Posttraining glucose in inhibitory avoidance facilitates memory consolidation in rats

YOKO OKAICHI and HIROSHIGE OKAICHI
Doshisha University, Kyoto, Japan

The present research examines the effects of glucose on memory consolidation in rats over time. Several groups of rats were trained on inhibitory avoidance tasks and were then injected with 500 mg/kg of glucose or saline intraperitoneally under different footshock-drug injection intervals. Memory-facilitating effects were observed only at intervals of less than 30 min, suggesting that glucose affects the memory consolidation process. Our results with rats agree with the results of Kopf, Opezzo, and Baratti (1993), who worked with mice. The present research also showed that glucose does not have nonspecific effects on memory but does facilitate the memory of a shock experience.

The authors are grateful to two anonymous reviewers and Shizuhiko Nishisato for their useful comments. Correspondence should be addressed to Y. Okaichi, Department of Psychology, Doshisha University, Kyoto 602, Japan (e-mail: hokaichi@mail.doshisha.ac.jp).
Glucose facilitates memory consolidation

## GENERAL METHOD

### Subjects

Experimentally naive male Wistar rats obtained from Shimize Lab Animals (Kyoto) were used. They were housed in individual wire cages with free access to water. The animal room was maintained at 23 ± 1°C, with a 12:12-h light:dark cycle starting from 7:00 a.m. Training and testing were conducted between 11:00 a.m. and 3:00 p.m.

### Apparatus

The training apparatus was an acrylic box (47 × 23 × 22 cm) with a stainless steel grid floor. The steel grids were connected to the shock generator (Biomedica Inc., Osaka, BSG-107), which could deliver a scramble footshock. The apparatus was divided into two compartments by a wooden wall at the middle. Into this wall, a 10- × 10-cm opening was cut from the floor, and a guillotine door separated the compartments. One compartment was white and was illuminated by a 10W light bulb; it served as the startbox. The other compartment was dark, and a foodcup was attached to its far wall, 1.5 cm above the floor. A pair of photocells placed on the side walls created a photo beam crossing 1 cm above the foodcup to permit measurement of time from the placement of a rat in the startbox to the moment it reached the foodcup.

### Drugs

Glucose solution was prepared daily. Fifty grams of D(−)-glucose, amphoteric (Nacalai Tesque, Inc., Kyoto), was dissolved in 100 ml of saline. For the glucose animals, a volume of 1.32 ml/kg of glucose solution was injected intraperitoneally so that a dose level of 500 mg/kg of glucose was achieved. For saline animals, the same volume of saline was injected intraperitoneally.

### Procedure

Prior to the training, rats were reduced to approximately 85% of ad lib feeding weights and handled for a total of 15 min. As habituation, each rat was placed individually in the apparatus with the guillotine door open for 5 min or until it consumed eight, 45-mg food pellets (P. J. Noyes Co., Inc., Lancaster, New Hampshire) from the foodcup, whichever came first.

Beginning the following day, rats received three trials of training per day for 5 days. In the training and subsequent retention test trials, five pellets were supplied for each trial. For each trial, animals were placed in the white compartment with the guillotine door open, and the door was closed when they entered the dark compartment. When animals ate food pellets, they were taken out of the apparatus. After the 4th day's training, rats were divided into subgroups in such a way that the 4th day's mean latency for each subgroup was counterbalanced. On the third trial of the 5th day, a footshock (0.5 mA, 1.2 sec) was delivered, except to rats in the nonshocked group, through the floor grids at the moment they broke the photo beam above the foodcup. The rats were then removed from the apparatus and received drug injections intraperitoneally according to the experimental design. Twenty-four hours later, they were put through three retention test trials. The retention test trials were the same as the training trials: Rats were placed in the apparatus and allowed to consume food pellets. On each training trial and test trial, the latency to the foodcup was measured. The cut-off time was 300 sec. Rats that did not enter the dark compartment within 300 sec were removed from the apparatus and received a score of 300 sec.

### Data Analysis

Group medians were used considering skewed distributions of statistics. Mann-Whitney U and Kruskal-Wallis tests for the between-groups comparisons and the Wilcoxon signed-ranks tests for the within-subject comparisons were employed for each of three trials on the final training day and the retention test day.

## EXPERIMENT 1

Glucose administration immediately after a shock experience in rodents lengths the latency to the goal in retention testing (Gold, 1986; Rodriguez et al., 1994). In Experiment 1, we tried to determine whether glucose enhances the memory of a shock experience, resulting in stronger resistance to entering the place where the shock was received, or whether the longer latency was due to nonspecific effects of glucose.

### Method

Forty rats, 2.5 months old with a mean weight of 307.1 g (SD = 13.6 g) at the beginning of the experiment, served as subjects. After 4 days of training, they were divided into four groups (10 rats in each group): nonshocked saline, nonshocked glucose, shocked saline, and shocked glucose. On the 5th day, animals in shocked groups received a footshock at the moment they reached the foodcup on their third trial. They were then taken out of the apparatus and immediately administered saline or glucose. For animals in nonshocked groups, the experimental procedure was the same except that no shock was given on their third trial. On the following day, all subjects received three retention test trials.

### Results

Median latencies to the foodcup for the four groups on the retention test trials were compared with those on the final training day (Figure 1). Nonshocked animals, both
Figure 2. (a) Median latencies for each group on the final training day. Drugs were administered immediately (0 min), 10 min, 30 min, or 60 min after the final training trial. (b) Effects of delayed injections of glucose on the retention tests 24 h later. Three bars with the same legend in a group indicate the group median latencies with quartiles on the first, second, and third trials.

those administered saline and those administered glucose, exhibited almost the same running performance on the final training day as on the test day. Their median latencies were very similar, indicating that there is no effect of glucose on the test trials when shock is not given on the training day. In contrast, both groups of shocked animals showed longer latencies on the test day than on the final training day \[Ts(n = 10) = 0, ps < .001\] for both groups on each of three trials. When the shocked saline and the shocked glucose animals were compared on the test day, the glucose-administered rats showed longer latencies \[Us(10 and 10) = 16, 15, and 24; ps < .01 , .01, and .10 for the first, second, and third trials, respectively\]. These statistical analyses confirmed shock effects on the retention tests conducted 24 h later, with shock effects being greater for the glucose group than for the saline group.

**EXPERIMENT 2**

Experiment 2 examined how the effects of glucose changed as a function of shock–glucose intervals.

**Method**

Sixty-four rats, 2.5 months old and weighing an average of 303.9 g \((SD = 17.8 \text{ g})\) at the beginning of the experiment, were used as subjects. On the third trial of the 5th training day, all subjects received a footshock when they reached the foodcup. Then, either saline or glucose was injected immediately (0 min), 10, 30, or 60 min after the footshock, according to the grouping design decided on the previous day. Saline-administered groups contained 6 rats each, and glucose-administered groups contained 10 rats each. All subjects received three retention test trials on the following day.

**Results**

Figure 2 shows the median latencies on the final training day and the retention test day. On the final training day (Figure 2a), the eight groups performed in a very similar and stable manner. As compared with the final training day, the latencies on the test day (Figure 2b) were significantly longer for all groups on every trial except the saline-10-min and the saline-30-min groups on the second trial. However, on the retention tests, the latencies for the saline groups and for the glucose groups revealed different tendencies. The latencies for all saline groups were approximately the same regardless of the length of the intervals \[Hs(3) = 4.22, 3.56, and 0.62; all n.s. on every trial\]. In contrast, the latencies for the glucose groups changed depending on intervals. As the interval became longer, the latency became shorter \[Hs(3) = 15.26, 10.44, and 11.62; ps < .01, .05, and .01 for the first, second, and third trials, respectively\].

When the latencies for the retention tests were compared between the saline group and the glucose group of matching intervals, significant differences were found only at 0- and 10-min intervals \[Us(6 and 10) = 2, 9, and 18; ps < .01, .05, and .20 (n.s.) for the first, second, and third trials at the 0-min interval; Us(6 and 10) = 7, 10, and 7; ps < .05 for the first, second, and third trials at the 10-min interval, respectively\]. When the intervals were longer than 10 min, the effect of glucose disappeared on every trial.

**DISCUSSION**

In the present research, we assessed the memory consolidation effects of glucose in inhibitory avoidance tasks using rats that had received 500-mg/kg glucose in-
jections after a trial in which a footshock was delivered. Many researchers have reported that glucose administered immediately after a shock experience in inhibitory avoidance tasks lengthens the latency to the goal in a retention test conducted 24 or 48 h later (Gold, 1986; Kopf & Baratti, 1994; Lee et al., 1988; Stone et al., 1988). Experiment 1 confirmed the results of these experiments. At the same time, Experiment 1 indicated that the longer latency in the retention test was not due to the nonspecific effects of glucose, but rather that glucose enhanced the memory of the shock experience.

In Experiment 2, we tried to determine whether there exists a shorter time limit than 1 h for memory enhancement by glucose. The results showed that the effect of glucose decreases as a function of the shock–glucose interval and that the effect disappears when the interval is longer than 30 min. These results indicate that the time required to consolidate the memory of0.5-mA footshock for 1.2 sec—is less than 30 min and that glucose administered during the consolidation period facilitates memory consolidation. The results also suggest that glucose does not affect memory after the completion of consolidation. The present results on the time course of memory consolidation agree with Kopf et al.'s (1993) results with mice, suggesting that rats and mice undergo similar memory consolidation processes.

Many researchers recognize that this glucose effect is dose dependent. In studies of rats, reported effective doses differ widely, from 10 mg/kg (Gold, 1986) to 2g/kg (Messier & White, 1987), although doses of 100 to 250 mg/kg have most often been found effective (Gold et al., 1986; Rodriguez et al., 1993; Stone et al., 1990). To the best of our knowledge, this is the only study in which 500 mg/kg has been found to be effective. As Gold et al. showed, the footshock intensity is an important variable for inhibitory avoidance learning. The footshock we gave used by other researchers (Gold, 1986; Gold et al., 1986), and the duration is also within the range used (from 0.7 sec in Gold, 1986, and Gold et al., 1986, to until the animal escapes in Rodriguez et al., 1994). Therefore, it is not likely that the duration of our footshock can be the reason why 500 mg/kg was effective. This effectiveness may be due to the difference in the strain of rats used. Gold and his colleagues used Sprague-Dawley rats, and White and his colleagues used hooded rats. Our rats were of the Wistar strain. Another variable that might have affected the result is the reward we used during training. We used food pellets, which contain some amount of starch. Gold and his colleagues often used water as reward in their inhibitory avoidance training, and Kopf et al. (1993) employed a step-through method, which does not use reward at all. These differences in the experimental design need to be examined more fully before the difference in the effective dose in our experiments can be explained.

In summary, on the basis of the results of the present experiments and other studies on time-dependent character-istics of memory processing, we conclude that one effect of posttraining glucose, among others, is the facilitation of the consolidation of the memory of an experience. We also conclude that glucose exerts such a facilitatory effect when it is administered within 30 min after the experience.

REFERENCES
BENTON, D., OWENS, D. S., & PARKER, P. Y. (1994). Blood glucose influences memory and attention in young adults. Neuropsychologia, 32, 595-607.

CRAFT, S., MURPHY, C., & WEMSTROM, J. (1994). Glucose effects on complex memory and nonmemory tasks: The influence of age, sex, and glucoregulatory response. Psychobiology, 22, 95-105.

GOLD, P. E. (1986). Glucose modulation of memory storage processing. Behavioral & Neural Biology, 45, 342-349.

GOLD, P. E., VOGT, J., & HALL, J. L. (1986). Glucose effects on memory: Behavioral and pharmacological characteristics. Behavioral & Neural Biology, 46, 145-155.

KOPF, S. R., & BARATTI, C. M. (1994). Memory-improving actions of glucose: Involvement of a central cholinergic muscarinic mechanism. Behavioral & Neural Biology, 62, 237-243.

KOPF, S. R., OPEZZO, J. W., & BARATTI, C. M. (1993). Glucose enhancement of memory is not state-dependent. Behavioral & Neural Biology, 60, 192-195.

LEE, M. K., GRAHAM, S. N., & GOLD, P. E. (1988). Memory enhancement with posttraining intraventricular glucose injections in rats. Behavioral Neuroscience, 102, 591-595.

LENNARTZ, R. C., & GOLD, P. E. (1995). Glucose does not reverse impairments on spontaneous alternation induced by the noncompetitive NMDA antagonist MK-801. Neurobiology of Learning & Memory, 63, 107-110.

MANNING, C. A., RAGGOZZINO, M. E., & GOLD, P. E. (1993). Glucose enhancement of memory in patients with probable senile dementia of the Alzheimer's type. Neurobiology of Aging, 14, 523-528.

MEANS, L. W., & FERNANDEZ, T. J. (1992). Daily glucose injections facilitate performance of a win-stay water-escape working memory task in mice. Behavioral Neuroscience, 106, 345-350.

MESSIER, C., & DESTRADE, C. (1988). Improvement of memory for an operant response by post training glucose in mice. Behavioral Brain Research, 31, 185-191.

MESSIER, C., & WHITE, N. M. (1987). Memory improvement by glucose, fructose, and two glucose analogs: A possible effect on peripheral glucose transport. Behavioral & Neural Biology, 48, 104-127.

PACKARD, M. G., & WHITE, N. M. (1990). Effects of posttraining injections of glucose on acquisition of two appetitive learning tasks. Psychobiology, 18, 282-286.

PARSONS, M. W., & GOLD, P. E. (1992). Scopolamine-induced deficits in spontaneous alternation performance: Attenuation with lateral ventricle injections of glucose. Behavioral & Neural Biology, 57, 90-92.

RAGGOZZINO, M. E., & GOLD, P. E. (1991). Glucose effects on mecamylamine-induced memory deficits and decrease in locomotor activity in mice. Behavioral & Neural Biology, 56, 271-282.

RAGGOZZINO, M. E., UNICK, K. E., & GOLD, P. E. (1996). Hippocampal acetylcholine release during memory testing in rats: Augmentation by glucose. Proceedings of the National Academy of Sciences, 93, 4691-4698.

RODRIGUEZ, W. A., HORNE, C. A., MONDRAJON, A. N., & PHELPS, D. D. (1994). Comparable dose-response functions for the effects of glucose and fructose on memory. Behavioral & Neural Biology, 61, 162-169.

RODRIGUEZ, W. A., VAN AUSDLE, L. R., DHANENS, K., & MONDRAJON, A. N. (1993). Glucose modulates recently reactivated memories. Psychobiology, 21, 93-100.

STONE, W. S., CROUL, C. E., & GOLD, P. E. (1988). Attenuation of scopolamine-induced amnesia in mice. Psychopharmacology, 96, 417-420.

STONE, W. S., RUDD, R. J., & GOLD, P. E. (1990). Amphetamine, epi-
nephrine, and glucose enhancement of memory retrieval. *Psychobiology, 18*, 227-230.

**Stone, W. S., Rudd, R. J., & Gold, P. E. (1992).** Glucose attenuation of scopolamine- and age-induced deficits in spontaneous alternation behavior and regional brain [3H]2-deoxyglucose uptake in mice. *Psychobiology, 20*, 270-279.

**Stone, W. S., Walser, B., Gold, S. D., & Gold, P. E. (1991).** Scopolamine- and morphine-induced impairments of spontaneous alternation performance in mice: Reversal with glucose and with cholinergic and adrenergic agonists. *Behavioral Neuroscience, 105*, 264-271.

**Winocur, G. (1995).** Glucose-enhanced performance by aged rats on a test of conditional discrimination learning. *Psychobiology, 23*, 270-276.

(Manuscript received December 5, 1996; revision accepted for publication April 18, 1997.)