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Consummatory Successive Negative Contrast in Mice

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Mice exposed to a 32% sugar solution during 10 daily sessions later rejected a 4% solution significantly more than control mice always given access to the 4% solution. With 3-h access to the solutions, Group 32-4 consumed significantly less than Group 4-4 during the first postshift session; however, an increase in the consumption level in Group 4-4 from the last preshift to the postshift sessions may have inflated the size of the effect. The phenomenon was clearly demonstrated when mice received 1-h access to the solutions (Experiments 2 and 3). This effect was eliminated by he administration of diazepam (4 mg/kg) during the postshift period (Experiment 3). This is the first demonstration of successive negative contrast in a consummatory response situation with mice. The species generality of successive negative contract in consummatory and instrumental response systems is discussed.

First reported independently by Elliott (1928) and Tinklepaugh (1928), the phenomenon known as successive negative contrast (SNC) has played a key role in illustrating the interplay between cognitive, motivational, and emotional factors in the control of learned behavior. In Elliott's (1928) experiments, the maze performance of rats shifted from a more preferred food type (bran mash) to a less preferred, but acceptable, food (sunflower seeds) exhibited a significant deterioration relative to a control condition always reinforced with sunflower seeds. Similar results were reported by Tinklepaugh (1928) in rhesus monkeys trained in a choice situation, by Crespi (1942) in rats trained in a runway situation and subjected to a quanti-

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tative downshift in the amount of food reward, and by Weinstein (1970) in rats reinforced for lever pressing with sucrose solutions.

SNC can also be obtained in consummatory responses. For example, Vogel, Mikulka, and Spear (1968) exposed rats to either 32 or 4% sucrose solutions in a 5-min-long sessions for 11 days and then shifted the 32% group to a 4% solution. Shifted rats showed a transient suppression of licking behavior below the level exhibited by the 4% control rats that lasted two to four postshift sessions. Consummatory SNC has been the subject of intensive research by Flaherty and his colleagues using rats as subjects (for a review, see Flaherty, 1996), but relatively little is known about the species generality of this effect. For example, newborn babies exhibit less sucking of water after exposure to a 15% sucrose solution (Kobre & Lippsit, 1972). Consummatory SNC has also been reported in two species of didelphid marsupials, also given access to 32 and 4% sucrose solutions in a procedure analogous to that used with rats (Papini, Mustaca, & Bitterman, 1988). There is also a report involving consummatory behavior in a nonmammalian species, the goldfish *Carassius auratus* (Couvillon & Bitterman, 1985). In this experiment, a shift from a more preferred food (fish diet) to a less preferred food (the same food adulterated with quinine) led to no change in consummatory behavior. The absence of consummatory SNC in goldfish parallels similar results obtained in instrumental situations with nonmammalian species (Lowes & Bitterman, 1967; Papini, 1997; Papini, Muzio, & Segura, 1995; Pert & Bitterman, 1970; Schmajuk, Segura, & Ruidiaz, 1981). We report here a successful demonstration of consummatory SNC in mice, *Mus musculus.*

EXPERIMENT 1

Preference tests were run to determine how much fluid intake could be obtained per day and whether the 32 and 4% sucrose concentrations commonly used in experiments with rats could also be employed with mice. Two bottles were left in the animal's cage for a 24-h period containing either water or a 32 or 4% sucrose solution (commercial sugar mixed with tap water, w/v). Each mice was tested between 5 and 8 days in the same two-bottle test; the relative position of the bottles was shifted across days. The results of these pilot observations are shown in Table 1. Mice preferred the 32% solution to the 4% solution, the latter to water, and also the former to water. Therefore, mice exhibit both discrimination between the two concentrations used in the present experiments and preference for the sweeter of the two solutions. Based on these pilot observations, the same solutions used in experiments with rats were adopted in the present studies. Animals were given 3-h-long sessions of access to the solutions in their living cages and without any specific food deprivation procedure. Food and water were continuously available in the cage, except during the session.

TABLE 1
Mean Daily Consumption (ml) in Two-Bottle Preference Tests: Pilot Results

Comparison	n	Consumption (ml)
32% vs. 4%	2	8.0 vs. 1.0
4% vs. water	4	4.0 vs. 2.9
32% vs. water	2	9.4 vs. 0.6

Note. Tests were conducted over 24-h periods, each mouse was tested between 5 and 8 days on the same two solutions, and the relative positions of the bottles were changed across days.

Method

Subjects. The subjects were 12 3-month-old female mice (BALB/c strain), all experimentally naive and weighing between 20 and 27 g. They were housed in individual cages in a room subject to a 12:12 h light:dark cycle (light on from 7:00 to 19:00 h). No special deprivation procedure was implemented in this experiment, except that food and water were taken away from the individual cages during the 3 h of exposure to the solution.

Apparatus. Training sessions were administered in the living cages. The mice used in the experiment were located in a separate room, apart from the rest of the colony. Each aluminum cage was 20 cm wide, 30 cm long, and 12 cm high, with a metallic lid. Water and food were administered through the lid. The sugar solutions were administered in graduated cylinders placed in the outside of the front wall of each cage. The spout was located approximately 5 cm from the floor.

Procedure. Pairs of mice matched in weight were randomly assigned to Groups 32-4 and 4-4 ($n = 6$). The solutions (commercial sugar mixed with tap water, w/v) were placed in the cages from 16:00 to 19:00 h each day (i.e., the last 3 h of light in the light:dark cycle). There were 10 preshift days in which animals received access to either the 32 or 4% solution, depending on the group, followed by 2 postshift days in which all animals were given access to the 4% solution. The amount of solution drunk by each animal was recorded at the end of each 3-hr period. In addition, during days 10, 11, and 12 the amount consumed was recorded at 1-h intervals during the 3 h of exposure to the solutions.

Results

Daily mean consumption of the 32% solution during the preshift period increased from 2.6 ml in day 1 to 4.3 in day 10. The same values for the 4% solution were 2.4 and 2.16 ml, indicating that it was consumed less than the 32% solution and that there was little or no change in consumption across days. A statistial analysis yielded a significant group by days interaction

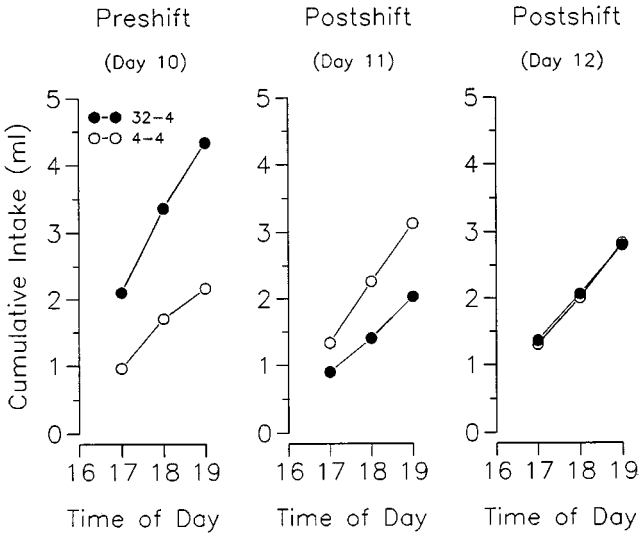

FIG. 1. Cumulative fluid intake of Groups 32-4 and 4-4 during the last preshift day (day 10) and the 2 postshift days (days 11 and 12). Solutions were refilled or shifted, depending on the condition, at 16:00 h. Fluid intake was recorded at the end of each hour, for a 3-hr period beginning at 17:00 h and ending at 19:00 h.

[$F(9, 90) = 9.177$; $p < .001$], as well as a significant group effect [$F(1, 10) = 210.033$; $p < .001$] and a significant days effect [$F(9, 90) = 3.050$; $p < .003$].

Figure 1 shows the results of the hourly record of fluid intake over the 3 key days of the experiment, the last preshift day (day 10), and the 2 postshift days (days 11 and 12). Two analyses were computed, one on total consumption over the 3-h period and a second on the amount consumed after 1 h of exposure to the solutions. For day 10 (last preshift day), fluid intake was significantly larger in Group 32-4 than in 4-4 both over the 3-h period [$F(1, 10) = 199.291$; $p < .001$] and after the first hour of exposure to the solutions [$F(1, 10) = 78.108$; $p < .001$]. In day 11 (first postshift day), the amount consumed by Group 32-4 dropped below the level of the 4-4 control. The overall consumption [$F(1, 10) = 180.090$; $p < .001$] and also the amount consumed during the first postshift hour [$F(1, 10) = 10.138$; $p < .001$] were significantly lower in Group 32-4 than in Group 4-4. By day 12, the second postshift day, shifted mice consumed as much fluid as unshifted controls; groups differed neither in the overall measure nor after the first hour (Fs < 1).

Figure 1 suggests that the difference between groups in day 11 (the critical first postshift day) may have been inflated by an increase in consumption in the unshifted control group. Analyses of total intake and first-hour intake in

Group 4-4 across days 10–12 indicated a significant change across sessions for both measures [Fs(2, 10) $\geq$ 6.852; ps $<$ .02. Pairwise Scheffe tests showed that, in both measures, intake was significantly lower in day 10 than in both days 11 and 12 (ps $<$ .05); the difference between days 11 and 12 was nonsignificant. Similar tests for Group 32-4 also demonstrated significant differences across days [Fs(2, 10) $>$ 88.214; ps $<$ .001]. In this group, however, consumtpion differed significantly in each pairwise Scheffe test (ps $<$ .05).

EXPERIMENT 2

The differences between Groups 32-4 and 4-4 in the previous experiment was already significant after an hour of exposure to the solutions in the first postshift session. Therefore, the present experiment explored the implementation of 1-h-long sessions from the start of training.

Method

The subjects were 14 male mice of the same strain, age, and weight as in the previous experiments. They were all experimentally naive and maintained under the same conditions described in Experiment 1, including the light:dark cycle and the house cages. Pairs matched in body weight were randomly assigned to Groups 32-4 and 4-4 (n = 7). The procedure was exactly as described in Experiment 1 with only one exception. The solutions were presented at 16:00 h, every day, and withdrawn at 17:00 h; the amount consumed was recorded at the end of the daily session.

Results

Figure 2 shows the average daily consumption of each solution during both periods of the experiment. Preshift consumption increased across days mainly in Group 32-4, an observation supported by a significant group by day interaction [F(9, 108) = 15.416; p $<$ .001]. Significant simple main differences across groups [F(1, 12) = 14.928; p $<$ .002] and days [F(9, 108) = 27.19; p $<$ .001] were also obtained in the analysis. As Experiment 1, Group 32-4 showed a significantly lower intake of the 4% solution than Group 4-4 in day 11, the first postshift session [F(1, 12) = 5.444; p $<$ .03], but not in day 12, the second postshift session (F $<$ 1).

In this experiment, the consumption level of the mice in the unshifted control condition was very stable, as shown in fig 2. An analysis of fluid intake during days 10–12 (from the last preshift day onward) in Group 4-4 indicated a nonsignificant session effect (F $<$ 1). Therefore, the SNC effect observed under the present conditions was not affected by variations in the dependent measure in the unshifted controls, as it was the case in the previous experiment. A similar analysis for the data of Group 32-4 indicated a highly significant days effect [F(2, 12) = 44.354; p $<$ .001]. Scheffe pairwise comparisons further demonstraed that fluid intake was significantly larger in day

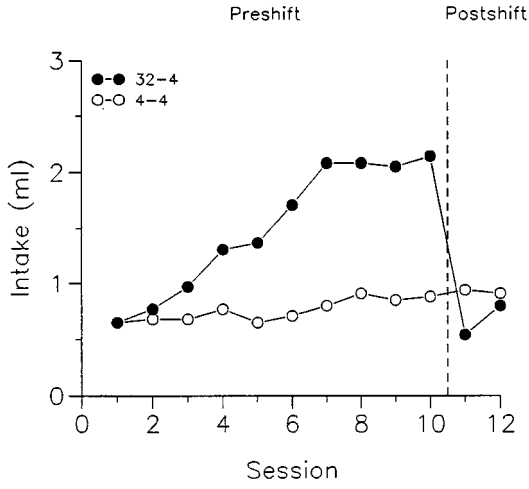

FIG. 2. Daily average intake of sucrose solutions in Groups 32-4 and 4-4 during the preshift (days 1–10) and postshift periods (days 11 and 12). Access to the solutions was restricted to an hour per day, from 16:00 to 17:00 h.

10 than in days 11 and 12 ($ps < .05$) which, in turn, did not differ from each other.

EXPERIMENT 3

Despite the low amounts of fluid consumed by mice in 1 h of exposure to sucrose solutions, the consummatory SNC effect found in the previous experiment was surprisingly strong. However, the question remains as to whether the type of SNC found under the present conditions is based on the same underlying mechanisms as that more typically studied in rats. We provide an initial answer to the question of whether the mechanisms of consummatory contrast observed in mice and rats are homologous by studying the effects of the benzodiazepine drug diazepam on contrast under the conditions described in Experiment 2. Benzodiazepine drugs (e.g., chlordiazepoxide, midazolam, and flurazepam) are among the most effective drugs in reducing consummatory SNC in rats (see review in Flaherty, 1991). Diazepam itself has not been studied in consummatory contrast, but it is known to have anxiolytic effects in a variety of aversively motivated tasks in rats (Flaherty, 1991).

Method

Subjects and apparatus. The subjects were 28 male, experimentally naive mice of the same strain, age, and weight as those used in previous experiments. The conditions of housing and maintenance were as described above, including the cages and the light:dark cycle.

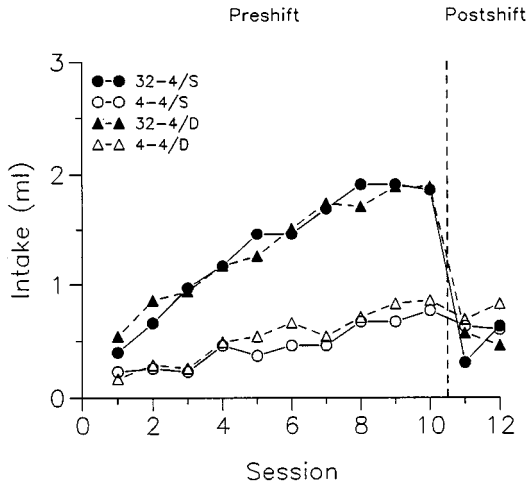

FIG. 3. Daily average intake of sucrose solutions in groups exposed to either 32 or 4% solutions during the preshift period (days 1–10) and treated with either diazepam (D, 4 mg/kg) or an equal volume of saline solution (S) during the two postshift sessions (days 11 and 12). All groups receive access to the 4% sucrose solution during the postshift sessions. Sessions lasted 1 h and were run between 16:00 and 17:00 h each day.

Procedure. Quadruplets of mice matched on weight were formed and individuals assigned randomly to one of four conditions ($n = 7$), depending on whether they received exposure to 32 or 4% solution during the preshift period and whether they were injected with 4 mg/kg of diazepam (d) or an equal volume of saline (S) solution during the postshift days. Group labels were, therefore, 32-4/D, 4-4/D, 32-4/S, and 4-4/S. The training procedure was exactly as that described in Experiment 2.

Results

One animal in group 4-4/S became ill in the course of the acquisition phase and was thus dropped from the experiment, leaving that group with an $n = 6$. Figure 3 shows the results of this experiment. The preshift period replicated the results obtained in the previous experiment with similar statistical outcomes. Since no drug treatment was administered during this phase and the groups to be treated with diazepam and saline were obviously similar in consumption, animals were pooled in two groups depending on the concentration of the sucrose solution received during these sessions for statistical purposes. The progressively larger amount of fluid consumption in the 32% groups versus the 4% groups gave rise to a significant group by day interaction [$F(9, 225) = 13.133$; $p < .001$]. Consumption of the 32% solution was significantly above that of the 4% solution ($F(1, 25) = 83.461$; $p < .001$], and there was also a significant increase in fluid intake aross days [$F(9, 225) = 66.111$; $p < .001$].

The two postshift days show three important results. First, there was an SNC effect that replicated the results of the prior experiment. Second, this SNC was eliminated by diazepam treatment. Third, diazepam did not affect drinking per se, as shown by a comparison of the 4-4 control groups. A Preshift (32%, 4%) by Drug (diazepam, saline) by Day (11, 12) analysis provided support for this pattern in terms of a highly significant triple interaction: $F(1, 23) = 13.656$, $p < .005$. The Preshift factor was also signficant [$F(1, 23) = 13.635$; $p < .005$], but none of the other effects reached a significant level. Pairwise tests for the results of day 11 (first postshift day) corroborated the main results. The SNC effect was evidenced in the significant difference between Groups 32-4/S and 4-4/S [$F(1, 12) = 10.368$; $p < 0.01$]. The effects of diazepam on contrast were evidenced in terms of both a significant difference between Groups 32-4/D and 32-4/S [$F(1, 12) = 8.100$; $p < .05$] and a nonsignificant difference between Groups 32-4/D and 4-4/D ($F < 1$). The lack of differences ($F < 1$) between Groups 4-4/D and 4-4/S indicates no evidence for an effect of diazepam upon drinking itself.

As in the previous experiment, Fig. 3 shows relatively stable levels of fluid intake in the two unshifted groups. Separate analyses were performed on these data over the last three sessions of the experiment. Nonsignificant day effects were obtained for both Group 4-4/D and 4-4/S [Fs$(2, 10) \leq 2.333$; ps $\geq .14$], suggesting that the interpretation of the SNC effect is not clouded by daily fluctuations in consumption in the unshifted controls. Group 32-4/D demonstrated significant changes in consumption across days [$F(2, 12) = 73.137$; $p < .001$]. While consumption was significantly higher in day 10 than in days 11 and 12 (Scheffe pairwise tests: ps $< .05$), no differences were observed between the 2 postshift days. By contrast, consumption differed across days in Group 32-4/S [$F(2, 12) = 116.333$; $p < .001$], with each pairwise Scheffe test demonstrating a significant difference (ps $< .05$).

GENERAL DISCUSSION

Despite its intensive use as a tool to understand the interplay between learning, motivation, and emotion, the consummatory SNC situation has not been studied intensively in many species besides the laboratory rat. Several isolated experiments with human newborns and didelphid marsupials reported positive results (Kobre & Lipsitt, 1972; Papini et al., 1988), but a series of experiments with goldfish provided no evidence of consummatory SNC, despite clear evidence of reward discrimination (Couvillon & Bitterman, 1985). Similar species differences were reported in experiments involving a related SNC effect based on instrumental performance (see the introduction for references), leading to the suggestion that the mechanisms underlying SNC may be uniquely mammalian (Papini, 1997). Therefore, the extension of contrast research to new species bears particular significance for an understanding of the commonalties and differences in learning mechanisms across vertebrates.

The most important contribution of the present series of experiments is the development of a suitable preparation for studying contrast effects in mice. The SNC effect demonstrated in the present experiments was particularly strong when male mice were given 1-h access to the experimental solutions (Experiments 2 and 3). The present demonstration is similar to the SNC effect described in rats in being both transient and vulnerable to a benzodiazepine drug. Unlike consummatory experiments with rats, the present experiments with mice did not involve explicit food deprivation procedures. In rats, satiation diminishes the licking rate induced by the 32% solution, but a shift to 4% reduces licking rate further, leading to a SNC effect (Flaherty, Coppotelli, & Potaki, 1996a). A lower preshift licking rate appears to be inconsistent with the significantly larger quantities consumed by mice before the shift in the present experiments. However, such a conclusion would be based on the assumption that licking rate and solution intake are positively correlated, for which we have no information.

The vulnerability of SNC to diazepam in mice implies that this form of contrast is based on similar neurochemical mechanisms to those underlying this effect in rats. The effects of benzodiazepine anxiolytics on consummatory SNC in rats is notorious for the degree of specificity of the results. Chlordiazepoxide, for example, reduces the SNC effect on the second postshift session, but has no effect when administered during the first postshift session (Flaherty, Grigson, & Rowan, 1986). In rats, however, sessions lasts for 5 min counting from the first lick, which is much much shorter than the shortest session in the present series (i.e., 1 h, as in Experiments 2 and 3). It seems plausible that benzodiazepine drugs act only after the animal has had some experience with the downward shift in concentration and comes to anticipate the aversiveness of the new experience. The hour-long session would have allowed the mice in Experiment 3 enough experience with the downshifted solution to learn to anticipate the aversive consequences of drinking. This is consistent witht he fact that, in rats, chlordiazepoxide does reduce SNC in the first session when this session is lengthened to 20 min (Flaherty et al., 1986) or after repeated downward shifts (Flaherty, Clarke, & Coppotelli, 1996b). Now that a suitable preparation has been developed for the study of consummatory contrast in mice, it may be useful to measure licking responses, in addition to consumption. Licking responses recorded in real time would allow for a more precise description of the temporal dynamics characterizing the emergence of the consummatory SNC effect.

The present results are consistent with the view that the mechanisms underlying consummatory SNC effects are the same in rats and mice, despite obvious procedural differences. The training procedure described in these experiments may be applied to the study of transgenic and knockout strains of mice, some of which have shown diferences in anxiety tests and aggressive behavior (e.g., Brunner, Buhot, Hen, & Hofer, 1999). For example, unpub-

lished results from our laboratory using the same procedure described in Experiment 1 have produced a normal SNC effect in knockout mice lacking the gene coding for the serotonin receptor 1B.[1] Similarly, the procedure developed in the present experiments may be usefully applied to other species of small mammals (e.g., insectivore species) in which the procedure used with rats may not work very effectively.

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[1] This unpublished experiment involving KO1B and wild-type groups of mice was run in collaboration with Dani Brunner and Brian L. Thomas. Four groups resulting from a cross between preexposure solution (4%, 32%) and strain (KO1B, wild type), received 15 3-h-long sessions of preexposure followed by a single postshift session. Unlike the case in Experiment 1, fluid intake in the two unshifted control groups was relatively constant from the end of the preshift to the postshift session. In both strains, a shift to the 4% solution caused a significant, but equal, suppression in fluid intake.

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