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# Prevention of memory loss for a brightness change in adult and middle-aged rats by postacquisition treatment with glucose

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**Abstract**

Following 2 h of unrestricted access to a black and a white arm of a Y maze, 8-month-old "adult" and 21-month-old "middle-aged" rats were injected intraperitoneally with 0 (vehicle), 50 or 100 mg/kg D-glucose. Twenty-four hours later they were allowed free access to two black arms. When treated with vehicle, there was no evidence of any memory for change as determined by first and total entries of, and time spent in the changed arm. However, the adult "younger" rats showed significant awareness of the changed arm following treatment with the higher dose of glucose. It was concluded that, for adult rats only, treatment with glucose prevented forgetting of their pretreatment experience with the maze arms thereby enabling detection and choice of the changed alternative. It was also suggested that the experimental procedure might have potential as a measure of memory, in particular, one not dependent on deprivation and reinforcement, in the absence of current effects of glucose or other enhancing agents.


*Keywords:* Glucose; Responsiveness to change; Novelty; Memory; Age-related effects

## 1. Introduction

It has been reported that the ability of 16-month-old rats to recognize a novel Y-maze arm, which had changed in brightness from earlier visual exposure to both arms (without being able to enter either) was increased for females only, when all subjects were treated with D-glucose before both exposure and choice trials (Hughes, 2002). Although this outcome appeared to support earlier reports of glucose-enhanced memory (Gold, 1995; Korol and Gold, 1998), timing of the treatment did not rule out the possibility of facilitated attention or encoding alone (Parkes and White, 2000). Therefore, in a second study (Hughes, 2003), 4- and 18-month-old rats were treated with glucose in between being able to enter and explore both arms, and their opportunity to show recognition of the changed novel arm. Although the older animals, unlike their younger counterparts, were able to recognize which arm had changed in brightness, this ability was not enhanced by glucose. Clearly, the outcome for younger rats could not be interpreted as glucose-enhanced attention to or encoding of information about the maze arms before a brightness change was introduced. Rather, it would appear to involve improvements in consolidation or retrieval.

However, in both studies (Hughes, 2002, 2003), all rats' choices were recorded while they were still under the influence of glucose. This raised the possibility that the compound had affected some process other than memory, such as preferences for novelty. It was therefore reasoned that a clearer picture of glucose's effects on memory processes would require the rats to be tested after the state induced by the compound had worn off (Hughes, 2003). The present study was designed to investigate responsiveness to change in rats of two ages that, after exposure to the arms of a Y maze were administered glucose, and then tested 24 h later when it was unlikely that the glucose state was still directly effective. This procedure mimicked posttraining administration, followed by testing up to 24 h later, adopted in studies with rats involving learning (e.g., Gold, 1986; Packard and White, 1990).

## 2. Methods

### 2.1. Animals

The subjects were 20 male and 20 female experimentally naïve Long–Evans hooded rats bred in the Psychology

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Department of the University of Canterbury. At the commencement of testing, 10 males and 10 females were 8 months old (adult), and 12 males and 8 females were 21 months old (middle-aged). They were kept in cages containing three or four same-sexed animals with ad libitum food and water in 12-h light/12-h dark conditions, and an ambient temperature of 20 ± 1 °C. The rats were not handled prior to experimentation except for the purposes of routine cage cleaning and the identification of individuals through applying nontoxic sheep branding dyes to their fur. They were all tested during the dark phase of their light/dark cycle.

Care of the rats and their experimental treatment were in compliance with Parts 5 (Codes of Welfare) and 6 (Use of Animals in Research, Testing, and Teaching) of the New Zealand Animal Welfare Act, 1999, and were approved by the Animal Ethics Committee of the University of Canterbury.

### 2.2. Apparatus

The apparatus comprised one of six identical wooden Y mazes with painted aluminum arm inserts described earlier (Hughes, 2002). Briefly, the maze consisted of two 45-cm-long arms set at an angle of 120° to each other, and a 30-cm-long stem. The arms and stem were 10 cm wide and 14 cm high and were covered by hinged transparent Perspex lids. The first 15 cm of the stem comprised a start area that was the same height and width as the rest of the apparatus, and was covered by a wooden lid. For all exposure and testing sessions, each arm contained a removable black or white insert (consisting of a floor, an end wall and two side walls) that occupied the width, height and the last 40 cm of the length of the arm. All internal surfaces of the maze not occupied by a painted insert comprised clear-varnished wood. The apparatus sat on a 1-m-high table and was evenly illuminated by dim fluorescent overhead lighting.

### 2.3. Procedure

To allow sufficient time for exposure to the brightness characteristics of the maze arms, individual rats were allowed free access to both arms of a maze for a 2-h acquisition trial. Each arm contained an insert—for one it was white, and for the other it was black. For half of each sex within each age group, the acquisition trial was briefly interrupted half way through while the rat was taken out of the apparatus and then returned. Because in earlier unpublished work it was noted that a number of rats fell asleep after about 1 h, the interruption was an attempt to evaluate the effects of reviving any consequent loss of attentiveness to the maze arms. At the end of the acquisition trial, the rat was removed from the apparatus and received a 1 ml/kg ip injection of 0 (vehicle only), 50 or 100 mg/kg D-glucose dissolved in distilled water, and returned to its home cage to await its choice trial 24 h later. [Unpublished pilot observations had earlier revealed that 18 untreated female middle-aged rats were able to recognise a brightness change 24 h after a 2-h acquisition trial as determined by the proportion of time spent in the changed arm i.e., mean (± S.E.M) percent = 59.00 (± 3.72), one-sample $t(17) = 2.38$, $P < .03$.] Each rat was then reintroduced into the same maze after both inserts had been exchanged for clean black replacements, and the stem and choice area had been thoroughly washed. This procedure was designed to remove olfactory cues left earlier by the rat that could determine its choice trial behavior. It also meant that the rat was faced with two black arms, one of which had changed from white, thereby avoiding any influence of aversions to white arms (Hughes, 2001).

During choice trials, the observer sat behind the start box and, by means of a PC computer and keyboard, recorded the first arm entered by all four feet and the time taken from leaving the start box to entering this arm (choice latency). Then, for exactly 60 s, the total numbers of entries of each arm, subsequent to the first entry, and the time spent in them were also recorded. These latter two measures enabled calculation of the percent entries of and time spent in the changed (or novel) arm i.e., percent novel entries and percent novel time.

All rats in both age groups experienced a total of six acquisition followed by six choice trials. They were administered each of the three glucose levels twice in a nonsystematic fashion, with 2 or 3 days intervening between a choice trial and the next acquisition trial. For one of each pair of choice trials, the novel arm was on the left and for the other it was on the right.

## 3. Results

Due to its death before completion of experimentation, the incomplete data from one middle-aged male rat were excluded from the results. The remaining rats' averages of each measure for their two choice trials following administration of each of the three glucose levels were used in all statistical analyses, except for first entries of the novel arm. In this case, totals were used. As preliminary two-tailed $t$ tests failed to reveal any significant differences for any measure between rats whose exposure trials had or had not been briefly interrupted, this variable was excluded from further analyses.

### 3.1. Responsiveness to both maze arms

Mean ± S.E.M values of latency to enter an arm, and total entries of and total time spent in both arms for each independent variable, and results of separate Glucose × Age × Sex ANOVAs are outlined in Table 1.

Glucose treatment did not significantly affect any of these measures. However, adult rats made more entries of both arms but spent less time in them than older subjects. Female rats entered an arm significantly faster, made more

Table 1
Mean (± S.E.M.) choice latencies, and total entries of and time spent in both arms/day 24 h after treatment with each of three doses of glucose ($n=39$), and for adult ($n=20$), middle-aged ($n=19$), male ($n=21$) and female rats ($n=18$), and results of $F$ tests

| Glucose dose (mg/kg) | 0 | 50 | 100 | $F$ (2,70) | $P$ |
|---|---|---|---|---|---|
| Choice latency (s) | 17.97 (5.56) | 21.59 (6.88) | 19.64 (5.50) | 0.09 | .916 |
| Total entries | 2.39 (0.13) | 2.37 (0.13) | 1.44 (0.09) | 1.27 | .286 |
| Total time (s) | 25.32 (1.46) | 24.60 (1.57) | 27.39 (1.25) | 1.34 | .270 |
| Age | Adult | Middle-aged | $F$(1,35) | $P$ | |
| Choice latency (s) | 14.78 (4.40) | 24.96 (7.43) | 1.01 | .321 | |
| Total entries | 2.71 (0.14) | 2.17 (0.09) | 9.79 | .004 | |
| Total time (s) | 24.77 (0.98) | 26.81 (1.85) | 5.42 | .026 | |
| Sex | Males | Females | $F$(1,35) | $P$ | |
| Choice latency (s) | 30.23 (7.21) | 7.49 (1.10) | 7.85 | .008 | |
| Total entries | 2.27 (0.15) | 2.65 (0.09) | 4.25 | .047 | |
| Total time (s) | 21.87 (0.84) | 30.32 (1.37) | 43.99 | .000 | |

entries of both arms and spent less time in them than males. No interactions were significant.

### 3.2. Responsiveness to the novel changed arm

While the glucose main effects were not significant for first entries of the novel arm [$F(2,70)=1.28$, $P>.2$] and percent entries of the novel arm [$F(2,70)=0.28$, $P>.7$], there were significant Glucose × Age interactions for both measures [$F(2,70)=3.71$, $P<.035$; $F(2,70)=7.66$, $P<.002$, respectively], as illustrated in Figs. 1 and 2.

Further post hoc analyses revealed that the glucose effect on the former measure was significant for adult rats [$F(2,70)=3.56$, $P<.035$] due to higher scores following treatment with 100 mg/kg, but not so for middle-aged subjects [$F(2,70)=1.54$, $P>.2$]. Significant preferences for entering the novel arm first by adults were also evident following both doses of glucose but not after treatment with vehicle. A similar pattern emerged with percent entries of the novel arm during the 1-min observation period namely, a significant glucose effect for adult animals [$F(2,70)=4.22$, $P<.02$] because of increases with 100 mg/kg, but not for older rats [$F(2,70)=2.56$, $P>.07$]. Adult (but not older) rats showed a significant preference for entering the novel arm only after treatment with 100 mg/kg glucose.

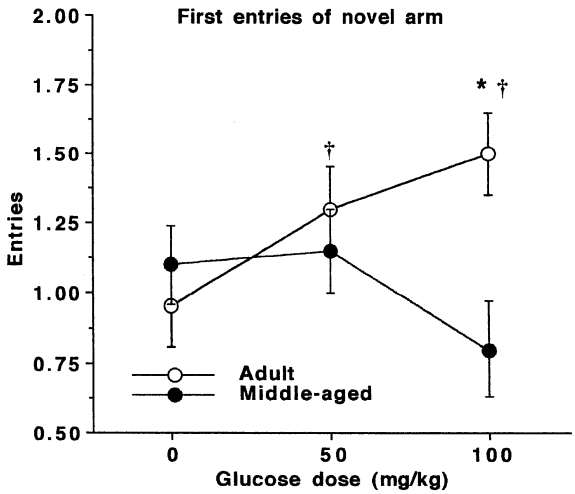


Fig. 1. Effects of two doses of D-glucose on mean ± S.E.M first entries of the novel arm for adult ($n=20$) and middle-aged rats ($n=19$) separately. * Significantly different (Scheffé tests, $P<.05$) from the 0 mg/kg control condition. †Significantly greater (one-sample $t$ tests, $df=19$, $P<.05$) than a chance expectancy of 1.00.

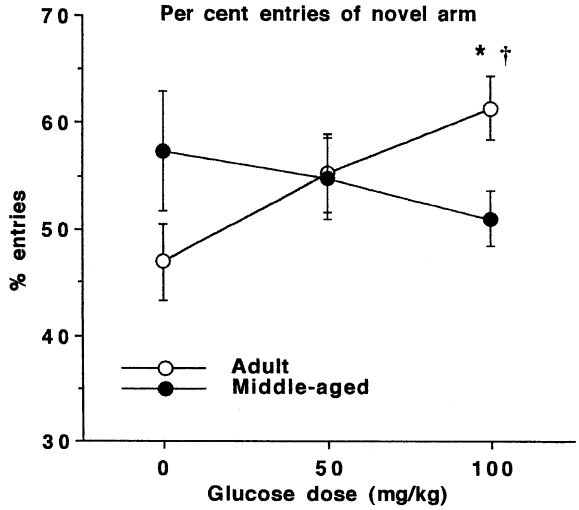


Fig. 2. Effects of two doses of D-glucose on mean ± S.E.M percent entries of the novel arm for adult ($n=20$) and middle-aged rats ($n=19$) separately. * Significantly different (Scheffé test, $P<.05$) from the 0 mg/kg control condition. †Significantly greater (one-sample $t$ test, $df=19$, $P<.005$) than a chance expectancy of 50%.

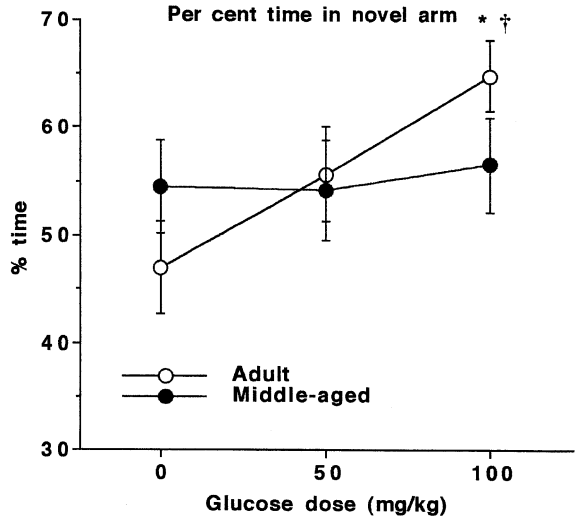


Fig. 3. Effects of two doses of D-glucose on mean ± S.E.M percent time spent in the novel arm for adult ($n=20$) and middle-aged rats ($n=19$) separately. * Significantly different (Scheffé test, $P<.05$) from the 0 mg/kg control condition. †Significantly greater (one-sample $t$ test, $df=19$, $P<.0001$) than a chance expectancy of 50%.

Table 2
Mean ( ± S.E.M.) first entries of, percent entries of and percent time spent in the novel arm for adult ($n = 20$), middle-aged ($n = 19$), male ($n = 21$) and female rats ($n = 18$), and results of $F$ tests

| Age | Adult | Middle-aged | $F(1,35)$ | $P$ |
|---|---|---|---|---|
| First entries | 1.25 (0.09) | 1.05 (0.10) | 2.13 | .153[a] |
| Percent entries | 54.51 (2.00) | 55.44 (2.68) | 0.24 | .628[a] |
| Percent time | 55.81 (2.70) | 53.74 (2.59) | 0.21 | .647 |
| **Sex** | **Males** | **Females** | | |
| First entries | 1.16 (0.07) | 1.15 (0.12) | 0.04 | .849 |
| Percent entries | 53.95 (2.43) | 56.24 (2.18) | 0.60 | .444 |
| Percent time | 54.64 (2.86) | 54.98 (2.33) | 0.01 | .937 |

[a] Age × Glucose interaction significant (see text and Figs. 1 and 2).

Although the main effect of glucose was significant for percent time spent in the novel arm [$F(2,70) = 3.37$, $P < .04$], the Glucose × Age interaction did not reach significance [$F(2,72) = 1.92$, $P > .1$]. However, in view of the age-related effects of glucose observed for the other two measures of responsiveness to the novel arm and because of similar age-related effects for this measure reported previously (Hughes, 2003), it was believed that there were good grounds for examining outcomes for the two age groups separately (see Fig. 3).

It was accordingly shown that, while the glucose effect was again significant for adults [$F(2,70) = 5.27$, $P < .008$] due to an increase in time spent in the novel arm with the higher glucose dose, there was no significant glucose effect for middle-aged rats [$F(2,70) = 0.23$, $P > .8$]. Adults alone showed a significant preference for occupying the novel arm only after they had been treated with the higher dose of glucose.

As can be seen from Table 2, neither the age nor sex of the rats significantly affected any of the three measures of responsiveness to change.

## 4. Discussion

It is clear from results for the three measures of responsiveness to change that, 24 h after experience with the two maze arms, treatment with glucose immediately following this experience increased later recognition of a brightness change, in an age-related manner. While the effect was evident with adult rats, it was entirely absent in middle-aged animals. Although it was extremely unlikely any direct action of glucose was still operating during testing, this could have been confirmed by the inclusion of a delayed injection condition. However, it should be noted that previous investigators have failed to observe any effects of glucose up to 24 h later when rats were treated 1 or 2 h after acquisition trials (Gold, 1986; Packard and White, 1990). It is therefore likely that the results of the present experiment were due to age-related effects of glucose on memory, rather than preferences for novelty (such as anxiety-related novelty avoidance, Hughes, 2003). Nevertheless, future research of this nature should include a delayed injection condition to discount the possibility of nonspecific proactive effects of glucose on performance.

Contrary to the results of an earlier study (Hughes, 2002), but in line with a more recent investigation (Hughes, 2003), effects of glucose on responsiveness to change were not related to the sex of the animals. Again this may have been due to the adoption of "active" exploratory rather than "passive" visual exposure to the maze arms (Hughes, 2003; Łukaszewska and Dławichowska, 1985) during acquisition trials.

As responsiveness to the novel arm by all rats was no greater than expected by chance following treatment with vehicle, it appears that, contrary to what occurred during pilot trials with middle-aged rats, all experiences with the maze arms were forgotten during the intervening 24 h. The reasons for such forgetting remain to be determined. However, treatment with glucose (especially 100 mg/kg) appeared to prevent memory loss for adults, but had no comparable effect on middle-aged animals. This failure for glucose to apparently enhance memory in older rats in favor of effects for younger animals is contrary to earlier findings (Winocur, 1995). Although serum glucose levels were not measured in the present study, it is nevertheless possible that, because of aging, the middle-aged rats had become hyperglycemic (Gold and Stone, 1988; Messier and Gagnon, 2000). Consequently, any lack of effect for them could have been due to rises in their glucose levels beyond the point where, in an inverted U-shaped fashion, any memory enhancement might have been expected (Gold, 1986; Messier and Destrade, 1988). It is also possible that insulin-mediated uptake of glucose into the brain or CNS effects of insulin itself (Park, 2001) were minimized in the older animals because of their likely higher insulin resistance.

The lack of any effects of glucose on responsiveness to both arms of the maze that were unrelated to the brightness change by either age group indicates that the results discussed above were unlikely to have arisen from any effects on performance factors, such as general activity. However, while middle-aged rats made fewer entries of both arms than younger animals, they spent more time in them. This outcome may have been due to less locomotor activity in the older subjects (Goodrick, 1966) accompanied by more exploration of both arms that, as a result of having forgotten their characteristics experienced 24 h earlier, may have appeared equally novel. Female rats' shorter latencies than males to enter an arm and their greater number of entries of both arms probably reflected their higher levels of general activity (Archer, 1974). It is likely that the longer total time spent in both arms by females also arose from the greater number of entries they made of each, rather than from forgetting-related exploration. This is supported by a significant positive correlation between the two measures for all adult rats [$r(18) = .61$, $P < .01$], but not for older subjects [$r(18) = .40$].

Contrary to earlier reports (Hughes, 2002, 2003), it is now possible to conclude with more certainty that glucose-induced increases in adult rats' responsiveness to brightness change are due to effects on memory between exposure and testing. It is likely that this outcome arises from facilitation of central cholinergic activity (Kopf and Baratti, 1996), principally in the hippocampus (Ragozzino et al., 1996). The responsiveness to change procedure exploits rats' natural curiosity about novelty (Dember, 1956; Hughes, 2001) and, with the modifications adopted in the present study, could be a viable, quick alternative to other methods involving conditioning (along with the possible confounding influence of associated deprivation states), when investigating memory-enhancing effects of glucose and other substances. In some situations, it might therefore be preferable to the spontaneous alternation procedure frequently used in memory research. This involves measuring alternating (or nonrepeating) choices of T- or Y-maze arms and is believed by many to reflect the operation of spatial working memory (Lalonde, 2002). It accordingly relies on animals being tested while the agent is still pharmacologically active, thereby implicating other processes in addition to or instead of memory (Gerlai, 2001; Hughes, 1998). However, as the task in the responsiveness to change procedure adopted in the present and earlier experiments (Hughes, 2002, 2003) was to recognize which of two black arms had changed from white, the rats also had to remember the position of this arm. The procedure therefore involved memory for the position as well as the brightness of the changed arm that was learnt during acquisition trials. Further research is obviously needed to determine the relative importance of each.

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