Saccharin and saccharin-glucose ingestion in two inbred strains of *Mus musculus**

PATRICK J. CAPRETTA
Miami University, Oxford, Ohio 45056

In one experiment 10 C57BL/6J and 10 DBA2/J mice received saccharin in daily ½-h sessions for 19 days while hungry or satiated. A second experiment involved giving these same mice saccharin or glucose and saccharin plus glucose for 24-h periods with or without solid food. It was found in Experiment 1 that saccharin consumption is related to drive; hungry mice at first drank more than did nonhungry mice until the 11th day, after which the former group dropped off and was equalled by the latter. The effect was more pronounced for C57 than for DBA mice—apparently related to strain differences in natural preferences for saccharin. Both strains drank large amounts of saccharin plus glucose in Experiment 2, especially when hungry. The results are discussed within the context of research on food habits.

This study consists of two experiments somewhat different in purpose. One is an extension of research (Smith & Capretta, 1956; Capretta, 1962, 1964) showing that laboratory rats with ample food in their stomachs ingest considerably more saccharin after several ½-h drinking sessions than do food-deprived rats. Here the Ss are two inbred strains of mice, one known to prefer relatively high concentrations of saccharin and the other not (Cooper, 1966).

Experiment 2, on the other hand, is a further probe into the phenomenon of polydipsia, or excessive drinking, as brought on by the synergistic action of glucose and saccharin. Valenstein, Cox, & Kakolewski (1967) found that laboratory rats ingest significantly more of a solution combining saccharin and glucose than equivalent solutions of these substances presented separately. It is the purpose of this second experiment to investigate the development of polydipsia in two inbred mouse strains known to have different preferences for saccharin.

METHOD

Ten male C57BL/6J mice and 10 male DBA/2J mice obtained from the Jackson Laboratory Production Colony at 8 weeks of age served as Ss in both experiments. The solutions in the first experiment consisted of 0.77% sodium saccharin in tap water for the C57s and 0.20% sodium saccharin for the DBAs. These concentrations are approximately at the point of maximum preference for the inbred strains used, judged by the fact that these Ss drink less of solutions with either much higher or lower amounts of saccharin (Cooper, 1966). The glucose-saccharin solution used in the second experiment consisted of 0.77% sodium saccharin plus 3.0% glucose mixed in tap water for the C57s and 0.20% sodium saccharin plus 3.0% glucose for the DBAs. This meant that each 100 ml of solution contained either 0.77 or 0.20 g of saccharin and 3 g of glucose. All solutions were freshly prepared at the start of each day of testing.

The Ss were housed individually in plastic containers, each approximately 25 x 25 x 12 cm deep. Water and solution bottles rested on the top of the container, the dripless spouts fitting through the numerous holes in the metal cover of the cage. After 5 days of habituation to ad lib feeding and daily weighing, the Ss were given saccharin-water to drink for ½-h periods once daily for 19 days. Each strain was randomly divided into groups of five Ss each; one subgroup was given saccharin 1½ h after eating and the other 22 h following its food rations. At this time, the Ss were on a feeding schedule lasting approximately 2 h per day. The Ss were weighed daily throughout the experiment.

After a week of maintenance on ad lib food and plain water, the same Ss were used in a second experiment to determine the course of glucose, saccharin, glucose plus saccharin, and plain water drinking under conditions of hunger and satiation. In all, fluid ingestion data were collected over 16 days. For the first four 24-h periods, the Ss were given ad lib food and tap water. Starting on Day 5, conditions were changed such that each S had access to two bottles, one containing either glucose or saccharin and the other containing a mixture of glucose plus saccharin. Half of each mouse strain was given a choice between saccharin (in different concentrations for the two strains) and glucose plus saccharin, while the others had glucose and glucose plus saccharin. Food was also available at all times (except for the time taken to weigh the Ss and make fresh solutions) during Days 5 through 10. On Days 11 and 12 the Ss again experienced ad lib food and tap water, followed by 2 days (13 and 14) when all solid food and tap water were removed and the Ss were given glucose and saccharin and glucose plus saccharin. Finally, on Days 15 and 16 the Ss had both food and glucose or saccharin and glucose plus saccharin. Weights of Ss and solutions consumed were taken to the nearest 0.10 g.

RESULTS

Figures 1 and 2 present the data collected in Experiments 1 and 2, respectively. In the first figure, it is seen that the amount of saccharin consumed varied with mouse strain and motivational conditions. The hungry C57 Ss drank consistently more saccharin than did their satiated counterparts until Day 12, after

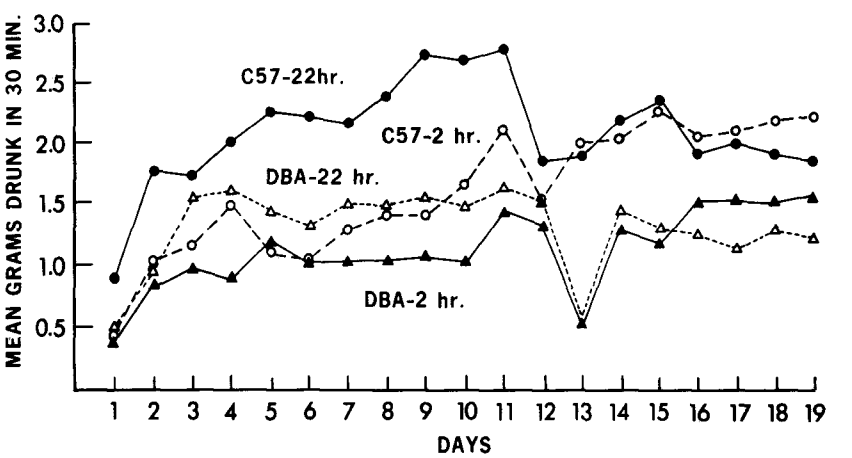

Fig. 1. Experiment 1: Ingestion of saccharin-water over 19 daily ½-h periods for two inbred strains of mice. One-half of each strain received the solution 2 h after eating, while the other half had it 22 h following their daily rations.

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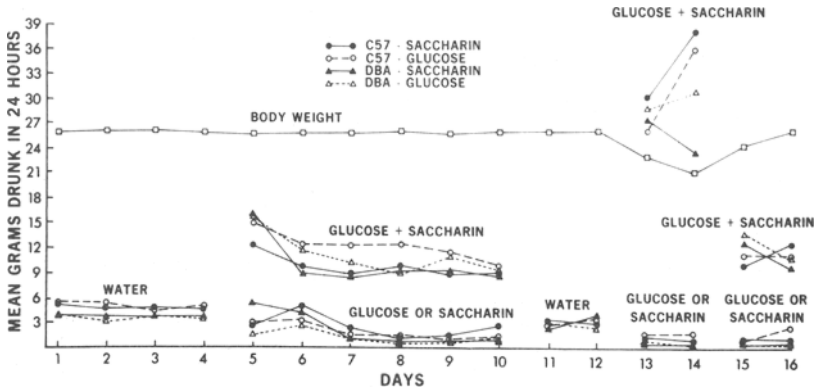

Fig. 2. Experiment 2: Ingestion of water, glucose, saccharin, or glucose + saccharin over 16 daily 24-h periods for two inbred strains of mice. Food and plain tap water were available at all times for Days 1-4; food but no plain water on Days 5-10; food and plain water for Days 11-12; neither food nor plain water on Days 13-14; food but no plain water on Days 15-16. Since no reliable differences appeared among the various groups in body weight, daily means are given for all Ss combined.

which time consumption dropped for the hungry Ss and equalled that for the satiated Ss who continued at a level they had reached earlier. Saccharin consumption curves for the two DBA groups are generally lower than those for the C57 strain and show a similar, though less pronounced, change in relative standing (i.e., the hungry Ss drank more at first and then were surpassed by the satiated group toward the end of training). The overall significance of these differences is indicated in the results of a 2 by 2 by 19 factorial analysis of variance of the data in Fig. 1. The Days by Strain and Days by Deprivation interactions were both significant ($F = 6.90$, $df = 18/288$, $p < .01$; $F = 4.40$, $df = 18/288$, $p < .01$, respectively). Post hoc tests of the mean differences between motivational states for each strain were made by calculating grand means for Days 1-11 and Days 12-19. Differences over the first 11 days were significant for the C57/22-h vs C57/2-h groups ($t = 5.52$, $df = 17$, $p < .01$) but not for the DBA/22-h vs DBA/2-h groups ($p > .05$). No significant t values were found for either strains over Days 12-19. It is also noteworthy that there were no discernible differences in mean body weight for the various groups over the entire 19 days of testing.

It is apparent in Fig. 2 that the combination of glucose and saccharin resulted in excessive drinking in both strains, regardless of whether the other solution available was plain glucose or saccharin. The polydipsic effect in this second experiment was sharply accentuated under conditions of hunger, when the majority of Ss drank more than their body weights in 24 h. No consistent findings appeared between subgroups, except, perhaps, under conditions of hunger when the C57 Ss drank appreciably more glucose plus saccharin than did the DBA Ss on the second day of solid food deprivation (Day 14).

DISCUSSION

Smith & Capretta (1956) and Capretta (1962, 1964) found that saccharin consumption in rats is influenced somewhat by the S's state of hunger. If rats are given a saccharin-water solution for ½-h periods before their daily food rations, consumption of this nonnutritive substance is considerably lower by the fourth or fifth day than it is for Ss receiving saccharin 1 or 2 h after feeding. Furthermore, the effectiveness of saccharin as a reward for T-maze learning was found to decrease as a function of the food deprivation at which it had been consumed in the past. The first experiment in the present study provides additional data consistent with these earlier findings for the laboratory rat. Although similar results were obtained across species, the effect of diminished saccharin ingestion under conditions of hunger became manifest much later for the mouse strains (after 12 days vs 5 or 6 days for rats). A difference is also seen between mouse strains in that the C57s (saccharin preferrers) showed the effect much more clearly than did the DBAs (nonpreferrers). It is interesting that by Day 19 the satiated DBAs were drinking almost as much as the hungry C57 Ss.

There are at least two possible explanations for the results in Experiment 1. One has to do with the nature of saccharin as a reinforcement agent. Smith & Capretta (1956) suggested that (1) saccharin solutions may lose some reinforcement value after a number of experiences as long as consumption is restricted to a time at which S is very hungry and no nutritive material is available, and (2) saccharin solutions may gain in reinforcement value after a number of experiences as long as consumption occurs at a time when S's stomach contains nutritive material. In short, the preference for saccharin increases or decreases depending on whether the feedback is beneficial or not (see Revusky & Garcia, in press). A second possibility, not necessarily incompatible with the first though more consistent with a stand taken by Pfaffmann (1960), is that the saccharin taste acquires secondary aversive properties because of its direct association with unreduced hunger in the animal. The fact that body weights did not differ for groups of mice receiving saccharin under various conditions of hunger argues against an interpretation predicated on differences in adjustment to the food schedules. I lean toward the explanation which stresses the aversive conditioning of tastes.

The second experiment produced data similar to those reported by Valenstein et al (1967) and Kakolewski, Cox, & Valenstein (1968). These Es found that saccharin and glucose in combination act together to induce excessive drinking (polydipsia) in both male and female rats. The fluid consumption in 24 h occasionally exceeded the body weight of food-nondeprived Ss. Reference is made by them to hungry male rats which more than doubled their average daily intake of the saccharin plus glucose solution. Both mouse strains in the present experiment show comparable results, although the polydipsic effect seems even more pronounced under conditions of starvation (there being a threefold increase). Also, there appeared to be a separation in the curves on Day 14, with the saccharin-preferring C57s increasing their consumption dramatically while the saccharin-nonpreferring DBAs remained more or less at the same level they had reached on Day 13. Additional testing in the absence of food could probably have been carried out, but I was worried by the steady decline in body weight seen in Fig. 2.

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Age differences in the development of the conditioned emotional response

R. L. BRUNNER, THOMAS G. ROTH, and RONALD R. ROSSI*
University of Cincinnati, Cincinnati, Ohio 45221

Rats aged 25, 50, or 120 days were tested for suppression of drinking behavior during the presentation of a fear-producing CS. The youngest group did not show any evidence of drinking suppression when compared to more mature rats or to a pseudoconditioning control group of the same age. These results are discussed in terms of fear conditioning and the ability of young rats to inhibit consummatory responses.

Three recent studies have indicated that there are maturational differences in the ability of rats to inhibit a punished response (Brunner, 1969; Riccio et al, 1968; Riccio & Schulenburg, 1969). One hypothesis suggests that a developmental change in a general inhibitory mechanism may be affecting performance (Campbell & Thompson, 1968). For example, weanling rats fail to show above chance spontaneous alternation (Kirkby, 1967) and internal inhibition is weak in very young birds (Vince, 1959). Douglas & Kowal (1968) have advanced the view that such age-related changes in inhibitory ability may be related to the postnatal development of the limbic structures of the brain, which are thought to be involved in the ability to withhold responding in the presence of changed environmental contingencies (Kimble, 1969).

The experiment reported here continues the investigation of age differences in fear conditioning. The conditioned emotional response (CER) is known to be markedly attenuated by disruption of the limbic system (Brady & Conrad, 1960; Goddard, 1965; Trafton, 1967). It is hypothesized that immature rats will tend to continue making a consummatory response in the presence of a stimulus previously associated with shock.

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SUBJECTS

Thirty-six naive hooded rats of the Long-Evans strain were assigned to three age groups. The adults (120 days) were obtained from a commercial breeder. The 25- and 50-day-old rats were bred from a colony maintained at the University of Cincinnati. Each group consisted of six males and six females. The Ss were weaned at 20 days of age and maintained in group cages with water and rat chow continuously available.

APPARATUS

The apparatus was a 9.5 x 8.5 x 11 in. operant-conditioning chamber. The opposite walls of the box were either Plexiglas or aluminum and the cover was Plexiglas. The chamber was housed in a ventilated sound-attenuated enclosure. The ventilation system also provided a masking noise, and a 6-W lamp provided illumination. A one-way window permitted observation of S. A Richter bottle was available through one wall and was connected through a touch-sensitive relay to a counter which recorded the number of licks. The conditioned stimulus (CS) was a moderate intensity, 10-kHz tone. The unconditioned stimulus (US) was a 1-mA scrambled footshock delivered through the grid floor of the chamber. The CS and US durations were controlled by interval timers.

PROCEDURE

The procedure followed the one-trial CER paradigm of Leaf & Muller (1965) and Lubow & Siebert (1969). The water bottles were removed from the home cages, and 24 h later each S was placed in the test chamber with the water tube available. Each S was allowed to make 200 licks. At the end of this session, water bottles were replaced in the home cage. On the following day (Day 2), each S was placed in the test chamber, and the drinking spout was not available.

One-half of the Ss in each age group received one pairing of tone and shock (Group E). The paired stimuli were presented 60 sec after S had been placed in the chamber. The CS duration was 10 sec and the last second overlapped with the onset of the 1-sec footshock. The Ss were then immediately removed from the chamber and returned to the home cage. Control Ss (Group C) received an unpaired presentation of tone and shock. Sixty seconds after S was placed in the chamber, the US came on for 1 sec. Ten seconds

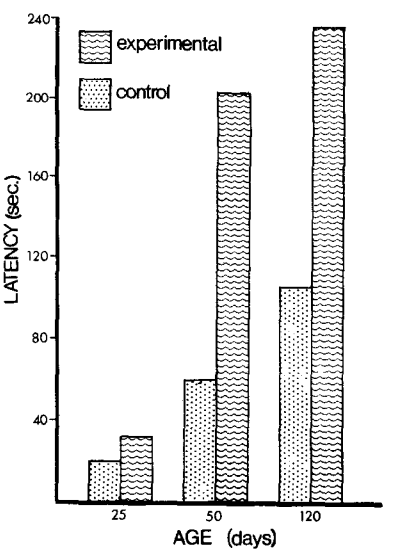

Fig. 1. Mean latencies to first 10 licks after CS presentation for conditioned (experimental) and pseudoconditioned (control) Ss.