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Author	石井, 拓(Ishii, Taku)
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8 Patterns of Consumption of Glucose Water Solutions in Common Marmosets *Taku Ishii*¹ ' Center for Advanced Research on Logic and Sensibility (CARLS), Keio University

I. Introduction

The common marmoset has recently come to be regarded as a useful laboratory animal for several reasons. First, they are a kind of primate and genetically closer to human beings than other common laboratory animals such as rats or mice. Second, they are smaller than most primates and thus are economical to maintain and easier to take care of. They have previously been used in neurological research (e.g. Ando, 2004) and in behavioral research (e.g. Rosati, Stevens, & Hauser, 2006).

However, a standard operant technique necessary for use of marmosets in behavioral research extensively has not yet been developed. In particular, we lack essential knowledge on suitable reinforcers: What is an effective reinforcer, how intense it should be, and how many times can we use it in a daily experimental session? In the present experiment, in order to answer these questions, I measured how many times marmosets licked glucose solutions in 30-min sessions.

In addition, I examined within-session patterns of consumption of glucose solutions. Generally, a rate of consumption, f(t), at time, t, from the beginning of a session decreases as a cumulative amount of consumption, F(t), increases. Aoyama (1998) reported a linear relationship between them:

$$f(t) = b - aF(t), \quad (1)$$

where a and b are slope and intercept, respectively. In one condition of his experiment, every lever-press response of food-deprived rats was reinforced by the presentation of a food pellet. Aoyama measured rates of lever pressing in 3-min blocks and plotted them against cumulative numbers of pellets earned. This plotting is equivalent to plotting rates of consumption against cumulative amounts of consumption. Because the fit of Equation 1 to Aoyama's data was good, we infer that the equation may be used to describe consumption patterns of glucose solutions in the present experiment.

II. Method

1. Subjects

One female (I3441F) and one male (I3613M) common marmoset (*Callithrix jacchus*) served as subjects, both of which were approximately 17 months old at the beginning of the experiment. They were housed in individual cages with 24 h water access. Experimental sessions were conducted individually before a daily ration of food was provided once a day in their cages at around noon.

2. Apparatus

One operant chamber ($W \times D \times H$, $33.5 \times 31.5 \times 31.5$ cm) was used. The front wall was made of aluminum and the other three walls, ceiling, and floor were made of stainless grids. A water bottle was held behind the front wall, the nozzle of which could be inserted into the chamber through a hole located at the center of the front wall. A capacitance sensor was attached to the nozzle in order to detect marmosets' licking at the tip of the nozzle, which was at 11.5 cm from the floor and projected 1.2 cm into the chamber. The body of the nozzle (except for its tip) was covered by a rubber tube and insulated. A white LED was located above the hole for the

nozzle presentation, 15 cm from the floor. This white LED was kept lit during the period when the nozzle was inserted into the chamber. Two slits for inserting retractable response levers and two stimulus lights were also set in the front panel but were not used in the present experiment. Two straight arrays of white LEDs were attached to the ceiling for providing general illumination. During experimental sessions, white noise was presented from two speakers placed outside the chamber, 50 cm away from the rear wall. The chamber itself was placed in a dark room. A personal computer, which was placed in the same room, controlled stimulus presentation and recorded the marmosets' licking responses to the nozzle.

3. Procedure

3. 1 Preliminary observation

The nozzle of the water bottle was presented to the marmosets for 30 min in each session, and their behavior was monitored through a video camera to ensure that they reliably licked the nozzle and to roughly estimate how many times they licked it in a session. The water bottle contained a 50% glucose solution. The LED in the front wall and the ceiling lights were always on during a session, and white noise was continuously presented. In eight sessions, the subjects came to start licking the nozzle soon after the beginning of a session.

3. 2. General procedure

A session consisted of a 30-min free access period following a 3-min habituation period. During the habituation period, the ceiling lights were on, and white noise was presented, but the marmosets could not access the nozzle of the water bottle. At the beginning of the free access period, the nozzle was inserted into the chamber and the LED above the nozzle was turned on, after which marmosets' licking responses to the nozzle were recorded.

3. 3. Experimental conditions

In each of the two 10-session blocks, glucose solutions were used in different combinations of concentrations: 50% and 20% solutions were used in the first block, while 20% and 10% solutions were used in the second. Within a session, only one solution was in use, and solutions of different concentrations were alternated between sessions in a pseudo-random order. Specifically, the order was determined according to one of the Gellerman series (Gellerman, 1933) so that each solution in the pairs was used in five sessions in a block. Both the marmosets experienced the same series of presentation of the different solutions.

III. Results

First, patterns of changes in cumulative numbers of licks over time (measured from the beginning of a session) were examined. Each line in Figure 1 corresponds with data from an individual session. In most cases, licking occurred at high rates early in a session, slowed down, and then stopped at some point during the session. In some cases, a high rate of licking was restored after a long pause. The rates of licking of the 50% solution of glucose slowed earlier than those of the 20% solution. However, there was no discernible difference between the rates of licking of the 20% solution of glucose and those of the 10% solution, at least from visual inspection.

The level at which each cumulative record of licks reaches the right ends of the panels in Figure 1 indicates the total number of licks in a session. Figure 2 shows averages of those total numbers for each condition of glucose solutions, along with standard deviations. Both the marmosets licked less of the 50% solution of glucose than the 20% solution. The results of a Welch two-sample t-test showed that these differences were statistically significant. (Because separate tests were conducted for each of the two marmosets, an alpha level of .025, instead of .05, was used.) On the other hand, although both the marmosets licked slightly more of the 10% solution of glucose than the 20% solution, on average, these differences were not statistically significant.



Figure 1. Cumulative number of licks as a function of time from the beginning of a session: Each line corresponds with data from an individual session. The top two panels are for the female marmoset (I3441F), and the bottom two panels are for the male marmoset (I3613M). The left panels are for the first 10-session block when 50% (solid lines) and 20% (dashed lines) solutions of glucose were used, whereas the right panels are for the second block when 20% (dashed lines) and 10% (solid lines) solutions were used.



Figure 2. Average and standard deviations of total number of licks: The top two panels show data for the female marmoset (I3441F), and the bottom two panels data for the male marmoset (I3613M).



Figure 3. Numbers of licks in 3-min bins as a function of cumulative numbers of licks: All the data shown are taken from the first 10 minutes from the beginning a session, averaged across sessions. Straight lines are best-fitting functions of Equation 1.

Next, relationships between the rates of licking and cumulative numbers of licks were examined. For this purpose, the 30-min period during which the nozzle was presented was divided into 30-s bins, and a rate of licking was calculated for each bin. These rates were averaged across sessions and plotted against the cumulative numbers of licks that were calculated from the averaged rates of licking (Figure 3). Only the data from the first 20 bins were plotted because thereafter, the data became highly noisy due to high rates of licking restored after a long pause; this condition obviously violated Equation 1. Equation 1 was fitted to the data from the 20th bin. The data from the first bin was omitted from this fitting because a rate of licking could sometimes increase, not decrease, early in a session because of sensitization (McSweeney &

		50% vs. 20%				20% vs. 10%			
	I34	I3441F		I3613M		I3441F		I3613M	
	50%	20%	50%	20%	20%	10%	20%	10%	
Intercept (b)	36.17	81.83	47.88	125.94	60.02	64.57	104.06	117.93	
Slope (a)	0.13	0.16	0.16	0.22	0.17	0.13	0.14	0.12	
VAC*	0.92	0.94	0.78	0.95	0.90	0.78	0.88	0.88	

Table 1. Results of fitting for Equation 1

* Proportion of variance accounted for

Murphy, 2000). The results of the fitting are summarized in Table 1.

Equation 1 fitted the data well and accounted around 90% of variance of the data, with two exceptions. The intercepts for the 50% condition were much smaller than those for the 20% condition, whereas there was no systematic difference between the intercepts for the 20% and 10% conditions. On the other hand, the slopes were around .15 regardless of the concentration of the glucose solutions, with one exception.

IV. Discussion

Results showed that the marmosets made hundreds of licks to the nozzle, suggesting that the glucose solutions were highly effective reinforcers. In future behavioral research, we may offer marmosets an opportunity to lick glucose solutions many times to reinforce their other behaviors. Because the 50% solution was consumed less than the 20% solution, moderate concentrations may be more effective than high ones, although a future study may reveal that an effective concentration would be dependent on required behavior to produce an opportunity to consume a glucose solution.

Equation 1 fitted well to the relationship between the rates of licking and the cumulative numbers of licks, at least in the first 10 min of a session: The rate of licking decreased linearly as the cumulative number of licks increased. As Aoyama (1998) interpreted, this means that each reinforcer consumed has the same effect of slowing down the speed of consumption. Although Aoyama (1998) did not mention it, Equation 1 (see



Figure 4. Schematic representation of the relationships between time (t), a rate of consumption (f(t)), and a cumulative amount of consumption (F(t)): The left panel shows the relationship between f(t) and F(t) shown by Aoyama (1998). The center panel shows f(t). The shaded area corresponds with F(t). The right panel shows F(t). The slope of the tangential line of F(t) corresponds with f(t).

also Figure 4, left panel) is consistent with the earlier proposal by Bousfield (1935) that a cumulative amount of consumption can be described as a function of time by the following equation:

$$F(t) = p(1 - e^{-qt}),$$
 (2)

where p and q are constants. To derive Equation 2 from Equation 1, note that a cumulative amount of consumption is an integral of a rate of consumption from the beginning of consumption to a certain time in a session:

$$F(t) = \int_0^t f(t)dt, \quad (3)$$

Inserting Equation 3 into Equation 1 yields,

$$f(t) = b - a \int_0^t f(t) dt$$
, (4)

and differentiating both sides of Equation 4 yields,

$$f'(t) = -af(t), \quad (5)$$

Equation 4 and 5 are satisfied with the following exponential function for f(t) (Figure 4, center panel):

$$f(t) = be^{-at}, \quad (6)$$

Inserting Equation 6 into Equation 1 yields Equation 7 (Figure 4, right panel), which is equivalent to Equation 2:

$$F(t) = (b/a)(1 - e^{-at}), \quad (7)$$

In both Equations 1 and 7, the parameter *a* corresponds to the rate of decrease of the speed of consumption. The fact that the value of *a* (slopes in Table 1) did not vary very much with the variety of solution concentrations means that the rate of decrease in the speed of consumption may be independent of the caloric content of glucose solutions. This is consistent with the notion that within-session changes of consumption are better explained by the effect of habituation than the effect of other factors of satiation, such as caloric content or stomach distention (McSweeney & Murphy, 2000). Future studies should test if this is true with other concentrations of glucose solutions or other kinds of reinforcers.

The limitation of both Equations 1 and 7 is obvious. These equations cannot explain an increase in the rate of consumption observed in an initial part of a session, or high rates of licking restored after a long pause in a later part of a session. The former can be explained by the effect of sensitization to the stimuli, such as the appearance of the nozzle or the taste characteristics of the glucose solutions. For the latter, I do not have any explanation at present. In fact, Aoyama (1998) did not observe a similar high rate of response restored later in a session. A major difference between the present and Aoyama's experiment, other than species and types of reinforcers, is whether the subjects were deprived of food. Because the rats used in Aoyama's experiment were maintained at 85% of ad lib weight, they did not stop responding for food pellets even 45 min after the beginning of a session. On the other hand, in the present experiments, the marmosets could always access food and water in their home cage, and they stopped or paused licking the glucose solutions at a certain point in a session. Therefore, when we offer marmosets an opportunity to lick a glucose solution to reinforce their other behavior for a different experimental purpose, we can just allow them to make a smaller

number of licks that would not make them stop responding. However, examining whether there is any pattern in the restoration of high response rates would be an interesting research topic itself.

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