The role of the gustatory thalamus
in the anticipation and comparison of rewards over time in rats

1Pearl Lee Schroy, 1Robert A. Wheeler, 1Collin Davidson, 2Giuseppe Scalera,
1Robert C. Twining, and 1Patricia S. Grigson

1Department of Neural and Behavioral Sciences
Penn State University
College of Medicine
Hershey, PA 17033

2Dipartimento di Scienze Biomediche
Sez. Fisiologia
Universita di Modena & Reggio Emilia
Via Campi, 287, 41100 Modena, Italy

Running Head: The thalamus and reward comparison

Address Correspondence to:
Patricia Sue Grigson
Department of Neural and Behavioral Sciences
Penn State University College of Medicine
Hershey, PA 17033
Phone: 717-531-5772
FAX: 717-531-6916
E-mail: psg6@psu.edu
ABSTRACT

Rats reduce intake of a palatable saccharin solution when followed by access to a preferred sucrose solution. This phenomenon, referred to as an anticipatory contrast effect (ACE), is thought to occur because the value of the saccharin conditioned stimulus (CS) pales in comparison to the highly rewarding sucrose unconditioned stimulus (US) expected in the near future. Although relatively little is known about the underlying neural substrates, lesions of the gustatory thalamus (THLX) fully disrupt the phenomenon (38, 40). The present set of experiments revisited this issue to determine the nature of this deficit. Rats with bilateral ibotenic acid lesions of the gustatory thalamus were given 3 min access to 0.15% saccharin and, after a zero sec or 5 min interval, were given 3 min access to either the same saccharin solution or a highly preferred 1.0 M sucrose solution. In Experiment 1, ACE testing began with the 5 min interstimulus interval (ISI) and then switched to the zero sec ISI. For Experiment 2, the order of ISI testing was reversed. The results show that axon-sparing, neurotoxic lesions of the gustatory thalamus prevent ACEs when using a zero second ISI and lead to a reversal (i.e., a reinforcement effect) when using a 5 min ISI. Taken together, the results suggest that the lesion leads to a specific reward comparison deficit whereby the rats fail to compare the value of an available reward with the memory of a preferred reward that is anticipated in the near future.

Key Words: Anticipatory contrast, memory, sucrose, drugs of abuse, reinforcement effect, taste
Primary taste neurons in the nucleus of the solitary tract (NST) terminate in the medulla, making up the first leg of the polysynaptic taste pathway (32, 34). From the NST, axons project to the pontine parabrachial nuclei (PBN) and then rostrally via two different pathways. The first pathway, the dorsal taste pathway, projects from the PBN to the parvicellular component of the ventral posteromedial nucleus (VPMpc) of the thalamus (also referred to as the gustatory thalamus) and then continues to the agranular insular cortex (27). The second pathway, the ventral taste pathway, includes several diverse projections from the PBN to the substantia innominata, the lateral hypothalamus, the central nucleus of the amygdala, and the bed nucleus of the stria terminalis (21, 33, 35, 44). The present experiments are concerned with the role of the VPMpc, the third relay in the dorsal taste pathway, in the comparison of natural rewards over time.

One type of reward comparison, anticipatory contrast, occurs when rats reduce intake of an otherwise palatable saccharin solution, for example, when it comes to predict access to a more preferred sucrose solution over repeated daily pairings. The reduction in intake of the saccharin cue is measured relative to control conditions where access to the saccharin cue is followed by access to more saccharin (7, 9, 28). This anticipatory contrast effect (ACE) has been interpreted within the context of classical conditioning where the conditioned stimulus (CS) is the saccharin cue and the unconditioned stimulus (US) is the preferred sucrose solution (16). The phenomenon is thought to occur because the value of the saccharin cue is reduced as it comes to predict the availability of the highly rewarding sucrose US that is anticipated in the near future (7, 9, 18) (but see 8).

Although this phenomenon is fundamental to understanding how animals compare and choose rewards over time, relatively little is known about its underlying neurocircuitry. It has been shown, however, that bilateral lesions of the gustatory thalamus (VPMpc) disrupt ACE without
affecting innate taste preferences or basic associative learning processes (38-41, 45). Specifically, Reilly and Pritchard (39) used long-term intake tests and Scalera et al. (45), short-term intake tests, to show that rats with lesions of the gustatory thalamus (THLX) display minimal deficits in response to the 4 basic taste stimuli. Furthermore, thalamic lesions did not affect the development of a conditioned taste preference, a conditioned odor aversion, or a LiCl-induced conditioned taste aversion (19, 40, 45). The same lesion, however, fully prevented the development of an ACE following daily saccharin-sucrose pairings when using the standard zero sec interstimulus interval (38, 40) and reversed the ACE (i.e., induced a reinforcement effect) when rats with electrolytic lesions of the taste thalamus had to wait 5 min for access to the preferred sucrose reward (i.e., when using a 5 min interstimulus interval, ISI).

The overall disruptive effect of the thalamic lesion on the development of an ACE, then, is fairly well established. Even so, the cause of the lesion-induced deficit and the role of the gustatory thalamus in the comparison of rewards over time remain unclear because anticipatory contrast is not a simple, but a multistage, phenomenon. The establishment of an ACE requires that the rat: [1] appropriately detect and respond to the saccharin and sucrose solutions, [2] associate the saccharin cue with the sucrose US, [3] remember the ‘value’ of the sucrose US upon CS presentation, [4] compare the value of the saccharin cue with the memory of the sucrose US that is anticipated in the near future, and [5] reduce intake of the saccharin cue as a result of this reward comparison process.

As mentioned, published data have shown that the lesion-induced deficit is not likely due to a failure to respond to the taste properties of the gustatory stimuli (39, 45). Nor is an intact gustatory thalamus required to make general CS-US associations, as taste-taste, odor-illness, and taste-illness associations are preserved following the lesion (19, 40, 45). As such, the
reinforcement effect obtained in the Reilly and Pritchard (40) ACE study is a key finding because it suggests that the lesion-induced deficit also is not due to a failure to make the specific saccharin-sucrose association or for the presentation of the CS to elicit the memory of the sucrose US. That is, when using a 5 min ISI, the thalamic lesioned rats clearly increased licking for the saccharin cue in anticipation of the availability of the impending, preferred sucrose reward (i.e., the rats behaved as though they had a clear US representation following presentation of the saccharin CS).

While these final conclusions are essential to our understanding of the role of the gustatory thalamus in the anticipation and comparison of rewards over time, they must be tempered because in the published experiment described, the lesions were induced with electrical current (40), making it impossible to determine whether the behavior change was attributable to damaged cell bodies within the VPMpc and/or to damaged fibers of passage. In a more recent report, the thalamic lesion was induced with ibotenic acid, but only the zero sec ISI was used and the magnitude of the reinforcement effect (the critical feature for the interpretation of the data) was only marginal (38). The present set of studies, then, used the neurotoxin, ibotenic acid, to test the role of taste cells in the VPMpc in the expression of an anticipatory contrast effect or a reinforcement effect using both a zero sec and a 5 min ISI. In Experiment 1, ACE testing began with a 5 min ISI in Phase I and then was switched to the zero sec ISI condition in Phase II. In Experiment 2, ACE testing in a naïve set of THLX rats began with a zero sec ISI in Phase I and was then switched, in Phase II, to the 5 min ISI condition.

**METHOD**

*Subjects.* All experimental procedures employed have been reviewed and approved by the Institutional Animal Care and Use Committee at the Penn State College of Medicine. The subjects
were 35 and 29 naive male Sprague-Dawley rats from Charles River and body weights initially ranged from 305 – 508 g. They were housed individually in standard wire-mesh cages in a colony room with temperature, humidity, and ventilation automatically controlled. The rats were maintained on a 12/12h light/dark cycle with all experimental manipulations conducted 4 hours into the light phase of the cycle. Food and water were available *ad libitum*, except where noted otherwise. In Experiment 1, 19 rats received bilateral, electrophysiologically-guided ibotenic acid lesions of the gustatory thalamus (Group THLX) and 16 rats served as control subjects (Group SHAM): Eight of these SHAM rats received vehicle infusions of sodium phosphate buffer (PBS) into the taste thalamus and 8 served as non-surgical controls (NSC). In Experiment 2, 17 rats served in Group THLX and 12 rats served in Group SHAM: 6 PBS and 6 NSC.

**Apparatus.** The rats were trained and tested in four identical modular operant chambers (MED Associates, Inc., St. Albans, VT) measuring 30.5 x 24.0 x 29.0 cm (length x width x height). All chambers had a clear Plexiglas top, front, and back wall. Side walls were made of aluminum. The grid floors consisted of nineteen 4.8-mm stainless steel rods spaced 1.6 cm apart (center to center). Each chamber was equipped with two retractable sipper tubes that could enter the chamber through 1.3-cm diameter holes spaced 16.4 cm apart (center to center). In the advanced position, the tip of the sipper tube was aligned in the center of the hole, flush with the right end wall. A lickometer circuit (0.3 uA) was used to monitor licking. A shaded bulb, which reflected light off the ceiling, was located on the right of the cage and a white-noise speaker was on the left-end wall, opposite to the sipper tubes. Each chamber was housed in a light- and sound-attenuated cubicle that was fitted with a ventilation fan and a white-noise source that provided a background noise level of 75 dB. Control of events in the chamber and collection of the data were carried out
on-line using a 33-MHz computer. Programs were written in the Medstate notation language (MED Associates, Inc.).

**Surgery.** Twenty min prior to anesthesia, the rats were injected intraperitoneally (ip) with atropine sulfate (0.25 mg/rat) and Gentamicin (6 mg/rat). They were then anesthetized with sodium pentobarbital (50 mg/kg, ip) and supplemented as necessary throughout surgery. Body temperature was maintained at $37 \pm 1^\circ$C. The rat’s head was then mounted in a stereotaxic instrument, using non-traumatic earbars, with the skull level between bragma and lambda. The skin over the skull was cleaned with Betadine and opened with a mid-line incision. Using a 4 mm diameter trephine, a hole was drilled in the skull on either side of the midline, 3.0 mm posterior to bregma. The dura matter was left intact and kept moist throughout surgery with physiological saline.

Gustatory neurons in the VPMpc were located by recording multiunit activity through a glass-insulated tungsten microelectrode ($Z=1.0 - 1.5$ Mohms at 1 kHz) while stimulating the anterior part of the tongue with 0.3 M NaCl. The tongue was rinsed with distilled water (dH$_2$O) both before and after each NaCl stimulation. Neural activity was amplified and continuously monitored using an oscilloscope and an audio-monitor. The coordinates for electrode penetrations ranged from -3.5 to -4.1 mm posterior to bregma; $\pm 1.1$ to $\pm 1.4$ mm lateral to the midsagittal suture; -5.5 to -6.8 mm below the skull surface. Testing began when the electrode penetrated to about 2 mm dorsal to the level of the target, and the spontaneous neural activity dropped to a low level that is characteristic as the electrode tip passes from the hippocampus into the dorsal thalamus.

Once the gustatory area had been located on both sides, which required an average of about 2 penetrations, the search microelectrode was replaced with a double barreled micropipette/electrode (M/E, o.d. 50 - 60 mm; $Z=0.5 - 1$ Mohms at 1 kHz) one lumen of which was
glued directly onto the needle of a 1.0 µl Hamilton microsyringe. The microsyringe and its attached micropipette were filled with mineral oil. The other lumen was filled with an etched tungsten wire constructed in a manner identical to the search electrode. The ibotenic acid was drawn in through the tip of the M/E immediately prior to making the injections. It was then lowered directly into the hole in the dura that was left by the penetration producing the best response to NaCl. The taste area was relocated electrophysiologically and 0.2 µl (20 μg/µl) of ibotenic acid was infused over 10 min. After the injection, the M/E remained in place for an additional 10 min, before repeating the procedure on the opposite side. The PBS rats were treated identically except that 0.2 µl of PBS (pH = 7.40), rather than ibotenic acid, was infused into the VPMpc. After removal of the M/E, the holes in the skull were filled with Gelfoam and the wound was closed with wound clips. The animals recovered within 2-3 days and body weight returned to presurgical levels within a week.

**Procedure.** Food Deprivation & Habituation. For both experiments, the rats were deprived to 82% of their free-feeding body weight, maintained by a once a day feeding with water freely available. Once all rats reached the target weight, they were habituated to the operant conditioning chambers for 5 min/day for 3 days. Both the house light and the white noise were on, but the tubes were empty and out of the reach of the animals.

**Testing.** All animals were placed into the dark operant chamber with the white noise on. Immediately thereafter, the house light was turned on and the first bottle was advanced containing a 0.15% saccharin solution (the CS). Following 3 min access, the first bottle was retracted. An ISI of zero sec or 5 min was implemented, followed by the advancement of a second bottle. The second bottle (the US) contained either the same 0.15% saccharin solution or a 1.0 M sucrose solution. After 3 min access to the second bottle, the bottle retracted, the house light turned off, and the rats were removed from their chambers and returned to their home cages. In Experiment
1, there was one taste-taste pairing a day for 14 days using a 5 min ISI (Phase I) and, after an 18 day interval, 10 additional daily taste-taste pairings using a zero sec ISI (Phase II). The 18 day interval was employed in an effort to reduce possible carry-over effects from the 5 min ISI to the zero sec ISI condition. In Experiment 2, there was one taste-taste pairing a day for 16 days using a zero sec ISI (Phase I) and, immediately thereafter, 8 additional daily taste-taste pairings using the 5 min ISI (Phase II). Dependent measures included the latency (sec) to first lick and the number of licks made for the first and second bottle. The number of licks did not differ between the PBS and the NSC rats so the data from these two groups were collapsed. These subjects are hereafter referred to as group SHAM.

**Histology.** At the end of all behavioral tests, the rats were given an overdose of Pentobarbital sodium (100 mg/kg, ip) and, once deeply anesthetized, were perfused transcardially for 5 min with physiological saline, immediately followed by 10% buffered-formalin for 25 min. The brains were removed and stored in a solution of 30% sucrose + 10% buffered-formalin for at least a week. They were then frozen and cut coronally in 50 μm sections. One series of alternate sections was stained for cell bodies with cresyl Lecht violet, the other for fibers with the Weil procedure. The adequacy of the lesions was judged by comparing the acellular areas in the brains that had been injected with ibotenic acid with comparable areas in the brains of the PBS injected controls. The boundaries of the VPMpc were defined from neuroanatomical and electrophysiological data from this and other laboratories (27, 32, 34, 36).

**Results and Discussion**

**Histology.** In Experiment 1, the data from 4 THLX rats were eliminated because of inadequate lesion placement while the data from 4 other THLX rats were eliminated because they failed to lick in the apparatus. Thus, in Experiment 1, 11 rats served in the THLX group and 16 rats
served in group SHAM (8 PBS and 8 non-surgical controls). In Experiment 2, the data from 8 rats were eliminated from the analyses, 7 because of misplaced lesions and 1 due to a failure to lick in the apparatus. Thus, 9 THLX rats and 12 SHAM rats contributed data to Experiment 2.

Lesions were assessed at four levels that correspond roughly to Figures 31 - 34 in Paxinos and Watson (37). Level I is considered pre-taste and occurs at the final point where the medial lemniscus splits. Level II demarks the point at which taste cells first spill into the thalamus. The fasciculus retroflexus (FR) is just evident. Level III is thought to fall right in the middle of the thalamic taste area. At this level the FR and the parafascicular nucleus (PF) are frankly evident. Level IV is best demarkated by the FR, which runs in long parallel lines dorsal to ventral, and is thought to be the location of the most posterior taste cells in this nucleus. An example of the brain of a PBS treated control at Level III of the gustatory thalamus is shown in Figure 1, Panel A. As demonstrated here, the PBS injected brains sustained no evidence of damage, aside from an occasional electrode track.

All 20 rats in the THLX group that contributed data in Experiments 1 and 2 had complete bilateral damage to Levels II, III, and IV of the VPMpc. Most lesions extended beyond the boundaries of the VPMpc to include parts of the reuniens (RE), centromedial (CM), ventromedial (VM), paracentral (PC), centrolateral (CL), ventral posteromedial (VPM), posterior (Po), parafascicular (PF), subparafascicular (SPF), and mediodorsal (MD) thalamic nuclei. Damage to these structures was, at most partial, with the exception of the centromedial nucleus which was damaged completely and bilaterally in all but a few lesioned rats. The VPMpc, then, is the only structure that sustained complete bilateral damage in all lesioned subjects. The data from one THLX rat in Experiment 1 and Experiment 2 are shown in Figure 1, Panel B and Panel C, respectively. The subject shown in
Panel B had bilateral damage to the VPMpc that was both symmetric and complete. Damage extended into the VPM and, to a lesser extent, into the Po. The PF nucleus also sustained partial damage, but only on the left. Otherwise, neighboring nuclei were largely spared. Damage to the VPMpc also was bilateral and complete for the subject shown from Experiment 2 in Panel C. The Po and PF nucleus were spared on the left, but partially damaged, along with the VPM, on the right. The CM was fully damaged on the right and partially damaged on the left.

**Behavior (Experiment 1).** The intake data (total licks/3 min) and the latency (sec) to make the first lick were averaged into two-day blocks for bottle 1 and for bottle 2 and were analyzed using 2 x 2 x 7 repeated measures analyses of variance (ANOVAs) varying lesion (SHAM vs. THLX), US (saccharin vs. sucrose), and blocks (1 – 5 or 7)). Post hoc tests were conducted, where appropriate, using Newman Keuls tests with alpha set at .05.

**CS Intake (Bottle 1):** The SHAM rats suppressed intake of the saccharin CS when paired with the preferred sucrose reward, whether 5 min or zero sec elapsed between CS and US presentation. The suppressive effect of the sucrose US, however, was reversed in rats with bilateral ibotenic acid lesions of the gustatory thalamus when testing began in Phase I with a 5 min ISI. When switched after an 18 day interval to the zero sec ISI in Phase II, the anticipatory contrast effect obtained in the SHAM rats was eliminated, but was not reversed in the THLX rats. These conclusions were supported by the following statistical analyses.

**Phase I: 5 min ISI.** When tested in the 5 min ISI condition, an anticipatory contrast effect was demonstrated by the SHAM animals, see Figure 2, left panel.

------------------ Insert Figure 2 About Here ------------------
The SHAM rats that were given a sucrose US (Group Sac-Suc) consumed less first bottle saccharin than the SHAM rats in the Sac-Sac condition. The THLX rats, on the other hand (see Figure 2, right panel), demonstrated a reinforcement effect such that the rats in the Sac-Suc condition actually licked more of the first bottle 0.15% saccharin solution than the Sac-Sac THLX controls. These effects were confirmed by post hoc Newman Keuls tests of a significant Lesion x US x Block interaction, $F_{(6,138)} = 3.28, p < .005$. Thus, SHAM rats in the Sac-Suc condition made fewer licks for first bottle saccharin than their Sac-Sac controls and this effect attained statistical significance on blocks 2, 4, and 5, $p_s < .05$. Rats in the THLX group, in comparison, made significantly more licks for first bottle saccharin when it predicted access to the preferred sucrose reward across blocks 2 – 7, $p_s < .05$. Finally, it should be noted that the THLX rats in the Sac-Sac condition made signficantly fewer licks of first bottle saccharin than the SHAM rats in the same condition and this effect was significant on blocks 2 – 7, $p_s < .05$.

**Phase II: Zero sec ISI.** The contrast effect that was obtained when using the 5 min ISI in the SHAM rats in Phase I persisted when the ISI was swtiched to a zero sec ISI in Phase II of the experiment, see Figure 3, left panel.

The reinforcement effect obtained in the THLX rats, on the other hand, was eliminated, see Figure 3, right panel. Post hoc tests of a signficant Lesion x US interaction, $F_{(1,23)} = 5.51, p < .03$, confirmed that the contrast effect remained signficant in the SHAM rats (i.e., SHAM rats in the Sac-Suc condition made fewer licks for the saccharin cue overall than did SHAM rats in the Sac-Sac condition). The reinforcement effect in the THLX rats, however, was abolished, $p > .05$. Taken together, these data show that cells intrinsic to the VPMpc, rather than fibers of passage, are required for the comparison of disparate natural rewards over time. Bilateral ibotenic acid lesions
of the gusatory thalamus reversed the ACE when using a 5 min ISI and eliminated the ACE when using a standard zero sec ISI. The occurrence of the reinforcement effect in Phase I with these THLX rats is important as it stands as evidence that the lesion-induced deficit in ACE is not due to a simple inability to associate the CS with the US.

**Latency to Lick (Bottle 1):**

*Phase I: 5 min ISI.* Analysis of the latency (sec) to lick bottle 1 saccharin showed that neither the SHAM nor the THLX rats demonstrated a significant contrast effect or reinforcement effect in latency (see Table 1). This conclusion was supported by a non-significant Lesion x US x Block interaction, $F < 1$.

*Phase II: Zero sec ISI.* As in Phase I, rats failed to evidence either a contrast or a reinforcement effect in the latency to initiate licking the saccharin cue as evidenced by a nonsignificant Lesion x US interaction, $F (1,23) = 2.18, p < .15$, and Lesion x US x Block interaction, $F (4,92) = 1.0, p < .4$ (see Table 1).

**US Intake (Bottle 2 Licks):**

*Phase I: 5 min ISI.* The main effect of US was significant, $F (1,23) = 160.5, p < .0001$, indicating that both SHAM and THLX rats made more licks for second bottle 1.0 M sucrose than 0.15% saccharin overall, see Figure 4.

The main effect of lesion also was significant, $F (1,23) = 6.08, p < .03$, showing that the THLX rats made fewer licks than the SHAM rats overall. Neither the Lesion x US, $F (1,23) = 2.96, p = .09$, nor the Lesion x US x Block, $F (6,138) = 1.48, p = .19$, interaction attained statistical significance. Even so, the significant main effect of lesion on intake appears to be influenced by differences in the ingestion of second bottle saccharin. As a consequence, the number of licks made for second
bottle saccharin was reanalyzed across the SHAM and the THLX rats and the results of this analysis showed a highly significant Lesion x Block interaction, \( F(6,72) = 4.70, p < .0004 \). Post hoc tests revealed that the THLX rats made fewer licks for second bottle saccharin than the SHAM rats on blocks 2 – 7, \( ps < .05 \). A similar analysis of the number of licks made for second bottle 1.0 M sucrose, in comparison, found no differences in intake between the SHAM and THLX rats, \( F_s < 1 \).

**Phase II: Zero sec ISI.** Again, these patterns of behavior persisted when the rats were switched to the zero sec ISI, see Figure 5.

The main effect of lesion was significant, \( F(1,23) = 9.60, p < .006 \), with the THLX rats consuming less than the SHAM rats overall. The main effect of US also was statistically significant, \( F(1,23) = 278.1, p < .0001 \), such that all rats made more licks for second bottle sucrose than for second bottle saccharin. Neither the Lesion x US, \( F < 1 \), nor the Lesion x US x Block, \( F(4, 92) = 2.43, p < .053 \), interaction was significant. Once again, given that the significant main effect of lesion appeared to be influenced by differences in saccharin intake, an additional 2 x 5 ANOVA was conducted on second bottle saccharin intake varying lesion and blocks. The results of this analysis revealed a significant interaction, \( F(4,48) = 4.63, p < .003 \), and post hoc tests showed that the THLX rats in the Sac-Sac condition made fewer licks for second bottle saccharin than the SHAM rats in the Sac-Sac control group on Blocks 2, 4, and 5, \( ps < .05 \). A similar analysis of second bottle sucrose intake also found a significant 2 x 5 interaction, \( F(4,44) = 6.09, p < .0005 \), and post hoc tests indicated that THLX rats made fewer licks for second bottle sucrose on blocks 4 and 5, \( ps < .05 \), than the similarly treated SHAM rats. This decline in licks made for the 1.0 M sucrose
solution during the terminal blocks was carried by one sick animal. These data were not eliminated from the analysis, however, because they did not affect the interpretation of the results.

Latency to Lick (Bottle 2):

*Phase I: 5 min ISI.* Analysis of the bottle 2 latency data revealed a significant main effect of US, $F_{(1,23)} = 5.02, p < .05$, whereby both control and THLX rats initiated licking more quickly for second bottle sucrose than for second bottle saccharin (see Table 1). Neither the main effect of lesion, $F_{(1, 23)} = 1.37, p = .25$, nor any interaction thereof, was statistically significant, $Fs < 1$

*Phase II: Zero sec ISI.* As in Phase I, a significant main effect of US, $F_{(1,23)} = 6.39, p < .02$, showed that both control and THLX rats were faster to make the first lick of second bottle sucrose than second bottle saccharin (see Table 1). Other factors and interactions, however, were not significant including: the main effect of lesion, $F_{(1,23)} = 3.15, p = .09$, the Lesion x US interaction, $F_{(1,23)} = 1.82, p = .19$, and the Lesion x US x Block interaction, $F < 1$ The latency data parallel the intake data by showing that the THLX rats, like the SHAM rats, can appropriately detect and respond to the absolute rewarding properties of the saccharin and sucrose US. Thus, while the THLX rats drank less second bottle 0.15% saccharin than the SHAM rats, both groups exhibited a clear magnitude of reinforcement effect by making more licks for 1.0 M sucrose than 0.15% saccharin and by initiating licking more quickly for the stronger solution.

Behavior (Experiment 2).

**CS Intake (Bottle 1):** As with the results of Experiment 1, lesions of the thalamus eliminated, but did not reverse the ACE when testing began with a zero sec ISI in Phase I. When switched to the 5 min ISI in Phase II, however, a reinforcement effect in CS intake became immediately apparent in these same experimental subjects (i.e., the reinforcement effect became
evident following a single CS-US pairing). Statistical support for these conclusions is provided below.

**Phase I: Zero sec ISI.** An anticipatory contrast effect was demonstrated by the SHAM animals when testing began with the zero sec ISI, see Figure 6, left panel, and this ACE was eliminated by lesions of the gustatory thalamus.

These conclusions were confirmed by post hoc tests of a significant Lesion x US interaction, $F(1,17) = 14.55$, $p < .002$, and Lesion x US x Block interaction, $F(7,119) = 4.59$, $p < .0002$. Post hoc tests of the 3-way interaction showed that SHAM rats in the Sac-Suc condition made fewer licks for first bottle saccharin than their Sac-Sac controls on blocks 3 – 8, $p < .05$. Rats in the THLX group, on the other hand, showed no significant differences in licks generated for first bottle saccharin as a function of US condition, $p > .05$. Finally, it should be noted that the THLX rats in the Sac-Sac condition, once again, made significantly fewer licks for first bottle saccharin than the SHAM rats in the same condition and this effect was statistically significant on blocks 3 – 8, $p < .05$.

**Phase II: 5 min ISI.** As alluded to above, the contrast effect obtained in the SHAM rats in Phase I (zero sec ISI) persisted when the ISI was switched to 5 min in Phase II of the experiment. The ACE in similarly treated THLX rats, on the other hand, tended to be reversed, see Figure 7.

Post hoc tests of a significant Lesion x US interaction, $F(1,17) = 10.74$, $p < .005$, confirmed that the contrast effect remained significant in the SHAM rats (i.e., SHAM rats in the Sac-Suc condition made fewer licks for the saccharin cue overall than did the SHAM rats in the Sac-Sac condition). Post hoc tests on this same interaction, however, confirmed that the reinforcement effect did not
attain statistical significance for the THLX rats, p > .05. Neither the main effect of US, F (1,17) = 1.24, p < .28, nor the Lesion x US x Block interaction, F (3,51) = 1.7, p < .17, was statistically significant. Finally, as has been the case previously, additional post hoc tests of the significant Lesion x US interaction showed that THLX rats in the Sac-Sac condition made fewer licks for first bottle saccharin than did their SHAM lesioned counterparts, p < .05.

In order to more closely investigate intake of first bottle saccharin in the SHAM and THLX rats, these same data were unblocked and graphed across each of the 8 individual trials, see Figure 8, right panel.

The data were analyzed using a 2 x 2 x 8 ANOVA varying lesion, US, and trials (1-8). The results of this analysis revealed a significant Lesion x US interaction, F (1,17) = 10.74, p < .005, and post hoc tests confirmed that, while the SHAM rats exhibited a significant contrast effect, the THLX rats demonstrated a significant reinforcement effect overall, ps < .05. The Lesion x US x Trials interaction did not attain statistical significance, p > .05. Even so, in an effort to track the rapidity with which the reinforcement effect emerged, a separate 2 x 2 ANOVA was conducted for the THLX rats varying US and trials (1 – 2 only). Post hoc Neuman Keuls tests of a highly significant US x Trials interaction, F(1,7) = 14.77, p< .007, confirmed that the THLX animals in the Sac-Suc condition increased licks for the first bottle saccharin solution from Trial 1 to Trial 2, p < .05. Intake of the same solution for the THLX rats in the Sac-Sac condition, on the other hand, did not change from Trial 1 to Trial 2, ps < .05. Indeed, on Trial 2, the THLX animals made more licks for first bottle saccharin when it predicted access to sucrose than when it predicted access to more saccharin, p < .05. Thus, once having experienced the 5 min ISI for the first time on Trial 1, the THLX rats demonstrated a reinforcement effect as of Trial 2.
Latency to Lick (Bottle 1):

*Phase I: Zero sec ISI.* Analysis of the latency (sec) to lick first bottle saccharin showed that neither the SHAM nor the THLX rats demonstrated a contrast effect or a reinforcement effect (see Table 1). This conclusion was supported by a non-significant Lesion x US x Block interaction, $F < 1$. A significant main effect of Lesion, $F(1,17) = 5.22$, $p < .04$, however, indicated that THLX rats were slower to initiate licking than were control rats overall.

*Phase II: 5 min ISI.* As in Phase I, there was no contrast effect in latency for either group. To the contrary, post hoc tests of a significant Lesion x US x Block interaction, $F(3,51) = 4.20$, $p < 0.01$, indicated that, on Block 2, THLX animals were, in fact, faster to make the first lick for first bottle saccharin when it predicted access to sucrose than when it predicted access to more saccharin (see Table 1). Neither the main effect of Lesion, $F(1,17) = 1.12$, $p = .3$, nor the Lesion x US interaction, $F(1,17) = 1.88$, $p = .19$, was statistically significant. This reinforcement effect in the latency to lick first bottle saccharin when it predicts subsequent access to sucrose provides further evidence that rats with lesions of the gustatory thalamus can associate the saccharin CS with the sucrose US and can anticipate the availability of the US upon CS presentation.

US Intake (Bottle 2):

*Phase I: Zero sec ISI.* The main effect of US was significant, $F(1,17) = 134.1$, $p < .0001$, indicating that all rats made more licks for second bottle 1.0 M sucrose than for 0.15% saccharin overall, see Figure 9.

The main effect of Lesion was not significant, $F < 1$, confirming that there was no difference in overall consumption between the THLX and the SHAM rats. Furthermore, neither the Lesion x US, $F < 1$, nor the Lesion x US x Block, $F < 1$, interaction attained statistical significance. As in
Experiment 1, the number of licks made for second bottle saccharin was analyzed alone for the SHAM and the THLX animals. In this case, neither the main effect of Lesion, $F(1,8) = 1.46$, $p < .26$, nor the Lesion x Block interaction, $F < 1$, was statistically significant. A similar analysis of the number of licks made for second bottle 1.0 M sucrose also found no differences in intake between the SHAM and the THLX rats, $F_s < 1$. Thus, the THLX animals detected and responded appropriately to both saccharin and sucrose.

**Phase II: 5 min ISI.** Again, these patterns of behavior persisted when the rats were switched to the 5 min ISI, see Figure 10.

As in Phase I, the main effect of Lesion was not statistically significant, $F < 1$, showing that there was no difference in consumption between the THLX and the SHAM rats overall. In addition, neither the Lesion x US nor the Lesion x US x Block interactions reached statistical significance ($F_s < 1$). Once again, the number of licks made for second bottle saccharin and second bottle sucrose was analyzed separately across lesion condition. The results of these analyses found that neither the main effect of lesion nor the Lesion x Block interaction was significant, $F_s < 1$. Thus, at least in this instance, the THLX rats made as many licks for second bottle saccharin and second bottle sucrose as did the SHAM rats.

**Latency to Lick (Bottle 2):**

**Phase I: Zero sec ISI.** Analysis of the bottle 2 latency data revealed a significant main effect of US, $F(1,17) = 9.14$, $p < .008$, whereby all rats were found to initiate licking more quickly for second bottle sucrose than for second bottle saccharin (see Table 1). Consistent with the findings from Experiment 1, these data show that the THLX rats, like the SHAM rats, can appropriately detect and respond to the absolute rewarding properties of the saccharin and the sucrose US. The
main effect of lesion, the Lesion x US interaction, and the Lesion x US x Block interaction all failed to attain statistical significance, $F_s < 1$.

**Phase II: 5 min ISI.** As in Phase I, a significant main effect of US, $F(1,17) = 12.73, p < .003$, showed that both the SHAM and the THLX rats were faster to make the first lick of second bottle sucrose than second bottle saccharin (see Table 1). Once again, the main effect of Lesion, $F < 1$, the Lesion x US interaction, $F < 1$, and the Lesion x US x Block interaction, $F(3, 51) = 1.58, p = .21$, were not statistically significant.

**General Discussion**

In two experiments, SHAM rats reduced intake of a saccharin cue when it predicted access to a preferred 1.0 M sucrose solution. This anticipatory contrast effect was evident in the SHAM rats whether testing began or ended with a zero second or a 5 min ISI. As such, these data are consistent with published data showing that intact rats will suppress intake of a saccharin cue whether it predicts immediate (zero or 15 sec) or delayed (e.g., 5, 10, or 30 min) access to a preferred sucrose reward (2, 7, 10). It should be noted, however, that while the SHAM rats in our experiment appropriately reduced intake of a saccharin cue when having to wait 5 min for access to the preferred sucrose reward, the magnitude and the persistence of the ACE was somewhat reduced for these subjects in the 5 min, relative to the zero sec, ISI condition. That is, while for SHAM rats, the ACE was significant on Blocks 3 - 8 when testing began with the zero sec ISI in Experiment 2, the ACE was significant only on Blocks 2, 4, and 5 when testing began with the 5 min ISI in Experiment 1. Similarly, when switched from the zero sec to the 5 min ISI in Experiment 2, the ACE failed to attain statistical significance on the 4th two-day block in these subjects. A similar, slight diminuation in the magnitude of the ACE has been reported for intact rats (3, 7, 29) when access to a saccharin CS was followed by access to a sucrose US across increasing ISI
conditions. Thus, even in intact rats where the ACE is robust, the magnitude of the effect can be slightly reduced by a reinforcement effect when hungry rats have to wait for access to a preferred sucrose reward.

Bilateral ibotenic acid lesions of the gustatory thalamus fully prevented the development of the ACE when access to the saccharin CS was immediately followed by access to the preferred sucrose reward (i.e., when tested with the zero sec ISI). Moreover, the ACE was not only eliminated, but actually was reversed, when hungry THLX rats had to wait 5 min for access to a preferred sucrose solution. This pattern was obtained when testing began with the 5 min ISI in Experiment 1 and, though somewhat smaller in magnitude, when switched to the 5 min ISI in Experiment 2. Thus, in two experiments, bilateral ibotenic acid lesions of the gustatory thalamus eliminated the ACE when testing began or ended with the zero sec ISI and reversed the ACE when testing began or ended with the 5 min ISI. Damage to cell bodies in the taste thalamus, then, can lead to either an elimination or to a reversal of the ACE and the reversal of the effect (the reinforcement effect) is most reliable and most robust when hungry THLX rats are required to wait 5 min for access to the preferred sucrose reward.

Before considering why rats with bilateral lesions of the gustatory thalamus might exhibit a reinforcement effect when tested with a 5 min ISI, it is reasonable to address the default condition whereby this lesion fully prevents the establishment of an ACE when access to the saccharin cue is immediately followed by access to the preferred sucrose reward. As described, the establishment of an ACE requires that the rat: (1) appropriately detect and respond to the saccharin and sucrose solutions, (2) associate the saccharin cue with the sucrose US, (3) remember the ‘value’ of the sucrose US upon CS presentation, (4) compare the value of the saccharin cue with the memory of the sucrose US, and (5) reduce intake of the saccharin cue as a
result of this reward comparison process. First, although saccharin intake has not been assessed across a range of concentrations in THLX rats, the disruption in contrast is not likely due to either a detection or a perception deficit. A detection deficit would seem an unlikely possibility because, as discussed, rats with similar lesions have been found to respond appropriately to representatives of the four basic tastants in both short and longer term intake tests (20, 39, 45). While there is some support for the alternative hypothesis that the THLX rats might suffer from a perceptual deficit (e.g., they may make fewer licks for saccharin than the SHAM rats because they perceive the solution as more aversive than intact rats), it should be noted that the failure to reduce intake was not due to a floor effect because there remained ample room for suppression by the THLX rats in the Sac-Suc condition. In addition, Reilly et al. (38) reported a similar lesion-induced deficit in ACE in THLX rats that did not evidence a reduction in baseline licking for saccharin. Finally, ACEs readily develop even when the saccharin CS is adulterated with an aversive quinine solution (10). Thus, it would appear unlikely that the failure to reduce CS intake by the lesioned rats relates to simple differences in the detection or perception of either the saccharin CS or the sucrose US.

Second, data argue against the conclusion that the THLX rats failed to suppress intake of the saccharin cue because they failed to associate the saccharin CS with the sucrose US or because, once having made the association, the saccharin CS failed to elicit the memory or the ‘representation’ of the sucrose US. As discussed above, published data show that rats with bilateral lesions of the gustatory thalamus readily form taste-taste, taste-illness, and odor-illness associations (20, 39, 45). Further, the THLX rats in the present report exhibited a clear reinforcement effect in both bottle 1 licks and latency when having to wait 5 min for access to the preferred sucrose reward. This stands as evidence that these animals can associate the saccharin cue with the sucrose consequence and that presentation of the saccharin cue must elicit
anticipation of (i.e., the memory for) the preferred sucrose reward. There is, then, no support for an associative deficit or for the suggestion that the saccharin CS fails to elicit the memory of the preferred sucrose reward that is anticipated in the near future.

A third consideration is that rats with bilateral lesions of the gustatory thalamus fail to exhibit an ACE because, once having associated the CS with the US, they fail to compare the relative value of the two disparate rewards. In support, rats with lesions of the gustatory thalamus do not only fail to demonstrate an ACE, but also a successive negative contrast effect which occurs when intact rats are unexpectedly shifted from a greater to a lesser reward (40, 42, 43). In this case, intake of the lesser 0.15% saccharin reward is reduced in the lesioned rats when downshifted from a 1.0 M sucrose solution, but only to the level of the unshifted saccharin controls (43). Oddly, while fully impaired in these two contrast paradigms, rats with similar lesions of the gustatory thalamus exhibit perfect simultaneous contrast effects when given the opportunity to compare the same saccharin and the sucrose solutions closely in time in the same daily session (41). Indeed, the clear lack of dependence upon the gustatory thalamus in this basic reward comparison paradigm is underscored by the fact that decerebrate rats (rats with an intact brainstem, but no neural connection between the brainstem and the forebrain) also exhibit normal simultaneous contrast effects (22). The gustatory thalamus, then, does not appear to be the seat of reward comparison, per se.

If the gustatory thalamus is not the “seat” of reward comparison, then why do rats with lesions of this structure fail to exhibit an ACE? An answer to this question might be provided, in part, by considering that which is common between an ACE and a successive negative contrast effect (both disrupted by the lesion) and how, in turn, these phenomena differ from simultaneous contrast effects (which are not disrupted by the lesion). Each of these phenomena involves the
comparison of disparate levels of reward in food deprived rats. Indeed, in each case, the role of 
the gustatory thalamus has been assessed when comparing 0.15% saccharin to 1.0 M sucrose. In 
the simultaneous contrast paradigm, the rewards are compared within a daily session over a 
relatively short time frame (5, 14). Specifically, the rats are given a total of 3 alternating 60 sec 
access periods to each of the two levels of reward within a daily session (41). Intake during these 
alternating trials is then compared to intake on other days when the rats are given 6 repeated 60 
sec access periods to only one of the levels of reward (referred to as continuous trials). A 
simultaneous negative contrast effect occurs when intake of the lesser reward (e.g., 0.15% 
saccharin) is reduced on trials when alternated with access to the greater reward (e.g., 1.0 M 
sucrose) compared to continuous trials where only the lesser of the two rewards is presented. This 
phenomenon does not appear to be due to simple receptor adaptation because simultaneous 
contrast effects persist when as many as 8 min elapse between successive stimulus 
presentations(38). In addition, Grigson, Cornelius, and Reich (unpublished data) found that 
simultaneous contrast effects also persist unchanged when rats are required to rinse their tongues 
with water between each successive stimulus presentation and when access is alternated 
between two gustatory stimuli (i.e., sucrose and Polycose) that bind to two distinct receptor 
populations in the oral cavity (31, 46). Thus, when taken together, the evidence suggests that 
simultaneous contrast is a brainstem mediated reward comparison process involving short-term 
memory, not adaptation of peripheral taste receptors (22).

As discussed briefly, a successive negative contrast effect is a multistage process that 
occurs when rats, with a history of experience (e.g., 5 min/day for 10 days) with a highly preferred 
sucrose solution, such as 32% sucrose, are unexpectedly downshifted to a lesser reward, such as 
4% sucrose or 0.15% saccharin (for discussion see 4, 43). Stage I, which typically occurs on the
first postshift day, involves detection, rejection, and a search for the missing reward. According to Flaherty (4), the shifted rats appear to “detect” the lesser reward in 10 – 20 sec after which licking stops and the rats begin to search for the missing reward. At this point, contrast is associated with an increase in the number of bursts initiated (a burst was defined as a run of licks without a 500 msec pause), but fewer licks per burst (24), an increase in arm entries in a radial arm maze, and an immediate approach to a second arm where the preferred reward previously was presented (4). The second stage, which generally occurs on the second postshift day, involves conflict where a hungry rat (having failed to find the missing reward) must choose whether to accept a reward of lesser value. In accordance, it is during this stage that circulating corticosterone levels are elevated (6, 30) and treatment with anxiolytics is effective in attenuating contrast (12, 15). The final stage occurs over the next couple of postshift days and involves recovery from contrast (i.e., acceptance of the lesser reward).

The differences between a simultaneous contrast effect and a successive negative contrast effect are not difficult to see. As stated, the simultaneous contrast effect depends upon comparing the two disparate levels of reward within a single daily session involving, at most, short-term memory processes. The successive negative contrast effect, on the other hand, is a multistage phenomenon that depends upon comparing an available level of reward (4% sucrose or 0.15% saccharin) with the memory of a preferred sucrose reward (32% or 1.0 M sucrose) that was received 24 h earlier. A simple short-term/long-term memory distinction, however, likely will not account for the dissociation in the lesion data as Reilly and Trifunovic (42) showed that thalamic lesions disrupted successive negative contrast effects even when the downshift occurred only 7.5 min (rather than 24 h) after the final access period to the preferred 1.0 M sucrose reward and simultaneous negative contrast effects, in turn, remain intact in THLX rats even when as many as 8
min elapsed between alternating access periods to the two disparate levels of reward within a given daily session (38).

At first blush, there also are several differences between ACE and successive negative contrast, making it difficult to see why the thalamic lesion might disrupt both phenomenon. Successive negative contrast involves a loss of an expected reward (a retrograde comparison) and anticipatory contrast (see 16) involves a decrease in responsiveness for the lesser reward in anticipation of the future availability of the preferred sucrose reward (an anterograde comparison). That is, while the memory for a preferred reward received 24 h earlier can, under some circumstances, reduce intake of the saccharin CS (47), the reduction in CS intake in the ACE paradigm is primarily carried by anticipation of the availability of the preferred reward that is expected in the very near future (16). Successive negative contrast effects are associated with conflict, as the hungry rat considers whether to accept a lesser reward. As stated, it is at this point that corticosterone levels are elevated and benzodiazepines attenuate the successive negative contrast effect. There is no evidence that anticipatory contrast involves conflict and, in accordance, treatment with benzodiazepines is not effective in reducing the magnitude of an anticipatory contrast effect (2). Rats recover from successive negative contrast effects over trials, where anticipatory contrast effects develop throughout testing. Finally, as further evidence that these two phenomena differ, rats bred for sensitivity to successive negative contrast were not found to differ when tested in the anticipatory contrast paradigm (13). Strain differences, however, may have been revealed if tested using a more sensitive (i.e., less robust) anticipatory contrast paradigm (25).

In spite of the clear differences between ACE and successive negative contrast, two similarities can be noted. A first similarity between the two phenomena relates to foraging. When
an animal suddenly receives a lower reward than expected in the successive negative contrast paradigm, the animal begins to search for the missing reward. Flaherty (4) described this when his rats, after having been shifted from 32% to 4% sucrose, increased entries into other arms of an 8-arm radial maze and rapidly entered one particular arm where they had, on occasion, received the preferred 32% sucrose reward. A similar behavioral response was described in 1928 by Tinklepaugh (49) when his monkey searched about after having unexpectedly received a piece of lettuce in place of a piece of banana. Although different from the searching behavior described in successive negative contrast, the anticipatory contrast paradigm has been described as an animal model of foraging (28, 48). Specifically, “when prey frequency in the current patch falls below the expected average or frequency of prey, the animals may give up on the current patch and move on to other locations” (for a discussion see 11, page 80). This is not to suggest that the ACE is due solely to competing responses because, although the effect is larger when the CS and US are presented at separate locations, ACEs still occur in the experimental chambers when the CS and the US are presented at the same spatial location or ‘patch’ (7, 11). When presented in opposite arms of a T-maze, however, the reduction in saccharin intake in the left arm is accompanied by (actually preceded by) an increase in time spent in the right arm where the preferred sucrose reward will be presented (11). Thus, both phenomena involve a giving up of the available, lesser reward to search for the missing (successive negative contrast) or the anticipated (anticipatory contrast) preferred reward. In light of these data, one might hypothesize that rats with lesions of the gustatory thalamus may be able to compare the available reward with the memory of the preferred reward, but they simply fail to “give up” the lesser reward in search of the greater reward (i.e., they may be impulsive). This hypothesis (which is readily testable) would have to assume, however, that simultaneous contrast effects (which are not disrupted by the lesion) either do not
depend upon foraging/searching or that an intact gustatory thalamus is not necessary for “giving up” an available lesser reward when alternated over very similar time periods with access to the preferred reward in the same daily session. While not impossible, this seems an unlikely conclusion.

Second, although the comparison processes are different (one retrograde and one anterograde), both anticipatory contrast and successive negative contrast involve comparison of an available reward with some ‘engram’ or memory. In successive negative contrast, the rat adjusts its response to a lesser reward (4% sucrose or 0.15% saccharin) because it expects (on the basis of prior experience) to have received access to a highly preferred 1.0 M sucrose solution. In anticipatory contrast, the rat adjusts its response to the lesser reward because it is anticipating (on the basis of prior experience) access to the preferred 1.0 M sucrose solution in the near future. In each case, then, the response to an available reward changes as it is compared to the memory of an alternative reward. As such, the lesion may disrupt anticipatory contrast and successive negative contrast because an intact gustatory thalamus is necessary to compare the value of an available reward with the long-term memory of an alternative reward. This interpretation would have to suggest that THLX rats failed to exhibit a successive negative contrast effect even when downshifted 7.5 min following a final access period to the preferred 1.0 M sucrose reward (42) because contrast, in this case, depended upon comparison with the long-term memory of the preferred reward, not with the characteristics of the stimulus that was received 7.5 min earlier. Were the important comparison between the available reward and the characteristics of the stimulus received 7.5 min earlier, then this would have been a simultaneous, rather than a successive, contrast paradigm and the contrast behavior of the THLX rats should not have been impaired (41). According to this account, while the taste cells in the VPMpc are not critical for
memory (THLX rats demonstrate a reinforcement effect) or for reward comparison (the lesion does not disrupt simultaneous contrast), per se, intact taste cells in the VPMpc are essential for comparing the value of an available gustatory stimulus with the long-term memory of a preferred reward. As such, the lesion-induced deficit is very specific.

Finally, consideration must be given to the overresponding that was obtained in the lesioned rats when using the 5 min ISI. Why do the lesioned rats overrespond under these circumstances and what does it mean? As discussed, overresponding to the CS has been obtained in intact rats in the ACE paradigm, but only under very specific circumstances. In the first example (18), a reinforcement effect occurred when the CS had no intrinsic reward value (e.g., when a hungry rat licked an empty spout or water prior to gaining access to the preferred sucrose reward). In the second case (10), intact Sprague-Dawley rats overresponded for a 2% sucrose CS when, after a 15 sec ISI, an 8% sucrose reward was presented. This finding showed that overresponding can occur when using a short ISI, but only when value of the CS approaches that of the US. A similar trend was obtained when access to 0.125% saccharin predicted access to 0.15% saccharin (17). A final instance of overresponding for the CS in the anticipatory contrast paradigm occurred when the rat was hungry, the CS had caloric value (i.e., 2% sucrose), and the animal had to wait 5 min for access to a preferred 32% sucrose reward (10).

The first of these three options (use of a neutral CS) does not provide a ready explanation because, while the THLX rats consumed less of the 0.15% saccharin solution than the SHAM rats, the THLX rats made many more licks for this solution than were reportedly made for water or on an empty bottle (18). The saccharin solution does not appear to be a neutral stimulus. The second possibility that overresponding occurred in the THLX rats because the value of the saccharin cue approached that of the sucrose reward also is not plausible because the 2nd bottle lick data show
that the THLX rats, like the SHAM rats, clearly distinguished between the two stimuli by making
many more licks for 2\textsuperscript{nd} bottle sucrose than for 2\textsuperscript{nd} bottle saccharin. Finally, though not perfectly
concordant, there is overlap with the third instance as outlined by Flaherty et al. (10). The rats in
the present report were food deprived and they had to wait 5 min for access to the preferred
sucrose reward. The saccharin CS, however, did not have caloric value. The thalamic lesioned
rats, then, were more sensitive to induction (i.e., more likely to exhibit a reinforcement effect) when
food-deprived and when having to wait 5 min for access to the preferred sucrose reward, even
when the CS was devoid of calories. Again, why might this be so? By way of explanation, Flaherty
and Grigson (9) obtained evidence for competition between the expression of a reinforcement
effect and the expression of an anticipatory contrast effect. Indeed, evidence of such competition
was provided here, as the ACE in the SHAM rats was slowly degraded by an apparent
reinforcement effect in the 5 min ISI condition. Thus, the lesion-induced disruption in contrast may
contribute to overresponding in the 5 min ISI condition because, in the absence of contrast, the
reinforcement effect stands unopposed.

In summary, bilateral ibotenic acid lesions of the gustatory thalamus eliminate the
development of an anticipatory contrast effect when tested using a zero sec ISI and reverse it
when tested with a 5 min ISI. These data verify that cell bodies in the gustatory thalamus, not
fibers of passage, are essential for the establishment of an ACE. In addition, the data suggest that
the failure to suppress intake of the saccharin cue following saccharin-sucrose pairings in THLX
rats is not due to a failure to detect or respond appropriately to either the saccharin CS or the
sucrose US. The disruption also is not due to an associative deficit. The reinforcement effect in
bottle 1 licks and latency confirms that the THLX rats are able to use the saccharin cue to predict
access to the preferred sucrose reward. The gustatory thalamus is not thought to be essential for
reward comparison, per se, because these same lesioned rats can readily compare rewards when presented in close temporal proximity in the simultaneous contrast paradigm. Finally, the ‘engram’ is assumed to be intact because the THLX rats over-consume the saccharin cue in anticipation of the future availability of sucrose – suggesting that they remember the stimulus characteristics of the coming reward. Although the lick and latency data suggest that the memory for the US is likely intact, the complexion of data suggest that rats with lesions of the gustatory thalamus fail to demonstrate an ACE (and a successive negative contrast effect) because the rats fail to compare the available lesser reward with the memory of the preferred reward. It is, therefore, both a reward comparison and a memory deficit.

An intact gustatory thalamus, then, is essential for comparing the value of different rewards over time, particularly when comparing the value of an available reward with the memory of a preferred reward. Indeed, the involvement of this structure is not limited to the comparison of sapid stimuli (i.e., natural rewards). Rats with these same lesions also fail to avoid intake of a saccharin CS when it predicts the future administration of morphine (23). These data are consistent with other reports where head direction cells in the anterodorsal thalamus anticipate the future direction of the head (1), cells in the posterior thalamus anticipate the value of a sucrose or an intracranial self-stimulation reward on the basis of the presentation of auditory and visual cues (26), and abnormal methylphenadate-induced activation of the thalamus (possibly the mediodorsal nucleus) as measured by PET is associated with greater cocaine craving (anticipation of cocaine) in cocaine-dependent subjects (50). Taken together, the data suggest that the thalamus is involved in the anticipation and the comparison of rewards over time and this reward comparison process, which can be retrograde (expectancy) or anterograde (anticipation), serves to amplify differences in perceived reward value in an effort to affect the appropriate selection of behavior over time.
Acknowledgements

This research was supported by U.S. Public Health Service Grants DA09815, DA12473, DA15261, DA05932, and DA16512. We thank Han Li and Kathy Matias for their technical support, Anne Baldwin for having read a draft of the manuscript, and Ralph Norgren for his support. These data were first presented at the meeting of the Society for Neuroscience, New Orleans, 2002.
Figure Captions

Figure 1. Low power (2 X) photomicrograph of the gustatory thalamus (tissue was sliced in 50 um sections and stained with cresyl Lecht violet) in a vehicle injected rat (Panel A) and in two rats treated bilaterally with the neurotoxin, ibotenic acid (Panels B & C). The margins of the gliosis are marked in Panels B and C with dotted lines. The arrow in Panel A indicates the track produced by the electrode-pipette assembly. The scale in Panel A equals 1.0 mm and applies to Panels A – C. VPMpc = ventroposteromedial nucleus of the thalamus pars compacta, CL = centrolateral nucleus, VPM = ventral posteromedial nucleus, Po = posterior nucleus, PF = parafascicular nucleus, SPF = subparafascicular nucleus, MD = mediodorsal nucleus, PVP = paraventricular nucleus, FR = fasciculus retroflexus, and 3V = third cerebral ventricle.

Figure 2. Mean (+/- S.E.M.) licks/3 min of first bottle 0.15% saccharin in SHAM and thalamic lesioned (THLX) rats when, in Phase I, daily access to first bottle saccharin was followed 5 min later (5 min ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 7 two-day blocks. * indicates p < .05.

Figure 3. Mean (+/- S.E.M.) licks/3 min of first bottle 0.15% saccharin in SHAM and thalamic lesioned (THLX) rats when, in Phase II, daily access to first bottle saccharin was followed immediately (zero sec ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 5 two-day blocks.

Figure 4. Mean (+/- S.E.M.) licks/3 min of second bottle saccharin or sucrose in SHAM and thalamic lesioned (THLX) rats when, in Phase I, daily access to first bottle saccharin was followed
5 min later (5 min ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 7 two-day blocks.

Figure 5. Mean (+/- S.E.M.) licks/3 min of second bottle saccharin or sucrose in SHAM and thalamic lesioned (THLX) rats when, in Phase II, daily access to first bottle saccharin was followed immediately (zero sec ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 5 two-day blocks.

Figure 6. Mean (+/- S.E.M.) licks/3 min of first bottle 0.15% saccharin in SHAM and thalamic lesioned (THLX) rats when, in Phase I, daily access to first bottle saccharin was followed immediately (zero sec ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 8 two-day blocks. * indicates p < .05.

Figure 7. Mean (+/- S.E.M.) licks/3 min of first bottle 0.15% saccharin in SHAM and thalamic lesioned (THLX) rats when, in Phase II, daily access to first bottle saccharin was followed 5 min later (5 min ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 4 two-day blocks.

Figure 8. Mean (+/- S.E.M.) licks/3 min of first bottle 0.15% saccharin in SHAM and thalamic lesioned (THLX) rats when, in Phase II, daily access to first bottle saccharin was followed 5 min later (5 min ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 8 daily trials.
Figure 9. Mean (+/- S.E.M.) licks/3 min of second bottle saccharin or sucrose in SHAM and thalamic lesioned (THLX) rats when, in Phase I, daily access to first bottle saccharin was followed immediately (zero sec ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 8 two-day blocks.

Figure 10. Mean (+/- S.E.M.) licks/3 min of second bottle saccharin or sucrose in SHAM and thalamic lesioned (THLX) rats when, in Phase II, daily access to first bottle saccharin was followed 5 min later (5 min ISI) by 3 min access to either the same saccharin solution (Sac-Sac) or a preferred 1.0 M sucrose solution (Sac-Suc) over 4 two-day blocks.

Table 1. Table of the mean latency to lick (sec) +/- sem for 1st bottle 0.15% saccharin or 2nd bottle 0.15% saccharin or 1.0 M sucrose for SHAM and thalamic lesioned (THLX) rats in the saccharin-saccharin or saccharin-sucrose condition. The data are shown for Experiment 1a and Experiment 1b, using a 5 min and a zero sec interstimulus interval (ISI), respectively, and for Experiment 2a and Experiment 2b, using a zero sec and then a 5 min ISI.
Phase I: 5 min ISI

**SHAM**
- Sac-Sac
- Sac-Suc

**THLX**
- Sac-Sac
- Sac-Suc

**Mean Bottle 1 Saccharin Intake (licks/3min)**

**2 DAY BLOCKS**

*Figure 2: Schroy et al.*
Phase II: Zero sec ISI

Mean Bottle 1 Saccharin Intake (licks/3min)

Figure 3: Schroy et al.
Figure 4: Schroy et al.

Phase I: 5 min ISI

SHAM

- Sac-Sac
- Sac-Suc

THLX

- Sac-Sac
- Sac-Suc

Mean Bottle 2 Intake (licks/3min)

2 DAY BLOCKS
Phase II: Zero sec ISI

SHAM

Sac-Sac

Sac-Suc

THLX

Sac-Sac

Sac-Suc

Mean Bottle 2 Intake (licks/3min)

2 DAY BLOCKS

Figure 5: Schroy et al.
Figure 6: Schroy et al.
Figure 7: Schroy et al.

Phase II: 5 min ISI

Mean Bottle 1 Saccharin Intake (licks/3min)

2 DAY BLOCKS

SHAM

Sac-Sac

Sac-Suc

THLX

Sac-Sac

Sac-Suc

Figure 7: Schroy et al.
Phase II: 5 min ISI

Figure 8: Schroy et al.
Figure 9: Schroy et al.
Figure 10: Schroy et al.
### Table 1

#### Exp 1a: 5 Min ISI

<table>
<thead>
<tr>
<th>Latency to lick Bottle 1 (sec)</th>
<th>BL 1</th>
<th>BL 2</th>
<th>BL 3</th>
<th>BL 4</th>
<th>BL 5</th>
<th>BL 6</th>
<th>BL 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHAM</strong> sac-sac mean</td>
<td>31.07</td>
<td>6.44</td>
<td>5.98</td>
<td>5.94</td>
<td>5.29</td>
<td>3.86</td>
<td>6.17</td>
</tr>
<tr>
<td>sem</td>
<td>9.92</td>
<td>1.95</td>
<td>1.51</td>
<td>1.17</td>
<td>1.32</td>
<td>0.74</td>
<td>2.05</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>46.31</td>
<td>8.71</td>
<td>8.47</td>
<td>9.38</td>
<td>3.61</td>
<td>7.14</td>
<td>9.14</td>
</tr>
<tr>
<td>sem</td>
<td>14.96</td>
<td>1.71</td>
<td>2.12</td>
<td>3.46</td>
<td>0.53</td>
<td>1.63</td>
<td>4.12</td>
</tr>
<tr>
<td><strong>THLX</strong> sac-sac mean</td>
<td>20.60</td>
<td>8.96</td>
<td>16.28</td>
<td>12.41</td>
<td>6.82</td>
<td>13.45</td>
<td>16.01</td>
</tr>
<tr>
<td>sem</td>
<td>6.07</td>
<td>1.57</td>
<td>4.02</td>
<td>3.65</td>
<td>2.67</td>
<td>5.97</td>
<td>9.32</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>52.81</td>
<td>10.21</td>
<td>6.46</td>
<td>12.92</td>
<td>5.33</td>
<td>5.41</td>
<td>4.14</td>
</tr>
<tr>
<td>sem</td>
<td>12.55</td>
<td>3.60</td>
<td>2.74</td>
<td>4.82</td>
<td>0.77</td>
<td>0.90</td>
<td>0.88</td>
</tr>
</tbody>
</table>

#### Latency to lick Bottle 2 (sec)

<table>
<thead>
<tr>
<th>Latency to lick Bottle 2 (sec)</th>
<th>BL 1</th>
<th>BL 2</th>
<th>BL 3</th>
<th>BL 4</th>
<th>BL 5</th>
<th>BL 6</th>
<th>BL 7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHAM</strong> sac-sac mean</td>
<td>40.98</td>
<td>34.12</td>
<td>13.88</td>
<td>11.45</td>
<td>8.16</td>
<td>4.27</td>
<td>3.48</td>
</tr>
<tr>
<td>sem</td>
<td>7.19</td>
<td>8.02</td>
<td>3.30</td>
<td>4.65</td>
<td>3.48</td>
<td>0.99</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>42.28</td>
<td>18.15</td>
<td>5.61</td>
<td>4.09</td>
<td>3.35</td>
<td>3.08</td>
<td>2.03</td>
</tr>
<tr>
<td>sem</td>
<td>11.56</td>
<td>3.67</td>
<td>1.59</td>
<td>1.46</td>
<td>0.77</td>
<td>0.62</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>THLX</strong> sac-sac mean</td>
<td>44.51</td>
<td>29.50</td>
<td>24.12</td>
<td>22.24</td>
<td>14.30</td>
<td>15.33</td>
<td>7.79</td>
</tr>
<tr>
<td>sem</td>
<td>13.35</td>
<td>7.76</td>
<td>8.35</td>
<td>8.59</td>
<td>4.28</td>
<td>5.98</td>
<td>2.62</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>37.06</td>
<td>12.93</td>
<td>8.89</td>
<td>4.78</td>
<td>1.73</td>
<td>5.10</td>
<td>4.18</td>
</tr>
<tr>
<td>sem</td>
<td>13.05</td>
<td>4.66</td>
<td>3.61</td>
<td>0.90</td>
<td>0.23</td>
<td>0.50</td>
<td>1.60</td>
</tr>
</tbody>
</table>

#### Exp 2a: Zero Sec ISI

<table>
<thead>
<tr>
<th>Latency to lick Bottle 1 (sec)</th>
<th>BL 1</th>
<th>BL 2</th>
<th>BL 3</th>
<th>BL 4</th>
<th>BL 5</th>
<th>BL 6</th>
<th>BL 7</th>
<th>BL 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHAM</strong> sac-sac mean</td>
<td>18.93</td>
<td>14.92</td>
<td>7.35</td>
<td>4.66</td>
<td>3.42</td>
<td>5.71</td>
<td>4.11</td>
<td>4.50</td>
</tr>
<tr>
<td>sem</td>
<td>2.76</td>
<td>5.23</td>
<td>1.70</td>
<td>1.70</td>
<td>0.86</td>
<td>1.43</td>
<td>1.20</td>
<td>0.85</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>29.86</td>
<td>13.93</td>
<td>11.98</td>
<td>9.90</td>
<td>6.83</td>
<td>2.93</td>
<td>5.32</td>
<td>3.73</td>
</tr>
<tr>
<td>sem</td>
<td>8.14</td>
<td>3.77</td>
<td>3.40</td>
<td>2.79</td>
<td>1.69</td>
<td>0.64</td>
<td>1.18</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>THLX</strong> sac-sac mean</td>
<td>37.96</td>
<td>17.99</td>
<td>18.72</td>
<td>18.64</td>
<td>11.83</td>
<td>9.48</td>
<td>4.95</td>
<td>15.88</td>
</tr>
<tr>
<td>sem</td>
<td>19.01</td>
<td>4.45</td>
<td>7.61</td>
<td>7.40</td>
<td>3.69</td>
<td>3.73</td>
<td>1.48</td>
<td>9.58</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>43.93</td>
<td>26.93</td>
<td>32.71</td>
<td>26.21</td>
<td>7.52</td>
<td>8.13</td>
<td>4.87</td>
<td>4.20</td>
</tr>
<tr>
<td>sem</td>
<td>20.87</td>
<td>16.10</td>
<td>24.39</td>
<td>13.80</td>
<td>2.15</td>
<td>2.87</td>
<td>1.14</td>
<td>1.02</td>
</tr>
</tbody>
</table>

#### Latency to lick Bottle 2 (sec)

<table>
<thead>
<tr>
<th>Latency to lick Bottle 2 (sec)</th>
<th>BL 1</th>
<th>BL 2</th>
<th>BL 3</th>
<th>BL 4</th>
<th>BL 5</th>
<th>BL 6</th>
<th>BL 7</th>
<th>BL 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHAM</strong> sac-sac mean</td>
<td>62.34</td>
<td>54.53</td>
<td>46.90</td>
<td>43.21</td>
<td>32.25</td>
<td>9.59</td>
<td>11.75</td>
<td>11.58</td>
</tr>
<tr>
<td>sem</td>
<td>17.11</td>
<td>21.63</td>
<td>14.09</td>
<td>12.96</td>
<td>10.28</td>
<td>3.00</td>
<td>3.06</td>
<td>2.87</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>106.86</td>
<td>22.60</td>
<td>4.54</td>
<td>5.24</td>
<td>4.93</td>
<td>4.11</td>
<td>2.27</td>
<td>1.99</td>
</tr>
<tr>
<td>sem</td>
<td>21.03</td>
<td>11.37</td>
<td>1.54</td>
<td>1.30</td>
<td>1.52</td>
<td>1.63</td>
<td>0.51</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>THLX</strong> sac-sac mean</td>
<td>57.50</td>
<td>57.82</td>
<td>60.02</td>
<td>59.49</td>
<td>32.82</td>
<td>29.79</td>
<td>13.11</td>
<td>19.71</td>
</tr>
<tr>
<td>sem</td>
<td>21.73</td>
<td>18.31</td>
<td>11.94</td>
<td>19.12</td>
<td>3.38</td>
<td>10.82</td>
<td>3.68</td>
<td>6.00</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>75.98</td>
<td>27.73</td>
<td>22.32</td>
<td>30.21</td>
<td>7.02</td>
<td>3.51</td>
<td>4.38</td>
<td>1.96</td>
</tr>
<tr>
<td>sem</td>
<td>31.56</td>
<td>15.01</td>
<td>14.50</td>
<td>22.07</td>
<td>2.11</td>
<td>0.74</td>
<td>1.57</td>
<td>0.35</td>
</tr>
</tbody>
</table>

#### Exp 2b: Zero Sec ISI

<table>
<thead>
<tr>
<th>Latency to lick Bottle 1 (sec)</th>
<th>BL 1</th>
<th>BL 2</th>
<th>BL 3</th>
<th>BL 4</th>
<th>BL 5</th>
<th>BL 6</th>
<th>BL 7</th>
<th>BL 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SHAM</strong> sac-sac mean</td>
<td>6.27</td>
<td>3.54</td>
<td>4.33</td>
<td>3.91</td>
<td>1.42</td>
<td>1.10</td>
<td>1.19</td>
<td>0.67</td>
</tr>
<tr>
<td>sem</td>
<td>1.42</td>
<td>1.10</td>
<td>1.19</td>
<td>0.67</td>
<td>2.59</td>
<td>4.54</td>
<td>4.15</td>
<td>4.55</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>2.59</td>
<td>4.54</td>
<td>4.15</td>
<td>4.55</td>
<td>0.34</td>
<td>0.74</td>
<td>0.72</td>
<td>1.14</td>
</tr>
<tr>
<td>sem</td>
<td>0.34</td>
<td>0.74</td>
<td>0.72</td>
<td>1.14</td>
<td>3.69</td>
<td>9.68</td>
<td>5.62</td>
<td>6.54</td>
</tr>
<tr>
<td><strong>THLX</strong> sac-sac mean</td>
<td>3.89</td>
<td>4.00</td>
<td>2.95</td>
<td>4.03</td>
<td>0.70</td>
<td>0.98</td>
<td>0.82</td>
<td>0.64</td>
</tr>
<tr>
<td>sem</td>
<td>0.70</td>
<td>0.98</td>
<td>0.82</td>
<td>0.64</td>
<td>16.25</td>
<td>10.34</td>
<td>8.34</td>
<td>4.64</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>16.25</td>
<td>10.34</td>
<td>8.34</td>
<td>4.64</td>
<td>4.45</td>
<td>3.30</td>
<td>2.71</td>
<td>1.98</td>
</tr>
<tr>
<td>sem</td>
<td>4.45</td>
<td>3.30</td>
<td>2.71</td>
<td>1.98</td>
<td>3.03</td>
<td>3.55</td>
<td>2.29</td>
<td>1.48</td>
</tr>
<tr>
<td><strong>THLX</strong> sac-sac mean</td>
<td>9.40</td>
<td>16.87</td>
<td>8.16</td>
<td>5.63</td>
<td>3.79</td>
<td>9.36</td>
<td>1.91</td>
<td>2.65</td>
</tr>
<tr>
<td>sem</td>
<td>9.40</td>
<td>16.87</td>
<td>8.16</td>
<td>5.63</td>
<td>3.79</td>
<td>9.36</td>
<td>1.91</td>
<td>2.65</td>
</tr>
<tr>
<td><strong>sac-suc</strong> mean</td>
<td>2.59</td>
<td>2.72</td>
<td>4.59</td>
<td>2.22</td>
<td>0.74</td>
<td>0.75</td>
<td>0.81</td>
<td>0.51</td>
</tr>
<tr>
<td>sem</td>
<td>2.59</td>
<td>2.72</td>
<td>4.59</td>
<td>2.22</td>
<td>0.74</td>
<td>0.75</td>
<td>0.81</td>
<td>0.51</td>
</tr>
</tbody>
</table>
References


