SALINE PREFERENCE IN RATS DETERMINED BY CONTINGENT LICKING¹

G. L. FISHER²

BROWN UNIVERSITY

The albino rat's preference for .1 molar NaCl solution when paired with distilled water was measured using a lick contingent method. The method precluded position bias and revealed some fluid-deprivation influences in the resulting measured preference. Rats were trained to lick at a drinking tube containing water in order to obtain another tube containing the saline solution, under several levels of water deprivation. When tube contents were varied, the patterns of licking varied concurrently. Preference data was collected at low levels of fluid deprivation. It was shown that the saline solution was the primary controlling stimulus.

This study was undertaken to examine the feasibility of requiring an animal to lick at one solution to make another solution available, as a means of measuring taste preferences where licking a solution is treated as an operant. The measurement of taste preferences with a minimum of confounding by nontaste factors presents certain difficulties. The widely-used two-bottle test requires counterbalancing of taste and comparison solutions to control for the strong position habits exhibited by the animals; in addition, there is often no attempt to separate the portion of the measured preference under taste-stimulus control and the portion resulting from nontaste factors (Fisher, 1964, 1965). In many instrumental taste preference situations, the animals are under thirst or hunger deprivation and the resulting preference reflects, at least, the interaction of taste and deprivation.

This work attempts to evaluate measured taste preference for .1 molar NaCl in albino rats under conditions in which position habit influences are attenuated and deprivation factors are considered.

METHOD

Subjects and Apparatus

Eight male albino rats of Charles River stock, about 200 g in weight at the beginning of the experiment were used.

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²Now at the University of Maryland.

The apparatus consisted of a Plexiglas chamber (8 in. by 8 in. by 16 in.) contained within a converted refrigerator shell (Fig. 1).

Fig. 1. Cutaway view of the apparatus. BC, bead chain; CW, counter weight; DT, drinking tube; F, fixed tube; G, grid floor; R, retractable tube; SM, synchronous motor; T, portion of vertical track; TCW, testing chamber wall; TT, test tube; TTH, test tube holder.

Two $\frac{1}{2}$ in. apertures (3 in. between centers) were positioned on one wall 2 in. above a grid floor. Two inverted 70 ml test tubes, with standard stainless steel drinking tubes (1.5 mm orifices) through rubber stoppers, were located behind the apertures. The meniscus was $\frac{3}{8}$ in. from the inside surface of the chamber to insure that the animal broke contact with the tubes at each tongue-lap. One tube (fixed) remained in its position behind the aperture during the entire experimental session. The other tube (retractable) was mounted on a vertical track and could be lowered (1 in. in .5 sec) to a position where the tube orifice was accessible to the animal and returned to its raised, inaccessible position by a synchronous motor. The duration of the tube's accessibility was controlled by an electric timer. The test chamber was illuminated by a 7.5 w white house light and white noise (85 db) was provided to mask distracting sounds. A 60 CFM blower provided ventilation. Two Grason-Stadler drinkometers (model E4690 A) were activated by tongue contact with the drinking tubes. Sodeco electromechanical counters rated at 25 imp/sec and a Gerbrands cumulative recorder were used to record the data.

The .1 molar NaCl solution was made with distilled water. The saline and water solutions were at room temperature when presented to the animals. Subjects were maintained on Purina Laboratory Chow throughout the experiment.

Part 1. The eight rats were deprived of water 24 hr before each training session. Session duration never exceeded 15 min; previous unpublished data showed that rats under *ad libitum* drinking conditions would lick steadily at a tube containing .3 molar sucrose for 15 to 20 min. Tube training consisted of lowering the retractable tube containing .1 molar NaCl for short intervals until the animal licked from the tube 100 times. The fixed tube, containing distilled water, was then introduced and the retractable tube remained at its upper (inaccessible) position until the fixed tube was licked. Initially, one lick at the fixed tube lowered the retractable one for 15 sec. After 10 presentations of the retractable tube, the ratio requirement on the fixed one was increased to 10 licks, *i.e.*, 10 licks at the fixed tube presented the retractable one for 15 sec dur-

ing which time the animal could continue licking at the fixed tube or shift its licking to the other. All animals were then required to complete two criterion sessions. The animals were required to lick at least 100 times at the retractable tube to satisfy the session criterion. All the animals met the criterion in two sessions.

The testing sequence was run as follows:

A. Fixed tube containing water, retractable tube containing saline (W→S). Three 15-min sessions, at four water-deprivation levels, were run in the following order: 23 hr, 6 hr, 1 hr, and 3 hr.

B. Fixed tube containing saline, retractable tube containing water (S→W). Three 15-min sessions at three levels of water-deprivation were run in the following order: 3 hr, 23 hr, and 1 hr.

Each animal was run one session a day at the same time each day. Deprivation was controlled by presenting tap water to the rats in their home cages, for 1 hr per day, at the appropriate time before the test session.

Upon completion of Part 1, the animals were maintained on *ad libitum* food and water. Experimentation was resumed after examination of the data drawn from Part 1.

Part 2. The animals were run as before except (1) the order of water deprivation levels was varied and (2) both tubes contained water in sub-part B.

A. Fixed tube containing water, retractable tube containing saline (W→S). Three 15-min sessions at each of the following levels of water deprivation were run in the following order: 1 hr, 3 hr, 6 hr, and 23 hr.

B. Both tubes containing water (W→W). Three 15-min sessions at each of two levels of water deprivation were run in the following order: 1 hr and 23 hr.

RESULTS

Part 1. Lick-rates were determined by dividing the total number of licks at a tube by the time the tube was available to the animal, *i.e.*, the number of licks on the fixed tube was divided by the session duration; the number of licks on the retractable tube was divided by the total duration of tube availability each session. The median was used as a measure to eliminate the influence of extreme individual data points caused

by uncontrolled factors, *e.g.*, an animal not licking at all during a given session for reasons unknown. The median lick rates of all animals on both tubes for the last session under each level of deprivation are presented graphically in Fig. 2. Each pair of bars (Fig. 2) indicates the median lick rates on the two tubes, left-hand bar the fixed tube and right-hand bar, the retractable one. The unfilled bars indicate water-licking and the filled ones, salt licking. Early in training (first W→S phase) there was a decline in the amount of licking applied to the fixed tube containing water and an increase in licking at salt. The progression of the decline is in the order of running at the different levels of deprivation. The first lines of cumulative records in Fig. 3 and 4 present the licking at both tubes during the early portion of the running (Part 1A). The stepping pen marked each lick at the fixed tube (regardless of contents) and the event marker, every tenth lick at the retractable tube. Note the high rates of licking on the fixed tube during high water deprivation (early in training) and the decline with lower

deprivation and continued training. Since there was no earlier data on this behavior, there was no way to anticipate the eventual form of the data. After a complete run (at all levels of water deprivation) tube contents were exchanged to see if the behavior would be affected by a change of NaCl location in the tubes. The S→W phase of Fig. 2 shows the resulting median lick rates. The bottom portions of Fig. 3 and 4 present licking records of this phase. Note the high rates of licking at the fixed tube containing the saline solution. The rates of licking at the fixed tube declined with level of water deprivation. Rates of licking at the retractable tube decline substantially when compared with previous W→S licking at 3-hr and 1-hr levels of deprivation. Note that the decline of lick-rates at the fixed tube is similar during the W→S and S→W phases. At all levels of deprivation rates of licking at the fixed tube were higher during the S→W phase than during the second W→S phase.

Part 2. The second running of the W→S phase resulted in a general decline in licking

Fig. 2. Median rates of licking for all animals on the third day of each level of water deprivation. The filled bars indicate saline licking; the unfilled bars indicate water licking. W→S indicates that licking at the water tube resulted in presentation of saline tube. S→W indicates that licking at the saline tube presents the water tube. W→W indicates that licking at the water tube resulted in presentation of another water tube.

F-3

Fig. 3. Sample licking records of F-3. The Stepping pen registered licks at fixed tube. Reinforcement marks indicate 15-sec presentation of retractable tube. Every tenth lick at the retractable tube is shown by a deflection of the event marker. Upper portion of figure presents record of third day at each level of water deprivation: 23 hr, 6 hr, 3 hr, and 1 hr for W→S. Lower portion of figure presents record of third day of each level of water deprivation: 23 hr, 3 hr, and 1 hr, for S→W.

at the fixed tube (containing water) when compared with the first W→S and S→W (Fig. 2). There is also a decline in licking at the fixed tube with descending level of water deprivation. Rates of licking at the tube containing NaCl are similar during S→W and the second W→S. The animals did not remain at the fixed tube as they did when it contained NaCl and, in fact, shifted their licking to the retractable tube, now containing NaCl. The plots in Fig. 2, S→W and the second running of W→S, show that the animals licked at higher rates when the fixed tube contained NaCl than when it contained water. Licking at the retractable tube was higher at 23 hr deprivation (W→S) than during (S→W) at that same level of

water deprivation. Licking at the water-filled tube at the 3-hr and 1-hr levels of deprivation in both S→W and the second W→S were similar. The licking records in Fig. 5 and 6 (upper line of records) show a stepwise character to the licking at the fixed tube containing water and a great deal of licking at the retractable tube containing NaCl. When both tubes contained water (W→W) the rates of licking at the fixed tube increased and at the retractable tube decreased (Fig. 2, 5, and 6). Overall, the following points may be made:

1. Licking rates on the fixed tube declined with level of deprivation whether the tube contained NaCl or water. This statement is supported by the left-hand bars of each pair

in Fig. 2. The only exception occurs at either the 23-hr or 6-hr level of deprivation during the second $W \rightarrow S$.

2. Hypotonic NaCl elevated the rate of licking at both tubes regardless of whether they were fixed or retractable, in both the $S \rightarrow W$ and second running of $W \rightarrow S$. In addition, the location of the NaCl (fixed or retractable tube) determined whether the animal concentrated its licking at the fixed tube or shifted over to the retractable tube when presented. The increase in lick rates shown in Fig. 2 during the second running of $W \rightarrow S$ could have been accomplished only by an increase in shifting to the retractable tube when presented. Therefore, the .1 molar NaCl was the primary factor determining whether the animals would shift to a retractable tube or remain on the fixed tube.

By removing NaCl from the situation, *i.e.*, the rat licked water and a water tube was presented ($W \rightarrow W$), the extreme stepwise char-

acter of the cumulative records in Fig. 5 and 6 was modified. The steplike records were straightened at 23 hr deprivation and less regular at 1 hr deprivation. This is clear when the upper and lower portions of Fig. 5 and 6 are compared. Note also, the reduced density of deflections along the event marker line in these figures during $W \rightarrow W$. There was less shifting to the retractable tube when it contained water.

DISCUSSION

Two main points may be developed. First, these data show that hypotonic NaCl can support an instrumental behavior in the white rat without known sodium deficits. Second, this technique, presentation of one solution contingent upon licking at another, offers certain advantages over alternative procedures. These points and others are developed below.

F-5

Fig. 4. Sample lick records of F-5. See Fig. 2 for explanation.

F-3

Fig. 5. Sample lick record of F-3. Same explanation as Fig. 2 with the exception that W→W indicates that licking at water tube presented another water tube.

Prior to this study, there was a lack of unequivocal evidence that an instrumental response may be trained and maintained by certain concentrations of hypotonic NaCl without specific hunger engendered by peritoneal dialysis, sodium restricted diets and adrenalectomy (Falk, 1961; Lewis, 1960; Wagman, 1963). In this study, there was an increased rate of licking at the retractable tube when it contained .1 molar NaCl. Every animal in this study reacted to the transposition of tube contents during S→W and the second W→S by licking primarily at the fixed tube when it contained .1 molar NaCl and shifting most licking to the retractable tube when it contained .1 molar NaCl. Purina Lab Chow is reported by its manufacturers to be

nutritionally complete. Since the animals were on an *ad libitum* food schedule in their home cages, it is fair to assume that they would make up any sodium deficit by increased food intake; these animals may be assumed to be free of any metabolic needs.

Very little can be said about the behavior during the first W→S period. It is apparent that the behavior was not stable and a discussion of the interrelation between water deprivation and reinforcing characteristics of hypotonic saline is unwarranted. However, beginning with the S→W period, the rats licked at very high rates on the fixed tube after they produced stepwise lick records on the fixed tube during the first W→S, e.g., Fig. 3 and 4.

A brief analysis of the elements of this behavior may aid in understanding the results. The animals are trained originally to lick at a tube, filled with .1 molar NaCl, upon its presentation. Each lick is reinforced by a small amount of the NaCl solution. Licking at the retractable tube is the final member of a behavioral chain. When the fixed tube is introduced, the chain is being lengthened by a second member; a fixed ratio of 10 licks leads to presentation of the final member where each lick results in reinforcement. Since licking at the fixed tube (initial member of the chain) also brings with it liquid, applied to the tongue's surface, the nature of that liquid has the potential to vary the behavior on either member of the chain. For instance, if the liquid applied to the tongue's surface from the initial member (fixed) is more reinforcing than that applied

by the rat licking at the final member (retractable), then the animal may be expected to lick primarily at the initial member of the chain. On the other hand, if the solution delivered by the final member of the chain is more reinforcing than that brought by the initial, the rat may be expected to concentrate its licking at the final member, the retractable tube. Since presentation of the retractable tube is contingent upon 10 licks at the fixed tube, the animal might be expected to lick no more than is necessary at the fixed tube to deliver the retractable one and to commence licking at the retractable one upon its presentation. If the animal licks entirely at the fixed tube (even though the retractable one has been presented) the data will yield high rates of licking at the fixed tube and a zero rate at the retractable one. If, on the other hand, the animal shifts to the retract-

F-5

Fig. 6. Sample lick record of F-5. See Fig. 5 for explanation.

able tube when presented, high rates of licking at the retractable tube and somewhat lower lick-rates at the fixed one should be seen. The time the animal is at the retractable tube is time during which it cannot be at the fixed one; this results in a somewhat lower rate at the fixed one. Therefore, high lick rates at the first member, accompanied by lower lick rates at the second member, indicate that the solution applied to the tongue by the first member (retractable tube) is more reinforcing than the solution applied by the second member.

Returning to the data, Fig. 2, $S \rightarrow W$, high lick rates at the fixed tube are accompanied by low lick rates at the retractable one, indicating that the solution in the fixed tube is more reinforcing or preferable to the solution in the retractable one. In the second $W \rightarrow S$ phase, (Fig. 2), lower lick rates at the fixed tube are accompanied by high lick rates at the retractable one. Since .1 molar NaCl was contained in the tube receiving high-rate licking in both phases, it may be concluded that .1 molar NaCl is the more reinforcing solution. Since the lick rate at the retractable tube declined when it contained water (during $W \rightarrow W$), it may also be concluded that the decrement is due in part to the absence of .1 molar NaCl.

Lewis (1960) was unable to condition rats to press a bar for hypotonic NaCl reward until she adrenalectomized the animals. In the present work, animals were conditioned to direct their licking at a tube containing hypotonic NaCl or lick through water to present a tube containing NaCl for licking. A major difference between the two studies is the choice of the response. Bar pressing has no direct bearing on the ingestion of liquids. Licking does. In the study above, the licking response, with its very high operant rate, was relatively simple to bring under stimulus control, *e.g.*, the licking response followed the tube containing the .1 molar NaCl. Bar-pressing and licking involve different physiological mechanisms. The critical difference is the fact that the sensory receptors for gustation reside in the tongue.

There are two advantages in this method of determining the rats' preference for one of two solutions. The chain of behavior leading to preferred intake is determined by the experimenter. The same behavior is required

of all animals. When two tubes are presented simultaneously in the two-bottle test, the animal may prefer licking at one because of the tube contents, because of a position habit, because it was the first one presented or for reasons not apparent. Even in those cases where a fairly clear preference develops, these other factors are partial determinants. The extent of these nontaste influences cannot be determined because the behaviors leading to the preference measurement are not instrumented. A second advantage of the lick-contingent presentation of solution is that the animal is forced to ingest both solutions throughout the term of the preference determination. In a two-bottle test the animal may taste both initially, and spend the remaining time on only one solution. By remaining near a slightly preferred solution, the resulting measured preference may, for any given animal, be disproportionate with the intrinsic reinforcing properties of the solution. This is most relevant where a preference is very slight but the animal simply remains near the preferred tube and produces data indicating a very great preference for that solution.

The tendency for rates of licking to comply with level of deprivation on the fixed tube supports the findings of Stellar and Hill (1952). They found an overall orderly relationship between lick rates and deprivation in a single tube situation. In the present two-tube situation, it is not unlikely that the rats first reduced thirst by remaining at the tube immediately available; after reducing the stronger effects of water deprivation, the rats began to shift licking to the now more reinforcing saline tube. The cumulative records in Fig. 6, $W \rightarrow S$, support this view. Note the high rates of licking at the fixed tube containing water at the beginning of the session and the gradual development of stepwise records later in the session.

Although this study was not conducted to evaluate Deutsch's "dilute water" theory, the data are directly relevant. Zotterman (1956) found that the application of water to the rat's tongue reduced the level of spontaneous electrical activity in the chorda tympani salt fibers. The rat has no positive fiber response to water. Deutsch and Jones (1960) believe that rats ingest greater volumes of hypotonic saline when paired with water because of the

reduced decrement in the spontaneous discharge of the salt fibers. The rat drinks more saline because it is summing a sparse water signal. This view cannot explain the fact that in the present study, the rats licked at water (fixed tube) and shifted to lick the hypotonic saline when presented via the retractable tube. Deutsch's formulation would predict that the rats maintain their licking at the fixed tube in $W \rightarrow S$, i.e., reduce the spontaneous activity of the salt fibers. The opposite results were obtained in this study.

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