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PAVLOVIAN CONDITIONING OF
THE HUMAN SALIVARY RESPONSE

by

BARBARA A. SHULTZ

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

2001

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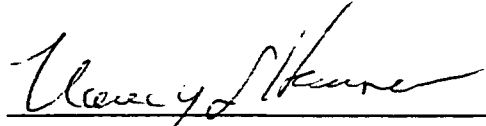
Barbara A. Shultz

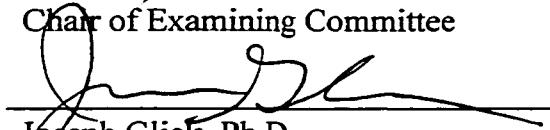
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This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

4/16/01
Date

4/18/01
Date


Nancy Hemmes, Ph.D.
Chair of Examining Committee


Joseph Glick, Ph.D.
Executive Officer

Claire Poulson, Ph.D.

Bruce Brown, Ph.D.

David Roll, Ph.D.

Bertram O. Ploog, Ph.D.

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK

Abstract

PAVLOVIAN CONDITIONING OF
THE HUMAN SALIVARY RESPONSE

by

Barbara A. Shultz

Adviser: Professor Nancy Hemmes

This study examined the magnitude (weight in grams) of the human salivary conditioned response (CR) during baseline, acquisition, and extinction trials when under a Pavlovian conditioning procedure. The compound conditioned stimulus (CS) was a 15-s tone and 0.1 ml of a vanilla solution, and the unconditioned stimulus (US) was 3 ml of lemonade placed in the mouth. Five subjects, between the ages of 29 and 45, participated. A within-subject-reversal design was used to assess individual response patterns for both the acquisition and extinction of the conditioned response. All subjects showed an increase in conditioned salivary responding on test trials during the Acquisition Phase when compared with level of salivary responding during the Baseline Phase, regardless of their initial levels of salivation during the Baseline Phase. Additionally, four of the five subjects showed a decrease in conditioned salivary responding during the Extinction Phase when compared with salivary responding on test trials during the Acquisition Phase. Salivary responding was variable between subjects as indicated by the mean, range, and standard deviation of salivary responses and by the speed of acquisition and extinction of salivary responses. The intersubject variability of

the current study supports the use of a within-subject design and presentation of individual data over successive trials. Group data may obscure the results of individual subjects and may partially account for the inconsistent findings in the literature. In contrast, the present individual-subject data permit increased precision for analysis of the behavior processes involved in conditioned salivary responding.

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Pavlovian Conditioning of the Human Salivary Response

Several researchers have studied Pavlovian conditioning of the human salivary response (e.g., Brown & Katz, 1967; Holland & Matthews, 1970; Jansen, Boon, Nauta, Van Den Hout, 1992; Wells & Feather, 1968). Although these researchers have reported positive results, other researchers have had difficulty demonstrating the acquisition (Conduit & Coleman, 1998; Jones, 1939) and extinction (Razran, 1935) of the human salivary response.

Although methodology has differed among experiments, one consistent finding reported by many researchers is that the human salivary response is highly variable, both within and between subjects (Conduit & Coleman, 1998; Finesinger, Sutherland, & McGuire, 1942; Razran, 1935). For example, Conduit and Coleman (1998) showed that prior to conditioning, unconditioned salivary responses varied considerably between subjects. For the first trial during the baseline condition, the amount of salivation by weight collected during a 2-min period ranged from approximately 0.2 g to approximately 2.6 g between subjects. Furthermore, the initial level of salivary responding may influence whether conditioning occurs. Feather (1967) reported that subjects who displayed a higher salivary level (levels of salivation during baseline were higher than 0.0015 cc) as compared to lower level salivators (levels of salivation during baseline were lower than 0.001 cc) showed a conditioned salivary response to the reinforced conditioned stimulus (CS+) but not to the nonreinforced conditioned stimulus (CS-) following a discrimination training procedure. When the

contingencies were reversed for the two stimuli, the results showed that the CS+ elicited significantly higher levels of salivation than the CS- for the high level salivators but not for the lower level salivators.

In the past, variability in the data has been obscured as many researchers presented data in terms of averages for a condition or session after using either a between-subject design (e.g., Brown & Katz, 1967; Feather, 1967; Jones, 1939) or a within-subject design (Jansen et al., 1992; Razran, 1935). Although averaged data for each condition for an individual subject are a better representation of behavioral processes than averaged data presented for several subjects, any averaged data remain an imprecise description of the behavioral processes. To increase precision in describing highly variable behavior processes, data for each trial must be presented. Through the use of a single-subject design, individual subject variability, including the treatment effect, will not be obscured by group averages. For example, in a single-subject-reversal design, there is a continuous evaluation of responding over trials. With each subject serving as his/her own control, the effects of the conditioning procedure can be evaluated against a stable baseline for each subject. By using this design and presenting individual data rather than group means, the experimenter can evaluate individual response patterns for both the acquisition and extinction of the conditioned response. Additionally, through further single-subject experimental manipulations, the experimenter may be able to identify and control for extraneous variables that cause variability in the data (Sidman, 1960). Furthermore, through visual analysis

rather than statistical analysis of individual subject data, the experimenter may make more conservative decisions as to whether changes in performance occurred after the introduction of the independent variable (Parsonson & Baer, 1986). Changes in performance evaluated by visual analysis need to be relatively larger than changes in performance evaluated by statistical analysis, as statistical tests are more sensitive to slight changes in performance (Kazdin, 1982).

Two studies (Conduit & Coleman, 1998; Holland & Matthews, 1970) that have used a within-subject design to demonstrate the acquisition and extinction of the human salivary response have presented only limited single-subject data. Conduit and Coleman (1998) presented data for three baseline trials, one test trial following acquisition training, and one test trial following extinction. Holland and Matthews (1970) reported one data point for the baseline, acquisition, and the test trial condition for one subject. Because of the limited data reported in both of these studies, intrasubject variability could not be assessed. In addition, responding over successive trials for the baseline or extinction condition could not be evaluated; therefore, any changes in responding cannot be compared to an adequate control condition. A review of studies evaluating the control conditions used in human salivary studies published from 1969-1998 is contained in Appendix A.

Prior to the present study, the author conducted research with 12 pilot subjects using a within-subject-reversal design. The purpose of that study and the present study was to demonstrate Pavlovian conditioning of the human salivary response

(weight of saliva in grams) and to assess changes in the conditioned response (CR) after each trial. For the first 3 pilot subjects, the delay conditioning procedure consisted of a minimum of 8 Baseline Trials (CS-Alone); 24 Acquisition Trials (CS offset immediately followed by US), and 4 Extinction Trials (CS-Alone). One of eight Test Trials (CS-Alone) were presented randomly within each consecutive block of three Acquisition Trials. The conditioned stimulus (CS) was a 15-s tone and the unconditioned stimulus (US) was 0.20 ml of lemon juice placed under the tongue. The intertrial interval (ITI) was 75 seconds. A screen blocked the subject from viewing the experimenter and apparatus located on the other side of the screen. Five seconds prior to the presentation of any trial, subjects were told to swallow once and then not to swallow again. Subjects were told that when they hear the tone, they should open their mouths with their tongues lifted and move their mouths to a round opening in the screen until they are given another instruction. For CS-Alone Trials subjects were told to spit into a cup three times to empty their mouths immediately after the CS offset. For Acquisition Trials subjects received lemon juice under their tongues immediately after the CS offset. Thirty seconds later, subjects were told to spit into a cup three times to empty their mouths. The cup with the saliva was weighed. In either condition, subjects were then handed 20 ml of water to rinse their mouths and then they were told to relax and swallow normally during the following ITI. The use of this procedure did not result in the elicitation of conditioned responding by the tone for the three subjects. See

Appendix B for a more complete description of the method and results of this study.

This delay conditioning procedure was modified for the next nine pilot subjects, based on research by Wooley and Wooley (1973) and Wooley, Wooley and Dunham (1976) indicating that the expectation to eat increased salivation in human subjects. For the first three pilot subjects, the following events prior to participation in the experiment may have increased expectancy of tasting the US: Subjects (a) read the criteria to participate on the human subject participation bulletin board that specifically informed them that they could not have allergies to lemons, limes or oranges; (b) read the informed consent form and in signing the form indicated that they agreed to participate and did not have allergies to lemons, limes or oranges; and (c) heard from the experimenter that a taste stimulus would be placed under the tongue. If salivation increased due to expectancy effects, when subjects were presented with CS-Alone trials, results would be similar whether the trials were presented during the Baseline, Acquisition, or Extinction phase. Consequently, if conditioning had occurred, it would appear as if it did not occur during or following Acquisition trials. Therefore, to reduce anticipatory salivation due to expectancy during the Baseline and Extinction Phases, the nine subjects in the next pilot group were informed prior to each phase whether or not to expect a lemon stimulus under the tongue. Six subjects participated in this experiment in the laboratory, and three subjects participated at home. Results indicated that the tone elicited conditioned salivation for only three of the nine

subjects. The weight of the salivary response for these three subjects was significantly higher on CS-Alone Test Trials during the Acquisition Phase than during CS-Alone Trials during the Baseline Phase and was significantly lower on CS-Alone Trials during the Extinction Phase than on CS-Alone Test Trials during the Acquisition Phase. Three of the nine subjects displayed significantly higher salivary weights on CS-Alone Test Trials during the Acquisition Phase than on CS Alone Trials during the Baseline Phase; however, salivary responding did not return to baseline levels during the Extinction Phase. See Appendix C for a more complete description of the method and results of this study.

One possible basis for the failure to demonstrate Pavlovian conditioning in 6 the nine pilot subjects may relate to the aversive qualities of the lemon juice and instructions given to the subjects to try to minimize mouth movements during the experiment. Both may have encouraged subjects to limit the area of the mouth that the lemon contacted. Perhaps conditioned responding would have been more likely to occur if the unconditioned stimulus were more palatable and made contact with the entire mouth on Acquisition Trials.

Research has shown that the acidity of a solution affects the amount of salivation produced by a person. Feather, Delse and Bryson (1967) reported that a less acidic solution may produce a greater salivary response than a more acidic solution. Their results showed that 3 cc of an acetic acid solution with a 3.1 pH level placed on the tongue produced greater salivation to the CS than a solution with a 3.0 pH level (a more acidic pH level). Thus, the stimulus used as the US

for the pilot subjects, 0.20 ml of lemon juice with a pH level of 2.6, was replaced with 3 ml of lemonade with a pH level of 3.1. Also, this stimulus was applied on the tongue and then made contact with the entire mouth, rather than the limited area under the tongue.

In addition to the acidity of the US, it is possible that the type of CS used was not conducive to conditioning. It has been reported that stimuli that affect the same sensory modality are associated more quickly than those that affect different sensory modalities (Garcia & Koelling, 1966; Testa, 1975). Accordingly, a gustatory CS consisting of 0.1 ml of 0.04% (vol/vol) solution of vanilla extract in water was presented immediately following the onset of the tone CS that was presented in the pilot experiments.

In another departure from the pilot study, the number of extinction trials was increased from between 4 to 8 trials to 16 trials to increase the opportunity for the response to extinguish.

Finally, the instructions informing subjects of whether or not to expect the lemon stimulus were eliminated. The results from the second pilot study indicated that under those conditions, conditioned responding occurred for only 3 of the 9 subjects.

In summary, five changes from the pilot study were made for the present experiment. These changes were as follows: (1) the US consisted of 3 ml of Lemonade placed on the tongue rather than 0.20 ml lemon juice placed under the tongue; (2) the subjects were instructed to move the stimulus over the entire

mouth rather than to limit contact to under the tongue; (3) the CS consisted of a 15-s tone and a 0.04% vanilla solution placed on the tongue and moved over the entire mouth rather than only a 15-s tone; (4) the number of extinction trials was increased to 16 trials rather than 4 to 8 trials; (5) the instructions stating whether or not to expect a lemon stimulus under the tongue were eliminated.

It was anticipated that with these changes, the acquisition of a conditioned response would be demonstrated. Because individual subject data were presented, the levels and trends of salivary responding were evaluated over successive trials.

Method

Subjects

One female and four male subjects, between the ages of 29 and 45, participated. Each subject who participated was a staff member recruited by word-of-mouth at Family Residences and Essential Enterprises, Inc. (FREE). Each subject met the following criteria: He or she (a) reported that he/she did not have a hearing impairment; (b) had been of stable weight (± 10 lbs) over the past 2 months; (c) had not received or was not currently receiving treatment for an eating disorder during the past year; (d) was not taking any medications; (e) did not have a disorder or disease that could affect salivation, and (f) was not allergic to lemonade or vanilla extract. Each subject was asked to refrain from eating and drinking for the 2-hour period before the 2 ½-hour session. The Queens College Institutional Review Board of the City University of New York gave approval for the procedures used in this study.

Settings

Each subject sat 20 cm from a white folding cardboard screen that measured 101.2 cm in width and 81.8 cm in height to block his/her view of the experimenter and apparatus located on the other side of the screen. A round opening (5.5 cm in diameter) in the screen was located 38.2 cm from the top and 13 cm from the left side of the screen, approximately at the level of the subject's mouth. A rectangular opening (9 cm in width and 11.6 cm in height) was located at the bottom and 26.3 cm from the left side of the screen for the subject to pass a cup through the opening. For two subjects, the experiment was conducted in their homes, and for three subjects, the experiment was conducted at Family Residences and Essential Enterprises, Inc. These settings were selected because the subjects reported that they would feel more comfortable participating in familiar surroundings rather than participating in a laboratory setting on the college campus.

Subject S1. The session was conducted from 9-11:30 AM in the subject's office. The office measured 4.87 m in width, and 6.45 m in length, and 2.44 m in height. The screen was placed on the subject's desk and the subject sat behind the screen in his usual chair. The subject answered one telephone call and spoke for 2 minutes prior to the 27th trial. No other interruptions occurred and the environment was quiet during the session except for occasional comments by the subject.

Subject S2. The session was conducted from 5-7:30 PM in a different office

from his own office. The office measured 2.26 m in width, 3.35 m in length, and 2.44 m in height. The screen was placed on a desk and the subject sat in a chair behind the screen. The subject answered one telephone call and spoke for 1 minute prior to the 16th trial and made one telephone call and spoke for 5 minutes prior to the 17th trial. No other interruptions occurred, although the environment was noisy as construction began in an adjacent area after the 18th trial.

Subject S3. The session was conducted from 9-11:30 AM in the kitchen of the subject's home. The kitchen measured 3.51 m in width, 5.51 m in length, and 2.44 m in height. The screen was placed on the kitchen table and the subject sat in a chair behind the screen. The screen prevented the subject from viewing any food. No interruptions occurred during the session although soft music was played by a downstairs tenant during the 3rd - 12th trials and 18th - 25th trials.

Subject S4. The session was conducted from 9-11:30 AM in the subject's office. The office measured 2.21 m in width, 2.90 m in length, and 2.44 m in height. The screen was placed on the subject's desk and he sat behind the screen. During the 5th, 9th, 21st, and 33rd trials announcements could be heard over the intercom system. Otherwise, no other interruptions occurred and the environment was quiet during the session except for occasional comments by the subject.

Subject S5. The session was conducted from 8-10:30 PM in the kitchen/dining area of the subject's home. The kitchen/dining area measured 3.05 m in width, 6.71 m in length, and 2.44 m in height. The screen was placed on the kitchen bar and the subject sat in a chair behind the screen. The screen prevented the subject

from viewing any food. During the session, interruptions included the dog barking during the 3rd, 16th, 24th - 26th, and 34th trials, the subject telling the dog to stop barking during the following intertrial interval, and the housemate entering the kitchen area during the 18th- 19th trials and the 52nd- 53rd trials. The subject also made occasional comments during the session.

Stimuli, Materials, and Apparatus

The compound CS consisted of a 15-s auditory stimulus, produced by an electronic piano (Yamaha Model # PSS-14) with a 64 dB re SPL (C weighted) (Bruel & Kjaer Sound Level Meter, Type 2230) that was heard from a distance of approximately 26 cm, and 0.1ml of a 0.04% (vol/vol) vanilla solution (Poland Spring Water and McCormick Pure Vanilla Extract). The US was 3 ml of lemonade (Minute Maid Lemonade). The pH of the lemonade was 3.1, and the pH of the vanilla solution was 4.8 as measured by a pH meter (Hanna Instrument pHel Electrode). All taste stimuli were placed in the subject's mouth by a syringe dropper (Apothecary Products EZY Dose Oral Syringe). Saliva was collected in paper cups (Dixie, James River Corp). Paper cups were weighed (to the nearest 0.001 g) using an electronic balance (Ohaus Model TS200S Electronic Balance). Water (Poland Spring) was used for the first 100-ml drink given to subjects and for the 20-ml rinse during ITIs. All time intervals were measured by a manual stopwatch (Radio Shack LCD Multi-Function Stopwatch, Cat. No. 63-5013).

Measurement of Saliva

Prior to the beginning of each trial, an empty paper cup was weighed.

Immediately after the subject spit into the cup, the paper cup was weighed again. The weight of saliva equaled the difference between the two values minus the weight of the liquid stimulus presented for that trial.

Experimental Design

An ABA reversal design was used to evaluate the magnitude of the CR during test trials. The magnitude of the CR was determined by weight to the nearest 0.001 g.

Procedure

Fifteen minutes prior to testing, the subject read and signed a consent form. The subject entered the testing area and sat approximately 20 cm behind a screen to block the subject's view from the experimenter and apparatus. The subject was then given 100 ml of water to drink while the protocol was read (see Appendix D for the protocol).

Experimental Conditions

Baseline. Each subject was presented with a minimum of eight trials of the compound CS (tone and solution). The subject was instructed that upon the onset of the tone, to open his/ her mouth and move it to the round opening in the screen. Once the mouth appeared at the round opening, 0.1 ml of the vanilla solution was placed in the mouth with a syringe. The subject was instructed to distribute the solution over the entire mouth during the 15-s interval. The subject was instructed that upon the offset of the tone, to open his/ her mouth and move it to the round opening in the screen. A syringe was placed near the subject's mouth

(as if a solution were going to be placed in mouth). The subject was then asked to spit three times into the cup provided. The subject was handed 20 ml of water through the rectangular opening to rinse the mouth. A 75-s fixed time ITI followed the completion of rinsing. The subject was instructed to relax and swallow normally during the ITI. The purpose of this condition was to assess the baseline level of responding to the CS. The presence of the syringe on each trial functioned as a control procedure for the presence of the syringe during the CS-US trials. After a minimum of eight trials during the Baseline condition, the criteria for a phase change were that the last four data points obtained during the Baseline condition did not show a systematic increase as determined by visual inspection of the plotted data (Kazdin, 1982), and that each of the last two data points was lower than the prior data point.

Acquisition. Each subject was presented with 24 acquisition trials in which the compound CS was presented. The subject was instructed that upon the onset of the tone, to open his/her mouth and move it to the round opening in the screen. Once the mouth appeared at the round opening, 0.1 ml of the vanilla solution was placed in the mouth with a syringe. The subject was instructed to move the solution over the entire mouth. The subject was instructed that upon the offset of the tone, to open his/her mouth and move it to the round opening in the screen. Once the mouth appeared at the round opening, 3 ml of lemonade was placed in the subject's mouth with a syringe. The subject was instructed to distribute the solution over the entire mouth. After a 30-s period elapsed, the subject was asked

to spit into a paper cup three times, rinse the oral cavity with 20 ml spring water. The subject was then presented with a 75-s fixed time ITI. The subject was instructed to relax and swallow normally during the ITI. The beginning of the ITI was delayed prior to Trial 27 for Subject S1 and prior to Trials 16 and 17 for Subject S2 until the telephone conversation was over and the subjects were sitting quietly for 15 seconds. On test trials, the compound CS was presented alone. One test trial occurred randomly within a block of 3 CS-US trials and was presented after the 3rd, 4th, 9th, 11th, 14th, 18th, 19th, and 23rd acquisition trial to assess changes in CR magnitude over successive CS-US trials. The procedure for the test trials was identical to the procedure used in Baseline.

Extinction. Each subject was presented with 16 trials of the compound CS alone. The procedure for the presentation of the compound CS was identical to the procedure used in Baseline. The purpose of this phase was to assess CR magnitude during extinction trials.

Results

Each subject showed a systematic increase in salivary responding on CS-alone trials after three or more CS-US pairings during the Acquisition Phase when compared with salivary responding during the Baseline Phase. Additionally, each subject showed a systematic decrease in salivary responding to CS-alone trials after the removal of the US during the Extinction Phase. Salivary responses for all trials are listed to the nearest 0.001 g in Appendix E. Figures 1 through 5 show the weight of salivation in grams for each trial during the Baseline, Acquisition,

Figure Caption

Figure 1. Weight of salivation in grams for S1 during baseline, acquisition, and extinction conditions for consecutive trials.

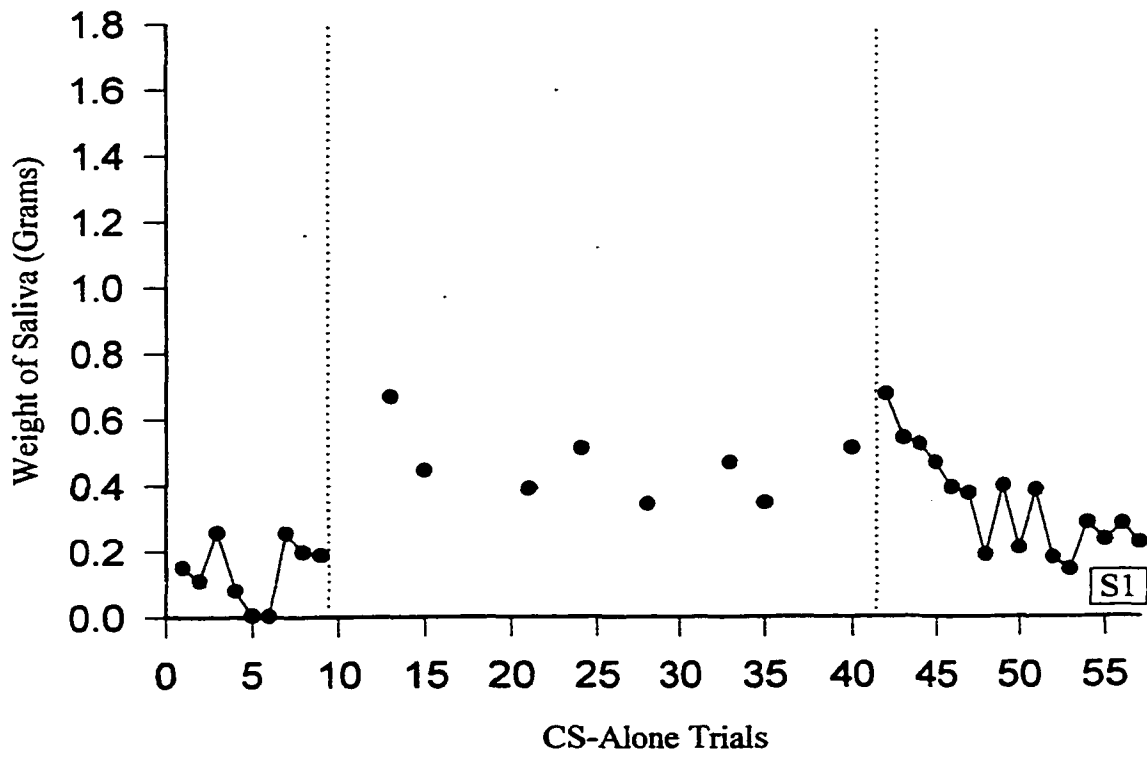
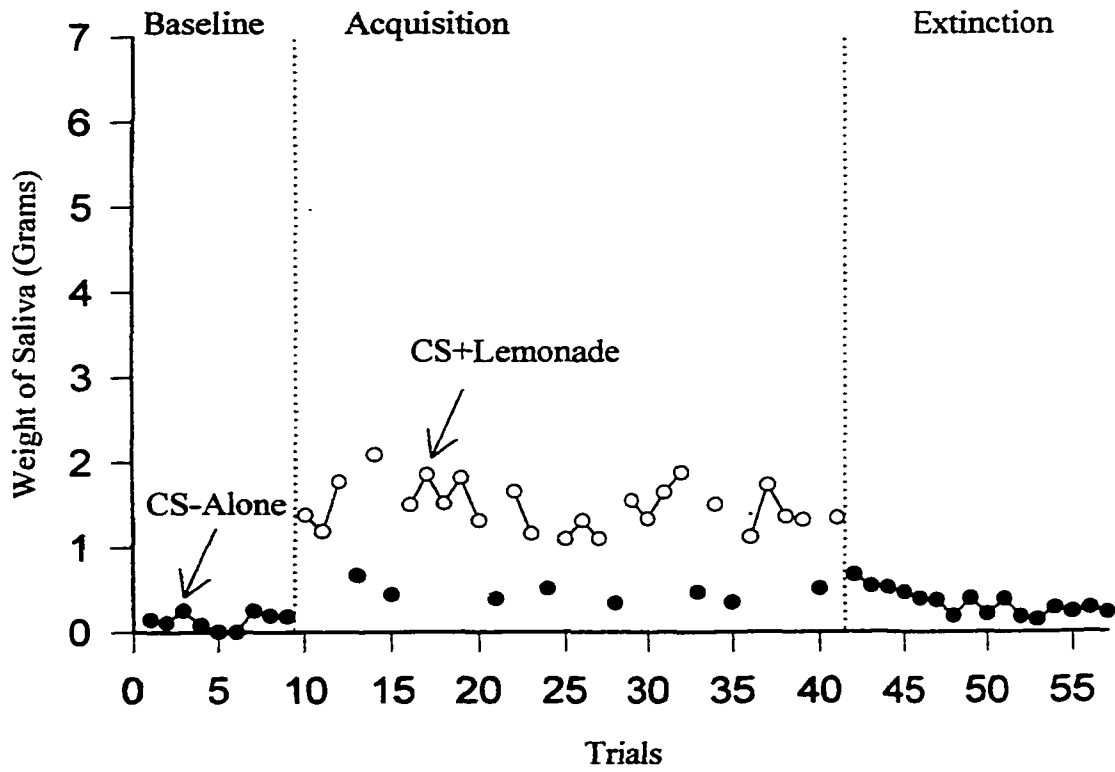
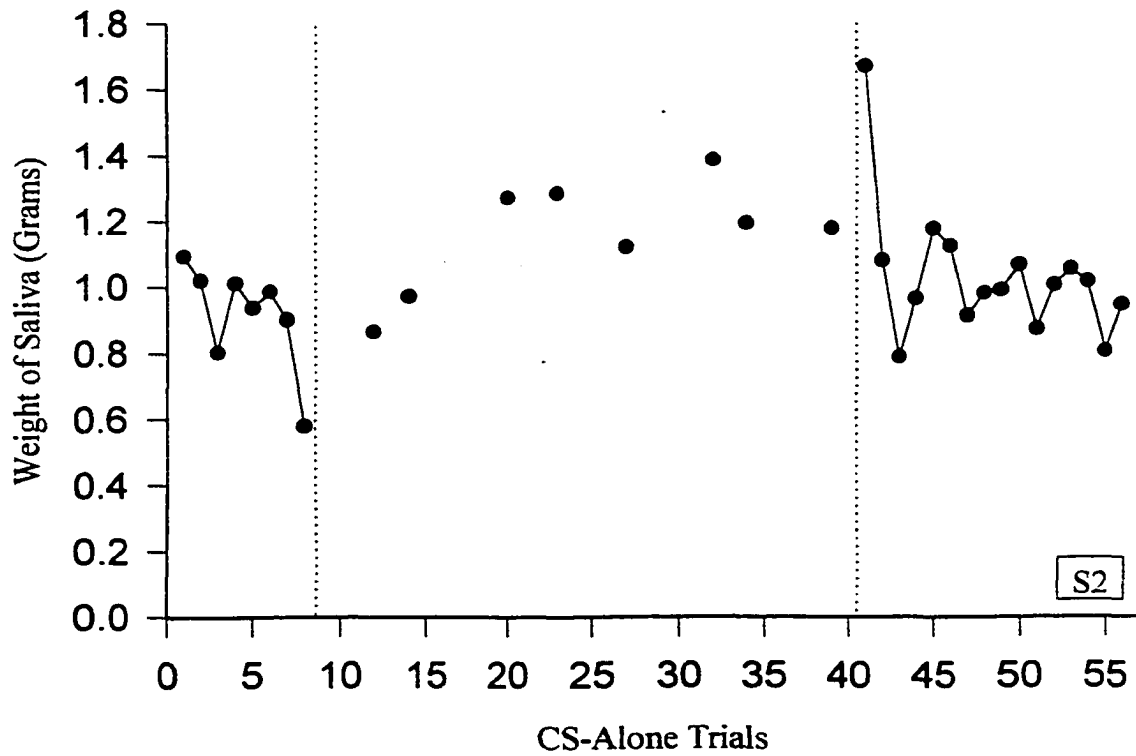
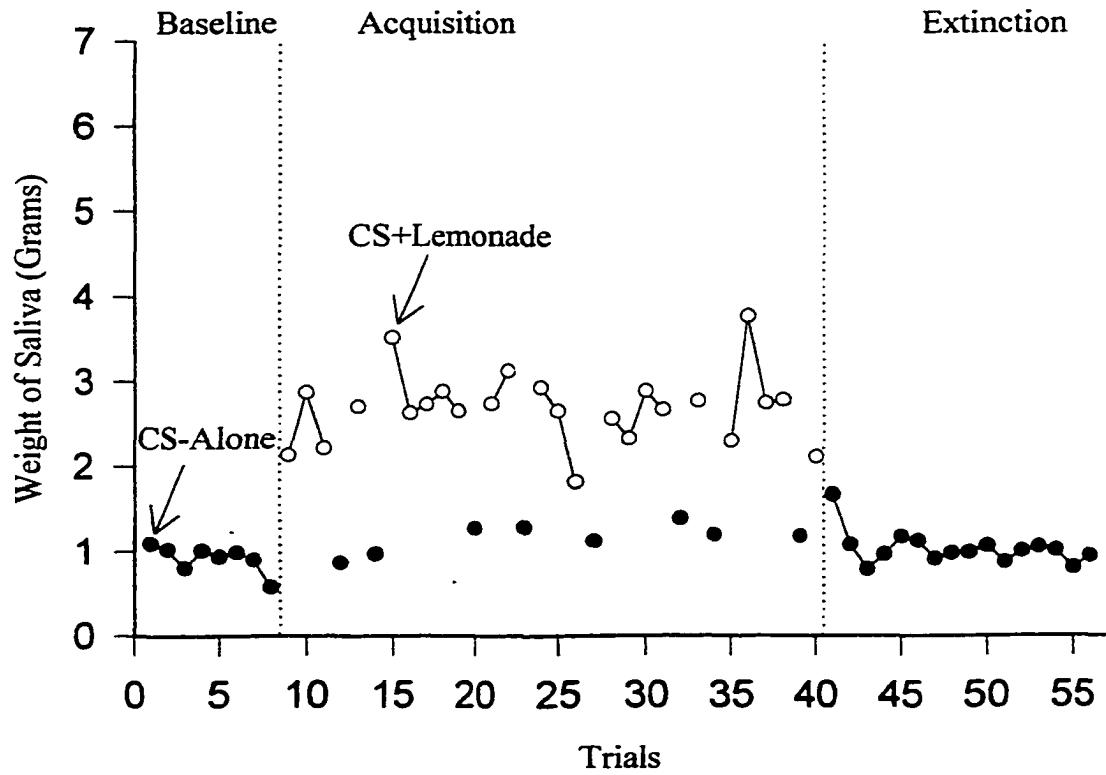


Figure Caption

Figure 2. Weight of salivation in grams for S2 during baseline, acquisition, and extinction conditions for consecutive trials.



S2

Figure Caption

Figure 3. Weight of salivation in grams for S3 during baseline, acquisition, and extinction conditions for consecutive trials.

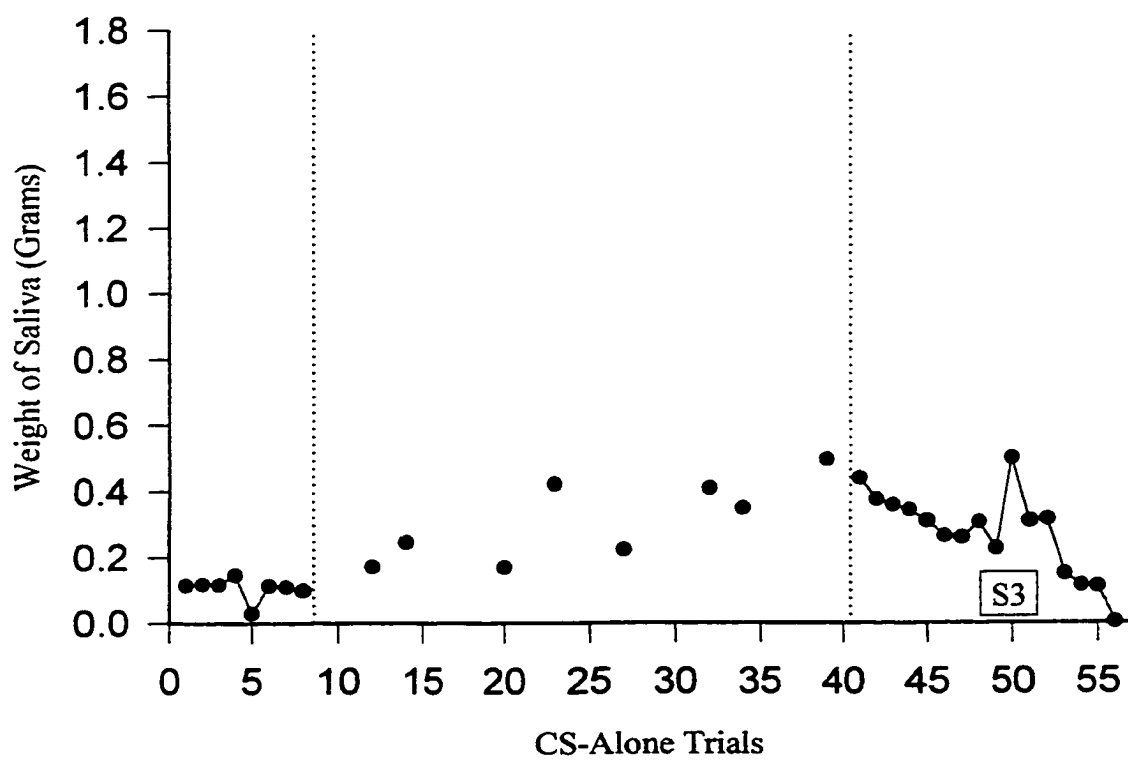
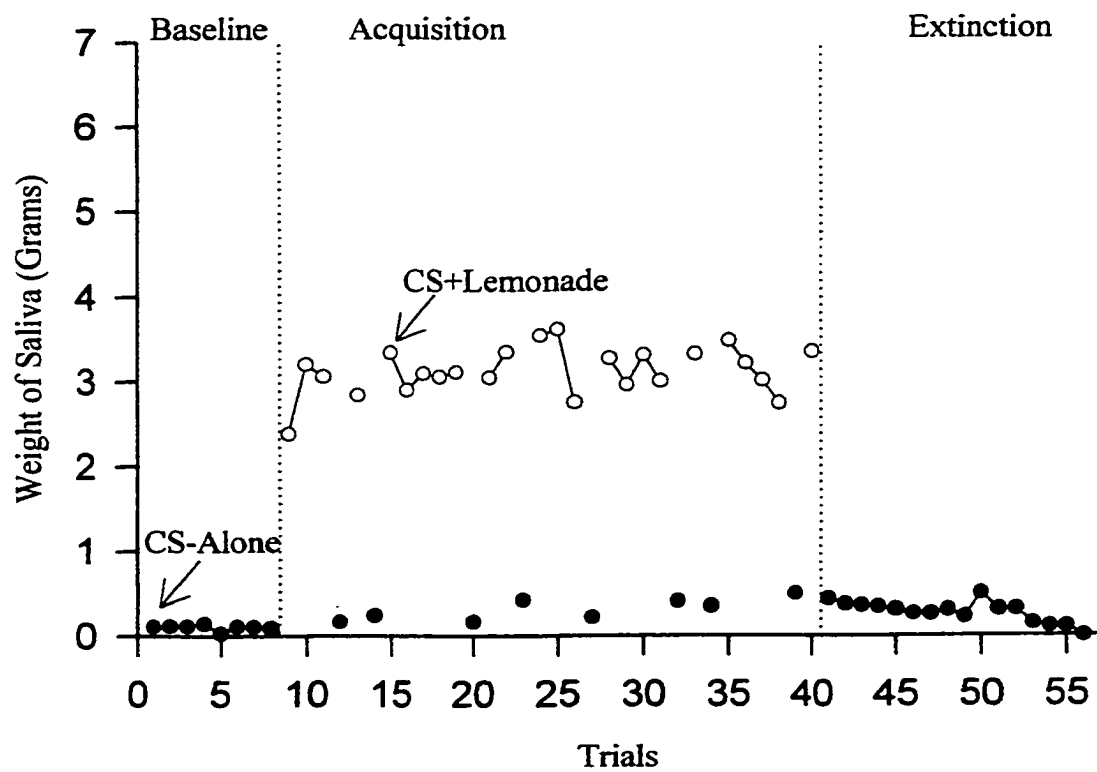


Figure Caption

Figure 4. Weight of salivation in grams for S4 during baseline, acquisition, and extinction conditions for consecutive trials.

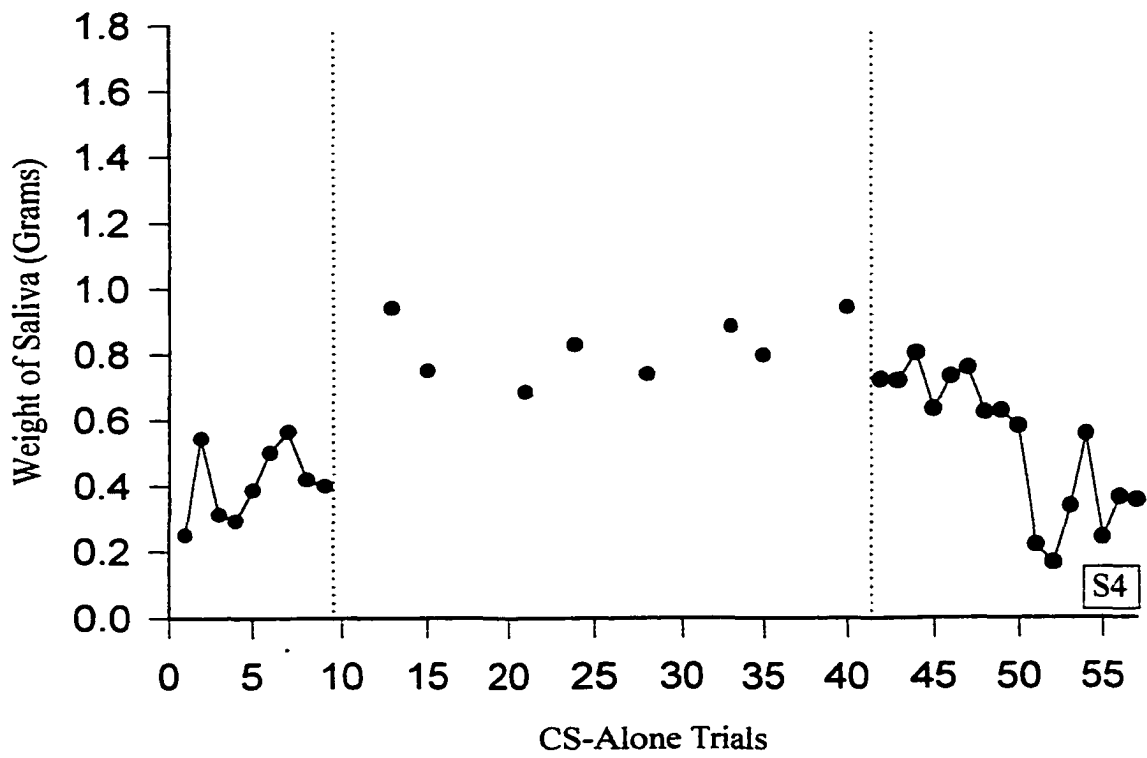
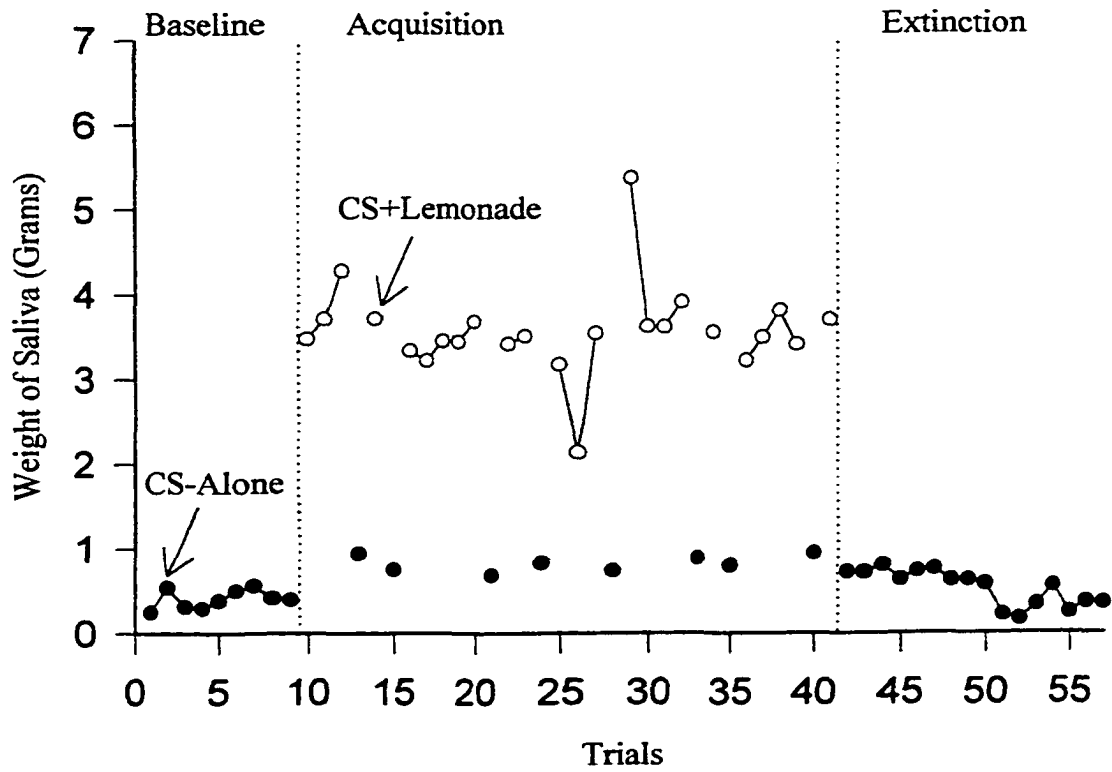
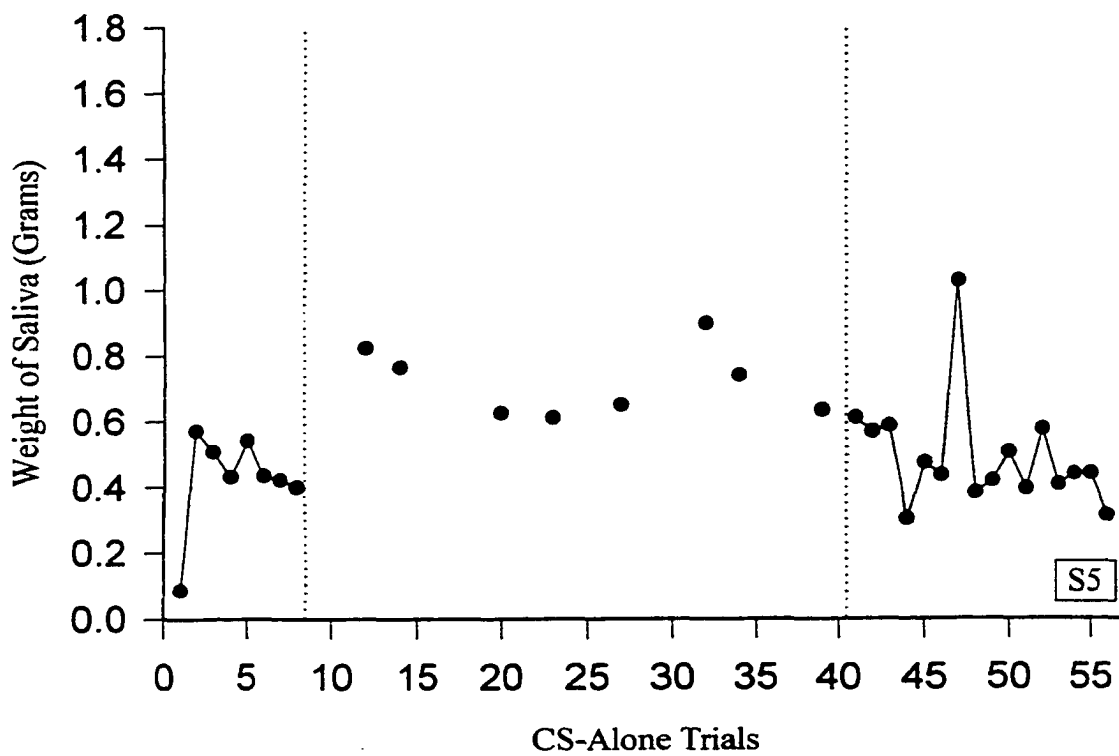
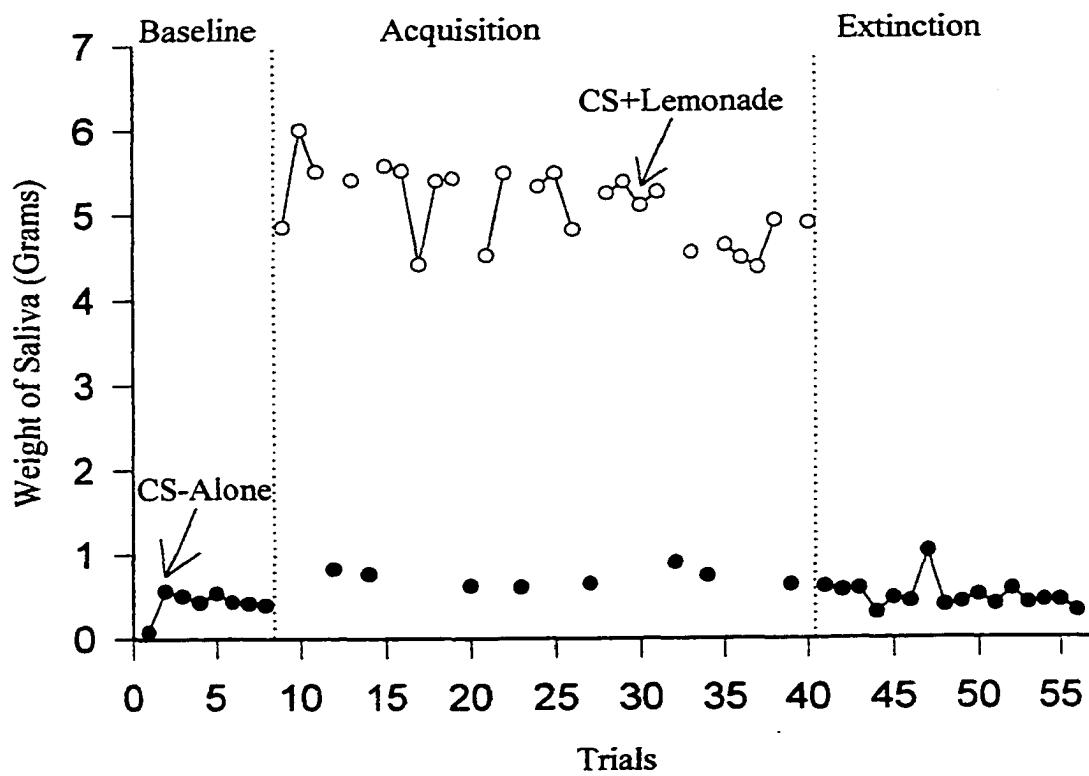


Figure Caption

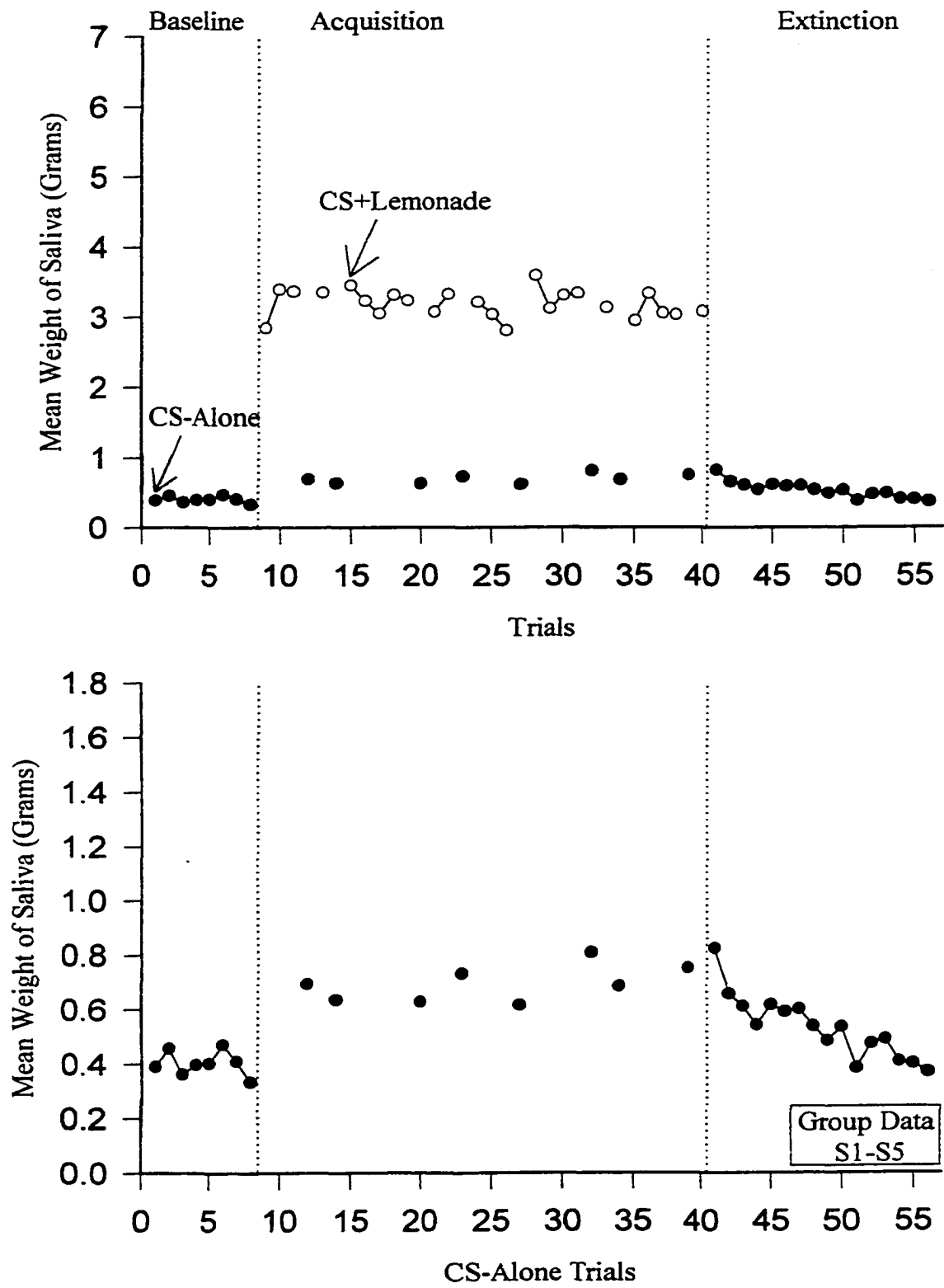
Figure 5. Weight of salivation in grams for S5 during baseline, acquisition, and extinction conditions for consecutive trials.



S5

Figure Caption

Figure 6. Weight of salivation in grams for group data (S1-S5) during baseline, acquisition, and extinction conditions for consecutive trials.



and Extinction Phases for each subject, and Figure 6 shows group mean data. Owing to differences in the number of baseline trials presented to each subject, the baseline data in Figure 6 consist of only the last eight trials for each subject. The top graph in each figure displays performance for CS-Alone trials during Baseline, Acquisition and Extinction Phases, and paired CS + Lemonade trials during the Acquisition Phase. Performance on CS-Alone trials during each phase is re-plotted in the bottom graph with a re-scaled ordinate axis so that changes in responding could be more readily observed.

The top graph in each figure shows that for all subjects, responding to the CS - US trials exhibited a near zero trend across trials. Subjects S1 and S3 showed a fairly stable level of responding and Subjects S2, S4, and S5 show a more variable level of responding to the CS - US trials.

Figures 1, 4 and 5 show that for Subjects S1, S4 and S5, the level of conditioned salivary responding on test trials showed an abrupt increase after 3 CS-US pairings during the Acquisition Phase when compared to salivary responding during the Baseline Phase. For these three subjects, when there was a return to baseline conditions, the level of conditioned salivary responding on CS-alone trials showed a gradual decrease and returned to baseline levels over the 16 extinction trials.

Figures 2 and 3 show that for Subjects S2 and S3, the level of conditioned salivary responding on test trials showed a gradual increase over the 24 CS-US pairings during the Acquisition Phase when compared to salivary responding

during the Baseline Phase. For Subject S2, when there was a return to baseline conditions, the level of conditioned salivary responding on CS-alone trials showed an abrupt decrease after the second extinction trial. In contrast, for Subject S3, the level of conditioned salivary responding gradually decreased after the removal of the US and returned to baseline levels over the 16 extinction trials.

In summary, for each subject, the introduction of the CS-US pairing occasioned increases in slope and/or level of conditioned responding in comparison with baseline. Return to baseline conditions was followed by a decrease in one or both of these measures.

The Mann-Wald time series analysis (Jones, Vaught, & Weinrott, 1977) was used to determine whether there were statistically significant differences in the mean level and slope of the functions of two consecutive conditions (i.e., baseline and acquisition or acquisition and extinction) for each subject. An alpha level of .05 was used for all tests. The results of these tests are presented in Tables 1 and 2. As shown in Table 1, there were significant increases in the mean level between baseline and acquisition CS-alone trials for all subjects whether the change in level occurred immediately, as for Subjects S1, S4, and S5 (Figures 1, 4, and 5) or gradually, as for Subjects S2 and S3 (Figures 2 and 3). As shown in Table 2, there were significant increases in the slope between baseline and acquisition CS-alone trials for Subjects S2 and S3 (Figures 2 and 3), and no significant change in slope for the remaining three subjects. When acquisition and extinction CS-alone trials were compared, there were significant decreases in

Table 1

The Mean Level of Salivary Responding Measured in Grams on CS-Alone Trials.

Subjects	Baseline	Acquisition	Extinction
1	.14	.46*	.35
2	.92	1.20*	1.02
3	.11	.31*	.27
4	.41	.82*	.52*
5	.43	.72*	.49*

*Indicates a statistically significant difference between two consecutive conditions based on the Mann Wald Time Series Analysis.

Table 2

The Slope Measure in each Condition

Subjects	Baseline	Acquisition	Extinction
1	.01	-.02	-.03
2	-.04	.04*	-.02*
3	.00	.04*	-.02*
4	.02	.01	-.04*
5	.02	-.007	-.012

*Indicates a statistically significant difference between two consecutive conditions based on the Mann Wald Time Series Analysis.

the mean level (see Table 1) for two of the five subjects, S4, and S5 (Figures 4 and 5) with the change in level occurring gradually for these subjects. There were also decreases in the mean level for the remaining three subjects although the differences were not significant. The change in level between the acquisition and extinction CS-alone trials occurred abruptly for Subject S2 (Figure 2) and gradually for Subjects S1 and S3 (Figures 1 and 3). Significant decreases in the slope (see Table 2) between acquisition and extinction CS-alone trials were found for three of the five Subjects, S2, S3 and S4 (Figures 2, 3, and 4). For two of the five subjects, Subjects S1 and S5 (Figures 1 and 5) there were increases in the negative slope between acquisition and extinction CS-alone trials, although these increases were not significant. When considering both mean level and slope measures, the acquisition and extinction of conditioned responding was demonstrated in four of the five subjects. Two of the five subjects, S4 and S5, showed significant changes in mean level and two of the five subjects, S2 and S3, showed significant changes in slope when comparisons were made between two consecutive conditions (i.e., baseline and acquisition conditions and acquisition and extinction conditions).

Figure 6 shows the group data for all subjects. The level of conditioned salivary responding on test trials showed an abrupt increase after 3 CS-US pairings during the Acquisition Phase when compared to salivary responding during the Baseline Phase. Figure 6 also shows that level of performance decreased gradually after the removal of the US and returned to baseline levels

after 16 extinction trials. A paired comparison t-test was used to determine whether there were statistically significant differences in the mean level and slope for the functions between two consecutive conditions (i.e., baseline and acquisition or acquisition and extinction) for the group data. An alpha level of .05 was used for this test. The mean salivation levels for the baseline, acquisition, and extinction conditions were: 0.40, 0.70, and 0.53, respectively. The increase in mean level between baseline and acquisition CS-alone trials was significant $t(4) = 8.85$, $p = .001$, as was the decrease in mean level between acquisition and extinction CS-alone trials $t(4) = 3.80$, $p = .02$. The difference in slope between baseline and acquisition CS-alone trials was not significant $t(4) = .50$, $p = .65$, although the decrease in slope between the acquisition and extinction CS-alone trials was significant $t(4) = 3.03$, $p = .04$.

When comparing performance between Baseline and Extinction Conditions, performance to the CS for the first two extinction trials was higher than performance to the CS for the baseline trials. Also, performance to the CS for the last two extinction trials was similar to performance to the CS for the baseline trials. A paired comparison t-test was used to determine whether there were statistically significant differences between the mean level of salivation from CS-alone trials during baseline and the first and last two CS-alone trials during extinction. There was a significant increase in the mean level between baseline and the first two extinction CS-alone trials $t(4) = 5.94$, $p = .004$, but not a significant difference in the mean level between baseline and the last two

extinction CS-alone trials $t(4) = -5.64, p = .11$.

The Pearson product-moment correlation and the Spearman's rho correlation analysis was used to assess whether there was a relationship between the initial level of salivation to the CS-alone and the level of salivation to the CS-alone following one or more acquisition trials. The Pearson correlation analysis showed a significant correlation ($r = .98, p < .01$) between the mean level of salivation for baseline and acquisition CS-alone trials. However, this analysis did not show a significant correlation between the mean level for baseline CS-alone trials and the change in mean level for CS-alone trials following acquisition trials ($r = .14$) or a significant correlation between the mean level for baseline CS-alone trials and the first CS-alone test trial during the acquisition condition ($r = .61$). The Spearman correlation analysis showed comparable values that led to the same conclusions.

Table 3 displays the mean, standard deviation and range of salivary responding to CS-Alone trials for each subject for each phase. As shown in Table 3, intra-subject responding appeared fairly stable within phases although the level of responding varied substantially between subjects. Table 4 displays the mean, standard deviation, range, and slope for the CS-US trials for each subject during the Acquisition Phase. As shown in Table 4, intra-subject responding appeared fairly stable for Subjects S1 and S3 (Figures 1 and 3) and slightly more variable for Subjects S2, S4, and S5 (Figures 2, 4, and 5), although all functions showed near zero trends. Tables 3 and 4 also shows that the level of responding varied substantially between subjects.

Table 3

Mean (M), Standard Deviation (SD), and Range of Salivary Responding
Measured in Grams on CS-Alone Trials for Each Subject Across Experimental
Conditions.

Subjects		Baseline	Acquisition	Extinction
S1	<u>M</u>	.14	.46	.35
	<u>SD</u>	.10	.11	.15
	<u>Range</u>	.00-.26	.35-.68	.14-.54
S2	<u>M</u>	.92	1.20	1.02
	<u>SD</u>	.16	.16	.20
	<u>Range</u>	.58-1.10	.87-1.67	.79-1.18
S3	<u>M</u>	.11	.31	.27
	<u>SD</u>	.03	.12	.13
	<u>Range</u>	.03-.15	.17-.50	.00-.50
S4	<u>M</u>	.41	.82	.52
	<u>SD</u>	.11	.10	.21
	<u>Range</u>	.25-.57	.69-.95	.17-.81
S5	<u>M</u>	.43	.72	.49
	<u>SD</u>	.15	.11	.17
	<u>Range</u>	.09-.57	.61-.90	.30-1.03

Table 4

Mean (M), Standard Deviation (SD), Range, and Slope of Salivary Responding Measured in Grams on CS+Lemonade Trials for Each Subject During the Acquisition Condition.

Subjects	<u>M</u>	<u>SD</u>	Range	Slope
S1	1.48	.28	1.09-2.09	-.01
S2	2.69	.43	1.82-3.77	.00
S3	3.13	.28	2.38-3.61	.01
S4	3.58	.54	2.14-5.37	.00
S5	5.11	.45	4.37-6.00	.00

Discussion

The present results showed the acquisition of a conditioned response for all five subjects and the extinction of a conditioned salivary response for four of the five subjects regardless of the amount of initial salivation produced during baseline. For three of the five subjects, there was a systematic increase in conditioned responding after the first 3 CS-US pairings, whereas for the remaining subjects, there was a systematic increase in conditioned responding over the 24 CS-US pairings. For four of the five subjects, conditioned responding extinguished gradually over the 16 extinction trials whereas for one of the five subjects, conditioned responding extinguished more rapidly after the US was removed. The results of the group data also showed the acquisition and extinction of the conditioned salivary response.

The results of the present study extend the research in the area of human salivary conditioning because they demonstrate the acquisition and extinction of human salivary responding by presenting individual data over successive trials. Variability found with this response, such as the pattern in acquisition as reflected by the slope, emphasizes the value of using single-subject methodology and presenting individual subject data. In addition, group data may obscure the actual results for individual subjects. For example, in the present study, the group data showed both the acquisition and extinction of the conditioned response. However, for one subject, (S1), although there was a significant increase in mean level for CS-alone trials during the acquisition phase when compared to the

baseline phase, there was not a significant decrease in responding on test trials during the acquisition phase when compared to responding during the extinction phase. In addition, there were not significant changes in slope between two consecutive phases (Tables 1 and 2).

Previous studies have reported data averaged across subjects when using a between-subject design (Brown & Katz, 1967; Feather, 1967) or a within-subject design (Jansen et al., 1992). Reporting data that are averaged across subjects may obscure the variability in the data and may not accurately reflect individual subject data. Thus, this may account for some inconsistent results found in studies of human salivary conditioning. For example, inconsistent results have been found regarding the number of acquisition trials required to obtain a CR. Conduit and Coleman (1998) reported that for some subjects, more than 40 CS-US trials were needed to meet the criterion for a conditioned response whereas Feather (1967) reported that conditioned responding occurred after as few as five CS-US pairings. Similar to Feather's results, the results from the present study show that acquisition of the conditioned response can occur after as few as three CS-US pairings. Feather claimed that immediate acquisition occurred only for subjects who were high salivators (levels of salivation during baseline were higher than 0.0015 cc) as compared to low salivators (levels of salivation during baseline were lower than 0.001 cc of saliva) during baseline. In the present study, although there was a correlation between level of salivation in the baseline and acquisition phases, there was no relation between baseline levels of responding and speed of

acquisition measured as weight of salivation. One subject (S1) who initially produced almost no measurable salivation on two trials during the Baseline Phase, displayed a significant increase in mean level of salivation on CS-alone trials during the Acquisition Phase as compared to CS-alone trials during the Baseline Phase. Furthermore, he showed a decrease in mean level of salivation on CS-alone trials during the Extinction Phase as compared to CS-alone trials during the Acquisition Phase, though this decrease was not significant. Because neither Conduit and Coleman (1998) nor Feather (1967) reported individual data for each trial, the differences in the development of the CR for each subject could not be evaluated.

Further, researchers have reported varied results regarding the extinction of the conditioned salivary response. Razran (1935) reported that the conditioned salivary response was difficult to extinguish whereas Feather (1967) and Butvick (1996) reported that the conditioned salivary response extinguished after as few as 5 CS-alone trials and 10 CS-alone trials, respectively. The results of the present study showed that for one subject, the conditioned salivary response showed substantial extinction after the first extinction trial; however, for four of the five subjects, the response gradually extinguished over 16 extinction trials.

In contrast to the current results with human subjects, the acquisition and extinction of the conditioned salivary response in dogs was more gradual, requiring a greater number trials and daily sessions. For example, Wagner, Siegel, Thomas, & Ellison (1964) presented 200 acquisition trials (10 trials presented for

20 sessions, 1 session per day) and 60 extinction trials (10 trials presented for 6 sessions, 1 session per day). Similarly, Fitzgerald (1963) presented a greater number of trials than the present study, 240 acquisition and extinction trials (24 trials presented for 10 sessions, 1 session per day). The data were presented in group averages, therefore, the pattern of conditioned responding during acquisition and extinction conditions could not be assessed for individual subjects.

The results of the current study support the use of a single-subject research design that presents individual data over successive trials rather than solely presenting group data. See Appendix A for a complete review describing inconsistent findings in human salivary studies that primarily used group designs published from 1969-1998.

Procedural Changes Made in the Current Study

The present data contrast with the difficulties in demonstrating acquisition and extinction of conditioned salivation using the procedures described in the pilot experiments. In the current experiment, several changes were made to the procedures. The first two changes involved both the type and amount of US presented. In the present experiment, 3 ml of lemonade was placed in the subject's mouth with the instruction to move the solution over the entire mouth whereas in the pilot experiments, 0.2 ml lemon juice was placed under the subject's tongue with the instruction to hold the solution under the tongue. Thus, the successful results in the present experiment may be partially explained by an

increase in US magnitude. Past researchers (Wagner, Siegel, Thomas, & Ellison, 1964) have demonstrated that an increase in US magnitude, such as increasing the amount of a food US given to dogs, resulted in a greater amount of conditioned salivation. In addition, Ost and Lauer (1965) also showed that the acquisition of conditioned salivation was sensitive to the magnitude of the US. Their results showed that the magnitude of a CR in dogs was greater at a higher acid concentration of the US, although at an extreme acid concentration, the magnitude of the CR decreased. Similarly, Feather et al. (1967) showed that this effect also occurred in human subjects.

The third change in the current procedure was in the type of putative conditioned stimulus. In the current experiment, a gustatory stimulus was combined with an auditory stimulus whereas in the pilot experiments, only the auditory stimulus was used as the neutral CS. The same auditory stimulus was used in both experiments. Garcia and Koelling (1966) have shown that presenting a CS and US that belonged to the same class of stimuli, that is, stimuli that are activated either internally (e.g. taste, nausea) or externally (e.g. light, tone and shock), may be more conducive to producing a CR than when a CS and US each belong to a different class. Using rats as subjects, they evaluated the effects of presenting a gustatory CS (saccharin) or an exteroceptive CS (light and tone) with either lithium chloride (nausea) or shock (pain) on the rate of licking a tasteless solution. The results showed greater reduction of licking behavior to the taste CS than to the audiovisual stimulus after it was paired with the lithium chloride.

Further, the results showed greater reduction of licking behavior to the audiovisual CS than to the taste CS after it was paired with shock. These results suggest that the addition of the taste CS to the current experiment may explain the successful results, although other researchers, such as Wells and Feather (1968), have reported that after conditioning trials, an auditory stimulus presented alone elicited conditioned salivation.

The fourth change in the procedure was the increase in the number of extinction trials. In the current experiment, 16 extinction trials were presented whereas in the pilot experiments, between 4 and 8 extinction trials were presented. This change was beneficial as the conditioned salivary response extinguished gradually; thus, the increased number of trials was needed to show this effect.

The fifth change was the elimination of instructions informing subjects whether or not to expect a lemon stimulus under the tongue. Results from the second pilot study indicated that only 3 of the 9 subjects, 33%, developed a conditioned response under those conditions. In the current experiment, 4 of the 5 subjects, 80%, developed a conditioned response indicating that the procedures used in the current experiment were more favorable for human salivary conditioning.

Suggestions for Future Research

Future research in the area of human conditioning of the salivary response could systematically evaluate each of the parameters that varied between the pilot

research and the current study to determine what variables were responsible for differences in the degree of salivary conditioning.

In addition, future researchers could evaluate other phenomena involving the human salivary response, such as the UR diminution effect. This effect occurs during acquisition trials (CS-US pairings) when the UR diminishes as the CR develops and is restored when the stimulus conditions are modified by omitting the CS (Kimble & Ost, 1961; Kimmel & Pennypacker, 1962; Baxter, 1966). Although this effect has been studied in the galvanic skin response of humans (Baxter, 1966; Kimmel & Pennypacker, 1962) and in the eyeblink response of humans (Kimble & Ost, 1961) and rabbits (Donegan, 1981; Hupka, Kwaterski, & Moore, 1970), this effect has not been studied in the salivary response of humans or animals. In the present study the UR was not measured directly. Instead, both the UR and the CR were measured together following each acquisition (CS-US) trial. The data show that the trend across acquisition trials is fairly stable for all subjects. Thus, for subjects S2 and S3 (Figures 2 and 3) whose data showed a gradual increase in the CR across CS-alone trials, it is presumed that the UR decreased across acquisition trials, suggesting UR diminution. For subjects S1, S4, and S5 (Figures 1, 4, and 5) whose data showed an abrupt increase in the CR after three reinforced trials and a near zero trend on CS-alone trials, it is presumed that the UR did not decrease across acquisition trials but remained stable across trials. More recently, it has been demonstrated that UR diminution does not always occur as the CR develops. Donegan & Wagner (1987) demonstrated that

there may be a facilitation of the UR when US intensity is varied. Two theories that attempt to account for changes in responding to the US are the priming theory (Wagner, 1976) and the sometimes opponent-process theory (SOP) (Wagner, 1981). The priming theory states that a prerepresented stimulus in memory, such as the presentation of the CS during CS-US trials, increases the expectancy of a subsequent stimulus, such as the US. When expectancy increases, this results in a decrement in the activation of US representation, and a consequent reduction in responding to the US. Alternatively, the SOP theory states that CS and US activate different systems within memory and that this activation may result in differential responding to the CS and US. Whether or not the facilitation or diminution of the UR occurs in human salivary conditioning situation could be evaluated with the addition of US-alone trials to the CS-alone and CS-US paired trials presented in the present study.

Other areas that future researchers could study are the parameters that affect the CR, such as number of conditioning trials. For example, Ellison (1964) demonstrated that when dogs were presented with 26 CS-US pairings for eight days, the conditioned salivary response shifted from closely following CS onset to just prior to US onset. This observation is consistent with Pavlov's (1927/1960) theory of inhibition of delay that proposes that under certain conditions the CR is inhibited during the early part of the CS-US interval. Ellison's finding is not unique to conditioned salivation in dogs as it has also been found to occur with conditioned salivation in humans (Feather, 1967). The present study did not

assess for this effect. Further research in this area could assess whether individual subjects differ in their temporal discrimination of the early versus late segments of the CS (Ellison, 1964) by increasing the number of acquisition trials (Fitzgerald, 1963) or increasing the ITI (Feather, 1967) and evaluating the CR independently from the UR during these trials.

In addition, future researchers could consider using the random control procedure (Rescorla, 1967) rather than the CS-alone control procedure used during both the Baseline and Extinction Conditions in the current experiment. In the random control procedure, the same number of CS and US presentations occur as in the conditioning procedure, however, these stimuli are presented randomly so that only the contingent relation between the CS and US is eliminated, though there may still be chance occurrences of a CS-US pairing. All other factors are held constant between the experimental and control procedures. The random control procedure could not be used in the current procedure because the beginning of each trial was signaled with the onset of the tone followed by the subject's opening his\her mouth and moving it to the opening in the screen to receive a stimulus. In a subsequent experiment, if a stimulus were applied to the mouth via tubing, the necessity of a tone signal will be eliminated. This would then allow the US to be delivered independently of the CS. According to Rescorla (1967), the random control procedure is the only adequate control procedure to use in Pavlovian conditioning procedure; however, subsequent research (Balsam & Tomie, 1985) has shown that when the random control procedure is used prior

to acquisition training, there is greater retardation of acquisition to the CS than when the CS-alone control procedure is used prior to acquisition training. In other words, the random control procedure may also present shortcomings (see Appendix A for a review of the control procedures used in human salivary experiments from 1969 - 1998).

The CS-alone control procedure used in the current study may have a less deleterious effect on acquisition than the random control procedure. Moreover, through the use of the single-subject-reversal design (ABA design) used in the current study, adequate control conditions were present. Comparisons of performance can be made over several consecutive conditions, i.e. baseline, treatment, return to baseline, etc. Each condition allows for the prediction of current and future performance as well as for the assessment of the accuracy of past predictions. Repeated reversals within the same subject increase the reliability of the treatment's effect. If, after the replication of reversals, performance varies as a function of the condition presented, there is increased confidence that the independent variable is responsible for the change in performance. Therefore, it is unnecessary to use the random control procedure if a single-subject reversal design with adequate replications is used.

Although Rescorla (1967) would argue that an increase in responding to the CS-alone may be the result of sensitization by the US or pseudoconditioning, the results of the current study are inconsistent with the results suggested by these two phenomena. If sensitization were to occur, there would have been an increase in

performance for the first few CS-US trials and then a subsequent decrease in responding. Additionally, if pseudoconditioning were to occur, this same pattern of responding would have occurred during the CS-alone test trials during the Acquisition Condition. In contrast, the results of the current experiment show that responding to the 24 CS-US trials remained stable and responding to the 8 CS-alone trials either remained stable or increased gradually. Moreover, the gradual decrease in performance to the CS-alone during the second Baseline Condition (Extinction Condition) are inconsistent with both of these phenomena. The current procedure included only two reversals. Future researchers may want to add a second intervention (B) condition to this design. The addition of the condition would assess whether a second presentation of the independent variable had the same affect on performance as during the previous intervention phase and would increase the reliability of the treatment's effect.

Another area that could be assessed is that of CS specificity. Garcia and Koelling (1966) reported that stimuli that affect the same sensory modality are associated more quickly than those that affect different sensory modalities. Through the use of the transfer test, such as an overshadowing procedure, researchers could evaluate the extent to which each separate stimulus of the compound CS elicited a conditioned salivary response. In the present experiment, during the Acquisition Condition, test trials of the tone stimulus could have been presented. However, in the current procedure, the presentation of the gustatory component of the CS would have been met with the same difficulty as the

presentation of the US alone. That is, the beginning of each CS trial was signaled with the onset of the tone. Therefore, the procedure would have to be modified so that a presentation of a gustatory CS could occur independently without a signal, such as through plastic tubing.

Research on the human salivary response is not limited to questions regarding the experimental demonstration of conditioned responding but also includes other types of questions. For example, how does the human salivary response correlate with self-report ratings of hunger or how does the human salivary response vary between those with and without eating disorders? Researchers assessing these types of questions have reported conflicting results, thus making it difficult to draw definitive conclusions. (See Appendix A for a review of the literature from 1969-1998.)

In conclusion, it is apparent that future basic experimental research is needed to understand the parameters that influence human conditioned salivation. Future research in these areas may also want to consider using a single-subject experimental design and presenting individual data to avoid obscuring results in averaged data and to reveal differences and similarities among subjects.

Appendix A

Pavlovian Conditioning of Human Anticipatory
Physiological Responses and Human and Animal Eating Behavior:

A Review and Critique

Barbara A. Shultz

Queens College and The Graduate Center
of the City University of New York

Abstract

A review of studies obtained from PsycINFO (1998) and MEDLINE (1998) computer search from the years 1969-1998 in the area of Pavlovian conditioning of human anticipatory physiological responding or human and animal meal initiation was conducted. One purpose was to determine whether a Pavlovian relation was merely assumed to have occurred or was experimentally demonstrated. When a relation was experimentally demonstrated, the adequacy of the control procedures used was evaluated. A second purpose was to review objective measures of eating behavior. It is concluded that future research should focus on systematic experimentation using appropriate Pavlovian conditioning procedures, including adequate control procedures, and an initially neutral conditioned stimulus. In addition, objective measures, such as amount eaten, rather than subjective measures, such as self-report, can be used to evaluate conditioned responding.

Pavlovian Conditioning of Human Anticipatory
Physiological Responses and Human and Animal Eating Behavior:
A Review and Critique

Pavlov's seminal research in the area of conditioned salivation in dogs (1927/1960) lead researchers to evaluate conditioned salivation in humans (Brown & Katz, 1967; Feather, 1965; Finesinger & Sutherland, 1939; Finesinger, Sutherland & McGuire, 1942; Jones, 1939; Razran, 1935a, 1935b, 1939, 1949; Wells & Feather, 1968). Responses to stimuli previously associated with food stimuli were evaluated between lower organisms and more advanced organisms. As interest increased in the area of anticipatory physiological responses, researchers began to study other types of anticipatory physiological responses using Pavlovian conditioning procedures, such as insulin secretion and gastric acid in rats (Weingarten & Powley, 1981; Woods, Vasselli, Kaestner, Szakmary, Milburn, & Vitiello, 1977) and blood glucose and insulin secretion in humans (Fehm-Wolfsdorf, Gnadler, Kern, Klosterhalfen, & Kerner, 1993; Overduin & Jansen, 1997). More recently, interest in studying the extent to which cephalic responses become classically conditioned to stimuli that precede the ingestion of food, and their subsequent influence in the eating behavior of humans, evolved (e.g., Bellisle, Louis-Sylvestre, Demozay, Blazy, & Le Magenen, 1983; Broberg & Bernstein, 1989). Cephalic responses are defined as autonomic and endocrine responses that include the salivary, insulin, and gastric secretions that occur in the presence of food-related stimuli prior to, rather than during or after, the ingestion

of food (Powley, 1977).

One purpose of this paper is to review literature in the area of food-related anticipatory physiological responses in humans to determine for each study in which a Pavlovian relation was being examined whether appropriate control procedures were used (Rescorla, 1967). This paper will also review studies that addressed other types of questions related to eating behavior while assuming that anticipatory physiological responding in humans was the result of classical conditioning between food-related stimuli and the ingestion of food in the natural environment.

A second purpose of this paper is to review objective measures used to evaluate meal initiation, as these measures may be an alternative to using self-report ratings of hunger or craving to measure conditioned responding. Many studies have investigated whether anticipatory physiological responding is a behavioral correlate to the constructs of hunger or craving by measuring whether there is an increase or decrease in physiological responding and self-report measures in the presence and absence of food-related stimuli (Christensen & Navazesh, 1984; Jansen, Boon, Nauta & Van Den Hout, 1992; Lappalainen, Sjoden, Karhunen, Gladh, & Lesinska, 1994; Nirenberg & Miller, 1982; Wisniewski, Epstein & Caggiula, 1992). These constructs were typically measured through the use of self-report rating scales; however, the results from these scales have been inconsistent. Although self-report scales allow subjects to describe their private experiences, self-report ratings, in comparison to direct

measures of observable behavior, are also under greater control of the subject and are more apt to be influenced by response biases (Kazdin, 1982). Because it has been demonstrated that classically conditioned appetitive stimuli can increase eating behavior in both sated animals (Weingarten, 1983) and children (Birch, McPhee, Sullivan, and Johnson, 1989), it may be worthwhile to consider using objective measures, such as amount eaten, rather than subjective measures, such as self-report, to evaluate conditioned responding. Articles over the past 30 years, from 1969 - 1998, obtained from PsycINFO (1998) and MEDLINE (1998) computer searches in the area of human anticipatory physiological responding to food-related stimuli and human and animal meal initiation to food-related stimuli are included in this review if they met the following criteria: (1) the authors attempted to experimentally demonstrate Pavlovian conditioning of an anticipatory physiological response to a stimulus in humans, (2) the authors referred to an anticipatory physiological response as either an "elicited" or a conditioned response in the introduction or discussion of the article, or (3) the authors suggested that the stimulus presented was predictive of an opportunity to eat food because of the subject's prior history. An exhaustive review of the research falling into these three categories is presented in the following sections.

Studies Where the Pavlovian Relation is Not Manipulated

In a number of studies, subjects were presented with a stimulus assumed to function as a CS for food, owing to the subjects' prior experience. After the CS was presented, one or more anticipatory physiological responses were measured.

These responses included the following: salivation (Bulik, Sullivan, Lawson, & Carter, 1996; Christensen & Navazesh, 1984; Durrant, 1981; Guy-Grand & Goga, 1981; Johnson & Wildman, 1983; Karhunen, Lappalainen, Tammela, Turpeinen, & Uusitupa, 1997; Lappalainen et al., 1994; Nirenberg & Miller, 1982; Sahakian, Lean, Robbins, & James, 1981; Staats & Hamond, 1972; Wardle, 1987; Wooley & Wooley, 1973; Wooley & Wooley, 1981; Wooley, Wooley, & Dunham, 1976), insulin secretion (Bellisle et al., 1983; Broberg & Bernstein, 1989; Johnson & Wildman, 1983; Karhunen et al., 1997; Lucas, Bellisle, & Di Maio, 1987; Simon, Schlienger, Sapin, & Imler, 1986; Sjostrom, Garellick, Krotkiewski, Luyckx, 1980), and glucose secretion (Karhunen et al., 1997; Simon et al., 1986; Sjostrom et al., 1980). All conditional stimuli used to produce a presumed conditioned salivary response included one or several aspects of food--namely, the sight of food (Christensen & Navazesh, 1984); the sight and smell of food (Durrant, 1981; Guy-Grand & Goga, 1981; Nirenberg & Miller, 1982; Sahakian et al., 1981; Wooley & Wooley, 1973; Wooley et al., 1976); instructions to think of food (Wooley & Wooley, 1973); the sight, smell, thought, and taste of food (Lappalainen et al., 1994); and the auditory presentation of names of food (Staats & Hammond, 1972). Similarly, studies that evaluated insulin, free fatty acid, or glucose levels all used food-related conditional stimuli--such as the sight or smell of food (Bellisle et al., 1983; Broberg & Bernstein, 1989; Johnson & Wildman, 1983; Karhunen et al., 1997; Simon et al., 1986; Sjostrom et al., 1980), instructions to think of food (Johnson & Wildman, 1983), instructions that the

subject would eat soon (Karhunen et al., 1997), and instructions to eat food (Lucas et al., 1987). In these studies, the authors assumed that Pavlovian conditioning had previously occurred in the natural environment with the food-related stimuli. Thus, the authors concluded that the physiological responses produced in the presence of food-related cues were either “elicited” responses or conditioned responses, though there was no experimental manipulation of variables using a Pavlovian procedure.

Human Salivation Studies

The human salivary studies reviewed that assume Pavlovian conditioning had occurred between a food-related cue and food in the natural environment primarily used a between-groups design and focused on differences in salivary responses to presumed CSs between normal-weight subjects and those who were overweight (Guy-Grand & Goga, 1981; Johnson & Wildman, 1983; Wooley et al., 1976), those who had bulimia nervosa (Bulik et al., 1996), or those who were obese with a binge-eating disorder (Karhunen et al., 1997). The independent variables in these studies included the presentation of food-related words compared with non-food-related words (Staats & Hammond, 1972), varying levels of deprivation (Christensen & Navazesh, 1984; Staats & Hammond, 1972; Wooley & Wooley, 1973; Wooley et al., 1976), pre-exposure followed by exposure to a food stimulus (Guy-Grand & Goga, 1981; Nirenberg & Miller, 1982; Sahakian et al., 1981; Wooley & Wooley, 1973; Wooley et al., 1976), exposure to food stimulus versus imagining food (Wooley & Wooley, 1973),

instructions that subjects would or would not eat in the presence of a food stimulus (Wooley & Wooley, 1973; Wooley et al., 1976), the prior ingestion of different amounts of calories prior to the presentation of a food stimulus (Durrant, 1981; Wardle, 1987), and tasting versus not tasting food while in the presence of a food stimulus (Lapalainen, et al., 1994).

In addition to measuring the salivary response, several studies assessed subjective feelings of hunger (Guy-Grand & Goga, 1981; Johnson & Wildman, 1983; Karhunen et al., 1997; Lapalainen et al., 1994; Nirenberg & Miller, 1982; Sahakian et al., 1981; Wardle, 1987; Wooley & Wooley, 1973; Wooley et al., 1976), craving (Lapalainen et al., 1994; Nirenberg & Miller, 1982), desire (Kahunen et al., 1997), palatability (Christensen & Navazesh, 1984), or appeal (Sahakian et al, 1981; Wooley & Wooley, 1973) through the use of a visual analog scale. For example, subjects were typically asked to rate their feelings of hunger on a scale of "0" to "100", zero indicating no sensations of hunger and 100 indicating maximum sensations of hunger. The self-reported ratings and the salivary response were measured in an attempt to find a behavioral correlate to the self-report measures.

Several researchers reported that the human salivary response increased when subjects were exposed to a food-related stimulus. Viewing or smelling a food stimulus (Guy-Grand & Goga, 1981; Nirenberg & Miller, 1982; Sahakian et al., 1981; Wardle, 1987; Wooley & Wooley, 1973; Wooley et al., 1976) as well as thinking of (Wooley & Wooley, 1973) or tasting the food stimulus (Lappalainen

et al., 1994) resulted in increased responding. In addition, subjects who consumed more calories prior to the experiment showed increased salivary responding than those who consumed fewer calories (Durrant, 1981; Wardle, 1987).

On the contrary, several researchers reported varied results in many areas, including the effects of food deprivation, expectations about eating, and self-report measures (Wooley & Wooley, 1973; Wooley et al., 1976). For example, Wooley and Wooley (1973) and Staats and Hammond (1972) demonstrated that food-deprived subjects had a greater salivary response in the presence of a food stimulus or when presented with an auditory food-related word than subjects who were not food-deprived. Furthermore, it was demonstrated that deprivation increased the salivary response of nonobese subjects, but not obese subjects (Wooley et al., 1976) whereas Christensen and Navazesh (1984) demonstrated that deprivation had no effect on the salivary response in nonobese subjects. In another example, Wooley and Wooley (1973) and Wooley et al. (1976) found that when subjects were initially told they would eat following the presentation of a food stimulus, the salivary response increased in nonobese and obese subjects, whereas Christensen and Navazesh (1984) found no effect on the salivary response of nonobese subjects. Also, correlational results between self-reported measures and the salivary response have been inconsistent. Wooley and Wooley (1973) and Wardle (1987) reported a positive correlation between increased salivation and increased hunger ratings whereas Guy-Grand and Goga (1981), Nirenberg and Miller (1982), and Sahakian, et al. (1981) reported either a

negative correlation or no correlation between salivation and self-reported ratings of hunger.

One explanation for the inconsistent results found among studies is that in these studies, the authors assumed rather than experimentally demonstrated that conditioning occurred between the food-related stimuli and food in the natural environment. Because the experimenter was unable to control for the past learning history of each subject, this variable may interact with the presence of food-related stimuli. As a result, it is difficult to identify the influence of Pavlovian conditioning on anticipatory salivary responding. By assuming that classical conditioning between food-related stimuli and anticipatory physiological responding had previously taken place in the subjects' history, researchers can ask more advanced questions, such as what are the differences in responding between those with and without an eating disorder. These researchers are interested in asking these questions because saliva serves many important functions in the digestive system. For example, it initiates digestion of fat and starch and lubricates and neutralizes acids in the mouth, pharynx, and esophagus. In addition, depressed salivary responses have been implicated in gastroesophageal reflux disease (Valdez & Fox, 1991). Nevertheless, without assessing the variables responsible for behavior change in these studies, it becomes difficult to interpret the results.

Human Insulin Studies

Similar to the studies measuring salivary responding, several studies measured

the insulin response after exposure to a food stimulus that was assumed to function as a CS owing to past learning experiences in the natural environment. These studies were primarily designed to explore whether exposure to the food stimulus resulted in a differential change in insulin levels between normal weight individuals and those who were obese (Johnson & Wildman, 1983; Sjostrom et al., 1980), overweight (Simon et al., 1986), had a binge-eating disorder (Karhunen et al., 1997), or had been diagnosed with anorexia nervosa (Broberg & Bernstein, 1989).

Conditions under which the insulin response was measured included presentations of visual and olfactory stimuli (Sahakian et al., 1981; Simon et al., 1986; Sjostrom et al. 1980), presentations of visual and olfactory stimuli and instructions to imagine food (Johnson & Wildman, 1983), and visual presentations of high- and low- palatability food stimuli prior to ingestion (Lucas et al., 1987).

The studies concur in showing that when food was presented prior to ingestion, insulin responding increased and the increase was greater in the obese (Johnson & Wildman, 1983; Simon et al., 1986; Sjostrom et al., 1980) and those diagnosed with anorexia nervosa (Broberg & Bernstein, 1989) than in nonobese individuals. Normal weight individuals also displayed an increase in insulin responding after a food presentation but to a lesser extent than those with obesity (Johnson & Wildman, 1983; Simon et al., 1986) and anorexia (Broberg & Bernstein, 1989). Individuals diagnosed with a binge-eating disorder displayed patterns in insulin

responding that were similar to those with obesity (Karhunen et al., 1997).

Self-report measures of hunger (Johnson & Wildman, 1983) and hunger and craving (Karhunen et al., 1997) were evaluated, but not to the extent that they have been in studies evaluating the human salivary response. Hunger ratings increased for both obese and normal weight subjects after both a visual and olfactory presentation of a food stimulus (Johnson & Wildman, 1983). Those diagnosed with a binge-eating disorder scored similarly in hunger ratings to those without the disorder.

Human Blood Glucose Responses

Studies found consistent results when assessing blood glucose. Blood glucose levels did not change when normal weight (Simon et al., 1986; Sjostrom et al., 1980), obese (Karhunen et al., 1997; Sjostrom et al., 1980), or overweight individuals (Simon et al., 1986) were presented with a food-related stimulus.

To be certain that a response elicited by a conditioned stimulus (CS) is a conditioned respondent (CR), there are two requirements: (a) that the presumed CS begin as a neutral stimulus that is perceptible by the subject and that it is paired with an unconditioned stimulus (US), minimally once (Flaherty, 1985), and (b) experimental control must be demonstrated to rule out nonassociative effects (Rescorla, 1967). Simply making the assumption that responding to a food-related stimulus is attributable to prior Pavlovian conditioning ignores these criteria.

Studies Where the Pavlovian Relation is Manipulated

The following experiments are attempts to demonstrate that a digestive secretory response was an elicited CR in the presence of a newly established CS. This attempt is found in research measuring conditioned salivation (Conduit & Coleman, 1998; Holland & Matthews, 1970; Jansen et al., 1992; Powers, Holland, Miller, & Powers, 1981), conditioned blood glucose (Fehm-Wolfsdorf, et al., 1993; Overduin & Jansen, 1997) and conditioned insulin (Overduin & Jansen, 1997). Although all experiments listed below include conventional control procedures, none used the random control procedures as suggested by Rescorla (1967). All experiments (Conduit & Coleman, 1998; Fehm-Wolfsdorf et al., 1993; Holland & Matthews, 1970; Jansen et al., 1992; Overduin & Jansen, 1997; Powers et al., 1981) fall short on one or both of the requirements for an adequate demonstration that responding to the presentation of a stimulus is attributable to classical conditioning.

According to Rescorla, demonstration of experimental control by the contingency between the CS and US requires that control by nonassociative effects be ruled out. It is necessary to demonstrate that presumed conditioned responding is attributable to the contingency rather than to the contiguous relationship between the CS and US. In the random control procedure, the same number of CS and US presentations occur as in the conditioning procedure, however, these stimuli are presented randomly so that only the contingent relation between the CS and US is eliminated, though there may still be chance

occurrences of a CS-US pairing. All other factors are held constant between the experimental and control procedures. Programming the random presentation of the CS and US can be done in several different ways. Rescorla suggested presenting either the CSs or USs as they are presented in the experimental condition and then to randomly place the USs or CSs within the session. Another way to arrange this procedure is to first determine the length of a cycle (the time between the CS onset to the end of the intertrial interval) and then divide the cycle time into equal time segments. For instance, if the length of the cycle is 5 minutes, it can be divided into 20 15-s intervals. The locations of one CS and of one US are independently and randomly placed within a 15 s interval.

Unlike the conventional control procedures, such as the CS-alone, explicitly unpaired, and discrimination control procedures, the random control procedure rules out nonassociative effects such as sensitization and habituation as well as other associative effects, such as inhibition. The CS-alone control procedure does not rule out sensitization effects from the US. The subject's experience with the US is different on CS alone trials and CS-US trials. Therefore, the increase in responding to the CS alone following CS-US pairings as compared to responding to the CS alone prior to CS-US pairings may be the result of the presence of the US during CS-US pairings. That is, just the presence of the US, rather than the CS-US contingency, may increase the eliciting effects of the CS. When this occurs, the increase in responding to the CS is called pseudoconditioning. Moreover, the CS-alone procedure does not rule out habituation effects of the CS.

Repeated presentations of the CS alone may result in greater habituation of responding than if both the CS and US were presented in a control condition. Epstein, Rodefer, Wisniewski, and Caggiula (1992) demonstrated that an additional stimulus presented during habituation trials maintained salivary responses. Because the random control procedure removes only the contingency between the CS and US rather than also removing the US, any change in responding to the CS after conditioning trials can be attributed to the CS-US contingency rather than to sensitization or habituation. In addition to the CS-alone control procedure, the explicitly unpaired control procedure and the discrimination control procedure are inadequate control conditions. Although the positive contingency between the CS and US is eliminated in these two procedures, a negative contingency between the two stimuli is arranged. As a result, the CS signals the absence of the US resulting in the CS acquiring inhibitory properties. By using the random control procedure instead of the explicitly unpaired control procedure or the discrimination control procedure, the CS neither signals the occurrence nor absence of the US. Because it is neither excitatory nor inhibitory, Rescorla (1967) claims that it is the only appropriate control condition. Research in the areas of conditioned salivation, conditioned blood glucose, and conditioned insulin that used these three conventional control procedures are described next.

Human Salivation

Researchers who have studied conditioned salivation have attempted to

demonstrate acquisition and extinction (Holland & Matthews, 1970; Powers et al., 1981) of the conditioned response. In addition, Jansen et al. (1992) attempted to evaluate whether there was a correlation between conditioned salivation and hunger. In all these studies, the procedures were presented while the subjects were awake. More recently, Conduit and Coleman (1998) attempted to demonstrate that conditioned responding to a CS could occur during REM sleep after prior conditioning trials when the subjects were awake. A brief review of these four studies are described in the following paragraphs.

Holland and Matthews (1970) initially presented subjects with six trials of the CS alone during the first phase (baseline trials) and three trials of the CS alone during the third phase (test trials). Ten trials of the CS and US presented simultaneously occurred during the second phase (acquisition trials). The compound CS consisted of both a buzzer and light. The US consisted of 1 ml of orange drink placed on the tongue. Holland and Matthews concluded that the increased responding from near 0 ml/min during baseline to over 20 ml/min following 10 acquisition trials was a measure of conditioned responding. However, when using the CS-alone procedure the number of US presentations differed between phases; therefore, any change in responding between two consecutive phases may be the result of the subject's different experiences with the US during those two phases (Rescorla, 1967).

Later studies attempted to demonstrate conditioned salivation to a compound stimulus that included either the sight and smell of food (Powers et al., 1981) or

the ingestion of candy (Jansen et al., 1992), together with a neutral stimulus. Both authors reported an increase in salivary responding after acquisition trials. It is questionable whether Powers et al. (1981) and Jansen et al. (1992) actually demonstrated classical conditioning, as both failed to show that the putative conditional stimuli had been previously neutral--that is, both involved the sight of food, and Jansen et al. (1992) included ingested candy as part of the compound CS. In addition, both studies used conventional control procedures.

Powers et al. (1981) presented two subjects with a discrimination training procedure. The CS+ condition consisted of the subjects' wearing sunglasses while instructed to think about, smell, and look at food. During the CS+ trials, subjects were told they would eat the US (bite of food) after each trial. The CS- condition consisted of the subjects' not wearing glasses while instructed to think about, smell and look at food. During the CS- trials, subjects were told they would not eat after the trial. After 20 to 35 discrimination trials for each of five sessions, six salivary test trials were presented. Results showed that salivary responding was greater in the presence of the CS+ than in the presence of the CS-. Following the test trials, a control condition in which random pairings of the CS+ and the CS- trials with the instruction that they would or would not eat were presented. Also, during this condition, the CS+ and CS- were randomly paired with deceptive instructions. That is, subjects were told at times that they would eat when they actually would not be given the opportunity to eat, or they were told that they would not eat but then were given food. The authors neither

described the random procedure in detail nor indicated the number of extinction trials. The results showed that responding after extinction trials was not significantly different between the CS+ condition and the CS- condition. Although the authors indicated that during the control condition there were random presentations of the CS+ and CS- followed by the US, this was not similar to the random control procedure described by Rescorla (1967). The authors (Powers et al.) presented trials where the CS+ was programmed specifically to follow the US. As a result, the contingency between the CS+ and the US was maintained. Therefore, according to Rescorla, this would not be an adequate control condition. Additionally, the use of discrimination control procedure does not serve as an adequate control (Rescorla, 1967) because an explicitly unpaired CS- becomes a cue for the nonoccurrence of the US, thus producing a new contingency between the CS- and the US. This new contingency might result in inhibitory responding to the CS-; therefore, responding to the CS- would be an inadequate baseline to compare responding to the CS+. When comparing responding to the CS+ and the CS-, changes in responding to the CS+ may appear greater because responding to the CS- may be lower due to inhibitory factors.

Similar to Powers et al. (1981), Jansen et al. (1992) used the sight of food in addition to the taste of food as a compound CS. This compound CS consisted of three elements, a 5-min red light, the sight of 30 g of chocolate, and the taste of 1.5 g of chocolate. Subjects were instructed to suck on the 1.5 g of chocolate at the beginning of the CS period. The US consisted of the intake of 30 g of

chocolate. In their experiment, Jansen et al. used a within-subject design. The three phases of the experiment were as follows: (a) three habituation trials with the CS presented alone with a 2-min ITI, (b) eight acquisition trials with the CS followed by the US, each separated by a 30-min ITI and (c) four extinction trials with the CS presented alone with 2-min ITI. Results from the second test trial following the acquisition phase indicated that salivary responding was greater than from the first test trial following the baseline phase. Although the authors concluded that the results showed that conditioned responding has occurred, these conclusions cannot be drawn from using the CS-alone procedure (Rescorla, 1967). In this procedure, the number of US presentations differ between phases; therefore, changes in responding may have been due to the subject's experience with the US as discussed previously, rather than attributable to classical conditioning.

In addition, the authors attempted to demonstrate the acquisition of a CR to a CS using a to-be-conditioned stimulus consisting of three components--namely, a 5-min red light, the sight of 30 g of chocolate, and the taste of 1.5 g of chocolate. This compound stimulus may not have been neutral because it consisted of both visual and gustatory presentations of a compound stimulus. Although the experiment initially included three habituation trials to the CS that presumably allowed for the use of stimuli that may have initially elicited a response (Flaherty, 1985), the effects of spontaneous recovery and external disinhibition of the CR to the CS (sight and taste of food) are unknown.

Spontaneous recovery is defined as the recovery of an extinguished response after a rest period has elapsed following extinction (Kimble, 1961). The 30-min ITI in Jansen's et al. (1992) experiment during acquisition trials may have functioned as a rest period. Pavlov (1927/1960) found that spontaneous recovery in both the magnitude and the latency of conditioned salivation in dogs occurred after a 23-min period. Further, Corty and Coon (1995) found that spontaneous recovery of conditioned salivation in humans occurred after a 60-min period. Therefore, it is unknown whether conditioned responding in Jansen et al.'s study was attributable to the conditioning process or whether it was the result of spontaneous recovery.

It is also possible that an increase in responding during the second test trial was due to external disinhibition (Schwartz, 1984). Disinhibition is defined as the inhibition of the CS inhibition that may occur during extinction trials. During extinction trials, the repeated presentations of a conditioned stimulus without an unconditioned stimulus results in the extinguishing of a response. If the decrement of responding is because of inhibition, then the presentation of a novel stimulus should increase responding. Perhaps the ingestion of 30 g of chocolate following the CS presentation functioned as a novel stimulus and resulted in the disinhibition of the inhibition to the compound CS. Again, it is unknown whether conditioned responding was attributable to the conditioning process or whether it was partially attributable to spontaneous recovery or external disinhibition.

More recently, Conduit and Coleman (1998) attempted to demonstrate that

conditioned responding would occur not only while the subject was awake but also during REM sleep. Using a within-subject design, subjects were initially exposed to a baseline sleep condition in which they were presented with a 2-min red light stimulus attached to eye pads followed by a 2-min mouth suction period. Next, while awake, subjects were presented with 20 conditioning trials in which they were presented with the CS (red light stimulus attached to eye pads) followed by 5 ml of citrus juice. The duration of the CS was randomly chosen from the following values: 5, 10, 20 or 40 seconds. After the conditioning trials, the subjects were presented with one CS-alone test trial followed by three trials where no CS was presented. If the amount of the salivary response to the CS-alone test trial was more than half of the amount of the salivary response for trials where no CS was presented, the authors concluded that conditioned responding had occurred. Until the amount of the salivary response met this criterion, subjects were presented with additional sets of 20 conditioning trials followed by one CS-alone test trial and three trials where no CS was presented. Once the magnitude of the salivary response met this criterion, subjects were exposed to one sleep test trial. The data show that for all 7 subjects, responding was greater during this sleep test trial than during the baseline sleep trial. Finally, during the extinction phase, there were 20 unpaired presentations of the CS and the US while the subject was awake. After the extinction trials, a CS-alone test trial was presented while the subject was awake and asleep. Results indicated that salivation produced during these two trials were not significantly different from baseline

levels, indicating that salivation had extinguished to the CS. The authors concluded that they had demonstrated the acquisition of conditioned salivation while the subject was awake and asleep. The control procedure used during the extinction phase was an explicitly unpaired procedure and as mentioned previously, according to Rescorla (1967), this is not an adequate control condition.

Human Insulin Study

In an attempt to establish a conditioned insulin response and blood glucose response to a CS, Overduin and Jansen (1997) manipulated a presumably neutral CS (flavor and fragrance of peppermint) and a US (50 g of oral glucose). The experimental group received the US consisting of oral glucose and the control group received a US consisting of aspartame. A third control group received a US consisting of water. Self-report ratings of craving, liking of the US and sweetness of the US were also evaluated. The experiment failed to establish conditioned blood sugar responding and conditioned insulin responding. There were no significant differences in the self-reported ratings of craving or sweetness of the US after conditioning for either the experimental or control groups, although there was a significant increase in self-reported rating of liking the US after conditioning for the experimental group, but not the control groups. The control procedure chosen by the authors involved each subject's taking home a beverage and being given a specific time to drink it. This control procedure does not meet the basic definition of the random control procedure, that is, presenting

both the CS and US randomly during a specified amount of time. In addition, removing the subject from the experimental context introduces new factors that cannot be controlled by the experimenter. Perhaps the failure to demonstrate conditioning may have been partially due to contextual control by the home setting after ingesting the beverage at home.

Human Blood Glucose Response

In a more recent study, Fehm-Wolfsdorf et al., (1993) attempted to demonstrate acquisition of a conditioned blood glucose response to neutral stimuli. During acquisition trials, subjects in the experimental group were presented with a compound CS consisting of a light, noise, and odor together with an injection of insulin. Subjects in the control group were presented only with the injection of insulin. After four acquisition trials were presented, a fifth trial (the test trial) was presented. During the test trial, experimental subjects who had received the compound CS and insulin injection received the compound CS and saline injection. The subjects in the control group received the saline injection during the test trial. The results indicated that the compound stimulus presented with a saline injection produced a response similar to the responses produced during acquisition trials for 7 of the 14 subjects in the experimental group. Four of the seven subjects in the control group responded to the saline injection with a change in blood glucose level. The author's concluded that the changes in blood glucose were due to the association between the compound stimulus and the injection of insulin. Although this is true for half of the subjects in the

experimental group, more than half of the subjects in the control group responded to the control condition with similar changes in their blood glucose level. Perhaps the context or the injection served as a compound CS for the control subjects. It is also possible that responding to the compound CS by the experimental and control subjects may have been due to other factors, such as sensitization to the US rather than due to Pavlovian Conditioning. Without the use of the random control condition, a baseline level of responding to the CS cannot be assessed.

Discussion

The results thus far indicate that anticipatory physiological responses can become classically conditioned to external stimuli. Researchers should continue empirical research to investigate basic learning processes underlying conditioned responding, perhaps modifying the experimental design to include a neutral stimulus (Flaherty, 1985) and the random control procedure (Rescorla, 1967). Researchers attempting to answer more sophisticated questions also need to realize that disregarding past learning histories may in fact impede progress, as is clearly supported by the conflicting results found in human salivary research that assumed Pavlovian conditioning had occurred between a food-stimulus and food in the natural environment.

Although research has been conducted with several anticipatory physiological responses, there is interest in determining whether the salivary response is a behavioral correlate to self-report measures of hunger or craving and in determining the influence of stimuli on reported feelings of hunger or craving.

Because of the biases present in self-report measures, it may be beneficial to use a more objective measure that includes actual eating behavior, such as amount of food eaten or latency to eat.

Pavlovian Conditioning Procedures in which

Meal Initiation Responses (Food Getting or Eating) are Measured

Physiological changes within the organism that signal energy depletion have been implicated in the regulation of eating behavior. Pavlovian processes that result in the organism's learning an association between environmental stimuli and food have also been implicated in the regulation of meal initiation (Weingarten, 1985). There are several ways to measure learning in a Pavlovian conditioning experiment, for example, measuring the latency or magnitude of the CR regardless of whether it is similar or dissimilar to the UR (Schwartz, 1984). The experiments reviewed in this section evaluate the learning of an association through one type of behavioral assay, that is the measurement of meal initiation responses in the presence of an appetitive CS.

The meal initiation responses that are measured in animals under Pavlovian Conditioning procedures have included the following: amount eaten (Berthoud, Sterner, Powley, 1984; Weingarten, 1983, 1984; Weingarten & Martin, 1989; Zamble, 1973), latency to eat (Berthoud et al., 1984; Weingarten, 1983, 1984; Weingarten & Martin, 1989), food magazine approach (Detke, Brandon, Weingarten, Rodin & Wagner, 1988; Weingarten, 1983, 1984; Weingarten & Martin, 1989), and duration of eating (Weingarten, 1984). Only one study

evaluated human eating behavior under a Pavlovian procedure (Birch et al., 1989). In this study latency to eat and amount eaten were measured in children. Below are brief descriptions of their experimental procedures and results. In addition, the conventional control procedures used for each experiment will be discussed.

Zamble (1973) assessed the effects of presenting a CS (15-min metronome) that had been previously paired with feeding behavior on subsequent amounts eaten. In the experiment, eighteen deprived rats were divided into two groups, the Forward group (a 15-min metronome sounded prior to feeding) and a Backward group (a 15-min metronome sounded immediately after feeding). Results showed that subjects in the Forward group consumed significantly more than in the Backward group. In addition, under greater deprivation, subjects in the Forward group increased eating behavior, whereas subjects in the Backward group either decreased eating behavior or eating patterns remained the same. The authors concluded that presenting subjects with a CS that signals food delivery increases eating behavior. Although these results are positive, Rescorla (1967) would argue that the backward conditioning control group introduces subjects to a negative contingency between the US and CS, that is, the CS predicts the absence of the US rather than introducing no contingency between the CS and US. Similar to the inadequacies of the explicitly unpaired control procedure and the discrimination control procedure, that backward control procedure may have produced inhibition. Thus, the results obtained by the Backward group cannot be

considered an adequate control condition, according to Rescorla (1967).

In another study, Weingarten (1983) demonstrated that eating behavior could also be elicited by a conditioned stimulus in sated rats. Using a delay conditioning procedure, Weingarten (1983) presented 7 rats with a 4.5- min compound CS+ consisting of a buzzer and light followed by the presentation of the US consisting of 8 ml of liquid diet during the last 30 seconds of the compound stimulus. Intermeal intervals were 3.5 hours. An intermittent tone served as a CS- and was presented halfway through each intermeal interval. The CS- was not followed by food. After 66 acquisition trials (6 trials per day), the rats were presented with 21 CS+ and 21 CS- test trials (each stimulus presented once per day). Prior to each test trial, the rats were free fed until they stopped eating. The test trial consisted of either the CS+ or the CS- stimulus followed by the US. The US was presented following both the CS+ stimulus and the CS- stimulus to insure that a decrease in latency to eating was not the result from arousal to the US. Results indicated that the latency to eat decreased from 60 seconds on the first day of conditioning to 5 seconds by the sixth day. During test trials, the latency to eat was less than 5 seconds for all CS+ trials as compared to between 10-60 seconds for all CS- trials. Results also indicated that there were no differences in the time spent with the nose in the food dish or in feeding time between the training versus test trials. In fact, sated animals ate 20% more when placed in the testing situation than in their home cage. However, they compensated by subsequently reducing the amount eaten while in the home cage

to an equal amount eaten from ad libitum food on the previous day. Similar results were found from Weingarten's (1984) systematic replication of the experiment by Weingarten (1983). In addition, Weingarten (1984) demonstrated that when stale milk was substituted for fresh milk, sated rats continued to eat the same amount of stale milk as fresh milk when presented with the CS+. The results described by Weingarten (1983, 1984) may not be solely attributable to Pavlovian conditioning because the discrimination control procedure rather than a random control procedure was used.

In another similar study, Berthoud et al. (1984) evaluated conditioned feeding behavior. Rats were randomly assigned to one of three groups as follows: signaled group (visual-acoustic CS presented for 4 minutes preceding a meal), nonsignaled group (CS dissociated from meal delivery) and sated group (never exposed to the CS). Details in this note are limited, but the reported results support previous findings that only subjects in the signaled group displayed conditioned feeding as compared to the subjects in the nonsignaled and sated groups. All procedures were not described fully so it is not known whether adequate control procedures were used.

Other research has evaluated whether contextual cues would affect approach to the food cup (Detke et al., 1988). Contextual cues may signal a specific CS-US relationship, that is, whether or not a specific CS is followed by a US, or they may become part of a compound stimulus with the CS (Gordon, 1989). Using a within-group design, four rats were presented with the following conditions: 14

trials of Ax+ (35-s auditory stimulus, (A), followed by a 5-s light stimulus, (x), with A and x coterminating and then followed by 1 ml of food), and 14 trials of Bx- (35-s auditory stimulus, (B), followed by a 5-s light stimulus, (x), with B and x coterminating but not followed by food). The ITI was 60 seconds. During test trials, each stimulus was presented alone. It was found that rate of responding was greater to the x stimulus in the presence of the "A" stimulus than to the x stimulus in the presence of the "B" stimulus or the x stimulus alone. The authors concluded that the presence of the "A" stimulus enhanced food-related activity. As previously mentioned, Rescorla (1967) would argue that the discrimination control procedure used was not an adequate control condition.

Further systematic experimentation (Weingarten & Martin, 1989) assessed consummatory behavior, such as eating, and food-related anticipatory behavior, such as head poking and latency to eat, following taste aversion conditioning, manipulation of dopamine and endorphin systems, and an intra-gastric nutrient preload in sated rats. Prior to these three experimental manipulations, all rats were exposed to 72 conditioning trials. In these trials, 4 minutes after the onset of a 4.5-min compound CS consisting of a buzzer and light, the US consisting of food was delivered. In this procedure, there were no control conditions. Therefore any responding to the CS+ may be due to nonassociative factors, such as sensitization to the US. After individual manipulations were conducted for each of the three experiments, the authors concluded that consummatory behavior and food-related anticipatory behavior function under two separate systems. The

results that support this conclusion were as follows: (a) Following taste aversion conditioning, the CS+ was presented to the subjects. The subjects in the experimental group displayed significantly lower consummatory behavior when compared with subjects in the control group. There were no significant differences between the experimental group and the control group in food-related anticipatory responding; (b) Following an injection of a dopamine blocker, the CS+ was presented to the subjects. When subjects were injected with the dopamine blocker, they displayed significantly lower head poking behavior to the CS+ than when injected with a saline solution. There were no significant differences between the two conditions when measuring latency to eating or consummatory behavior; and (c) Following an injection of an opioid blocker, the CS+ was presented to the subjects. When subjects were injected with the opioid blocker, they displayed significantly lower consummatory behavior to the CS+ than when injected with a saline solution. There were no significant differences between the two conditions when measuring food-related anticipatory behavior. Although Weingarten and Martin (1989) also found that nutrient preloads had similar effects on both consummatory and food-related anticipatory responses, they concluded that an appetitive and consummatory distinction is important when studying food intake. In the future, an adequate control procedure, such as the random control procedure (Rescorla, 1967) should be used to insure that responding to the CS+ is actually a conditioned response.

The influence of Pavlovian conditioning on human eating behavior was

evaluated by Birch et al. (1989). Seven preschool children were presented with 10 conditioning sessions, one 20-min session per day. In a snack room children played with toys while they were presented with a CS+ that consisted of a 4 to 5 minute rotating red light with music (clapping song). Thirty seconds after its onset, food was presented. During the CS- condition, children went to the same snack room to play and were presented with a CS- that consisted of a 4 to 5 minute traffic light stimulus with music (Music Box Hymnals), but no food was presented. After the 10 conditioning sessions were completed, test trials for each condition were presented once a week for two weeks. Snacks were freely available during all test trials. After the children finished eating the snacks, they were brought to a different room and were either presented with the CS+ or CS- stimulus. Results showed that children ate more during the CS+ condition than during the CS- condition, but not a significantly different amount. Next, the authors analyzed data to determine whether children who could identify the stimuli that predicted food ate significantly more than children who could not identify stimuli that predicted food. The results showed that those who could correctly identify the cues associated with eating ate significantly more during the CS+ versus the CS- condition.

In a second experiment (Birch et al., 1989), the same procedure was conducted, only the CS+ was presented in a different room from the CS- during training trials. Prior to the test trial, a third room was used where the children ate until satiated. The children then went to either the CS+ or the CS- room for the test

trial. During testing, it was found that latency to eat after the visual and auditory cues began was significantly different to the CS+ than the CS-. Latency to eat was defined as the amount of time that elapsed from when the visual and auditory cues began until each child began to eat. Although the authors did not describe the measurement procedure, they reported the mean latency to eat for all CS+ and CS- test trials for all children. The latency to eat in the presence of the CS+ was less than 1 s for all children, whereas the latency to eat in the presence of the CS- was less than 1 s for only 3 out of 15 children. It was also found that during testing trials, children ate significantly more in the presence of the CS+ than in the presence of the CS-. As previously discussed, the discrimination control procedure that was used in this study was not an adequate control procedure according to Rescorla (1967).

It appears from the research that both meal initiation and food-related anticipatory behavior may be elicited by a CS. Future research should use the random control procedure as suggested by Rescorla (1967) to insure that only the contingency between the CS and US is eliminated, rather than introducing a new contingency that may produce inhibitory conditioning or introducing other nonassociative factors, such as sensitization.

In any case, these experiments suggest that it is possible to design an experiment that can incorporate a more objective measure than self-report, such as amount eaten, in addition to the reflexive salivary response. The use of more objective measures will eliminate the bias associated with self-report measures.

However, it is possible that there is a difference between acquisition and performance of what was learned when measuring food-related responding. The development of an association between the CS and US may or may not be initially reflected in measurable behavior. That is, subjects may learn the contingent relationship between the CS and US without actually displaying the conditioned response.

Conclusions

Research evaluating the influence of classical conditioning on anticipatory physiological responses has evolved from Pavlov's (1927/1960) studies on conditioned salivation in dogs to studies on both conditioned salivation and other conditioned anticipatory responses, such as insulin and glucose secretion (Overduin & Jansen, 1997), in humans. Researchers interested in eating disorders have also attempted to demonstrate that anticipatory responses are a behavioral correlate to self-report measures of feelings of hunger. More recently, rather than focusing on internal feelings of hunger, researchers have studied the influence of classical conditioning on actual eating behavior in rats (Weingarten, 1983,1984; Detke et al., 1988) and in humans (Birch et al., 1989).

Consequently, hunger has been studied from two different perspectives, as both an internal feeling and an overt response. In the early 20th century, hunger had been simply defined as a dull ache that was a signal for eating (Cannon & Washburn, 1912). Since that time, research has revealed that hunger is defined by a more complex interaction of stimuli than simply a biological need (Kassel &

Shiffman, 1992; Weingarten, 1985). It has been suggested that internal stimuli along with environmental stimuli influence hunger and the initiation of eating. In fact, in a recent survey (Tuomisto, Tuomisto, Hetherington & Lappalainen, 1998), over 43% of individuals surveyed reported that they begin to eat because of environmental stimuli, such as seeing others eating, smelling food, etc. Only 30% of those surveyed reported a physiological reason to begin to eat, such as feeling hungry or weak. Perhaps further systematic experimentation will clarify the role of Pavlovian conditioning on both anticipatory physiological responses and eating behavior.

This review outlines four suggestions that future researchers may want to consider when evaluating the influence of classical conditioning on anticipatory physiological responses or meal initiation. The first two suggestions are as follows: (a) to use an initially neutral CS, and (b) to use the random control procedure as suggested by Rescorla (1967). Although the benefits of using the random control procedure have been presented, there is also an associated risk. Balsam and Tomie (1995) have demonstrated that the use of this procedure during the initial baseline phase can result in greater retardation of acquisition to the CS than when a CS-alone control procedure is used prior to acquisition training. Therefore, the third suggestion is to consider the use of other experimental designs, such as a single-subject reversal design. When using this design, individual subject data are presented for each trial so that changes in performance can be assessed on a trial by trial basis. Also, systematic changes in performance

are evaluated after the introduction of different experimental conditions for a subject. Repeated reversals of conditions within the same subject increase the reliability of the treatment's effect. Through this level of analysis, changes in responding characteristic of sensitization effects or pseudoconditioning, such as an abrupt increase in performance after the first few CS-US trials and then a subsequent decrease in responding, would be evident if it were to occur. The fourth suggestion for future researchers is related to the measure of hunger. When evaluating this hypothetical construct, researchers may want to consider using an objective measure, such as amount eaten, rather than self-report measures as the conditioned response.

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Appendix B

Description and Results of Research with 3 Pilot Subjects

The purpose of this pilot research was to demonstrated the acquisition and extinction of the conditioned salivary response.

Method

Subjects

Three female subjects, between the ages of 18 to 45, served as pilot subjects. Each subject was recruited through the Human Subjects Pool of the Psychology Department at Queens College. Each subject received credit toward a class requirement for 2 hours of participation in this study. Subjects were selected on the basis of the following criteria: He or she (a) reported that he/she did not have a hearing impairment; (b) had been of stable weight (± 10 lbs) over the past 2 months; (c) had not received or was not currently receiving treatment for an eating disorder during the past year; (d) was not taking any medications; (e) did not have a disorder or disease that could affect salivation, and (f) was not allergic to lemons. Each subject was asked to refrain from eating and drinking for the 2-hour period before the 2-hour session. The Queens College Institutional Review Board of the City University of New York gave approval for the procedures used in this study.

Setting

The experiment took place in a psychology laboratory at Queens College. The laboratory measured 5.85 m in width and length and 6.35 m in height. Each

subject sat 20 cm behind a white folding cardboard screen that measured 101.2 cm in width and 81.8 cm in height to block his/her view of the experimenter and apparatus. A round opening (5.5 cm in diameter) in the screen was located 38.2 cm from the top and 13 cm from the left side of the screen, approximately at the level of the subject's mouth. A rectangular opening (9 cm in width and 11.6 cm in height) was located at the bottom and 26.3 cm from the left side of the screen for the subject to pass a cup through the opening.

Stimuli, Materials, and Apparatus

The CS consisted of a 15-s auditory stimulus, produced by an electronic piano (Yamaha Model # PSS-14) with a 64 dB re SPL (C weighted) (Bruel & Kjaer Sound Level Meter, Type 2230) that was heard from a distance of approximately 26 cm. The US was 0.20 ml of lemon juice (Borden). The pH of the lemon was 2.6 as measured by a pH meter (Hanna Instrument pHel Electrode). All taste stimuli were placed in the subject's mouth by a syringe dropper (Apothecary Products EZY Dose Oral Syringe). Saliva was collected in paper cups (Dixie, James River Corp). Paper cups were weighed (to the nearest 0.001g) using an electronic balance (Ohaus Model TS200S Electronic Balance, Serial #SND07633161). Spring water (Poland Spring) was used for the 100-ml preload and for the 20-ml rinse during ITIs. All time intervals were measured by a manual stopwatch (Radio Shack LCD Multi-Function Stopwatch, Cat. No. 63-5013).

Measurement of Saliva

Prior to the beginning of each trial, an empty paper cup was weighed.

Immediately after the subject spit into the cup, the paper cup was weighed again. The weight of saliva equaled the second measure minus the weight of the cup and the lemon stimulus.

Experimental Design

An ABA reversal design was used to evaluate the magnitude of the CR during test trials. The magnitude of the CR was determined by weight to the nearest 0.001 g.

Procedure

Fifteen minutes prior to testing, the subject read and signed a consent form. The subject entered the testing area and sat behind the screen. The subject was then given 100 ml of spring water to drink while the following protocol was read:

During the session, try to minimize your movement and remain in the chair at all times. In some cases, you may be presented with a tone stimulus. When you hear the tone, open your mouth and move it to the opening in the screen with your tongue lifted. You may have a stimulus placed in your mouth. Try to minimize your mouth movements. Next, you will be handed a cup and asked to spit into it three times to empty your mouth. At times, you will then be handed a cup of water to rinse your mouth and should discard the remains into a waste container that will be located on your right. There may be times that you will be given no instructions, and you should remain seated and rest quietly while swallowing normally. Do you understand the procedure so far?
[After the subject acknowledged that he or she understood the experiment, the

subject was given the following instructions] Please sit quietly, minimize your body movement and keep your head tilted slightly forward in a comfortable position at all times and relax and swallow normally. We will get started in a few minutes. [The subject was given the option to read materials unrelated to food. After the 10-minute period elapsed, the subject was given the following instructions] We are ready to begin now, I'll take the reading material from you. Please sit quietly, minimize your body movement and keep your head tilted slightly forward in a comfortable position at all times. [Instructions for CS-alone Trials] Swallow once and then do not swallow again. [The 15-s tone begins] Please try to spit into this cup three times to empty your mouth [subject is handed a cup]. Please rinse out your mouth [subject is handed water, rinses mouth, and returns cup to experimenter] You can relax and swallow normally. [Instructions for Acquisition Trials] Swallow once and then do not swallow again. [The 15-s tone begins, after offset of tone, .20 ml of lemon placed under the tongue for 30 seconds] Please try to spit into this cup three times to empty your mouth [subject is handed a cup]. Please rinse out your mouth [subject is handed water, rinses mouth, and returns cup to experimenter]. You can relax and swallow normally.

Experimental Conditions

Baseline. Each subject was presented with a minimum of eight trials in which the CS was presented. The subject was instructed that once the tone began, to open his/her mouth and move it to the round opening in the screen. The subject

was then asked to spit three times into the cup provided. The subject was handed 20 ml of water through the rectangular opening in the screen to rinse the oral cavity and then was presented with a 75-s fixed time ITI. During the ITI the subject was instructed to relax and swallow normally. The purpose of this phase was to assess the baseline level of responding to the CS. The second phase began after a minimum of eight data points had been obtained and no positive linear trend was noted.

Acquisition. Each subject was presented with 24 acquisition trials in which the CS was presented. The subject was instructed that once the tone began, to open his/her mouth and move it to the round opening in the screen. Upon the offset of the tone, 0.20 ml of lemon was placed in the subject's mouth for a 30-s period. After a 30-s period elapsed, the subject was asked to spit into a paper cup three times and rinse the oral cavity with 20 ml spring water. The subject was then presented with a 75-s fixed time ITI. During the ITI the subject was instructed to relax and swallow normally. On test trials, the compound CS was presented alone to assess the magnitude of the CR. The procedure for the presentation of the compound CS was identical to the procedure used in Baseline. Test trials were presented after the 3rd, 4th, 9th, 11th, 14th, 18th, 19th, and 23rd acquisition trial.

Extinction Subjects S1A and S2A were presented with 4 CS-alone trials and Subject S3A was presented with 5 CS-alone trials. The procedure for the presentation of the compound CS was identical to the procedure used in Baseline. The purpose of this phase was to assess CR magnitude during extinction trials.

Results

No subjects showed an increase in conditioned salivary responding on test trials during the Acquisition Phase or a decrease in conditioned salivary responding during the Extinction Phase, when compared with salivary responding on baseline trials. Figures 1A through 3A show the weight of salivation in grams for each trial during the Baseline, Acquisition, and Extinction Phases for each individual subject. The top graph in each figure displays performance for CS-Alone trials during Baseline, Acquisition and Extinction Phases, and paired CS + Lemon trials during the Acquisition Phase. Performance on CS-Alone trials during each phase is re-plotted in the bottom graph; the ordinate axis is re-scaled so that changes in responding could be more readily observed.

The Mann-Wald time series analysis (Jones, Vaught, & Weinrott, 1977) was used to determine whether there were statistically significant differences in the mean level and slope of the functions of two consecutive conditions (i.e., baseline and acquisition or acquisition and extinction) for each subject. An alpha level of .05 was used for all tests.

The Mann-Wald time series analysis indicated that for CS-alone trials between the baseline and acquisition conditions, there was a significant increase in mean level for Subject S2A (Figure 2A), a significant decrease in mean level for Subject S1A (Figure 1A), no significant difference in mean level for Subject 3A (Figure 3A), and no significant differences in slope for any of the three subjects. When comparing the CS-alone trials between the acquisition and extinction conditions,

Figure Caption

Figure 1A. Weight of salivation in grams for SIA during baseline, acquisition, and extinction conditions for consecutive trials.

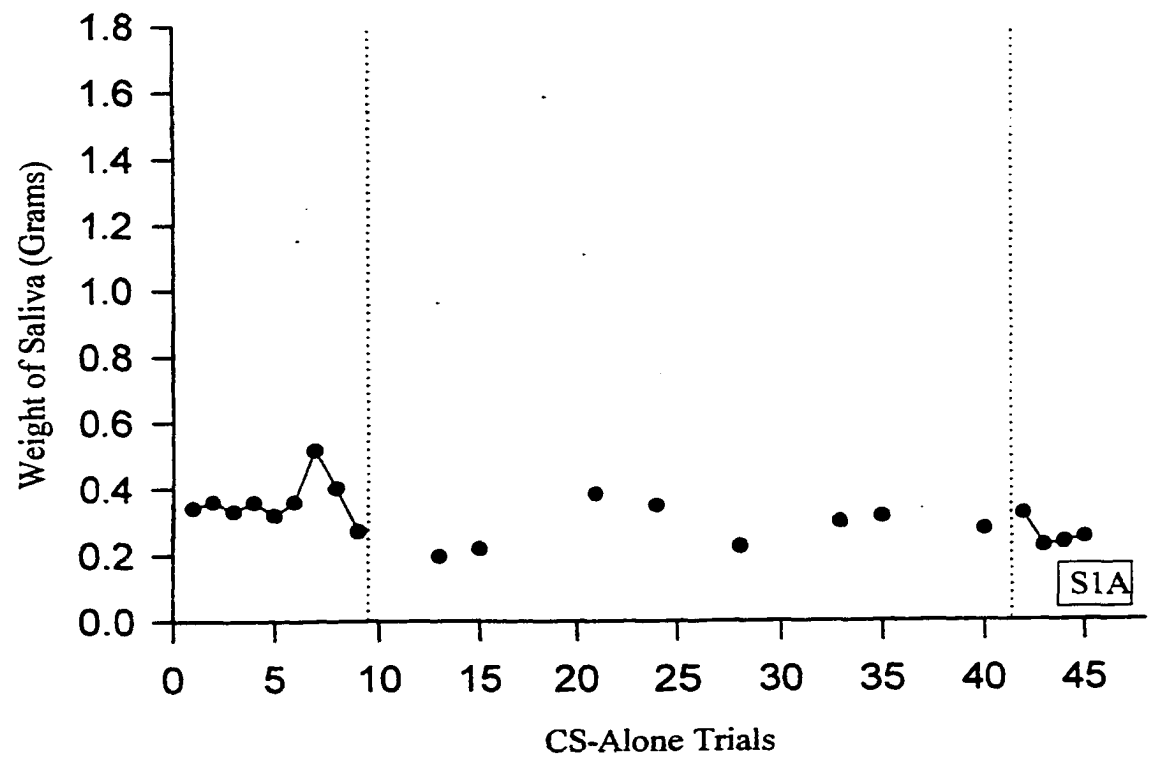
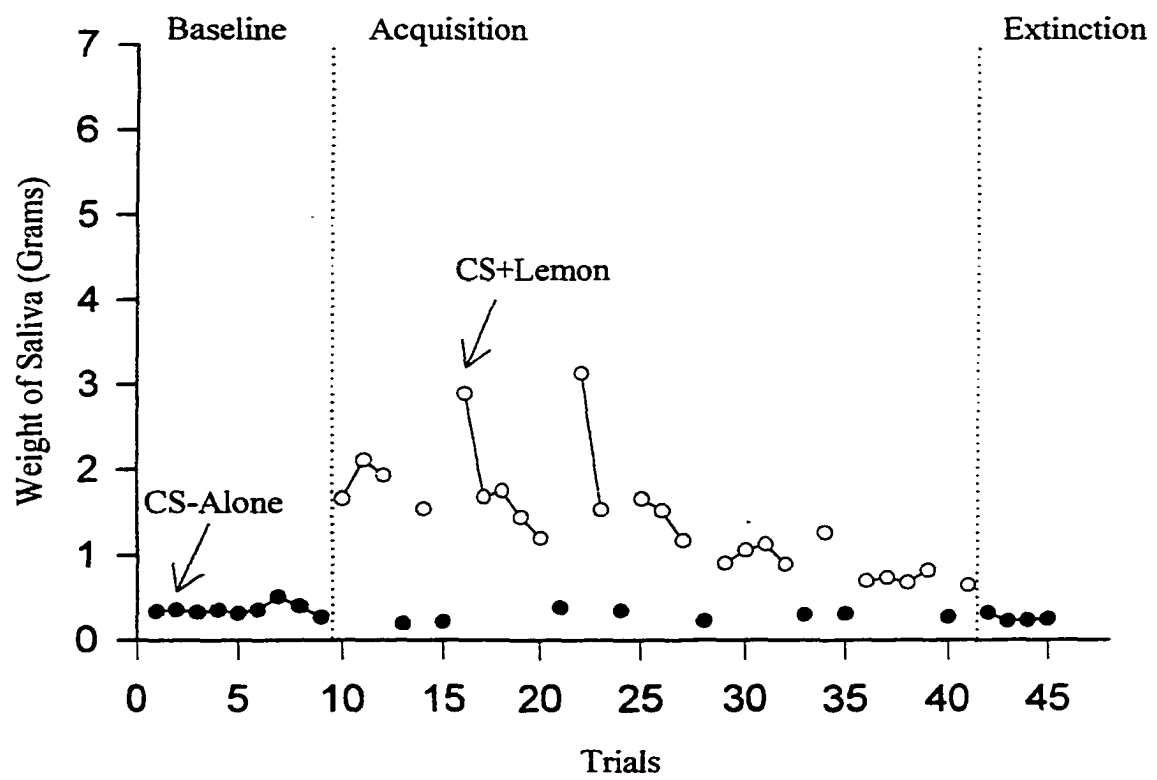


Figure Caption

Figure 2A. Weight of salivation in grams for S2A during baseline, acquisition, and extinction conditions for consecutive trials.

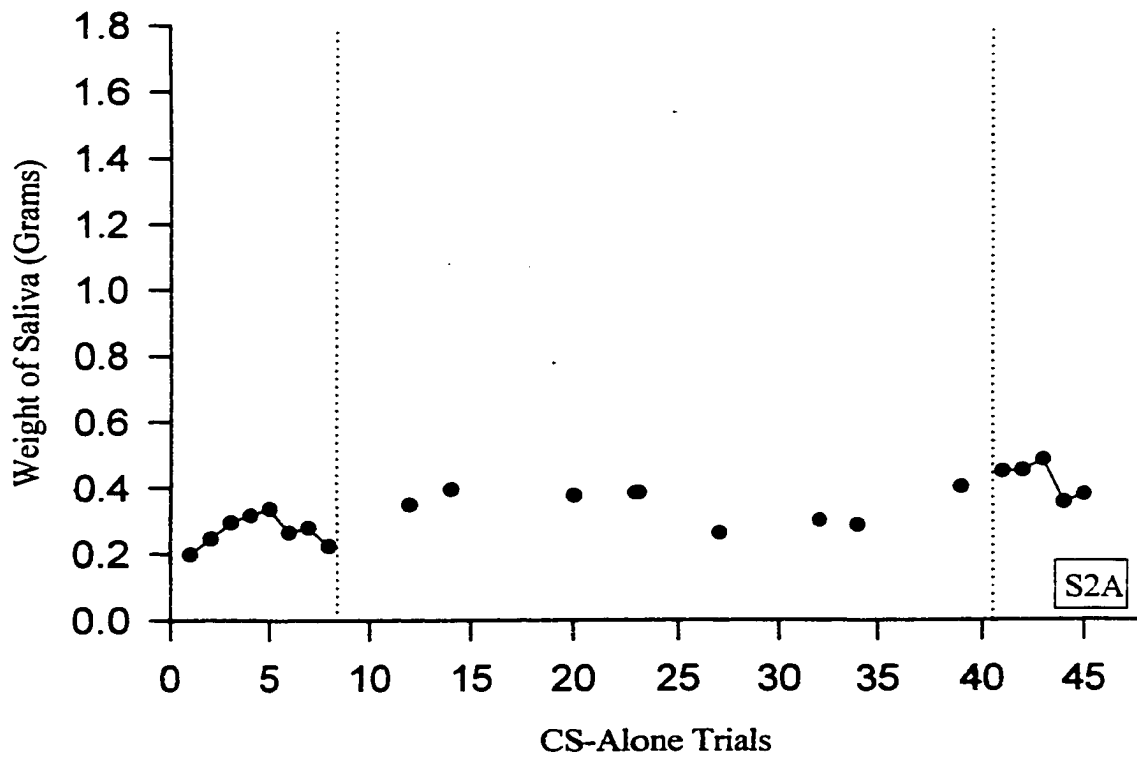
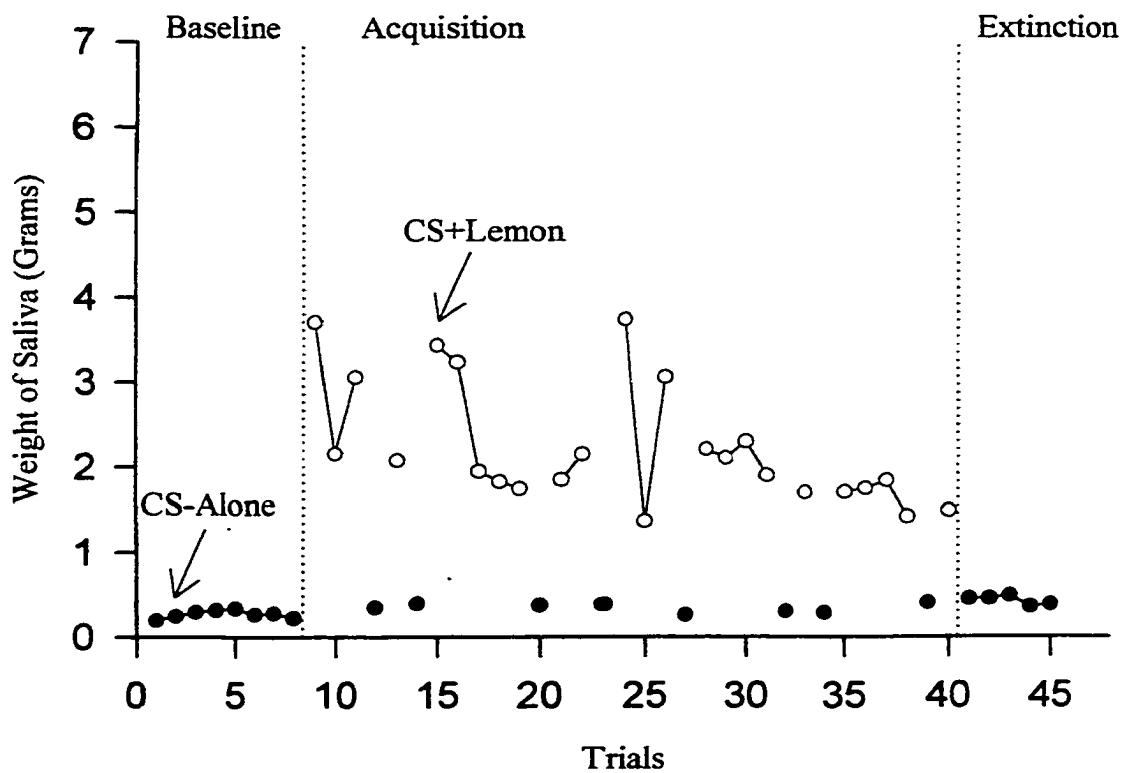
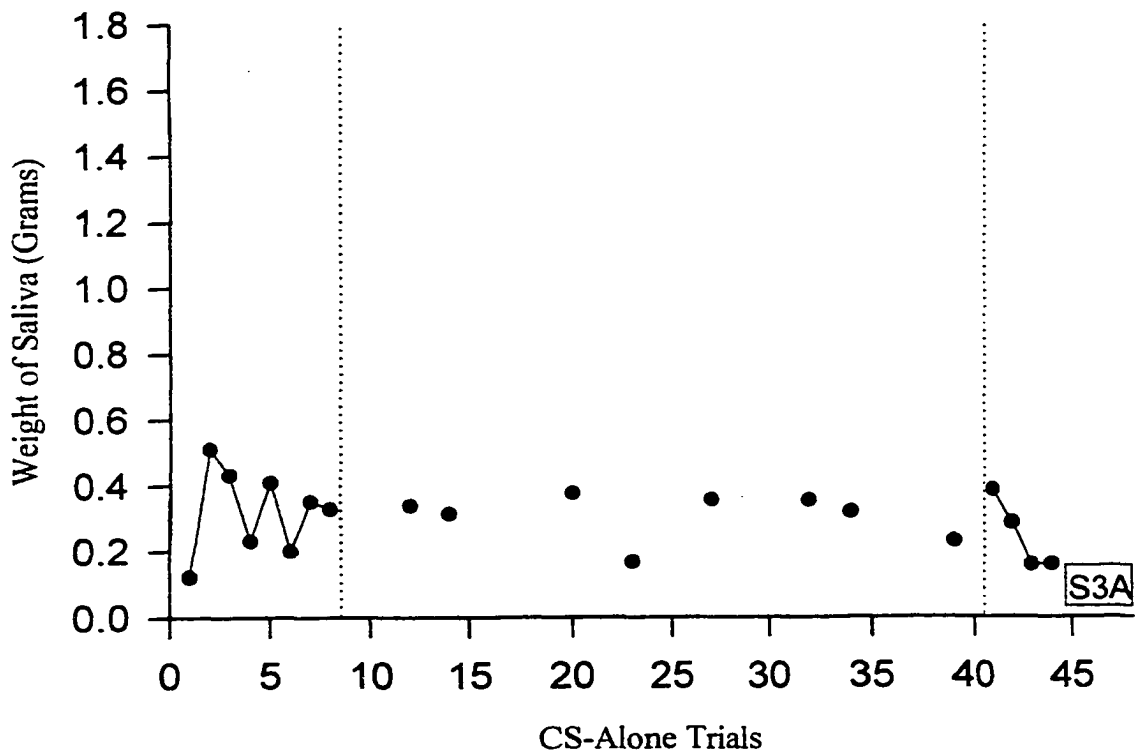
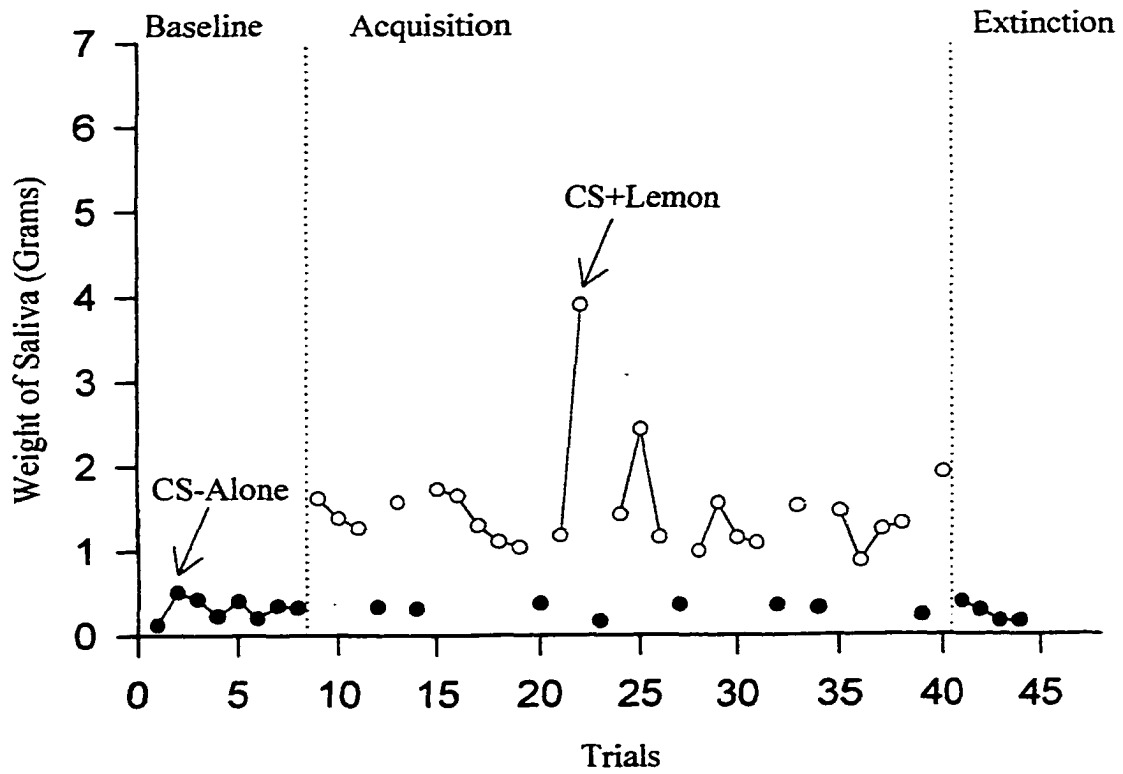


Figure Caption

Figure 3A. Weight of salivation in grams for S3A during baseline, acquisition, and extinction conditions for consecutive trials.



the Mann-Wald time series analysis indicated that there was a significant increase in mean level for Subject S2A (Figure 2A) and no significant differences in mean level for Subject 1A or 3A (Figures 1A and 3A). Also, when comparing the CS-alone trials between the acquisition and extinction conditions, the Mann-Wald time series analysis indicated that there was a significant increase in slope for Subject 3A (Figure 3A) and no significant differences in slope for Subjects 1A and 2A (Figures 1A and 2A).

Discussion

The results from the first 3 pilot subjects did not show the acquisition of a conditioned salivary response. It is possible that several events prior to participation in the experiment may have increased the expectancy of tasting the US. These events included the following: Subjects' (a) reading the criteria to participate on the human subject participation bulletin board that specifically informs them that they cannot have allergies to lemons, limes or oranges; (b) reading the informed consent form and signing the form indicating that they agree to participate and do not have allergies to lemons, limes or oranges; and (c) hearing from the experimenter that a stimulus will be placed under the tongue. If salivation increased due to expectancy effects, when subjects were presented with CS-Alone trials, results would be similar whether the trials were presented during the Baseline, Acquisition, or Extinction phase. Consequently, it would appear as if conditioning did not occur during or following Acquisition trials. Therefore, to reduce anticipatory salivation due to expectancy during the Baseline and

Extinction Phases, the procedure was modified for the next 9 pilot subjects.

These subjects were informed prior to each phase whether or not to expect a lemon stimulus under the tongue.

Appendix C

Description and Results of Research with 9 Pilot Subjects

The purpose of this pilot research was to demonstrate the acquisition and extinction of the conditioned salivary response. The procedure was modified for the next nine subjects to reduce anticipatory salivation due to expectancy during the Baseline and Extinction Phases. To reduce expectancy effects, all subjects were informed prior to each phase whether or not to expect a lemon stimulus under their tongue.

Method

Subjects

Eight female subjects and one male subject, between the ages of 18-45, served as pilot subjects. Six subjects were recruited through the Human Subjects Pool at Queens College and received credit toward a class requirement for 2 hours of participation in this study. Three subjects were staff members who were recruited by word-of-mouth at Family Residences and Essential Enterprises, Inc. (FREE). Each subject met the criteria to participate that were described for the first pilot study (Appendix B). The Queens College Institutional Review Board of the City University of New York gave approval for the procedures used in this study.

Setting

The setting was the same as in the first pilot study (Appendix B) for Subjects S4A through S9A. The settings for Subjects S10A through S12A were as follows:

Subject S10A. The session was conducted in the kitchen of the subject's brother. The kitchen measured 3.04 m in width, 6.10 m in length, and 2.44 m in height. The screen was placed on the kitchen table and the subject sat in a chair behind the screen. The screen blocked the subject from viewing any food. Interruptions occurred when a person entered the kitchen area during the 21st trial. The subject also made occasional comments during the session.

Subject S11A. The session was conducted in the kitchen of the subject's home. The kitchen measured 2.43 m in width, 4.88 m in length, and 2.44 m in height. The screen was placed on the kitchen table and the subject sat in a chair behind the screen. The screen blocked the subject from viewing any food. Interruptions occurred during the session as follows: a dog barking during the 5th - 6th trial, 15th - 16th trial, 30th - 31st trial, and 33rd trial; a jet engine during the 35th trial; a boat engine during the 41st trial.

Subject S12A. The session was conducted in the kitchen of the subject's home. The kitchen measured 6.10 m in width, 6.10 m in length, and 2.44 m in height. The screen was placed on the kitchen table and the subject sat in a chair behind the screen. The screen blocked the subject from viewing any food. There were frequent interruptions by the telephone prior to trials 12, 15, 18 and 25. After one phone call, the subject informed the experimenter that she would be receiving a delivery within the next 20 minutes and would need to leave the table for while. As a result, the Acquisition Phase was prematurely discontinued so that the Extinction Phase was completed prior to the disruption.

Stimuli, Materials, and Apparatus

All stimuli, materials and apparatus were the same as used in the first pilot study (Appendix B).

Experimental Design and Procedure

The same experimental design and procedure was used as in the first pilot study (Appendix B) except for one change. In this experiment, subjects were informed prior to each phase whether or not to expect a lemon stimulus under the tongue. Prior to the Baseline and Extinction Phases, the subjects were told that no lemon would be put under the tongue. Prior to the Acquisition Phase, the subjects were told that on some trials, lemon would be put under the tongue.

Results

Figures 4A through 12A show the weight of salivation in grams for each trial during the Baseline, Acquisition, and Extinction Phases for each individual subject. The top graph in each figure displays performance for CS-Alone trials during Baseline, Acquisition and Extinction Phases, and paired CS + Lemonade trials during the Acquisition Phase. Performance on CS-Alone trials during each phase is re-plotted in the bottom graph; the ordinate axis is re-scaled so that changes in responding could be more readily observed.

Subjects S4A, S7A and S11A (Figures 4A, 7A, and 11A, respectively) showed increases in conditioned salivary responding on test trials during the acquisition condition when compared with salivary responding during the baseline condition and decreases in conditioned salivary responding during the extinction condition

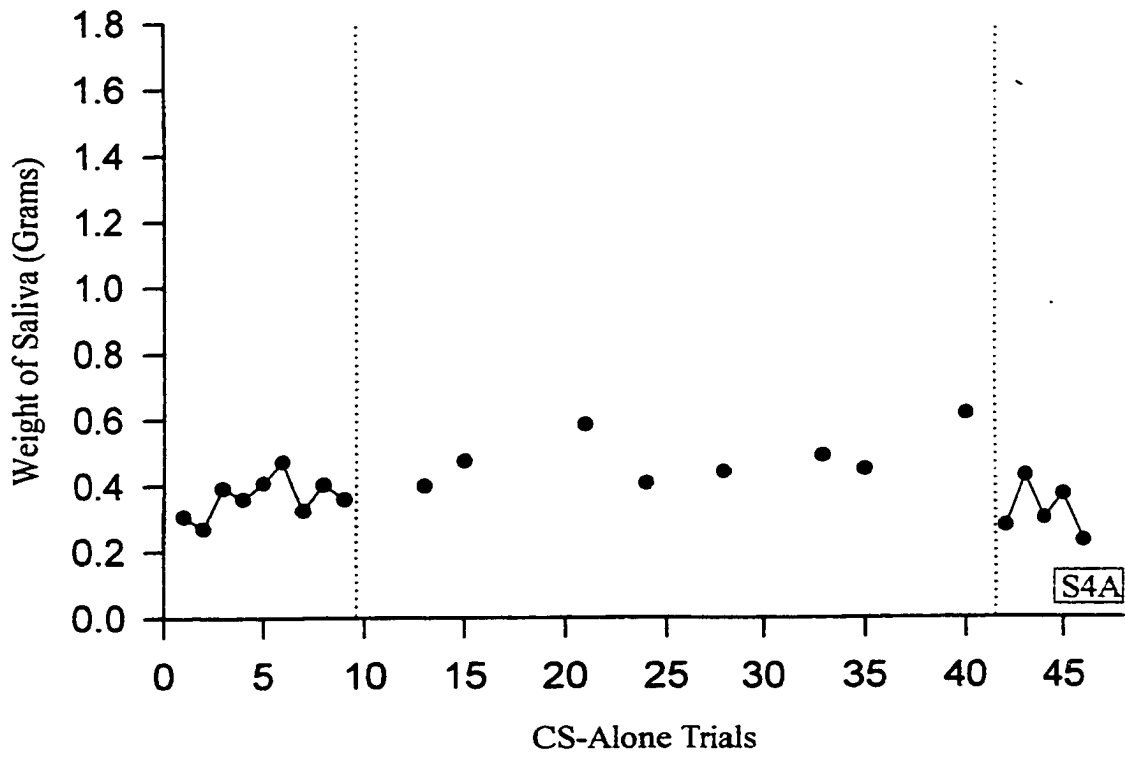
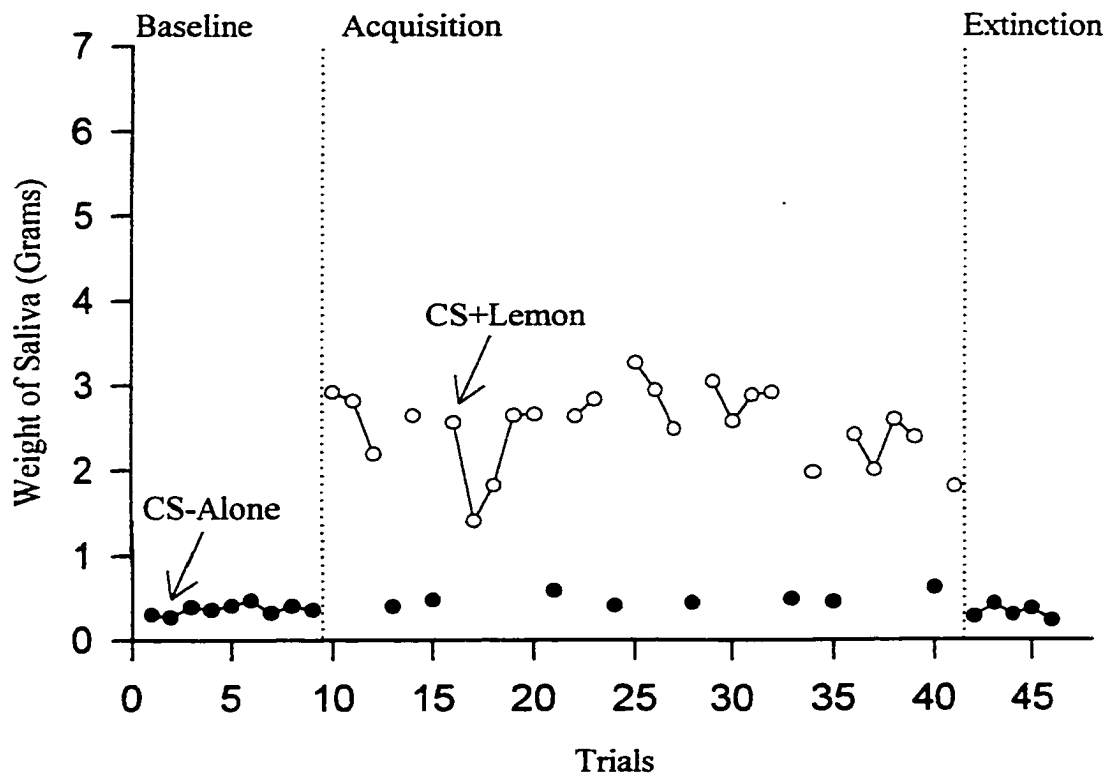
when compared with salivary responding on test trials during the acquisition condition. Subjects S6A, S8A, and S10A (Figures 6A, 8A, and 10A, respectively) showed increases in salivary responding on test trials during the acquisition condition when compared with salivary responding during the baseline condition, but they did not show decreases in salivary responding on test trials during the extinction condition when compared with salivary responding during the acquisition condition. Subject S9A (Figure 9A) showed a decrease in salivary responding on test trials during the extinction condition when compared to salivary responding on test trials during the acquisition condition.

The Mann-Wald time series analysis (Jones, Vaught, & Weinrott, 1977) was used to determine whether there were statistically significant differences in the mean level and slope of the functions of two consecutive conditions (i.e., baseline and acquisition or acquisition and extinction) for each subject. An alpha level of .05 was used for all tests.

The Mann-Wald time series analysis indicated that there were significant increases in the mean level between baseline and acquisition CS-alone trials for Subjects S4A, S6A, S7A, S8A, S10A, and S11A (Figures 4A, 6A, 7A, 8A, 10A, 11A, respectively) but not for the other three subjects. No subjects showed significant differences in slope between baseline and acquisition CS-alone trials. When comparing the CS-alone trials between the acquisition and extinction conditions, there were significant decreases in mean level for Subjects S4A, S7A, S9A, and S11A (Figures 4A, 7A, 9A, and 11A, respectively) but not for the

Figure Caption

Figure 4A. Weight of salivation in grams for S4A during baseline, acquisition, and extinction conditions for consecutive trials.



S4A

Figure Caption

Figure 5A. Weight of salivation in grams for S5A during baseline, acquisition, and extinction conditions for consecutive trials.

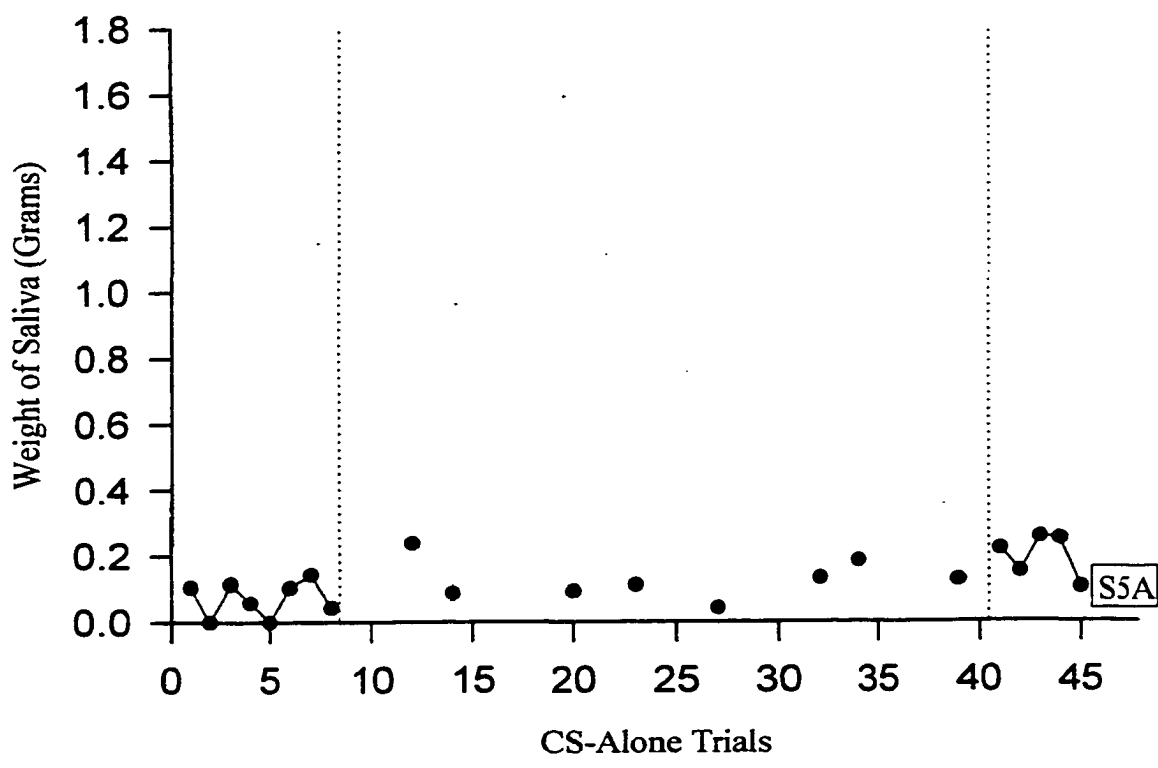
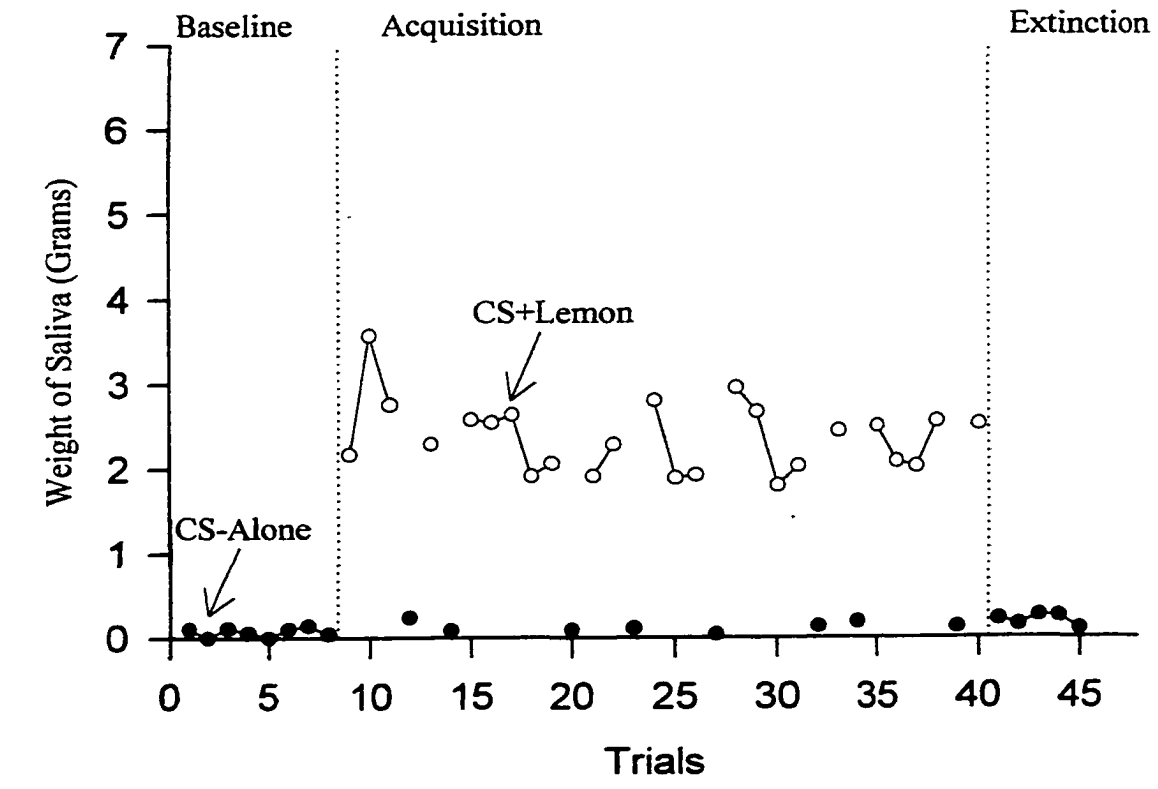
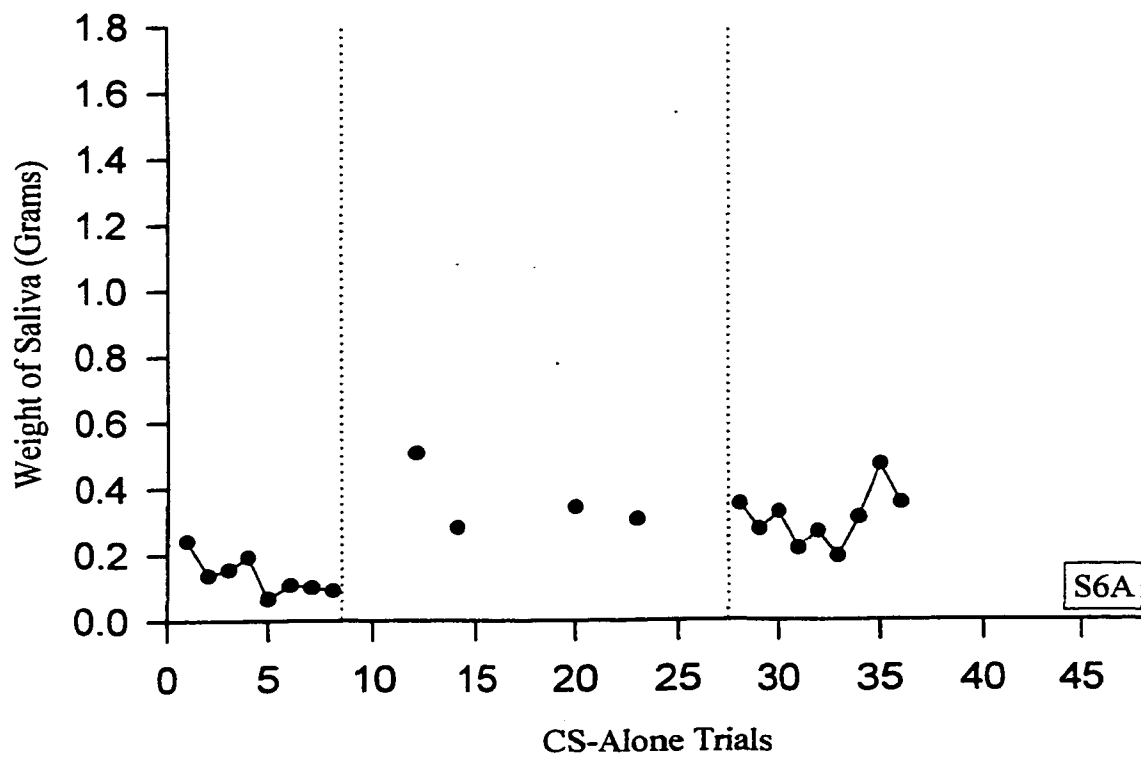
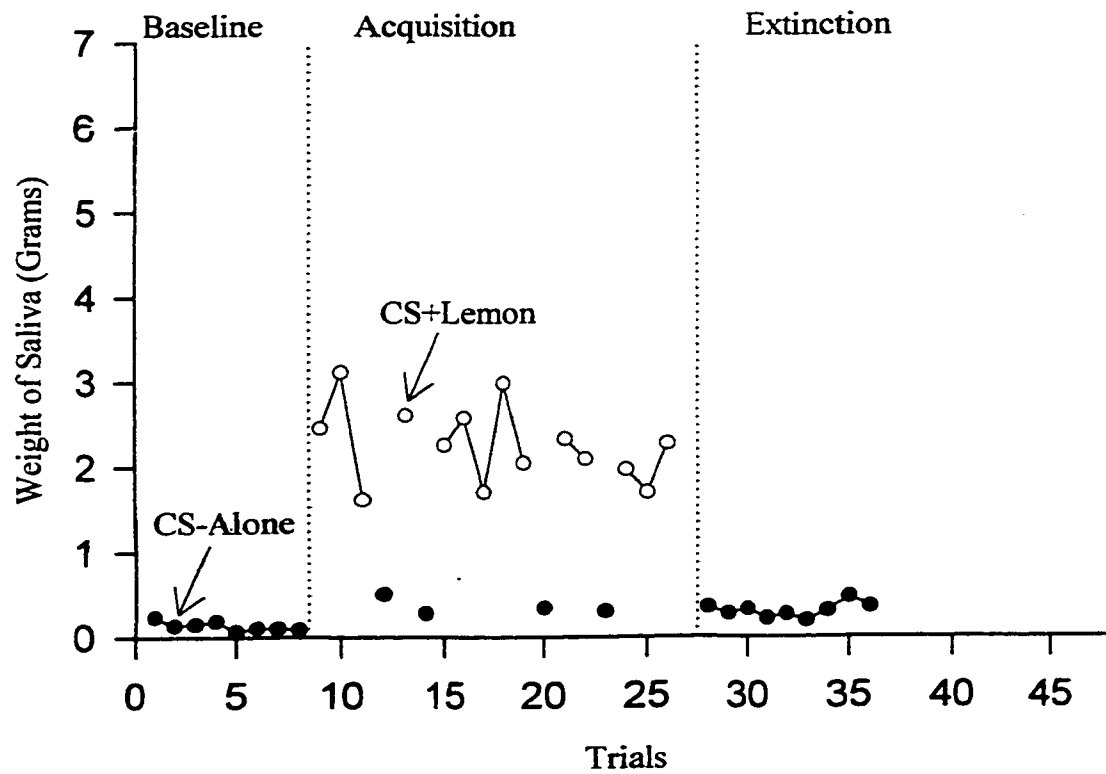


Figure Caption

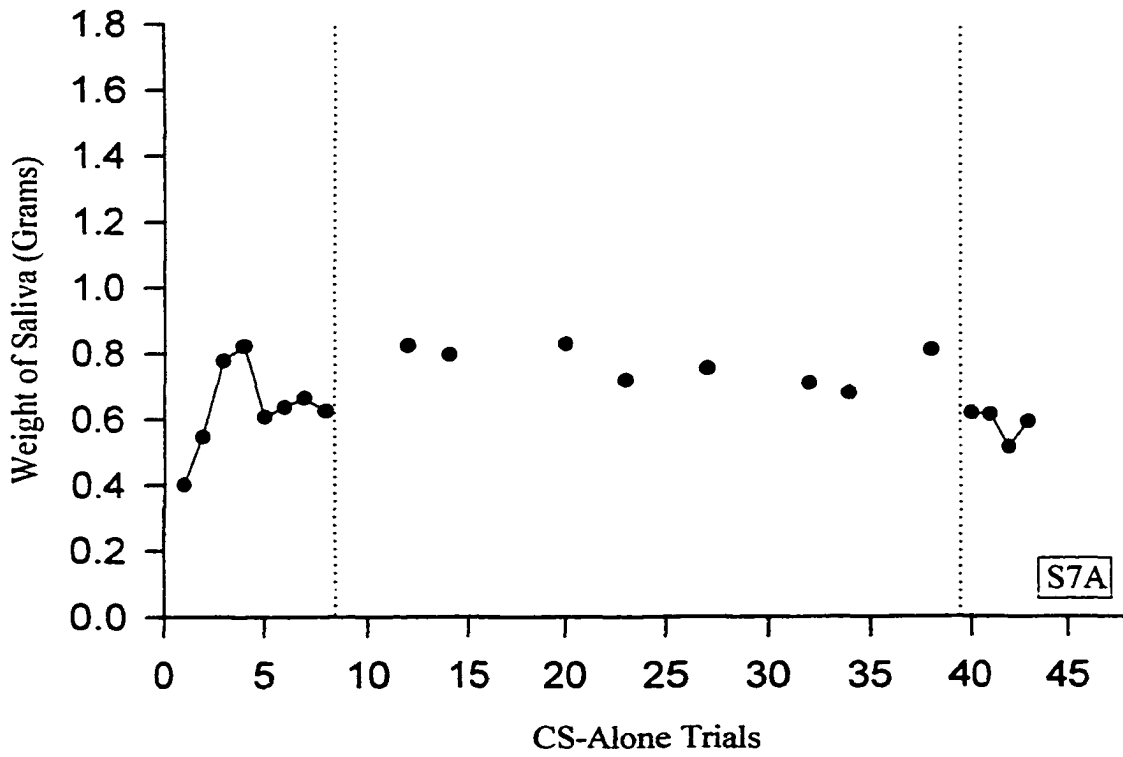
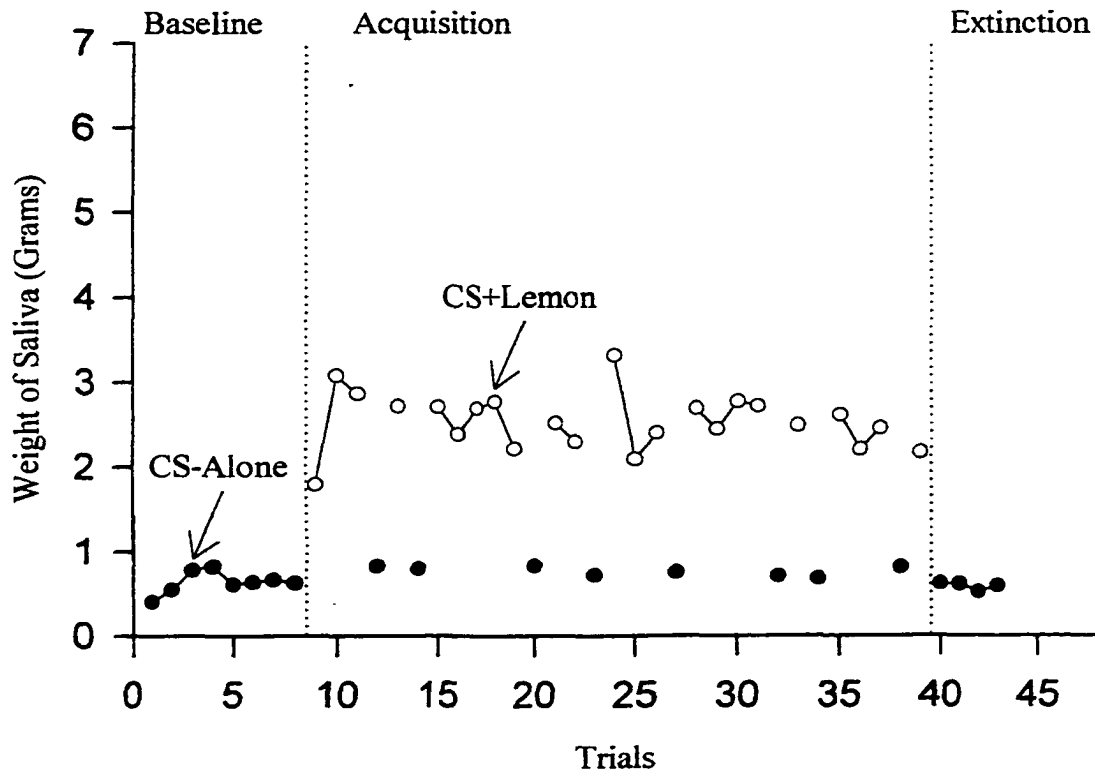
Figure 6A. Weight of salivation in grams for S6A during baseline, acquisition, and extinction conditions for consecutive trials.



S6A

Figure Caption

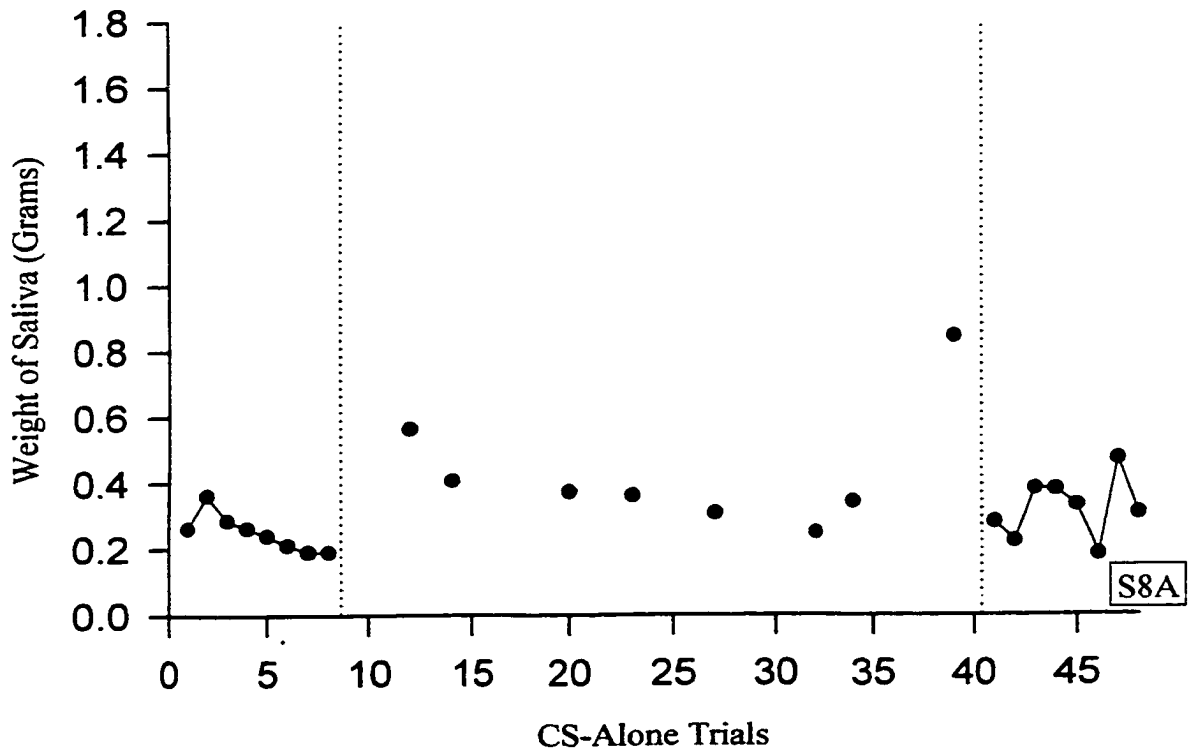
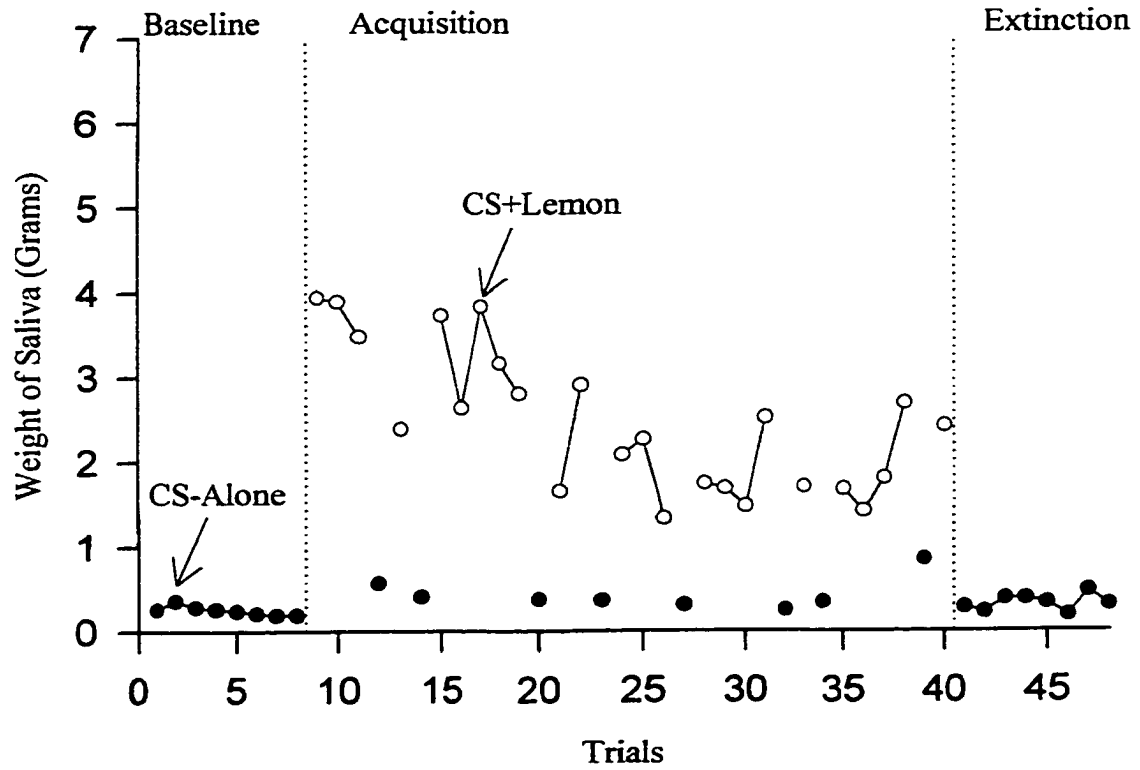
Figure 7A. Weight of salivation in grams for S7A during baseline, acquisition, and extinction conditions for consecutive trials.



S7A

Figure Caption

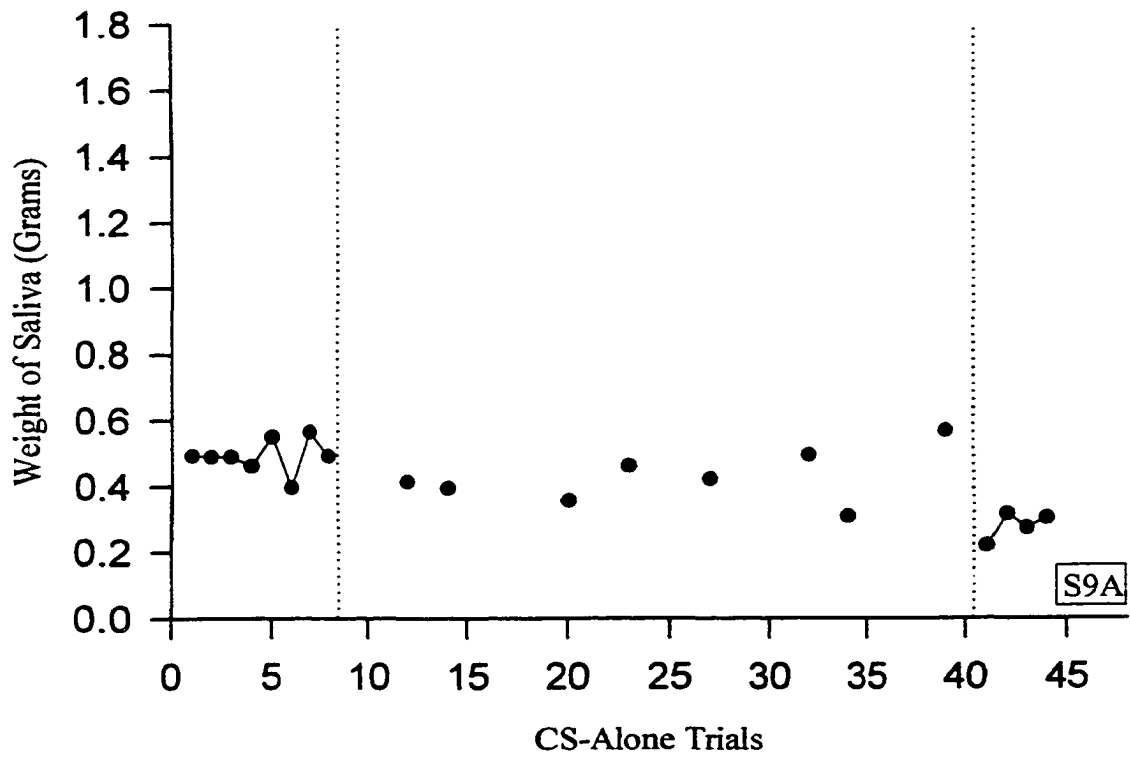
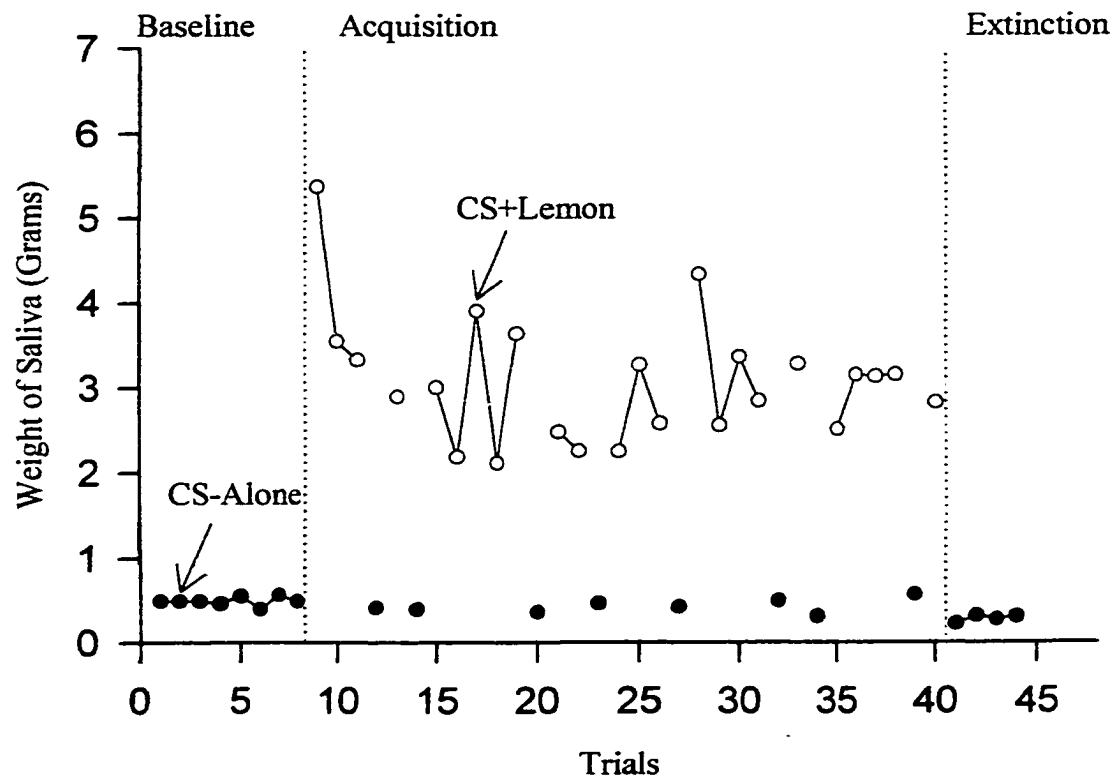
Figure 8A. Weight of salivation in grams for S8A during baseline, acquisition, and extinction conditions for consecutive trials.



S8A

Figure Caption

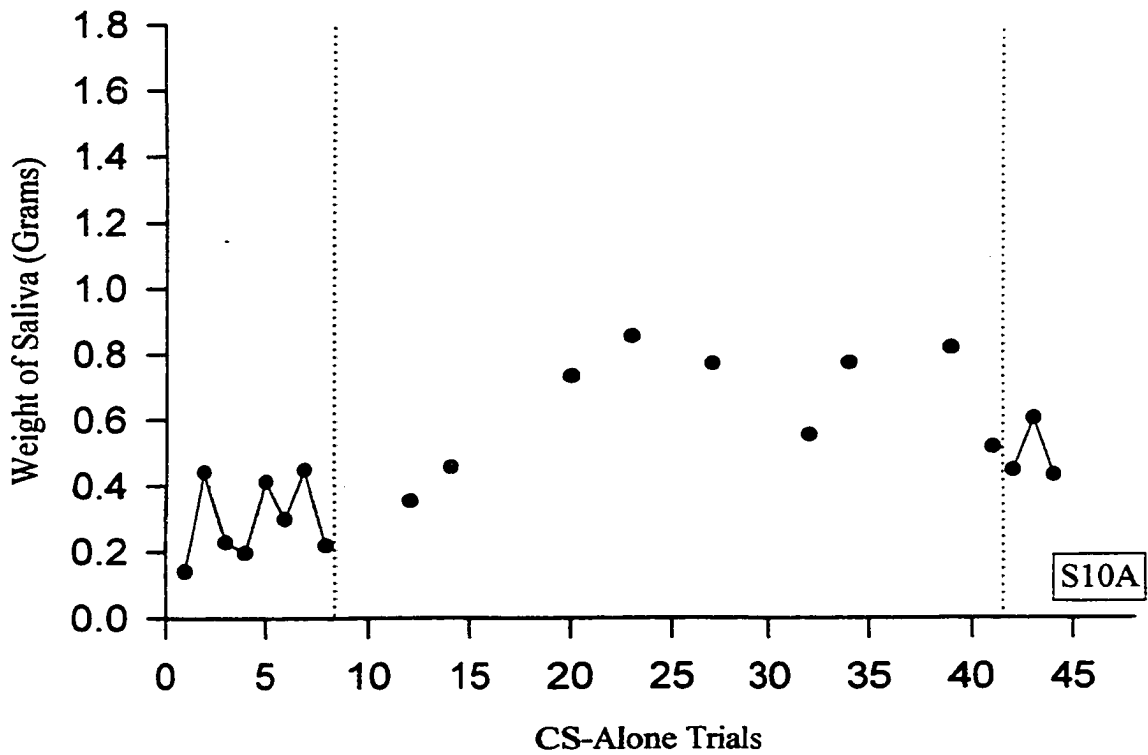
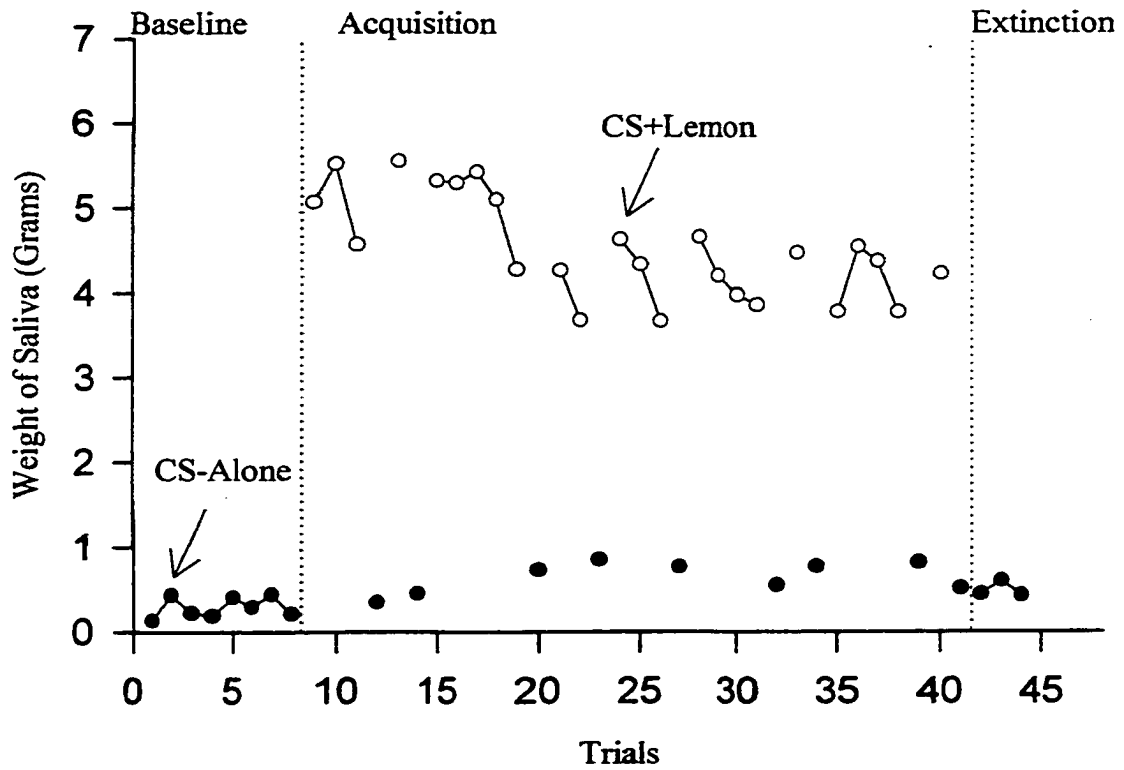
Figure 9A. Weight of salivation in grams for S9A during baseline, acquisition, and extinction conditions for consecutive trials.



S9A

Figure Caption

Figure 10A. Weight of salivation in grams for S10A during baseline, acquisition, and extinction conditions for consecutive trials.



S10A

Figure Caption

Figure 11A. Weight of salivation in grams for S11A during baseline, acquisition, and extinction conditions for consecutive trials.

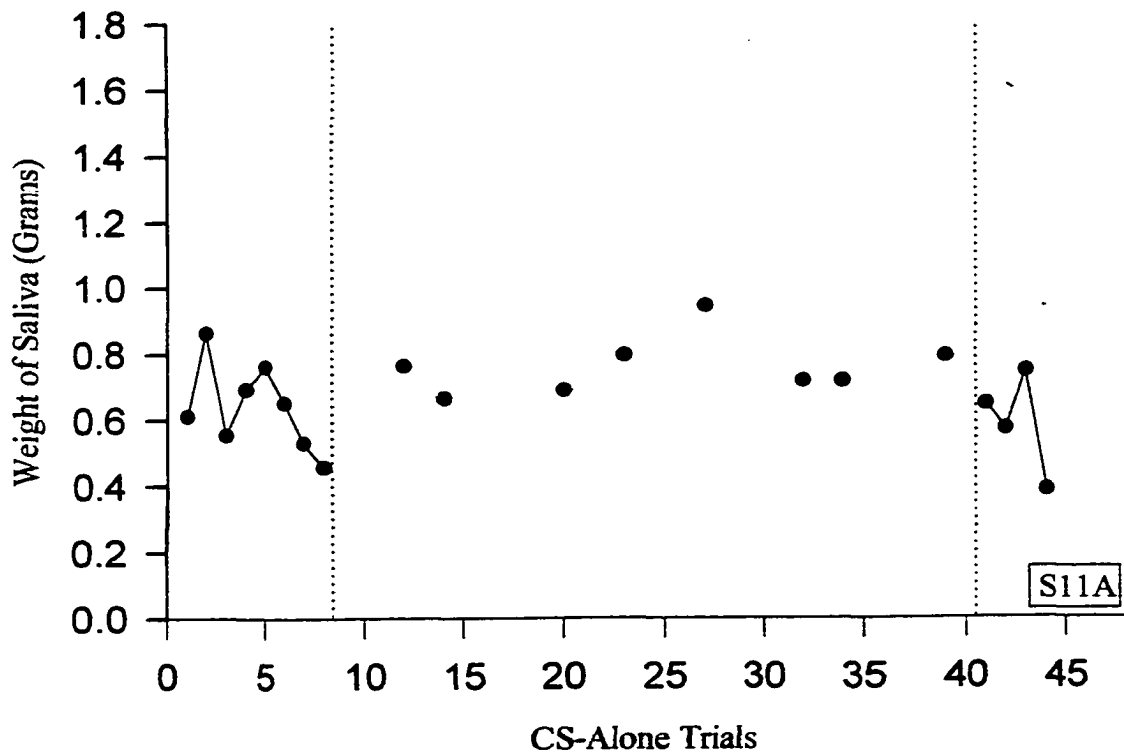
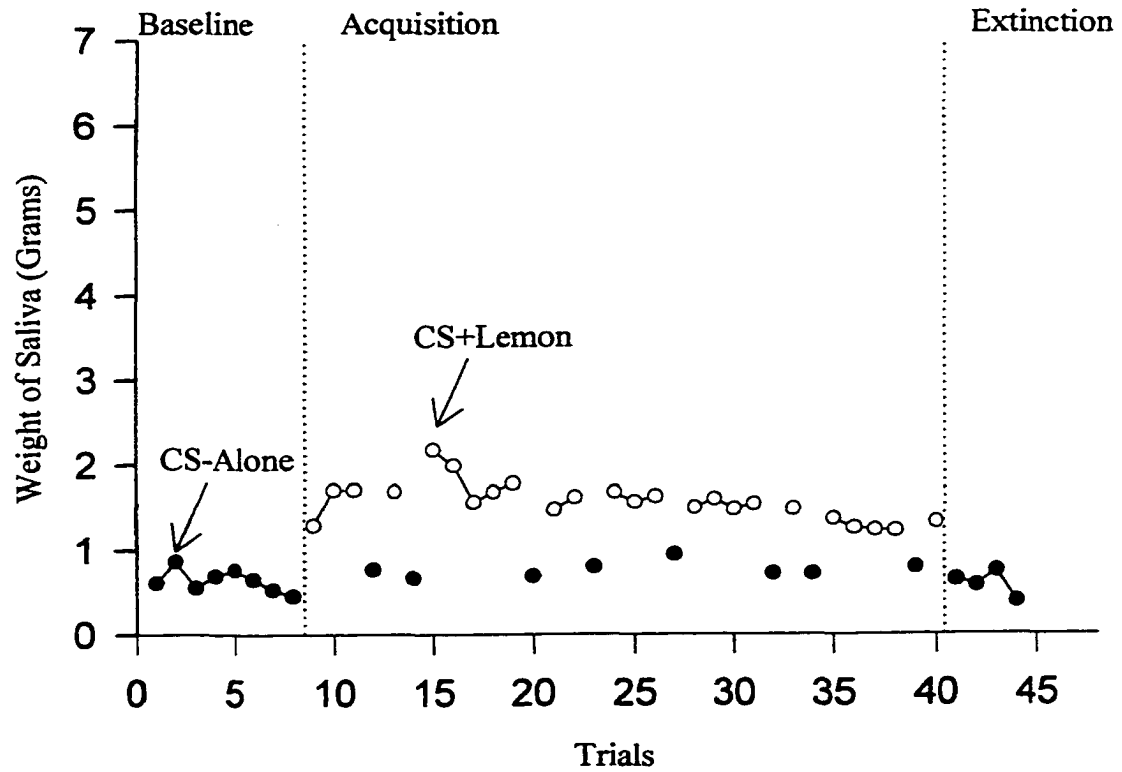
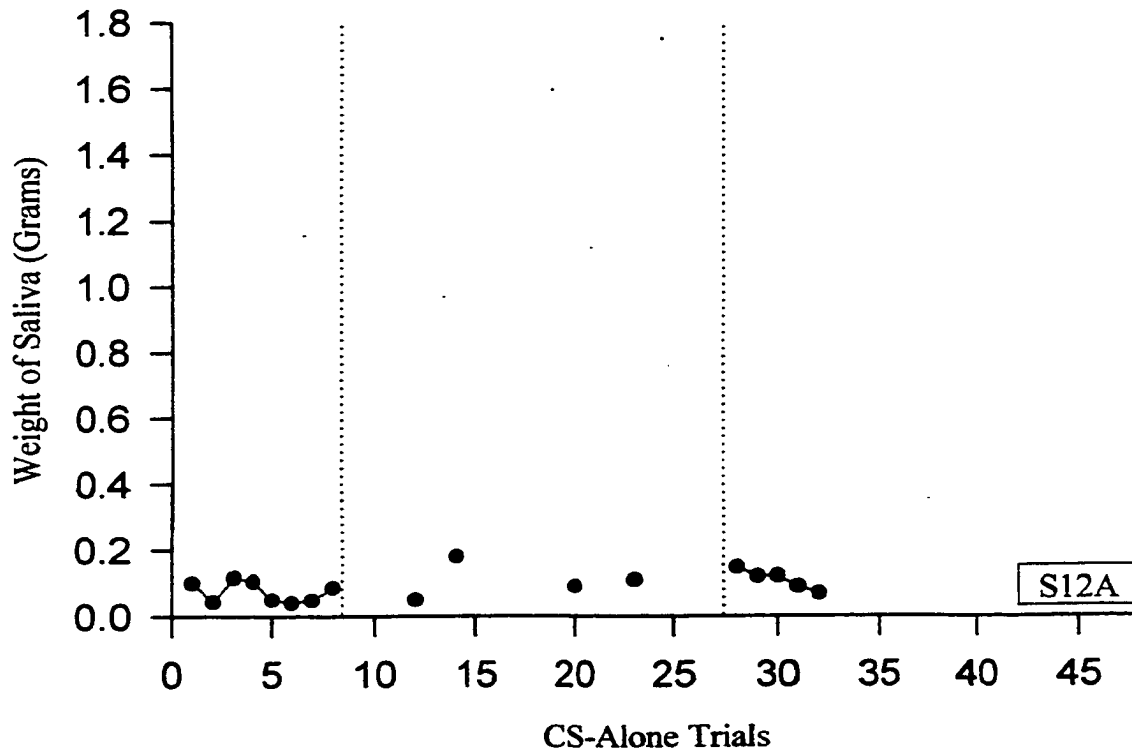
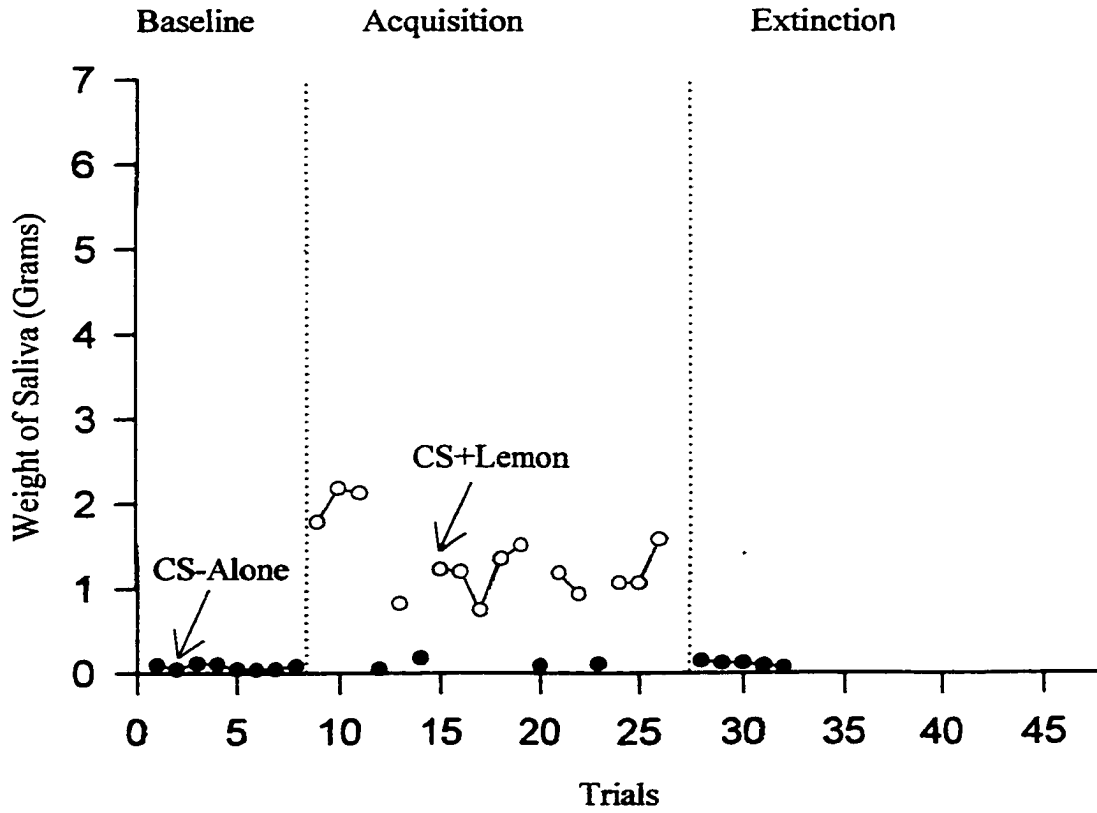


Figure Caption

Figure 12A. Weight of salivation in grams for S12A during baseline, acquisition, and extinction conditions for consecutive trials.



S12A

other five subjects. No subjects showed significant differences in slope between acquisition and extinction CS-alone trials.

Discussion

The present results showed the acquisition and extinction of a conditioned salivary response for three subjects. As a result of the difficulty in demonstrating conditioned salivary responding, five changes were made from this pilot study for the present experiment. These changes were as follows: (1) the US consisted of 3 ml of lemonade placed on the tongue rather than 0.20 ml lemon juice placed under the tongue; (2) the subjects were instructed to move the stimulus over the entire mouth rather than to limit contact to under the tongue; (3) the CS consisted of a 15-s tone and a 0.04% vanilla solution placed on the tongue and moved over the entire mouth rather than only a 15-s tone; (4) the number of extinction trials was increased to 16 trials rather than 4 to 8 trials; (5) the instructions stating whether or not to expect a lemon stimulus under the tongue were eliminated. It was anticipated that with these changes, the acquisition of a conditioned response would be demonstrated.

Appendix D

Instructions to Subjects

During the session, try to minimize your movement and remain in the chair at all times. In some cases, you may be presented with a tone stimulus. When you hear the tone, open your mouth and move it to the opening in the screen. You may have a stimulus placed in your mouth, and if so, move the stimulus so that it makes contact with your entire mouth. Continue to move the stimulus in your mouth until the offset of the time. Upon the offset of the tone, move your mouth to the opening in the screen again. Another stimulus may be placed in your mouth and if so, move the stimulus so that it makes contact with your entire mouth. Continue to move the stimulus in your mouth until you are given another instruction. Next, you will be handed a cup and asked to spit into it three times to empty your mouth. At times, you will then be handed a cup of water to rinse your mouth and should discard the remains into a waste container that will be located on your right. There may be times that you will be given no instructions, and you should remain seated and rest quietly while swallowing normally. Do you understand the procedure so far? After the subject acknowledged that he or she understood the experiment, the subject was given the following instructions: Please sit quietly, minimize your body movement and keep your head tilted slightly forward in a comfortable position at all times and relax and swallow normally. We will get started in a few minutes. (The subject was given the option to read materials unrelated to food). After the 10-minute period elapsed, the

subject was given the following instructions: We are ready to begin now, I'll take the reading material from you. Please sit quietly, minimize your body movement and keep your head tilted slightly forward in a comfortable position at all times.

Instructions for CS-alone Trials: Swallow once and then do not swallow again. (The 15-s tone begins, vanilla solution presented, after offset of tone for CS-Alone Trials the syringe is placed near the subjects mouth as if a solution was going to be presented.) Please try to spit into this cup three times to empty your mouth (subject handed cup). Please rinse out your mouth (subject handed water, rinses mouth, and returns cup to experimenter). You can relax and swallow normally.

Instructions for Acquisition Trials: Swallow once and then do not swallow again. (The 15-s tone begins, vanilla solution presented, after offset of tone, 3 ml of lemonade placed in mouth for 30 seconds) Please try to spit into this cup three times to empty your mouth (subject handed cup). Please rinse out your mouth (subject handed water, rinses mouth, and returns cup to experimenter). You can relax and swallow normally.

Appendix E

Table E1

Individual trial data for Subject S1.

Trial #	Data	Trial #	Data	Trial #	Data
1	.151	21	.391	41	1.342
2	.110	22	1.654	42	.676
3	.259	23	1.160	43	.543
4	.082	24	.513	44	.524
5	.005	25	1.093	45	.469
6	.003	26	1.310	46	.394
7	.256	27	1.100	47	.376
8	.197	28	.345	48	.189
9	.187	29	1.548	49	.399
10	1.381	30	1.330	50	.209
11	1.188	31	1.646	51	.387
12	1.770	32	1.878	52	.180
13	.669	33	.471	53	.144
14	2.087	34	1.504	54	.287
15	.447	35	.349	55	.235
16	1.500	36	1.120	56	.283
17	1.853	37	1.729	57	.226
18	1.513	38	1.357		
19	1.814	39	1.317		
20	1.311	40	.513		

Appendix E

Table E2

Individual trial data for Subject S2.

Trial #	Data	Trial #	Data	Trial #	Data
1	1.096	21	2.738	41	1.673
2	1.022	22	3.125	42	1.083
3	.805	23	1.288	43	.791
4	1.014	24	2.932	44	.967
5	.938	25	2.654	45	1.180
6	.988	26	1.822	46	1.127
7	.903	27	1.124	47	.916
8	.580	28	2.559	48	.984
9	2.133	29	2.326	49	.993
10	2.870	30	2.886	50	1.070
11	2.213	31	2.669	51	.876
12	.868	32	1.389	52	1.008
13	2.703	33	2.769	53	1.057
14	.973	34	1.196	54	1.018
15	3.521	35	2.298	55	.808
16	2.635	36	3.773	56	.948
17	2.732	37	2.748		
18	2.885	38	2.783		
19	2.650	39	1.181		
20	1.274	40	2.114		

Appendix E

Table E3

Individual trial data for Subject S3.

Trial #	Data	Trial #	Data	Trial #	Data
1	.115	21	3.045	41	.440
2	.119	22	3.347	42	.377
3	.117	23	.423	43	.360
4	.147	24	3.545	44	.345
5	.030	25	3.616	45	.311
6	.114	26	2.754	46	.265
7	.110	27	.224	47	.260
8	.099	28	3.270	48	.307
9	2.384	29	2.957	49	.227
10	3.206	30	3.311	50	.500
11	3.063	31	3.001	51	.311
12	.172	32	.409	52	.316
13	2.844	33	3.321	53	.150
14	.246	34	.350	54	.115
15	3.343	35	3.481	55	.112
16	2.901	36	3.211	56	.004
17	3.097	37	3.009		
18	3.057	38	2.740		
19	3.110	39	.496		
20	.170	40	3.491		

Appendix E

Table E4

Individual trial data for Subject S4.

Trial #	Data	Trial #	Data	Trial #	Data
1	.251	21	.688	41	3.701
2	.545	22	3.420	42	.725
3	.314	23	3.512	43	.722
4	.294	24	.832	44	.360
5	.388	25	3.173	45	.635
6	.502	26	2.137	46	.734
7	.566	27	3.544	47	.761
8	.419	28	.741	48	.624
9	.400	29	5.368	49	.629
10	3.478	30	3.620	50	.582
11	3.716	31	3.617	51	.221
12	4.278	32	3.912	52	.166
13	.943	33	.887	53	.338
14	3.720	34	3.555	54	.558
15	.752	35	.800	55	.243
16	3.339	36	3.221	56	.365
17	3.220	37	3.496	57	.355
18	3.453	38	3.812		
19	3.446	39	3.411		
20	3.689	40	.945		

Appendix E

Table E5

Individual trial data for Subject S5.

Trial #	Data	Trial #	Data	Trial #	Data
1	.087	21	4.519	41	.612
2	.573	22	5.492	42	.568
3	.509	23	.613	43	.586
4	.433	24	5.331	44	.303
5	.543	25	5.488	45	.474
6	.436	26	4.821	46	.435
7	.423	27	.652	47	1.027
8	.401	28	5.247	48	.382
9	4.858	29	5.382	49	.418
10	6.006	30	5.104	50	.505
11	5.515	31	5.260	51	.394
12	.825	32	.899	52	.575
13	5.413	33	4.546	53	.407
14	.766	34	.741	54	.439
15	5.580	35	4.632	55	.440
16	5.519	36	4.480	56	.312
17	4.413	37	4.368		
18	5.399	38	4.924		
19	5.427	39	.635		
20	.627	40	4.895		

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