

POSTCONDITIONING MANIPULATION OF CONTEXT ASSOCIATIVE  
STRENGTH ON CONDITIONED RESPONDING IN CONDITIONED TASTE  
AVERSION

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

POSTCONDITIONING MANIPULATION OF CONTEXT ASSOCIATIVE  
STRENGTH ON CONDITIONED RESPONDING IN CONDITIONED TASTE  
AVERSION

Presented by Shawn Smith

A candidate for the degree of Master of Arts

And herby certify that in their opinion it is worthy of acceptance

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## DEDICATION

To my amazing wife Melissa, my best accomplishment was finding you.

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I would like to thank the assistance and guidance of my advisor, Dr. Todd Schachtman, who has helped me through this entire process. Additionally, I would like to thank my committee members: Drs. Dennis Miller, Tom Piasecki, and Agnes Simonyi for their support.

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## ABSTRACT

These experiments examined the effect of manipulating context associative strength on conditioned responding to a conditioned stimulus using a conditioned taste aversion procedure. In Experiments 1 and 3, subjects were given one or two flavor-LiCl pairings in a distinct context followed by context-US pairings. Rats that received context-US pairings were predicted to show a lower CR to the flavor at test than rats that received either context-US pairings in an irrelevant context or rats that received no context-US pairings. However, no difference was observed between these treatments. Experiments 2 and 4 examined the effects of postconditioning context-alone exposures. Subjects received context-US conditioning trials as well as one or two CS-US pairings during initial training, followed by context-alone exposures. It was predicted that these context exposures would increase the CR at test. Rats in these experiments also showed no differences in CR, indicating that postconditioning context-alone exposures did not affect the CR to the CSs.



## CHAPTER 1: INTRODUCTION

Contextual cues (i.e., usually defined as the static apparatus cues present during experimental procedures conducted with the target conditioned stimulus) have been shown to have an influence on classical conditioning (see Balsam & Tomie, 1985; Gordon & Klein, 1994). The influence of contextual cues has been examined using many classical conditioning procedures, including latent inhibition, blocking, and overshadowing (e.g., Gordon & Weaver, 1988; Lovibond, Preston, & Mackintosh, 1984). Contextual cues have been shown to attenuate (e.g., Gordon & Weaver, 1988) as well as enhance (e.g., Gordon & Mowrer, 1980) learning about a conditioned stimulus (CS).

One method of examining the effects of contextual cues on responding to a CS during classical conditioning is to manipulate the associative strength of the context before, during, or after pairing the CS with an unconditioned stimulus (US) in the context. Manipulation of context strength after CS-US pairings includes increasing and decreasing the associative strength of the context. “Context inflation” refers to increasing the strength of the context after CS-US pairings (Miller, Hallam, & Grahame, 1990). This typically involves multiple postconditioning (i.e., after CS conditioning) pairings of the context alone with a US. “Context deflation” refers to decreasing the strength of a context after CS-US pairings.

Different learning processes predict different effects of context manipulation on conditioned responding to a CS trained in that context. A weaker conditioned response (CR) to the CS produced by inflating contextual associative strength is predicted by comparator theories (e.g., Miller & Schachtman, 1985). According to these theories, an

association is formed between the CS and the US during CS-US pairings. Also, a context-US association is also formed due to the presence of the US in that context. Conditioned responding to a CS is, then, directly related to the strength of the CS-US association and inversely related to the strength of the context-US association. That is, at test subjects compare the associative strength of the conditioning context with the associative strength of the CS. To the extent that the strength of the CS-US association exceeds that of the context-US association, the CR to the CS is stronger. Hence, weaker conditioned responding to the CS is predicted if the context associative strength has been “inflated,” and a stronger CR is expected if the context has been “deflated.”

Another effect of inflating the associative strength of a context is that it may influence second-order conditioning supporting the CR to the CS as mediated by the contextual cues (Marlin, 1982; Rescorla, Durlach, & Grau, 1985). Typically (that is, the usual case in which second-order conditioning involves two CSs), second-order conditioning occurs when an initial CS is paired with the US, and then a second CS (the target CS) is paired with the first CS. The second (or target) CS then comes to elicit the CR because, presumably, this second CS now activates the representation of the first (or mediating) CS because of the CS-CS association (e.g., Rescorla, 1980; Hittesdorf & Richards, 1978; Rashotte, Griffin, & Sisk, 1977). Contextual cues can mediate second-order conditioning to a CS (Marlin, 1982) such that CS-context and context-US associations can be formed, and the CR to the CS can be enhanced by strengthening the latter association. If contextual cues mediate the CR to a target CS trained in that context (i.e., second-order conditioning), then “inflation” of the strength of the contextual cues will result in an increase in the CR to the CS. Hence, comparator processes and second-order conditioning predict different outcomes from a context inflation treatment (see

Bills, Smith, Myers & Schachtman, 2003). Comparator theories predict that context inflation will decrease the CR to the CS and a second-order conditioning process predicts it will increase the CR.

Context extinction is another method for influencing the associative strength of a context (Stout & Miller, 2004; Bouton, 1994, 2004). The context can be extinguished following CS-US pairings (“context deflation”). Comparator theories and a second-order conditioning process also predict effects of context deflation. Deflating the associative strength of the contextual cues, according to comparator theories, will result in an increase in the CR to the CS. This occurs due to a weaker context-US association promoting a stronger CR to the CS. However, if second-order conditioning occurs to the CS as mediated by contextual cues, then decreasing the associative strength of a context will also cause a decrease in the CR to the CS, since such extinction will attenuate the effects of a contributor to the CR.

Previous research by Bills et al. (2003) examined postconditioning extinction of contextual cues using a conditioned taste aversion (CTA) procedure. CTA involves pairing a novel flavor CS with a nausea-inducing US (typically LiCl). Later test trials typically show that rats learn to associate the flavor with illness. Bills et al. (2003) found that, following a short-duration conditioning session (i.e., 15 min), extinguishing the contextual cues decreased the CR. This result is consistent with second-order conditioning in which context-US associations support the CR to the CS that would typically occur if the deflation manipulation had not occurred.

Similar to the approach of Bills et al. (2003), the present study used a CTA procedure. By manipulating the postconditioning strength of the context-US association, the influence of the context on the CR to a CS trained in that context was examined.

## CHAPTER 2: METHODS AND RESULTS

### Experiment 1

This experiment explored the effect of increasing context associative strength following CS-US pairings. All subjects were given flavor-LiCl pairings in a distinctive context. One group of subjects then received context-US pairings in the context, while another group received context-US pairings in an irrelevant context. A third group received no context inflation. Subjects were then tested in the home cage with the flavor CS. Although either a comparator effect (a decrease in CR) or a second-order conditioning effect (an increase in CR) was possible, it was predicted that increasing the associative strength of the context would result in a decreased CR to the CS.

#### *Method*

*Subjects.* Twenty-four male Sprague-Dawley rats ranging in weight from 210 to 253g and purchased from Sasco Co. (Indianapolis, IN) served as subjects. Each rat was individually housed in hanging, stainless-steel wire mesh cages measuring 24 x 17.7 x 18.2 cm (l x w x h) with ad libitum access to rat chow on a 16/8-hr light/dark cycle. The rats were handled a few times prior to the start of the experiment, and were placed on a graded water-deprivation schedule resulting in 15 min of water access per day. Water access occurred in the home cage after each day's treatment, approximately 23.5 hours prior to the experimental manipulations of the next day. The rats were maintained in this fashion in subsequent experiments.

*Apparatus.* The experiment used two distinctly different experimental contexts.

Context A included clear, plastic boxes with a sloping ceiling constructed of parallel stainless-steel rods that were spaced 0.9-cm apart. They measured 36.1 x 31.3 x 17 cm (l x w x h) at the largest height of the chamber. There were approximately 2.5 cm of aspen wood shavings lining the floor of these chambers. These chambers were located in a dimly illuminated room with the odor of methyl (Vicks Vaporub, Richardson-Vicks Inc., Shelton, CT), and a soft white noise present. Context B consisted of small [measuring 28.8 x 17.9 x 12.2 cm (l x w x h)], off-white, nearly opaque boxes made of polycarbonate plastic with a lid constructed of parallel, stainless-steel rods spaced 0.8-cm apart. The room was brightly illuminated and quiet, with another distinctive odor present (Glade Hawaiian Breeze, SC Johnson, Racine, WI). Hence, these contexts differed in brightness, shavings, odor, and background noise. Rats have been shown to be able to discriminate contexts highly similar to these chambers (Chelonis, Calton, Hart, & Schachtman, 1999; Bills et al., 2003).

Flavored solutions were presented in a plastic drinking tube (a modified, inverted 50ml centrifuge tube with a metal lick tube in a rubber stopper attached). All tubes were weighed to the nearest 0.1g to assess the amount of solution consumed. LiCl was administered using a 25-ga, 1.59-cm hypodermic needle.

*Solutions.* LiCl (Sigma Co., St. Louis, MO) at concentrations of 0.3M and 0.15M at 1.0% body weight (bw) was injected. The flavor solutions (CS) were 0.1% saccharin (Sac, Sigma Co., St. Louis, MO) and 0.75% coffee (Coff, Sanka, General Foods, White Plains, NY) solutions.

*Procedure.* The procedure for Experiment 1 is shown in Table 1. On Day 1, all rats were given a 20-min exposure to one of the two contexts (A or B) with water

available in the drinking tubes in order to allow the rats to acclimate to the chambers. Half of the rats were placed in Context A for 20 min and the other half were placed in Context B for 20 min. On Day 2 all rats received a similar exposure, except to the context not exposed on Day 1.

On Days 3 and 9, half of the rats were placed in Context A, and the other half were placed in Context B. After 10 min, drinking tubes filled with Sac were placed on the cages in Context A for 15 min, and tubes filled with Coff were placed on the cages in Context B. The drinking tubes were then removed and all rats received an ip injection of 0.3M LiCl at 1.0% bw. The rats were then returned to their respective contexts for 60 min. Hence, sessions lasted 85 min. On Days 6 and 12, all rats were given a similar conditioning trial, but using the opposite context/flavor pairing, such that rats conditioned on Days 3 and 9 with Sac in Context A were conditioned on Days 6 and 12 with Coff in Context B, and vice versa. No treatments were given on Days 4, 5, 7, 8, 10, 11, 13 and 14 to allow recovery from conditioning.

Inflation of the associative strength of the contextual cues occurred on Days 15-36. On Days 15, 18, 21, 24, 27, 30, 33, and 36 two groups of rats received context inflation trials. Half of these rats were placed in Context A, and the other half were placed in Context B. For half of the rats receiving inflation, this context corresponded to where conditioning of the target CS (i.e., the CS to be tested) occurred (Group Inflate,  $n = 8$ ); the other half of the rats receiving inflation treatment did so in a different context from where target conditioning occurred (Group Inflate Control,  $n = 8$ ). After 10 min, rats in Contexts A and B were injected with 0.15M LiCl at 1.0% bw and placed back in their respective context (A or B) for 60 min and then placed back in the home cages. No

Group	Phase 1	Phase 2	Test
Inflate	A[X-US] B[Y-US]	A[US]	X
Inflate Control	A[X-US] B[Y-US]	B[US]	X
Home Cage	A[X-US] B[Y-US]	-	X

Table 1. Two X-US trials and two Y-US trials occurred in Contexts A and B in Phase 1; Eight US inflation sessions occurred in Contexts A or B in Phase 2. “X” refers to 0.1% saccharin solution; “Y” refers to 0.75% coffee solution.

treatments occurred on Days 16, 17, 19, 20, 22, 23, 25, 26, 28, 29, 31, 32, 34, 35, 37 and 38 to allow recovery. A third group of rats (Group Home Cage,  $n = 8$ ) did not receive any context inflation treatment, and remained in the home cage on these days. On Days 39-44, all rats were given a 15-min test presentation of Sac in the drinking tubes in the home cage. Based on pilot data collected in our laboratory, it was predicted that inflating the associative strength of contextual cues would result in a decreased CR to the CS - an outcome consistent with comparator theories. This would result in an increase in flavor consumption at test for Group Inflate. Group Home Cage was expected to exhibit a lower amount of flavor consumption (i.e., a stronger CR) relative to Group Inflate. Group Inflate Control was expected to consume a similar amount of flavor (since these rats received inflation treatment in a control context) as Group Home Cage.

### *Results & Discussion*

Means and SEMs of CS consumption on the conditioning trials are shown in Tables 2 and 3. Two 3 (Group) x 2 (Conditioning Trial) Analyses of Variance (ANOVAs) performed for each flavor's respective conditioning trials revealed significant differences in flavor consumption across conditioning trials  $F_s > 34.08$ ,  $p_s < 0.001$ , but no significant main effects of Group,  $F_s < 1.2$ ,  $p_s > 0.30$ , indicating that all groups acquired taste aversions readily from the first to the second conditioning trial, and that conditioning occurred to the flavors.

The test results of Experiment 1 are shown in Figure 1. A 3 (Group) x 8 (Test Trial) ANOVA revealed a significant main effect of Test Trial,  $F(7, 147) = 44.05$ ,  $p < 0.001$ , but no significant main effect of Group,  $F(2, 21) = 1.47$ ,  $p = 0.25$ , indicating that



Group	Cond 1	Cond 2	Cond 3	Cond 4
Inflate	5.2 ( $\pm$ 0.8)	6.1 ( $\pm$ 1.1)	0.6 ( $\pm$ 0.1)	1.3 ( $\pm$ 0.7)
Inflate Control	5.8 ( $\pm$ 1.0)	4.8 ( $\pm$ 1.2)	1.0 ( $\pm$ 0.4)	1.0 ( $\pm$ 0.3)
Home Cage	6.6 ( $\pm$ 1.1)	7.5 ( $\pm$ 1.4)	2.0 ( $\pm$ 0.8)	1.0 ( $\pm$ 0.6)

Table 2. Means ( $\pm$  SEM) of flavor consumption on the conditioning trials. Values indicate flavor consumption of either Sac or Coff. Cond 1 CS was identical to Cond 3 CS (Sac or Coff). Cond 2 CS was identical to Cond 4 CS (Coff or Sac).

Group	Cond 1 – Sac	Cond 2 – Sac	Cond 1 – Coff	Cond 2 – Coff
Inflate	4.8 ( $\pm$ 1.1)	1.0 ( $\pm$ 0.6)	6.5 ( $\pm$ 0.7)	0.9 ( $\pm$ 0.3)
Inflate Control	3.8 ( $\pm$ 1.1)	0.9 ( $\pm$ 0.3)	7.3 ( $\pm$ 0.7)	1.1 ( $\pm$ 0.3)
Home Cage	6.7 ( $\pm$ 1.5)	1.0 ( $\pm$ 0.4)	7.5 ( $\pm$ 1.0)	2.4 ( $\pm$ 0.9)

Table 3. Means ( $\pm$  SEM) of flavor consumption on the conditioning trials. Values indicate flavor consumption of either Sac or Coff.

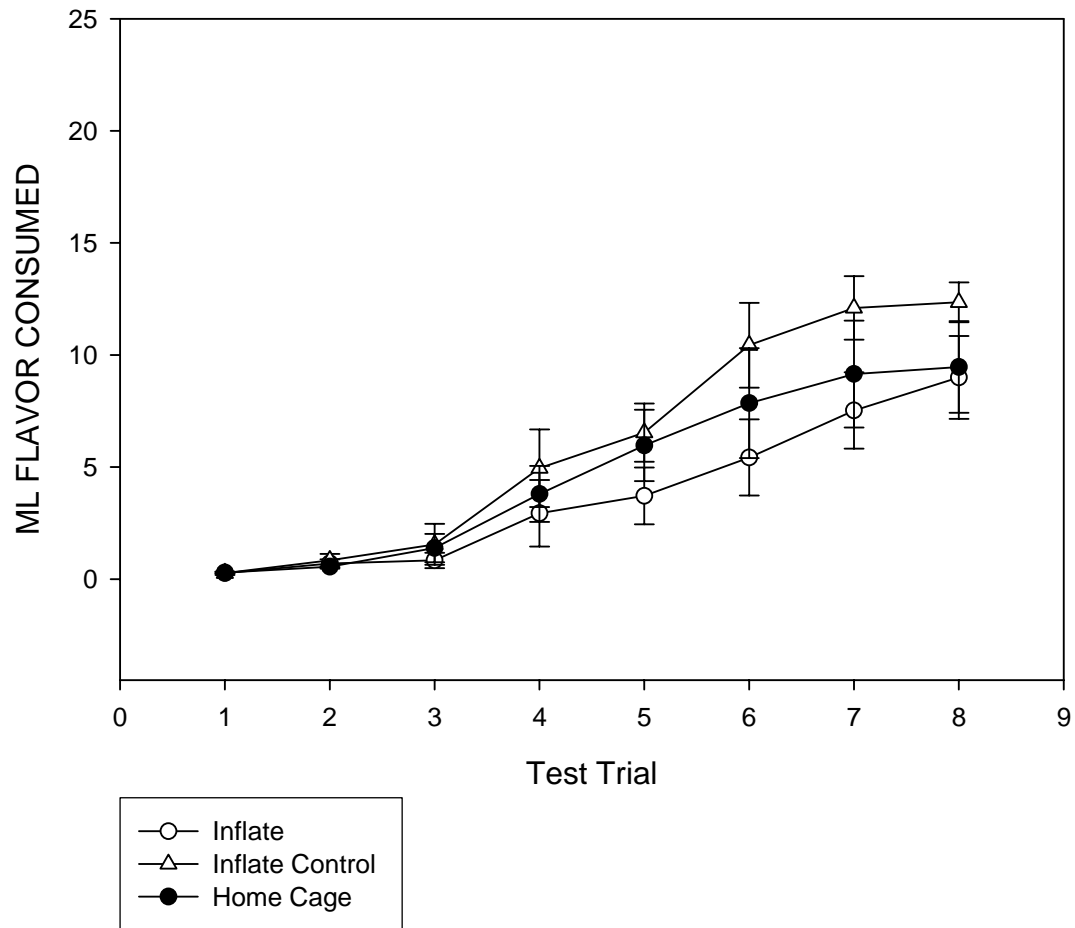


Figure 1. Mean levels ( $\pm$  SEM) of flavor consumption for Experiment 1. Group Inflate was tested on the flavor associated with the inflated context; Group Inflate Control was tested on the flavor associated with the non-inflated context; Group Home Cage was not given context inflation.

the groups consumed similar amounts at test. Additionally, significant group differences were not found when One-Way ANOVAs were conducted for comparisons involving all three groups on each test trial, as well as any combination of two of the three groups on each trial,  $F_s < 4.30$ ,  $p_s > 0.05$ . It should be noted that Group Inflate exhibited a marginally significant increase in CR relative to Group Inflate Control on Test Trial 6,  $F(1, 14) = 3.89$ ,  $p = 0.07$  and Test Trial 7,  $F(1, 14) = 4.72$ ,  $p = 0.06$ . Such differences, although modest, are in the direction of the inflation of contextual cues supporting an increased CR to the CS, an outcome predicted by a second-order conditioning effect. However, the lack of significant differences between Groups Inflate and Inflate Control, when compared to Group Home Cage, indicate that second-order conditioning processes may not be responsible for this difference. In sum, increasing the associative strength of a context did not convincingly impact the CR to the CS trained in that context.

## Experiment 2

Experiment 2 sought to influence the CR to a CS after extinction of the contextual cues. In this experiment, subjects were conditioned in a distinct context with a flavor CS. Half of the rats then received extinction trials with those contextual cues and the other half received no context extinction. Subjects then were tested in the home cage on the flavor to assess the influence of the context manipulation. If a deflation effect was obtained, context specificity of the effect would be explored in a subsequent experiment.

### *Method*

*Subjects and Apparatus.* The source of the sixteen adult subjects, the apparatus,

and the flavor (Sac) was identical to that used in Experiment 1. Sixteen rats ranging in weight from 178 to 227g were used. Only Context A chambers were used, since no context deflation control condition was included.

*Procedure.* The procedure of Experiment 2 is shown in Table 4. The rats were given a single 20-min exposure to Context A on Day 1 to allow them to acclimate to the context. No treatment occurred on Day 2, with the rats remaining in their home cages. On Days 3 and 7, all rats were placed in Context A. After a period of 10 min, drinking tubes containing Sac were placed on the cages for 15 min, followed by an ip injection of 0.3M LiCl at 1.0% bw. All rats then remained in their contexts for an additional 60 min, after which they were returned to their home cages. Days 5 and 9 were identical to 3 and 7, except that no Sac was given. The purpose of these US presentations was to ensure strong context-US associations so as to increase sensitivity to obtaining an effect of subsequent context-alone treatments (Kaspro, Schachtman, & Miller, 1987). No treatments occurred on Days 4, 6, 8, and 10 to allow the rats to recover from conditioning.

Half of the rats (Group Deflate,  $n = 8$ ) then received context deflation (extinction) trials on Days 11-18. These rats were placed in Context A for 60 min, after which they were returned to their home cages. A second group received no context deflation trials (Group No Deflate,  $n = 8$ ), and remained in the home cage during these days. On Days 19-23, all rats were presented with Sac in the drinking tubes in their home cages for 15 min to assess possible effects of context deflation on conditioned responding to the CS. Either a comparator effect (an increase in CR to the CS stemming from the context manipulation) or a second-order conditioning effect (a decrease in CR due to extinction

Group	Phase 1	Phase 2	Test
Deflate	A[X-US] A[US]	A	X
No Deflate	A[X-US] A[US]	-	X

Table 4. Two X-US trials and two A-US trials occurred in Context A in Phase 1. Eight deflation sessions occurred in Context A in Phase 2 for Group Deflate. “X” refers to 0.1% saccharin solution.

of the context) was possible. Similar to the prediction made for Experiment 1, it was expected that deflation of contextual cues would result in a comparator effect - an increase in CR to the CS. Group Deflate, therefore, was expected to consume a lower amount of compared to Group No Deflate.

### *Results & Discussion*

Means and SEMs of CS consumption on the conditioning trials are shown in Table 5. A 2 (Group) x 2 (Day) ANOVA conducted on the data from Days 3 and 7 (conditioning trials) revealed no significant main effect for Group,  $F(1, 11) = 2.93, p = 0.12$ . There was also no significant main effect of Day,  $F(1, 11) = 1.15, p = 0.31$ . Additionally, the interaction between Group and Day was not significant,  $F(1, 11) = 2.95, p = 0.11$ . This indicates that there was no difference in consumption of Sac between groups on the conditioning trials.

The test results of Experiment 2 are shown in Figure 2. A 2 (Group) x 5 (Test Trial) ANOVA conducted for Days 19-23 showed a significant main effect for Test Trial,  $F(4, 52) = 18.93, p < 0.001$ , but not for Group  $F(1, 12) = 0.16, p = 0.69$ , indicating that both groups consumed a similar amount on the test trials. Additionally, no significant Group x Test Trial interaction was found  $F(4, 52) = 0.50, p = 0.74$ . Additional ANOVAs conducted for each test trial revealed no significant effect of Group on any of those days  $F_s < 1, p_s > 0.10$ . Deflating the associative strength of a context had no effect on the CR to the CS associated with that context.

Group	Cond 1	Cond 2
Deflate	1.1 ( $\pm$ 0.6)	1.7 ( $\pm$ 0.8)
No Deflate	4.8 ( $\pm$ 1.5)	1.6 ( $\pm$ 0.8)

Table 5. Means ( $\pm$  SEM) of Sac consumption on the conditioning trials.



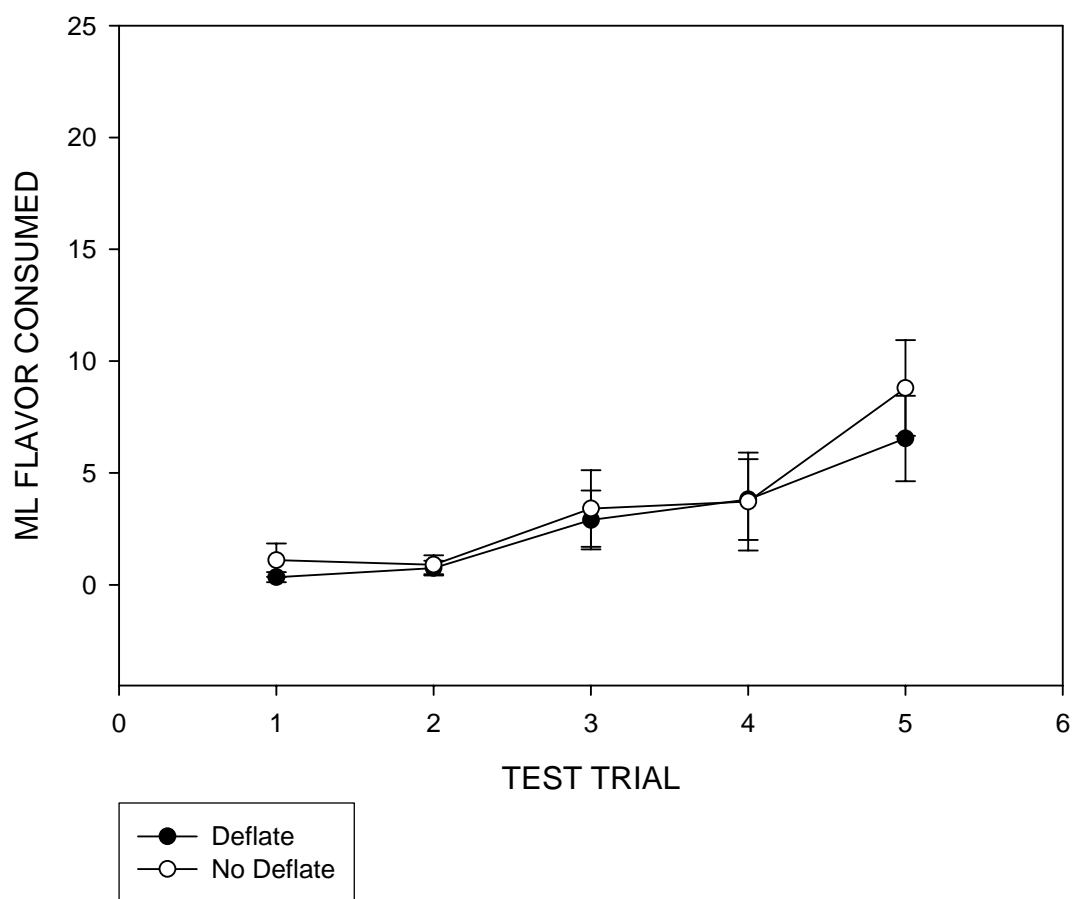


Figure 2. Mean ( $\pm$  SEM) levels of Sac consumption for Experiment 2. Group Deflate was given context extinction and Group No Deflate was not.

### Experiment 3

The results of Experiment 1 did not produce a between-group difference in flavor consumption during the test as a result of the context inflation manipulation. Experiment 3 used a within-subject design to assess if inflating the associative strength of a context influenced the CR to the CS associated with that context relative to another CS associated with a non-inflated context. It was anticipated that a within-subject design might be more sensitive to obtaining an effect on context inflation by enhancing the discrimination of contextual cues, thereby increasing sensitivity to the contingencies. All subjects received CS-US pairings with two CSs in two distinct contexts, such that subjects received one conditioning trial per flavor-context pair. Subjects were given context-US pairings in only one of those contexts, and then tested with both CSs in the home cage.

#### *Method*

*Subjects and Apparatus.* The source of the subjects and the apparatus was identical to that used in Experiment 1. Sixteen rats ranging in weight from 176 to 202g were used in this experiment. A 12% sucrose (Suc) solution was substituted for Sac (to match the flavor used in earlier pilot work from our laboratory) and the coffee (Coff) concentration was changed to 1.5%. A stronger dose (2%) of LiCl was also used since one conditioning trial was used for each flavor.

*Procedure.* The procedure for Experiment 3 is shown in Table 6. On Day 1, all rats were given a 20-min exposure to one of the two contexts (A or B) with water available in drinking tubes in order to allow the rats to acclimate to the chambers, with half of the rats being placed in Context A for 20 min and the other half being placed in Context B for 20

min. On Day 2 all rats received a similar exposure, except to the context not exposed on Day 1.

On Day 3, half of the rats were placed in Context A, and half were placed in Context B. After 10 min, drinking tubes filled with Suc were placed on the cages in Context A for 15 min, and tubes filled with Coff were placed on the cages in Context B. The drinking tubes were then removed and all rats received an ip injection of 2.0% LiCl at 0.5% bw. Rats were then returned to their respective contexts for 60 min. Hence, the sessions lasted 85 min. No treatments were given on Day 4 to allow recovery from conditioning. On Day 5, all rats were given a similar conditioning trial, but using the opposite context/flavor pairing, such that rats conditioned on Day 3 with Suc in Context A were conditioned on Day 5 with Coff in Context B, and vice versa.

On Days 7, 9, 11, and 13 both groups of rats received context inflation trials. Half of these rats were placed in Context A ( $n=6$ ), and the other half were placed in Context B ( $n=6$ ). Note that Context A had been the context where Suc had been conditioned for half of the rats placed there on these days, whereas Coff had been conditioned there for the remaining rats placed there on these days. The same was true for Context B. After a period of 10 min, the rats were injected with 2.0% LiCl at 0.5% bw and placed back in their respective context (A or B) for 60 min. The rats were then removed from the context and placed back in the home cages. No treatments occurred on Days 8, 10, 12 and 14 to allow recovery from illness.

Testing of both flavors occurred on Days 15-22. On Days 15, 17, 19, and 21, half of the rats were given a 15-min presentation of Suc in the drinking tubes in the home cage, and the other half were given Coff. On Days 16, 18, 20, and 22 rats were tested on the other

Phase 1	Phase 2	Test
A[X-US] B[Y-US]	A[US] or B[US]	X and Y

Table 6. One X-US trial and one Y-US trial occurred in Contexts A and B in Phase 1; Four US inflation sessions occurred in Contexts A or B in Phase 2. “X” refers to the 12% sucrose solution; “Y” refers to the 1.5% coffee solution.

flavor (Suc or Coff), such that each flavor was tested a total of 4 times for each subject.

Similar to the proposed outcome of Experiment 1, it was predicted that inflating the associative strength of contextual cues would result in a decreased CR to the CS associated with a context - an outcome consistent with comparator theories. This would result in an increase in flavor consumption at test for the flavor associated with the inflated context (A or B) relative to the flavor associated with the non-inflated context.

### *Results & Discussion*

Means and SEM's of CS consumption on the conditioning trials are shown in Tables 7 and 8. Four rats were lost for failing to drink greater than 1ml of both flavors on the initial conditioning trials.

A 2 (Day) x 2 (Context) x 2 (Flavor) ANOVA conducted on the two initial conditioning trials revealed a significant main effect of Day,  $F(1, 11) = 22.9, p < 0.01$ , indicating that subjects differed in their consumption of flavors across conditioning trials. No main effects of Context, Flavor, nor interactions of any of these factors were significant,  $F_s < 2.9, p_s > 0.12$ . Additional ANOVAs conducted on each day found no significant effects of Context or Flavor,  $F_s < 2.6, p_s > 0.10$ , indicating that although subjects did differ in their flavor consumption across conditioning trials, this increase did not interact with either Flavor or Context. Therefore, the subjects conditioned similarly on the two flavors and in the two contexts.

The test results of this experiment are shown in Figure 3. A 2 (Treatment) x 2 (Flavor) x 2 (Context) x 4 (Test Trial) ANOVA revealed a significant main effect of Test Trial,  $F(3, 57) = 33.80, p < .0001$ . However, no significant main effects were found for

Treatment	Cond 1	Cond 2
INF-Suc/ NI-Coff	5.2 ( $\pm$ 1.4)	12.1 ( $\pm$ 0.9)
INF-Coff/ NI-Suc	7.5 ( $\pm$ 1.3)	11.7 ( $\pm$ 1.3)

Table 7. Mean ( $\pm$  SEM) Suc or Coff consumption on the conditioning trials.

Treatment	Cond – Suc	Cond – Coff
INF-Suc/ NI-Coff	9.2 ( $\pm$ 2.4)	7.0 ( $\pm$ 1.1)
INF-Coff/ NI-Suc	11.4 ( $\pm$ 1.2)	7.8 ( $\pm$ 1.6)

Table 8. Mean ( $\pm$  SEM) Suc or Coff consumption on the conditioning trials.

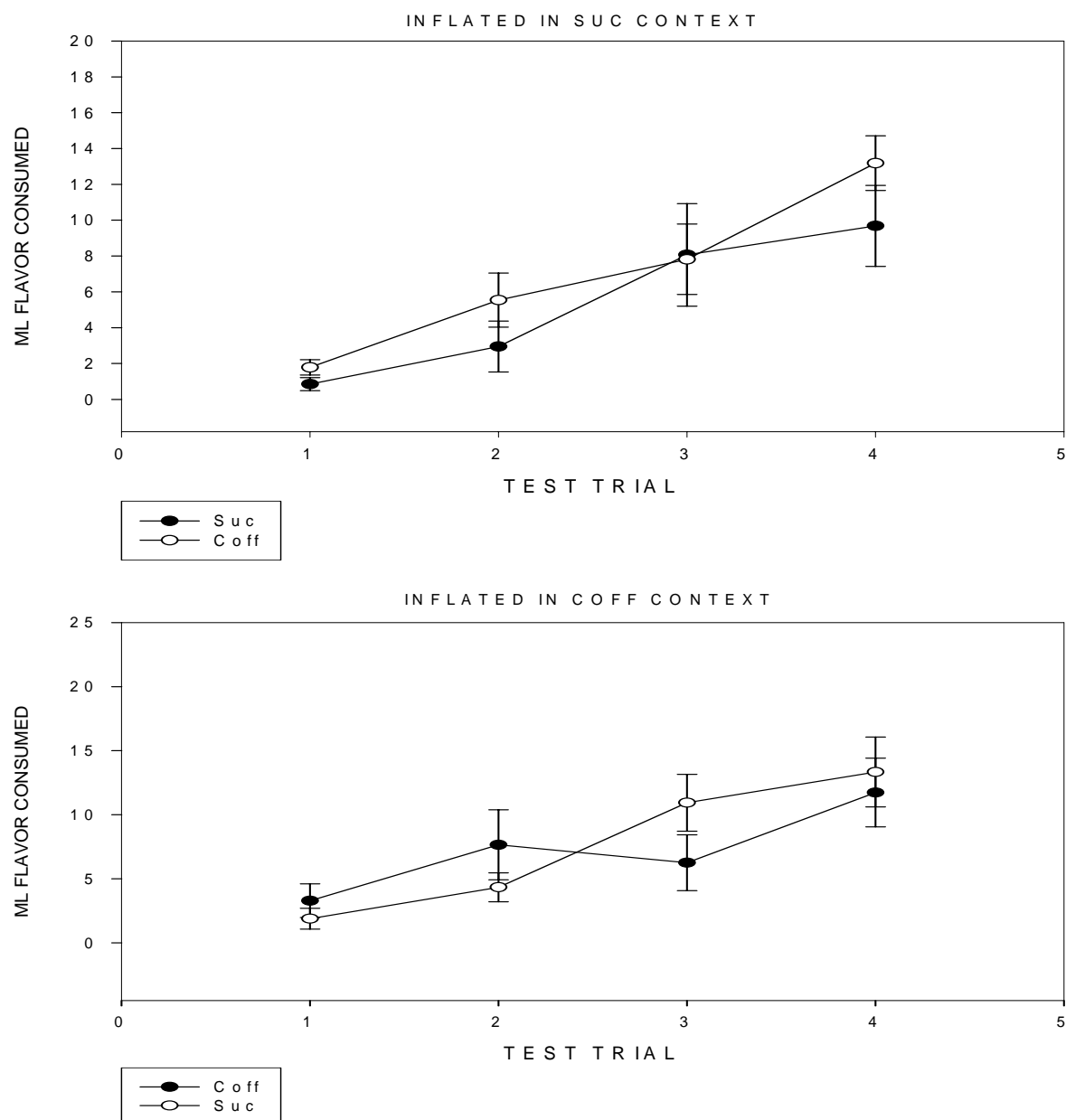


Figure 3. Mean levels ( $\pm$  SEM) of flavor consumption for both treatments in Experiment 3. Rats given context inflation in the context where Suc was conditioned are shown in the top figure. For these rats, Coff was conditioned in the non-inflated context. Rats given context inflation in the context where Coff was conditioned are shown in the bottom figure. Suc was conditioned in the non-inflated context for these rats.



Treatment (Inflated or Non-Inflated) Context, or Flavor,  $F_s < 1$ . There were also no significant interactions between variables,  $F_s < 1.97$ ,  $p_s > 0.50$ . Additional ANOVAs conducted for each test trial revealed no additional significant main effects for Treatment, Context, or Flavor, as well as no significant interactions of these variables,  $F_s < 2.95$ ,  $p_s > 0.10$ . This indicates that context inflation had no effect on the CR to the CS; additionally, there was no effect of which flavor was paired with either context. Between-group analyses comparing inflated Suc to non-inflated Suc obtained no group difference,  $F(1, 10) = 1.79$ ,  $p > 0.20$ , and inflated Coff to non-inflated Coff found no group difference,  $F < 1$ .

In order to further explore within-subject effects, two 2 (Treatment) x 4 (Test Trial) ANOVAs were then conducted within-subjects. These analyses compared the inflated flavors to their respective non-inflated flavors, since each subject was exposed to both treatments (i.e., inflated Suc compared to non-inflated Coff and inflated Coff compared to non-inflated Suc). These analyses revealed a significant main effect of Test Trial,  $F_s > 24$ ,  $p_s < 0.0001$ , but no significant main effect of Treatment,  $F_s < 1$ , or a significant interaction between the two factors,  $F_s < 1$ . No significant effect of Treatment was found for any individual test trial,  $F_s < 2.27$ ,  $p_s > 0.10$ .

These results indicate that subjects drank relatively similar amounts of both flavors across all test trials regardless of treatment condition. Inflating the associative strength of a context, therefore, had no effect on the CR to the flavor associated with it. Flavors associated with contexts that were later inflated were consumed at test at similar rates as those associated with contexts which were not later inflated.

## Experiment 4

The results of Experiment 2 also did not show a between-group difference in flavor consumption during test as a result of context deflation. The present experiment used a within-subject design as a follow-up to Experiment 2 to assess if deflating the associative strength of a context influences the CR to the CS associated with that context. All subjects received CS-US pairings using two CSs in two distinct contexts. Subjects were given context-alone exposures in only one of those contexts, and then tested with both CSs in the home cage.

### *Method*

*Subjects and Apparatus.* The source of the sixteen subjects and the apparatus were identical to those used in Experiment 3. A 1.0% vinegar (Vin) solution was substituted for Coff and a 0.2% saccharin (Sac) solution was substituted for Suc (to match the flavors used in earlier pilot work from our laboratory).

*Procedure.* The procedure of Experiment 4 is shown in Table 9. Context preexposures, CS-US pairings, and US-alone exposures occurred similarly to Experiment 2, except that two CSs were conditioned in two contexts and US-alone exposures occurred in two contexts. The rats were given a single 20-min exposure to Context A or B on Days 1 and 2 to allow them to acclimate to the context. On Day 3 all rats were placed in either Context A or B. After a period of 10 min, drinking tubes containing Sac or Vin were placed on the cages for 15 min, followed by an ip injection of 2.0% LiCl at 0.5% bw. The rats then remained in their contexts for an additional 60 min, after which they were returned to their home cages. On Day 5 the rats received identical treatment, but with the opposite flavor in

Phase 1	Phase 2	Test
A[X-US] B[Y-US]	A or B	X and Y

Table 9. One X-US trial and one Y-US trial occurred in Contexts A and B in Phase 1; Eight A-only or B-only deflation sessions occurred in Contexts A or B in Phase 2. “X” refers to the 0.2% Sac solution. “Y” refers to the 1.0% Vin solution.

the second context. Days 7 and 9 were identical to 3 and 5, except that no Sac was given. No treatments occurred on Days 4, 6, 8, and 10 to allow the rats to recover from conditioning.

On Days 11-18, the rats received context deflation trials. Half of these rats were placed in Context A, and the other half were placed in Context B for 60 min. The rats were then removed from the context and placed back in the home cages. Testing of both flavors occurred on Days 19-26. On Days 19, 21, 23, and 25 half of the rats were given a 15-min presentation of Sac in the home cage, and the other half were given Vin. On Days 20, 22, 24, and 26 rats were tested on the alternate flavor (Vin or Sac), such that each flavor was tested a total of 4 times for each subject. Again, it was predicted that decreasing the associative strength of contextual cues would result in an increased CR to the CS associated with a context, resulting in decreased flavor consumption at test for the flavor associated with the deflated context.

### *Results & Discussion*

Means and SEMs of CS consumption on the conditioning trials are shown in Tables 10 and 11. Two rats were removed from the study for failure to drink enough solution on the conditioning trials. A 2 (Day) x 2 (Context) x 2 (Flavor) ANOVA conducted on the two conditioning trials revealed significant effects of Day,  $F(1, 11) = 7.65, p = 0.018$ , and Flavor,  $F(1, 11) = 6.66, p = 0.026$ , as well as a significant interaction between the two,  $F(1, 11) = 9.26, p = 0.011$ . Context and any interactions involving Context had no significant effects,  $F_s < 1.5, p_s > 0.28$ . Additional ANOVAs conducted on the individual conditioning trials revealed a significant effect of Flavor on Day 1 of conditioning,  $F(1, 11) = 19.7, p = 0.001$ , as well as a Context x Flavor interaction,  $F(1, 11) = 5.15, p = 0.04$ . These significant

Treatment	Cond 1	Cond 2
Def-Sac/ND-Vin	6.6 ( $\pm$ 1.2)	6.1 ( $\pm$ 0.7)
Def-Vin/ND-Sac	7.6 ( $\pm$ 1.9)	4.8 ( $\pm$ 0.9)

Table 10. Mean ( $\pm$  SEM) Sac or Vin consumption on the conditioning trials.

Treatment	Cond – Sac	Cond – Vin
Def-Sac/ND-Vin	7.9 ( $\pm$ 1.0)	4.7 ( $\pm$ 0.5)
Def-Vin/ND-Sac	9.7 ( $\pm$ 1.0)	3.5 ( $\pm$ 0.6)

Table 11. Mean ( $\pm$  SEM) Sac or Vin consumption on the conditioning trials.

differences on this single conditioning trial occurred largely because the rats conditioned on Sac drank more flavor, especially on the conditioning trial in Context B. Rats given Sac in Context A, Sac in Context B, Vin in Context A, and Vin in Context B consumed (respectively) 8.1 ml, 11.1 ml, 5.8 ml, and 3.0 ml. Hence, all rats at least had adequate consumption of the flavor on the first conditioning trial, as well as the second conditioning trial which used the other flavor (and for which no significant differences in flavor consumption occurred).

The results of the test trials of Experiment 4 are shown in Figure 4. A 2 (Flavor) x 2 (Treatment) x 2 (Context) x 4 (Test Trial) ANOVA were conducted. A significant main effect of Test Trial,  $F(3, 60) = 6.86, p < 0.001$  was obtained, but no main effects of Treatment, Context, or Flavor was found to be significant,  $F_s < 1.3$ . No significant interaction between any of these variables was found either,  $F_s < 3.1, p_s > 0.09$ . Additional ANOVAs conducted for each test trial revealed a significant main effect of Treatment on Test Trial 3,  $F(1, 20) = 5.13, p = 0.035$ , such that deflation increased the CR (consistent with comparator theory), but no effect occurred on Test Trials 1, 2, or 4,  $F_s < 1$ . Context and Flavor had no significant effects on any one of the test trials,  $F_s < 2.3, p_s > 0.10$ . It is interesting to note that although there was a significant Treatment x Context x Flavor interaction on Test Trial 3,  $F(1, 20) = 5.89, p = 0.025$ , no other interaction between these variables was significant on any of the test trials,  $F_s < 1.9, p_s > 0.18$ .

Comparisons of flavors with the same treatment (i.e, Sac that was associated with the deflated context compared to Vin associated with the deflated context) revealed no significant differences on any test trial,  $F_s < 1$ . In contrast, a significant difference was found for flavor consumption between the non-deflated treatments on Test Trials 2 and 3,  $F_s$

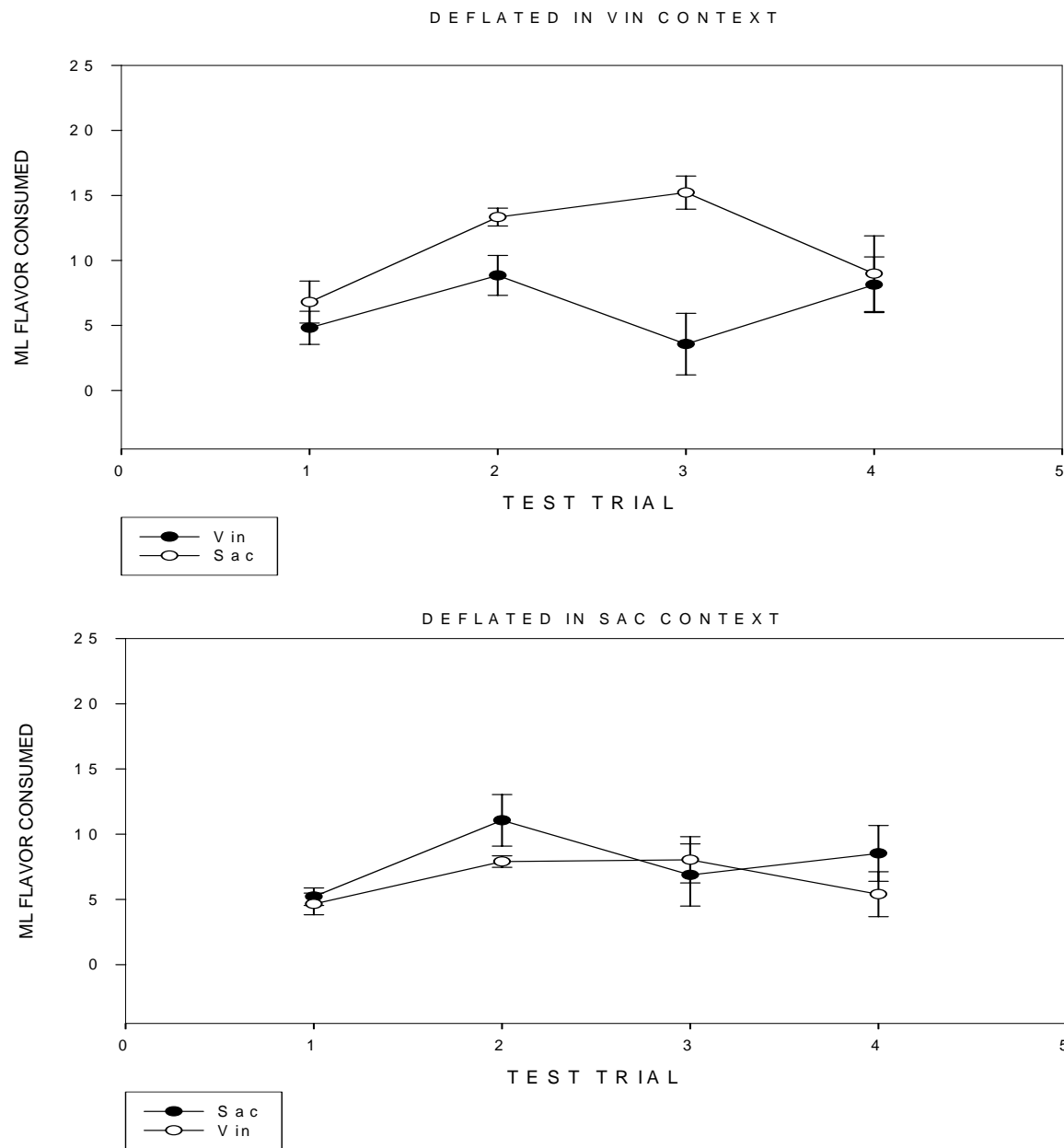


Figure 4. Mean levels ( $\pm$  SEM) of flavor consumption for Experiment 4. Rats given context deflation in the context where Vin was conditioned are shown in the top figure. For these rats, Sac was conditioned in the non-deflated context. Rats given context deflation in the context where Sac was conditioned are shown in the bottom figure. Vin was conditioned in the non-deflated context for these rats.



$> 9.4$ ,  $ps < 0.01$ , but not on Test Trials 1 and 4,  $F_s < 1.63$ ,  $ps > 0.10$ .

When each flavor was examined across treatment conditions (i.e, Sac associated with the deflated context vs. Sac associated with the non-deflated context; Vin associated with the deflated context vs. Vin associated with the non-deflated context), only Sac consumption on Test Trial 3 differed across treatments,  $F(1, 12) = 7.8$ ,  $p = 0.016$ . Vin consumption did not differ for deflated and non-deflated treatments on any test trial,  $F_s < 1$ . In sum, these results indicate that only Sac consumption in the non-deflated treatment showed a significantly decreased CR to the CS.

Additional ANOVAs were conducted comparing each deflated flavor to its respective non-deflated flavor (i.e., within-subjects). These again revealed significant main effects of Test Trial,  $F_s > 5$ ,  $ps < 0.007$ . A significant main effect of Treatment was found only for the treatment whose context deflation was associated with Vin (i.e., Sac was non-deflated),  $F(1, 10) = 5.8$ ,  $p = 0.037$ , as well as a significant Treatment x Test Trial interaction,  $F(3, 30) = 5.96$ ,  $p = 0.003$ . ANOVAs conducted for individual test trials of treatments (within-subject) revealed significantly elevated Sac consumption relative to Vin on Test Trials 2 and 3,  $F_s > 7$ ,  $ps < 0.025$ , but not on Test Trials 1 and 4,  $F_s < 1$ . A significant effect of deflation was not present for the other treatment (i.e., when the context associated with Sac was deflated),  $F < 1$ . Taken together, these results do not support the hypothesis that deflating the associative strength of a context affects the CR to the CS associated with that context.

## CHAPTER 3: GENERAL DISCUSSION

This series of experiments sought to further explore manipulations of context associative strength after CS conditioning. Comparator processes and second-order conditioning effects make opposing predictions regarding the effects of manipulations of context strength on the CR to the CS. Specifically, comparator processes predicted outcomes such that a stronger association between the context and the US would diminish the CR to the CS, and weaker context-US associations would strengthen the CR. Second-order conditioning effects predicted outcomes wherein increasing the associative value of the context would produce a likewise change in the CR to the CS in the same direction as the associative strength of that context. It was predicted that the manipulations used in these experiments would produce results that were consistent with comparator theories: Inflating the associative strength of the context would result in a reduced CR to the CS, and deflating the associative strength of the context would result in an increased CR to the CS. However, this series of experiments found little effect of changing the associative strength of a context using a CTA procedure.

These experiments provide some insight into processes influencing posconditioning associations when compared to previous research as well as data collected previously by our laboratory. Previous experiments by Miller, Hallam and Grahame (1990) were not able to find an effect of increasing the associative strength of a context by postconditioning pairings of the context with the US in multiple experiments using a fear conditioning procedure. Pilot data collected by our laboratory, however,

found that postconditioning increases in the associative strength of context produced by subsequent context-US pairings reduced the CR to the CS (consistent with comparator theory). Unfortunately, the group in this pilot experiment that received context-US pairings showed a decreased CR to the CS, regardless of whether these pairings occurred in the CS's training context or a nontarget control context. While increased flavor consumption was an outcome consistent with those predicted by comparator processes, the generalization of this effect across contexts is not readily explained, and is indicative of some process other than comparator mechanisms. Experiments 1 and 3 in the present project failed to confirm the findings of these pilot data.

Methodological limitations may have contributed to the present effects. For instance, the subjects in Experiment 1 were conditioned twice with each flavor. CTA itself is a robust procedure, and typically only requires a single trial for subjects to be able to associate the CS with the US. Two conditioning trials per flavor may have produced such strong conditioning that it reduced sensitivity to obtaining any effects of postconditioning context-US pairings. An additional methodological concern could be that comparator processes and second-order effects could conceivably cancel each other out, although this actually occurring seems unlikely.

The data from Experiment 1 show that subjects were slow to extinguish to the CS, with some of the rats failing to extinguish at all across all 8 test trials because conditioning was so strong. Experiment 3 was designed with two significant changes in mind: Subjects received one conditioning trial per flavor-context pair, and it was thought that a within-subject design might be more sensitive to the effects of inflation of a context on the CR to the flavor associated with that context. However, the results of Experiment

3 corroborate those found in Experiment 1: Inflating the strength of contextual cues does not decrease the CR to the CS. Even though a decrease in CR is predicted by comparator theories, finding such an effect in this direction has not been readily accomplished, and as such, these experiments further support the notion posited by Miller, Hallam, and Graham (1990) that asymmetrical differences may exist between postconditioning manipulation of context associative strength by inflation and deflation of a context. That is, Miller et al. found that it was fairly easy to obtain an effect of postconditioning extinction of the contextual cues on conditioned responding to a CS, but postconditioning inflation of the associative strength of the contextual cues was difficult to achieve experimentally.

Experiments 2 and 4 examined decreasing the associative strength of contextual cues. The nonreinforced postconditioning presentations of the context in Experiments 2 and 4 were not able to produce an increased CR to the CS. As in Experiment 1, rats in Experiment 2 were conditioned twice with the CS. As such, these multiple conditioning trials may have reduced detection of any effects of context deflation. However, previous pilot data collected in our laboratory support the results of Experiments 2 and 4, in that postconditioning context-alone presentations had no effect on the CR to the CS. These findings differ from the results found in previously published experiments from R.R. Miller's laboratory (which support the comparator hypothesis), such that context-alone presentations should have increased the CR to the CS.

In regards to context deflation, modifications of the initial conditioning procedure (such as increasing the number of context-US trials) may produce stronger preliminary context-US associations to the effect that deflation may be easier to observe. Increasing

the strength of the initial association between contextual cues and an aversive US could benefit later assessment of deflating the associative strength of the contextual cues. Another helpful procedural change when examining the effects of deflation would be to decrease the number of initial CS-US pairings. This sort of modification could decrease the association formed between the CS and the US, which could enhance sensitivity to detection of a deflation effect. However, Experiment 4 used a within-subject design and only one CS-US association for each flavor, and yet no deflation effect was found.

The results of Experiments 2 and 4 can also be considered with respect to previously published research completed by our laboratory. Bills et al (2003) found that extinction of a context where a CS was conditioned decreased the CR to the CS when the CS conditioning sessions were of short duration (15 min) – a result indicative of second-order conditioning. The results of Experiments 2 and 4 did not show this effect; however, longer sessions were used and the significant effect found in Experiment 4 was in the opposite direction (such that the CR was increased for the flavor associated with the deflated context) – a result suggestive of a comparator process. Corroborated by pilot data collected by our laboratory, it can be concluded that context-mediated second-order conditioning does not influence the CR to the CS using the present CTA procedure.

Taken together, these experiments show that an effect of postconditioning manipulation of the associative strength of a context is not easily obtained using a CTA procedure. With regard to previously published research evaluating the comparator hypothesis, the lack of an effect of inflation in this set of experiments was anticipated. Previous pilot data collected by our laboratory, though, suggested that it was possible to obtain an effect of context inflation in the direction predicted by the comparator

hypothesis. The absence of a deflation effect on the CR to the CS was surprising, given previous published research by our laboratory showing second-order conditioning effects produced by context deflation. While both the comparator hypothesis and second-order processes are of great interest and importance in classical conditioning, their relationship to the modulation of conditioned responding using a CTA procedure unfortunately remains unclear.

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