

Primacy vs. Recency in Sugar-Reinforced Flavor Preference Learning

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### Primacy vs. Recency in Sugar-Reinforced Flavor Preference Learning

If rats experience a specific flavor, say cherry, mixed with sugar, they later prefer that flavor over an alternative flavor (e.g., grape) even when the cherry no longer contains the sugar. That is, even when both flavors are presented in plain form, rats still choose the one they used to contain the sugar. This indicates that they remember which flavor had contained the sugar, and prefer that flavor. The present experiment will ask the following question: What if one flavor (e.g., Cherry) was sugared for an initial week of flavor training, but then the alternate flavor (e.g., Grape) was instead sugared for a second week of training? In a subsequent two-bottle unsugared choice test, would rats choose the flavor that originally contained sugar (primacy effect) or the one that most recently did so (recency effect).

## Method

### Subjects

The subjects for this study were eleven female rats of the Long-Evans strain, obtained from Charles River Laboratories in Wilmington, MA. They were approximately 50 days old at the beginning of the experiment. Each rat was housed in a separate, hanging wire mesh cage (10" long, 8" high, 8" wide) alongside other rats in a standard cage rack. Food (Mazuri rat chow) was accessible through a mesh-faced food hopper, and tap water was provided through a 250-ml glass bottle equipped with a stainless-steel sipper tube. Both were attached to the front of each cage.

### Conditioning solutions

Both flavored solutions were made by mixing one two-quart size paper packet of unsweetened Cherry or Grape Kool-Aid with 2000 ml of either plain tap water (the PL solution) or sugared water (the SUC solution). The sugared water was created by dissolving 200 grams of

sucrose (table sugar) into 300 ml of warm water, and then adding enough cold water to reach 2000 ml. This produced a 10% sugar solution.

### **Procedures**

**Conditioning Procedure.** The goal of conditioning was to present Subjects with sugar-reinforced Cherry & Grape Kool-Aid solutions. The procedure spanned two weeks, with the first week starting Monday, 2 October 2017, introducing one sugar-reinforced flavor solution and one plain solution of the opposing flavor. On any given day Subjects were only able to consume one flavor, that was either sweetened or plain, which was determined by a schedule. On the second week, starting Monday, 9 October 2017, we swapped the sugar-reinforcer to the other flavor. For example, if during week 1 the sugar-reinforced flavor was Grape and the plain flavor Cherry, in week 2 Grape would become plain while Cherry would become sugar-reinforced. Through the two weeks, every Subject became paired to two sugar-reinforced flavors. It is already known that the Subjects will prefer the sugar-reinforced flavor, however we investigated if they would prefer the first or last sugar-reinforced flavor (primacy vs. recency).

Subjects were in one of two groups, either G[C] or C[G]. The first letter indicates the sugar-reinforced flavor during week 1, whereas the second letter in brackets indicates the sugar-reinforced flavor for week 2. The method with which Subjects were placed into their conditioning groups was through random assignment. There were two groups to see if the Subjects preferred a flavor due to something inherent about it, or if was due to primacy or recency. Meaning, that by having two groups we could see if, for example, Subjects consistently picked Grape over Cherry; for if that were the case, then there would be something unique about the Grape flavor that caused this phenomenon. However, what we primarily investigated in this study was primacy vs. recency in sugar-reinforced learning. Throughout the two weeks of the

conditioning procedure, Subjects were on different drinking schedules depending on if it was a weekday (Monday through Thursday) or during the weekend (Friday through Sunday).

Throughout the entirety of the conditioning process, Subjects always had access to a full hopper of food.

***Weekday Conditioning Procedure.*** Starting on Monday, 2 October 2017, and repeating every day until Thursday, Subjects were placed into individual cages each with their own food hopper and bottle. The bottle with a straight nozzle rested on the right side on the front of the cage resting in a nook attached to the hopper. The hopper slid on the left on the front of the cage and held food pellets for Subjects to consume. Once a day, every weekday, between 8 AM to 4 PM, Subjects had their hoppers filled to the max and bottles tended to by undergraduate students. There were approximately 24 hours between every time the Subjects were tended to.

The solution schedule for week 1 can be seen in Figure 1, where the G[C] group has sugar-reinforced Grape, on Monday & Wednesday, and plain Cherry on Tuesday & Thursday. In week 2, the G[C] group will have sugar-reinforced Cherry on Monday & Wednesday, and plain Grape on Tuesday & Thursday (see Figure 1 for full drinking schedule). Regardless of whether or not the solution was sugar-reinforced, Subjects were always given 100 ml of flavored solution. Undergraduate students went into the laboratory and measured the amount of flavored solution consumed by Subjects, then after washing the bottles filling them with a new flavor solution. The amount consumed was measured by pouring the remainder solution into a 250-ml beaker, then subtracting from 100 the amount of ml remaining. Flavors would alternate between sugar-reinforced and plain. This procedure would be repeated every day until Friday, when the Subjects drinking schedules changed over to the weekend schedule; which is not shown in Figure 1, but is instead described in the next section.

<i>Figure 1. Week 1</i>	<u>Monday</u>	<u>Tuesday</u>	<u>Wednesday</u>	<u>Thursday</u>
Day's Flavor	Grape	Cherry	Grape	Cherry
G[C]	<b>Sugar</b>	Plain	<b>Sugar</b>	Plain
C[G]	Plain	<b>Sugar</b>	Plain	<b>Sugar</b>
Week 2	<u>Monday</u>	<u>Tuesday</u>	<u>Wednesday</u>	<u>Thursday</u>
Day's Flavor	Grape	Cherry	Grape	Cherry
G[C]	Plain	<b>Sugar</b>	Plain	<b>Sugar</b>
C[G]	<b>Sugar</b>	Plain	<b>Sugar</b>	Plain

***Weekend Conditioning Procedure.*** Starting on Friday, Subjects had their drinking schedules changed to a free drinking schedule. The schedule change was made sometime between 8 AM through 4 PM, the same way Subjects were fed on the weekdays. Subjects had their bottles filled with approximately 250 ml of tap water (the bottles cannot hold more than 250 ml), while the hoppers were maintained the same way they were during the weekdays, always full of food. Subjects would not have their hoppers or bottles changed out or refilled until Monday, when the change back to their weekday drinking schedule was made.

***Testing Procedure.*** After two weeks of conditioning, the Subjects entered the testing phase. On 16 October 2017, Subjects had their cages readjusted for a Two-Bottle Test, testing to see if the Subjects had preferred the first (primacy) or last (recency) flavor. Every Subject was caged the same way they were during conditioning. For the testing phase, cages were set up as follows. Instead of having a hopper on the left on the front of the cage, with the bottle on the right resting in the nook of the hopper, there was no hopper and two bottles. The bottles each had

curved nozzles and were suspended by springs, one bottle on the left and the right on the front of the cage. Since there was no hopper for Subjects to consume food from, the Subjects were each given approximately 30 grams of food placed towards the back of the inside of their cage.

In addition to all of this, each cage had two spill cups placed below each bottle in the droppings tray. The spill cups were used to give us a measure of whether the Subjects consumed a lot of one flavor, or if they had just merely shaken the nozzle a lot. Throughout the designated week of testing, the water dummy test happened Monday to Tuesday, then the flavor test occurred Tuesday to Wednesday. After the water dummy test and flavor test, all testing stopped. The water dummy test occurred to accustom the Subjects to the presence of the spill cups and new cage setup. When the Subjects first saw the spill cups, they played with them rendering them ineffective. During the flavor test Subjects did not tamper with the spill cups enough to render them ineffective.

***Dummy Water Test Procedure.*** The dummy water test occurred on Monday, 16 October 2017. The goals of this ‘test’ were to accustom Subjects to the new cage setups and presence of spill cups. The conditioning procedure cage setups were taken off and the testing procedure setups were placed on the cages, sometime between 8 AM through 4 PM. There were two bottles, each bottle was filled with 100 ml of tap water. Data was not recorded from this test, it was only to accustom Subjects to the new cage layout of testing. The water dummy test lasted for approximately 24 hours.

***Flavor Test Procedure.*** The flavor test occurred on Tuesday, 17 October 2017. The goal of this test was to test if Subjects had a preference to either the first or last flavor they were introduced to with sugar. The bottles and spill cups from the dummy water test were exchanged for new bottles and spill cups for the flavor test between 8 AM through 4 PM. Large food pieces

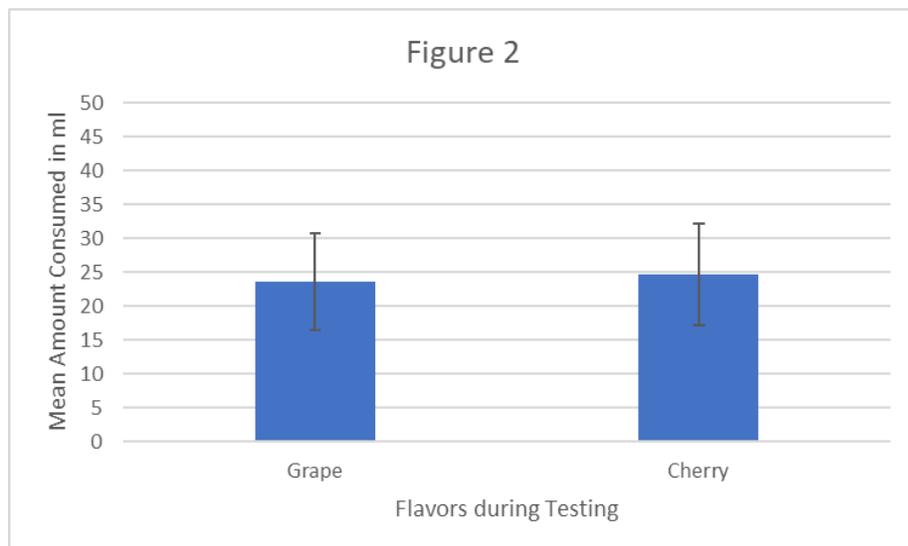
were put inside the back of the cage to total eight. The bottle on the left on the front of the cage was filled with plain Cherry, while the bottle to the right was filled with plain Grape. Each bottle was filled with 100 ml of the solution. The flavor test lasted for approximately 24 hours.

Between the hours of 8 AM through 4 PM on 18 October 2017, the bottles and spill cups were poured into two 250 ml beakers to measure the amount of solution consumed. If there was any excess debris in the spill cups, it was removed to measure only the solution in the spill cups.

**Results**

**Flavor Test Results**

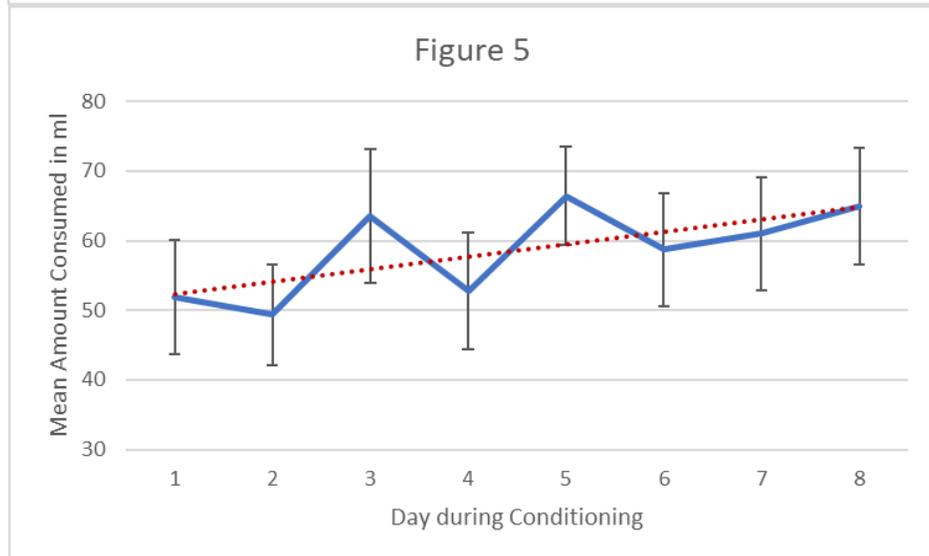
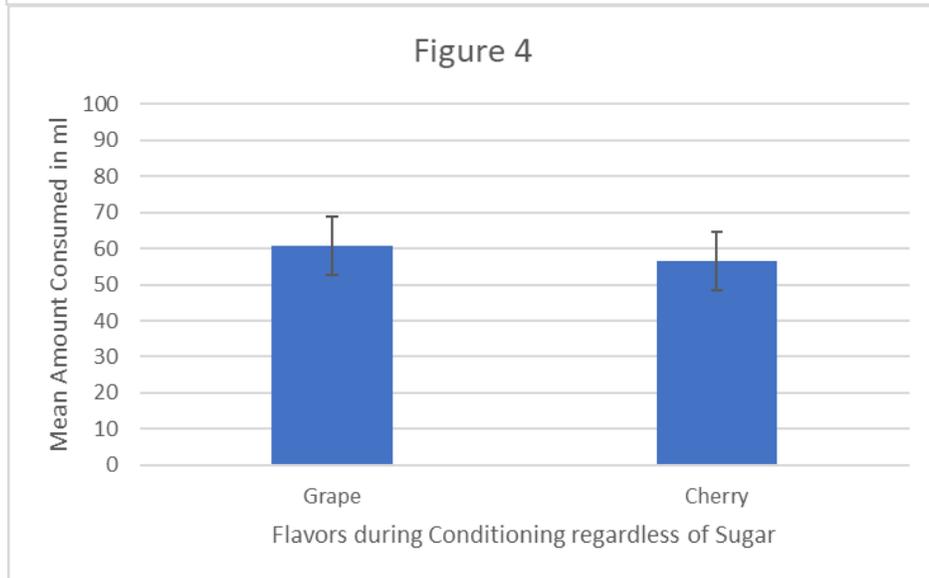
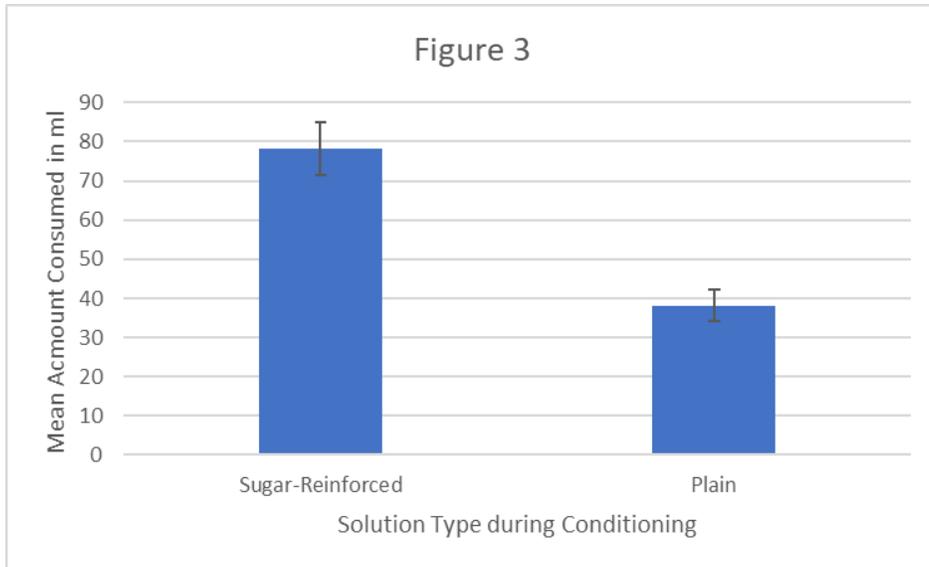
The results of the flavor test showed that Subjects did show a preference to the primary flavor. The mean primary preference ratio ( $M = .54$ ) was greater than chance ( $M = .50$ ). The primary preference ratio is the percentage of all solutions consumed by Subjects that were the flavor matching the first introduced sugar-reinforced flavor. On average, the amount of the primary flavor consumed was 25.91 milliliters; while the amount of the recency flavor consumed was 22.45 milliliters. This difference was considered significant,  $t(10) = 2.44, p = .03$ . There did appear to be a slight unlearned bias towards Cherry ( $M = 24.73$  ml) over Grape ( $M = 23.64$  ml) during testing (see Figure 2).

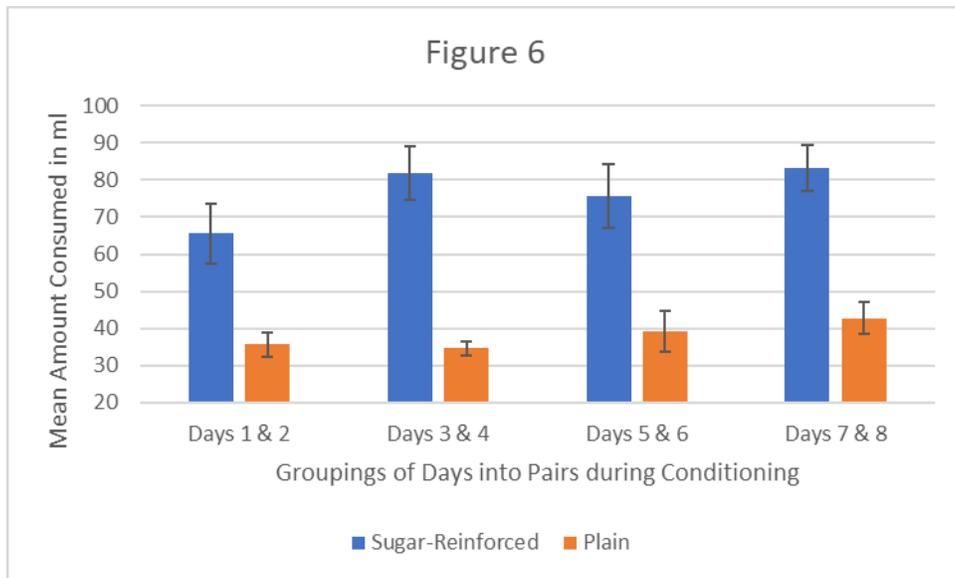


### **Intake of both flavors during conditioning**

The results of the conditioning show that there was a strong preference to the sugar-reinforced flavor, which was expected. The mean sugar-reinforced preference ratio ( $M = .66$ ) was greater than chance ( $M = .50$ ). In other words, all rats always consumed more of the sugar-reinforced flavor rather than the plain flavor. On average, the amount of the sugar-reinforced flavor was 78.23 milliliters; while the amount of the plain flavor consumed was 38.11 milliliters (see Figure 3). This difference was considered extremely significant,  $t(10) = 8.93$ ,  $p < .0001$ . The p-value showed us that the results would not likely be by chance alone. During conditioning, there was an unlearned bias towards Grape ( $M = 60.73$  ml) over Cherry ( $M = 56.48$ ), the same during testing phase (see Figure 4).

Over time, there was an increase in the consumption of all flavor solutions (see Figure 5). There also seemed to be strong relationships between the 1<sup>st</sup> and 2<sup>nd</sup> pairs of days ( $r = .55$ ), and the 3<sup>rd</sup> and the 4<sup>th</sup> pairs of days ( $r = .73$ ). Where in pairs 1 and 3 there was the introduction of a new sugar-reinforced flavor; however, in pairs 2 and 4 there was the reintroduction of the previous sugar-reinforced flavor. The relationship that occurred was an increase in consumption of sugar-reinforced flavors *if it had been introduced beforehand* (see Figure 6). In other words, pair 1 had a mean consumption of 65.55 ml, whereas pair 2 had a mean consumption of 81.73 ml. This increase was also found between pairs 3 and 4, with means of 75.58 ml and 83.18 ml respectively. However, although the correlations between pairs was strong, they were not statistically significant. Pairs 1 & 2 were almost significant,  $t(10) = -2.21$ ,  $p = .052$ , whereas pairs 3 & 4 were certainly insignificant,  $t(10) = -.17$ ,  $p = .87$ .





### Discussion

We found that Subjects preferred the first sugar-reinforced flavor that they were introduced to in the test. Throughout conditioning, we reaffirmed that Subjects would vastly prefer the flavor that was sugar-reinforced rather than the plain flavor. Throughout conditioning Subjects had a trend of consuming more solution over time, and consumed more between certain pairs of days (1 to 2, and 3 to 4). We hope that the findings of this project can serve as a basis for further study into the field of flavor preference learning.