

Effects of glucose on responsiveness to change in young adult and middle-aged rats

Robert N. Hughes*

Department of Psychology, University of Canterbury, PB 4800, Christchurch, New Zealand

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Abstract

After a 0.5-, 15-, or 30-min intertrial interval, first entries of a novel Y-maze arm that had changed in brightness, percent entries of the arm, and percent time spent in this arm during a 1-min period were recorded in 4-month-old hooded rats following 6 or 30 min of free exploration of both arms. From the results, it was concluded that maximal responsiveness to the changed (or novel) arm occurred after 6 min of exploration and a 30-min intertrial interval. In a second experiment, responsiveness to change was assessed in young adult (4 months) and middle-aged (18 months) rats after 6 min of free exploration followed by an intraperitoneal injection of distilled water, or 50 or 100 mg/kg *d*-glucose before testing 20 min later. While glucose increased first entries of the changed arm in all rats, longer-term responsiveness in the form of percent entries of the novel arm and time spent in the novel arm was increased only for young adults. Although the results suggested age-specific glucose-enhanced consolidation or retrieval of change-related information, it was also possible that the treatment had differentially increased preferences for novelty in the two age groups. This possibility should be addressed in future research.

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1. Introduction

Recently, young adult and middle-aged rats were given the opportunity to visually inspect, but not enter, the two arms of a Y-maze by means of a transparent barrier across each arm [1]. For this exposure trial, one arm was black and the other was white. When later given a choice trial without the barriers in place and faced with two black arms, only the younger animals showed any ability to recognise the arm that had changed from white to black. In a second experiment, female, but not male, middle-aged rats were able to recognise the changed arm when they had received an intraperitoneal injection of glucose before their exposure to the black and white arms. Because responsiveness to change is regarded by some as a way of assessing recognition memory [2–4], it is possible that glucose may have led to memory improvements for female middle-aged rats in a fashion similar to that described by previous authors for elderly males [5–7]. If so, such

effects were likely due to facilitation of the synthesis and release of the memory-related neurotransmitter, acetylcholine [8,9].

More recently, it has been suggested that the main effect of glucose on short-term memory is facilitation of attentional or encoding processes, rather than retrieval [10]. However, in studying memory via responsiveness to change, it is not possible to distinguish between effects on attention or encoding, on one hand, and effects on retrieval (or consolidation), on the other hand, when glucose is administered both before rats' exposure to the maze arms and their subsequent opportunity to choose the changed arm. One way of making such a distinction would involve administering the compound between exposure and choice trials. But to enable this, sufficient time must be allowed for glucose to take effect while also ensuring that intertrial intervals are short enough to prevent significant memory fading. The first experiment was therefore designed to determine the results of varying the interval between exposure to the two arms and the opportunity to choose between the arm that was of the same brightness as it was earlier, and the arm that had changed in brightness. In addition, this experiment inves-

* Tel.: +64-3-364-2879; fax: +64-3-364-2181.

E-mail address: r.hughes@psyc.canterbury.ac.nz (R.N. Hughes).

tigated the effects of two durations of exposure in case the optimum intertrial interval for retention and glucose onset was dependent on exposure time. In order to maximise exposure to the maze arms, the rats were able to freely enter and explore each of them during their exposure trials. Such active exposure has been shown to increase subsequent responsiveness to change presumably by somehow improving memory for characteristics of the arms [4,11,12].

Following production of a procedure for enabling a distinction between attention or encoding, and subsequent memory processes without significant fading, a second experiment aimed to assess the effects of glucose on responsiveness to change in male and female rats. Because in the earlier study only middle-aged rats were investigated [1], glucose effects on younger animals were also studied. While glucose-induced memory improvements in elderly humans and rodents are well established, there is less agreement about the compound's effects on younger subjects [13].

An additional aim of the study was to further assess the viability of the modified "response to change procedure" [14] as a relatively quick potential measure of memory that avoids any possible confounding effects of food or water deprivation, or electric shock, which typify experimental paradigms based on animals being trained to learn responses.

2. Experiment 1

2.1. Subjects

The subjects were 20 male and 20 female Long–Evans hooded rats approximately 4 months old at the commencement of testing. They were caged in groups of three or four same-sexed animals with free access to food and water under reversed 12 h light/12 h dark conditions and an ambient temperature of 20 ± 1 °C. All rats were tested during the light phase of their light/dark cycle.

2.2. Apparatus

The apparatus was a Y-maze with painted metal arm inserts described earlier [1]. Briefly, it comprised two 45-cm-long arms set at an angle of 120° to each other, and a 15-cm-long stem to which was attached a 15-cm-long start box. All parts of the maze were 10 cm wide and 14 cm high and were covered by hinged transparent Perspex lids over the arms and stem, and a wooden lid over the start box. Into each arm was placed a removable black or white aluminum insert (consisting of a floor, an end wall, and two side walls) that occupied the arm's width, height, and the last 40 cm of its length. The apparatus sat on a 1-m-high table with the observer seated behind the start box. It was illuminated by light reflected from two fluorescent

tubes directed at a white wall approximately 1 m in front of the arms.

2.3. Procedure

Each rat was given access to the stem of the maze and allowed to freely enter and explore both arms, one of which contained a black insert and the other contained a white insert. On each occasion, this occurred during 6 min for half the rats and during 30 min for the remaining animals. The rat was then returned to its home cage during an intertrial interval of 0.5, 15, or 30 min while both inserts were replaced with washed and dried black ones. (Replacement of both inserts ensured that subsequent behavior was not guided by olfactory cues arising from the rat's earlier exploration of the arms.) It was allowed back into the stem for a choice trial where it was faced with two identical black arms, but with one that had now changed from white. The changed arm was always black to avoid possible contamination of the rat's choices by effects of aversions to white arms described earlier [15].

By means of a PC computer and keyboard, the first arm entered by all four feet and the time taken (choice latency) to enter this arm were recorded. Then, for exactly 60 s, the total number of entries of each arm and the time spent in them were also recorded. From the latter two measures, it was possible to later calculate the percent entries of this arm and percent time spent in the changed (or novel) arm (i.e., percent novel entries and percent novel time, respectively).

All rats in each of the two exposure groups experienced a total of six choice trials (i.e., two trials after each of the three intertrial intervals). For one of these two trials, the novel arm was on the left and for the other it was on the right. Only one exposure and one choice trial were experienced in each experimental session. The presentation of intertrial

Table 1

Mean (\pm S.E.) scores per day on all measures for each sex after two active exposure periods; results of *F* tests and (for novel arm choice data only) one-sample *t* tests

Sex	Males	Females	<i>F</i> (1,36)	<i>P</i>
Choice latency (s)	72.43 (16.07)	23.36 (4.98)	8.86	.005
Total entries	1.71 (0.16)	2.68 (0.15)	22.25	<.001
Total time (s)	21.72 (2.06)	29.75 (1.28)	11.06	.002
First novel entries/6	3.35 (0.32)	3.35 (0.25)	0.00	1.00
Percent novel entries	57.37 (2.44)*	57.99 (1.78)*	0.04	.837
Percent novel time	57.83 (4.33)	59.32 (2.80)*	0.08	.778
Exposure period	6 min	30 min	<i>F</i> (1,36)	<i>P</i>
Choice latency (s)	62.71 (13.17)	33.08 (12.24)	3.23	.081
Total entries	1.95 (0.17)	2.43 (0.19)	5.56	.024
Total time (s)	24.67 (2.19)	26.79 (1.62)	0.77	.240
First novel entries/6	3.20 (0.25)	3.50 (0.32)	0.52	.474
Percent novel entries	59.90 (2.09)*	55.46 (2.07)*	2.17	.149
Percent novel time	60.52 (2.96)*	56.63 (4.19)	0.55	.464

* Significantly greater (one-sample *t* tests, *df*=19, *P*<.05) than a chance expectancy of 50%.

intervals was randomised with 2 days intervening between sessions.

3. Results and discussion

Main effects of sex, exposure, and intertrial interval on each rat's average frequencies per day for all measures (apart from the arm first entered; i.e., first novel entries, for which totals for all the six trials in each condition were used) are outlined in Tables 1 and 2. Results of separate 2 (Sex) \times 2 (Exposure) \times 3 (Intertrial interval) ANOVAs are also displayed in the tables.

Male rats took significantly longer to enter an arm and, during the 60-s observation period, made fewer entries of and spent less time in both arms than females. As shown previously [1], these latter two measures were significantly positively correlated for all rats combined ($r[38]=.56$, $P<.001$), thereby indicating that the more entries they made of the arms, the longer the rats spent in them. Consequently, the sex differences in these measures suggested the often-reported higher activity levels of female rats [16]. While neither sex entered the novel changed arm first more often than predicted by chance, they both repeatedly reentered it at above chance levels. However, only females spent significantly more time in the novel arm than expected by chance.

The only significant difference between rats exposed to both arms for 6 min and those exposed for 30 min was the greater number of entries of both arms made by the latter group. However, while both groups repeatedly reentered the novel arm at above chance levels, only those that were exposed for 6 min spent more time in this arm than predicted by chance.

As shown in Table 2, the length of the intertrial interval did not affect any measure, but it was only after an interval of 15 min for first entries of the novel arm,

and 15 and 30 min for both percent entries of this arm and time spent in this arm that significant choices occurred. For some as yet inexplicable reason, an intertrial interval of only 0.5 min appeared to interfere with either memory of the arm that had changed, or preference for it.

There were no significant interactions amongst any of the three factors investigated.

Overall, ability to recognise the changed arm was still apparent after an exposure trial of 6 min and intertrial interval of either 15 or 30 min. It was therefore clear that, provided the time period between injection and testing fell between the latter two intervals, significant fading of exposure trial experiences should not occur. This would enable a 20-min period for the onset of glucose action adopted previously [1] to be used in subsequent experimentation.

4. Experiment 2

If the effects of glucose on short-term memory are primarily due to facilitation of attention or encoding [10], intertrial treatment with the compound should have little influence on consolidation or retrieval of the maze arm characteristics and, thus, minimal effects on responsiveness to change. However, if these latter processes are also affected, then enhanced recognition of the novel changed arm should follow intertrial administration of glucose. This second experiment was designed to assess the effects of glucose on responsiveness to change in both young adult as well as middle-aged rats when it was unlikely that the compound's main effect had been on attention or encoding.

4.1. Materials and methods

The subjects were 20 male and 20 female rats. When tested, half of these were 4 months old (young adult) and the other half were 18 months old (middle-aged). They were kept in the same cages and conditions, tested in the same apparatus, and, apart from the addition of glucose administration, experienced the same general experimental procedure as for Experiment 1.

Following an exposure period of 6 min with free access to a black and a white arm, each rat received an intraperitoneal injection (1 ml/kg) of vehicle (0) and 50 or 100 mg/kg *D*-glucose dissolved in distilled water before being returned to a holding cage for 20 min. Both arm inserts were replaced with clean black ones before the rat was allowed access to the arms again. The same measures were recorded as for Experiment 1. The apparatus and inserts were washed and dried before the next rat's exposure trial.

All rats were tested once a day with an interval of 2 or 3 days between tests. They were administered each of the three glucose levels twice in a nonsystematic fashion. For one

Table 2
Mean (\pm S.E.) scores per day on all measures following three intertrial intervals; results of *F* tests and (for novel arm choice data only) one-sample *t* tests

Measure	Intertrial interval (min)			<i>F</i> (2,72)	<i>P</i>
	0.5	15	30		
Choice latency (s)	56.65 (12.42)	45.19 (10.03)	41.85 (8.49)	1.24	.295
Total entries	2.22 (0.17)	2.16 (0.16)	2.19 (1.15)	0.08	.923
Total time (s)	26.02 (2.12)	24.71 (1.63)	26.48 (1.80)	0.37	.696
First novel entries/2	1.00 (0.11)	1.23 (0.11)*	1.12 (0.11)	1.02	.365
Percent novel entries	52.56 (3.18)	60.71 (3.58)*	59.78 (3.06)*	1.45	.241
Percent novel time	54.32 (4.13)	63.23 (3.97)*	58.18 (4.05)*	1.29	.281

* Significantly greater (one-sample *t* tests, $df=39$, $P<.05$) than a chance expectancy of 50%.

Table 3

Mean (\pm S.E.) choice latencies, total entries of both arms, and time spent in both arms per day following treatment with three doses of glucose, the effects of age and sex, and the results of *F* tests

Glucose dose (mg/kg)	0	50	100	<i>F</i> (2,72)	<i>P</i>
Choice latency (s)	11.93 (3.03)	18.00 (5.89)	20.45 (4.94)	0.90	.411
Total entries	2.47 (0.11)	2.56 (0.15)	2.65 (0.15)	0.76	.471
Total time (s)	24.63 (1.09)	27.08 (1.28)	27.04 (1.59)	0.76	.471

Age	Young adult	Middle-aged	<i>F</i> (1,36)	<i>P</i>
Choice latency (s)	15.62 (3.92)	17.97 (4.67)	0.21	.649
Total entries	2.54 (0.14)	2.58 (0.15)	0.03	.854
Total time (s)	28.80 (1.46)	23.70 (1.00)	8.25	.007

Sex	Males	Females	<i>F</i> (1,36)	<i>P</i>
Choice latency (s)	27.18 (5.01)	6.40 (0.95)	16.48	<.001
Total entries	2.21 (0.06)	2.91 (0.06)	15.11	<.001
Total time (s)	25.85 (1.59)	26.65 (1.12)	0.20	.655

experience with each, the changed arm was on the left, and for the other it was on the right.

5. Results

5.1. Responsiveness to both maze arms

Main effects of glucose, age, and sex on averages for the 2 days of testing for latency to enter an arm, total entries of the arm, and total time spent in both arms, as well as the results of ANOVAs, are outlined in Table 3.

The only significant effects were longer choice latencies and fewer entries of both arms for male rats than for females,

and less time spent in both arms by middle-aged rats than their younger counter parts. No interaction was significant.

5.2. Responsiveness to the novel changed arm

Effects of glucose treatment on total first entries of the novel arm in each condition for all rats are shown in Fig. 1.

As shown by a two-way ANOVA, the Glucose effect was significant [*F*(2,72)=4.61, *P*<.02] because of a higher number of choices of the novel arm following treatment with 100 mg/kg than with vehicle alone. Choices of the novel arm significantly exceeded chance expectancies at each treatment level. However, neither sex, age, nor any interaction was significant.

Although the main Glucose effect was significant for both percent entries of the novel arm [*F*(2,72)=9.50, *P*<.0002] and percent time spent in the novel arm during the 1-min choice trials [*F*(2,72)=6.32, *P*<.005], these

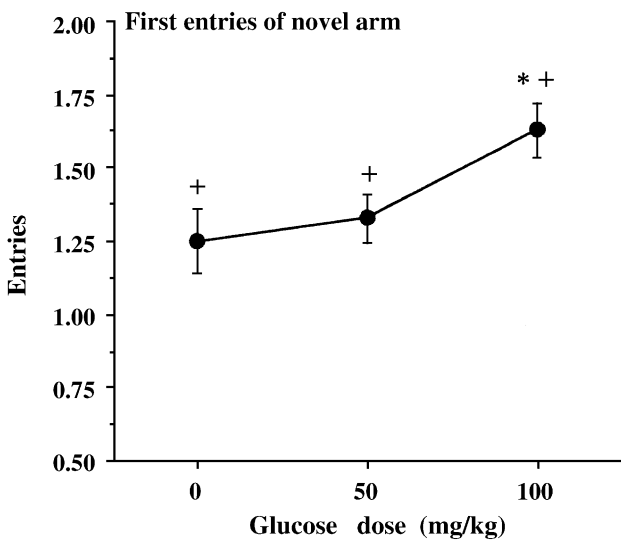


Fig. 1. Effects of two doses of *d*-glucose on mean \pm S.E. first entries per day of the novel arm for all rats combined. * Significantly different (Scheffé tests, *P*<.05) from the 0 mg/kg control condition. + Significantly greater (one-sample *t* tests, *df*=39, *P*<.05) than a chance expectancy of 50%.

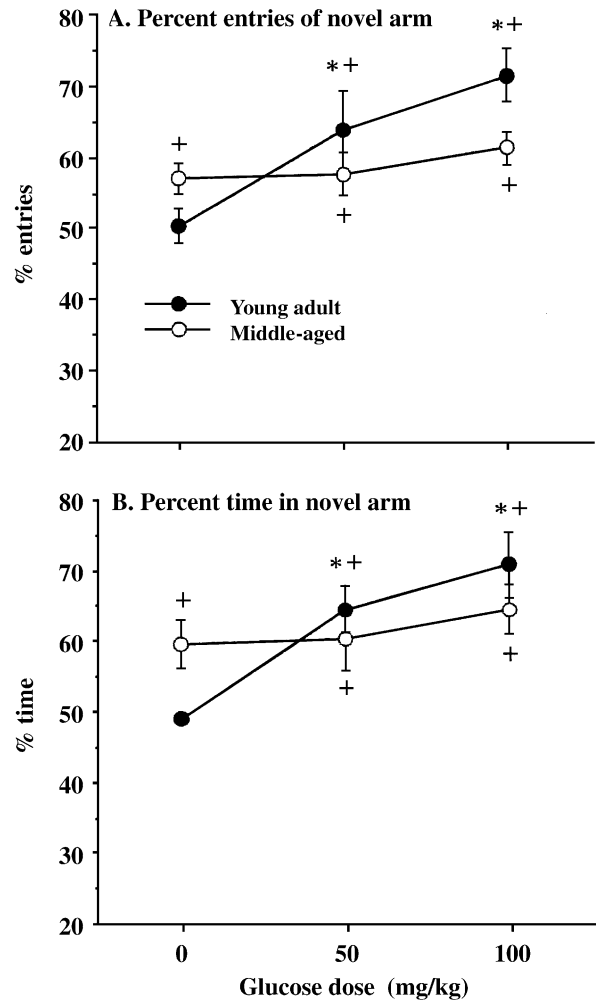


Fig. 2. Effects of two doses of *d*-glucose on mean \pm S.E. (A) percent entries and (B) time spent in the novel arm for young adult and middle-aged rats separately. * Significantly different (Scheffé tests, *P*<.05) from the 0 mg/kg control condition. + Significantly greater (one-sample *t* tests, *df*=19, *P*<.05) than a chance expectancy of 50%.

results are more appropriately considered in the light of significant Age \times Glucose interactions that occurred for both measures, that is, entries [$F(2,72)=4.64, P<.02$] and time [$F(2,72)=3.16, P<.05$] (see Fig. 2).

The interactions reflected significant increases in responsiveness for young adults following treatment with both 50 and 100 mg/kg glucose, but not for middle-aged rats. While for the latter subjects entries of and time spent in the novel arm significantly exceeded chance expectancies with each treatment level, significant percentages occurred for young adults only when treated with the two doses of glucose.

No sex difference or interaction involving sex was significant for either entries of or time spent in the novel arm.

6. Discussion

The main results of this experiment showed that glucose administration increased initial responsiveness to change (first entries of the novel arm) in all rats as reported previously for middle-aged females but not males [1]. The lack of a sex-related effect on this measure in the present investigation could have been due to the presence of young adults in the sample (which were not included in the previous study), or to the different procedures used. Whereas subjects in the present experiment were able to freely enter both arms during their exposure trial, they were earlier [1] prevented from doing this by means of transparent barriers positioned across the arm entrances. Because prior “active exploration” is known to further increase responsiveness to change following “passive exploration” [11], it is possible that the lower responsiveness of vehicle-treated middle-aged females observed earlier [1] was raised to nearer that of middle-aged males in the present experiment. And, in fact, mean \pm S.E. scores for vehicle-treated middle-aged males and females, respectively, in the previous study were 1.4 ± 0.21 (70%) and 0.5 ± 0.28 (50%), whereas in the present study they were 1.4 ± 0.22 (70%) and 1.2 ± 0.22 (60%). While sex-related outcomes were no longer apparent, an overall glucose effect nevertheless still occurred, in spite of differences between the two studies mentioned above.

It might appear from first entries of the novel arm that, because the rats were not treated until after their prechange opportunity to explore the maze arms, glucose had improved consolidation or retrieval of information, rather than attention or encoding. But this interpretation is problematic when effects of the compound on the two longer-term measures of responsiveness are considered, namely, repeated entries of the novel arm and time spent in the novel arm. Glucose has been shown to improve memory in older rats and humans [17] often to a greater extent than in younger individuals. Such age-related sensitivity could be due to poorer glucose regulation [18,19] or higher insulin resistance in older subjects [20,21]. However, in the present study, glucose enhancement of repeated entries of the novel arm and time spent in the novel arm in young adults only

suggests that they were more sensitive to the treatment than older rats. If so, it is not unlikely that plasma glucose levels in the middle-aged possibly hyperglycemic animals were elevated in a curvilinear fashion beyond the point where enhancement would have occurred [22,23]. Alternatively, if insulin-mediated glucose uptake were responsible for the behavioral effects, the possibly more insulin-resistant older rats might have been expected to be less affected by glucose treatment than young adults. A similar outcome would also follow if treatment-induced increases in insulin alone were responsible for the behavioral results via effects of the hormone on associated central nervous system activity [24]. Unfortunately, it is not possible to conclusively assess the involvement of glucose regulation or insulin in the present findings as plasma glucose and insulin levels were not measured after the subjects had been treated with glucose. Clearly, this should occur in future research.

Although effects of glucose on consolidation or retrieval might appear to be the most likely explanation for the results obtained, another interpretation could be preferable. Because the rats encountered the novel arm only 20 min after injection and thus still under the immediate influence of glucose, it is possible that the treatment modified preferences for novelty independently of any effects on memory. This possibility is supported by the lack of any significant tendency for young adults to repeatedly enter and spend time in the novel arm following treatment with vehicle only, thereby suggesting reluctance to engage in a longer-term encounter with novelty. Because first entries of the novel arm exceeded chance expectancies when all rats were treated with vehicle, it is unlikely that members of either age group were incapable of recognizing which arm had changed. Could the aversive nature of the vehicle injection procedure have interfered with the younger rats' longer-term preference for this arm in the same way that other aversive experiences can disrupt novelty preferences [25,26]? (Saline injections have been shown to reduce locomotor activity in mice [27], thereby suggesting that, by themselves, they can be aversive [28].) If so, then perhaps via calming [29], tension reducing [30], or distress relieving [31] effects of elevated plasma glucose, treatment with the compound counteracted the aversive nature of the procedure and led to increases in preferences for novelty. But why middle-aged rats should not have been similarly affected is difficult to determine unless, in view of age-related differences in forgetting [32], any aversive impact of vehicle injections wore off more rapidly for the older than for younger animals. But then, any failure for glucose to increase novelty preferences could have been due to their plasma levels becoming elevated beyond the point for enhancement, as discussed above for possible effects on memory mechanisms. Another possibility for the age-related effects might have involved epinephrine- and corticosterone-induced increases in plasma glucose [33–36] following the putatively stressful injection procedure. Because of middle-aged rats' likely poorer glucose regulation, such increases could

have caused their plasma glucose levels to rise beyond the optimum for enhancement [22,23] following glucose administration (contrary to young adults).

While it is clear that treatment with glucose after pre-change exploration of the maze arms had significant effects on responsiveness to change, it cannot be concluded with any certainty that the effects observed were all due to improvements in memory. Given the possibility that age-related changes in preferences for novelty might have been partially or fully responsible for the results, it is important to try and ensure in the future that responsiveness to change is assessed only when acute effects of the compound have worn off. This would then enable a clearer demonstration of the role of memory by effectively mimicking posttraining treatment that characterizes other more demanding procedures involving learning (while also avoiding potentially confounding influences of deprivation states or electric shock). By ruling out any immediate postadministration influence of glucose, its effects on consolidation and retrieval might then be investigated without the possibility of unconditioned preferences contaminating experimental outcomes in responsiveness to change research.

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