.99, p<0.02; block, F(6, 132)=28.98, p<0.0001; lesion × block interaction, F<1, p=0.82 ]. When comparing the latency to B2 of the L-L groups for the control and lesions, the latency became shorter across blocks in all groups [block, F(6, 132)=2.84, p<0.02], but there were no significant differences between each other [lesion, F<1, p=0.87].
Corn oil ACE

Control. The control rats developed an ACE while using 1.5% as low and 25% corn oil as high concentration (Fig. 5.10A). Two-way ANOVA comparing average licks on B1 showed an effect of block [F(6, 84)=3.86, p<0.002] and an interaction [F(6, 84)=9.75, p<0.0001]. The B1 licks increased across blocks for the L-L group, and became significantly more than the L-H group from block 5 to 7 (post hoc, p<0.03). It should be noticed that the B1 licks at block 1 were significantly higher in the L-H than in the L-L group (post hoc, p<0.006). This may be due to the fact that rats in the two groups had different experience. The L-L group was the L-H group in the sucrose ACE experiment, and their licking to B1 was suppressed. Conversely, the L-H group did not suppress their B1 lick during the sucrose ACE experiment, and so their average licks in block 1 were significantly higher than the L-L group. A second phenomenon was that 4 of the 10 rats in the L-L group did not lick consistently through out the 14 days period. Most of the days, they made less than 20 licks on B1 and B2 and occasionally above 100 licks. These data was excluded from the statistic analysis so the subject number for the L-L group decreased from 10 to 6. This phenomenon also occurred in the TOAx rats (see below). It appears that the phenomenon can be observed when the stimuli were switched from sucrose to corn oil, and particularly in the group that switched from the L-H condition (the group that suppresses B1 intake after ACE training) to the L-L condition.

When comparing the B1 and B2 licks (Fig. 5.10 A & B), the average licks of both bottles increased [block, F(6, 60)=5.1, p<0.0003] but did not differ from each other across trials for the L-L group. In contrast, the L-H group licked more B2 than B1 from block 2 to 7. There was an effect of bottle [F(1, 18)=61.62], block [F(6, 108)=5.93] and a bottle × block interaction [F(6, 108)=18.37] with p<0.0001. Unlike the sucrose experiment, there was no significant effect of latency to first lick of either B1 (data not shown) or B2 (Fig.5.13, control).

Parabrachial nucleus lesions. The PBNx rats again did not show an ACE for oil when comparing the average B1 licks of the L-L and L-H groups (Fig. 5.11A). Two-way ANOVA showed a block effect, F(6, 78)=3.08, p<0.01, indicating that the licks increased over the first few blocks. The within-group comparisons also showed an block effect in both L-L and
Fig. 5.10. **Control.** Anticipatory contrast effect for 1.5% and 25% corn oil comparisons. (A) The control rats showed an ACE for corn oil in bottle 1 licks. (B) Comparisons of bottle 2 licks between the L-L (n=6) and L-H (n=10) groups. Mean (± SEM) number of 3 min licks (sham intake) made for the B1 (1.5% corn oil) or B2 (1.5% or 25% corn oil) over 7 2-day blocks for rats in the L-L or L-H condition. (Note the symbols for the L-L and L-H groups have reversed from the sucrose figures because the symbols follow the rats.)

L-H groups, the licks to both bottles increased across trials [L-L, F(6, 84)=3.81, p<0.003; L-H, F(6, 72)=3.40, p<0.006; Fig 5.11 A and B] but the average licks did not differ between bottles. These results indicated that the PBNx rats failed to show a contrast effect in B1 licks, and that the L-H group consumed equivalent amounts of 1.5% and 2.5% corn oil emulsion. In fact, the licks of 25% oil for the PBNx were significantly lower than for the control and TOAx (post hoc, p<0.003). This was further demonstrated by between and within group comparisons. For the L-H group, the latency to B2 was not only slower than to
B1 \[F(1, 12)=19.30, p<0.0009\], but also slower than the latency to B2 for the L-L group \[F(1, 13)=10.11, p<0.008; \text{Fig. 5.13}, \text{PBNx}\]. There was no significant effect of latency to B1 when comparing the data for the L-L and L-H groups.

Fig. 5.11. **PBNx.** Anticipatory contrast effect for 1.5% and 25% corn oil comparisons. (A) The PBNx rats failed to show an ACE for corn oil in bottle 1 licks. (B) Comparisons of bottle 2 licks between the L-L (n=8) and L-H (n=7) groups. Mean (± SEM) number of 3 min licks (sham intake) made for the B1 (1.5% corn oil) or B2 (1.5% or 25% corn oil) over 7 2-day blocks for rats in the L-L or L-H condition. (Note the symbols for the L-L and L-H groups have reversed from the sucrose figures because the symbols follow the rats.)

*Thalamic orosensory lesions.* The TOAx rats showed similar effects during the corn oil comparisons as during the sucrose comparisons. Licking of B1 1.5% corn oil was significantly higher in the L-L than the L-H group from block 3 to 7 (post hoc, p<0.05; Fig. 5.12 A). Although the main effect of group was not significant \[F(1, 10)=4.92, p=0.051 \],
Fig. 5.12. TOAx. Anticipatory contrast effect for 1.5% and 25% corn oil comparisons. (A) The TOAx rats showed an ACE for corn oil in bottle 1 licks. (B) Comparisons of bottle 2 licks between the L-L (n=5) and L-H (n=7) groups. Mean (± SEM) number of 3 min licks (sham intake) made for the B1 (1.5% corn oil) or B2 (1.5% or 25% corn oil) over 7 2-day blocks for rats in the L-L or L-H condition. (Note the symbols for the L-L and L-H groups have reversed from the sucrose figures because the symbols follow the rats.)

The ANOVA showed an effect of block, F(6, 60)=4.27, p<0.002, and a group × block interaction, F(6, 60)=9.79, p<0.0001. Rats in the L-L and L-H groups were switched from the prior sucrose trials, but their licks in block 1 were not significantly different. This was different from the controls, which showed more licks for the L-H than for the L-L group in block 1. The control and the TOAx rats were similar in that some of the rats in the L-L group failed to licked consistently so the number of subjects included for data analysis in the corn oil experiment was less than in the sucrose experiments. The data for B1 licks...
demonstrated that the TOAx rats formed an ACE for corn oil. These rats also responded to the switching of stimuli (e.g. from sucrose to corn oil) and experimental condition (e.g. from L-L to L-H and vice versa) similarly with the control rats.

When comparing the licks of B1 1.5% oil and B2 25% oil within group (Fig. 5.12 A and B), the L-L group increased licking to both bottles across blocks \([F(6, 48)=6.48, p<0.0001]\). The number of licks on the two bottles, however, was not significantly different \((F<1, p=0.74)\). The L-H group increased licks across trials, and the licks of B2 were significantly higher than on B1 from block 2 to 7 \([\text{bottle, } F(1, 12)=13.41, p<0.004; \text{block, } F(6, 72)=5.34, p<0.0002; \text{interaction, } F(6, 72)=11.23, p<0.0001; \text{post hoc, } p<0.0002]\). The licks of 25% oil for the TOAx were significantly less than for the controls, but more than for the PBNx \([\text{lesion effect, } F(2, 21)=12.41, p<0.0003]\). Unlike the control groups, which had no significant latency effect, the L-H group of the TOAx rats approached B2 more slowly than the L-L group in the first block \((\text{post hoc, } p<0.02, \text{Fig. 5.13, TOAx})\). There was a group \(\times\) block interaction \([F(6, 60)=2.58, p<0.03]\) without significant effects for either group \([F(1, 10)=2.95, p=0.12]\) or block \([F(6, 60)=1.81, p=0.11]\).

The ANOVA comparisons of the B1 licks for the L-L groups across control and lesion groups showed an effect of block \([F(6, 96)=12.71, p<0.0001]\) and a lesion \(\times\) block interaction \([F(12, 96)=2.73, p<0.004]\) without an effect of lesion \([F<1, p=0.43]\). The number of B1 licks for the control and TOAx groups did not differ from each other. The B1 licks for the control in the 6th block \((\text{post hoc, } p<0.05)\) and for the TOAx in 7th block \((\text{post hoc, } p<0.002)\) were significantly higher than those for the PBNx rats. Comparisons of the B1 licks for the L-H groups showed an effect of lesion \([F(2, 21)=4.08, p<0.04]\) and a lesion \(\times\) block interaction \([F(12, 126)=3.1, p<0.0008]\) without an effect of block \([F(6, 126)=1.52, P=0.18]\). The B1 licks of the L-H condition for the controls were significantly higher than those for the TOAx rats in blocks 1, 2, 5 and 7 \((\text{post hoc, } p<0.03)\), but were significantly higher than those for the PBNx rats only in block 1 \((\text{post hoc, } p<0.0001)\). The B1 licks of the L-H condition for the PBNx and the TOAx were not significantly different except that those for the PBNx were significantly higher in block 5 than for the TOAx \((\text{post hoc, } p<0.02)\). These data indicate that the suppression of the low corn oil concentration (B1) in the L-H condition was more in the TOAx rats than in the controls. Furthermore, although the L-H
group of the PBNx rats failed to suppress their B1 licks, they did not make more licks on B1 than did the L-H groups of the control and the TOAx rats.

Fig. 5.13. Corn oil. Latency to first lick of B2 for the control, PBNx, and TOAx. Mean (± SEM) second of latency to lick for the B2 over 7 2-day blocks for rats in the L-L (1.5% corn oil) and L-H (25% corn oil) groups. Except for the PBNx groups, there was no difference in approaching B2 between the L-L and L-H groups. (Note the symbols for the L-L and L-H groups have reversed from the sucrose figures because the symbols follow the rats.)

The latency to B1 of the L-L condition among the control and lesion groups was not different from each other, and neither was that of the L-H condition. The ANOVA analysis of latency to B2 for the L-H groups showed significance in lesion [F(2, 21)=21.59, p<0.0001], block [F(6, 126)=3.13, p<0.007] and the lesion × block interaction [F(12, 126)=2.19, p<0.02]. The L-H group of the PBNx had longer latency to B2 than of the control in block 1 to 3, 6 and 7 (post hoc, p<0.03) as well as longer than that of the TOAx in block 6 and 7 (post hoc, p<0.0001). In contrast to the L-H groups, there were no significant
differences between the control and lesions when comparing the latency to B2 for the L-L groups.

Overall, the results showed that the PBNx rats failed to develop an ACE for either sucrose or corn oil. The TOAx rats, in contrast, formed ACEs for both sucrose and corn oil as the control rats did.

Discussion
This study demonstrated that lesions of the PBN disrupted the development of an ACE for not only sucrose but also corn oil; lesions of the TOA spared an ACE for either sucrose or corn oil. The results support our hypothesis that the gustatory PBN is required for sucrose reward comparison, but reject that the PBN is not necessary for corn oil reward comparison. The results also failed to support that the TOA is required for corn oil reward comparison. While the results did not support our hypothesis, they provided further information for understanding the role of the PBN and the TOA in reward comparisons of orosensory stimuli. First, although the PBNx prevented the development of ACE for both sucrose and corn oil, the effects did differ. When the CS solution was 0.06M sucrose, the PBNx rats showed an induction rather than a suppression effect on the intake of B1 0.06M sucrose in the L-H condition. In contrast, the PBNx rats showed neither an induction effect nor a suppression effect when the CS was 1.5% corn oil. Second, the result that lesions of the TOA displayed an ACE for sucrose is inconsistent with previous result that lesions centered in the taste thalamus disrupted an ACE for comparing saccharin and sucrose (Reilly and Pritchard, 1996; Reilly et al., 2004; Schroy et al., 2005). The following discussion will focus on the two findings.

A successful development of an ACE requires the rats perform appropriately for several steps. First, the rats must be able to detect and respond to the CS and US. Then they have to associate the CS cue with the following US. Third, they remember and compare the hedonic value of the CS cue and the US. Finally, they reduce the intake of the CS cue as a result of the reward comparison process (Schroy et al., 2005). Although the taste detectability is decreased (Spector et al., 1995), lesions of the PBN allow concentration dependent response
of different taste stimuli (Spector et al., 1993). In this study, the PBNx rats made fewer licks of the 0.06M sucrose CS and 1.0M sucrose US than the control and TOAx rats. This phenomenon was probably not due to the deficit of sensory detection, but to the food restriction regime. In several studies, it has been shown that the response curves of taste stimuli in PBNx rats varies depending on the deprivation state. When comparing with intact animals, the concentration dependent intake among PBNx animals increases slightly under water deprivation and decreases under food restriction (Spector et al., 1993; Grigson et al., 1994; Sclafani et al., 2001). That said, it should be noted that the PBNx rats in this study were able to show an induction effect for sucrose comparisons. The fact that the PBNx rats showed an induction effect has several implications. First, the PBNx rats were able to detect and respond to the CS and US, and the reduced consummatory expressed in the PBN rats was not a general motor or motivational deficit. Second, the PBNx rats in this study were able to associate the weak sucrose CS with the strong sucrose US because they showed over-responding instead of a suppression effect. If the rats could not remember or associate the CS with the US, they would have showed neither effect. Therefore, that the PBNx rats showed an induction effect indicates two possible deficits. One is that the PBNx rats were unable to modify their behaviors based on experience e.g. to reduce the CS intake. The other is that they could not make appropriate comparison of two rewards over time. Before more discussion about the two issues, the data for corn oil comparisons need to be examined and decide whether the two sets of data from sucrose and corn oil contrast experiments lead to the same specific issues.

In contrast to the results of sucrose comparisons, the PBNx rats failed to show either an induction or a contrast effect for corn oil comparison. During sucrose comparisons, the PBNx rats over-responded for the B1 0.06M sucrose in the L-H condition but not in the L-L condition. They respond equivalently for the B1 1.5% oil in the L-H and the L-L condition during corn oil comparisons. Moreover, the number of licks for 1.5% oil did not reach more than 250 licks whereas the same PBNx rats could make 360 lick at highest during sucrose comparisons. According to previous experiments with sweet tastes, the development of an ACE depends on the reward value of the CS cue (Flaherty et al., 1994) as well as the reward disparity between the CS and the US (Flaherty et al., 1991). The greater the hedonic value of
the CS and the greater the reward disparity between the CS and US, the sooner and greater magnitude of the contrast develops. The corn oil licking data of the PBNx rats indicate that the 1.5% corn oil might not have a strong enough hedonic value to drive behavior because the same rats were able to lick more 0.06M sucrose than 1.5% corn oil. Although both control and TOAx rats in the L-L condition licked more for the 1.5% corn oil than did the PBNx rats, it should be reminded that data from several animals in the control and lesion groups were withdrew because of failure to perform during corn oil comparisons. Thus, it is possible that the 1.5% corn oil was not a strong enough oral stimulus to drive licking behavior, particularly when the PBNx rats’ orosensory detectability was probably compromised.

The latency data further indicated that the 1.5% corn oil as the CS cue did not support an CS-US association for the PBNx rats. For the sucrose comparisons, the licking data showed that the PBNx rats were able to form a CS-US association. The fact was supported for their latency to B1 became shorter across blocks and did not differ from the control and TOAs rats. Although different from the intact control and the TOAx rats, the PBNx rats decreased their latency to start licking the US across blocks (Fig. 5.9). In the corn oil comparisons, however, the PBNx rats had a longer latency to lick B2. In particular, the latency to lick 25% corn oil US was longer and did not decrease across blocks compared with the control and the TOAx rats (Fig. 5.13). Thus, the latency data indicates that the PBNx rats could not incorporate the CS-US contingency in corn oil comparisons. It is not likely the deficit was a result of lesions in the PBN for the same animals could make CS-US association for sucrose comparisons. Nevertheless, the deficit was shown specifically in the PBNx rats, and it is still possible that the same CS cue was strong enough to support behaviors for the intact and TOAx rats but not for the PBNx rats. That said, the effects of the CS intensity on the conditioning learning for the PBN damaged rats have been shown in other conditioning tasks. When the CS cue is more obvious or more complex, the PBNx rats are able to show conditioned behaviors. The PBNx rats are able to learn a flavor-taste association and perform a conditioned flavor preference (CFP). In this task, the PBNx rats performed as well as the control rats when the CS contains a higher concentration (0.1875%) of Kool-Aid (Reilly et al., 1993; Grigson et al., 1998). The CFP was attenuated in the PBNx rats when
the concentration of the Kool-Aid was reduced to 0.05% (Sclafani et al., 2001). Overall, the data from the corn oil contrast experiments suggest that the concentration used was not ideal for producing an ACE for this stimulus. Furthermore, the suppression of a CS from one contrast experiment seems to affect the initial intake of another CS that has different orosensory properties. For future studies, it is worthwhile to compare the hedonic values of the sucrose and corn oil CS e.g. with two-bottle test as well as to conduct the contrast experiments in a reverse order. Indeed, the laboratory is currently conducting a study that uses higher concentration corn oil CS and the order of the contrast experiments will be in reverse order to this present study i.e. corn oil comparisons precede sucrose comparisons.

The data suggest that the possible deficits as a result of PBN lesions in ACE are whether the PBNx rats can make appropriate reward comparisons and modify their behaviors according to the chronic reward comparison experience. The L-H group of PBNx rats appeared to be able to modify their behavior as their licking of the 0.06M sucrose CS increased across blocks and became significantly more than the L-L group. In this occasion, rats with PBN lesions were able to modify their behavior based on experience, but the modification was in an opposite direction to intact rats. The question, then, is whether the PBNx rats can reduce their intake as a function of experience. From the literature, a number of consummatory behaviors in response to treatments are disrupted while some remain intact after the gustatory PBN is damaged. For example, in a standard conditioned aversion paradigm, PBNx rats failed to reduce a taste CS intake after it is paired with the injection of LiCl, which induces visceral illness (Flynn et al., 1991; Reilly et al., 1993; Reilly, 1999). They failed to reduce intake (neophobia) for a novel tastant (Reilly et al., 1993), but cannot increase intake of an otherwise unpalatable sodium chloride solution after salt depletion (Flynn et al., 1991). On the other hand, rats with PBN lesions were able to (a) increase intake of lab chow after an injection of insulin or 2-D-deoxy glucose (Flynn et al., 1991), (b) increase intake of a Kool-Aid solutions that have been paired with a palatable taste in a CFP task (Reilly et al., 1993), (c) decrease capsaicin intake after it has been paired with LiCl injections (Grigson et al., 1998), (d) avoid a CS if the CTA learning was acquired before the PBN damage (Grigson et al., 1997), (e) reduce the intake of LiCl when the LiCl is presented as a CS and US at the same time (Di Lorenzo, 1988). The summaries of the results above
indicate that the PBNx rats can increase or decrease their CS based on learning. Therefore, unable to reduce CS intake was probably not the deficit that result in an induction instead of suppression effect of PBNx rats. The puzzle then is under which condition can the PBNx rats use the CS cue to adjust their ingestive behaviors appropriately? Lesions of the PBN do not disrupt conditioning learning when the CS is not a taste e.g. capsaicin or a more complex stimulus e.g. Kool-Aid flavor. The implication from these facts is that the PBNx rats can perform a conditioning response normally when there is considerably more CS-US contiguity for the association to be formed e.g. CFP or LiCl as CS and US at the same time.

Moreover, it seems that rats with PBN lesions fail to make appropriate modification of behaviors as a function of experience when the adjustment of consumption involves adding or subtracting the reward value of a taste stimulus. As mentioned above, PBNx rats failed to switch the hedonic value of a taste from palatable to aversive in a CTA task. They could not add reward value to a previously non-preferred sodium chloride solution after salt depletion. Further, they failed to demonstrate contrast effect in another reward comparison, the successive negative contrast (SNC) paradigm. In this paradigm, a weak or strongly preferred sucrose solution is presented to two groups of animals over days. On the day that the strongly preferred sucrose is replaced with the weak solution, the animals consume significantly less of the weak sucrose than animals that experienced only the same weak solution. Rats with PBN lesions failed to show such a significant decrease of intake when the reward value was shifted from highly to less preferred (Grigson et al., 1994). Accordingly, it is possible that the impairment of the PBNx rat in showing an ACE for orosensory reward is due to the interference with a reward comparison process. Further studies are required to determine whether the interference is with an intermediate or chronic reward comparisons. That is, it will be worthwhile to determine whether the PBNx rats can show preference in a brief two-bottle test; or whether the PBNx rats can show a simultaneous contrast effect when two levels of reward solutions are briefly (within 60s) and alternatively presented for three times within a daily session (Flaherty and Rowan, 1986). Finally, previous studies have shown that damage of the gustatory cortex or the amygdala prevents reward contrasts (Becker et al., 1984; Gilbert and Kesner, 2002; Kesner and
Gilbert, 2007). It will be also of interest to investigate whether the interactions between the PBN and some forebrain structures are required for the reward comparison process.

The observation that the PBNx rats in this study were able to form a taste based CS-US association is inconsistent with previous findings. In the sucrose comparisons, the PBNx rats in the L-H condition increased instead of suppressed their B1 licks. They also began to lick the B2 high concentration sucrose sooner over trials. These results suggest that the PBNx rats can use the CS cue to predict the availability of the US. Substantial evidence from CTA have shown that lesions of the PBN disrupt the CS-US association specifically when the CS is a taste stimulus (Reilly et al., 1993; Grigson et al., 1998; Reilly, 1999), and so the PBNx rats are able to learn a conditioned aversion to a trigeminal stimulus, capsaicin (Grigson et al., 1998). The CS and US in the sucrose anticipatory contrast experiment were both taste stimuli, and a CS-US association was formed though not result in a contrast effect. To interpret this discrepancy, it is necessary to compare the procedure differences. The US in the CTA paradigm produces aversive effects whereas the US in the anticipatory contrast paradigm produces rewarding effects. That is, the association is taste-illness in the CTA, but is taste-taste (hedonic-hedonic) in the ACE. The interstimulus interval in a standard CTA task is from several minutes to half an hour while in this study the interstimulus interval was zero. Moreover, how the rats receive the CS and US is different in the two paradigms. The CS is approached and ingested and the US is passively given to the rat in a standard CTA paradigm whereas the CS and US are both actively consumed by the rat. These procedural differences point to a fact- there is considerably more CS-US contiguity in the anticipatory contrast than in the CTA task. Every aspect of the procedure including the type of stimulus, the effects produced by the stimulus and the way the stimulus is consumed in the anticipatory contrast is continuous for the rats. Thus, it is possible that the more CS-US contiguity in a task, the more likely that the PBNx rats are able to form a CS-US association. Supporting evidence comes from the data that PBNx rats were able to avoid 0.12M LiCl as it was offered as a CS and US at the same time and the PBNx rats actively drank it (Di Lorenzo, 1988). To further support this possibility, it is worthwhile to investigate whether rats with PBN lesions will be able to avoid a taste CS that has been added in the LiCl drinking solution. Moreover, it will also be interesting to determine whether increasing the
CS-US contiguity will not only support the CS-US association but also facilitate forming an ACE for the PBNx rats. In other words, it is of interest to study whether presenting the CS and US from the same licking location will facilitate or reverse the induction effect produced by the PBNx rats in the present study.

While showing an ACE for corn oil, the TOAx rats displayed a different degree of contrast effect from the control rats. Since the corn oil contrast experiment was conducted after the animals had completed the sucrose contrast experiment, the suppression effect on the sucrose CS could affect the intake of the 1.5% corn oil CS. Such a carry over effect was most obvious in the control groups in which the suppression effect on 0.06M sucrose appeared in the first block of 1.5% corn oil intake. In Fig. 5.10A, the licks of the 1.5% were significantly less for the L-L group, previously L-H group of the sucrose comparisons, than for the L-H group, previous L-L group of the sucrose comparisons. That is, the developed contrast effect from the sucrose comparisons appeared in the first block of the corn oil comparisons because the B1 1.5% oil licks for the L-L and L-H groups were significantly different. Further, the difference was according to their group assignment in the sucrose comparisons. As the training went on, the licks of the 1.5% corn oil for the two groups went in different directions, and eventually showed a contrast effect for corn oil comparisons. The robust carry over effect has been shown repeatedly in our laboratory when the stimuli for the anticipatory contrast experiments were switched from sucrose to corn oil in particular. Interestingly, this effect did not appear when the CS stimulus was switched from one concentration of corn oil to another (Liang et al., 2008b). As such, it seems the carry over suppression effect occurs when the CS is switched between stimuli that have different sensory properties. Carry over effects did not occur in the TOAx animals as no difference was shown in the first block licks. The L-L group of TOAx rats made the same number of B1 licks as did the L-L group of the controls. The L-H group of the TOAx rats, however, suppressed the B1 licks more than did the L-H group of the controls. These results suggest that lesions of TOA spared the ACE for sucrose but altered the degree of the contrast effect for corn oil.
Inconsistent with previous studies, lesions of the thalamic orosensory area spared the development of ACE. It has been repeatedly shown that chemical (Reilly et al., 2004; Schroy et al., 2005) or electrolytic (Reilly and Pritchard, 1996) lesions centered in the thalamic taste area eliminate an ACE for sucrose. It is intriguing that the thalamic lesions in the present study were larger than those in previous studies, but these larger lesions spared not only an ACE for sucrose, but also one for corn oil. Except for the feeding condition, real feeding in previous studies and sham feeding in this study, there are no serious procedural differences between the previous and present studies. The feeding condition, however, does not seem to alter the effects of lesions on behavior as demonstrated our previous study with operant tasks (Liang et al., 2008a). Since it has also been shown that gustatory thalamic lesions eliminate a similar contrast effect that is induced by drugs of abuse (Grigson et al., 2000), we tested the animals from the second iteration of this experiment with the drug induced contrast paradigm. In this paradigm, animals have 5-min access to a taste CS and then received an i.p. injection of saline or a drugs of abuse such as morphine or cocaine. After several trials, animals suppress their intake of the taste CS cue. This suppression effect has been interpreted as a result of reward comparison between the taste CS and the drug US (Grigson, 1997, 2000; Grigson and Freet, 2000). Previous results suggest that the gustatory thalamus is required in both ACE and drug induced intake suppression, and lesions of the gustatory thalamus disrupt both suppression effects (Grigson et al., 2000; Reilly et al., 2004). Using 0.03M polycose as the CS and 0.9% saline or 15mg/kg morphine as the US, we showed that lesions of the PBN or TOA both eliminated morphine-induced suppression of polycose intake (Fig. 5.14). This data is consistent with previous study (Grigson et al., 2000), and also is intriguing in that the TOA lesions spared ACE, but disrupted drug induced suppression of the CS intake. The result leads us to examine the area of both lesions. The major difference between this TOA damage and prior gustatory thalamic lesions is that the former was centered 500m lateral to the later. Bilateral lesions of the TOA in this study extended from lateral to medial of the VPM, and the lesions crossed the midline in 6 out of 19 brains. The damaged areas included the gustatory responsive zone (VPMpc) and VPM, and the shape of lesions was like a horizontal thin strip (Fig. 5.5D). The damages centered in the thalamic taste area in previous study crossed the midline of the brain, and the shape of lesions was like a rectangular box. Lesions included large portions of the central medial
Fig. 5.14. Mean intake of the 0.03M polycose CS in control, PBNx, and TOAx. In each group, the rats were divided into two conditions. In one condition, the rats received injections of saline, and in the other condition, the rats received injections of morphine. Bilateral lesions of PBN and TOA both disrupted morphine (15mg/kg) induced suppression of the CS intake. Mean (± SEM) 5 min intake over baseline water and 6 trials for rats received saline or morphine injection.

(CM), medial dorsal (MD), and central lateral (CL) thalamic nucleus. By comparison, the TOA lesions in this study included little of the CM, MD and CL thalamic nucleus. Therefore, it is likely that those spared regions in the TOAx rats are enough to supported the function for taste initiated contrast such as ACE and SNC, but not for the drug-induced suppression. It has been shown that lesions centered in the gustatory taste area also impaired the SNC effect (Reilly and Trifunovic, 1999a, 2003). To understand the neural mechanisms occurred in this conundrum, it is necessary to investigate whether lesions of the TOA will interfere with the SNC effect.

The results of this study are more consistent with an important role of the gustatory PBN in processing both oral sucrose and corn oil rewards. Rats with PBN lesions performed an
induction instead of a suppression effect for sucrose comparison, and did not develop an ACE for corn oil comparison. The TOAx rats, on the other hand, developed ACE for both sucrose and corn oil. Those results in combination with previous data from the CTA and operant tasks do not support the hypothesis that the gustatory PBN and the TOA is necessary for the processing of orosensory taste and trigeminal rewards, respectively. Bilateral lesions centered in the oral trigeminal thalamus spare CTA, operant responding, and ACE when the stimuli are either sucrose or corn oil. Except for a 100% corn oil CS, bilateral lesions of the PBN disrupt CTA, operant responding and ACE when the stimuli are sucrose or corn oil emulsions. Accordingly, the PBN is required for transmitting the hedonic value of not only sucrose, but also corn oil. Although the results argue against the hypothesis that the oral trigeminal thalamus is important in processing corn oil reward, a role of the trigeminal system in sensory detection and reward processing of oil cannot be discount. It is possible that other trigeminal relays in the brainstem such as the principal trigeminal sensory nucleus and the spinal trigeminal nucleus are involved in the reward produced by oral oil stimuli. In fact, these brainstem trigeminal relays project heavily to the gustatory PBN (Dallel et al., 2004). As such, it is possible that the PBN transmits oil reward by way of these trigeminal projections. Finally, this is the first study to explore and compare the effects of PBN and TOA lesions on the anticipatory contrast effect for sucrose and corn oil. Substantially more is known about the sensory property, detection, neural coding, and behavior effects for sucrose than for corn oil. The results of this study provide fundamental knowledge for the future studies of oral corn oil as well as of comparing neural mechanisms involved in the reward processing of the two basic macronutrients.
Chapter 6

General discussion

In chapter 2, dopamine (DA) levels in the nucleus accumbens during sham intake of 100% corn oil were measured using high performance liquid chromatography (HPLC). The results demonstrate that DA overflow in the NAc increased when the rats were sham feeding oil emulsions. Combined with previous observation that DA efflux in the NAc increases during sham feeding sucrose solutions (Hajnal et al., 2004), these results suggest that similar forebrain structures i.e. the NAc is involved in both oral sucrose and corn oil rewards. As mentioned in chapter 1, the hypothesis for processing sucrose reward was based on substantial studies from the recent three decades from our laboratory. The system needed for sensory detection of sucrose is the gustatory system (Lundy and Norgren, 2004). Furthermore, the secondary gustatory relay, the parabrachial nucleus (PBN), is suggested to be involved in the reward processing for sucrose (Scalera et al., 1997; Grigson et al., 1998; Hajnal and Norgren, 2005). On the other hand, the sensory pathway for fat and oil detection is not determined. Compared with the published data for sucrose, less is known about the neural coding for fats, and how animals respond to fat or oil in various conditions. It was assumed that sensory coding for fat depends on the trigeminal somatosensory system (Mindell et al., 1990; Waite, 2004). Therefore, the general hypothesis was that sapid sucrose and corn oil rewards are transmitted through different orosensory pathways – the gustatory and intraoral trigeminal pathway, respectively. Chapter 3, 4, and 5 included different behavioral tasks, conditioned taste aversion (CTA), operant tasks, and anticipatory contrast effect (ACE) to test the hypothesis. Specifically, the role of the gustatory PBN and the thalamic orosensory area (TOA) in the three conditioning tasks were tested. It was hypothesized that lesions of the PBN would disrupt behavior when using sucrose but not corn oil as the CS, and the converse effects would occur in lesions of the TOA. A summary of results is shown in Table 6.1.

The results of all three tasks only partially support the hypotheses. An intact TOA is not necessary for all three behavioral tasks. Rats with TOA lesions were able to learn a conditioned aversion to either sucrose or corn oil. They over-responded for sucrose solutions,
Table 6.1. Comparisons of lesions of different central orosensory nuclei on behavioral performance

<table>
<thead>
<tr>
<th>Rewarding effects of sucrose and corn oil are processed through different orosensory pathways.</th>
<th>CTA (conditioned taste aversion, LiCl)</th>
<th>PR (progressive ratio)</th>
<th>ACE (anticipatory contrast effect)</th>
<th>DIS (drug induced CS suppression, morphine)</th>
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<tbody>
<tr>
<td></td>
<td>Sucrose</td>
<td>Oil</td>
<td>Sucrose</td>
<td>Oil</td>
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<tr>
<td><strong>Normal</strong></td>
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<td>Real</td>
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<tr>
<td>Sham</td>
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<td>+</td>
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<tr>
<td><strong>PBN lesions</strong></td>
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<td>Real</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Sham</td>
<td>−</td>
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<tr>
<td><strong>Thalamic Orosensory lesions</strong></td>
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<td>Real</td>
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<td><strong>Thalamic Taste lesions</strong></td>
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This table is made based on data from the literature and studies in this thesis (gray areas). For normal rats, the behavioral tasks have been tested during real feeding. The gray marked areas indicate the results collected during this thesis project, which every task was first tested with sham feeding. (+: learn to perform based on the CS cue, -: performance is eliminated, +/-: performance reduced, ?: performance has not been tested)
and responded normally for corn oil emulsions when comparing with their surgical controls. Moreover, they displayed ACEs for sucrose and corn oil. The PBN, in contrast, is required for all behavioral performance when using sucrose as the CS or the reward. The results are promising and consistent with previous results – the rewarding effects of sucrose is predominantly transmitted to the ventral forebrain reward circuits via the PBN. Rats with PBN lesions spared a conditioned aversion to 100% corn oil, but failed to avoid sucrose after it had been associated with LiCl induced visceral illness. These rats greatly reduce sham intake of sucrose solutions, but consumed the same amount of oil emulsions as did their surgical controls during free access sessions in operant tasks. Their operant responses for sucrose were completely blocked, but responses for corn oil were modest at best. Finally, the PBNx rats could not develop an ACE for either sucrose or corn oil. Overall, the results confirm that the gustatory PBN is necessary for processing sucrose reward. The simplest and least complicated possible pathway for fat reward, however, was not supported. It appears that the neural pathways for oil reward are more complicated than those for sucrose reward. The results also suggest that the gustatory PBN is involved in transmitting oil reward to the forebrain.

Comparisons of real and sham feeding
The deprivation regime as well as the concentration of and the access interval for the stimulus affect the differences in the results for real and sham feeding. Many ingestive behaviors have been tested with sham feeding previously (Weingarten and Watson, 1982; Ackerman et al., 1992; Sclafani et al., 1994; Davis et al., 2000). However, it is not certain that the orosensory cue alone can support a learning process involved associating two stimuli (classical conditioning) or a behavior and a reward (operant conditioning). The purpose of this dissertation was to study the orosensory reward of sucrose and corn oil, and thereby all the experiments were designed with sham feeding to minimize the postingestive feedback. Only during the operant tasks was real feeding included in the experiments (chapter 4). We demonstrated that the rats can learn classical conditioning e.g. CTA and ACE and operant conditioning e.g. fixed and progressive ratio when receiving no caloric effect from the CS or the reward. Compared the data collected here with prior studies from our laboratory, it should be noted that the capability to learn and perform in the three behavioral tasks did not differ in
normal rats when real or sham feeding (Table 6.1). One would expect that the intakes of the 
sapid solutions be more during sham feeding than during real feeding because the satiety 
signals were excluded during sham feeding. The intakes of the CS in CTA and the number of 
licks in ACE, however, did not significantly differ when conducted in real or sham feeding. 
For CTA experiments in our laboratory, the access time for the CS is always 15 min. When 
the CS is 0.3M sucrose, the intakes during sham feeding are not different from the intake data 
collected previously during real feeding (unpublished observation). The absence of 
differences in intake is probably due to the fact that rats were both food and water-deprived in 
the current study whereas rats were only water-deprived in previous real feeding studies. 
According to the observation in our laboratory, rats tend to consume less when both food and 
water-deprived than when water-deprived alone. As a consequent, there were no differences 
in terms of the CS intake between real and sham feeding. For the anticipatory contrast 
experiments, the access time to each solution is 3 min and 6 min as the total. The 
postingestive feedback is minimized in real feeding condition with such short intake period, 
and so no significant differences for intake as comparing real (Flaherty et al., 1991; Reilly et 
al., 2004) and sham feeding experiments. For the operant tasks, the intakes and break points 
for weak solutions (e.g. 0.03M and 0.3M sucrose or 2.5% corn oil) did not differ between real 
and sham feeding. The major differences were that the intakes and break points for the high 
concentration reward solutions (e.g. 1.0M and 2.0M sucrose or 100% corn oil) were higher in 
sham feeding than in real feeding. This is consistent with previous investigations about 
mechanisms for sham feeding, which suggest that the concentration of sucrose affects the 
negative feedback that controls intake (Davis and Smith, 1990; Davis et al., 2000). 
Furthermore, the fact that animals had significantly longer access time to the CS in the 
operant tasks than in CTA and ACE experiments also indicate that the access time is 
important for distinguishing the results for real feeding and sham feeding. In sum, it is 
concluded that sham feeding influences the magnitude of performance for high concentration 
stimulus solution, and particularly when the time interval is long enough for the postingestive 
feedback to function. Data collected in this dissertation implicate that the orosensory cue 
without the metabolic consequence of a stimulus supports an association not only for classical 
conditioning but also for operant conditioning.
Based on the results of normal animals, it may seem that sham feeding is not a necessary procedure for the investigation of orosensory rewards. The study with lesions and anticipatory contrast, however, suggests that further studies are required to determine whether the mechanisms for reward during sham and real feeding differ. The results in chapter 5 demonstrated that lesions of the TOA spare an ACE for sucrose, which is inconsistent with previous studies showing that lesions of the gustatory thalamus disrupt ACEs for taste rewards (Reilly and Pritchard, 1996; Reilly et al., 2004; Schroy et al., 2005). As mentioned in chapter 5, it was suggested that this inconsistency could be due to different lesion areas were included in the present and previous studies. Compared with the thalamic lesions in prior studies, the lesions in the present study included more of the ventral posteromedial nucleus (VPM) but less of the dorsal part of the thalamus e.g. the central medial nucleus (CM) and medial dorsal nucleus (MD). Besides differences in lesions, it must be noted that the studies were performed in real feeding in the previous but in sham feeding in the present study for ACEs. Further, animals in the present study were tested with a drug of abuse induced suppressive effect on the CS in real feeding. The results showed that the same lesion animals that spared an ACE for sucrose failed to suppress a taste CS when the US is morphine (Fig. 5.14, chapter 5), which is consistent with previous real feeding study (Grigson et al., 2000). Therefore, it is not clear that the discrepancy in developing an ACE is due to the size or location of the lesion, the nature of the US, or the method of feeding. Finally, it is also possible that sham feeding affects reward processing in rats with but not without neurological damages.

**Corn oil emulsions as orosensory stimuli**

Comparing with sucrose ACE, mild food deprivation, longer access period, and more number of trials are required for corn oil ACE. Many studies have used either sucrose solutions or corn oil emulsions as orosensory stimuli (Cagan and Maller, 1974; Weatherford et al., 1988; Ackerman et al., 1992; Smith et al., 2000; Sclafani and Ackroff, 2003; Avena et al., 2006). However, it is not clear how the two orosensory qualities as conditioned stimuli initiate learning. This is the first time the two stimuli are compared in different behavioral tasks. The results from the anticipatory contrast studies found that sucrose solutions and corn oil emulsions support behavior differently. In an anticipatory contrast paradigm, two different
concentrations of the same orosensory quality stimulus are used. Initially, we studied whether rats with sham feeding can develop an ACE for sucrose (chapter 5). The access time for each stimulus was 3 min. The experimental group had a weak stimulus e.g. 0.06M sucrose from the first bottle, and immediately after received a stronger solution e.g. 1.0M sucrose from the second bottle (the L-H group). The control comparison had access sequentially to the same weak stimulus from two different bottles without interstimulus interval (the L-L group). The rats were trained once daily for 14 days in this paradigm. An ACE for sucrose during sham feeding was displayed with the first attempt. This ACE for 0.06M and 1.0M sucrose comparisons was significant and reliable, and we were able to replicate it consistently. When using corn oil emulsions as the stimuli, however, it took several experiments to establish the appropriate parameters for a corn oil ACE. Using 2.5% oil emulsion as the weak concentration and 25% oil emulsion as the strong concentration, the rats could form an ACE if they were deprived to 95% but not 90% of their free feeding weight. The studies conducted in our laboratory recently also found that it takes more trials for the rats to develop an ACE for oil when the access time was 3 min for each bottle. During sucrose contrast experiments, the rats began to show contrast by the 8th day of training. A recent study in our laboratory with 5% and 25% oil comparisons demonstrated that more than 12 days of training are required until the rats show a contrast effect for corn oil. The on going project for corn oil ACE also suggests that the training length can be shortened if the access period for each stimulus is longer e.g. 5 min.

The rats cannot adjust their licking behavior when the stimulus is switched from sucrose to corn oil in the anticipatory contrast experiments. The procedures for the ACE experiments involved switching the rats from the L-L to the L-H condition and vice versa from one contrast experiment to another. When the comparisons switched from sucrose to corn oil, about 50% of the rats switching from the L-H e.g. 0.06M-1.0M sucrose to the L-L e.g. 1.5%-1.5% corn oil condition did not make a reliable number of licks (below 50 licks) throughout 14 days training. This phenomenon was consistent whenever the switch was from sucrose to corn oil. It was interpreted that the suppression effect for the L-H condition during sucrose comparisons was carried over for the L-L condition during corn oil comparisons. However, the recent experiment from our laboratory found no carry over effect when the rats were
switched from 5%-25% corn oil to 0.06M-0.06M sucrose condition. It is unclear why the carry over effect only occurred from sucrose to corn oil but not vice versa. One possibility is that the disparity of reward value between 1.0M sucrose and 1.5% corn oil was more than between 25% corn oil and 0.06M sucrose. To support this possibility, it will be worthwhile to study whether the carry over effect occurs when the rats are switched from 0.06M-1.0M sucrose to 5%-5% corn oil comparisons. Furthermore, it could also be that 0.06M sucrose is more rewarding than 1.5%, 2.5% and 5% corn oil emulsions. The preference order for these stimuli can be obtained by a series of 2-bottle tests to compare a sucrose solution with a corn oil emulsion.

Overall, the results from the anticipatory contrast experiments suggest that sucrose and corn oil are different orosensory stimuli that initiate behavior differently. The results show that it takes the rats more brief access trials to begin consistent licking for diluted corn oil emulsions e.g. 1.5%, 2.5% and 5% than for diluted sucrose solutions e.g. 0.06M. Thus, it seems that it takes more exposure for the rats to show preference to corn oil than to sucrose. It has been demonstrated that preweanling rats increase their intake of sucrose and corn oil emulsions as a function of concentration in a 20 min test (Ackerman et al., 1992). Adult rats can distinguish 0.78% corn oil emulsion from water (Mindell et al., 1990) and drink above 30ml of 0.06M sucrose (Nissenbaum and Sclafani, 1987) in a 30 min sham feeding experiment. These results suggest that sucrose and corn oil are both inherently rewarding to the rodents (Weatherford et al., 1990; Smith and Greenberg, 1991). The results of the anticipatory contrast experiments, however, suggest that diluted corn oil emulsions e.g. 1.5% and 2.5% do not provide enough orosensory cues for the rats to develop preferences for them in a brief exposure. On the other hand, the results for using 0.06M sucrose in the contrast experiment suggest that the rats have enough orosensory cues from the weak sucrose solutions to perform consistently during brief and long access experiments.

**Possible pathways for corn oil reward**
While showing no deficits in operant tasks and ACE, the TOAx rats performed differently from the controls. The intraoral trigeminal somatosensory system bypasses the PBN and projects directly into the thalamocortical pathway (Waite, 2004). Based on this anatomical
character, it was hypothesized that the thalamic trigeminal orosensory area is necessary for the sensory coding and reward processing of oral corn oil. The results did not support this hypothesis because lesions of the TOA spared conditioned aversion to 100% corn oil, did not decrease the reward strength of corn oil emulsions, and finally did not disrupt an ACE for corn oil. That said, there are still differences in oil intake between the controls and the TOAx rats. The free access intakes of 25% corn oil during operant tasks were almost significantly more for the TOAx rats than for the controls (chapter 4, Fig. 4.4). In the anticipatory contrast experiment, the TOAx rats licked significantly less for the bottle 2 25% corn oil than did the controls (chapter 5, Fig. 5.10 and 5.12) while expressing an ACE for oil. Furthermore, the TOAx rats showed similar degree of suppression on 0.06M sucrose intake and drank similar amount of 1.0M sucrose as did the controls during sucrose anticipatory contrast experiment (Fig. 5.6 and 5.8). The same TOAx rats, however, appeared to have a different degree of suppression on 1.5% corn oil intake from those same controls. The number of 1.5% corn oil licks for the TOAx rats reduced from 146 licks at the first block to 57 licks at the last block, which was about 60.94% of suppression. In contrast, the number of 1.5% corn oil licks for the controls reduced from 316 licks to 185 licks, which was 41.46% of suppression. Additionally, the L-H group of the TOAx rats in the contrast experiment licked significantly less for both 1.5% and 25% corn oil than did the L-H group of the controls. It is not clear whether the stronger degree of suppression for the TOAx rats was a result of reward comparison, or the lesions of the orosensory thalamus actually compromised the intensity of the corn oil emulsions. The TOA is not necessary for learning classical conditioning (CTA and ACE) or operant conditioning (operant tasks), but it may be involved in modulating the magnitude of behavioral performance during the tasks.

If corn oil reward is processed via the trigeminal somatosensory system, the involvement of structures other than the TOA for processing corn oil reward should be investigated. Because the thalamic trigeminal orosensory area is not necessary for learning behaviors related to oil reward, other structures along the trigeminal somatosensory pathways should be considered. The structure required for corn oil reward could be the principal sensory trigeminal nucleus (Pr5) as lesions of this area have been shown to produce aphagia (Zeigler and Karten, 1974). Furthermore, the spinal trigeminal nuclei cannot be discounted for translating orosensory corn
oil to reward. The same strategy used here can be applied to test these possibilities. In addition, if the detection of fats and oils depends on the trigeminal system, data from neural coding and psychophysics should be consistent with this notion. For example, applying fats or oils in the oral cavity should produce neuronal activities at the peripheral and in the central trigeminal nucleus i.e. lingual nerve activity and Pr5 neuronal firing. The logic and methods used for the sensory pathways of sucrose can apply. It has been demonstrated that lesions of the gustatory NST flatten the concentration response function for sucrose solutions (Shimura et al., 1997). Damage to the brainstem trigeminal relays should blunt the concentration-dependent responses for fat solutions or oil emulsions in brief access tests. Moreover, sham intake of sucrose oil increases DA overflow in the NAc (Hajnal and Norgren, 2004), and lesions of the PBN blunt this effect (Hajnal and Norgren, 2005). As sham intake of corn oil also increases DA overflow in the NAc (Liang et al., 2006), similar blunt effect should also be shown in lesions of Pr5 or spinal trigeminal nuclei to demonstrate the involvement of the intraoral trigeminal system in processing corn oil rewards. In addition to that, those lesions should also influence learning a conditioned aversion, performing operant tasks, and developing an ACE for corn oil emulsions.

The results of this thesis favor a role of the gustatory PBN in sensory detection and reward processing for fats. The only result that supports the hypothesis that the PBN is necessary for sucrose but not corn oil reward is when lesions of the PBN spared conditioned aversion to 100% corn oil. When using diluted corn oil emulsions ranging from 1.5% to 25% in the progressive ratio and the anticipatory contrast studies, rats with PBN lesions failed to learn the behaviors. The results imply a concentration effect. In fact, preliminary data in our laboratory showed that both naïve and PBNx rats trained to avoid 100% corn oil did not generalize this aversion to 16% corn oil emulsions. It may be that the 100% corn oil provides more sensory cues than the diluted emulsions i.e. greasiness and olfaction. Thus, it would be worthwhile to use an oil emulsion such as 5% or 25% oil as the CS for a CTA experiment, and test whether rats with PBN lesions can learn conditioned aversions to the diluted emulsion. Further, if indeed the gustatory PBN is involved in processing corn oil reward, lesions of the PBN should blunt the DA overflow in the NAc during intake of corn oil as they did during intake of sucrose (Hajnal and Norgren, 2005).
Assuming the results from PBN lesions support the involvement of taste in oil processing, then the possibility that fats are detected by the gustatory system must be reconsidered. The most provocative results supporting gustatory mechanisms for the detection of fats come from the discovery of fat transporters on taste cells (Fushiki and Kawai, 2005; Laugerette et al., 2005), the demonstration of lingual lipase activity on rat tongue (Kawai and Fushiki, 2003), and the response of delayed rectifying K⁺ channels on taste cells to fatty acids (Gilbertson, 1998). Studies that support a role of gustatory system in fat detection most frequently use free fatty acids as stimuli. From those results, it is certain that animals can detect fatty acids at very low concentrations, and also fatty acids such as linoleic acid can be a CS for learning a CTA (Tsuruta et al., 1999; Fukuwatari et al., 2003; McCormack et al., 2006). While oil solutions are included often along with anosmic rats, the results depend on the concentration of oil solutions. It appears that above 5% corn oil, neither a gustatory nor an olfactory cue is necessary for sensory detection (Takeda et al., 2001; Saitou et al., 2009). Data collected in this dissertation, however, showed that PBN lesions compromise learning oil reward such as 25% oil emulsions. Therefore, more studies comparing behavioral responses to fatty acid and oils as well as gustatory neuronal activity to fatty acid and oils are required to support gustatory mechanisms in sensory detection for fats.

Besides the direct involvement of the PBN in corn oil reward, another alternative mechanism is that the PBN processes oil reward via its anatomical and physiological connections with the trigeminal system. Anatomical evidence demonstrates connections between the central gustatory and intraoral trigeminal systems. First, the anterograde tracer horseradish peroxidase in the lingual branch of the trigeminal nerve transport to the lateral NST just rostral to the obex, and the labeling diminished once reaches the forth ventricle (Jacquin et al., 1983; Hamilton and Norgren, 1984; Takemura et al., 1987). Secondly, neurons of the paratrigeminal nucleus project ipsilaterally to the PBN and the rostral NST, and contralaterally to the VPM (Saxon and Hopkins, 1998). Furthermore, there are reciprocal projections between the Pr5 and the PBN. Neurons from the caudal two thirds of the PBN project bilaterally to the trigeminal sensory nuclear complex (TSNC) with an ipsilateral dominance whereas ascending neurons from the TSNC project to the PBN with a ipsilateral
dominance (Slugg and Light, 1994; Feil and Herbert, 1995; Yoshida et al., 1997; Dallel et al., 2004). Finally, the Pr5 project contralaterally (Emmers, 1975) and spinal trigeminal nucleus oralis project bilaterally (De Chazeron et al., 2004) to the gustatory thalamus, VPMpc.

Neuronal recording also supports interactions between the gustatory and the intraoral trigeminal systems. Peripherally, the lingual and chorda tympani nerves both innervate the fungiform papilla on the anterior two-thirds of the tongue (Whitehead et al., 1985; Suemune et al., 1992; Lundy and Norgren, 2004). Four physiological classes of fibers including mechanoreceptors, thermoreceptors, nociceptors, and proprioceptors are included in the lingual nerve (Pittman and Contreras, 1998). The lingual nerve modulates chorda tympani activities to chemical stimulation (Wang et al., 1993, 1995; Pittman and Contreras, 1998) and in some neurons, such modulation depends on the temperature of the stimulus (Pittman and Contreras, 1998). After chorda tympani damage or chorda tympani-lingual nerve damage, taste buds are reinnervated by fibers from the ipsilateral lingual nerve or from the contralateral lingual nerve (Kinnman and Aldskogius, 1988). Centrally, tactile or mechanical stimulation in the oral cavity activates neuronal activities in the gustatory NST (Halsell et al., 1993; Travers and Norgren, 1995), the PBN (Ogawa et al., 1982; Ogawa and Hayama, 1984; Ogawa et al., 1984; Halsell and Travers, 1997), and the VPMpc of thalamus (Nomura and Ogawa, 1985; Ogawa et al., 1987). Further, the responsive areas for taste and tactile stimulation overlaps but distinguishes within the NST and the PBN (Halsell et al., 1993; Travers and Norgren, 1995; Halsell and Travers, 1997). Therefore, it is possible that both the gustatory and the trigeminal somatosensory systems are involved in processing sucrose and oil reward.

**Forebrain involvement of reward mechanisms**

Normal performance of reward-related behaviors requires the involvement of forebrain structures. The results in this thesis demonstrate that lesions of the PBN but not the TOA have profound effects on the acquisition of ACE, CTA and operant behaviors. A previous study demonstrates that lesions of the PBN, but not the thalamic taste relay, blunt DA release in the NAc during sucrose intake (Hajnal and Norgren, 2005). The combination of these results suggests that the connections between the PBN and the forebrain substrates are important for
transmitting the rewarding effects of oral food reward. The PBN has direct projections to several forebrain areas including the central nucleus of amygdala (CeA), the lateral hypothalamus (LH), and the bed nucleus of the stria terminalis (BNST). There are also reciprocal connections between each of the central gustatory relays, cortical or subcortical (Lundy and Norgren, 2004). These connections provide the anatomical basis of the PBN-ventral forebrain pathway for processing reward. Indeed, several studies have shown that the forebrain structures are involved in the reward-related behaviors described in this thesis. Chronically decerebrate rats, i.e. rats with complete transections of the brain just rostral to the superior colliculus, failed to show voluntary preference and aversion (Grill and Norgren, 1978b) as well as a CTA (Grill and Norgren, 1978a). Studies with Fos expression also suggest the forebrain structures are involved in the neural mechanisms of CTA learning (Schafe et al., 1995; Tokita et al., 2007; Clark and Bernstein, 2009).

The effects of lesions of the limbic forebrain structures on CTA learning are not as profound as the effects of lesions of the PBN. Bilateral lesions of the gustatory cortex (Geddes et al., 2008a), LH, or BNST have little or no effect on the acquisition of CTA (Roman et al., 2006). While some studies report that lesions of the amygdala significantly disrupt CTA learning (Yamamoto and Fujimoto, 1991; Roldan and Bures, 1994; Schafe and Bernstein, 1996), others demonstrate little or no effect (Dunn and Everitt, 1988; Bermudez-Rattoni and McGaugh, 1991; St Andre and Reilly, 2007). Some studies distinguish the effects of the basolateral amygdala (BLA) and the CeA on CTA learning (Morris et al., 1999; Sakai and Yamamoto, 1999). From the results of excitotoxic lesions, it seems to be consistent that the BLA but not the CeA plays an essential role in the formation of CTA learning. Nevertheless, the conditioning method in these studies is spout presentation, in which rats initiate CS exposure voluntarily by drinking the CS solution from a bottle. It has been demonstrated that the role of the amygdala in CTA learning depends on the conditioning method (Schafe et al., 1998). Electrolytic and excitotoxic lesions of the CeA both disrupt a CTA learning when the CS solution is infused into the oral cavity of the animal, but at best attenuate a CTA in the bottle presentation procedure (Schafe et al., 1998). Besides the conditioning method, the number of the CS-US pairing trials also determines the results of studies with amygdala lesions. Since rats with amygdala lesions often increase intakes of the CS at the first exposure,
several trials are necessary to determine the lesion effect on the acquisition of a CTA (Reilly and Bornovalova, 2005). In fact, studies that show a disruption or attenuation of a CTA in rats with amygdala lesions often include only one CS-US pairing (Reilly and Bornovalova, 2005).

It may seem that the forebrain structures do not play a necessary role in CTA learning based on the results described in the previous paragraph. As mentioned, the CeA is necessary for a CTA learning in the IO infusion but not the bottle presentation conditioning method (Schafe et al., 1998). The PBN, on the other hand, is necessary for the acquisition of a CTA in either the IO infusion (Spector et al., 1992) or the bottle presentation (Reilly et al., 1993) method. These results suggest that the CeA or other forebrain structures involved in different aspects of CTA learning i.e. depends on the conditioning method. Besides the factor of conditioning method, the lack of disruption of CTA learning in rats with bilateral lesions of one forebrain structure maybe attributed to forebrain redundancy of function. This assumption can be tested with asymmetric lesions, which involve a lesion of one gustatory relay on one side and damage to one of its reciprocal targets on the other side, and thereby disrupt interactions between the two structures. The data support this hypothesis for lesions that disconnect one reciprocal connection only retard or do not affect the acquisition of a CTA (Norgren et al., 2008), but lesions that disconnect more than one reciprocal connection within the forebrain or between the forebrain and the PBN disrupt CTA learning (Yamamoto et al., 1995; Bielavska and Roldan, 1996).

The forebrain gustatory relays such as the gustatory insular cortex (GC) and the CeA, are involved in reward comparison. There are different forms of reward comparison tasks including the simultaneous contrast, anticipatory contrast, and successive negative contrast, all of which are described in chapter 5. The reward comparison paradigm applied in this dissertation is an anticipatory contrast task, and the results reveal that the PBN but not the TOA is required for forming ACEs for sucrose and corn oil. As mentioned, because lesions of the TOA included the thalamus taste area, the results are inconsistent with previous results that demonstrate an elimination of ACE in gustatory thalamus lesion rats (Reilly and Pritchard, 1996; Reilly et al., 2004; Schroy et al., 2005). Although the results are inconsistent, examination of the lesion areas suggests that areas more dorsal to the VPMpc e.g. the MD are
involved in the reward comparison process. In fact, a model involved several thalamic areas such as the taste thalamus, MD, and the paraventricular nucleus of the thalamus has been proposed for the integration of appetitive motivation and food reward (Kelley, 2004; Kelley et al., 2005a; Kelley et al., 2005b). Several pieces of evidence suggest the dorsal gustatory pathway, the thalamocortical connection, is important for comparing the relative properties of reward. First, the DA level in the NAc tracks the comparison of taste and drug rewards over time (Genn et al., 2004; Grigson and Hajnal, 2007). Second, it has been shown that bilateral GC lesions (Geddes et al., 2008a) and the asymmetric lesions of the gustatory thalamocortical loop (Geddes et al., 2005; Geddes et al., 2006; Geddes et al., 2007; Geddes et al., 2008b) both disrupt ACEs while using either sucrose or drugs of abuse as the US. Third, lesions of the gustatory thalamus block successive negative contrast in rats (Reilly and Trifunovic, 1999). Finally, the results in this thesis provide an insight to whether the major thalamic taste relay, VPMpc, or other areas such as the MD is responsible for reward comparisons for nature rewards.

The fact that lesions of the PBN but not the gustatory thalamus blunt DA release in the NAc during sham drinking sucrose suggests the inputs from ventral gustatory projections are important for the accumbens to report on the neurochemical response to primary food reward. Furthermore, the results presented in table 6.1. suggest that the gustatory PBN and possibly its connection to the limbic forebrain is important for transmitting not only the absolute but also the relative reward properties of food. In addition to the GC and the thalamocortical loop, studies have demonstrated that several other forebrain structures are involved in the neural mechanisms of reward comparison. Lesions of the medial amygdala included the CeA block a successive negative contrast effect (Becker et al., 1984). When measuring contrast effect with an instrumental task (e.g., the time taken to traverse a runway), rats being presented with a reward that is downshifted from palatable to weak (10 to 1 sucrose pellet) significantly slow their running speed toward the reward compared with rats with an unshifted reward (1 to 1 sucrose pellet) (Crespi, 1942; Flaherty, 1982). This instrumental negative contrast is totally eliminated by damages to the hippocampus and reduced by lesions of the NAc, amygdala, and dorsal striatum (Salinas et al., 1993; Salinas and White, 1998; Leszczuk and Flaherty, 2000). Interestingly, lesions of the NAc spare the consummatory negative contrast but reduce the
instrumental contrast effect. In order to determine the role of the PBN and the ventral
gustatory pathway in reward comparison, future studies should focus on comparing effects of
bilateral PBN lesions and asymmetric PBN-limbic forebrain lesions on different reward
comparison tasks. It will also be worthwhile to investigate whether different neural
mechanisms are involved in consummatory and instrumental contrast. It has been shown that
temporal inactivation of the CeA with lidocaine not only blunts the DA efflux in the NAc
during food reward (Ahn and Phillips, 2002, 2003) but also attenuates the instrumental
contrast effect in rats (Salinas et al., 1993). As a start, it seems appropriate to study the effects
of asymmetric lesions of the PBN and the CeA on reward comparisons.

The nucleus accumbens tracks both the absolute and relative reward properties of foods
(Grigson and Hajnal, 2007). In chapter 2, DA levels in the NAc was used as an index of the
primary corn oil rewards. Although the role of dopamine in reward is controversial (Berridge,
1996; Salamone et al., 2003; Smith, 2004; Berridge, 2007), there is no doubt that the nucleus
accumbens plays an important role in appetitive motivation and food reward. Neuronal
activity in the accumbens follows the palatability of rewards (Taha and Fields, 2005; Wheeler
et al., 2005). Manipulation of activities in the accumbens via neuronal damages (Bowman
and Brown, 1998; Hamill et al., 1999) or pharmacological methods (Zhang et al., 2003; Pratt
and Kelley, 2004; Baldo and Kelley, 2007; Cousens and Beckley, 2007) influence how
animals work and the break points in a progressive ratio for food reward. From those results,
it appears that dopamine and other neurotransmitters in the accumbens working as a concert
for rewarded behaviors. The study in this thesis shows that the PBN is required for the
acquisition of operant responding for sucrose and corn oil in operant tasks. Rats with PBN
lesions do not seem to distinguish their working effort for different concentrations of sucrose
or corn oil rewards. As the operant training was conducted post lesions in this thesis, it is
important to investigate how rats with PBN lesions made after operant training respond to oral
sucrose and corn oil reward in the same operant tasks. Given that the PBN is an important
orosensory input to the limbic forebrains and then to the accumbens. Similar asymmetric
PBN-limbic forebrain lesion method can be applied to study how the ventral gustatory
pathway is involved in transmitting the reward values of sucrose and corn oil in operant
behaviors.
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