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The Influence of Flavored Solution Concentration on the Poisoned-Partner Effect

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Two experiments are reported in which we attempted to replicate the poisoned-partner effect, varying the concentration of a novel-tasting solution. Unpoisoned rats exhibited an aversion to a saccharin solution that had been consumed previously in the presence of a poisoned rat when it had a concentration of 0.6% but did not when it was of 0.15%. These results seem to show that the poisoned-partner effect depends upon the intensity of the solution employed and to suggest that it may be facilitated by a low initial consumption.

The ease with which rats associate food-related stimuli (taste and odor) with internal consequences of ingestion is well known (see Baker, Best, & Domjan, 1977; Milgram, Krames, & Alloway, 1977). This ability allows rats to efficiently avoid poisonous food.

Lavin, Freise, and Coombes (1980) have shown that rats can also learn to avoid a novel-tasting solution if it has been consumed prior to or in the presence of a poisoned rat. This is called the poisoned-partner effect (PPE). The PPE seems to be a learned flavor aversion produced by association between the taste of the solution and the olfactory cues induced by the poisoned partner's illness (Stierhoff & Lavin, 1982). Given that several parameters, such as novelty of solution, doses of poison, preexposure, and delay, act in the same way for the PPE as for the normal flavor-aversion paradigm (see Coombes, Revusky, & Lett, 1980; Revusky, Coombes, & Pohl, 1982), the associative mechanism of the PPE seems similar to this latter.

However, although the PPE appears to be a robust phenomenon, since it has been confirmed repeatedly (Alonso & Alzate, 1986; Bond, 1982, 1984; Coombes et al., 1980; Iraola, 1992; Revusky et al., 1982; Stierhoff & Lavin, 1982), it is frequently small or even nonoccurring (see Iraola, 1992; Revusky, Coombes & Phol, 1981). In any case, the PPE is weaker than aversion produced by poisoning itself and extinguishes more quickly. This seems reasonable considering that the nonpoisoned partner (NPP) learns to avoid a flavored solution as a consequence of olfactory cues induced by its poisoned partner (PP)'s illness, and these cues are somewhat volatile and short-lived (Bond, 1984), while the PP avoids the flavor due to an illness that is long-lived.

However, there may be other reasons for this weakness. For instance, the PPE was found to be stronger for a less preferred flavor, such as a 0.02% quinine sulfate solution, than for a more preferred one, such as a 0.6% saccharin solution (Coombes et al., 1980; Lavin et al., 1980). Therefore, the strength of the PPE could depend on the initial level of preference, being greater to the extent that a solution is less preferred. The present experiments set out to test whether this factor influenced the PPE.

EXPERIMENT 1

The first experiment attempted to replicate the experimental design employed by Lavin et al. (1980), but using a different concentration of saccharin solution. We used a concentration of 0.15% instead of the 0.6% concentration used by them. The 0.15% saccharin solution is even more preferred than the 0.6% one (Iraola, 1992). If the PPE's strength depends on the level of initial preference for the flavored solution, as was stated above, then greater PPE to a less preferred solution should be observed.

Method

Subjects and Apparatus

Thirty, experimentally naive, male Wistar albino rats were used. They ranged in *ad lib.* weight from

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232 to 284 g just before the experiment began. They were kept individually in makrolon cages (15 × 27.5 × 27.5 cm) located in an air-conditioned temperature-controlled room on a 12-h light/dark cycle with light on at 2 AM. The experimental procedures were conducted in black metal cages (19 × 31.5 × 20 cm) located in a room adjacent to the home cages room during the dark portion of the cycle. Dry food was available throughout the experiment, but access to water was limited as indicated. There were three training groups (PP, NPP, and CONT) of 10 rats each that had been matched on water intake scores. The rats were randomly assigned to 15 pairs: individual rats from the PP group were paired with individual rats from the NPP group and individual rats from CONT group were paired with each other. Pairs remained constant throughout the experiment.

Procedure

Pretraining. Water was removed from all home cages 24 h before the experiment began. On the first 4 days (Days 1–4), rats were placed in pairs and allowed to drink unflavored water from glass bottles for 30 min daily. On Day 5, all rats, housed individually, were given access to 15 min of water. After water consumption was recorded, the bottles were placed back in the home cages until conditioning.

Training. On Day 6 conditioning took place. The rats were weighed and then placed with their partners. They were allowed to have access to a novel 0.15% saccharin solution (weight/volume) for 2 h. Rats were then removed and weighed, and their saccharin consumption was recorded. Immediately afterward, PP rats received an intraperitoneal injection of 10 ml/kg of 0.3 *M* LiCl. NPP and CONT rats did not receive an injection. All rats were then returned to the cage with their partners and allowed a further 2 h access to the saccharin solution. At the end of the second 2-h period, all animals were once again weighed and consumption of saccharin solution was noted.

Testing. On Day 7 the aversion test took place. All rats, housed individually, were given access to the saccharin solution for 15 min.

Consumption scores were determined by subtracting the postintake weights of the bottles from the preintake weights.

Results

Pretraining. On Day 5 the animals individually drank a mean of 13, 13.4, and 13.3 g of water for PP, NPP, and CONT groups, respectively. A one-way analysis of variance on these data revealed that differences among groups were not significant [$F(2, 27) = 0.23$, $p > 0.5$].

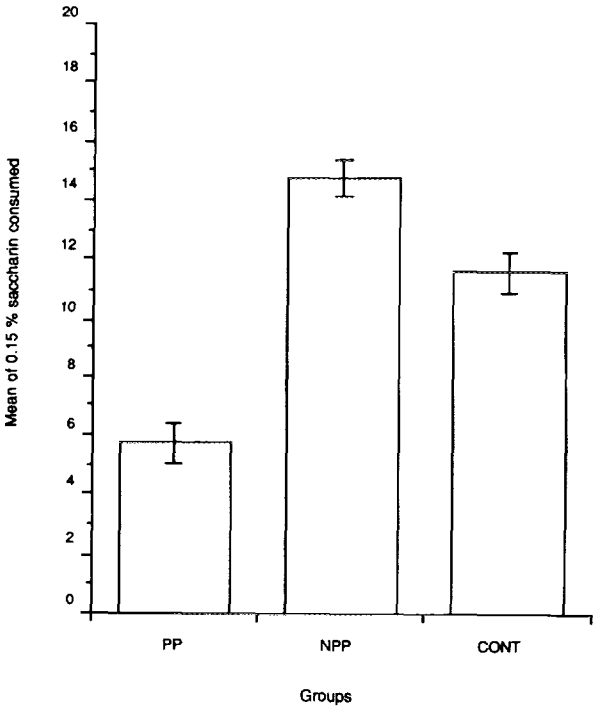

FIG. 1. Mean consumption of the 0.15% saccharin solution on the test day for each of the three groups in Experiment 1. PP stands for poisoned partner, NPP for nonpoisoned partner, and CONT for control.

Training. As expected, the PP–NPP (17.3 g) and CONT (16 g) pairs' saccharin intake did not differ during the first 2-h period of conditioning day [$t(13) = 0.36$, $p > .05$]. During that period, the animals gained a mean weight of 3.74 ± 0.4 g but there were no reliable difference in weight change among groups [$F(2, 27) = 1.6$, $p > .05$]. Although animals of PP–NPP pairs (7.15 g) drank less than CONT pairs (11 g) during the second 2-h period, the difference between them was not significant [$t(13) = 1.74$, $p > .05$]. However, the animals lost a mean of 1.94 ± 0.33 g in that period, differences among the groups being statistically significant [$F(2, 27) = 5.4$, $p < .01$]. The Newman–Keuls tests showed that PP rats lost more weight in relation to NPP and CONT rats ($ps < .01$), probably due to poisoning produced by the injection of LiCl. Moreover, saccharin consumption was significantly lower during the second period as compared to the first for paired PP–NPP animals [$t(9) = 4.7$, $p < .01$], but not for paired CONT animals [$t(4) = 1.4$, $p > .05$].

Testing. Figure 1 shows mean amounts of saccharin consumed on the test day for each of the three groups. As expected, the PP group displayed an aversion to saccharin, but the NPP group did not. The differences among groups (one-way analysis of variance) were statistically significant [$F(2, 27) = 48.9$, $p < .001$]. The Newman–Keuls tests showed that PP animals consumed significantly less saccharin than NPP and CONT animals ($ps < .01$), and NPP animals surprisingly consumed more than CONT animals ($p < .05$). Aversion to saccharin for the PP group is explained by the effect of poisoning, but the NPP group result is difficult to explain. We believe it must be attributed to chance. Saccharin consumption on Day 7 was also significantly lower than water consumption on Day 5 for the PP group [$t(9) = 8.4$, $p < .001$], nearly significantly greater for the NPP group [$t(9) = 2.17$, $p = 0.6$], and not significantly different for the CONT group ($t(9) = 1.56$, $ps > .05$).

These results did not replicate those obtained by Lavin et al. (1980), since we failed to obtain the PPE. As Bond (1984) suggested, the failure to replicate an experiment can be due to the altering of, seemingly, minor but important parameters. This could be the case; but this negative result seems to have important consequences. One modification in this replication was the place where the experiment was run: it was not in the home cages. However, we think that the essential difference between this experiment and that of Lavin et al. was the level of saccharin solution concentration employed. We used a low concentration of saccharin solution (0.15%), which is initially much more preferred by rats than a high concentration one (0.6%), such as they had used (Iraola, 1992). It may be that a highly preferred solution was considered "safe" and, in consequence, would not easily be associated with cues induced by the poisoned partner's illness. That could explain why nonpoisoned rats did not show an aversion to saccharin. If this is so, then the PPE would depend on the initial level of preference for the solution, being scarce or null when a highly preferred solution is employed.

EXPERIMENT 2

We failed to obtain the PPE in Experiment 1. That was probably due to our altering of some of the parameters often used, essentially the saccharin solution's level of concentration: 0.15% instead of 0.6%. A subsequent experiment was carried out in an aim to eliminate procedural differences, other than those apparently essential, and to test whether the PPE depends on the level of solution concentration by varying this.

Method

Subjects and Apparatus

Forty-eight, experimentally naive, male Wistar albino rats were used. They ranged in *ad lib.* weight from 303 to 388 g just before the experiment began. They were kept under the same conditions as those described for Experiment 1.

Procedure

The rats were pretrained, trained, and tested under similar conditions as those described in Experiment 1, except that all procedures were conducted in the home cages instead of the black cages, animals were distributed in six groups of eight rats each, and a 2 × 3 factorial design was employed. The level of saccharin solution concentration (0.6% vs 0.15% w/v) was one factor, and the training condition (PP, NPP, and CONT) was the other.

Results

Pretraining. On Day 5 the animals individually drank a mean of 15.4, 14.1, 16.9, 17.2, 15.1, and 15.4 g of water for the PP.15, PP.6, NPP.15, NPP.6, CONT.15, and CONT.6 groups, respectively. The differences among groups were not significant [$F(5, 42) = 2$, $p > .05$].

Training. The PP.15–NPP.15 (21.1 g), PP.6–NPP.6 (8.2 g), CONT.15 (22.2 g), and CONT.6 (9 g) pairs' saccharin intake significantly differed during the first 2-h period of the conditioning day [$F(3, 20) = 8.53$, $p < .01$]. Subsequent analysis showed that paired PP.6–NPP.6 and CONT.6 animals consumed less than paired PP.15–NPP.15 animals and CONT.15 animals ($ps < .05$). No other differences between groups were reliable ($ps >$. 05). As expected, this result seems to indicate that rats initially preferred the 0.6% saccharin less in relation to the 0.15% saccharin. During the first period, the animals lost a mean weight of 2 ± 0.19 g, but differences in weight change among groups were not significant [$F(5, 42) = 0.5$, $p > .05$].

The PP.15–NPP.15 (7.7 g), PP.6–NPP.6 (5.2 g), CONT.15 (19 g), and CONT.6 (10.9 g) pairs' saccharin intake also differed during the second 2-h period of the conditioning day. A 2 (saccharin concentration) × 2 (training pair) ANOVA revealed a significant effect for level of saccharin concentration [$F(1, 20) = 8.39$, $p < .01$], a significant effect for training pair [$F(1, 20) = 27.6$, $p < .01$], but no

interaction effect [$F(1, 20) = 2.95$, $p > .05$]. Subsequent analysis with the Newman–Keuls tests showed that paired PP.15–NPP.15, PP.6–NPP.6, and CONT.6 animals consumed less than paired CONT.15 animals ($ps < .05$) . The remaining comparisons were not significant ($ps > .05$). This indicated that animals exposed to poisoning and a poisoned partner consumed less saccharin independently of which saccharin solution was used, and animals not exposed to poisoning or a poisoned partner also consumed less if the saccharin had a concentration of 0.6%. During the second period, the animals lost 2.14 ± 1.75 g of weight and the differences among groups were significant [$F(5.42) = 3$, $p < .05$]. Weight change for PP.15, PP.6, NPP.15, and CONT.15 animals was greater than that for CONT.6 animals ($ps < .05$) The remaining comparisons were not significant ($ps > .05$).

Intragroup comparison revealed that paired PP.15–NPP.15 animals consumed significantly less saccharin solution during the second period than the first one [$t(7) = 4.2$, $p < .05$], and CONT.6 animals consumed significantly more during the second period than the first [$t(3) = 3.5$, $p < .05$]. There were no significant differences in the remaining pairs ($p > .05$).

Testing. Figure 2 shows mean amount of saccharin consumed on the test day for each of the six groups. As shown, rats that received the 0.6% saccharin solution consumed, on the whole, less than rats that received the 0.15% saccharin solution. Moreover, PP rats consumed less than the CONT rats, as did some but not all NPP rats. The visual impression was confirmed by statistical analysis. A 2 (saccharin concentration) × 3 (training conditions) ANOVA showed a significant effect for the level of saccharin solution concentration [$F(1, 47) = 43.6$, $p < .01$], a significant effect for training condition [$F(2, 47) = 64.7$, $p < .001$], and there was also an interaction between these main effects [$F(2, 47) = 3.67$, $p < .05$]. Subsequent analysis revealed that the PP.6 group consumed significantly less than the remaining groups ($ps < .05$); the PP.15 and NPP.6 groups consumed significantly less than the NPP.15, CONT.15, and CONT.6 groups ($ps < .05$); and the CONT.6 group also consumed less than the NPP.15 and CONT.15 groups ($ps < .05$). The remaining comparisons were not significant ($ps > .05$).

Finally, intragroup comparison revealed that saccharin consumption on Day 7 was significantly lower than water consumption on Day 5 for the PP.15, PP.6, and NPP.6 groups [$ts(7) > 7$, $ps < .05$],

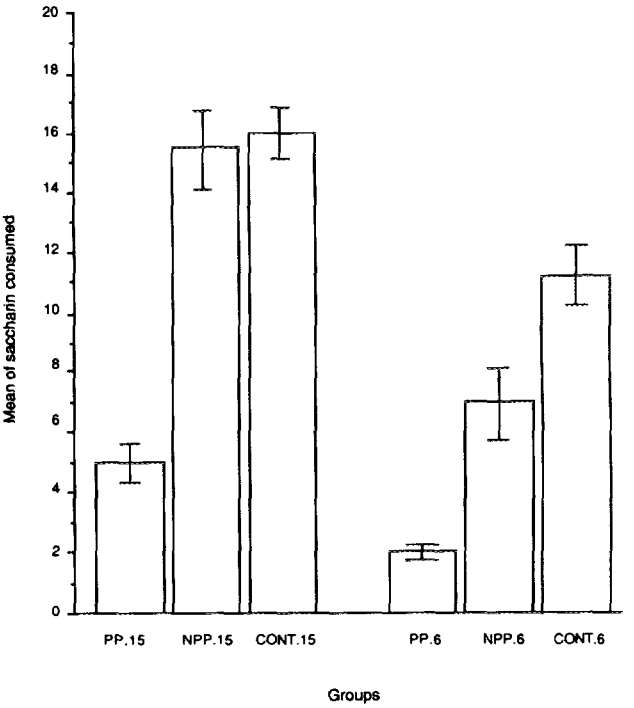

FIG. 2. Mean consumption of saccharin solutions on the test day for each group in Experiment 2.

and it approached an acceptable level of significance for the CONT.6 group [$t(7) = 2.1$, $p = .06$]. The remaining comparisons were not significant [$ts(7) < 2$, $ps > .05$]. This seems to indicate that a greater concentration of saccharin solution can produce greater conditioning by poisoning, a conditioned aversion by exposure to a poisoned partner, and an unconditioned aversion without poisoning and without exposure to a poisoned partner.

These results confirmed those found in Experiments 1 and also those of Lavin et al. (1980) and indicated that the differences between them were due to the different parameters employed, the essential difference being the level of saccharin concentration. The PPE was obtained only when the concentration of saccharin solution was of 0.6%, not when it was of 0.15%. That is to say, the effect of saccharin consumption in the presence of a poisoned partner on the NPP's posterior intake seems to depend on which saccharin solution is consumed. The most important point, however, is that these results seem to suggest that some unconditioned aversion to saccharin may need to be present in order to obtain the PPE. All rats that had had access to the 0.6% saccharin, including CONT rats, showed an apparent aversion for that solution in relation to the 0.15% one during the testing and the first 2-h period on conditioning day. Aversion was not shown

in any case when the saccharin was of 0.15%, except by the poisoned rats in testing.

DISCUSSION

The experiments reported here describe two conditions of saccharin concentration in the PPE paradigm that produced different results. The fluid consumption of a nonpoisoned rat was attenuated by the presence of a sick rat when a 0.6% saccharin solution was employed, but not when a 0.15% saccharin solution was. That is to say, the PPE was obtained with the more concentrated saccharin solution, but not with the less concentrated one. The general conclusion is that the PPE seems to depend on the concentration of the solution employed.

An explanation of these results might be based on the solution's salience. It is well known that flavor aversion learning is directly related to the intensity or salience of the solution (Dragoin, 1971). Thus, the more concentrated the solution, the greater its intensity or salience, and, consequently, the greater its associability or the possibility of becoming associated with the aversive consequences. This seems to have occurred with the 0.6% saccharin solution for poisoned and nonpoisoned rats, who showed more aversion to it than did their counterparts to the 0.15% one. The absence of the PPE with the less concentrated saccharin solution may have resulted from the combination of low salience and weakness of the effect.

However, this explanation may seem incomplete without taking into consideration the drinking behavior shown by control rats. Rats which were neither exposed to poisoning nor to a poisoned partner also consumed less 0.6% than 0.15% saccharin solution. That is, subjects generally drank less of the more concentrated saccharin solution, just as Domjan and Gillan (1976) previously reported. Therefore, the different consumption of saccharin, depending on the level of concentration, occurred in all cases and was found before and after the different trainings. All of this may be very important in understanding the results obtained.

The different initial consumption may be explained by either palatability or novelty of the solution. Although Kalat and Rozin (1970) found that solution palatability and salience are generally independent of each other, it could be that the more salient saccharin was less palatable in this case. On the other hand, the different initial consumption may be due to a relative novelty response (neophobia). At first sight, it may be considered that there are not reasons to expect a different initial neophobia to the different saccharin solutions, given that all rats experience a novel solution for the first time at the beginning of the conditioning day. However, it would be reasonable to think that the more diluted solution was more familiar, due to its greater similarity to familiar water, than the more concentrated one. Although no data have been presented here to support this hypothesis, neophobic response was found to be reduced faster under repeated exposure to the less concentrated solution condition (Iraola, 1992).

In any case, different initial consumption would have, in fact, resulted in a different exposure amount to the solution and, in turn, a different degree of familiarity. It is known that the amount of exposure to the solution is directly related to the reduction of neophobia (Domjan, 1975) and inversely related to the associability of the solution (Revusky and Bedarf, 1967). Therefore, the lesser familiarity with more concentrated saccharin solution may have produced less consumption of that solution and facilitated its association with the aversive consequences for poisoned and nonpoisoned rats. In contrast, the greater initial familiarity with the less concentrated saccharin solution may have produced a greater consumption and hindered its association with the aversive consequences, to the extent of preventing the association when it is weak itself, as occurred in the PPE.

However, the salience, determined by either intensity or novelty of the solution, does not seem sufficient to explain all of the results, as we have seen, without considering the weakness of the PPE. It could be that the PPE was such a weak phenomenon that it had to be facilitated by "extra conditions" to be observed, in contrast to usual flavor aversion learning. One "extra condition" comes from the fact that the PPE is only obtained when rats are not fluid deprived at the onset of conditioning. Lavin et al. (1980) indicated that the aversion is not found if rats go into the training situation while thirsty. The explanation offered by them eventually indicates that the critical condition is that, in order to obtain the PPE, rats must not consume too much of the flavored solution. In accordance with this is the fact that the PPE was not found for us in nonpoisoned rats that, while not deprived at the onset of conditioning, consumed a lot of the 0.15% saccharin solution. Thus, it could be that the extra condition, which would result in low consumption, was sufficient unconditioned aversion to the solution, produced by either its palatability or its novelty, the strength of the PPE being stronger as the initial aversion is greater. Experimental support for

this suggestion seems to come from the results obtained by Coombes et al. (1980) and Lavin et al. (1980), given that a stronger PPE was found with a flavor initially less preferred (from bitter-tasting 0.02% quinine sulfate) than a flavor initially more preferred (from sweet-tasting 0.6% saccharin solution). With a still more preferred solution, such as that used by us (0.15% saccharin solution), a smaller or even nonexistent PPE would be expected, as, in fact, occurred.

It should be noted that Lavin et al. excluded an explanation of those differences in terms of an enhanced neophobia produced by sensitization of the experience with the sick rat. Others (Bond, 1984; Coombes et al., 1980) also excluded that possibility as an explanation of the PPE and demonstrated that the PPE is an associative phenomennon as was previously indicated. However, since the experience with the sick rat is supposedly less aversive (at least is shorter) than the direct poisoning, it may be reasonable to think that the solution, in order to become associated with that consequence, were, to some extent, also aversive.

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