

Effect of posttraining injections of glucose on acquisition of two appetitive learning tasks

MARK G. PACKARD and NORMAN M. WHITE
McGill University, Montreal, Quebec, Canada

The present experiments were designed to examine the effect of posttraining glucose injections on the acquisition of two appetitive tasks in an 8-arm radial maze. On a win-stay visual discrimination task, the presence of food in four randomly selected maze arms was signaled by a light cue, and rats were required to visit each of the four lit arms twice within a trial. The animals were given one trial per day and injected immediately posttraining on Day 5. A dose of 2.0 g/kg glucose significantly improved win-stay acquisition relative to the performance of controls, but a dose of 100 mg/kg had no effect. On a win-shift task, rats were allowed to obtain food from four randomly selected maze arms, followed by a delay period in which they were removed from the maze. The animals were returned to the maze for a retention test, in which only the arms that had not been visited prior to the delay contained food. After training on shorter delays, 18 h were imposed between the first and second four choices; glucose was injected immediately after the first four choices. Glucose doses of both 2.0 g/kg and 100 mg/kg significantly improved retention, relative to that of controls. The results demonstrate that the memory-improving action of glucose generalizes to appetitive tasks, and they suggest that glucose can improve memory in appetitive tasks with different mnemonic requirements.

Several recent studies have shown that peripheral posttraining injections of glucose have a "reinforcing" (White, 1989) action in the rat (Gold, 1986; Gold, Vogt, & Hall, 1986; Messier & White, 1984, 1987). In these studies, retention of both one-trial inhibitory avoidance (Gold, 1986) and conditioned emotional response tasks (Messier & White, 1984) was improved by posttraining glucose administration. In both studies, the effect of glucose on memory was time-dependent: Delaying the injection by 2 h posttraining eliminated the memory-improving effect (Gold, 1986; Messier & White, 1984).

Our purpose in the present study was to extend these findings in two ways. First, we examined whether the memory-improving properties of glucose generalize to appetitively motivated tasks. The importance of this phenomenon would be enhanced by the demonstration that glucose can improve both aversive and appetitive learning. Second, animals were trained on two radial maze tasks, a *win-shift* task and a *win-stay* task, in which the mnemonic requirements are different—a suggestion that is supported by the finding that acquisition of the two tasks may be mediated by different brain structures (Packard, Hirsh, & White, 1989). Lesions of the fimbria-fornix, but not caudate nucleus, impaired acquisition of win-shift radial maze behavior. In contrast, lesions of the caudate nucleus, but not fimbria-fornix, impaired the acquisition of win-stay radial maze behavior (Packard et al., 1989).

We examined the effects of posttraining glucose on each of these tasks. Finally, two different doses of glucose have been shown to enhance retention. Both 2 g/kg (Messier & White, 1984) and 100 mg/kg (Gold, 1986) were shown to be optimal doses in different experiments. In the present study, we examined the effects of both of these doses.

EXPERIMENT 1

Win-Stay Radial Maze Task

Method

Subjects. The subjects were 44 male Long-Evans rats (275–300 g), housed individually in a temperature-controlled room with a 12:12-h light:dark cycle (7 a.m. to 7 p.m.). Animals were handled for 5 min per day on the first 5 days following arrival in the laboratory. All rats were given ad-lib access to water.

Apparatus. The apparatus was an elevated (60 cm) wooden radial maze painted flat gray. Each arm measured 60 × 9 cm. The center platform was 40 cm in diameter. Food cups were drilled into the outer end of each arm. Small 6-W light bulbs were attached to a 3 × 9-cm wooden strip above the entrance to each arm. The light bulbs faced away from the center platform and were controlled by the experimenter with a manual switchbox. A system of overhead tubing ran from the experimenter's location to the food cups in each arm, allowing for unobtrusive rebaiting. The maze was surrounded by blue curtains. A slanted overhead mirror above the maze was used to observe the animals from behind the curtains. Ceiling lights provided dim illumination.

Drug. The doses of glucose used (2.0 g/kg and 100 mg/kg) were selected for their ability to improve memory, as had been demonstrated in previous studies involving aversively motivated tasks (Gold, 1986; Messier & White, 1984). For animals in the 2 g/kg group, the injection volume was 4.0 ml/kg. For animals in the 100 mg/kg group, the injection volume was 1.0 ml/kg. The injections were administered subcutaneously on the dorsal surface of

Please address all correspondence to Mark G. Packard, Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec H3A 1B1, Canada.

the neck. Control animals for both groups were injected with an equal volume of demineralized water.

Procedure. Prior to training, animals were reduced to 85% of their ad-lib feeding weights. Each was individually placed on the maze for 5 min on 2 consecutive days, and allowed to explore the maze with no food available. On these 2 days, the animals were allowed to consume 10 45-mg Noyes Improved Formula A pellets in their home cages. Food trials began on Day 3. On each food trial, four randomly selected maze arms were illuminated and baited with a single food pellet in the food cup. On the initial food trial only, a few pellets were also placed on the center platform and on the edge of the food cup of each of the four baited arms. When an animal had obtained food from a lit arm and returned to the center platform, the arm remained lit and was rebaited. After the animal had obtained a second pellet from a given lit arm, the light was turned off and no further food was placed in that arm. Thus, the animals earned 8 food pellets within a trial by visiting each of the four illuminated arms twice. The animals were removed from the maze after 8 pellets had been obtained or after 10 min had elapsed. Records were kept of the arms entered. Visits to unlit/unbaited arms were scored as errors. Food trials were run once a day for 10 days. Testing occurred between 1 and 5 p.m. daily.

The animals were randomly assigned to one of three groups: demineralized water ($n = 8$), glucose 2.0 g/kg ($n = 8$), or glucose 100 mg/kg ($n = 8$). On Day 5 of training, the animals were removed from the maze and injected immediately with glucose (2 g/kg or 100 mg/kg) or water vehicle. For food, we used 45-mg Noyes Improved Formula A pellets containing 5% sucrose. In order to control for the possibility that the postingestional effect of sucrose in the pellets might confound the effect of the posttraining glucose injections (particularly at the 100 mg/kg dose), we trained additional groups of animals (water, $n = 4$; glucose, 100 mg/kg, $n = 8$) using 45-mg Noyes Traditional Formula A food pellets (which are made of rat chow with no additional sugar) as the reinforcer. An additional group of animals ($n = 8$) underwent identical training, but were injected with glucose (2 g/kg) after a 2-h delay. This dose was chosen after evaluation of the effects of the immediate post-training injections.

In previous work, we have observed that the rate of acquisition of control animals in this task does not improve measurably over the first 5 days of training (Packard & White, 1989). From Days 5-10, the number of correct choices gradually increases. This was the basis for our selection of Day 5 for the posttraining injections.

Results and Discussion

The effect of posttraining glucose injections on the acquisition of win-stay radial maze behavior is shown in Figure 1. The immediate posttraining injection of 2.0 g/kg of glucose significantly improved win-stay behavior relative to that of water controls, while 100 mg/kg of glucose had no effect. When the injection of 2.0 g/kg of glucose was delayed until 2 h posttraining, no improvement in memory was observed. A two-way, one repeated measure ANOVA computed on Trials 1-5 (i.e., preinjection trials) revealed no significant effect of group [$F = .430$, n.s.] or trial [$F = .158$, n.s.]. A two-way, one repeated measure ANOVA computed on Trials 6-10 (i.e., postinjection trials) showed a highly significant effect of group [$F(4,39) = 39.98$, $p < .001$]. In addition, the analysis revealed a significant group \times trial interaction [$F(4,16) = 3.69$, $p < .001$]. One-way ANOVAs computed by trial revealed significant group effects on each of the five postinjection trials. For Trial 6, the immediate postinjection trial, the group effect was highly significant [$F(4,39) = 24.4$, $p < .001$]. A post hoc Scheffé's test showed that only the 2.0-g/kg dose of glucose had a significantly different effect from water on Trial 6 [$F = 21.2$, $p < .05$]. This superiority of choice accuracy was maintained over Trials 7-10 (Figure 1).

These results show that a 2.0-g/kg dose of glucose improves acquisition of win-stay radial maze behavior,

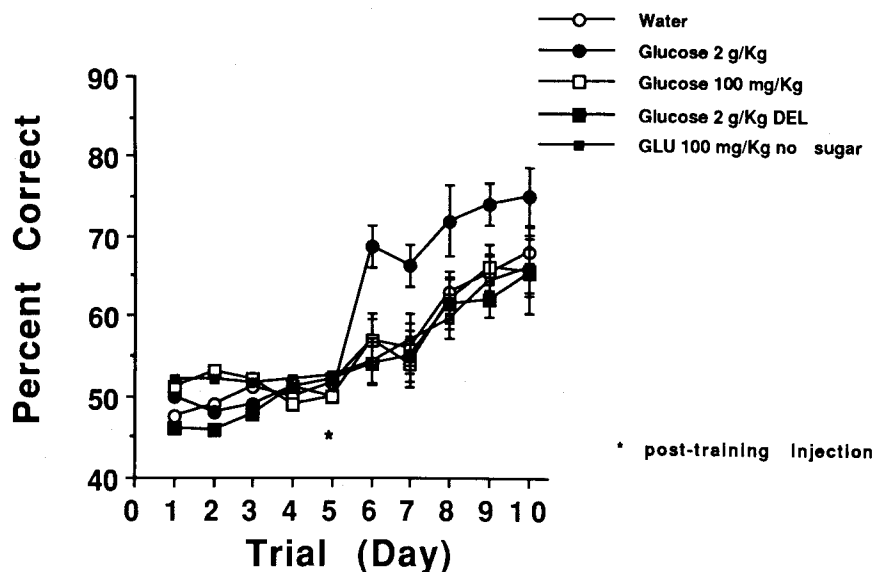


Figure 1. Effect of posttraining injection of glucose on win-stay radial maze acquisition. Animals received one trial per day and were injected posttraining on Day 5 (asterisk). Vertical bars represent the standard error of the mean. DEL = delayed injection. "No sugar" refers to the use of pellets containing no added sugar.

which requires animals to learn an approach response to illuminated maze arms. When the injection was delayed for 2 h after training, no enhancement of memory was observed, suggesting that the improvement in performance was not due to nonspecific proactive effects of glucose. This dose has previously been shown to improve retention in a conditioned emotional response task (Messier & White, 1984).

In contrast, 100 mg/kg of glucose failed to improve acquisition of win-stay radial maze behavior with either food pellets containing sugar or pellets containing no sugar. This suggests that a postingestional effect of sucrose in the Improved Formula A pellets did not confound the effect of the low dose of glucose. Previous studies have shown that 100 mg/kg of glucose improved retention of a one-trial inhibitory avoidance task, while doses of 1.0 and 500 mg/kg had no effect (Gold, 1986). Although the present results suggest that only a relatively high dose of glucose improves win-stay radial maze behavior, it would be necessary to examine the effects of a larger number of doses in the 100-mg/kg range before concluding that doses in this range do not affect acquisition of this task.

EXPERIMENT 2

Win-Shift Radial Maze Task

Method

Subjects. The subjects were 30 male Long-Evans rats (275-300 g) housed in conditions identical to those described in Experiment 1.

Apparatus. The apparatus was a radial maze of the same dimensions as in Experiment 1. However, neither the overhead tubing system nor the curtains were present, and the extramaze environment contained several cues, including wall posters, a table, a 25-W desk lamp, boxes, and the seated experimenter.

Drug. The drug and its preparation was identical to those described in Experiment 1.

Procedure. Prior to training, animals were reduced to 85% of their ad-lib feeding weights. All animals were individually habituated to the maze for 5 min on 2 consecutive days with no food available, and they were introduced to the reinforcer in their home cages on these 2 days. The rats were transported from the animal colony to a location in the testing room (which was visually secluded from the maze) by moving a rack that contained their home cages. The rack remained in the testing room for the duration of the experimental trials on each day. Food trials began on Day 3. On each food trial, four randomly selected arms were blocked by removable Plexiglas shields, and the other four were baited. The animals were allowed to obtain food from the four open arms. They were then removed from the maze and returned to their cages. After a delay, the animals were returned to the maze for a retention test. During the retention test, all eight arms were open; only the arms that had been blocked prior to the delay contained food. The animals were removed from the maze after the four baited arms had been chosen. Records were kept of the arms entered and the order of entry. Visits to unbaited arms on the retention test were scored as errors.

There were two training phases followed by a single test (i.e., drug) trial. To pass from one phase to the next, a rat had to make at least four correct responses in the first five retention test choices on 2 consecutive days. The delay in Phase 1 was 5 min; the delay in Phase 2 was 15 min. Once an animal had reached criterion at the 15-min delay, the test (i.e., drug) trial was given on the following day. On this trial, the animals were removed from the maze following the four pre-delay choices, injected immediately, and then

returned to their cages. The retention test was given after a delay of 18 h. In previous work, we showed that the performance of untreated animals trained to criterion with a 15-min delay gradually deteriorates as the test trial delay is extended from 4 h to 18 h (Packard & White, 1989). At the 18-h delay used for the drug injection trials in the present study, the performance of control animals is essentially random.

The animals were assigned to treatment groups using a rank-order method as each rat reached the 15-min criterion. In general, the rats acquired this task at an even rate, so that several animals were tested on any given drug trial. Thus, the use of a rank-order method in assigning animals to treatment groups assured that the number of trials to criterion was consistent across groups. Treatment groups were demineralized water ($n = 8$), glucose 2.0 g/kg ($n = 8$), and glucose 100 mg/kg ($n = 8$). The mean numbers of trials to criterion for the three groups were 12.2, 12.3, and 12.0, respectively (range, 11-14). An additional group of animals ($n = 8$) underwent identical training but received a posttraining injection of glucose (100 mg/kg) 2 h after the first four choices on the test trial (trials to criterion, 11.8).

Results and Discussion

The effect of posttraining glucose injections on retention in the win-shift task is shown in Figure 2. Both the 2.0-g/kg and the 100-mg/kg doses of glucose improved retention relative to that of water controls. A one-way ANOVA computed on the data in Figure 2 revealed a significant effect of group [$F(3,28) = 3.21, p < .05$]. Newman-Keuls post hoc tests revealed that both the 2-g/kg dose ($Q = 7.53$) and the 100-mg/kg dose ($Q = 6.82$) improved retention. When the injection of glucose (100 mg/kg) was delayed 2 h, no improvement in retention was observed, suggesting that the improvement produced by the immediate injection of this dose was not due to a nonspecific proactive effect of glucose.

These results demonstrate that posttraining injection of both 2.0 g/kg and 100 mg/kg of glucose improve retention of win-shift radial maze behavior. The finding that the 100-mg/kg dose of glucose improved retention in the win-shift task stands in contrast to the failure of this dose to improve win-stay acquisition (Experiment 1). Although the reasons underlying this task difference in effective doses is unknown, the results of Experiment 2 show that the ability of a 100-mg/kg posttraining injection of glucose to improve memory is not limited to aversively (Gold, 1986) motivated tasks.

GENERAL DISCUSSION

Taken together with previous studies demonstrating memory-improving effects of glucose on aversively motivated tasks (Gold, 1986; Messier & White, 1984), the demonstration that posttraining glucose injections improve memory in appetitive win-stay and win-shift radial maze tasks suggests a general ability of glucose to modulate memory processes. In a recent study, it was reported that posttraining glucose injections also reinforced (White, 1989) an appetitive leverpress response on a continuous reinforcement schedule in mice (Messier & Destrade, 1988). In addition, pretraining glucose administration has been found to improve the performance of young and old

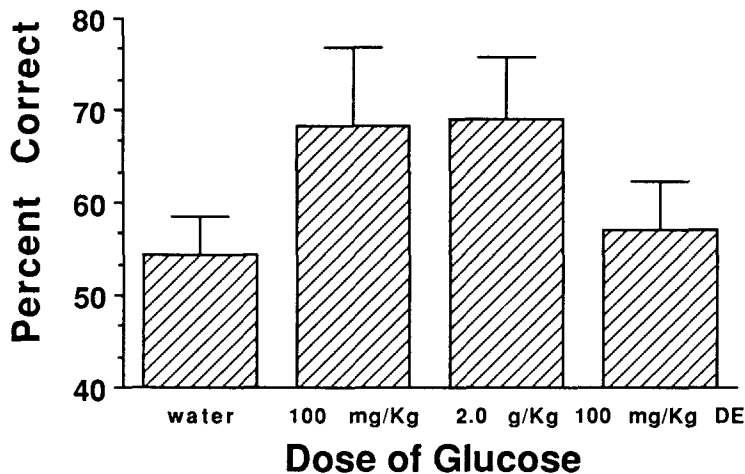


Figure 2. Effect of posttraining injection of glucose on win-shift retention (18-h delay). Vertical bars represent standard error of the mean. DE = delayed injection.

humans on modified versions of the Wechsler Memory Scale (Hall, Gonder-Frederick, Chewning, Silveira, & Gold, 1989). These findings suggest that the memory-improving properties of glucose may generalize across mammalian species.

The mnemonic requirements of the present win-stay and win-shift radial maze tasks may be fundamentally different. In the win-stay task, the presence of the reinforcer is consistently signaled by the light cue, so that performance on the task does not require animals to remember which maze arms have been entered within a trial. An animal need only acquire a response tendency to approach the illuminated maze arms in order to perform accurately in this task. In the animal learning literature, this type of memory process has been termed habit formation (Hirsh, 1974; Hull, 1943; Mishkin & Petri, 1984), taxon learning (O'Keefe & Nadel, 1978), or reference memory (Olton & Papas, 1979).

In contrast, accurate performance on the win-shift radial maze task requires animals to avoid revisiting rewarded arms by remembering which arms have been visited within a trial. This type of memory process is hippocampal-dependent (e.g., Olton & Papas, 1979), and it has been termed contextual retrieval (Hirsh, 1974), or working memory (Olton & Papas, 1979). In addition, it has been suggested that animals may operate upon a spatial "cognitive map" of the environment in performing win-shift radial maze behavior (O'Keefe & Nadel, 1978). As mentioned in the introduction, the finding that acquisition of the present win-stay and win-shift radial maze tasks is mediated by neural systems involving the caudate nucleus and the hippocampus, respectively (Packard et al., 1989), supports the hypothesis that the two tasks involve different memory processes. Therefore, the present results suggest that the memory-improving ability of posttraining glucose injections generalizes to fundamentally different "types" of memory processes.

The reasons underlying the failure of the 100-mg/kg dose to improve win-stay acquisition are unknown. This finding stands in contrast to the ability of this dose to improve retention of passive avoidance (Gold, 1986), conditioned emotional responding (White, unpublished data), and win-shift radial maze retention (the present study). In other work, we have observed that 100 mg/kg of fructose (which does not cross the blood-brain barrier) failed to improve retention of a conditioned emotional response (White, unpublished data). In contrast, 2.0 g/kg of fructose reinforced conditioned emotional response retention (Messier & White, 1987). The present effects of glucose on win-stay radial maze acquisition demonstrate a further difference between the low and high doses of glucose. One possible explanation is that low doses of glucose may act centrally to improve memory, but that higher doses may improve memory through a peripheral mechanism.

Although the present experiments do not reveal the mechanism by which systemic posttraining injections of glucose improve memory, several have been proposed, ranging from a peripheral "signaling" of the brain (Gold & Stone, 1988; Messier & White, 1987), to a direct action of glucose on a central substrate (Gold & Stone, 1988; Lee, Graham, & Gold, 1988). Further research is necessary to examine the mechanism(s) by which glucose exerts its memory-improving effects.

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