Neural Structures Involved in Sensory-Specific Associations in Flavor Preference Conditioning

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

2010

This manuscript has been read and accepted for the
Graduate Faculty in Psychology in satisfaction of the
dissertation requirement for the degree of Doctor of Philosophy

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Abstract

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Advisor: Dr. Andrew R. Delamater

This dissertation research examined the roles of the basolateral amygdala (BLA), orbitofrontal cortex (OFC), and gustatory cortex (GC) in the formation of sensory-specific associations in conditioned flavor preference and conditioned magazine approach paradigms using US devaluation and selective Pavlovian-instrumental transfer (PIT) procedures. Experiment 1 analyzed the effects of pre-training BLA and OFC lesions on the formation of sensory-specific flavor-nutrient associations in a US devaluation task, where flavor cues were paired either simultaneously or sequentially with nutrient rewards. Experiment 2 explored the effects of pre-training BLA and OFC lesions on the development of sensory-specific associations in a magazine approach paradigm where auditory and visual cues were used to predict the occurrence of the same nutrients as the ones used in Experiment 1. In this experiment selective PIT and US devaluation tasks were both used to assess the formation of sensory-specific associations. In Experiment 3 we examined the effects of pre-training lesions of the GC and BLA|GC disconnection lesions on the formation of sensory-specific flavor-nutrient associations in a conditioned

flavor preference paradigm. The results indicate that while none of these lesions impaired the formation of sensory-specific associations in a flavor preference paradigm as revealed by normal US devaluation effects in lesioned animals, both BLA and OFC lesions impaired the formation of such associations in the conditioned magazine approach paradigm as revealed by a loss of selective PIT in lesioned animals. In addition, the results of Experiment 2 also demonstrated that OFC, but not BLA, lesions abolished the US devaluation effect. These findings suggest that OFC lesions more completely prevent acquisition of sensory-specific associations than BLA lesions in magazine approach conditioning. In Experiment 3 it was found that both GC and GC|BLA disconnection lesions failed to impair the formation of sensory-specific flavor-nutrient associations as revealed by a normal US devaluation effect, but these lesions did result in (1) impaired learning of a discrimination between the devalued and nondevalued nutrients, and (2) impaired extinction of sensory-specific flavor-nutrient associations as revealed by a more long lasting US devaluation effect than was seen in non-lesioned controls. The overall patterns of these results allow for multiple interpretations of the neural processes involved in the formation of sensory-specific associations in various Pavlovian paradigms.

Acknowledgement

There are a number of people whom I would like to thank for their support. First, I would like to express my sincerest gratitude to my advisor, Dr. Delamater for his endless guidance and understanding. There are few people I hold in higher regard than he. Dr. Delamater helped me overcome my phobia of rats, helped me design and execute my studies, and taught me to believe in myself. I will forever be grateful to him for his support and patience.

Next, I would like to extend a special thank you to Dr. Khalid Touzani for all his assistance with lesioning and histological protocols and to Dr. Frank Grasso for his continuous encouragement. I would also like to thank Dr. Tony Sclafani and Dr. Robert Batsell for their support.

Other people to whom I would like to extend my deepest gratitude are Brooklyn College faculty: Aaron Kozbelt, Louise Hainline, Elisabeth Brauner, Laraine McDonough, Benzion Chanowitz, David Owen, Laura Rabin, and Jenny Basil; Brooklyn College staff: Maureen Ghans, Phyllis Gross, Denise Pucci and Brenda; as well my fellow Brooklyn College students: Melissa Dolese, Vincent Campese, Matt Fein, Natasha Nadler, Joanna Serafin, Katherine Nutter-Upham, Karla Felix, Rommel Robertson, Lyl Tomlinson, Latoya Lyn, and Michael Brown for helping me get through graduate school and for their endless support.

In addition, I would like to thank Lou DiBella for encouraging me to apply to graduate school as well as my parents, Sofya and Alik, my brother Michael, my cousin Alex, Larissa Mashensky, my grandfather David, Don and DeeDee Sims, and, Kathy Sims for believing in me. I would also like to thank my wonderful friends, Andrew Pagliughi, Eugene Zusman, Matt Bartels, Stephen Lanuto, Veronica Belitski, John Legarreta, and Raisa Gasparian for their love and friendship. Also, I would like to honor four very special people, Tahel Erez, Illese Alexander, Jake McDonough, and Peter Yelyash, who are no longer here but who will forever live in my heart.

Finally, I would like to thank my loving husband, Jay, for all the weekends, holidays, and sleepless nights he's been there for me as I was working on my dissertation. I am a better person because of him.

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INTRODUCTION

Classical (or Pavlovian) conditioning consists of pairing a motivationally neutral conditioned stimulus (CS, e.g. sound of a metronome) with a motivationally significant unconditioned stimulus (US, e.g. meat powder). Repeated presentations of such pairings result in subjects forming a conditioned response (CR, e.g. salivation) to the CS, which can occur even before the US is presented (Pavlov, 1927).

This form of learning has been extensively studied over the last century and research has shown this learning process to have profound impact over behavior in a number of different settings. For example, Watson and Rayner (1920) were able to condition a fear of rats by pairing a presentation of a white rat with a loud noise. Other studies have also demonstrated the importance of Pavlovian learning in drug conditioning (Logan, 1993; Siegel, Hinson, & Krank, 1978), sexual conditioning (Domjan, 1994) and conditioned taste aversion (Garcia & Koelling, 1966) studies. Despite the importance of Pavlovian learning and its demonstrated significance in a number of different domains a number of key issues remain to be fully understood. One such issue is the nature (or content) of Pavlovian learning.

The nature of CS-US associations has been of interest for many years (see Delamater & Oakeshott, 2007 for review). Some have suggested that over the course of training the CS becomes associated with the unconditioned response (UR, e.g. salivation) and that the US only strengthens that connection (the S-R view), and hence with extended training the CS produces the CR (Donahoe & Vegas, 2004; Guthrie, 1935; Thorndike, 1911; Watson, 1913). Alternatively, it has been suggested that the CS becomes associated

with the US and over the course of training the CS reminds the subjects of the US (the S-S view) thus resulting in the expression of the CR (Tolman, 1933).

A number of studies used a reinforcer revaluation (also known as US revaluation) technique to demonstrate that animal subjects acquire S-S associations (Holland & Rescorla, 1975; Tolman, 1933). US revaluation refers to changing the value of the US after the conditioning phase by pairing the US with another outcome (e.g. pairing meat powder with the emetic lithium chloride, see Delamater & LoLordo, 1991 for review). Of interest is determining whether the US revaluation treatment has an effect on conditioned responding in a subsequent test session where just the CS is presented. According to the S-S view, if, after the conditioning phase, the US was to be revalued (e.g. by being paired with illness), then the subjects should reduce their CRs to the CS. This would imply that the CS formed an association with the US during training. Thus, when the CS is presented again after the devaluation phase, it then reminds the subjects of the revalued US, causing the subjects to lower their CRs as compared to the training phase. Alternatively, the S-R view would predict that no difference in CRs would be observed after revaluation because the CS comes to elicit the CRs through a direct connection established between the CS and the UR. The current value of the US should not affect responding because this element is not assumed to be a part of the associative structure. In fact, when such an experiment was conducted, a reduction in CRs was observed thus supporting the S-S view (Delamater, Campese, LoLordo, & Sclafani, 2006; Holland, 1990a; Holland & Rescorla, 1975). For instance, Holland (1990a) taught rats to discriminate between two auditory CSs. When tone 1 (T1) was presented, the subjects received one flavored solution (Peppermint sucrose, F1) in the food cup. However, when

tone 2 (T2) was presented the subjects received a different flavored solution (Wintergreen sucrose, F2) in the same cup. Thereafter, the F2 was devalued, where the rats received an injection of an emetic lithium chloride (LiCl) after consumption of F2. During the test phase, food cup approach CRs were recorded during T1 and T2 test trials. Subjects approached the food cup on T1 trials more than they did on T2 trials. These results support the S-S view, suggesting that the T1 and T2 CSs became associated with the USs. When the T2 was presented during the test, it recalled the representation of its associate, F2, which was devalued, thus resulting in fewer food cup CRs.

Currently it is understood that both S-S and S-R relationships may take place during Pavlovian conditioning to different degrees in different circumstances (see Delamater & Oakeshott, 2007). Moreover, it is suggested that the CS becomes potentially associated with a number of different properties of the US, such as its sensory (e.g. gustatory or olfactory components of the US), hedonic (i.e. how appealing is the US), motivational (i.e. general motivational properties, such as food when the subject is hungry), and temporal properties (i.e. how much time elapses between the CS and the presentation of the US), as well as the overt response components of the S-R association (Delamater & Oakeshott, 2007). This dissertation will focus on the relationship between the CS and the sensory properties of the appetitive US in Pavlovian conditioning.

Chapter 1. Behavioral Evidence for Sensory-Specific Associations

Sensory-specific associations are the associations that form between the conditioned stimulus (CS) and the sensory properties of the unconditioned stimulus (US). The formation of sensory-specific associations has been studied using Pavlovian-instrumental transfer (Delamater & Holland, 2008; Kruse, Overmier, Konz, & Rokke, 1983), contingency degradation (Delamater, 1995; Yin, Ostlund, Knowlton & Balleine, 2005), potentiated feeding (Galarce, Crombag, & Holland, 2007; Holland & Petrovich, 2005), and US devaluation (Dwyer, 2005; Delamater, et al., 2006; Scarlet, Campese, & Delamater, 2009) tasks.

Evidence for sensory-specific associations has been studied using excitatory stimuli in a Pavlovian-instrumental transfer (PIT) task (Colwill & Motzkin, 1994; Colwill & Rescorla, 1990; Delamater & Holland, 2008). For example, Delamater and Holland, (2008) taught hungry rats to press a lever for one outcome (e.g. food pellet) and pull a chain for another outcome (e.g. sucrose) in the instrumental phase. In the Pavlovian phase, the subjects learned to expect a pellet after one CS (e.g. light) and sucrose after another CS (e.g. tone). In the transfer test the subjects were given a choice between the two instrumental manipulanda in the absence of the outcomes. It was found that when either CS was presented, the subjects produced significantly more of the instrumental responses which previously shared an outcome with that CS as compared to the instrumental response which did not. For example, if the lever-press response and the light CS were both previously reinforced with a food pellet, then when the light CS was presented during the transfer test, the subjects made more lever-pressing responses than

chain pulling responses (Delamater & Holland, 2008). These findings suggest that the conditioned stimuli formed an association with specific sensory aspects of each outcome, and that these specific sensory attributes had selective effects on instrumental choice.

Moreover, sensory-specific associations also can be observed in a PIT task with inhibitory cues (Delamater, LoLordo & Sosa, 2003; Kruse, et al., 1983). For example, Delamater, et al., (2003) taught hungry rats to press a lever for one US (e.g. food pellet) and pull a chain for a different US (e.g. sucrose) during the instrumental phase. In the following phase (Pavlovian backward conditioning) the subjects were presented with Pavlovian conditioned stimuli (i.e. tone and light) 10 sec after the USs (i.e. pellet and sucrose) were presented. The pellet US was always followed by one CS (e.g. tone), whereas the sucrose US was followed by the other CS (e.g. light). The subjects were then given a transfer test where both the lever and the chain were present and the subjects' instrumental responses on each during the tone and light CSs was measured in the absence of the pellet and sucrose USs. The results of the PIT test showed that the subjects produced significantly fewer instrumental responses when the response and CS shared its outcome than when they did not. For example, if the subjects learned to press the lever for a pellet US and if the pellet US later preceded the light CS, then during the transfer test the subjects reduced their lever pressing instrumental responses when the light CS was presented and instead showed more chain pulling instrumental responses during that time. These results can be interpreted to mean that the light CS entered into an inhibitory association with specific sensory properties of the US, hence when the light CS was presented, it reminded the subjects of the absence of the pellet thus guiding them to engage in the alternate instrumental response (i.e. chain pulling) in order to optimize their

chances of obtaining further reinforcement. If the CS was associated with some general motivational aspect of the US (e.g., frustration from the lack of food), then one might expect an overall reduction in responding when the CSs were presented. However, the stimulus-specific transfer observed in this experiment suggests that the associations formed between the CS and the US were sensory-specific and inhibitory in nature.

Further evidence for the formation of sensory-specific associations in Pavlovian learning was found in contingency degradation tasks (Delamater, 1995; Yin, et al., 2005). For example, Delamater (1995) taught the subjects to press a lever for one outcome (e.g. pellet) and pull a chain for another outcome (e.g. sucrose) in the instrumental phase. In the Pavlovian phase the rats were taught to associate a light CS with one outcome (e.g. pellet) and a noise CS with another outcome (e.g. sucrose). In addition, one of the USs (e.g. pellet) was also randomly presented during the inter-trial-intervals (ITIs) in order to degrade one of the CS-US contingencies. Thereafter the subjects were given a PIT test, where they were given a choice between the instrumental responses in the presence of either the light or the noise CSs in the absence of the outcomes. The results illustrated that during the Pavlovian phase the subjects showed significantly more magazine approach CRs to the CS the outcome of which was not randomly presented during the ITI. In the transfer test the subjects showed a good transfer effect (i.e. the subjects produced significantly more instrumental responses on the manipulandum which shared its outcome with the presented CS than of the manipulandum which did not) during the stimulus the contingency of which was not degraded. However, when the CS with a degraded CS-US contingency was presented, the subjects showed non-selectively lowered responding on both manipulanda. These results suggest that the CS whose

contingency was not degraded formed an association with the sensory-specific properties of the outcome, and this resulted in a selective PIT effect. However, when the CS-US contingency was degraded, the sensory-specific association and selective PIT was undermined. If the CSs formed associations with non-specific motivational or hedonic components of the outcomes, then one would expect to see a non-differential increase in responding whenever the contingent CS was presented. Hence, the results of this study support the view that the CS can form an association with the sensory-specific properties of the outcomes in Pavlovian learning tasks.

Evidence for the formation of sensory-specific associations in Pavlovian learning has also been found in cue-potentiated feeding tasks (Galarce, et al., 2007). For example, Galarce and colleagues (2007) taught hungry rats to associate a tone CS with one outcome (e.g. 4% sucrose) and a white noise CS with another outcome (e.g. 4% maltodextrin). The CSs were presented for 2 min with a variable intertrial interval (ITI), with the outcomes occurring at random times during each CS presentation. In the following phase the rats were sated on food chow in their home cages for one week. Thereafter the subjects were given six 10-min potentiated feeding tests, where the consumption of each outcome was measured during the consistent CS (i.e. cue, which previously predicted the occurrence of that US), inconsistent CS (i.e. cue, which previously predicted the occurrence of the opposite US) and during the no CS interval. During the pretest (five min) the subjects were given adlib access to the US (either sucrose or maltodextrin) in order to decrease overall consumption of that US. Thereafter, the subjects were allowed to consume that same US during a 5-min test during which one of the 2-min CSs was presented twice, separated by a 40 sec intertrial interval. The

results indicated that the consumption of the sated US was selectively potentiated by the consistent CS but not by the inconsistent CS (Galarce, et al., 2007). These results suggest that sensory-specific CS-US associations were learned in this paradigm; otherwise, the rats should have shown non-specific potentiation of feeding. More specifically, these results imply that each CS became associated with specific sensory aspects of the US which was paired with it during the acquisition phase, resulting in increased consumption of the sated US when the CS associate was presented but not when the other CS was presented.

US devaluation tasks have also been used to study sensory-specific associations in Pavlovian learning (Delamater, et al, 2006; Delamater, 2007; Dwyer, 2005; Holland, 1990a; Holland & Rescorla, 1975; Scarlet, et al., 2009; Tolman, 1933). For example, Dwyer (2005) taught the rats to associate a grape flavor CS with one outcome (e.g. 2% sucrose) and a cherry flavor CS with another outcome (e.g. 2% maltodextrin). In the following phase, one of the USs was presented alone followed by an injection of an emetic lithium chloride (LiCl). In the test phase, the rats were given a two-bottle choice test, where the two flavors were presented without their US associates. The rats avoided the flavor paired with the devalued US and consumed more of the flavor paired with the non-devalued US (Dwyer, 2005). Dwyer's (2005) findings once again support the notion that the conditioned stimuli form an association with the sensory-specific properties of the US. Hence, when one of the USs was devalued by being paired with LiCl, the rats selectively avoided the flavor paired with it, but not the other flavor. If the flavors had merely associated with non-specific motivational or hedonic properties of the outcomes, then one would expect no difference in flavor intakes during the two-bottle choice test.

Chapter 2. Factors that Affect Sensory-Specific Associations in Pavlovian Learning Tasks

Type of Conditioned Stimulus

While US devaluation tasks provide evidence for the formation of sensory-specific associations in Pavlovian learning tasks, the fate of these associations is dependent on the type of the conditioned stimulus used in the acquisition phase of the experiment. More specifically, when the CSs are auditory and visual, the sensory-specific associations are resistant to extinction (Delamater, 1996; Rescorla, 1996), whereas when the CSs are gustatory and olfactory, the control by sensory-specific associations will be reduced by extinction (Delamater, 2007). For instance, Rescorla (1996) taught hungry rats to associate a noise CS with one outcome (e.g. pellet) and a light CS with another outcome (e.g. 8% sucrose) during the Pavlovian conditioning phase. During the extinction phase, half of the subjects were presented with the CSs without the outcome (Group Ext.) whereas the subjects from another group were placed in the experimental chamber without any stimuli being present (Group NotExt). In the following phase the subjects were taught to associate both the light and the noise CSs with a third outcome (i.e. 15% Polycose) to equate the overall levels of magazine approach responding. During the devaluation phase half of the subjects in each group were devalued on pellets and half were devalued on sucrose by pairing it with LiCl. During the test phase the subjects were placed in the experimental chamber and their magazine approach CRs to each CS was recorded in the absence of the outcomes. The results indicated that extinguished and non-extinguished subjects alike equally produced less CRs to the CS that was paired with the

devalued outcome than to the CS paired with the nondevalued outcome. These results suggest that the sensory-specific associations are quite robust and are resistant to extinction.

On the other hand, Delamater (2007) found that when flavor CSs were used, the sensory-specific associations learned during the training phase were extinguished. In his experiment, Delamater (2007) taught thirsty rats to associate one flavor CS (e.g. banana) with one outcome (e.g. 8 % sucrose), and another flavor CS (e.g. strawberry) with the same outcome. Two additional flavors (almond and vanilla) were similarly paired with a different outcome (8% Polycose). In the extinction phase, one of the flavors previously paired with sucrose and one of the flavors previously paired with Polycose were extinguished whereby they were repeatedly presented in the absence of their associated outcomes. In the devaluation phase, one of the outcomes was devalued by being paired with lithium chloride (LiCl) in the absence of the flavor CSs. The testing procedure consisted of a two-bottle choice test where one flavor cue that was extinguished and another flavor cue that was not extinguished, both of which had been previously paired with the same outcome, were pitted against one another. The results illustrated that the rats preferred the flavor CS that was not extinguished to the CS that was extinguished when the associated outcome had not been devalued. However, when the flavors previously paired with the devalued outcome were pitted against one another, the subjects preferred the extinguished flavor CS to the non-extinguished one (Delamater, 2007). These results suggest that when the flavor cues are used as CSs, control by sensory-specific associations is diminished by an extinction procedure.

Amount of Training

In addition to the type of the conditioned stimulus used in the acquisition phase, the number of CS-US presentations has also been found to be quite pertinent in the formation of sensory-specific associations, especially in mediated food aversion tasks (Holland, 1990a; 1998; 2005). In a mediated food aversion design the CS is paired with illness (i.e. LiCl) and the effect of this on subsequent intake of the US is assessed. For example, Holland (1998, Experiment 1) taught hungry rats to associate a tone CS with one outcome (e.g. 0.2M of wintergreen-flavored sucrose solution) and white noise CS with another outcome (e.g. 0.2M of peppermint-flavored sucrose solution). One CS-US pair was trained minimally (16 trials), while another was trained extensively (64 trials). During the mediated aversion phase the subjects were presented with one of the CSs (e.g. tone) followed by a LiCl injection. On alternate days subjects were presented with the other CS (e.g. white noise) without LiCl. After the mediated aversion phase the subjects were given a consumption test, where on alternate sessions they were given one of the two flavored solutions to drink and their intake was measured. The results indicated that the subjects that received the pairings of the minimally trained CS with LiCl consumed significantly less of the solution paired with that CS than the solution paired with the other CS. These results suggest that when the CS is presented it evokes a neural representation of the sensory properties of the US. Therefore, when the CS was paired with LiCl, the value of the US decreased through its neural representation being associated with LiCl. However, this mediated aversion effect is abolished with extensive training.

Interestingly, the amount of training does not appear to influence the US devaluation effect (Holland, 1998; 2005; 2008). In a similar task to the one described above Holland (1998, Experiment 3) paired one of the USs (rather than one of the CSs) with LiCl and then presented both CSs in a magazine approach test, where the CRs were measured to each CS in the absence of the outcomes. The results illustrated that all subjects, despite the amount of training they received, produced significantly less CRs to the CS whose associated nutrient US was devalued than to the CS whose associated nutrient US was not devalued. These results suggest that the US devaluation and mediated aversion tasks might be fundamentally different from one another but that these differences only become apparent after extended training. After limited training, the CS may evoke a neural representation of the US that is indistinguishable from the US itself, i.e. an image of the US (Holland, 1990a, b). According to this interpretation, when the CS is presented alone and paired with illness after minimal training an aversion is established to the US because its image was directly paired with illness. On the other hand, after extended training, the CS may evoke a neural representation of the US that is also specific in its sensory content but which is distinguishable from the US itself, i.e., an expectancy of the US. This interpretation suggests that the mediated aversion effect should be lost after extended training, but not the US devaluation effect because the former depends upon the image process whereas the latter depends upon either the image or expectancy process.

Reversal Learning and Time

Another factor that has been shown to be effective in controlling the expression of previously learned sensory-specific associations is reversal learning and time since reversal training (Scarlet, et al., 2009). Reversal learning refers to the subjects learning to associate two pairs of CS-US associations in one phase and reversing those associations in another phase. In their experiment, Scarlet, et al., (2009) taught thirsty rats, for example, to associate an almond flavor CS with a sucrose US and a banana flavor CS with a Polycose US. In the following phase, the CS-US contingencies were reversed where the CS that was previously paired with sucrose was now paired with Polycose, and vice versa. After the reversal phase the subjects were devalued on one of the outcomes (either sucrose or Polycose) by pairing it with LiCl. The test phase consisted of a two-bottle choice test between the two flavor CSs in the absence of the outcomes. The results indicated that the subjects avoided the flavor CS most recently paired with the devalued outcome. Therefore, the preference was governed by the most recently learned sensory-specific associations. However, it was unclear whether the second phase erased the associations learned in the first phase or simply masked it. In order to answer this question, a 3-week retention interval was interpolated between the reversal and the devaluation phases. The results illustrated that these subjects avoided the flavor that was initially paired with the devalued outcome. These results suggest that the sensory-specific associations learned in the first phase were initially masked by those learned in the second phase and then recovered after a retention interval.

Chapter 3. Neural Structures Involved in Sensory-Specific Associations in Pavlovian Learning

While it is evident that sensory-specific associations occur in many different Pavlovian appetitive conditioning paradigms and under a variety of conditions, it is still unclear which neural structures are involved in this type of learning. Some research suggests that the basolateral amygdala (BLA) is involved in the formation of sensory-specific associations (Blundell, Hall & Killcross, 2001; Corbit & Balleine, 2005; Hatfield, Han, Conley, Gallagher, & Holland, 1996). Other studies point to the importance of the orbitofrontal cortex (OFC, Gallagher, McMahan, & Schoenbaum, 1999; Pickens, Saddoris, Setlow, Gallagher, Holland, & Schoenbaum, 2003). Some recent studies have also found behavioral (Balleine & Dickinson, 2000) as well as molecular (Saddoris, Geirut, Holland, & Gallagher, 2008) evidence of gustatory cortex (GC) involvement in the formation of sensory-specific associations. In addition, the ventral tegmental area (VTA) (Corbit, Janak, & Balleine, 2007) and dorsomedial striatum (Corbit & Janak, 2007; Yin, et al., 2005) have also been implicated in sensory-specific associations.

Basolateral Amygdala

The basolateral amygdala (BLA) has been studied extensively and recent experiments have identified it to be crucial for the formation of sensory-specific associations (Blundell, et al., 2001; Corbit & Balleine, 2005; Hatfield, et al., 1996). For instance, Hatfield and colleagues (1996) studied the role of the BLA in the formation of sensory-specific associations in a Pavlovian US devaluation task. Hatfield and colleagues

(1996) initially conditioned hungry rats to associate a light CS with the delivery of a food pellet US to the food cup. In the next phase, the food pellets were devalued by being paired with LiCl for one of the groups (Group Paired). The other group received food pellets and LiCl on separate occasions (Group Unpaired). The light CS was not presented in this phase. In the test phase, magazine approach CRs were measured during the light CS. The results showed that when the light CS was presented, the normal subjects in Group Paired had significantly fewer food cup approach CRs as compared to those of Group Unpaired. However, rats that received a BLA lesion prior to conditioning did not show a devaluation effect and continued to approach the food cup during the light CS regardless of whether the food pellet had been devalued or not. Furthermore, the lesioned rats reduced their food consumption during the US devaluation phase similarly to the controls, suggesting that the devaluation procedure was equally effective in both of these groups. These results can be interpreted to mean that the BLA lesion affected the rats' ability to form sensory-specific associations, suggesting that the formation of sensory-specific associations in Pavlovian conditioning is BLA dependent. Interestingly, similar results were not obtained using the same task when the BLA was lesioned prior to the US devaluation but after the conditioning phase had taken place (Pickens, et al., 2003). This suggests that sensory-specific CS-US associations require the BLA for their establishment, but that once these associations are established other structures are more critical in mediating a US devaluation effect.

The effects of BLA lesions on the formation of sensory-specific associations in Pavlovian learning have also been studied using Pavlovian-to-instrumental transfer tasks. For instance, Corbit and Balleine (2005) initially trained a group of rats to press the left

lever for outcome 1 (e.g. pellets) and the right lever for outcome 2 (e.g. sucrose). During the Pavlovian training phase of the experiment the subjects were conditioned to associate CS1 (e.g. tone) with O1 and CS2 (e.g. light) with O2. In addition, CS3 (e.g. white noise) was paired with outcome 3 (e.g. Polycose). The transfer test consisted of two extinction tests. During each test, only one lever was present. Each of the three CSs was presented at random times throughout the test without the outcomes. The results of the transfer test illustrated that when normal subjects were presented with CS1, they pressed the left lever more than the right lever (and vice versa for CS2). This phenomenon is referred to as the specific transfer effect and it suggests that during training each CS became associated with the sensory-specific properties of the particular US with which it was paired. However, when the stimulus that was paired with O3 was presented, responding on both levers was increased. This is referred to as the general transfer effect and it suggests that the CS became associated with some non-selective motivational (i.e. general activating) property of the outcome in addition to its specific sensory properties. Corbit and Balleine (2005) found that lesioning the basolateral amygdala (BLA) prior to the experiment resulted in the elimination of the specific, but not general, PIT effect. However, lesions of the central nucleus of the amygdala eliminated the general, but not the specific transfer effect. The authors concluded that the amygdala is involved in processing reward-based information, but that different parts of the amygdala provide diverse information about the reward. The BLA is involved in processing of sensory-specific information, whereas the central amygdala is involved in processing more general motivational properties of the reward (Corbit & Balleine, 2005; see also Blundell, Hall & Killcross, 2003).

Although the BLA appears to be involved in the formation of sensory-specific associations in first order Pavlovian conditioning (as revealed by US devaluation), and PIT tasks, it does not appear to be involved in the formation of such associations in sensory preconditioning (Blundell, et al., 2003). Sensory preconditioning tasks involve three phases. First, an initial pairing of two neutral stimuli (e.g. bell + light) occurs. Then, one of these stimuli (e.g. bell) is separately paired with a US (e.g. shock to the left forelimb), and this is then followed by a test in which responding is assessed to the other stimulus. In one early study, control subjects were trained with either a bell or light CS paired with shock in phase 2 (as was also true for experimental subjects), but controls did not previously receive bell + light pairings. In the test phase the subjects were all presented with the CS different from the one paired with shock and flexion responses were measured. Brogden (1939) found that when bell and light were presented simultaneously in the first phase, and then bell was paired with shock in a subsequent phase, the subjects showed fear CRs to the light CS in the test phase, even though it was never directly paired with the shock US (Brogden, 1939). However, control subjects showed little to no fear responding to the light CS in the test phase since it was not previously paired with the CS associated with shock. This result is usually interpreted to mean that specific sensory associations are formed between the two neutral CSs in the first phase. Thus, this result can be taken as another form of sensory-specific learning.

Blundell, et al., (2003; see also Dwyer & Killcross, 2006) explored the role of BLA lesions on a task that they described as sensory preconditioning. In their experiment, Blundell, et al., (2003) presented thirsty rats with two pairs of flavors (e.g. 10% sucrose + 0.01M hydrochloric acid and 0.16M saline + 60.0 μM quinine) in the

acquisition phase. Thereafter, one of the flavors from each of the pairs (i.e. either hydrochloric acid or quinine) was presented separately from its associate. One of these flavors (e.g. hydrochloric acid) was devalued by being paired with LiCl, while the other (e.g. quinine) was presented on alternate days without LiCl. The rats were then given a two-bottle choice test between the associates of the devalued and the nondevalued flavors (i.e. sucrose vs. saline). Normal subjects avoided the flavor that was previously paired with the devalued flavor. Interestingly, subjects that were BLA lesioned prior to the conditioning phase were not impaired on this task. Similar to the control subjects, the BLA lesioned rats avoided the flavor paired with the devalued flavor (Blundell, et al., 2003). The authors interpreted their results to mean that the BLA is not involved in the formation of sensory-specific associations in tasks where cues associate with motivationally neutral target events and suggested that the BLA is only involved in the formation of sensory-specific associations in tasks where cues associate with motivationally significant events. In this experiment 10% sucrose, for example, served as a predictive cue for a relatively less palatable HCL taste, which served as the target cue. It follows from this reasoning that if the predictive and target cues were to be reversed (e.g. where HCL was the predictive cue and 10% sucrose was the target cue), then one ought to observe that BLA lesions would produce deficits in the formation of sensory-specific associations in this task.

Further evidence to suggest that the BLA is only involved in tasks where motivationally significant events are used was provided by Dwyer and Killcross, (2006). In their experiment, Dwyer and Killcross (2006) taught thirsty and hungry rats to associate one arm of a Y-maze with 15% sucrose, another arm with 15% maltodextrin,

and a third arm with water (“neutral arm”). During a mediated conditioning phase subjects were placed in one of the nutrient-paired arms (“the target arm’) and allowed to consume water, after which they were injected with LiCl. Over the next 2 days the subjects were given one-bottle tests in their home cage and their intake of each nutrient was measured. On the next day the subjects received a place-preference test where they were given 5 min in the maze to go to the arm of their choice. The results indicated that the sham rats showed a good mediated aversion effect, where they consumed significantly less of the solution associated with “the target arm” as compared to the solution associated with “the non-target arm.” However, the BLA lesioned subjects did not display a preference during the consumption test. The authors interpreted this result to mean that the BLA lesion prevented the mediated flavor aversion to take place. In addition, the place preference test showed that all the subjects exhibited a preference for “the non-target arm” over the “neutral arm”, illustrating that the BLA subjects were not deficient because they were incapable of learning a preference. These results suggest the BLA is involved in the formation of sensory-specific associations in a task where neutral cues (arms of the Y maze) are paired with motivationally significant events (different nutrients).

Orbitofrontal Cortex

The orbitofrontal cortex (OFC) has also been found to be involved in the formation of sensory-specific associations in Pavlovian learning (Gallagher, et al., 1999; Pickens, et al., 2003). Lesions of the OFC have been reported to result in elimination of the US devaluation effect in a magazine approach paradigm. In fact, this effect was

observed regardless of whether the OFC was lesioned prior to conditioning (Gallagher, et al., 1999) or after conditioning, but prior to the US devaluation phase (Pickens, et al., 2005). Gallagher and colleagues (1999) taught hungry rats to associate a light CS with food pellet USs. In the following phase, the food pellets were devalued by being paired with LiCl. In the test phase, magazine approach CRs were recorded when the light CS was presented (in extinction). Gallagher and colleagues (1999) found that the OFC lesioned subjects failed to show the US devaluation effect, i.e. they continued to approach the magazine after the light CS was presented regardless of whether or not the pellet US had been devalued (Gallagher, et al., 1999). The same results were obtained when the OFC was lesioned after the training but before the devaluation phases (Pickens, et al., 2003).

While pre-training (Gallagher, et al., 1999) and post-training (Pickens, et al., 2003) OFC lesions were found to disrupt a US devaluation effect, only post-training but not pre-training OFC lesions were found to disrupt selective PIT (Ostlund & Balleine, 2007). In their experiment, Ostlund and Balleine (2007) taught hungry rats to associate one auditory CS (e.g. tone) with a pellet US and another auditory CS (e.g. white noise) with a sucrose US in the Pavlovian conditioning phase. In the instrumental phase, the subjects were taught to press the left lever for one US (e.g. pellet) and press the right lever for another outcome (e.g. sucrose). The subjects were then given a single PIT test, where their instrumental responses on each manipulandum were measured during each CS. It was found that the Sham lesioned rats showed a selective PIT effect, where they made significantly more “same” responses (i.e. instrumental response, which shared its outcome with the presented CS) than “different” responses (i.e. instrumental responses,

which did not share its outcome with the presented CS), suggesting that they were able to establish the sensory-specific associations. On the other hand, rats which received OFC lesions after the training but before the testing phases failed to display the selective PIT effect, suggesting that the OFC lesion disrupted the formation of sensory-specific associations in these subjects. However, subjects that received OFC lesions prior to the training phase resembled the Sham lesioned subjects as their selective PIT effect was not impaired (i.e. they made more "same" than "different" responses). These results suggest that the OFC is not involved in the formation of sensory-specific associations but are involved in the maintenance of these associations. However, Ostlund and Balleine (2007) suggested that the OFC lesions used in their task may have been incomplete, which could explain the lack of deficit seen in pre-training OFC lesioned subjects.

Gustatory Cortex

The gustatory cortex (GC) has also been implicated in the formation of sensory-specific associations in Pavlovian tasks (Saddoris, Geirut, Holland, & Gallagher, 2008; Saddoris, Holland, & Gallagher, 2009). In one of their experiments, Saddoris, et al., (2009) gave a set of thirsty rats a sucrose solution to drink for 5 min and 20 min later the subjects were given a second sucrose solution to drink for 5 min. The subjects were then perfused and stained for the *Arc* and *Homer1a* immediate early genes (IEG), which was measured in the dysgranular layer of the gustatory cortex. One of these genes (*Arc*) is activated by the most recently presented stimulus. The other gene (*Homer1a*) is activated by the less recent activity (see Vazdarjanova, McNaughton, Barnes, Worley, & Guzowski, 2002 for review). A large number of cells were identified that were stained for

both the *Arc* and *Homer1a* genes. This suggests that similar neural ensembles within the GC were activated by sucrose presentation at the two time points. In addition, Saddoris, et al., (2009) also taught thirsty rats to associate one odor (e.g. hexenol B gamma extra) with sucrose and another odor (e.g. isoamyl butyrate) with water. During the test phase, subjects were presented with the odor previously paired with sucrose, but now without sucrose and this was followed by sucrose twenty min later. In this case, a large number of cells were also identified in the GC that were stained for both *Arc* and *Homer1a* genes. Indeed, the number of double labeled cells in this test did not differ from that found in the sucrose-sucrose test. These results imply that similar neural ensembles in the GC are associatively activated by a cue for sucrose as is activated by sucrose itself.

In addition, there is also some evidence to suggest that the GC interacts with the BLA in conditioned taste aversion (Bielavska & Roldan, 1996; Yamamoto, Azuma, & Kawamure, 1984) and flavor preference learning (Saddoris, et al., 2008). For example, Saddoris, et al., (2008) performed unilateral lesions of the GC prior to training, thereby preventing communication between the BLA and GC in the lesioned hemisphere, and then examined cellular activation in the BLA in both hemispheres with *Arc* and *Homer1a* staining. After the surgery the subjects were taught to associate one olfactory cue (e.g. hexenol B gamma extra) with one nutrient (e.g. sucrose), a second olfactory cue (e.g. isoamyl butyrate) with another nutrient (e.g. maltodextrin), and a third olfactory cue (e.g. vanilla) with just plain water. During the test phase, all subjects were given one nutrient-paired odor presented in water (e.g. hexenol B gamma extra) and 20 min later they were given the other nutrient-paired odor presented in water (e.g. isoamyl butyrate) alone. The subjects were then perfused and stained for the immediate early genes for *Arc* and

Homer1a in the BLA. It was found that the flavor cues elicited different neural ensembles in the BLA in the intact side (contralateral to the GC lesion) because there were relatively few cells double stained for both *Arc* and *Homer1a*. However, on the ipsilateral side, there were more double labeled cells than in the intact side as well as more overall IEG activity. These results can be interpreted to mean that the gustatory cortex is involved in the formation of distinct associatively-activated representations of the nutrients in the BLA. When the GC was lesioned, the cells in the BLA presumably lost their specificity, thus, causing an increase in the number of double labeled cells (Saddoris, et al., 2008).

In addition to *Arc* and *Homer1a* IEG studies, *c-FOS* staining data suggests that all of the above mentioned structures, the BLA, OFC, GC as well as the nucleus accumbens are involved in coding of sensory-specific associations (Kerfoot, Agarwal, Lee, and Holland, 2007). In their study, Kerfoot, et al., (2007) paired a tone CS with an intraoral infusion of the sucrose US (8%) during the acquisition phase. In the following phase half of the subjects were injected with LiCl (group Devalue) after sucrose infusions, whereas the other group received unpaired presentations of sucrose and LiCl (group Maintain). On the following day, the rats were again presented with a tone and then were infused with water; their taste-reactivity responses (e.g. tongue protrusions and gaping) were measured. Following the test, the rats were perfused and stained for the expression of the immediate early gene *c-Fos*. The taste reactivity responses showed that the subjects in group Devalue engaged in more aversive taste-reactivity responses during the water infusion, whereas the subjects in group Maintain engaged in more appetitive taste-reactivity responses when they were infused with water. These results suggest that the subjects formed an association between the tone CS and the sensory-specific properties of

the US (likely the sweet taste of sucrose), which, if devalued, caused subjects to display aversive facial expressions (in group Devalue) but, if not devalued, resulted in appetitive facial expressions (in group Maintain). Of additional interest are the FOS expression results. It was found that subjects in group Devalue showed more FOS activity in the basolateral amygdala, orbitofrontal cortex, gustatory cortex, and posterior nucleus accumbens shell than did the subjects in group Maintain. These findings suggest that these structures are somehow involved in sensory-specific associations in Pavlovian learning tasks.

Nucleus Accumbens

Another area possibly involved in coding sensory-specific associations is nucleus accumbens (Corbit, Muir, & Balleine, 2001; Kerfoot, et al., 2007). Using IEG staining in a taste reactivity task described above, Kerfoot, et al., (2007) found that anterior and posterior regions of the nucleus accumbens shell were differentially involved in the expression of sensory-specific associations. More specifically, subjects in Group Devalue, showed higher *c-FOS* expression in posterior nucleus accumbens shell than the subjects in Group Maintain. On the other hand, the subjects in Group Maintain showed higher *c-FOS* activation in anterior nucleus accumbens shell than the subjects in Group Devalue. These results suggest that the anterior region of the nucleus accumbens shell may code for positive incentive value of an anticipated reward, whereas the posterior region of nucleus accumbens shell may code for negative incentive value of an anticipated reward. However, it is difficult to determine from this study whether differential *c-FOS* expression observed in these structures reflects cells that are coding

sensory specific aspects of learning or some other some other more non-specific aspect of learning (e.g., hedonic properties of the nutrient outcomes).

In another study, Corbit, et al., (2001) observed that the nucleus accumbens shell, but not core, is involved in the formation of sensory-specific associations in a PIT task. In their study, Corbit, et al., (2001) taught hungry rats to press a left lever to earn one outcome (e.g. pellet) and a right lever to earn another outcome (e.g. sucrose). Thereafter, the subjects were trained to associate a tone CS with one outcome (e.g. pellet) and a white noise CS with another outcome (e.g. sucrose). Then the subjects were given two PIT tests (one on each lever), where their instrumental responses were measured during each CS. Sham subjects and subjects with pre-training nucleus accumbens core lesions alike, made more "same" than "different" responses, whereas the subjects with pre-training nucleus accumbens shell lesions failed to exhibit the selective PIT effect and made equally low amounts of instrumental responses during each CS. These results suggest that the nucleus accumbens shell, but not core is involved in the formation of sensory-specific associations in a PIT task.

Dorsomedial Striatum

The dorsomedial striatum has recently been found to be involved in the formation of sensory-specific associations in Pavlovian conditioning (Corbit & Janak, 2007, 2010; Yin, et al., 2005). Corbit and Janak (2007) taught hungry rats to associate a clicker with one outcome (e.g. sucrose) and white noise with another outcome (e.g. Polycose). Thereafter the subjects were taught to press one lever for one outcome (e.g. pellet) and another lever for another outcome (e.g. sucrose). During a Pavlovian-instrumental

transfer test, one lever was available and the instrumental responses were recorded to each CS in the absence of the outcomes. Some subjects were infused with baclofen-muscimol into the dorsolateral striatum (DLS) or dorsomedial striatum (DMS) in order to temporarily deactivate it, whereas the controls were infused with saline. PIT results demonstrated that the control subjects displayed higher than baseline levels of the action which shared an outcome with the presented cue (specific transfer). However, baclofen-muscimol infusions in the DLS significantly attenuated this effect by decreasing responding to near baseline levels, thereby eliminating selective PIT. On the other hand, inactivation of the DMS also resulted in abolished selective PIT, but in this case subjects made equally high levels of both "same" and "different" responses (Corbit & Janak, 2007). These findings suggest that the DMS may be involved in the formation of sensory-specific associations. Hence, when the DMS was inactivated, the subjects lost the selective PIT effect. Nevertheless, when the stimuli were tested in these animals they still nonselectively energized responding above baseline levels. What could account for this general elevation? Perhaps these stimuli entered into an association with some general "motivational" aspect of reward, and this learning was unimpaired by DMS inactivation. In contrast, in DLS-inactivated subjects, since overall responding was very low the primary influence of this treatment was likely to impair learning involving the general motivational aspects of reward. Whether the DLS might also be involved in sensory-specific learning in less clear from these data.

Chapter 4. Neural Structures Involved in Sensory-Specific Associations in Instrumental Learning

Evidence has been obtained for the involvement of the BLA (Balleine, Killcross and Dickinson, 2003), prelimbic cortex (PL, Balleine & Dickinson, 1998; Balleine, Liljeholm, & Ostlund, 2009), DMS (Balleine, et al., 2009; Corbit & Janak, 2010), and medial dorsal thalamus (MDT, Balleine, et al., 2009) in the formation of sensory-specific response-outcome associations in instrumental learning. In addition, the GC (Balleine & Dickinson, 2000) and nucleus accumbens core (Corbit, et al., 2001), appear to be involved in another aspect of instrumental learning, namely, "incentive learning."

Basolateral Amygdala, Mediodorsal Thalamus, Prelimbic Cortex

The BLA appears to be involved in both Pavlovian (Hatfield, et al., 1996) as well as instrumental conditioning (Balleine, et al., 2003; Ostlund & Balleine, 2008). For example, Balleine, et al., (2003) lesioned the BLA for half of the rats prior to the study; the rest of the rats received a sham lesion. Once they had a chance to recover from the surgery, the rats were food deprived and taught to press a lever for one outcome (e.g. pellets) and pull a chain for another outcome (e.g. maltodextrin). In the next phase, the subjects were sated on one of the outcomes and given a devaluation choice test in the absence of the outcomes. The results illustrated that sham controls produced significantly more instrumental responses on the manipulandum the outcome of which had not been sated prior to the choice test. However, the BLA lesioned subjects failed to show a differential response during the test phase and responded equally low on both manipulanda. Furthermore, when the same subjects were trained on a contingency

degradation task, where one of the USs was presented non-contingently, the Sham and BLA lesioned subjects also differed in their performance on the instrumental choice test conducted in extinction conditions. Whereas the Sham subjects made significantly more instrumental responses on the manipulandum the contingency of which was not degraded, the BLA lesioned subjects responded equally high on both manipulanda. These results suggest that the BLA is involved in the formation of specific response-outcome (R-O) associations and that lesioning this structure prevented the formation these associations from occurring (Balleine, et al., 2003). Similar results were also observed in subjects with PL (Balleine & Dicksinson, 1998) and MDT (Corbit, Muir, & Balleine, 2003; Ostlund & Balleine, 2008) lesions suggesting that these structures are also involved in the formation of specific R-O associations (Balleine, et al., 2009).

Dorsomedial Striatum

Another structure shown to be involved in the formation of specific R-O associations is the DMS (Corbit & Janak, 2010). In their experiment, Corbit and Janak (2010) taught hungry rats to press one lever to earn one outcome and press another lever to earn another outcome. The subjects were then sated on one of the outcomes and given an instrumental choice in extinction conditions, while one third of the subjects received temporary inactivation of the DMS, one third received inactivation of the DLS and the rest did not receive either inactivation (Sham controls). Sham subjects and subjects with DLS deactivation alike, made more instrumental responses on the manipulandum the outcome of which they were not sated on. However, the subjects with DMS deactivation responded indiscriminately on each manipulandum. Furthermore, Yin, et al., (2005)

showed that DMS inactivation abolished the subjects' sensitivity not only to US devaluation but also to contingency degradation (see also Yin, Knowlton, & Balleine, 2005). These results suggest that DMS but not DLS is involved in the formation of specific R-O associations.

Gustatory Cortex

The gustatory cortex has been found to be involved in incentive learning in instrumental learning tasks (Balleine & Dickinson, 2000). For instance, Balleine and Dickinson (2000, Experiment 1) lesioned the gustatory cortex (GC) for some subjects, but not for the other subjects prior to the experiment. Then they trained all subjects to press a lever to earn one outcome (e.g. 20% maltodextrin) and pull a chain for another outcome (e.g. pellet). The subjects were then devalued on one of the outcomes, where they were presented with either the maltodextrin or the pellets until they were sated. The subjects then were given an instrumental response choice test in extinction conditions (i.e. the responses were not reinforced). The control subjects made more instrumental responses on the lever, the outcome of which was not devalued. For example, if the subjects were sated on maltodextrin, they lowered their lever press responses relative to the chain pull responses in the choice test if the lever press was previously paired with the maltodextrin outcome. However, the GC lesioned rats failed to show this selective satiation effect. Their response rates did not differ from one another. These results could suggest that the gustatory cortex is involved in specific response-outcome (R-O) associations in instrumental learning. However, by the same account GC lesions would be expected to impair the selective contingency degradation effect, but Balleine and

Dickinson (2000, Experiment 2) found that GC lesions had no effect on a contingency degradation task. Using the same subjects as in Experiment 1, Balleine and Dickinson (2000, Experiment 2) retrained the subjects on the response-outcome contingencies and then degraded one of the response-outcome contingencies by presenting one of the outcomes unpaired with the response. The subjects were then given a choice test between the two manipulanda in extinction conditions. Sham and GC lesioned subjects alike made more instrumental responses when the response-outcome contingency was not degraded than when it was degraded. These results suggest that the GC lesioned subjects were in fact capable of making response-outcome associations, and this suggests that the failure to observe devaluation effects in GC lesioned subjects in Experiment 1 was not due to impaired R-O associations . In their Experiment 3, Balleine and Dickinson (2000), trained hungry rats to press a lever for one outcome (e.g. pellet) and pull a chain for another outcome (e.g. sucrose) in the instrumental training phase. During a subsequent "incentive learning" phase, the subjects were exposed to one of the outcomes, without the response manipulanda available, in a high motivational state (i.e. hungry) and the other outcome in a low motivational state (i.e. sated). Exposure to an outcome in a low motivational state is thought to lower its incentive value when the subjects are later tested in that low motivational state. The test was conducted while the rats were in a low motivational state (under extinction conditions), and Sham control subjects made more of the instrumental response that was previously associated with the outcome exposed in a high than a low motivational state. However, the GC lesioned subjects made equally high amounts of both instrumental responses. These results suggest that GC lesions impair an incentive learning process whereby new incentive value may or may not be attached to a

rewarding outcome under various circumstances. The main implication for the earlier result in this series is that GC lesions impair the sensitivity of the instrumental response to outcome devaluation not by disrupting the formation of R-O associations but by disrupting the ability of the selective satiation manipulation to alter the value of the outcome through an incentive learning process (Balleine & Dickinson, 2000).

Nucleus Accumbens

Another structure shown to be involved in incentive learning is the nucleus accumbens core (Corbit, et al., 2001). In their experiment, Corbit, et al., (2001) taught hungry rats to press the left lever to earn one outcome (e.g. pellet) and the right lever to earn another outcome (e.g. sucrose). The subjects were then sated on one of the outcomes (e.g. sucrose) and were given instrumental choice tests where the two levers were presented but no outcomes could be earned. Sham and nucleus accumbens shell lesioned subjects alike, made significantly more instrumental responses on the manipulandum the outcome of which they were not sated on. However, the nucleus accumbens core lesioned subjects produced equally low responses on each manipulandum.

Although the nucleus accumbens core was shown to be involved in the US devaluation task (Corbit, et al., 2001, Experiment 1), it does not appear to be involved in contingency degradation (Corbit, et al., 2001, Experiment 2). Using a contingency degradation task identical to that of Balleine, et al., (2003) and Balleine and Dickinson, (2000), Corbit, et al., (2001, Experiment 2) found no effects of nucleus accumbens core lesions on contingency degradation. These results suggest that nucleus accumbens core,

similarly to the GC is involved in incentive learning rather than in the formation of specific R-O associations.

Orbitofrontal Cortex

Although the OFC plays an important part in the formation of sensory-specific associations in Pavlovian tasks (Gallagher, et al., 1999; Pickens, et al., 2003, Pickens, Saddoris, Gallagher, & Holland, 2005), it does not appear to be involved in outcome encoding in instrumental conditioning (Ostlund & Balleine, 2007). Ostlund and Balleine (2007) lesioned the OFC prior to the conditioning phase for some rats and after the conditioning phase for other subjects. Sham lesioned subjects served as controls in this experiment. During the conditioning phase hungry rats were taught to associate CS1 (e.g. tone) with one outcome (e.g. pellet, O1) and CS2 (e.g. white noise) with another outcome (e.g. sucrose, O2). In the following phase the subjects were given instrumental training in which a left lever press was rewarded with O1 and a right lever press was rewarded with O2. Thereafter the subjects were satiated on one of the two outcomes. This procedure is known to reduce the value of the sated outcome, thus making it less desirable (Balleine & Dickinson, 1998). Following satiation, the subjects were given a choice test between the two levers (in extinction). No CSs or USs were present at this time. The results illustrated that all subjects (OFC lesioned and shams) regardless of the time of lesion (before or after the conditioning phase) responded more to the lever associated with the non-devalued outcome as compared to the lever paired with the devalued outcome. This suggests that the OFC lesions had no effect on the formation of sensory-specific associations in instrumental conditioning (Ostlund & Balleine, 2007).

Summary of Neural Structures Involved in Pavlovian and Instrumental Learning

In summary, it appears that some structures, such as the BLA (Balleine, et al., 2003; Corbit & Balleine, 2005; Hatfield, et al., 1996; Ostlund & Balleine, 2008) and the DMS (Corbit & Janak, 2007, 2010) are involved in the formation of sensory-specific associations in both Pavlovian and instrumental tasks. On the other hand, while the OFC (Gallagher, et al., 1999; Ostlund & Balleine, 2007; Pickens, et al., 2003) and nucleus accumbens shell (Corbit, et al., 2001) are involved in the formation of sensory-specific associations in Pavlovian tasks, they do not seem to be involved in the formation of sensory-specific associations in instrumental tasks. In addition, GC (Balleine & Dickinson, 2000) and nucleus accumbens core (Corbit, et al., 2001) appear to be involved in incentive learning rather than in the formation of specific R-O associations in instrumental learning, per se. It should be noted, however, that while IEG staining data suggests that GC may be involved in the formation of sensory-specific associations in Pavlovian learning tasks (Kerfoot, et al., 2007; Saddoris, et al., 2008; Saddoris, et al., 2009), its involvement in the formation of such associations in Pavlovian tasks was never directly measured.

Chapter 5. Goals of the Present Research

Given some of the uncertainties noted in previous sections above concerning the importance of the BLA, OFC, and GC for the development of sensory-specific Pavlovian associations in a conditioned flavor preference paradigm, and given the more general importance of these structures in sensory-specific processes in both Pavlovian and instrumental learning, the goals of the present dissertation research were to explore the effects of pre-training lesions of these structures upon flavor-nutrient learning when motivationally significant nutrients are used. A US devaluation task was used to assess such learning. In addition, this research also examined whether the involvement of these structures in the formation of these associations depends on whether the stimuli were trained simultaneously or sequentially. Finally, we also analyzed the roles of BLA and OFC structures on the formation of these associations on US devaluation and selective PIT tasks in a magazine approach paradigm in order to provide more information on the possibility that learning involving flavor cues may differ, in some way, from learning involving exteroceptive cues.

<u>Experiment 1:</u> The effects of BLA and OFC lesions on the formation of sensory-specific associations in a conditioned flavor preference paradigm

Previous research found evidence to suggest that BLA and OFC structures are involved in the formation of sensory-specific associations in Pavlovian learning. These studies used Pavlovian-instrumental transfer (PIT) and US devaluation tasks to assess such learning where auditory and visual stimuli were paired with sucrose or pellet USs in hungry rats (Corbit & Balleine, 2005; Gallagher, et al., 1999, Hatfield, et al., 1996; Pickens, et al., 2003).

In addition, immediate early gene activation (IEG) studies suggest a role for the BLA, OFC, GC and posterior accumbens shell in sensory-specific associations in Pavlovian learning with auditory CSs (Kerfoot, et al., 2007) as well as a role for the BLA (Desgranges, Ramirez-Amaya, Ricaño-Cornejo, Levy, and Ferreira, 2010) and GC (Saddoris, et al., 2008; Saddoris, et al., 2009) with flavor CSs. For example, Desgranges, et al., (2010) taught thirsty rats to associate a flavor CS (i.e. almond) with a nutrient US (i.e. sucrose) for half of the subjects (Group Paired) while the rest of the rats were given unpaired presentations of the two solutions. Following training, all the subjects were tested with the almond solution for 5 min and 25 min later with the sucrose solution. Immediately following sucrose consumption all subjects were perfused and stained for the *Arc* gene using cytoplasmic (for the first stimulus) and nuclear (for the second stimulus) staining techniques. The results demonstrated that the subjects in the Paired Group showed a four-fold increase in the number of cells that had both, cytoplasmic and nuclear staining in the BLA as compared to Group Unpaired, suggesting that the sucrose

and sucrose-paired cue activated the same cells in Group Paired but not in Group Unpaired. These results were interpreted to mean that the BLA is involved in the formation of flavor-taste associations.

However, some recent research suggests that the BLA is not always involved in the formation of sensory-specific associations (Blundell, et al., 2003; Dwyer & Killcross, 2006). Recall that in their task, Blundell, et al., (2003) presented thirsty rats with two pairs of taste- taste mixtures (e.g. sucrose + HCL, NaCl + quinine) and then devalued one of the tastes (e.g. HCL) by pairing it with LiCl. The subjects were then given a choice between the two taste associates of the differentially valued cues (i.e. sucrose vs. NaCl). The results revealed that the BLA lesioned and the normal subjects alike avoided the taste that was previously paired with the devalued taste (e.g. sucrose). These results suggest that sensory-specific associations were formed between the tastes that were paired together and that the BLA lesions failed to disrupt the formation of these associations.

Blundell, et al., (2003) attempted to reconcile the conflicting US devaluation results in their task compared to other research (listed above) by suggesting that the BLA may not be involved in the formation of sensory-specific associations in tasks where predictive cues signal motivationally neutral target events, but is involved in tasks where predictive cues signal motivationally significant events. In their task, Blundell, et al., (2003) used 10% sucrose and 0.16M saline as predictive stimuli and 0.01M hydrochloric acid and 60.0µM quinine as target events. At these concentrations sucrose and NaCl are likely to be more valuable and, therefore, motivationally significant to a thirsty rat than HCL and quinine. It follows that if the more neutral tastes (e.g. HCL and quinine) served

as the predictive stimuli (i.e. as CSs) and the more valuable ones (e.g. sucrose and NaCl) served as target stimuli (i.e. as USs), the results would be expected to differ. More specifically, one would expect that unlike the sham controls, the BLA lesioned subjects would not show a preference in a two-bottle choice test where the two flavor associates are pitted against one another in a test of the US devaluation effect.

An alternate explanation for the discrepancy between Blundell, et al.'s, (2003) finding and those in magazine approach paradigms (Hatfield, et al., 1996) is that a procedure where the two events to be associated are presented simultaneously (such as the one implemented by Blundell, et al., 2003) may employ different neural structures than procedures in which the two events are presented sequentially, such as the ones used in traditional magazine approach paradigms (Corbit & Balleine, 2005; Gallagher, et al., 1999) and instrumental go-no-go tasks (Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003). There is evidence to suggest that learning differs both qualitatively (Higgins & Rescorla, 2004) and quantitatively (Mowrer & Aiken, 1954; Rescorla, 1980; Smith & Roll, 1967) in Pavlovian paradigms where a CS predicts the future presentations of the US (i.e. delay or trace conditioning) as opposed to ones where the CS and US are presented at the same time (simultaneous conditioning). Thus it is possible that simultaneous and sequential training procedures may result in learning that depends upon different neural mechanisms.

Simultaneous Training

Acquisition	Devaluation	Flavor Test	Nutrient Test
CS1 + US1	US1 -> LiCl	CS1 vs. CS2	US1 vs. US2
CS2 + US2	US2 -		

Sequential Training

Acquisition	Devaluation	Flavor Test	Nutrient Test
CS1 -> US1	US1 -> LiCl	CS1 vs. CS2	US1 vs. US2
CS2 -> US2	US2 -		

Table 1. Experimental Design for Experiment 1 for subjects that received simultaneous (top panel) and sequential training (bottom panel). The CSs were 1% almond and 1% banana McCormick imitation extracts. The USs were 10% sucrose and 10% Polycose. All flavor-nutrient pairings were counterbalanced across subjects. For subjects that received simultaneous training the flavor CSs and the nutrient USs were mixed in a solution. For subjects that received sequential training, the flavor CSs were presented first and was then was immediately followed by the nutrient USs.

The following experiment was designed in order to address the ideas described above. The general procedures of Experiment 1 are outlined in Table 1. Prior to the beginning of the experiment, one third of the subjects received BLA lesions, one third received OFC lesions and the rest received Sham BLA and OFC lesions. During the acquisition phase, two distinct neutral flavor CSs (1% McCormick almond and 1% McCormick banana extracts) were separately paired with different motivationally significant USs (i.e. 10% sucrose and 10% Polycose). We regard these nutrients to be motivationally significant for thirsty rats since as little as 8% (Albertella & Boakes, 2006)

and even 4% (Harris, Shand, Carroll, & Westbrook, 2004) concentration of sucrose has been found to establish a lasting preference for a flavor paired with sucrose over a flavor not paired with sucrose. Our rationale for using these particular nutrients was that there is evidence to suggest that these nutrients are distinctly different from one another, where an aversion to one of these nutrients does not generalize to the other nutrient (Nissenbaum & Sclafani, 1987). Half of the subjects in each group (BLA, OFC and Sham) received simultaneous presentations of flavors and nutrients, where the two were mixed in the same solution, for 15 min (simultaneous training conditions). The other half of the subjects were presented with the flavor solution for 5 min followed immediately by the nutrient solution for 10 min (sequential training conditions). Subsequently, one of the nutrients was devalued in all subjects by being paired with LiCl and the other nutrient was presented without LiCl injection. Finally, all subjects were given two-bottle flavor choice tests to assess sensory-specific flavor-nutrient learning and then two-bottle nutrient tests in order to validate that the two nutrients were differentially valued.

It was anticipated that during the two-bottle flavor choice test the sham control subjects would avoid the flavor associated with the devalued nutrient, as was also found by Blundell, et al., (2003). This selective aversion is our primary evidence that sensory-specific flavor-nutrient associations are learned. If each flavor cue evokes a representation of the nutrient with which it was paired, then it follows that if one of these nutrients was no longer valuable the flavor associate should also be avoided. Blundell, et al., (2003) suggested that the BLA would be involved in a task where the USs are motivationally significant. Assuming that 10% sucrose and 10% Polycose are motivationally significant USs, then BLA-lesioned rats should show a reduced US

devaluation effect. Moreover, this deficit might be seen equally in subjects given simultaneous and sequential training. Alternatively, if the BLA is only involved in tasks that allow for the subjects to predict the future occurrence of the US as is true in the magazine approach task, then one would expect that only BLA lesioned rats given sequential flavor-nutrient training would fail to show the devaluation effect and that the BLA lesioned rats given simultaneous flavor-nutrient training would not differ from the sham controls.

In addition to analyzing the effects of pre-training BLA lesions on the formation of sensory-specific flavor-nutrient associations, this experiment also analyzed the effects of OFC lesions on the formation of such associations. This experiment will extend previous research (Gallagher, et al., 1999, Pickens, et al., 2003) by analyzing the effects of OFC lesions on the formation of sensory-specific associations in a conditioned flavor preference task. If the OFC is involved in the formation of such associations in tasks where the outcomes are motivationally significant, then one would expect to find that the OFC lesions would impair the US devaluation effect regardless of whether the subjects received simultaneous or sequential flavor-nutrient training. However, if the OFC is only involved in tasks that allow the subjects to predict the future occurrence of the US, then one would expect that only the OFC lesioned subjects that received sequential training would be impaired on the US devaluation task, but that the subjects that received simultaneous training would not differ from Sham controls. Finally, we expected that the devaluation effect would be more pronounced in the sham subjects from Group Simultaneous than those in Group Sequential since there is evidence to suggest that

simultaneous training produces stronger associations than sequential training (Rescorla, 1980).

Method

Subjects

Subjects were 96 naïve Long Evans rats (24 male and 72 female), weighing 350-422 g (males) and 251-318 g (females) at the start of the experiment. The subjects were bred at Brooklyn College and derived from rats obtained from Charles River laboratory. The subjects were individually housed in stainless steel cages (24 cm × 18 cm × 17.5 cm) and maintained on a 14:10 light: dark cycle. Food chow (Lab Diet 5001) was available ad libitum throughout the study, but fluids were restricted to two, 15-min drinking sessions per day, which were always five hrs apart, starting four hrs after the lights came on in the colony rooms. All sessions were conducted in the rats' home cages.

Surgery

Some subjects received bilateral BLA lesions (n = 32), some received bilateral OFC lesions (n = 32), while the remainder received sham BLA (n = 16) or sham OFC (n = 16) lesions. Prior to surgery the subjects were anesthetized with sodium pentobarbital (50mg/kg; Sigma-Aldrich Laboratories, administered intraperitoneally). Fifteen min after receiving the anesthetic, the subjects were treated with atropine sulfate (0.54 mg/kg; Sigma-Aldrich Laboratories, administered intramuscularly) in order to maintain respiration. The subjects were then placed into the stereotax (Stoelting Co.). The subjects' eyes were treated with one drop of mineral oil (Rite Aid Pharmacy) in order to

prevent corneal damage. Incision was made with a scalpel blade in order to expose the skull. In order to ensure the accuracy of head placement, bregma and lambda coordinates were identified and compared to one another. If the DV coordinates of the two were different by more than 0.1 mm, the head was adjusted until the DV measurements matched. Holes were drilled at the proper locations calculated from bregma. The coordinates relative to bregma for the BLA lesioned subjects were as follows: -2.3, -3.0 (AP); +/- 5.2 (ML); -7.6 (DV). The infusion was made with an infusion pump (KD Scientific 53100) through a 1 µl Hamilton syringe. Neurotoxin (.25µl of NMDA; 20µg/µl mixed in distilled water) was infused into each hole at the rate of .10µl/min and to allow for diffusion the needle stayed in place for an additional 3 min prior to being extracted from the infusion site. The coordinates for the OFC lesioned subjects were as follows: +3.5 (AP); +/- 3.2 (ML); -4.7 (DV). NMDA (20µg/µl) was infused at the rate of .12 µl/min, in the amount of .50µl for each hole. After the injection, the needle remained in place for an additional 5 min before being extracted to allow for diffusion. Sham controls received the same treatment as the lesioned subjects except that after the needle was lowered into the appropriate location, the NMDA was not infused. After the infusions, the subjects were sutured and treated with Bacitracin (Rite Aid Pharmacy) applied to the site of damage and physiological saline (5 ml) was injected subcutaneously in order to hydrate the animal. After completion of the surgery the subjects were placed into a heated chamber and were monitored until they woke up. They were then placed back into their home cages, given ad libitum food (Lab Diet 5001 pellets) and water and allowed two weeks to recover prior to participating in the experiment.

Histology

After completing the experiment, the subjects were injected (i.p.) with 1 ml of diluted (10:1) Beuthanasia D, which resulted in each rat receiving 39 mg/ml of sodium pentobartbital (Schering Plough Corporation). Once non-responsive, the rats were perfused transcardially with physiological saline and 10% formalin. The brains were extracted and stored in a refrigerator (3^{o} C) in a solution of 30% sucrose and 10% formalin for 1 week. The brains were then placed in a Cryostat (Microm HM 505E) and allowed 15 min to freeze (-23^{o} C). The brains were then sectioned (40µm coronal sections) and mounted onto slides (Corning Frosted Micro Slides), which were pretreated with 2% gelatin (Gelatin Type B, Sigma Aldrich). The tissues were then Nissl stained (Goble, 2009), and coverslipped (Corning Cover Glass) using Permount histological mounting medium (Sigma Aldrich). The extent of lesion was assessed with a microscope (4, 10, 40, and 100 times zoom) depending on the amount of neuronal loss in the targeted area.

Solutions

The solutions were presented in 50 ml testing tubes and were attached to the outside of the cage using a metal spring. The flavor CSs consisted of 1% banana and 1% almond McCormick's imitation flavor extracts mixed with tap water. The nutrient USs were 10% sucrose (Domino) and 10% Polycose (Ross Laboratories) solutions prepared with tap water. These flavors and nutrients were either presented separately (Group Sequential) or mixed together (Group Simultaneous) depending on group assignment.

Procedure

Acquisition

In order to familiarize the subjects with the water deprivation schedule, they were given one-bottle water training, which lasted three days. During this phase, the subjects were presented with a single 50ml tube of tap water for 15 min twice a day, presented 5 hrs apart starting 5 hrs after the lights came on in the colony rooms. The next day after the water training was complete, the acquisition training began. During the acquisition phase the subjects were presented with one flavor CS (for example, banana) paired with a particular nutrient US, either sucrose or Polycose and the other flavor CS (for example, almond,) paired with the opposite nutrient. For counterbalancing purposes, half of the animals received one type of set of flavor-nutrient pairings and the other half received the other. The simultaneous groups (N = 36) received the flavor CS mixed in solution with a nutrient US for 15 min. The sequential groups (N = 60, run in two replications) received the flavor CS for 5 min followed immediately by the appropriate nutrient US for 10 min. One third of the subjects in each set of groups (simultaneous and sequential) were BLA lesioned, one third were OFC lesioned, and the remainder had sham lesions. Each flavor-nutrient pairing was presented once a day for 8 days, five hrs apart and was counterbalanced for order across days. Thus, each flavor CS was paired with a distinctive nutrient US on 8 occasions four times in the AM session and four times in the PM session. Intakes were recorded to the nearest 0.1g by comparing the weight of the bottles and its solution before and after consumption.

Devaluation

On the day following the final acquisition session, the animals entered a 6-day devaluation phase. The devaluation phase consisted of three, 2-day cycles, where on the first day of each cycle the subjects were presented with nutrient-LiCl pairings and on the following day they were presented with the other nutrient without LiCl. In particular, during the 15-min AM session on days 1, 3, and 5, the subjects were presented with one of the nutrients (without its associated CS). Immediately following consumption of the devalued nutrient, they were injected (i.p.) with LiCl (.3 M, 1% body weight). For half of the animals sucrose was devalued and for the other half Polycose was devalued. Subjects were given tap water for 15 min during the PM session of each day. During the AM session on days 2, 4, and 6, the subjects were presented with the alternate nutrient, but were not given LiCl. During the PM session on these days the subjects were again given tap water to drink for 15 min. Intakes of all the solutions were recorded following each session.

Testing

The day after the devaluation training was completed, the subjects were given one day of two-bottle water training in order to familiarize them with the testing procedure, where they were presented with two test tubes each containing tap water during the AM session. All two-bottle choice tests throughout all experiments were conducted in the following manner. The subjects were first given the solution on the left to sample. After they had tasted it, the solution was removed and they were allowed to taste the solution on the right. Once they sampled that solution, it was removed and both solutions were then simultaneously presented allowing the subjects to choose which

solution they wanted to drink for 15 min. During the PM session on this day the subjects were given tap water to drink for 15 min in a single bottle. On each of the two days following the two-bottle water training session, the subjects were given a two-bottle flavor choice test during the AM session. In these sessions, one bottle contained one flavor CS and the other bottle contained the other flavor CS, and these were pitted against one another without any nutrients for 15 min. On the following day the flavor CS that was presented on the right was presented on the left and vice versa in order to counterbalance the side positions. In the PM sessions on these days the subjects were given one bottle of tap water to drink for 15 min.

On the two days following the completion of the two-bottle flavor CS choice tests, the subjects were given 2, two-bottle nutrient choice tests in the AM sessions in order to verify that the nutrients were differentially valued. These tests were conducted exactly like the preceding flavor tests except that the two nutrient USs were pitted against one another (in the absence of the flavor CSs). In the PM sessions on these days the subjects were given one bottle of tap water to drink for 15 min. All intakes of the solutions were recorded during each session.

Statistical analysis

Here and throughout this dissertation, analysis of variance (ANOVA) techniques were used to analyze the data. Rodger's F table and post hoc methods (Rodger, 1974) were used to evaluate the data presented here because these methods are more powerful at detecting true effects than more conventional approaches. Throughout all the experiments a Type I error criterion of $p < 0.05$ was adopted.

Results

Histology

Figures 1 and 2 display the maximum and minimum damage as a result of BLA (Figure 1) and OFC (Figure 2) lesions of subjects that were included in the analysis for subjects trained in both the simultaneous and sequential conditions. The black areas represent minimum lesion and the grey areas represent the areas of maximum lesions. The lesion was considered to be appropriate if significant neuronal loss was found in the target area and was accompanied by gliosis (i.e. proliferation of astrocytes). BLA lesions typically caused neuronal loss in the anterior, posterior and ventral basolateral amygdala with some sparing of the tissue in dorso-lateral amygdala. OFC lesions extended to the ventral orbital medial orbital, and the lateral orbital cortexes. Subjects with unilateral lesions or with lesions outside of the target areas were excluded from the analysis.

After histological analysis, 4 BLA subjects in the simultaneous condition and 5 BLA subjects in the sequential condition were excluded due to inappropriate lesion. In addition, 5 additional BLA subjects and 2 OFC subjects from the sequential condition were excluded as the tissue became damaged during histology rendering it impossible to analyze. The data presented here include only the remaining subjects whose lesions were analyzed to be appropriate rendering the following number of subjects given simultaneous training: $n = 8$ for BLA, $n = 12$ for OFC, and $n = 12$ for Group Sham. For the subjects given the sequential training the final counts were as follows: $n = 10$ for BLA, $n = 18$ for OFC and $n = 20$ for Group Sham.

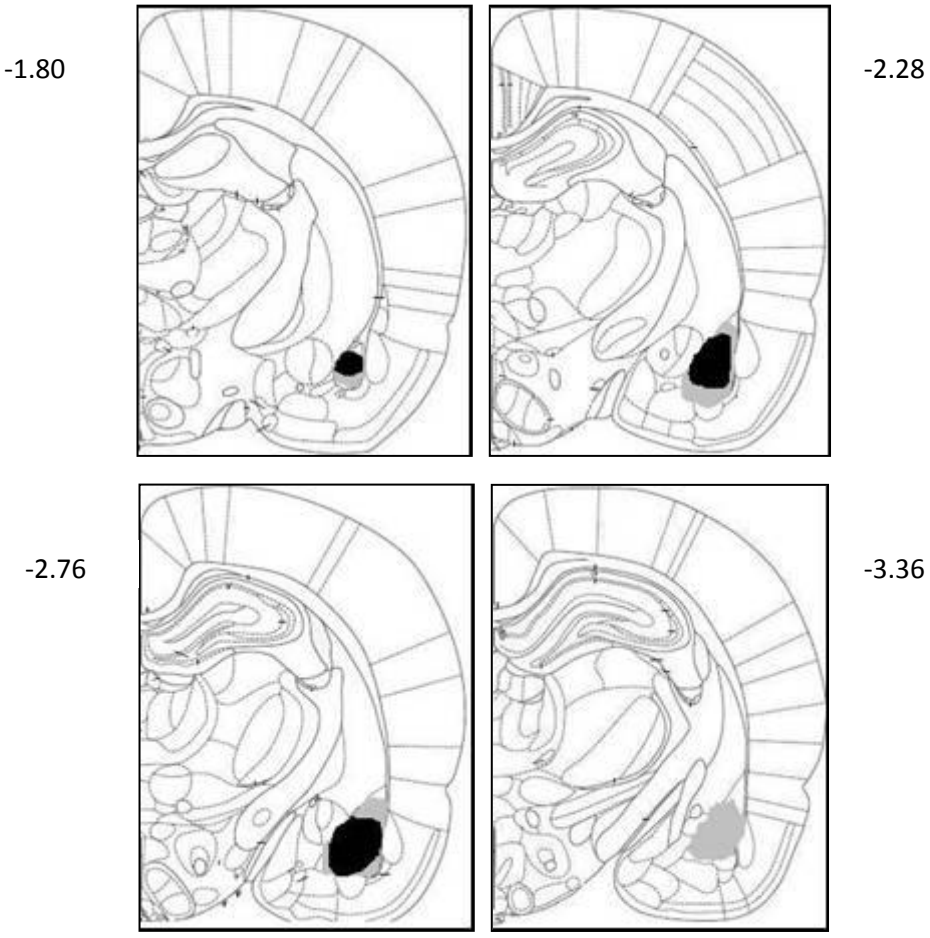

Figure 1. Experiment 1, BLA histology results. Diagrams of coronal sections (40 µM slices) illustrating the extent of the BLA lesions for the subjects that received simultaneous (*n* = 8) and sequential (*n* = 10) training ranging from -1.80 to -3.36 mm (posterior to bregma). The drawings illustrate the approximate extent of the lesions. The black areas represent minimum lesion and the grey areas represent the areas of maximum lesions. Images adapted from Paxinos and Watson, (2009).

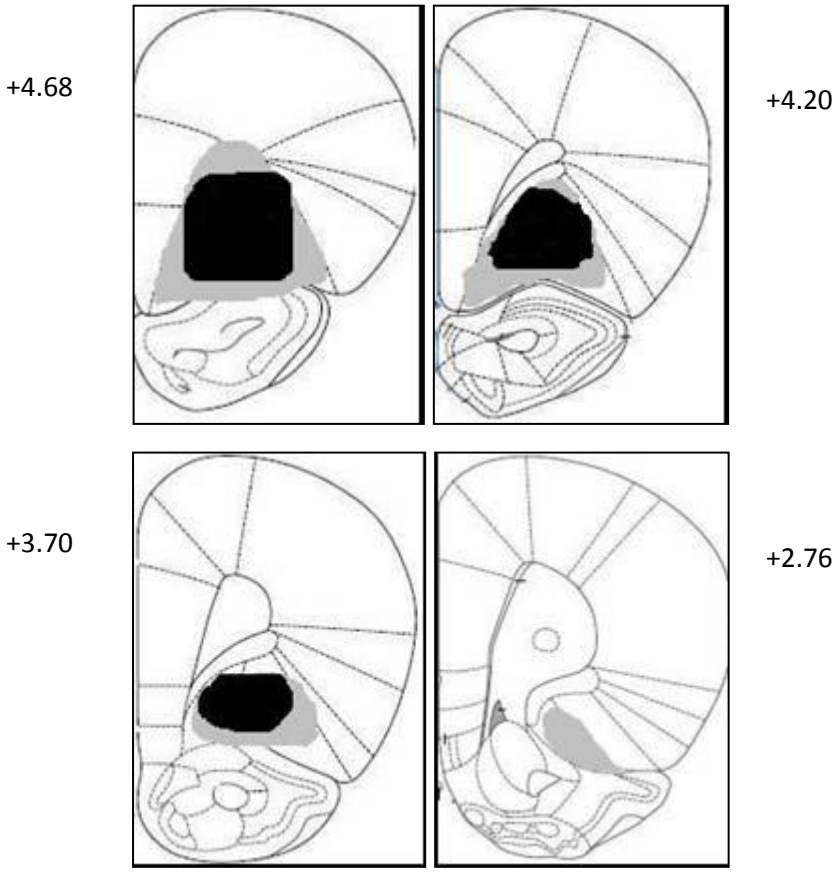

Figure 2. Experiment 1, OFC histology results. Diagrams of coronal sections (40 µM slices) illustrating the extent of the OFC lesions for the subjects that received simultaneous (n = 12) and sequential (n = 18) training ranging from 4.68 to 2.76 mm (anterior to bregma). The drawings illustrate the approximate extent of the lesions. The black areas represent minimum lesion and the grey areas represent the areas of maximum lesions. Images adapted from Paxinos and Watson, (2009).

Acquisition

Preliminary analyses found no differences between males and females throughout the experiment in any measure, so the data presented here were collapsed across sex. Acquisition intake data for subjects given simultaneous training are illustrated in Figure 3. The top panel shows the average intakes of the flavor + nutrient solutions for the subjects in Group BLA over the 8 acquisition cycles. The solutions are represented as

flavors paired with sucrose (Fs) and flavors paired with Polycose (Fp). The middle panel shows the same data for Group OFC and the bottom panel shows the same data for Group Sham combined across both sham conditions. The results suggest that all subjects increased their overall intakes over the course of training and that they consumed slightly more of the flavor + sucrose mixture than the flavor + Polycose mixture. A Flavor (sucrose vs. Polycose) x Lesion (BLA vs. OFC vs. Sham) x Cycle (1-8) ANOVA revealed significant main effects of Cycle, F (7, 203) = 58.17, and Flavor, F (1, 29) = 11.48, but no other main effects or interactions. In addition, separate t-tests were performed between the baseline water intake and the Fs and Fp solutions on the last 2 days of training. The results suggest that both Fs, t (62) = 10.35, and Fp, t (62) = 8.92, were consumed above baseline.

The acquisition data for subjects given sequential training are illustrated in Figure 4. The figure shows the average solution intakes during the acquisition phase for BLA (top panel), OFC (middle panel), and Sham (bottom panel) lesioned subjects over 8 training cycles. The flavor solution intakes are presented on the left panels and the nutrient solution intakes are presented on the right panels. Fs refers to the intake of the flavor associated with sucrose, whereas Fp refers to the intake of the flavor associated with Polycose. It was observed that all the subjects increased their intakes of the flavor as well as well as the nutrient solutions over the course of training. However, all the subjects consumed equally low amounts of Fs and Fp flavor solutions and equally high amounts of sucrose and Polycose nutrient solutions.

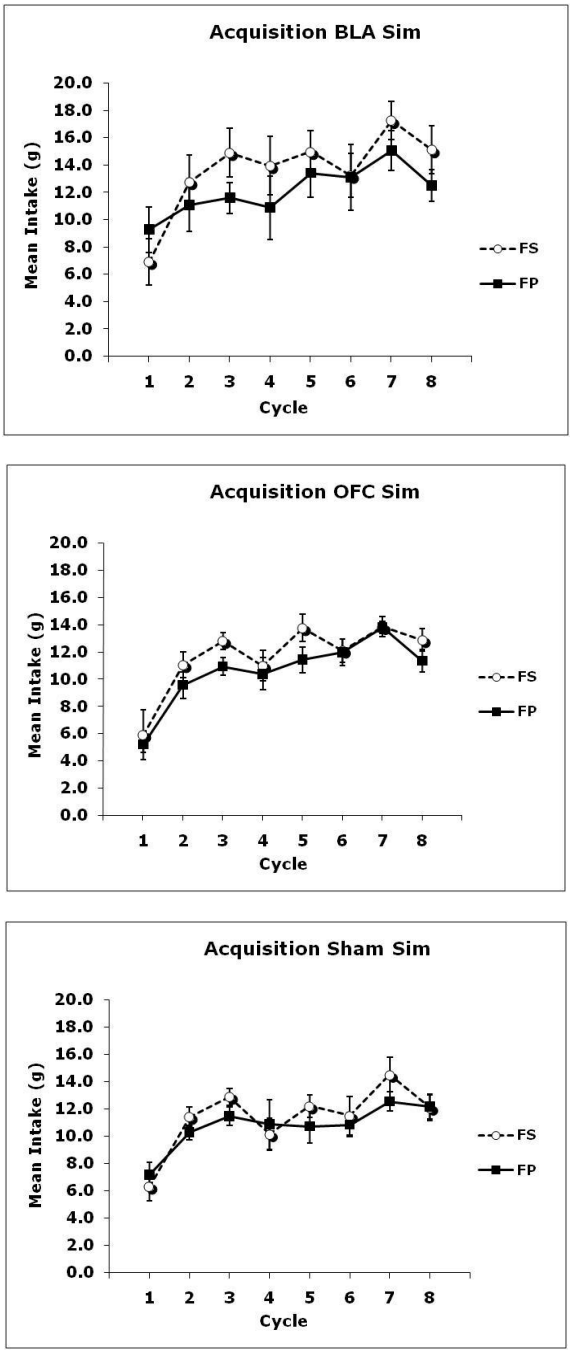

Figure 3. Experiment 1, Acquisition data for subjects with simultaneous training. Mean solution intakes (to the nearest 0.1g) during the acquisition phase for BLA (top panel), OFC (middle panel), and Sham (bottom panel) lesioned subjects over 8 training cycles in Group Simultaneous. The solutions consisted of 1% McCormick extract CSs (almond and banana) each mixed in a solution with different nutrient USs (10% sucrose and 10% Polycose). Fs refers to the intake of the solution where the flavor was paired with sucrose. Fp refers to the intake of the solution where the flavor was paired with Polycose.

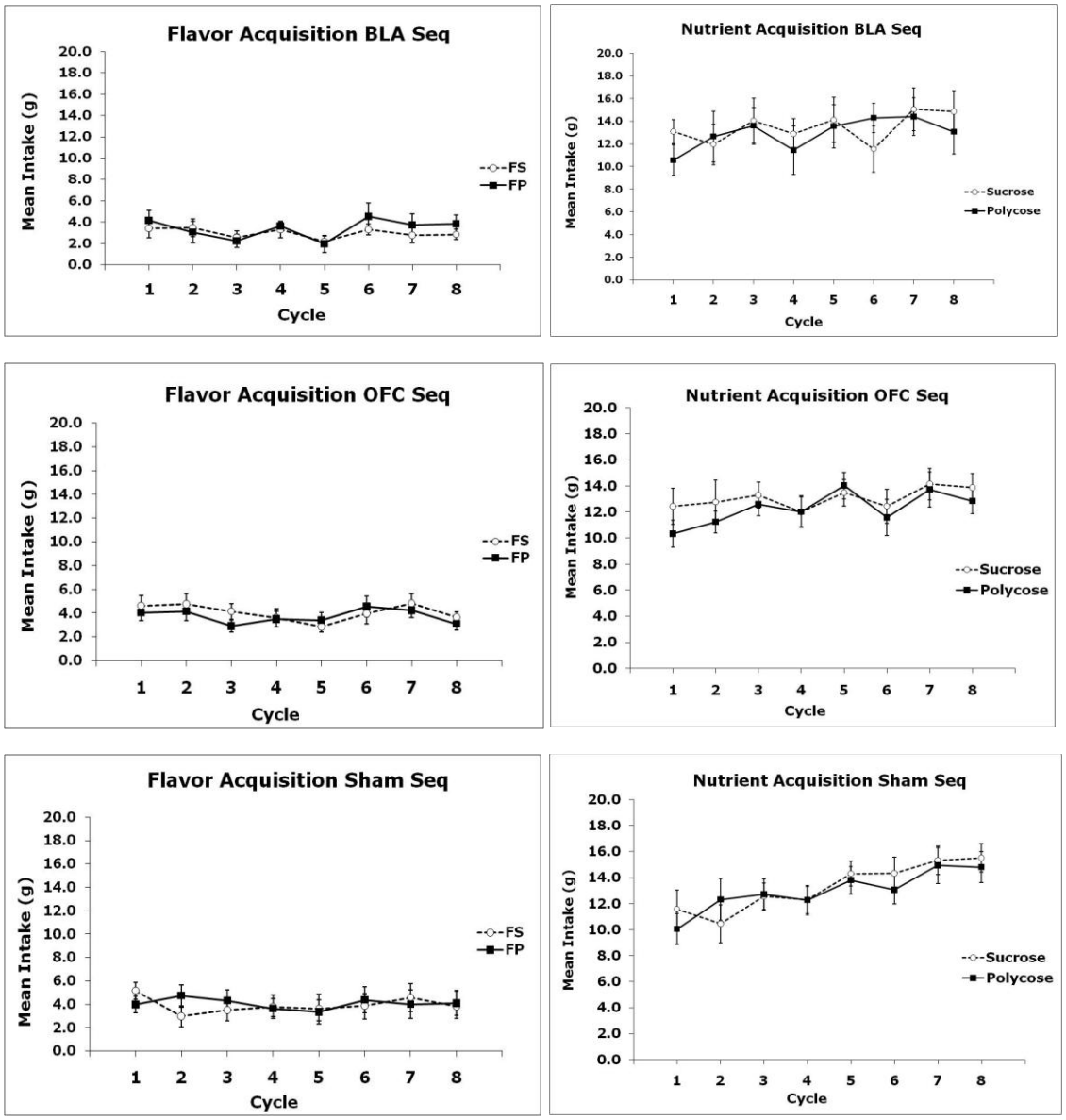

Figure 4. Experiment 1, Acquisition data for subjects with sequential training. Mean solution intakes (to the nearest 0.1g) during the acquisition phase for BLA (top panel), OFC (middle panel), and Sham (bottom panel) lesioned subjects over 8 training cycles in subjects with sequential training. The flavor solutions (presented on the left panels) consisted of 1% McCormick extract CSs (almond and banana) each presented for 5 min and then followed by one of the nutrients USs (10% sucrose and 10% Polycose, illustrated on the right panels) for 10 min. Fs refers to the intake of the flavor associated with sucrose. Fp refers to the intake of the flavor associated with Polycose.

A Flavor (Fs vs. Fp) x Lesion (BLA, OFC, or Sham) x Cycle (1-8) ANOVA performed on the flavor intake data revealed a main effect of Cycle, F (7, 315) = 18.90 but no other main effects or interactions. A Nutrient (Sucrose vs. Polycose) x Lesion (BLA, OFC, or Sham) x Cycle (1-8) ANOVA also revealed a significant main effect of cycle, F (7, 315) = 11.57, but no other main effects or interactions. Additional t-test analyses indicated that while the flavor solutions were not consumed above baseline levels, both sucrose, t (94) = 12.51 and Polycose, t (94) = 12.38 were consumed above the water baseline levels, providing some evidence that these nutrients were, indeed, motivationally significant.

Devaluation

The devaluation data are illustrated in Figure 5, where top panels represent BLA lesioned subjects, middle panels represent OFC lesioned subjects and bottom panels represent Sham subjects. The panels on the left illustrate data for the subjects given simultaneous training and the panels on the right illustrate the data for the subjects given sequential training. The figures display mean intakes of the devalued (dev) and the nondevalued (ndev) nutrients over the three devaluation cycles. The results demonstrate that initially there were no differences between the nutrient intakes but over the course of training all subjects learned to avoid the devalued nutrient but consumed more of the nondevalued nutrient. Since no intake differences were found to be dependent on whether the subjects were devalued on sucrose or Polycose in a preliminary analysis, the data were collapsed across the nutrient factor. Separate Cycle (1-3) x Devaluation (devalued vs. nondevalued) x Lesion (BLA, OFC, or Sham) ANOVAs were conducted on these data and this analysis revealed a significant Cycle x Devaluation interaction for both the

set of subjects given simultaneous training, F (2, 58) = 43.94 and those given sequential training, F (2, 52) = 67.70, however, these did not interact with Lesion. This result suggests that all subjects learned to selectively avoid the devalued nutrient and consume the nondevalued nutrient by the end of selective devaluation training regardless of lesion condition.

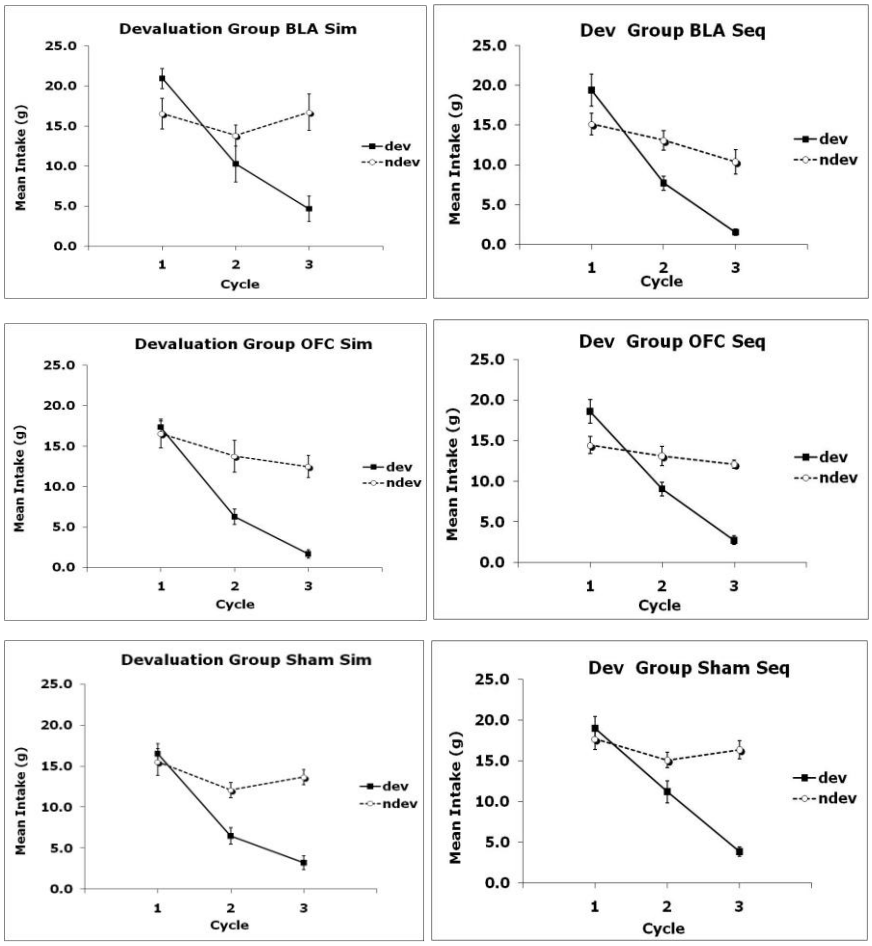

Figure 5. Experiment 1, devaluation training. Mean intakes of the devalued (dev) and the nondevalued (ndev) nutrients during the devaluation phase for BLA (top panel), OFC (middle panel) and Sham (bottom panel) lesioned subjects in with simultaneous (Sim) training (presented on left) and sequential (Seq) training (presented on right) over the three cycles. Devalued nutrients were paired with .3M of LiCl (1% bodyweight), whereas the nondevalued nutrients were not paired with LiCl. No flavor CSs were presented during this phase.

Flavor Tests

The data of most interest are shown in Figure 6, which illustrate the results of the 2, two-bottle Flavor Tests combined in the subjects given simultaneous training (top panel) and those given sequential training (bottom panel). Preliminary analyses did not find a difference between subjects devalued on sucrose and those devalued on the Polycose US, so the data were collapsed across the devalued nutrient factor. The figure illustrates the mean intakes of the flavor paired with the devalued nutrient (Fd) and the flavor paired with the nondevalued nutrient (Fnd) for each lesion condition. The results show that all subjects despite lesion condition or training procedure consumed more of the Fnd solution than of Fd . Separate Flavor (Fd vs. Fnd) x Lesion (BLA, OFC, or Sham) ANOVAs performed on the simultaneously and sequentially trained animals revealed a significant main effect of Flavor for the groups given simultaneous conditioning, F (1, 29) = 67.72, as well as for the groups given sequential conditioning, F (1, 45) = 10.20, but in neither case did these interact with Lesion.

Nutrient Tests

The nutrient test data collapsed across tests 1 and 2 for the groups given simultaneous (top panel) and sequential training (bottom panel) are illustrated in Figure 7, which shows the mean intakes of the devalued (Dev) and the nondevalued (Ndev) nutrient USs for each lesion condition. Since preliminary analyses did not find significant differences between subjects devalued on sucrose and those devalued on Polycose US, the data were collapsed across this factor. The results found that all the subjects consumed significantly more of the nondevalued than the devalued solution. Separate

Nutrient (Dev vs. Ndev) x Lesion (BLA, OFC, or Sham) ANOVAs yielded a significant main effect of Nutrient, F (1, 29) = 180.79 for the groups given simultaneous training and F (1, 45) = 280.22 for the groups given sequential training, but no Lesion main effect or Nutrient x Lesion interaction.

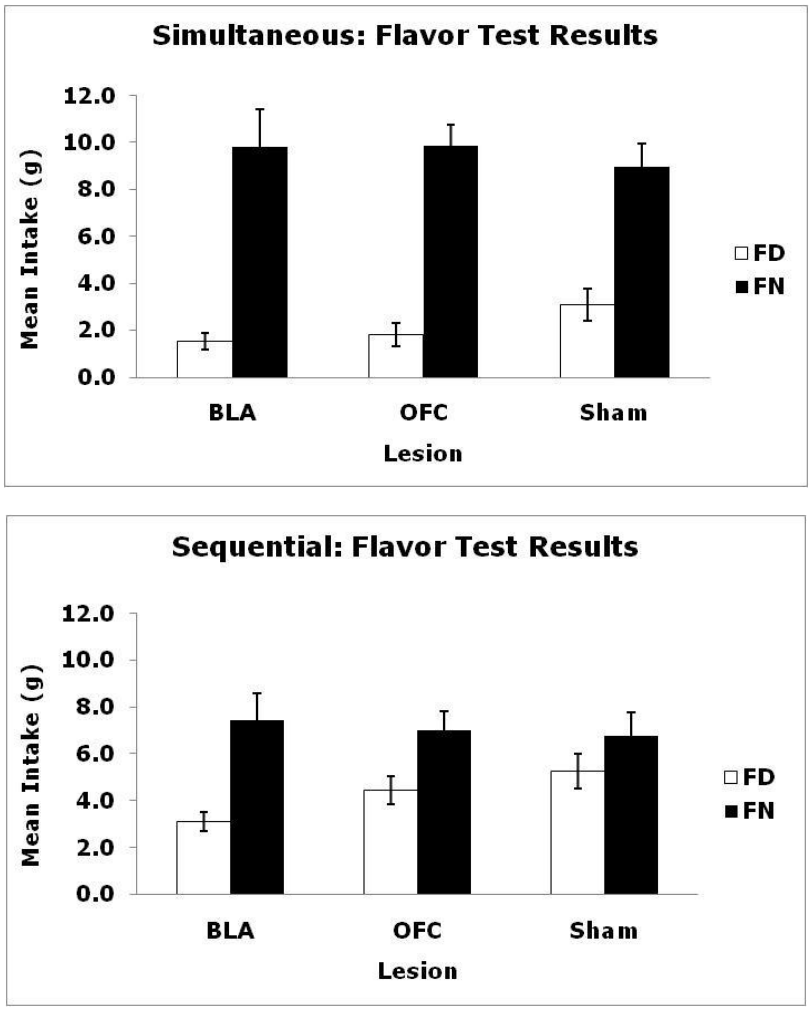

Figure 6. Experiment 1, flavor test results. Mean intakes of the flavor paired with the devalued nutrient (Fd) and flavor paired with the nondevalued nutrient (Fnd) collapsed across the two 2-bottle choice flavor tests, where the flavor CSs (i.e. 1% almond and 1% banana extracts) were pitted against each other without any nutrients present. The top panel illustrates the results of the two-bottle flavor choice tests for subjects with simultaneous training, whereas the bottom panel illustrates the results from the two-bottle flavor choice test for subjects with sequential training.

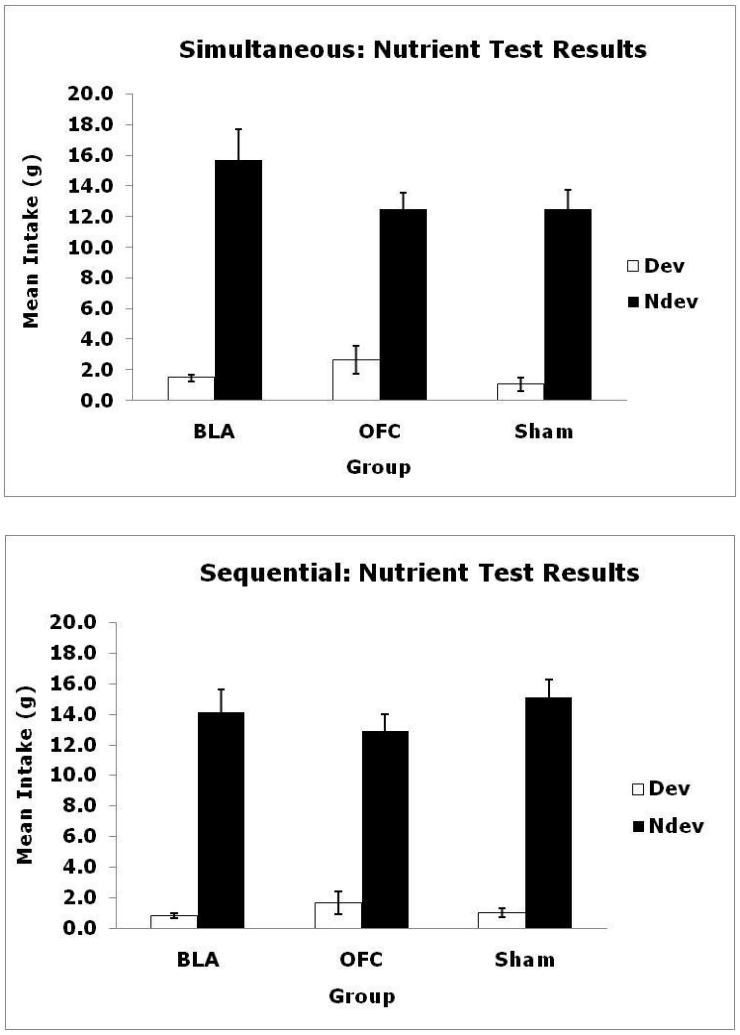

Figure 7. Experiment 1, nutrient test results. Mean intakes of the devalued nutrient (Dev) and the nondevalued nutrient (Ndev) collapsed across the two 2-bottle choice nutrient tests, where the nutrient USs (i.e. 10% sucrose and 10% Polycose) were pitted against each other without any flavor CSs present. The top panel illustrates the results of the two-bottle nutrient choice tests in subjects with simultaneous training, whereas the bottom panel illustrates the results from the two-bottle nutrient choice test in subjects with sequential training.

Discussion

The results of this experiment revealed that all the subjects regardless of lesion condition (BLA, OFC, or Sham) or training type (simultaneous or sequential) acted in a

similar fashion during the flavor preference test: all subjects consumed significantly more of the flavor paired with the nondevalued nutrient than the one paired with the devalued nutrient. These findings suggest that neither of these structures is crucial to establish sensory-specific flavor-nutrient associations even when neutral flavor CSs are trained with motivationally significant nutrient USs. Furthermore, whether the CSs predict the future occurrence of the USs or occur simultaneously with them is also not critical in determining the involvement of the BLA and OFC in the development of sensory-specific flavor-nutrient associations.

These findings therefore do not support the hypothesis that in order for the BLA to be involved in the formation of sensory-specific flavor-nutrient association, the flavor has to be presented before the nutrient, as we saw no differences between the groups that received simultaneous and sequential training. Nor do these results support Blundell, et al.'s, (2003) hypothesis that the BLA is only involved in tasks where predictive cues signal motivationally significant outcomes, since we used motivationally significant outcomes (i.e. 10% sucrose and 10% Polycose) and still failed to find BLA lesion effects on selective US devaluation. It is possible that the concentrations used here were not motivationally significant enough to involve these structures. However, Albertella and Boakes (2006) found that 8% sucrose was motivationally significant enough to support a long lasting flavor preference to a flavor previously paired with sucrose. Furthermore, Desgranges, et al., (2010) found that as little as 3.4% of sucrose was enough for the subjects to display a significant taste preference for a sucrose-paired odor (i.e. almond) over water, whereas subjects that were trained with unpaired presentations of almond and

sucrose demonstrated the opposite preference pattern in two-bottle choice tests. These findings suggest that our nutrients were, indeed, motivationally significant.

The results from this experiment could also be interpreted to mean that while the BLA and OFC may be involved in the formation of sensory-specific associations, neither alone is solely responsible for this type of learning. If one structure is damaged, then perhaps other structures could contribute to this type of learning. For example, Kerfoot and colleagues (2007) found higher *c-Fos* expression in the BLA, OFC, GC and posterior accumbens shell in subjects that were tested with a sucrose-paired cue following a US devaluation treatment compared to controls. These results imply that more than one structure is involved in this type of task. Therefore, it is possible that lesioning one of these structures results in compensatory plasticity in other structures whereby sensory-specific flavor-nutrient associations can still be learned properly. However, this hypothesis cannot explain why pre-training BLA and OFC lesions affect performance in some paradigms (e.g. magazine approach, PIT), but not in the present task. Potential explanations for this inconsistency are further addressed in the General Discussion section.

Before the results in Experiment 1 can be clearly evaluated, however, we need to rule out several other explanations for our failure to detect BLA and OFC lesion effects on the formation of sensory-specific flavor-nutrient associations. First, it is possible that our lesion techniques used in this study would not be behaviorally effective even in tasks expected to produce such effects. In order to verify the effectiveness of our lesions, a study using procedures that previously showed a lesion effect on sensory-specific

learning (e.g. PIT and US devaluation tasks in a magazine approach paradigm) would need to be performed. If doing such an experiment yields a lesion effect on sensory-specific learning, then we can be more confident that our lesion techniques were behaviorally effective. Second, it is possible that structures are only involved in the formation of sensory-specific associations in tasks where the subjects are maintained in a hungry motivational state. Other studies finding lesion effects explored this issue in hungry rats whereas our rats were thirsty throughout the experiment. If this variable were important, then the results of Experiment 1 would not be surprising. Finally, it is possible that lesioning the BLA and/or the OFC results in the US devaluation deficit only under special circumstances. In prior magazine approach conditioning studies that successfully found lesion effects (Gallagher, et al., 1999; Hatfield, et al., 1996), rats were given Pavlovian training with a single CS-US pair. In Experiment 1, our rats were given Pavlovian training with two distinct CS-US pairs. Training with multiple CS-US pairs might render the task more sensitive than training with a single CS-US pair at detecting US devaluation effects. If the number of CS-US pairs is important, then it is possible that Experiment 1 failed to find any lesion effects for this reason.

Experiment 2: The effects of BLA and OFC lesions on the formation of sensory-specific associations in a magazine approach paradigm

In Experiment 1 we failed to find an effect of BLA and OFC lesions on the development of sensory-specific flavor-nutrient associations. In order to determine if our lesion techniques are behaviorally effective, Experiment 2 examined the effects of BLA and OFC lesions on sensory-specific learning in thirsty rats using auditory and visual CSs to predict the same motivationally significant nutrient USs used in Experiment 1. This study extended the previous findings of Corbit and Balleine (2005) and Ostlund and Balleine (2007) by using a PIT task with two liquid reinforcers to assess the fate of sensory-specific associations in thirsty, as opposed to hungry rats. Furthermore, this study also extended the findings of Gallagher, et al., (1999) and Hatfield, et al., (1996) by using a US devaluation technique to assess the fate of sensory-specific associations in a design where two CS-US pairs, as opposed to one, were learned. If lesion effects can be documented with these more typically used procedures, then it would suggest that our failure to find such effects in Experiment 1 were not simply due to behaviorally ineffective lesions in that study.

The design for Experiment 2 is illustrated in Table 2. In this experiment thirsty subjects with pre-training BLA, OFC, or Sham lesions first learned to associate one CS (e.g. tone) with a 10% sucrose US and another CS (e.g. light) with a 10% Polycose US during the Pavlovian training phase. Then, during an instrumental training phase, the subjects learned to make one instrumental response (e.g. lever press) to earn the sucrose US and another instrumental response (e.g. chain pull) to earn the Polycose US.

Subsequently, during the Pavlovian-instrumental transfer test, the subjects were separately presented with each CS while choosing between the two instrumental responses under extinction conditions.

PIT Task

Pavlovian	Instrumental	Retraining	PIT Test
CS1 -> US1	R1 -> US1	CS1 -> US1	CS1: R1 vs. R2
CS2 -> US2	R2 -> US2	CS2 -> US2	CS2: R1 vs. R2

US Devaluation Task

Retraining	Devaluation	Devaluation Test	Nutrient Test
CS1 -> US1	US1 -> LiCl	CS1 vs. CS2	US1 vs. US2
CS2 -> US2	US2 -		

Table 2. Experimental Design for Experiment 2. The CSs consisted of tone (1500 Hz) and flash (6-W bulb). The USs were 10% sucrose and 10% Polycose. The instrumental responses were lever press and chain pull. All conditions were counterbalanced across subjects. During each of the two PIT tests, the subjects received 16 presentations of each CS and were allowed to make an instrumental response in either manipulandum without any USs present.

Based on prior work Sham subjects should make more instrumental responses when the stimulus and response were previously reinforced with the same as opposed to different US. Similar results are typically found in PIT tasks using normal subjects

(Colwill & Motzkin, 1994; Delamater & Holland, 2008; Kruse, et al., 1983) although to the best of my knowledge this has not been examined in rats trained and tested while thirsty. However, we anticipated that the BLA and possibly also OFC lesioned subjects would not show a selective PIT effect, as was previously found by Corbit and Balleine (2005), who used hungry subjects and pellet and liquid outcomes. On the other hand, Ostlund and Balleine (2007) reported that while post-training OFC lesions caused the subjects to become impaired on the PIT task, pre-training lesions failed to impair this effect. This finding suggests that the OFC is not critical in the formation of sensory-specific associations but becomes critical later on in training. However, Ostlund and Balleine (2007) speculated that their lesions may not have encompassed an area as extensive as that used by Gallagher, et al., (1999), which suggests that larger OFC pre-training lesions may result in a selective PIT impairment.

After the PIT tests, the subjects received additional Pavlovian training and then underwent a US devaluation phase, in which one of the nutrients USs was devalued by being paired with LiCl and the other nutrient US was not devalued. After selective devaluation training, the subjects were given two devaluation tests, where the two CSs were presented separately under extinction conditions. In this test Sham subjects should make more CRs during the CS whose associated nutrient US was not devalued than during the CS whose associated nutrient US was devalued. This would indicate that the two CSs had formed sensory-specific associations with their respective USs. However, based on prior work, BLA and OFC lesioned subjects should not demonstrate a significant difference in responding as was previously found by Gallagher, et al., (1999), and Hatfield, et al., (1996), assuming that our lesions are behaviorally effective and that

the results occurring with hungry rats apply to thirsty rats. Finally, if the BLA and OFC lesions only result in the US devaluation effect when one, but not two CS-US associations are formed, then we would not expect to find a deficit on the US devaluation effect in any subjects.

Method

Subjects

Subjects were 36 naïve Long Evans rats (21 male and 15 female). Some subjects received a BLA lesion (n = 12), some received an OFC lesion (n = 12), while the remainder received a sham BLA (n = 6) or OFC (n = 6) lesion. The rats were individually housed and maintained in conditions similar but not identical to those in Experiment 1. In particular, throughout the experiment all subjects were maintained with unlimited access to food, but their water access outside of the experimental session was restricted to 30 min/day (approximately 3 hrs after a given training session).

Surgery and histology

The surgery and histology methods were identical to those used in Experiment 1.

Apparatus

The apparatus consisted of two sets of eight identical conditioning chambers, which were encased in lightproof and soundproof wooden shells. The dimensions of the conditioning chambers were 30.5 cm long x 24.0 cm wide x 25.0 cm deep. The end walls were made from aluminum and the ceiling and the sidewalls were made from clear

Plexiglass. The food magazine measured 3.0 cm long x 3.6 cm wide x 2.0 cm deep and was located at the center of one of the end walls. The USs (0.1 ml droplet of 10% sucrose (Domino) or 10% Polycose (Ross Laboratories) was delivered into a well on the bottom of the food magazine. The floor was made from stainless steel rods (0.6 cm in diameter, 2.0 cm apart). An infrared detector and emitter were mounted on the magazine walls (and positioned at the entrance) to record magazine entry behavior. A response lever was 4 cm wide and was located 3 cm to the right of the food magazine and 8 cm above the floor level. While the lever was permanently mounted in the chamber, access to it was only available during appropriate instrumental training or test sessions. A chain manipulandum was located 3 cm to the left of the magazine. Unless used during the instrumental training and test sessions, the chain was not present in the chamber. A tone CS (1500 Hz) was produced by a speaker located approximately 22 cm behind the front wall of the conditioning chamber. This tone measured 4dB above background noise levels. A flashing light CS (6-W light bulb) was mounted on the bottom sidewall of the outer chamber and the light flashed with approximately equal on-off pulse durations at the approximate rate of 2/sec. Background noise and ventilation was provided by a fan, which was attached to the outer shell. Background noise was measured at 78dB. The equipment was controlled and data were recorded by a personal computer and interfacing equipment (Alpha Products), which was located in the same room as the experimental chambers.

Procedure

Magazine training

For two days thirsty subjects were placed in individual Skinner boxes and were taught to approach the food magazine. During each 40 min training session the subjects were given 20 presentations of one kind of US during the first 20 min of the session and this was followed by 20 presentations of the other US presented during the second half of the session. The USs were presented on a variable time 60-sec schedule. The two USs (i.e. 10% Sucrose and 10% Polycose) were delivered to different wells, adjacent to each other in the food magazine. On each of the two days, the order of US presentations within the session was counterbalanced. Access to the lever manipulandum was prevented with a sheet metal covering and the chain manipulandum was withdrawn during the magazine and Pavlovian training phases. The tone and flash CSs were not presented during the magazine training phase.

Pavlovian training

For eight days day following the completion of magazine training, the subjects were taught to associate the tone CS with one US (e.g. sucrose) and the flash CS with the other US (e.g. Polycose). Half of the subjects in each lesion condition (BLA, OFC, and Sham) had tone paired with sucrose and light paired with Polycose and the rest of the subjects received the alternate pairings. Each conditioning session was 1 hr and 7 min long, during which there occurred 6 presentations of each CS-US pair. The order of CS presentations was varied irregularly across days with the constant that no single CS could occur three or more times on a row. Each CS lasted for 30 sec and the appropriate US was delivered immediately at the offset of the CS. The intertrial intervals (ITS) varied between 180 and 420 sec and averaged 300 sec. The subjects were removed from the

chambers 1 min following the final conditioning trial. The number of magazine approach responses was recorded 30 sec before, during, and 20 sec after each trial.

Instrumental training

On the day immediately following the completion of Pavlovian training phase, the subjects were taught to press the lever to earn one outcome (e.g. sucrose) and pull the chain for the other outcome (e.g. Polycose) on a continuous reinforcement schedule (CRF). Half of the subjects in each lesion condition (BLA, OFC, and Sham) received lever paired with sucrose and chain paired with Polycose and the rest of the subjects received the alternate pairings. These assignments were orthogonal to the particular Pavlovian CS-US combinations. Each subject was trained on the CRF schedule until they made 50 responses on each manipulandum. After completing CRF training, all subjects were trained on steadily increasing variable interval schedules: VI 10 sec for 1 day, VI 30 sec for 2 days, and VI 60 sec for 2 days. On each day, subjects were given two 20-min training sessions, one with lever and one with chain. The order of these training sessions was counterbalanced across days. The number of responses was recorded during each session.

Pavlovian-instrumental transfer test

Two Pavlovian retraining sessions were given to all the subjects on the two days following the completion of instrumental training. The parameters of the two retraining sessions were identical to those presented during the Pavlovian training session. The first transfer test occurred the day after Pavlovian retraining. Both instrumental manipulanda

were present at this time but no USs were presented during this test. The test session lasted for 34 min, with alternating periods of 30 sec CS on and 30 sec CS off periods. Each PIT test began with a 2-min extinction period in order to familiarize the animals with the choice procedure as well as to lower the overall levels of responding before the transfer trials. Each CS was presented 16 times and the sequence was as follows: FTTFTFFTTFFTFTFTTFTFTFTFTFTFTFFT. A second transfer test was conducted the day after two additional instrumental (VI 60) retraining sessions were given with each response. The second PIT test was identical to the first one. Instrumental as well as magazine approach responses were measured 30 sec before and during each trial.

Outcome Devaluation Training

On the day following the Pavlovian-instrumental transfer tests the subjects received four Pavlovian retraining sessions. The parameters of these retraining sessions were identical to those presented during the initial Pavlovian training phase. After Pavlovian retraining sessions the subjects were placed in individual Skinner boxes without any CSs or instrumental manipulanda (i.e. lever and chain) present over 5, two-day cycles. Sucrose was presented on the odd numbered days and Polycose was presented on the even numbered days non-contingently on a VT 60 secs schedule. Each session was 20 min long and immediately after it was over half of the subjects (in each lesion condition) were injected with .3M LiCl (1% bodyweight) and placed back in their home cages on sucrose days, and the remaining animals received these injections on Polycose days. Thus, one half of the subjects received sucrose devaluation and the other half received Polycose devaluation training.

Devaluation Test

One the day following the completion devaluation training, the subjects were given two US devaluation test sessions. These sessions were identical to the Pavlovian training sessions except no USs were presented. The subjects' magazine approach responses were measured 30 sec before, during, and 20 sec after each CS presentation. The percent of time spent in the magazine was recorded for each subject as an indirect measure of drinking behavior.

Nutrient Test

The day after the devaluation tests were completed, the subjects were given one day of two-bottle water training in order to familiarize them with the testing procedure, identical to that in Experiment 1. Starting on the day following the water training the subjects were given 2, two-bottle nutrient choice tests in order to verify that the nutrients were differentially valued. These tests were identical to those in Experiment 1. All intakes of the solutions were recorded during each session.

Results

Histology

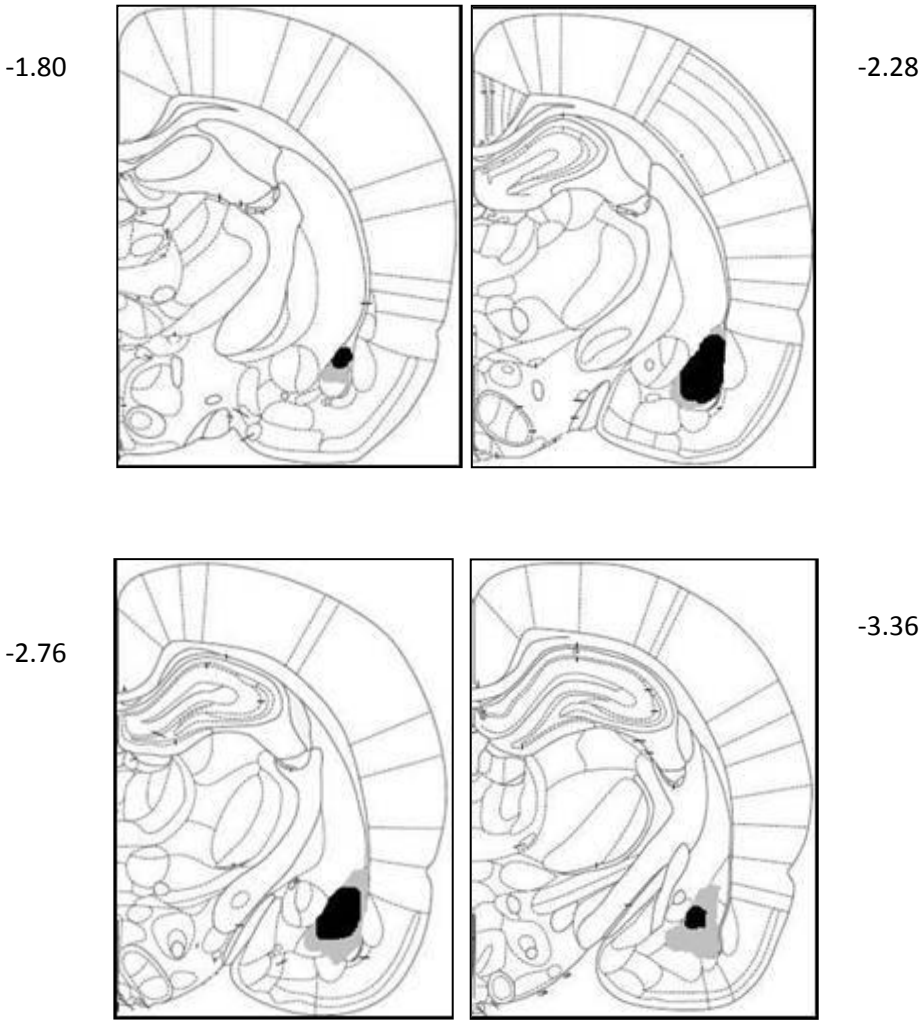

Figure 8. Experiment 2, BLA histology results. Diagrams of coronal sections (40 µM slices) illustrating the extent of the BLA lesions (n = 8) ranging from -1.80 to -3.36 mm (posterior to bregma). The drawings illustrate the approximate extent of the lesions. The black areas represent maximum lesion and the grey areas represent the areas of minimum lesions. Images adapted from Paxinos and Watson, (2009).

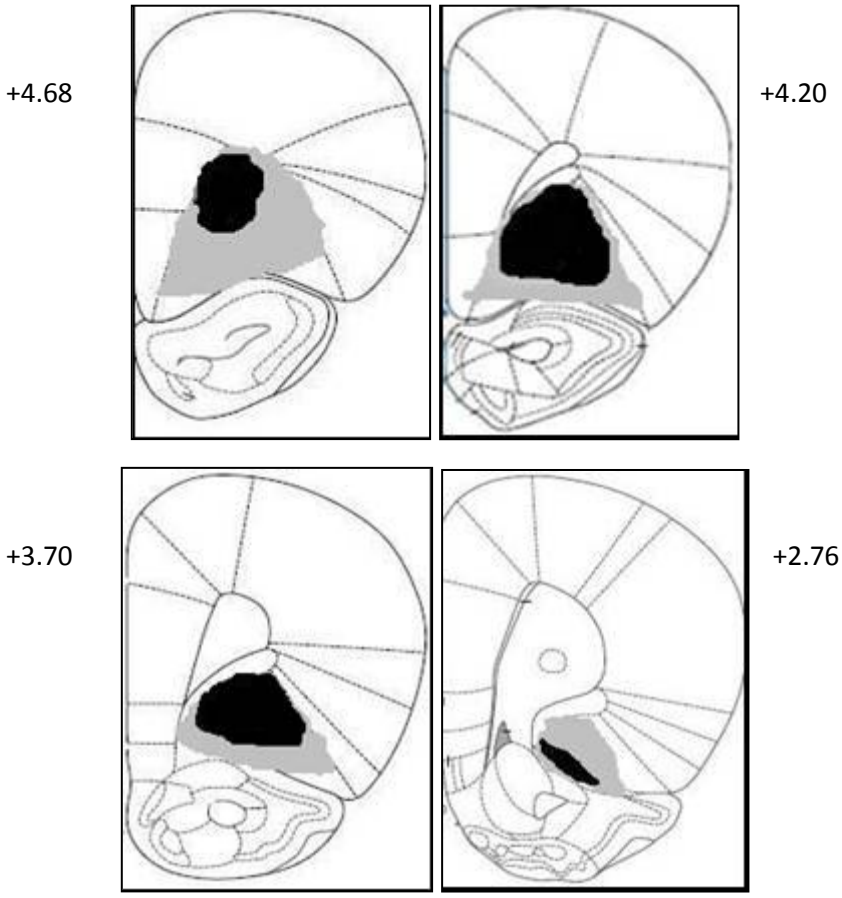

Figure 9. Experiment 2, OFC histology results. Diagrams of coronal sections (40 µM slices) illustrating the extent of the OFC lesions (n = 12) ranging from 4.68 to 2.76 mm (anterior to bregma). The drawings illustrate the approximate extent of the lesions. The black areas represent maximum lesion and the grey areas represent the areas of minimum lesions. Images adapted from Paxinos and Watson, (2009).

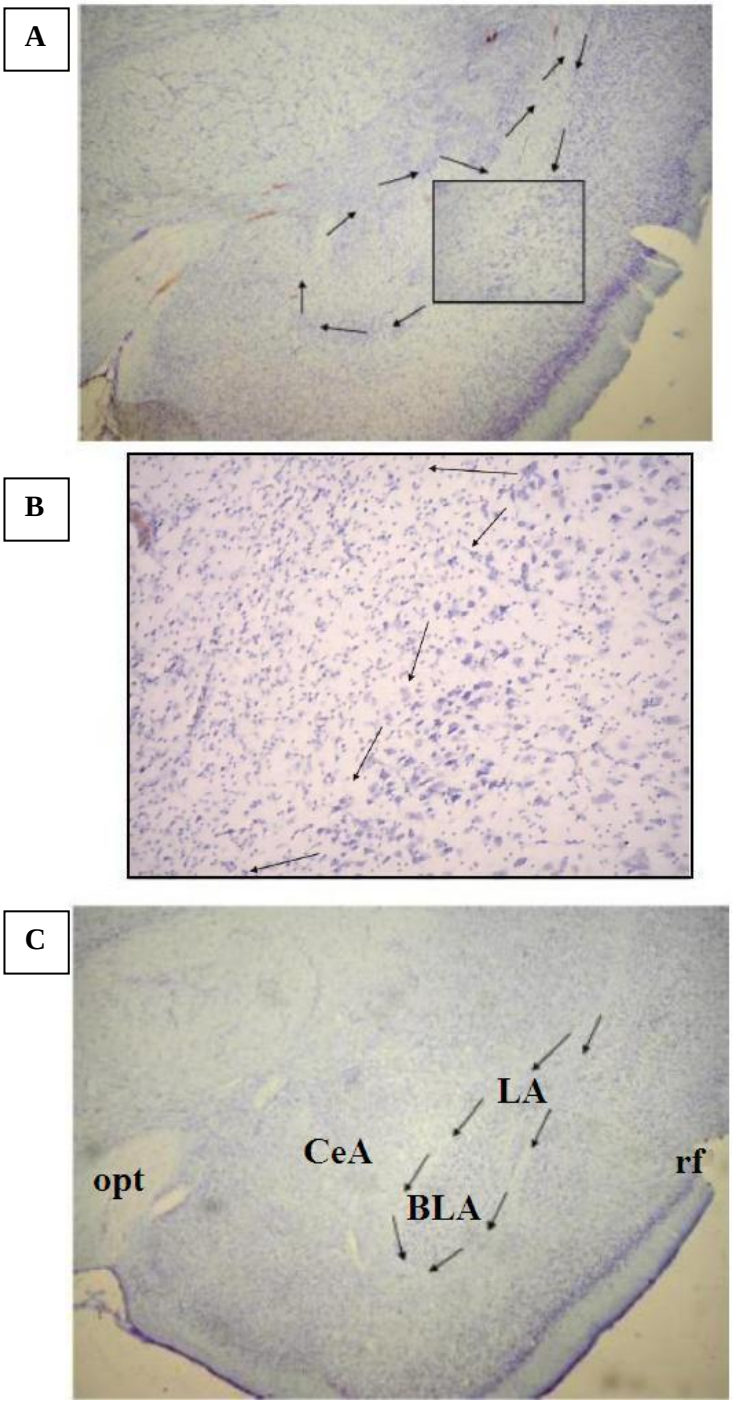

Figure 10. Experiment 2, Photomicrographs of BLA lesioned subjects (*A*) and BLA Sham controls (*C*) in low (4x) magnification (~2.28 mm posterior to bregma). *B* shows the rectangle shown in *A* in higher (10x) magnification. In lesioned structures the arrows represent the extent of the lesion and in the Sham structure the arrows outline the structure itself. BLA: basolateral amygdala; CeA: central amygdala; LA: lateral amygdala; opt: optic tract; rf: rhinal fissure.

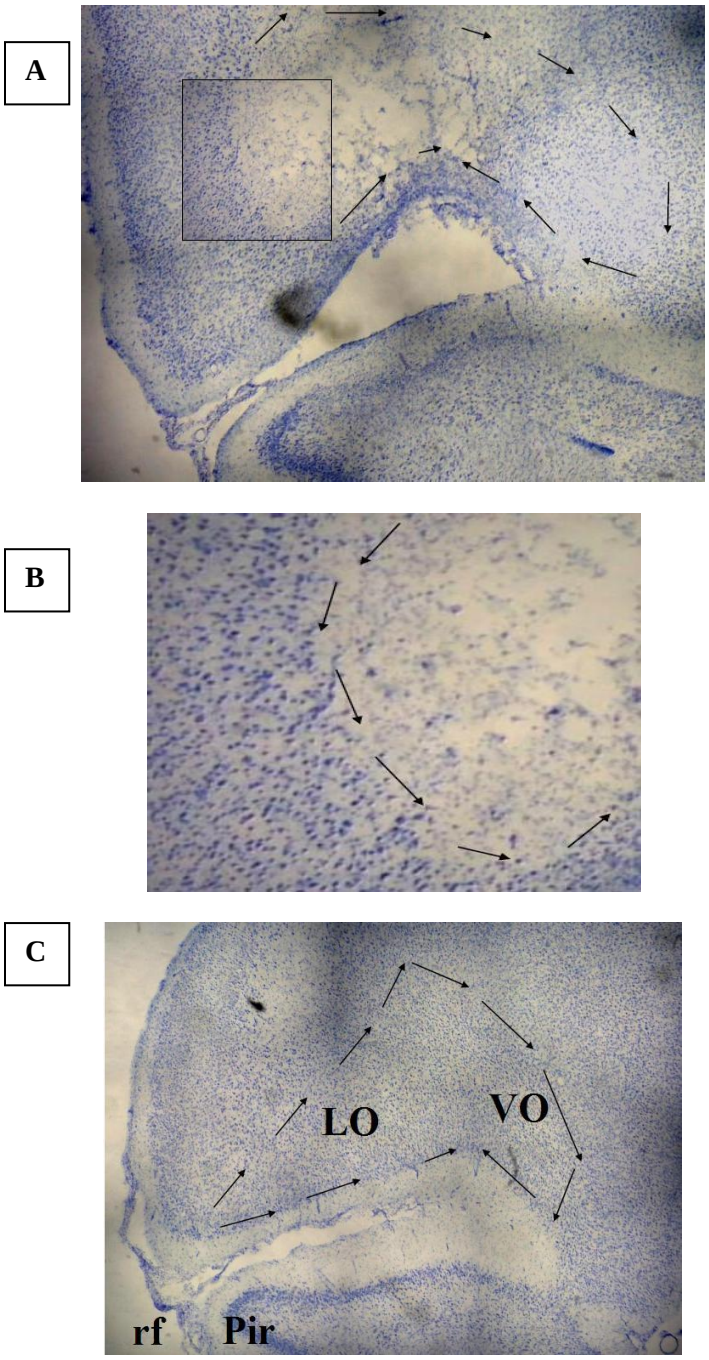

Figure 11. Experiment 2, Photomicrographs of OFC lesioned subjects (*A*) and OFC Sham controls (*C*) in low (4x) magnification (~4.20 mm anterior to bregma). *B* shows the rectangle shown in *A* in higher (10x) magnification. In lesioned structures the arrows represent the extent of the lesion and in the Sham structure the arrows outline the structure itself. LO: lateral orbital; VO: ventral orbital; Pir: piriform cortex; rf: rhinal fissure.

Figures 8 and 9 display the maximum and minimum damage as a result of BLA (Figure 8) and OFC (Figure 9) lesions of subjects that were included in the analysis. The black areas represent minimum lesion and the grey areas represent the areas of maximum lesions. The inclusion criteria were the same as that in Experiment 1. In addition, Figures 10 and 11 display photomicrographs from representative BLA (Figure 10) and OFC (Figure 11) lesioned (top panel) and Sham (bottom panel) subjects. After histological analysis, 4 BLA subjects and 3 OFC subjects were excluded from the analysis due to inappropriate lesions. The data presented here include only the remaining subjects whose lesions were analyzed to be appropriate rendering the following number of subjects in each group: n = 8 for BLA, n = 9 for OFC, and n = 12 for Group Sham. In addition, one of the BLA subjects died during the devaluation phase, rendering n = 7 for the BLA lesioned subjects during the US devaluation training, as well as US devaluation test and nutrient test.

Pavlovian training

Preliminary analysis found no differences between males and females throughout the experiment, so the data presented here are collapsed across sex. The acquisition results showed that the rats across all lesion conditions did not differ in their acquisition rates in magazine approach training. Pavlovian acquisition is illustrated in Figure 12, which shows the average rate of magazine approach CRs over the course of training (Days 1-8) collapsed across the two CSs, as well as for comparable Pre CS periods. Since no significant differences were found between CS Suc (CS paired with sucrose) and CS Poly (CS paired with Polycose), the data illustrated here are collapsed across the two CSs

for each group. The results for Pavlovian acquisition suggest that over the course of training all subjects significantly increased their conditioned responding to both stimuli and that they maintained high levels of responding to the stimuli during all retraining sessions (R1 – R6). A Cycle (1-8) x Interval (CS vs. Pre) x Lesion (BLA, OFC, or Sham) ANOVA found a significant main effects of Cycle, F (7, 182) = 2.30, and Interval, F (1, 26) = 65.03, as well as a Cycle x Interval interaction, F (7, 182) = 26.98, but no other main effects or interactions. These results suggest that over the course of training the subjects started making more CRs during the CS as compared to baseline (Pre-CS intervals). In addition, the retraining data yielded a significant Cycle (R1-R8) x Interval (CS vs. Pre) x Lesion (BLA, OFC, Sham) interaction, F (10, 130) = 1.14, suggesting that the OFC subjects made less CRs than the BLA and Sham subjects.

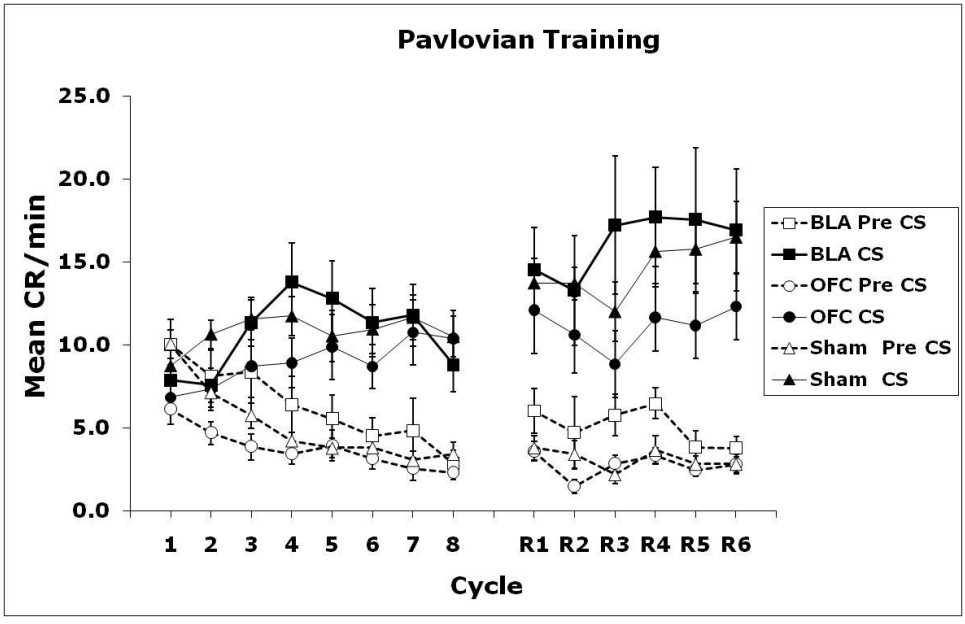

Figure 12. Experiment 2, Pavlovian conditioning. Mean conditioned responses CR/min prior and during CS presentations (collapsed across sucrose and Polycose paired CSs) in a Pavlovian training phase for BLA, OFC, and Sham lesioned subjects over the 8 training sessions and 6 retraining sessions (R1-R6), where R1 and R2 happened after the instrumental training and R3-R6 happened after the two PIT tests.

Instrumental training

Instrumental acquisition data are presented in Figure 13, which shows average instrumental responding/min (i.e. pressing the lever or pulling the chain) in order to earn sucrose or Polycose USs over 7 instrumental training sessions, where the first 5 sessions (i.e. VI10, VI30, VI30, VI60, and VI60) were conducted prior to the PIT tests and Ret1 and Ret2 were two retraining sessions (VI60) conducted after the first but before the second PIT test. The data show that over the course of training the subjects increased the number of instrumental responses and that while they showed lower overall instrumental responding during Ret1, they increased their instrumental responding during Ret2. A Cycle (VI10-VI60) x Lesion (BLA, OFC, or Sham) x Response (sucrose-paired vs. Polycose-paired) ANOVA yielded a significant main effect of Cycle, F (4, 104) = 2.85, but no other main effects or interactions.

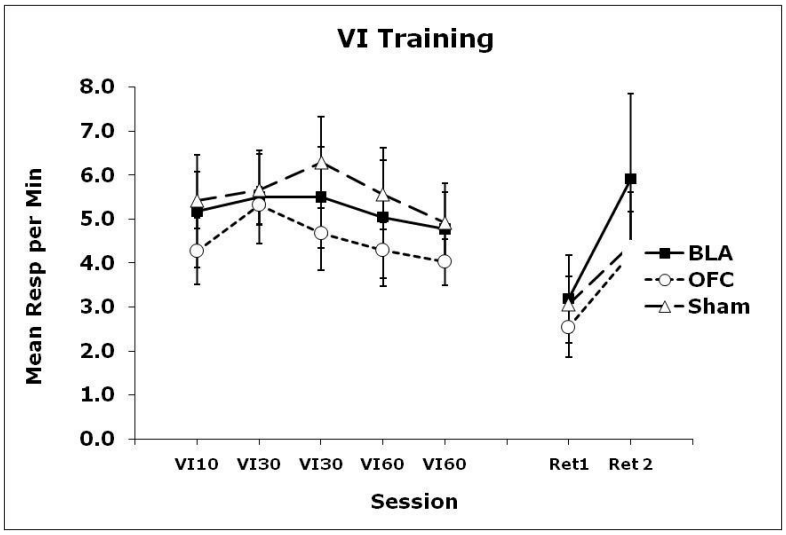

Figure 13. Experiment 2, instrumental training. Mean instrumental responses/min for in the instrumental training phase collapsed across sucrose and Polycose paired manipulanda (i.e. lever and chain) over the VI10, VI30 and VI60 training sessions and 2 VI60 retraining sessions (Ret 1 and Ret2), where Ret1 and Ret2 happened after the first PIT test.

Pavlovian-instrumental transfer tests

The results from the two PIT tests combined are illustrated in Figure 14. Illustrated here are the mean rates of instrumental responses expressed in terms of a CS-Pre difference score collapsed across the two PIT tests for each of the lesion conditions (i.e. BLA, OFC, and Sham). The "same" response was defined as the instrumental response that was previously reinforced with the same outcome as that signaled by the presented CS, whereas the "diff" response was defined as the instrumental response that was reinforced with the alternative outcome. For example, if the instrumental lever press response was previously reinforced with sucrose and the chain pull response was reinforced with Polycose and if the light CS was paired with sucrose and tone CS was paired with Polycose, then when the light CS was presented, the lever press response would be considered the "same" response and the chain pull response would be considered the "diff" response. The results showed that all subjects made less instrumental responding during the CS than they did during the Pre-CS period. However, the Sham subjects were less suppressed (i.e. made more instrumental responses) on the "same" manipulandum than on the "diff" manipulandum. A Lesion (BLA, OFC or Sham) x Response (same vs. different) ANOVA yielded a significant interaction, F (2, 26) = 3.01. Furthermore, a separate F_{max} test applied to the "same" – "different" difference scores showed that the homogeneity of variance assumption was violated, F_{max} (3, 11) = 4.96, due to increased variability in Group BLA (σ^2 = 2.73) compared to the other groups (σ^2 = 0.55 for OFC and σ^2 = 0.55 for Sham). Because of these unequal variances, we performed separate analyses directly comparing "same" vs. "different" responses for each of the three groups using each group's own error term rather than pooling over the three

groups' error terms. These follow-up tests revealed that the subjects in Group Sham made significantly more "same" than "diff" instrumental responses, F (1, 11) = 7.73, but that there were no corresponding differences in Group BLA and Group OFC.

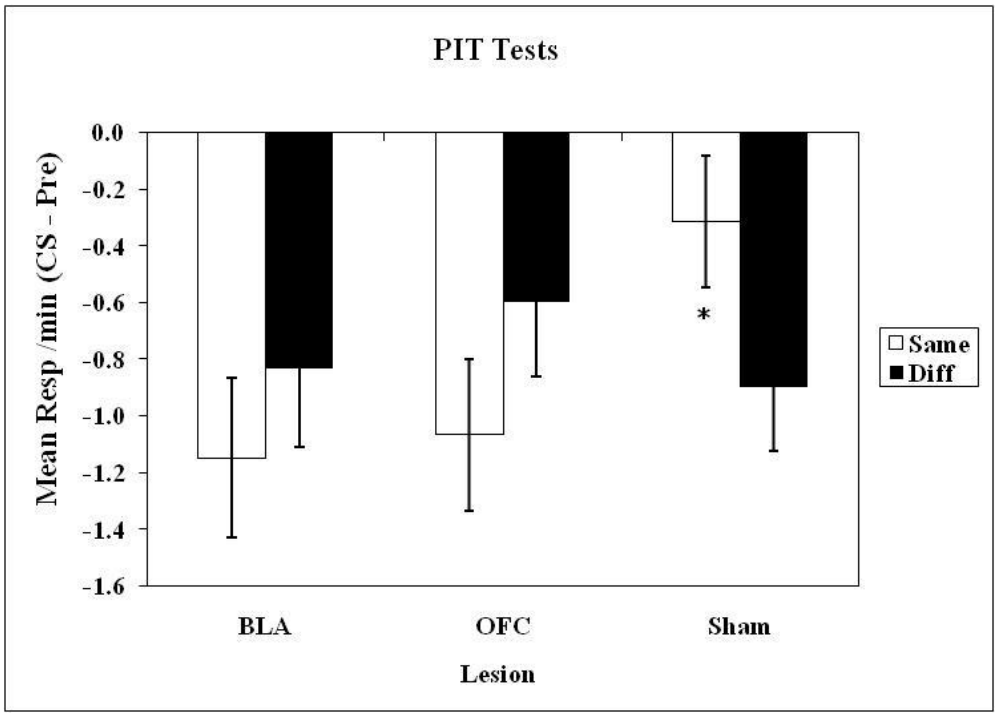

Figure 14. Experiment 2, PIT test results. Mean instrumental responses/min (CS-Pre) collapsed across the two Pavlovian-instrumental transfer tests for BLA, OFC and Sham lesioned subjects. During these tests, both manipulanda (i.e. lever and chain) were available but no USs could be earned. The instrumental responses on each manipulanda were measured before, during and after each CS (i.e. tone and flash) were presented. The "Same" responses were considered to be the instrumental responses, which shared an outcome with the presented CS, whereas the "Diff" responses were the instrumental responses, which did not share an outcome with the presented CS. The asterisks indicate instrumental response types, the rates of which were significantly different from the other response type for that group.

US Devaluation

The devaluation training data are illustrated in Figure 15. This figure displays % time during a 20 sec post-US interval that the subjects in each group spent in the magazine when either the devalued (Dev) or the nondevalued (Ndev) US was presented over the course of the 5 cycles of devaluation training. We evaluated % time spent in the magazine rather than magazine approach response rate because this measure provides a better assessment of drinking behavior after each US is presented. In particular, if the rats were drinking the nutrient, the % time they spent with their head in the magazine after that nutrient US was presented would be larger than if they did not consume the nutrient. The data show that over the course of training the subjects spent significantly more time in the magazine just after presentation of the nondevalued US compared to the devalued US. A Cycle (1-5) x US (devalued vs. nondevalued) x Lesion (BLA, OFC, or Sham) ANOVA found a significant main effect of US, F (1, 26) = 5.41 and a significant main effect of Cycle, F (4, 104) = 4.42 as well as a significant Cycle x US interaction, F (4, 104) = 3.13. These data were interpreted to mean that toward the later stages of training the subjects spent significantly less time consuming the devalued compared to the nondevalued US.

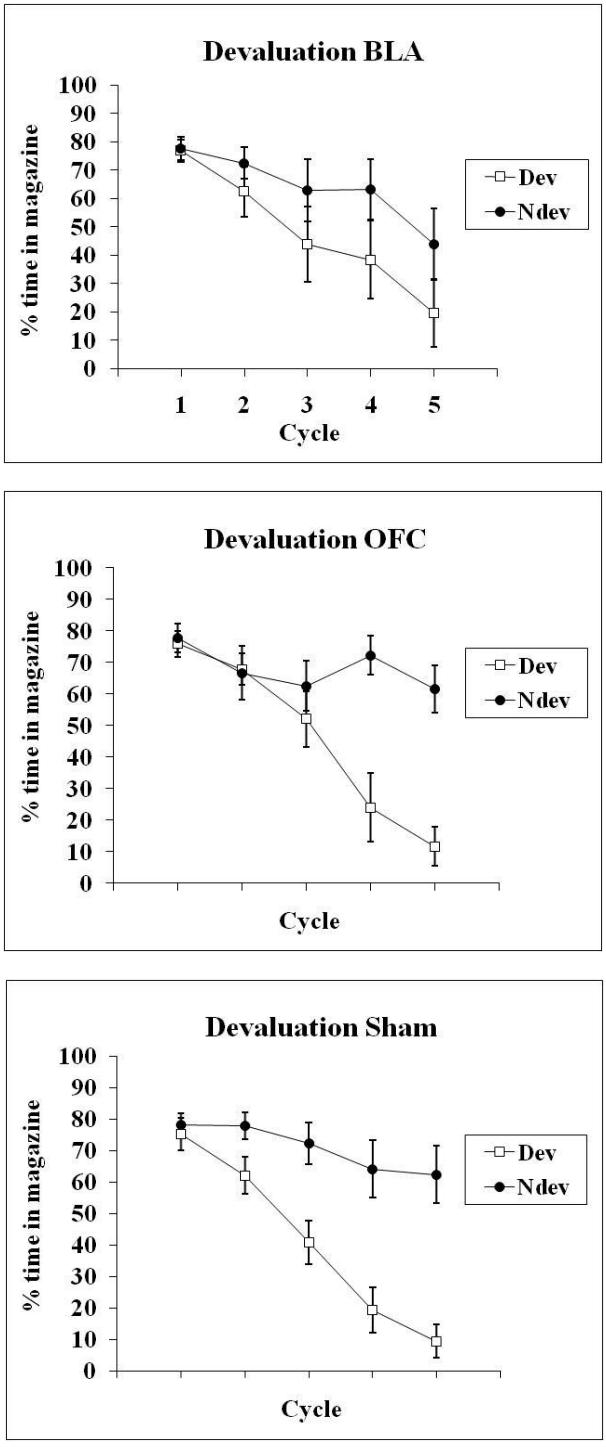

Figure 15. Experiment 2, devaluation training. Mean % time spent in the magazine when the devalued (Dev) and the nondevalued (Ndev) nutrient USs were presented for BLA (top panel), OFC (middle panel) and Sham (bottom panel) lesioned subjects. After the session where the Dev USs were presented, the subjects were injected with LiCl, but after the Ndev session they were not injected.

US Devaluation Test

The devaluation test data are depicted in Figure 16. This figure shows the mean rate of magazine approach CRs (CS-Pre) for each test (Tests 1 and 2) separately for the CS paired with the devalued nutrient (CSd) and the CS paired with the nondevalued nutrient (CSnd). Preliminary results indicated that there were no differences in magazine approach responding depending whether the subjects were devalued on sucrose or Polycose, so the data were collapsed across the devalued nutrient. The results illustrate that the BLA and Sham subjects made less CRs during the CSd than CSnd, whereas the OFC subjects made equally low amounts of CRs during each CS, suggesting that the OFC subjects were impaired on this task, but the BLA subjects, similarly to the Sham subjects, were not impaired. A Test (1 vs. 2) x Lesion (BLA, OFC, or Sham) x CS (devalued vs. nondevalued) ANOVA revealed a significant 3-way interaction, F (2, 25) = 3.63, $p < 0.05$. Separate one-way ANOVAs using a pooled error term were performed for each group to assess the nature of this interaction. Significant differences were seen in the BLA, F (3, 75) = 7.46 and Sham, F (3, 75) = 3.75 groups but not in the OFC group. Further post hoc tests were then performed for Group BLA and Group Sham and these revealed that the subjects in Group BLA made significantly less CRs (magazine approach responses) during the CSd than during the CSnd during both tests. The Sham subjects also made less CRs during the CSd than during CSnd but this effect was only significant during the first test.

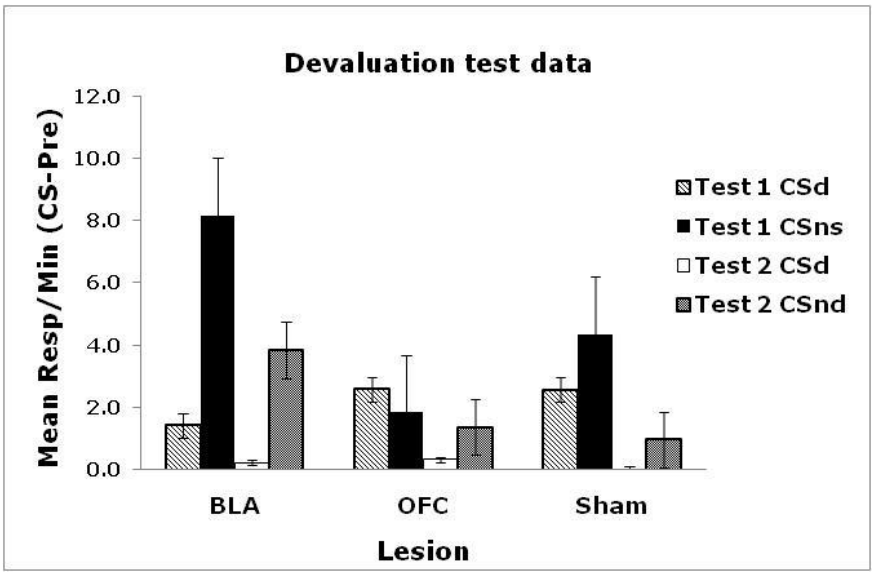

Figure 16. Experiment 2, devaluation tests. Mean CRs/min (CS-Pre) for the two devaluation tests for BLA, OFC and Sham lesioned subjects. CSd refers to the CS, whose outcome was devalued, whereas CSnd refers to the CS, whose outcome was not devalued. During the tests, the CSs were presented as described in the Procedure section of Experiment 2. No USs were presented during the devaluation tests. The asterisks indicate that the CRs (i.e. magazine approach responses) to one stimulus were significantly different from the CRs to the other stimulus on the given test day from the given group.

Nutrient Tests

The nutrient test results are illustrated in Figure 17, which shows the mean intakes of the devalued nutrient (Dev) and the nondevalued nutrient (Ndev) collapsed across the two, 2-bottle choice nutrient tests. The nutrient USs (i.e. 10% sucrose and 10% Polycose) were pitted against each other in the home cages. Since no intake differences were found to be dependent on whether the subjects were devalued on sucrose or Polycose in a preliminary analysis, the data were collapsed across nutrients. The results showed that all subjects consumed significantly more of the nondevalued nutrient than the devalued nutrient. A Devaluation (devalued vs. nondevalued) x Lesion (BLA, OFC, or Sham)

ANOVA found a significant main effect of Devaluation, F (1, 25) = 38.49, but not of Lesion and no Devaluation x Lesion interaction, suggesting that the selective devaluation treatment was equally effective in each group.

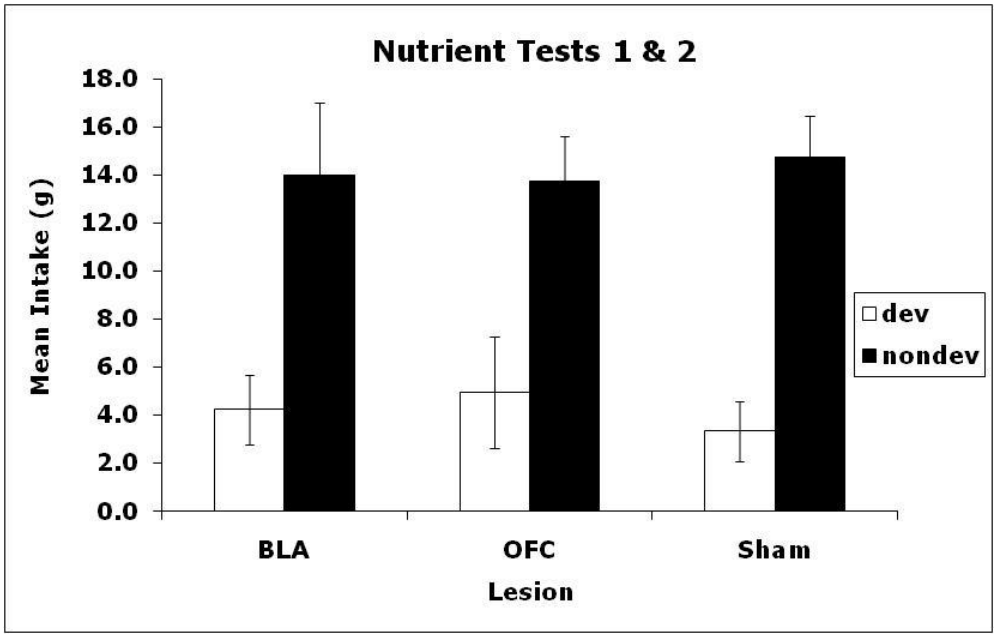

Figure 17. Experiment 2, nutrient test results. Mean intakes of the devalued nutrient (Dev) and the nondevalued nutrient (Ndev) collapsed across the two 2-bottle choice nutrient tests, where the nutrient USs (i.e. 10% sucrose and 10% Polycose) were pitted against each other without any flavor CSs present.

Discussion

The results of this experiment support the claim that our lesion techniques were behaviorally effective. In particular, they revealed that BLA and OFC lesions impaired the subjects' ability to form sensory-specific associations. Selective PIT was impaired by both BLA and OFC lesions and selective US devaluation was impaired by OFC lesions. Surprisingly, BLA lesioned rats displayed a normal selective US devaluation effect, implying that BLA lesions may not totally prevent the formation of sensory-specific

associations and that US devaluation is more sensitive than selective PIT at detecting any residual associative learning.

These results are consistent with those reported by Corbit and Balleine (2005) in terms of BLA being involved in selective PIT. Just like the present findings, Corbit and Balleine (2005) found that the BLA lesion resulted in the subjects being unable to display the specific PIT effect. However, whereas Corbit and Balleine (2005) found BLA lesion effects on PIT tasks using hungry subjects, we extended their findings by demonstrating a similar effect in thirsty subjects. On the other hand, these results are inconsistent with those reported by Ostlund and Balleine (2007) who reported that post-training, but not pre-training OFC lesions impaired selective PIT. We found that pre-training OFC lesions impaired the subjects' ability to display specific PIT. An explanation of this inconsistency could be that, as acknowledged by Ostlund and Balleine (2007), their pre-training OFC lesions might have been incomplete.

In addition, while the US devaluation results support the previous findings of Gallagher, et al., (1999) and Pickens, et al., (2003; 2005) regarding the role of the OFC in learning sensory-specific associations, they do not support the findings of Hatfield, et al., (1996) regarding the role of the BLA. Unlike Hatfield, et al., (1996), we found that BLA lesions did not eliminate the selective US devaluation effect. One explanation for this inconsistency is that the previous studies conducted Pavlovian training with one CS-US pair (Hatfield, et al., 1996), whereas we trained rats with two CS-US pairs. If OFC lesions completely prevent the formation of sensory-specific CS-US associations but BLA lesions merely attenuate such learning, then tasks that are more sensitive at

detecting such learning may more successfully reveal it. Because we trained rats with two CS-US associations, our procedures may merely have been more sensitive than those used by Hatfield, et al., (1996) at detecting sensory-specific associations.

The results from this experiment imply that the failure to find BLA and OFC lesion effects on the sensory-specific flavor-nutrient associations in Experiment 1 were not simply due to ineffective lesions since in the present experiment we were able to find that BLA and OFC lesions disrupted selective PIT and that OFC lesions disrupted the US devaluation effect. In addition, these results imply that our failure to find BLA and OFC lesion effects on the sensory-specific flavor-nutrient associations in Experiment 1 were also not due to weak concentrations of the USs, since these same concentrations (i.e. 10% sucrose and 10% Polycose) were used in the present experiment. Finally, these results suggest that our failure to observe a lesion effect in Experiment 1 was not due to the fact that we maintained our subjects in a thirsty rather than hungry motivational state, unlike Hatfield, et al., (1996) and Gallagher, et al., (1999).

The results of the present experiment found that BLA lesions did not impair the US devaluation effect. These results are consistent with those we found in Experiment 1, suggesting that the BLA lesion either has no effect on the formation of sensory-specific associations or that BLA lesion effects cannot easily be detected with our measure. The latter possibility is more likely since BLA lesions did undermine selective PIT. That suggests that the sensory-specific associations were at least partially compromised. However, as noted above, if the US devaluation task with two CS-US pairs is more sensitive at detecting residual associations, and if BLA lesions only incompletely prevent

sensory-specific association formation, then our results may be expected. Nevertheless, the present findings make the BLA results in Experiment 1 less surprising since we trained with two CS-US pairs in that study as well. On the other hand, it may also be possible that the neural structures involved in sensory-specific associations when flavor CSs are used may be different from those involved in tasks where auditory and visual CSs are used. In Experiment 2 we found that OFC lesions impaired the US devaluation effect in a magazine approach paradigm, suggesting that this structure is involved in the formation of sensory-specific associations. Based on this result, one would expect that OFC lesions should also impair the US devaluation effect in a flavor preference paradigm, such as the one used in Experiment 1, but we did not find this to be the case. This result suggests that the OFC is involved in the formation of sensory-specific associations in a magazine approach paradigm but not in a flavor preference paradigm. Further implications of these findings are addressed in the General Discussion section.

Experiment 3: The effects of GC lesion and GC-BLA disconnection on the formation of sensory-specific association in a conditioned flavor preference task

This experiment aimed to analyze the effects of GC lesions and GC-BLA disconnection lesions on sensory-specific learning in a flavor preference task. As reviewed in the general introduction, there is evidence from IEG staining and behavioral studies to suggest that the GC may be involved in sensory-specific learning (Balleine & Dickinson, 2000; Kerfoot, et al., 2007; Saddoris, et al., 2009). Briefly, Saddoris, et al. (2009) provided evidence suggesting that similar neural ensembles within the BLA were activated by a flavor associate of sucrose and by sucrose itself. Furthermore, these authors also provided evidence to suggest that when distinct flavor cues were associated with different nutrients, specific associatively-activated neural representations of the nutrients within the BLA depended upon input from the GC (Saddoris, et al., 2008).

Given that GC and GC-BLA interactions have been shown to play some role in the development of flavor-nutrient associative encoding, Experiment 3 was designed to determine if GC lesions or GC|BLA disconnection lesions would result in a significantly impaired US devaluation effect in a flavor preference task. In this experiment, one third of the subjects received a pre-training GC lesion, one third received pre-training GC|BLA disconnection lesions, and the rest received sham lesions. The experimental design for Experiment 3 was identical to that used in simultaneously-trained groups in Experiment 1 (See Table 1, top panel). It was anticipated that during the flavor preference tests, the Sham subjects would consume more of the flavor paired with the nondevalued nutrient than the one paired with the devalued nutrient. However, it was expected that if the GC is

involved in the coding of sensory-specific flavor-nutrient associations, subjects with GC and GC-BLA disconnection lesions would not show a US devaluation effect.

Method

Subjects

The subjects consisted of 47 naïve Long Evans rats (16 male and 31 female). The rats were individually housed and maintained as in Experiment 1.

Surgery & Histology

Some subjects received bilateral GC lesion (*n* = 16), some received GC-BLA disconnection (unilateral lesions of the BLA and GC on contralateral sides of the brain counterbalanced for side, *n* = 16), while the remainder received sham GC (*n* = 7) or sham disconnection (*n* = 8) lesion. The surgery and histology methods were identical to those of Experiment 1 with the following exceptions: the coordinates for the rats with the gustatory cortex lesions (*n* = 16), calculated from bregma, were +1.2 (AP), +/- 5.3 (ML) and -6 (DV). Infusions were made with NMDA (20µg/µl) in the amount of .5µl in each hole in the rate of .1µl/min. The needle was allowed to remain in place for an additional 5 min after the infusion to allow for diffusion of the drug. The rats with a disconnection between the BLA and the GC (*n* = 16) received the GC lesion on one side and the BLA lesion (see the coordinates from Experiment 1) on the contralateral side. Half of the subjects in this condition received a BLA lesion on the left side and the GC lesion on the right side of the brain, whereas the rest of the subjects received the BLA lesion on the

right side of the brain and the GC lesion on the left side of the brain. Sham controls (*n* = 16) underwent the same treatment as the lesioned rats but without the infusion of NMDA.

Solutions

Solutions were identical to those in Experiment 1.

Procedure

Acquisition and Devaluation

Acquisition and devaluation training were the same as the set of groups given simultaneous training in Experiment 1.

Testing

The day after the devaluation training was completed, the subjects were given one day of two-bottle water training in order to familiarize them with the testing procedure, identical to that in Experiment 1. The testing phase began the following day after the water training and it consisted of 4 cycles of 2, two-bottle and 2, one-bottle tests (totaling 16 tests). The two bottle choice tests in the first cycle were identical to those in Experiment 1. The single bottle tests consisted of a single bottle containing one of the flavor CSs being presented in the AM session for 15 min. The following day the alternate flavor CS was presented in the AM session for 15 min. Tap water was given to all subjects to drink for 15 min in the PM sessions. During each cycle of the two bottle choice tests the left-right positions of the solutions were counterbalanced across subjects and lesion conditions in an ABBA test sequence. In addition, the presentations of the

solutions during the single bottle tests were counterbalanced as well, in an ABBA test sequence.

Immediately following the completion of the flavor tests, the subjects were given 2, two-bottle nutrient choice tests in order to assess that the nutrients were differentially valued. These tests were identical to those in Experiment 1. All intakes of the solutions were recorded following each session.

Results

Histology

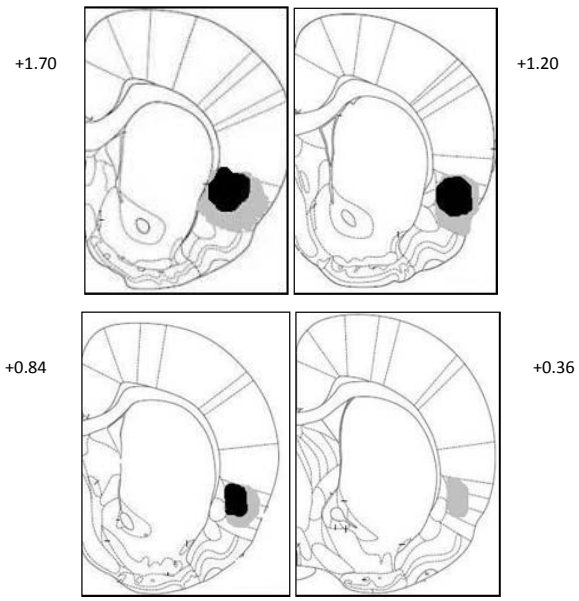

Figure 18. Experiment 3, GC histology results. Diagrams of coronal sections (40 µM slices) ranging from 1.70 to 0.36 mm (anterior to bregma). Illustrated is the extent of the GC lesions, which include GC subjects (*n* = 11), who received bilateral GC lesions on both sides of the brain and GC|BLA subjects (*n* =12), who received unilateral GC lesions on one side and BLA lesions on the other side of the brain. The drawings illustrate the approximate extent of the lesions. The black areas represent maximum lesion and the grey areas represent the areas of minimum lesions. Images adapted from Paxinos and Watson, (2009).

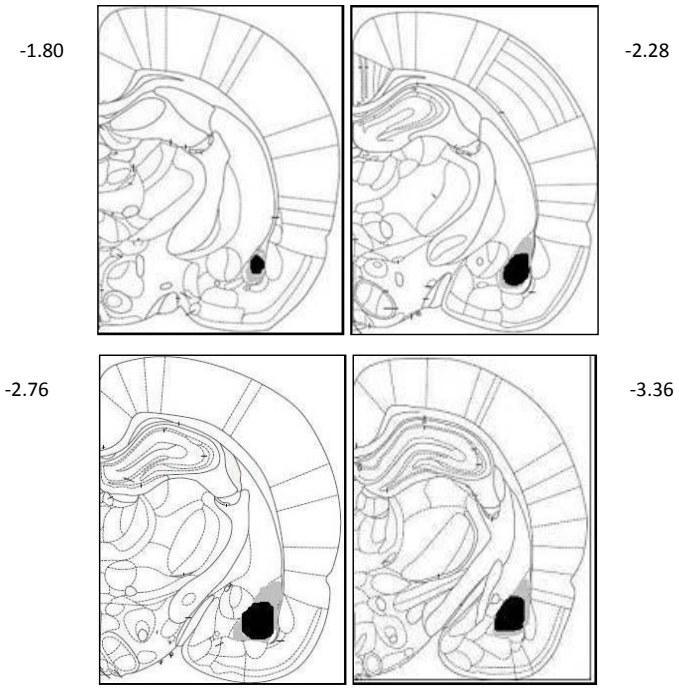

Figure 19. Experiment 3, BLA histology results. Diagrams of coronal sections (40 µM slices) illustrating the extent of the BLA lesions for Group GC|BLA, which received BLA lesions on one side and GC lesions on the other side of the brain (n = 12) ranging from -1.80 to -3.36 mm (posterior to bregma). The drawings illustrate the approximate extent of the lesions. The black areas represent maximum lesion and the grey areas represent the areas of minimum lesions. Images adapted from Paxinos and Watson, (2009).

After histological analysis, 5 GC subjects and 4 GC|BLA subjects were excluded from the analysis due to inappropriate lesions. The data presented here include only the remaining subjects whose lesions were analyzed to be appropriate rendering the following number of subjects: n = 11 for GC, n = 12 for GC|BLA, and n = 15 for Group Sham. Figures 18 and 19 illustrate the extent of GC (Figure 18) and BLA (Figure 19) lesions, whereas Figure 20 shows the micrographs from GC lesioned subjects using 4 x (top panel) and 10 x magnification (middle panel) and Sham subjects (bottom panel) using 4 x magnification.

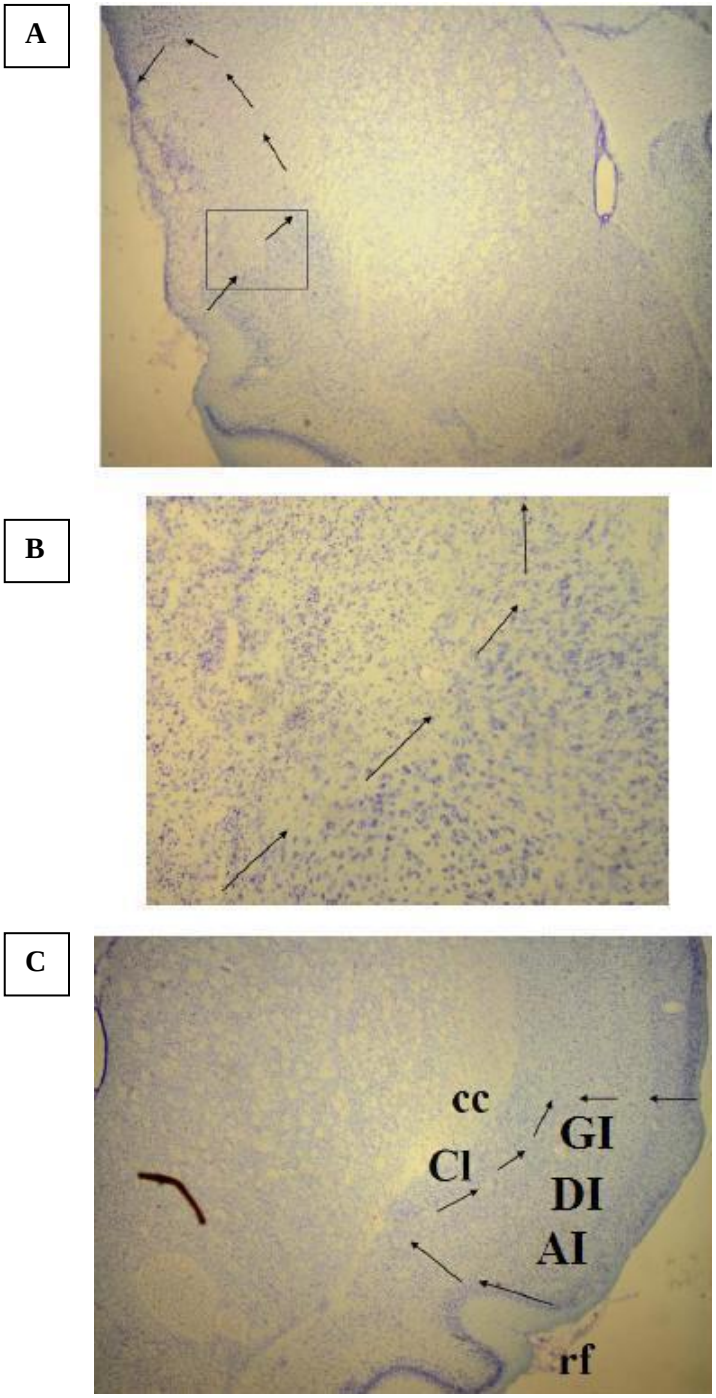

Figure 20. Experiment 2, Photomicrographs of GC lesioned subjects (*A*) and GC Sham controls (*C*) in low (4x) magnification (~2.28 mm anterior to bregma). *B* shows the rectangle shown in *A* in higher (10x) magnification. In lesioned structures the arrows represent the extent of the lesion and in the Sham structure the arrows outline the structure itself. GI: granular insular cortex; DI: dysgranular insular cortex; AI: agranular insular cortex; Cl: claustrum; cc: corpus callosum; rf: rhinal fissure.

Acquisition

Preliminary analyses found no differences between males and females throughout the experiment in any measure, so the data presented here are collapsed across sex. Acquisition data are illustrated in Figure 21. Represented here are the mean intakes of the flavor mixed in a solution with sucrose (Fs) and the flavor mixed in a solution with Polycose (Fp) over the 8 training cycles for the GC lesioned subjects (top panel), GC-BLA disconnection (GC|BLA) subjects (middle panel) and the Sham subjects (bottom panel). The results showed that the subjects in all groups increased their intake of all solutions over the course of training. In addition, the results of the acquisition data also showed the GC and the GC|BLA subjects consumed more of the Fs than the Fp solutions, whereas the Sham subjects consumed equal amounts of each. A Cycle (1-8) x Lesion (GC, GC|BLA, or Sham) x Flavor (Fs vs. Fp) ANOVA found a significant main effect of Cycle, F (7, 245) = 21.59 as well as a significant Flavor x Lesion interaction, F (2, 35) = 4.11. These data suggest that the GC and the GC|BLA subjects consumed more Fs than Fp, but that the Sham subjects did not differ in their intakes of these solutions. In addition, separate t-tests were performed between the baseline water intake and the Fs and Fp solutions on the last 2 days of training. The results suggest that both Fs, t (74) = 8.86, and Fp, t (74) = 7.47, were consumed above baseline.

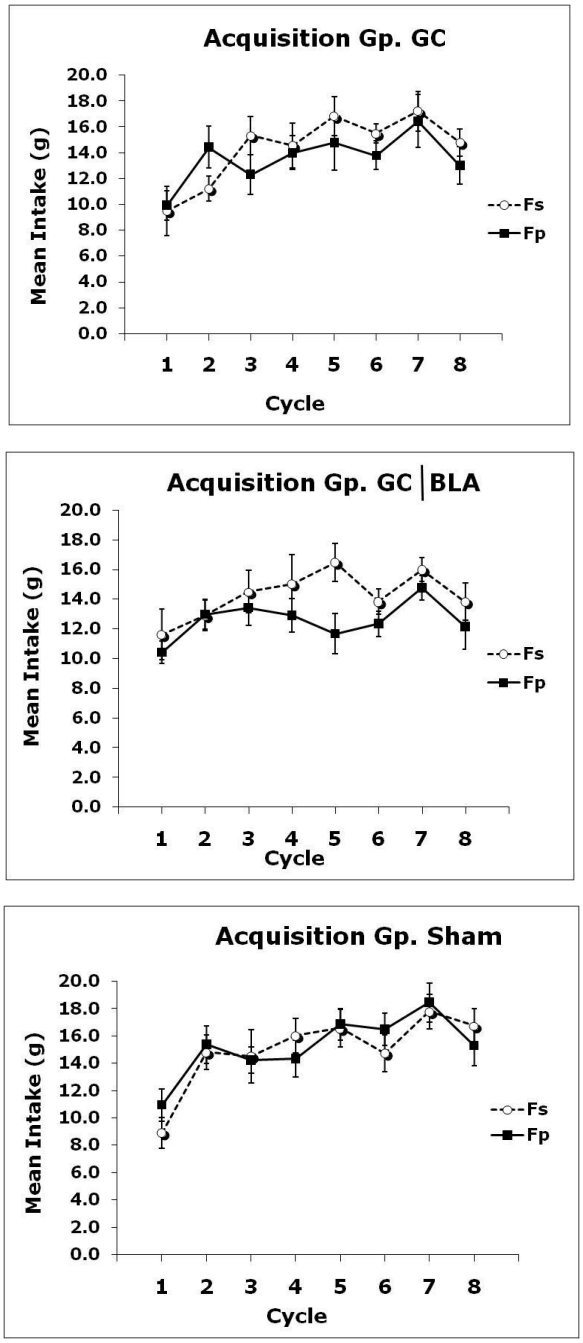

Figure 21. Experiment 3, acquisition data. Mean solution intakes (to the nearest 0.1g) during the acquisition phase for gustatory cortex (GC, top panel), disconnection (GC|BLA, middle panel), and Sham (bottom panel) lesioned subjects over 8 training cycles. The solutions consisted of 1% McCormick extract CSs (almond and banana) each mixed in a solution with one of the nutrients USs (10% sucrose and 10% Polycose). Fs refers to the intake of the solution where the flavor was paired with sucrose. Fp refers to the intake of the solution where the flavor was paired with Polycose.

Devaluation

The results from the devaluation phase (Figure 22) illustrate the mean intakes of the devalued (dev) and the nondevalued (ndev) nutrients over the 3 devaluation cycles in Group GC (top panel), GC|BLA (middle panel) and Sham (bottom panel) subjects. Since no intake differences were found to be dependent on whether the subjects were devalued on sucrose or Polycose in a preliminary analysis, the data were collapsed across nutrients. The results showed that, as in Experiment 1, by the end of devaluation training all the subjects consumed significantly more of the nondevalued nutrient than the devalued nutrient. However, the results also showed that the GC and GC|BLA subjects took longer to learn to distinguish between the devalued and the nondevalued nutrients. A Cycle (1-3) x Lesion (GC, GC|BLA, or Sham) x Devaluation (devalued vs. nondevalued) ANOVA yielded significant mains effect of Devaluation, F (1, 35) = 13.92 and Cycle, F (2, 70) = 108.77. In addition, the Devaluation x Lesion interaction was significant, F (2, 35) = 9.04, as was the Cycle x Devaluation interaction, F (2, 70) = 102.12. Separate Lesion x Devaluation ANOVAs using pooled error terms were then performed at each level of Cycle. These analyses yielded a main effect of Devaluation at each level of Cycle, F (1, 35) = 49.11 for Cycle 1, F (1, 35) = 16.33 for Cycle 2, and F (1, 35) = 114.56 for Cycle 3. More importantly, the Devaluation x Lesion interaction was significant for Cycle 2, F (2, 35) = 6.89 and Cycle 3, F (2, 35) = 4.80. Separate one-way ANOVAs analyzing the Devaluation effect in each Lesion condition for Cycles 2 and 3 revealed that during Cycle 2 only the Sham subjects significantly discriminated between the devalued and the nondevalued nutrients, F (1, 12) = 33.12, whereas during Cycle 3, all subjects drank significantly more of the devalued nutrient than the nondevalued nutrient, F (1, 12) =

20.88, for Group GC, F (1, 12) = 28.41, for Group GC|BLA, and, F (1, 12) = 82.29, for Group Sham.

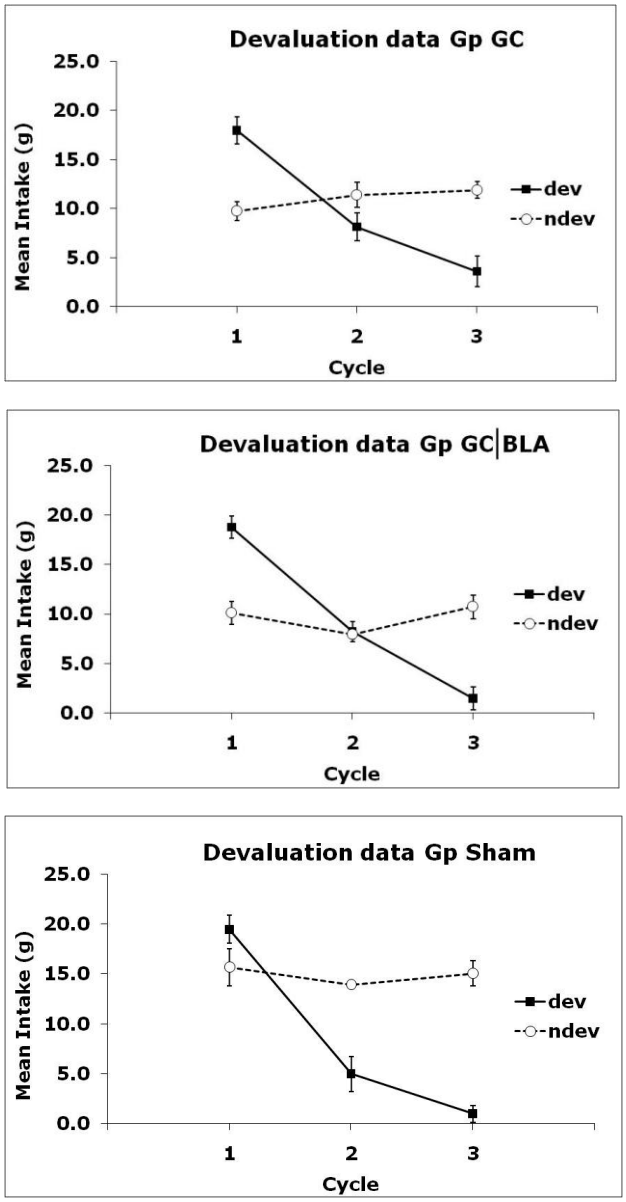

Figure 22. Experiment 3, devaluation data. Mean intakes of the devalued (dev) and the nondevalued (ndev) nutrients during the devaluation phase for GC (top panel), GC|BLA (middle panel) and Sham (bottom panel) lesioned subjects over the three cycles. Devalued nutrients were paired with .3M of LiCl (1% bodyweight), whereas the nondevalued nutrients were not paired with LiCl. No flavor CSs were presented during this phase. Asterisks indicate the intakes that were significantly different from each other.

Flavor Tests

The data of most interest came from the flavor tests. Figure 23 illustrates the average intakes of the flavor associated with the devalued nutrient (Fd) and the flavor associated with the nondevalued (Fn) nutrient for all three lesion conditions collapsed over the 4 cycles of 2, two-bottle choice tests (totaling 8, two-bottle choice tests in all). Preliminary analyses suggested that there were no differences depending on whether the subjects were devalued on sucrose or Polycose, so the data were collapsed across the devalued nutrient. The results revealed that overall the subjects consumed significantly more of the flavor associated with the nondevalued nutrient than the flavor associated with the devalued nutrient. A Flavor (Fd vs. Fnd) x Lesion (BLA, OFC, or Sham) ANOVA yielded a significant main effect of Flavor, F (1, 35) = 24.21, but no Lesion main effect or Flavor x Lesion interaction.

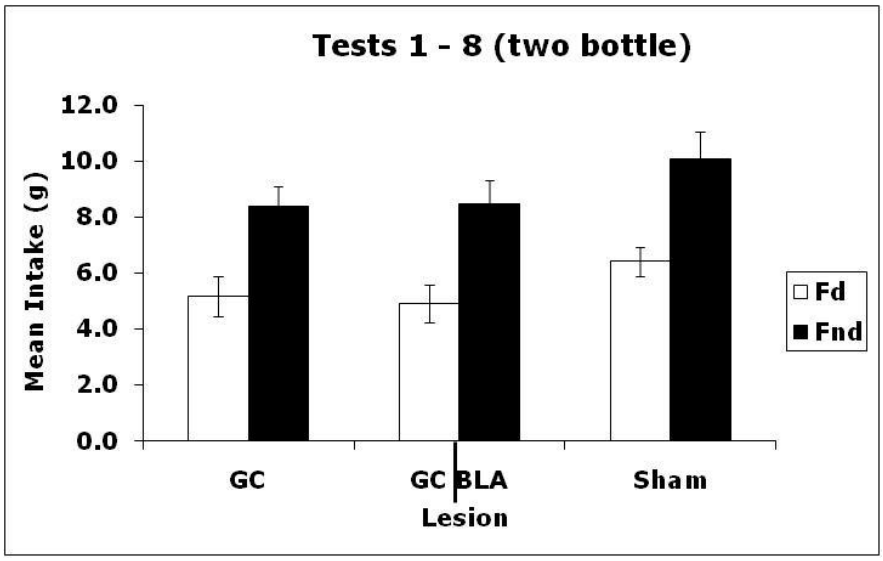

Figure 23. Experiment 3, flavor test results. Mean intakes of the flavor paired with the devalued nutrient (Fd) and flavor paired with the nondevalued nutrient (Fnd) collapsed across 4 cycles of 2, two-bottle choice flavor tests, where the flavor CSs (i.e. 1% almond and 1% banana extracts) were pitted against each other without any nutrients present.

In order to examine potential differences across extinction testing, the results were also analyzed without collapsing over the 4 extinction Cycles. Figure 24 illustrates the mean intakes of Fd and Fnd over the 4 Cycles of 2, two-bottle flavor choice tests for GC (top panel), GC|BLA (middle panel) and Sham (bottom panel) subjects. During the first Cycle the Sham control group displayed a somewhat larger preference for Fnd than Fd compared to the GC and GC|BLA groups. However, during the last 2 Cycles that preference extinguished in the Sham subjects but persisted in the GC and the GC|BLA subjects. A Flavor (Fd vs. Fnd) x Lesion (GC, GC|BLA, or Sham) x Cycle (1 - 4) ANOVA yielded significant main effects of Flavor, F (1, 35) = 23.93, Cycle, F (3, 105) = 18.25, and Lesion (2, 35) = 3.95, and significant Cycle x Flavor, F (3, 105) = 3.16 and Flavor x Cycle x Lesion, F (6, 105) = 1.65, interactions. Separate Flavor x Cycle ANOVAs using a pooled error term, were then performed on each group to determine the nature of the 3-way interaction. This analysis yielded significant main effects of Flavor for Group GC, F (1, 10) = 6.78, for Group GC|BLA, F (1, 11) = 7.80 for 25.61, and for Group Sham, F (1, 14) = 10.37. More importantly it revealed a significant Flavor x Cycle interaction only for Group Sham, F (3, 42) = 7.05. This supports the claim that all three groups displayed a selective devaluation effect but that the effect extinguished in Group Sham only.

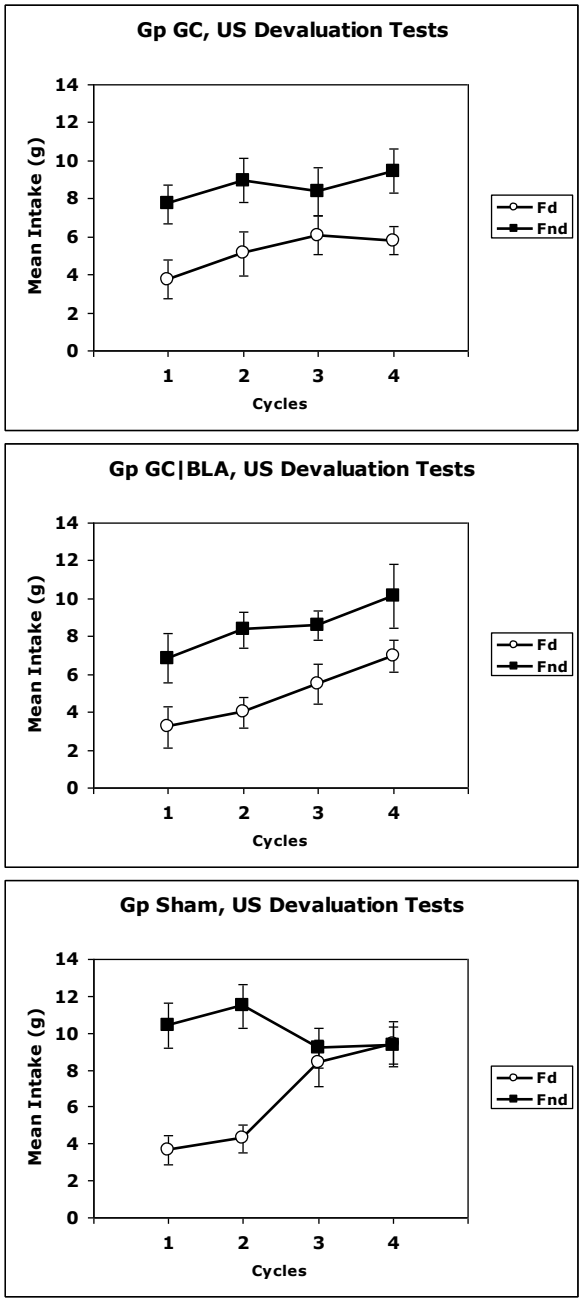

Figure 24. Experiment 3, extinction, two-bottle tests. Mean intakes of the flavor paired with the devalued nutrient (Fd) and flavor paired with the nondevalued nutrient (Fnd) over the 4 cycles of 2, two-bottle choice flavor tests for GC (top panel), GC|BLA (middle panel) and Sham (bottom panel) lesioned subjects, where the flavor CSs (i.e. 1% almond and 1% banana extracts) were pitted against each other without any nutrients present.

In order to determine whether the size of the devaluation effect may have differed among the groups prior to extinction, a separate Lesion x Flavor ANOVA was performed on the Cycle 1 data. This analysis yielded significant main effects of Flavor, F (1, 35) = 19.16 and Lesion, F (2, 35) = 3.72, but no Flavor x Lesion interaction. The lack of a significant Flavor x Lesion interaction was surprising given that subjects in Group Sham appeared to show a larger devaluation effect than subjects in Groups GC and GC|BLA. Whereas the main effect of Flavor indicates that the subjects in Group Sham consumed more of the solutions overall, a closer examination of the data suggests that this was only true for Fnd intake. To confirm this impression separate between-group ANOVAs were performed, with a pooled error term and Satterthwaite's (1946) correction for error degrees of freedom, to compare Fd and Fnd intakes across the groups. This analysis yielded a significant main effect of Lesion for Fnd, F (2, 56) = 3.15, but not for Fd. Subsequent post-hoc tests confirmed that Fd intake was higher in Group Sham compared to the two lesioned groups. These results suggest that during the first Cycle, Group Sham showed a larger discrimination between the Fd and Fnd Flavors than the other groups.

The data for the one-bottle tests were also analyzed and are illustrated in Figure 25. Presented here are the average intakes of the flavor associated with the devalued nutrient (Fd) and the flavor associated with the nondevalued nutrient (Fnd) for GC (top panel), GC|BLA (middle panel) and Sham (bottom panel) subjects over the 4 cycles of 2, one-bottle choice tests (totaling 8, one-bottle tests). A Flavor (Fd vs. Fnd) x Lesion (GC, GC|BLA, or Sham) x Cycle (1 – 4) ANOVA yielded significant main effects of Flavor, F (1, 35) = 10.03 and Cycle, F (3, 105) = 24.39, and Lesion (2, 35) = 3.34, as well as a significant Cycle x Flavor, F (3, 105) = 3.74, interaction. These results suggest that

initially all subjects consumed more of Fnd than Fd but that this discrimination was eliminated over the course of the 8 one-bottle choice tests.

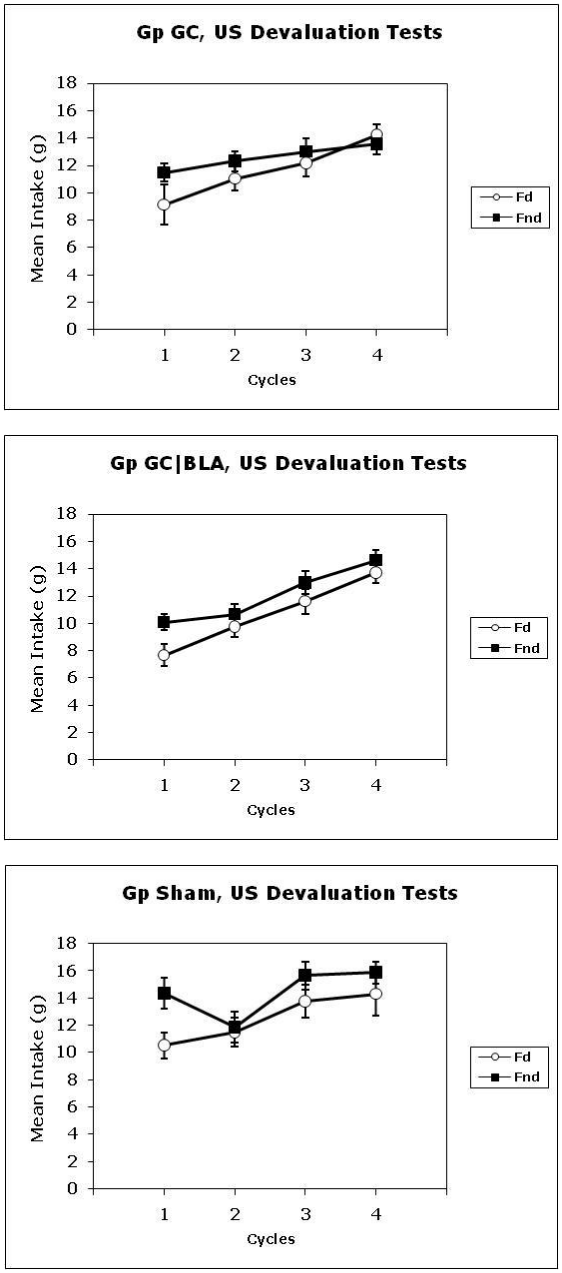

Figure 25. Experiment 3, extinction, one-bottle tests. Mean intakes of the flavor paired with the devalued nutrient (Fd) and flavor paired with the nondevalued nutrient (Fnd) over the 4 cycles of 2, one-bottle flavor tests for GC (top panel), GC|BLA (middle panel) and Sham (bottom panel) lesioned subjects, where the flavor CSs (i.e. 1% almond and 1% banana extracts) were pitted against each other without any nutrients present.

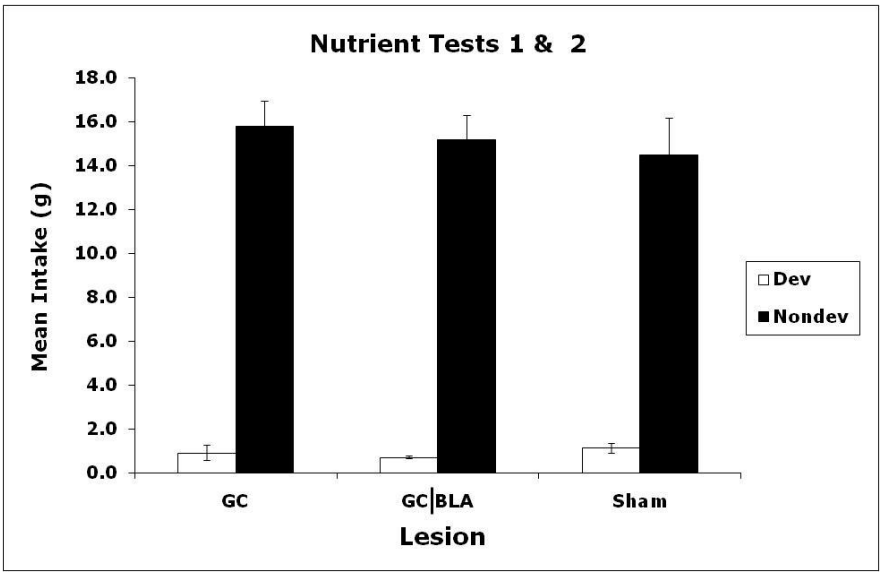

Figure 26. Experiment 3, nutrient tests. Mean intakes of the devalued nutrient and the nondevalued nutrient collapsed across the two 2-bottle choice nutrient tests, where the nutrient USs (i.e. 10% sucrose and 10% Polycose) were pitted against each other without any flavor CSs present.

Nutrient Tests

The nutrient test results are illustrated in Figure 26, which presents the average intakes of the devalued and the nondevalued nutrients during the two-bottle nutrient tests collapsed across the 2 nutrient tests for each group. The nutrient tests revealed that all subjects consumed more of the nondevalued nutrient than the devalued nutrient. A Devaluation (devalued vs. nondevalued) x Lesion (GC, GC|BLA, or Sham) ANOVA performed on these data yielded a significant main effect of Devaluation, F (1, 35) = 269.63, which did not interact with Lesion. In other words, at this time in the experiment all groups displayed equivalent selective aversions to the devalued nutrient.

Discussion

The results of this experiment showed that GC lesions and GC|BLA disconnection lesions both failed to impair the formation of sensory-specific flavor-nutrient associations. All subjects consumed more of the flavor paired with the nondevalued nutrient than the flavor paired with the devalued nutrient during the critical test sessions. However, during the devaluation training phase, it was evident that the subjects in Group GC and GC|BLA had a more difficult time distinguishing between the two nutrients and learning which nutrient was devalued and which was not devalued. Thus, the failure to observe lesion effects on preference for the two flavor associates cannot be attributed to behaviorally ineffective lesions in this experiment.

These results support the hypothesis that the GC and its communication with the BLA is not critical in the formation of sensory-specific flavor-nutrient associations but, nevertheless, may be involved in coding distinct neural representations of the nutrients. On the one hand, these results are consistent with the findings of Saddoris, et al., (2008; 2009) since we found that the GC and GC|BLA lesioned subjects took longer to learn a selective aversion to one of the nutrients during the devaluation training phase compared to the Sham control subjects. One explanation for this finding is that GC and GC|BLA disconnection lesions might have impaired the discrimination learning process responsible for enabling the rat to learn that one of the nutrients is paired with illness while the other is not. An alternative explanation for this finding is that these lesions might have resulted in enhanced generalization between the two nutrients, rather than impairing the discrimination process per se. According to this view, the GC and

GC|BLA lesioned subjects took longer than the Sham controls to learn the selective nutrient devaluation discrimination because there was a larger amount of overlap of the neural ensembles activated by the two nutrient USs in the BLA and/or the GC in lesioned rats. While we cannot definitively decide which of these views is correct on the basis of the present results it is worth pointing out that in the very first nutrient devaluation cycle the two lesioned groups appeared to consume less of the non-devalued nutrient on Day 2 of the cycle (the day after the first nutrient was paired with LiCl). This result would be expected if these subjects generalized more between the two nutrients. In any case, the present findings are consistent with Keifer and Braun's (1979) finding that GC lesioned rats took longer than the Sham controls to learn to discriminate between sucrose and NaCl taste cues when a conditioned taste aversion (CTA) was established to one of these cues (see also Kiefer, Leach, & Braun, 1984, but also see Lorden, 1976).

On the other hand, our results provide less support for Saddoris et al.'s (2008) finding that the associatively activated nutrient representations in the BLA were less specific when GC was lesioned compared to when it was not lesioned. Although we did not directly assess such neural representations, subjects with GC and GC|BLA disconnection lesions appeared to develop sensory-specific flavor-nutrient associations normally. While there was some evidence of a weaker US devaluation effect in the lesioned rats in this experiment during the first cycle of two-bottle tests compared to sham controls, this effect was only reliable when comparing overall intakes of the flavor associate of the nondevalued nutrient. If the neural representations of the nutrients, themselves, were somewhat less distinctive, then a slightly weaker US devaluation effect seen to the flavor associates would be expected if normal associative learning had taken

place. Nevertheless, learning of sensory-specific flavor-nutrient associations does not appear to depend exclusively on these structures. Other structures could very well compensate for the presumed loss of function when either the BLA or the OFC are lesioned.

Another interesting aspect of our results concerns the extinction data, which shows that the Sham subjects lost their aversion to the flavor paired with the devalued nutrient over the course of extinction whereas the GC and GC|BLA lesioned subjects were resistant to extinction. These results may be interpreted to mean that these structures are involved in the normal extinction of sensory-specific flavor-nutrient associations and that damage to these structures can result in resistance to extinction. This interpretation is consistent with the findings of Touzani and Sclafani (2007). In their experiment, Touzani and Sclafani (2007) presented hungry rats with one flavor CS (e.g. orange-saccharin Kool-Aid, CS+) paired with 8% fructose on some days and another flavor CS (e.g. lemon/lime-saccharin Kool-AID, CS-) paired with plain water on other days. Sixteen two-bottle choice tests under extinction conditions were given and it was found that controls and subjects with pre-training GC lesions both initially preferred CS+ to CS-, but that lesioned subjects displayed a significantly larger conditioned preference. Furthermore, while both groups ultimately lost their preference for CS+, the preference was lost sooner in the control group compared to lesioned subjects. Touzani and Sclafani (2007) interpreted these results to mean that GC lesions produced better preference learning but retarded extinction learning compared to Sham controls. However, since the GC subjects in their experiment showed a higher CS + to CS- preference initially, it is difficult to determine whether the rate of extinction was actually affected by GC lesions.

In other words, exactly these results would be expected even if the rates of extinction learning did not differ between the two groups. In contrast, in our experiment Sham subjects initially showed, if anything, a somewhat larger US devaluation effect than GC and GC|BLA lesioned subjects. However, this effect was more quickly lost in Sham controls compared to GC and GC|BLA lesioned subjects. If we take the US devaluation effect as a measure of sensory-specific associations, then these data suggest such associations are resistant to extinction when the GC is lesioned or when GC-BLA communication is prevented. Hence, our results provide evidence that GC and its communication with the BLA play a role in the extinction of sensory-specific flavor-nutrient associations.

It is noteworthy to point out that while extinction of flavor preference learning and sensory-specific associations appears to be retarded by GC (as well as GC | BLA disconnection) lesions, conditioned taste aversion (CTA) is enhanced by GC lesions (Fresquet, Angst, & Sandner, 2004). Fresquet, et al., (2004) taught thirsty rats to associate sucrose with LiCl and then gave these subjects two-bottle choice tests between sucrose and water. It was found that all subjects initially preferred water to sucrose but the GC lesioned subjects quickly extinguished the CTA consuming more sucrose solution with each extinction trial, whereas the Sham subjects extinguished at a much slower rate. These results suggest that GC is somehow involved in the recall of the nutrient-malaise associations and when the GC is lesioned, these associations are rapidly extinguished. Interestingly, we did not find this to be the case in our experiment. All subjects, regardless of lesion condition consumed significantly more of the non-devalued nutrient than the devalued nutrient. On the other hand, Fresquet, et al., (2004) reported that

differences between GC and Sham lesioned subjects did not become apparent until the later extinction trials. It is then possible that had we given our subjects multiple nutrient tests, we would have also observed differences in extinction rates between the lesioned and the Sham control subjects.

General Discussion

The overall results of this dissertation demonstrated that pre-training BLA, OFC, GC lesions and GC|BLA disconnection lesions did not impair the formation of sensory-specific flavor-nutrient associations in subjects given simultaneous training. Similar results were also found for the BLA and OFC lesioned subjects given sequential training. On the other hand, it was found that BLA and OFC lesions disrupted selective Pavlovian-instrumental transfer and that the OFC, but not the BLA, disrupted selective US devaluation in a magazine approach paradigm in thirsty rats. Furthermore, it was found that the GC and the GC|BLA disconnection lesions made it more difficult for the subjects to discriminate between devalued and nondevalued nutrients during a selective US devaluation training phase. Finally, it was found that the GC and the GC|BLA disconnection lesions resulted in the subjects showing more resistance to extinction of their selective US devaluation effect compared to Sham controls.

In Experiment 1 we explored the hypothesis that the BLA and the OFC are involved in the formation of sensory-specific associations in a conditioned flavor preference paradigm where motivationally significant outcomes were used. According to Blundell, et al., (2003) the BLA is involved in tasks where motivationally significant outcomes are used but not in tasks where motivationally neutral outcomes are used. In addition, the role of OFC in the formation of sensory-specific associations in a conditioned flavor preference paradigm was not previously explored. Although we used motivationally significant outcomes (i.e. 10% sucrose and 10% Polycose), we failed to detect the roles of either the BLA or the OFC lesions on the formation of sensory-specific

associations in Experiment 1, where flavor cues were paired with nutrient outcomes. This finding argues against the hypothesis proposed by Blundell, et al., (2003), who suggested that the BLA is involved in tasks with motivationally significant outcomes. Furthermore, these results demonstrate that the OFC is also not involved in the formation of sensory-specific associations in a conditioned flavor-preference task.

In addition, we explored the hypothesis that the BLA and the OFC are involved in tasks that allow for predictive learning (i.e. where the CS precedes the US), rather than in tasks where the two occur concurrently. We entertained this hypothesis in an attempt to understand why, on the one hand, pre-training BLA or OFC lesions undermines the US devaluation affect in magazine approach conditioning but, apparently, not in flavor preference learning. However, we found that neither the BLA nor the OFC lesions were effective at impairing the formation of sensory-specific flavor-nutrient associations as a function of training (i.e. simultaneous or sequential). More specifically, the subjects that were trained simultaneously or sequentially with either pre-training BLA or OFC lesions did not significantly differ from Sham controls on the US devaluation task.

The discrepancy between our results as well as those of Blundell, Hall, and Killcross, (2003) and Dwyer and Killcross, (2006) from the findings of Hatfield, et al., (1996), Gallagher, et al., (1999), and Corbit and Balleine, (2005) can be explained by the fact that perhaps there is something special about the flavor preference paradigm, which causes it to use different learning mechanisms than the magazine approach paradigm. More specifically, it is possible that these structures (i.e. BLA, OFC, GC), as well as communication between at least some of these structures, are not involved in the

formation of sensory-specific associations in a task where the CSs and the USs are both from olfactory and gustatory modalities but are only involved in tasks where auditory/visual stimuli are used. Blundell, et al., (2003) argued against this explanation by pointing out that Setlow, Gallagher, and Holland, (2002) found that BLA lesions failed to impair second-order conditioning in a magazine approach task involving auditory and visual CSs. However, it is worth noting that Setlow, et al., (2002) did not directly measure BLA lesion effects on the formation of sensory-specific second-order associations. Thus, Setlow, et al.'s, (2002) results may not be applicable to our findings.

However, our finding in Experiment 2 that BLA lesions had no influence on the US devaluation effect in a magazine approach task is also problematic for the hypothesis that suggests that the BLA is only involved in tasks with auditory/visual CSs since in this experiment the CSs were in fact auditory and visual. Nevertheless, in this study BLA lesions did undermine selective PIT, another measure of control by sensory-specific associations. Thus, it seems likely that some measures are more sensitive than others at detecting lesion effects on sensory-specific associations.

There are theoretical reasons to suppose that training with stimuli from olfactory-gustatory and auditory-visual CSs might be an important procedural difference. For instance, according to Kehoe, Horne, Horne, and Macrae, (1994) (see also Trost and Batsell, 2004), when conditioned and unconditioned stimuli are from olfactory-gustatory modalities, the representation of both events become integrated as a configural whole, whereas when the stimuli are from audio-visual and olfactory-gustatory modalities, these events are represented elementally. Consistent with this possibility, earlier work

demonstrated that second-order conditioning proceeds more rapidly when stimuli come from the same, as opposed to different, modality (Rescorla and Furrow, 1976). This interpretation suggests that different neural structures may be involved in tasks exploiting configural versus elemental learning processes. Our findings that OFC lesions impaired the formation of sensory-specific associations in a magazine approach task in Experiment 2 but not in a flavor conditioning task in Experiment 1 are consistent with this view if it is assumed that configural processes are engaged by flavor conditioning while elemental processes are engaged by magazine approach conditioning.

Another likely explanation for the failure to detect BLA, OFC, GC, and GC|BLA disconnection lesion effects on the formation of sensory-specific flavor-nutrient associations in our studies is that these may not be the only structures involved in the formation of these associations and when one structure is lesioned other structures may compensate for this deficit. Other structures thought to be involved in the formation of sensory-specific associations are the nucleus accumbens shell (Corbit, et al., 2001; Kerfoot, et al., 2007), nucleus accumbens core (Corbit, et al., 2001), mediodorsal thalamus (Corbit, et al., 2003), and dorsomedial striatum (Corbit & Janak, 2007; Yin, et al., 2005). Hence, it is possible that one or more of these structures compensated for the loss of BLA, OFC, or GC structures, or GC-BLA communication. Illustrated below is a diagram adapted from Dagher (2009) and Balleine, et al., (2009), which illustrates the neural network for appetitive behavior.

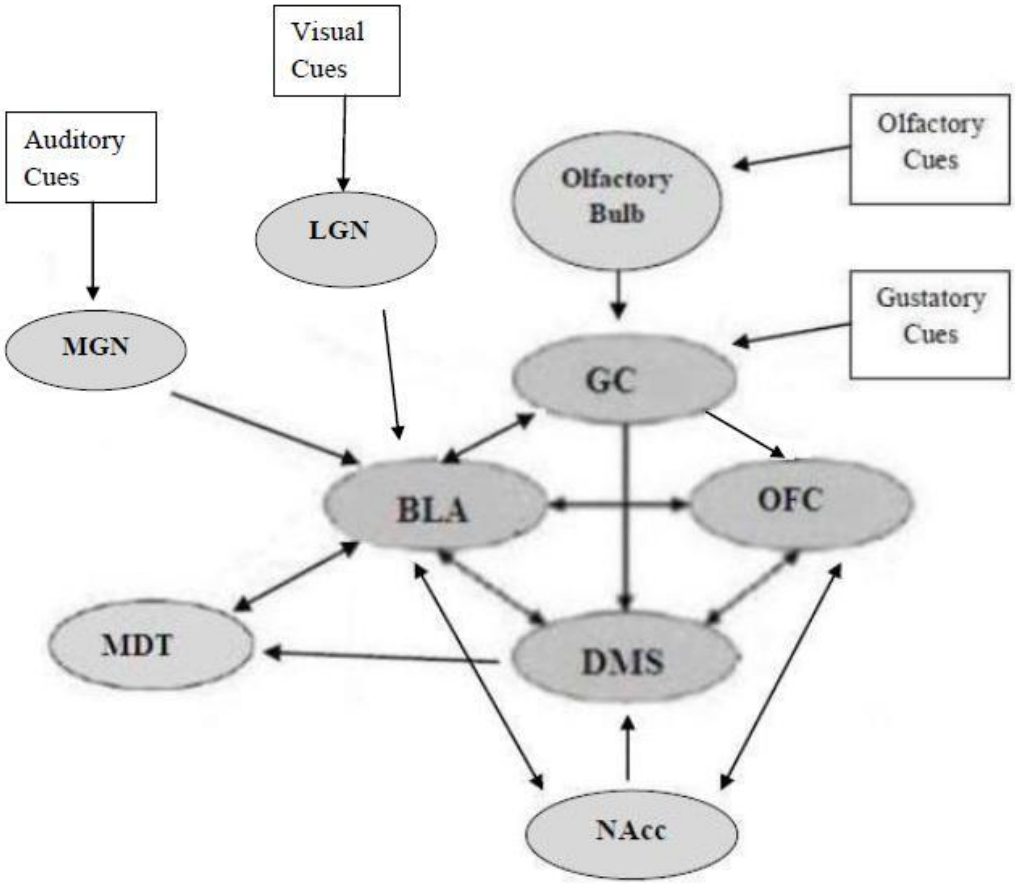

Figure 27. Neural network for appetitive behavior. Adapted from Dagher, (2009) and Balleine, et al., (2009). BLA: basolateral amygdala, OFC: orbitofrontal cortex, GC: gustatory cortex, DMS: dorsomedial striatum, NAcc: nucleus accumbens, MDT: mediodorsal thalamus, MGN: medial geniculate nucleus, LGN: lateral geniculate nucleus.

This figure suggests that olfactory cues are processed in the olfactory bulb and are then transmitted to the gustatory cortex, where the taste cues are processed as well. The GC, in turn, propagates the information about the olfactory and gustatory cues to the BLA, OFC, and the dorsomedial striatum (DMS). Some of these structures have two-way communication, meaning that they communicate back and forth. In addition, DMS also appears to communicate with mediodorsal thalamus (MDT) and nucleus accumbens

(NAcc). On the other hand, auditory and visual information is relayed from the medial geniculate nucleus (MGN) and the lateral geniculate nucleus (LGN) of the thalamus, respectively, to the BLA.

Given that these structures appear to be communicating to one another, it is not unreasonable to expect these to compensate for one another, in particular, when one of these structures is damaged. This type of neural compensation can explain why we failed to see lesion effects on the formation of sensory-specific associations in Experiments 1 and 3. If this is the case, there are several ways one can avoid this problem. For example, one can examine the effects of post-training lesions or inactivate a particular structure either during training or during testing, rather than conducting pre-training lesions, in order to minimize the opportunity for compensation by other structures. However likely this possibility seems, the compensation interpretation does not explain why there was a difference in the involvement of BLA and OFC in flavor preference and magazine approach paradigms.

Another explanation for our failure to find lesion effects in flavor conditioning may be related to various procedural differences between this paradigm and magazine approach. One potentially important variable is the amount of training given prior to selective nutrient devaluation. There is evidence to suggest that while amygdala lesions abolish learning and memory of fear conditioning in subjects given minimal training, they do not prevent fear conditioning when subjects are extensively trained (Maren, 1999). These results suggest that when the subjects receive extensive training, it can result in structures outside the amygdala participating in fear conditioning. It seems

possible that this might reflect a more general principle in the nervous system, i.e., that neural networks for learning become more widely distributed with additional training. It is then possible that with extended training the sensory-specific flavor-nutrient associations assessed in this dissertation become so widely distributed that pre-training lesions to any one particular structure is without effect. In Experiments 1 and 3 subjects received 8, 15-min exposures to each of two flavor-nutrient compound stimuli. Since other work in our lab has established that the US devaluation effect can be seen after a single flavor-nutrient pairing, the amount of training given here could be considered extensive. Future work will be needed to determine if this variable is critical.

Another interpretation of the effects of overtraining on the formation of sensory-specific associations is that while extensive training of a CS-US association does not impair a US devaluation effect, it can impair other qualitative aspects of learning (see Holland, Lasseter, and Agarwal, 2008). For example, Holland, et al., (2008) trained hungry rats to associate a tone CS with sucrose and then half of the subjects were devalued on sucrose (Group Devalue), while the rest were not devalued (Group Maintain). In addition, some of the subjects in each group were given extensive tone -> sucrose training, while others were given minimal tone -> sucrose training. Holland, et al., (2008) found that the subjects given either minimal or extensive training did not differ in their magazine approach CRs to tone (i.e. the subjects in Group Devalue produced significantly less CRs to tone than the subjects in Group Maintain). In addition to assessing the magazine approach responses to the tone CS, Holland, et al., (2008) also measured the subjects' taste reactivity responses. More specifically, after the subjects were presented with the tone CS, they were infused with water via oral cannulae and their

tongue protrusion behavior was videotaped, where long licks were categorized to represent appetitive taste reactivity responses and short licks represented the aversive taste reactivity responses. Results demonstrated that the minimally trained subjects in both group Devalue and Group Maintain displayed significantly more evaluative taste reactivity responses, where the minimally trained subjects in Group Devalue displayed more aversive taste reactivity responses than in Group Maintain, while the latter also displayed more appetitive taste reactivity responses than the former. However, in extensively trained subjects these differences were either not observed or were substantially attenuated. These results suggest that early in training the CS produces an image of the US, which is indistinguishable from water. However, with extensive training the CS produces an expectancy of the US, which in turn can be discerned from water. These results suggest that overtraining can alter the quality of Pavlovian learning.

Another procedural difference between our study and that of others showing lesion effects (Corbit & Balleine, 2005; Gallagher et al., 1999; Hatfield, et al., 1996) is that subjects in our studies were trained and tested in a thirsty motivational state. The studies that were successful at demonstrating a US devaluation deficit in BLA (Corbit & Balleine, 2005; Hatfield, et al, 1996) and OFC lesioned subjects (Gallagher, et al., 1999) used hungry rats. Blundell, Hall, and Killcross, (2003) and Dwyer and Killcross (2006), who failed to find a BLA lesion effect on US devaluation, also used thirsty subjects similar to the present studies. It is then possible that the BLA and OFC structures are not involved in sensory-specific learning that takes place in water-deprived states. However, the results from Experiment 2 suggest that this interpretation is inapplicable since we were able to detect BLA and OFC lesion effects on the formation of sensory-specific

associations in a magazine approach paradigm even when the subjects were trained and tested while thirsty.

Another procedural difference between our study and those who were successful at demonstrating the BLA (Hatfield, et al., 1996) and OFC (Gallagher, et al., 1999) lesion effects on US devaluation is that we trained our subjects to associate two CS-US pairs, whereas Hatfield, et al., (1996) and Gallagher, et al., (1999) used only one CS-US pair. Studies, similar to those reported here, that were unsuccessful at demonstrating BLA (Blundell, et al., 2003; Dwyer & Killcross, 2006) lesion effects on US devaluation, trained subjects to learn two distinct CS-US associations. We found that while neither the BLA nor the OFC were involved in the formation of sensory-specific associations in our conditioned flavor preference paradigm, both of these structures were shown to be involved in the formation of these associations in a magazine approach paradigm as detected by selective PIT task. However, only the OFC but not the BLA lesions impaired a US devaluation effect in the magazine approach paradigm. One explanation for this discrepancy is that perhaps the US devaluation task is more sensitive at detecting residual learning than the PIT task. It is then possible that the BLA is involved in the formation of sensory-specific associations but in order to detect the lesion effect, one needs to train the subjects to associate only one CS-US pair. An interesting follow-up experiment would be one where two different groups of subjects are trained with either one or two CS-US pairs, and their US devaluation effects are compared for BLA and Sham subjects. If the parameters of the US devaluation procedure used in our study were in fact too sensitive to detect the US devaluation effect, then one would

expect that with one CS-US pair we would be able to detect a lesion effect on US devaluation in BLA lesioned subjects.

On the other hand, the view that states that the US devaluation task trained with two CS-US pairs is too sensitive at detecting residual learning does not account for why we were able to detect a lesion effect with OFC lesioned subjects on both US devaluation and selective PIT tasks. One explanation for this is that OFC lesions might have more completely abolished the formation of sensory-specific associations in a conditioned magazine approach paradigm than the BLA lesions. There exists some evidence to support the view that OFC and BLA perform different functional roles in appetitive learning. For instance, Stalnaker, Franz, Singh, Schoenbaum, (2007) suggested that the OFC is involved in facilitating neural plasticity in the BLA that codes for cue-outcome associations. In addition, Hatfield, et al., (1996) and Gallagher, et al., (1999) found that while pre-training BLA and OFC lesions abolished the US devaluation effect in a magazine approach task with hungry subjects, Pickens, et al., (2003) demonstrated that the post-training lesions of the OFC but not the BLA abolished the US devaluation effect.

Another account for the dissociation between the effects of BLA and OFC lesions on PIT and US devaluation tasks in a magazine approach paradigm is that these two tasks might assess different components of learning. One of these components may be especially sensitive to OFC lesions whereas the other may be only partially sensitive to BLA lesions (see Dwyer and Killcross, 2006; Holland, 2004). However, in order to provide more evidence for this claim, one would need to find evidence for a double dissociation between the effects of lesions to two structures on performance in these two

tasks. In Experiment 2 BLA lesions impaired selective PIT but not US devaluation. One would also need to demonstrate that lesioning some other structure would impair US devaluation but not selective PIT. Only then could a strong claim be made that these two tasks assess different components of learning.

In addition to studying the BLA and OFC, we also analyzed the role of GC and its interaction with the BLA in the formation of sensory-specific associations in a conditioned flavor preference paradigm in Experiment 3. In the past, GC was found to be involved in incentive learning (Balleine & Dickinson, 2000), neophobia (Roman & Reilly, 2007), conditioned taste aversion (Desgranges, Sevelinges, Bonnefond, Levy, Ravel, & Ferreira, 2009; Kiefer and Braun, 1979), and, possibly, flavor-nutrient conditioning (Saddoris, et al., 2009). Our results suggest that while neither the GC, nor the GC-BLA communication, seem to be required for the formation of sensory-specific flavor-nutrient associations, they support earlier work suggesting that these structures may be involved in establishing distinct neural representations of different USs (e.g., Saddoris, et al., 2009; Saddoris et al., 2009). In these studies evidence was provided from IEG staining techniques to suggest that a cue for sucrose and sucrose itself come to activate similar neural ensembles in the GC, and that distinct associatively-activated neural ensembles of nutrient USs in the BLA depend upon input from the GC. However, the results from Experiment 3 suggest that while the ability to distinguish between two nutrient USs may be partially dependent upon GC and BLA, learning to associate flavor cues with these distinct nutrients does not depend upon these structures. However, one must be careful in interpreting Saddoris, et al.'s (2009) results because the exact

relationship between *IEG staining* in various brain structures and behavior remains unclear.

Other aspects of the results from Experiment 3 suggest that while GC and BLA may not play a substantial role in acquisition of flavor-nutrient associations, they may play a role in extinction. We found that GC and GC|BLA disconnection lesions result in subjects being resistant to extinction as compared to Sham controls. These results suggest that GC and GC|BLA disconnection lesions are involved in extinction of sensory-specific associations. In order to have a better understanding of the neural circuits involved in extinction of sensory-specific flavor-nutrient associations, a helpful first step might be to establish these circuits for acquisition of this form of learning. While the exact neural circuits involved in extinction of sensory-specific associations remain unknown, it appears that at the very least, GC-BLA communication is important for extinction learning in the flavor preference paradigm.

While we did not find the effects of BLA, OFC or GC lesions on the formation of sensory-specific associations in a flavor preference paradigm, it is possible that one or more of these structures might be involved in reversal learning. Schoenbaum, et al., (2003), who analyzed the effects of pre-training BLA and OFC lesions on reversal learning in an instrumental go-no-go task (with odor cues and liquid outcomes), found that neither BLA nor OFC lesions disrupted acquisition of the initial go-no-go discrimination, but that both of these structures affected the reversal of these contingencies. Behavioral studies using non-lesioned subjects in a conditioned flavor preference paradigm demonstrated that performance was governed by recently learned

associations immediately after a reversal phase (Scarlet, et al., 2009). In their experiment, Scarlet, et al., (2009) taught thirsty rats to associate two CS-US pairs. In the following phase, the CS-US contingencies were reversed and after the reversal phase the subjects were devalued on one of the outcomes by pairing it with LiCl. The test phase consisted of a two-bottle choice test between the two flavor CSs in the absence of the USs. The results indicated that the subjects avoided the flavor CS most recently paired with the devalued outcome. Therefore, the sensory-specific associations were governed by the most recently learned CS-US contingencies. It is possible that if the paradigm explored here was to be extended to include a reversal phase, one would find that BLA and OFC lesioned subjects might fail to acquire the reversal and be controlled by their initially acquired associations instead.

In summary, the present data demonstrate that while none of the structures we analyzed, the BLA, OFC, GC were found to be critical in the formation of sensory-specific associations in a conditioned flavor preference paradigm, we found that both the BLA and the OFC are critical for the formation of these associations in the magazine approach paradigm. While there is still a lot to learn about the nature of sensory-specific associations in Pavlovian learning paradigms and the neural mechanisms they involve, the present studies provide some additional information about the complicated mechanisms involved in this type of learning.

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