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Sex-related glucose effects on responsiveness to brightness change in middle-aged rats

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Abstract

Unlike younger animals, middle-aged hooded rats showed no evidence of a tendency to enter first and spend time in the arm of a Y-maze that had changed in brightness between an exposure and a choice trial. When treated with 50 or 100 mg/kg D-glucose, female (but not male) middle-aged rats entered first the novel changed arm, whereas male (but not female) subjects repeatedly entered both arms more often and spent longer in them following glucose administration. The female-specific glucose effects on first entries of the novel arm may have been due to changes in attention, novelty preference or memory possibly arising from interactions with estrogen levels. It was suggested that the male-specific results for entries of both arms may have reflected females' lower sensitivity to glucose effects because of their higher baseline activity levels.

Keywords: Glucose; Responsiveness to change; Sex differences

1. Introduction

There have been a number of reports indicating that treatment with glucose can enhance memory in rats, mice and humans (Gold, 1995; Korol and Gold, 1998) and, more particularly, ameliorate memory deficits in aged members of each species (e.g., Hall et al., 1989; Manning et al., 1992, 1998; Stone et al., 1992; Winocur, 1995; Winocur and Gagnon, 1998). It appears that such improvements in memory of aged subjects are due to facilitation of hippocampal cholinergic activity (Gold, 1991; Ragozzino et al., 1996).

The present study investigated the effects of glucose on older (but not aged) rats' responsiveness to a novel brightness change in the setting originally devised by Dember (1956) and recently further developed by the present author (Hughes, 2001). As subjects are required to detect which of two black Y-maze arms have had a different brightness on a preceding occasion, the task has been treated as a test of recognition memory by some authors (Becker et al.,1992; Łukaszewska, 1993; Poucet and Buhot, 1989). Because rats are not food-deprived, shocked or trained to acquire a particular response, measures of responsiveness to change involve minimal risk of drug and chemical effects interacting with such variables and thus influencing performance rather than underlying behavioural processes. However, it was first necessary to determine whether or not middle-aged rats differed from younger animals in their tendency to respond to a brightness change, before assessing effects of glucose. Although most studies of glucose effects on age-related memory impairments have involved elderly rats, there is evidence that middle-aged animals (10 months old) show lower memory-dependent tendencies to visit the novel side of an exploration box than rats half their age (Hughes, 1968).

The care of all rats used in the experiments and the experimental protocols 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. Experiment 1

This first experiment investigated whether or not tendencies to initially choose and then re-enter and spend time in a maze arm that had changed in brightness was affected by

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the age of the rats. Therefore, middle-aged hooded rats of both sexes were compared with younger adults.

2.1. Animals

The subjects were 20 male and 20 female Long–Evans hooded rats. At the time of testing, half of each sex was 6 months old (young adult), and the other half was 15 months old (middle-aged). All subjects were caged in groups of three same-sexed animals with ad libitum food and water in reversed 12-h light/dark lighting, an ambient temperature of 20±1 °C and relative humidity of 50%.

2.2. Apparatus

The apparatus was a clear-varnished wooden Y-maze. It consisted of two arms and a stem that were 45 and 30 cm long, respectively. The arms and stem were 10 cm wide and 14 cm high, and the angle between the arms was 120°. Half of the stem was made up of a 15-cm-long start box separated from the remainder by a wooden guillotine door and covered by a hinged wooden lid. The rest of the stem and the maze arms were covered by a hinged transparent Perspex lid. The brightness of each arm was determined by a removable black or white aluminum insert (comprising a floor, an end wall and two side walls) that occupied the arm's width, height and 40 cm of its length. A transparent Perspex barrier could be placed across each arm entrance by inserting it in vertical slots in the side walls, 5 cm from the stem. The maze sat on a 1-m-high table and was illuminated by reflected light from two fluorescent tubes directed at a cream-colored wall in front of the arms.

2.3. Procedure

All rats were tested during the dark phase of their light/dark cycle. For its exposure trials, each rat was confined to the stem of the maze where, because of the transparent barriers, it could see into but not enter the arms (one of which was black, and the other white). Six minutes later, it was returned to the startbox for approximately 30 s while the white insert was replaced with a black one, and the barriers removed. The startbox door was then raised (thereby commencing the choice trial) and, by means of a PC computer and keyboard, the latency (to the nearest 0.1 s) of leaving the startbox (emergence latency) was recorded. This was followed by a 1-min record of the time between leaving the startbox and entering either arm (choice latency), the arm first entered, the total number of repeated entries of each arm and the total time spent in each arm. From the entries and time data, it was possible to determine whether the changed (or novel) arm was entered first, and to calculate the percent entries of (percent novel entries) and percent time spent in the novel arm (percent novel time).

Every rat experienced an exposure and a choice trial on each of 2 days separated by a nontest period of 3 days. For one-choice trial, the novel arm was on the left, and for the other it was on the right.

3. Results and discussion

Individual rats' averages of each measure for the two 2 days were used in statistical analyses except first entries of the novel arm, for which totals were used. The data are summarised in Table 1 along with the results of Sex×Age ANOVAs performed separately on each measure.

There was a significant age effect for first entries of the novel arm arising from middle-aged rats making fewer such entries than their younger counterparts. Two-tailed one-sample *t*-tests showed that, while the young adults first entered the novel arm significantly more often than the chance expectancy of twice out of four opportunities [$t(19)=3.33$, $P<.005$], middle-aged rats responded at chance levels [$t(19)=1.00$, $P>.3$]. Although the age difference in percent novel time did not reach significance, again only the younger rats spent more time during the 1-min trial in the novel arm than the chance expectancy of 50% [$t(19)=3.51$, $P<.003$]. These results suggest that the older animals either could not distinguish between the novel changed and the unchanged arm, or did not prefer the former over the latter.

No other main effect or interaction was significant, although females made marginally more repeated entries

Table 1
Mean±S.E.M. scores per day on each measure for male and female young adult and middle-aged rats, and results of *F* tests for sex and age effects

Measure	Sex		*F*(1,36)	Age		*F*(1,36)
	Male	Female		Young adult	Middle-aged	
Emergence latency (s)	3.7±0.3	3.6±0.4	0.09	3.8±0.5	3.6±0.1	0.39
Choice latency (s)	7.0±0.8	6.6±1.0	0.12	6.9±0.7	6.8±1.1	0.01
First entries of novel arm[a]	1.2±0.8	1.1±0.1	0.24	1.5±0.1	0.9±0.2	8.64*
Total entries of both arms	3.8±0.4	4.7±0.3	3.57	4.5±0.4	4.0±0.3	1.23
Total time spent in both arms (s)	34.5±0.2	39.9±2.0	1.95	37.9±3.0	36.4±2.5	0.61
Percent novel entries	50.6±2.6	48.0±1.9	0.68	47.8±2.7	50.7±1.7	0.81
Percent novel time	57.1±2.6	55.3±3.3	1.90	58.9±3.6	53.5±1.1	2.12

[a] Totals for 2 days.
* $P<.006$.

of both arms combined than males ($P<.07$). This suggestive sex difference was consistent with many reports of female rats being more active than males (Archer, 1974).

The main conclusion to be drawn from this experiment is that the middle-aged rats were either incapable of remembering the location of the previously white arm, or had no significant preference for it. In other forms of behavior that were less directly related to the brightness change (such as latency to enter an arm and total time spent in both arms), they were no different from younger animals.

4. Experiment 2

It was established in the first experiment that middle-aged rats were no more responsive to a Y-maze arm that had changed in brightness from its appearance during an exposure trial than they were to the unchanged alternative. As the result may have been due to the rats' inability to remember which arm had changed, this second experiment explored the possibility that treatment with glucose might increase their responsiveness to change.

4.1. Method

The subjects were 40 rats (20 males, 20 females) that were 16 months old at the beginning of testing. They were maintained under the same conditions and tested in the same apparatus as for Experiment 1. The procedure was exactly the same as earlier with the addition that each rat received a 1 ml/kg ip D-glucose dissolved in distilled water at a dose of 0, 50 or 100 mg/kg 30 min prior to its 6-min exposure trial. All subjects randomly experienced each condition twice with an interval of 3 days between each test.

5. Results and discussion

There were no significant effects of glucose administration on emergence latency [$F(2,36)<1$, or choice latency, $F(2,36)<1$]. Nor were the sex differences in either of these cases significant [Fs$(1,18)<1$]. However, there were a number of significant main and interactive effects for other measures. Glucose effects on these measures for each sex separately are outlined in Figs. 1 and 2.

Although first entries of the novel arm were not significantly affected by either glucose treatment [$F(2,36)<1$, or sex, $F(1,18)$], the interaction between the two factors was significant [$F(2,36)=6.51$, $P<.004$]. Subsequent one-way analyses of glucose effects for each sex separately revealed a significant outcome for females [$F(2,18)=9.57$, $P<.002$], but not for males [$F(2,18)=1.41$, $P>.25$] (see Fig. 1a). Post hoc Scheffé tests ($P<.05$) showed that the effect for females was due to significantly more first entries following treatment with both levels of glucose, but no difference between the levels. While the sex difference (favoring males) was significant ($P<.05$), when the rats were administered saline only, there were no significant sex differences following

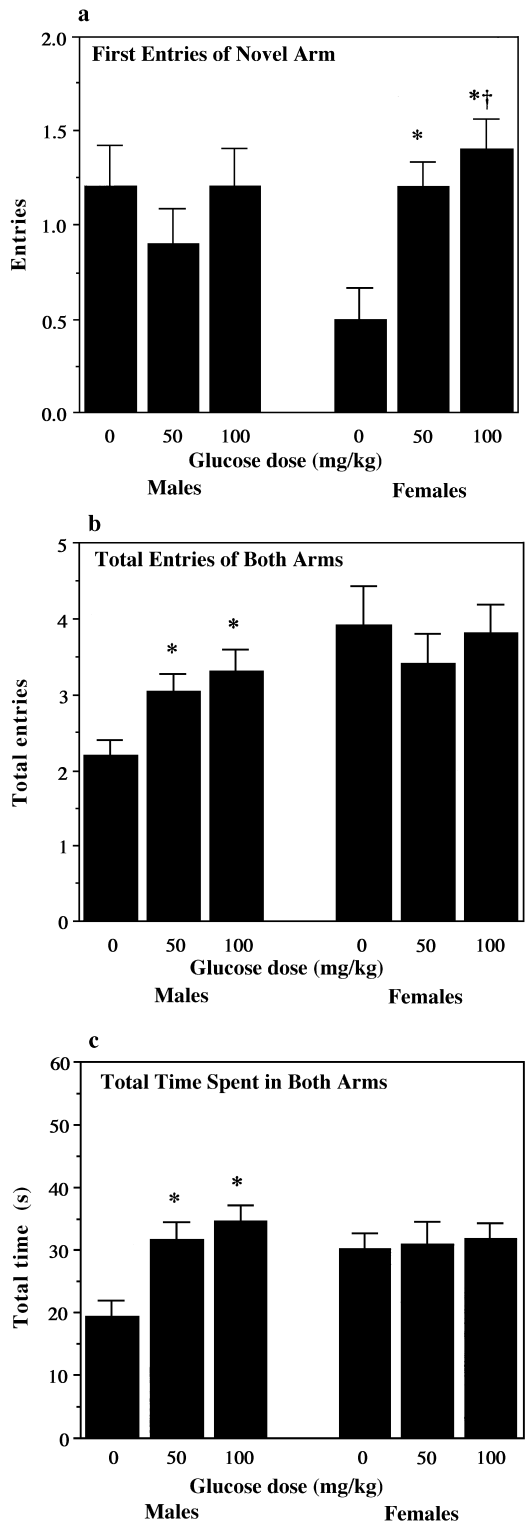

Fig. 1. Mean±S.E.M. (a) First entries of the novel arm/2, (b) total entries per day of both arms and (c) total time spent per day in both arms for male and female middle-aged rats following treatment with two doses of D-glucose. *Significantly different from 0 mg/kg, $P<.05$. †Level significantly exceeds chance expectation, $P<.04$; one-sample t test.

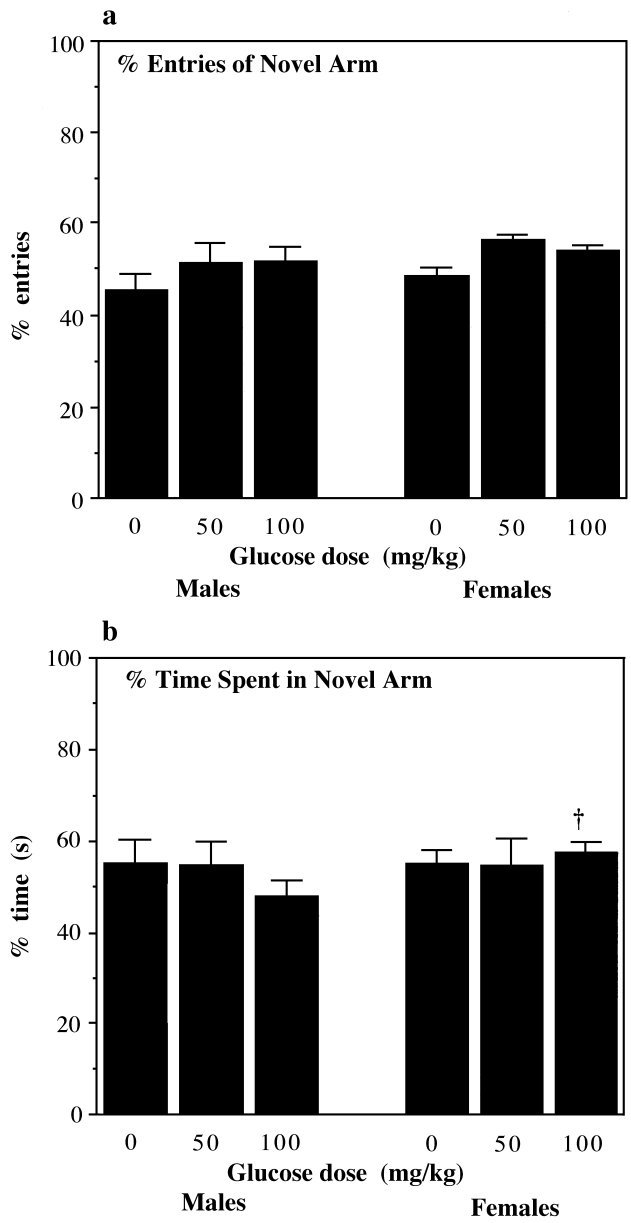

Fig. 2. Mean±S.E.M. (a) Percent entries per day of both arms and (b) percent time per day spent in both arms for male and female middle-aged rats following treatment with two doses of D-glucose. †Level significantly exceeds chance expectation, $P<.007$; one-sample t test.

treatment with either dose of glucose. According to two-tailed one-sample t tests, first entries of the novel arm did not exceed chance expectancies of 50% at any level of glucose for either sex except for females following treatment with 100 mg/kg [$t(9)=2.45$, $P<.04$].

Although total entries of both arms were unaffected by glucose [$F(2,36)=1.59$, $P>.2$], there was a significant sex effect [$F(1,18)=4.45$. $P<.05$], which is more appropriately considered in the light of a significant interaction between the two factors [$F(2,36)=3.89$, $P<.03$]. While it is clear from Fig. 1b that, overall, males made fewer entries of both arms than females, males were affected by glucose treatment [$F(2,18)=9.95$, $P<.002$], but not females [$F(2,18)<1$]. Both doses of glucose significantly increased total entries for males (Scheffé $P<.05$), but there was no significant difference between the doses.

Total time spent in both arms was affected by glucose [$F(2,36)=8.62$, $P<.001$], but not by sex [$F(1,18)<1$]. However, a significant Glucose×Sex interaction [$F(2,36)=5.94$, $P<.006$] indicated that, as with total entries of both arms, the glucose effect was significant for males [$F(2,18)=17.05$, $P<.0001$], but not for females [$F(2,18)<1$] (see Fig. 1c). Although total time for males was increased by both doses (Scheffé $P<.05$), they did not significantly differ from each other.

Neither glucose nor sex affected the longer-term measures of responsiveness to change, namely percent entries of and percent time spent in the novel arm (see Fig. 2). However, after treatment with 100 mg/kg glucose, females spent significantly more time in the novel arm than expected by chance [$t(9)=3.56$, $P<.007$] (Fig. 2b).

It can be concluded that the effects of glucose were dependent on the sex of the rats. Whereas only females showed evidence of glucose-enhanced responsiveness to change, behavior less obviously directed towards the brightness change was increased by the compound in males alone.

6. General discussion

Although middle-aged rats were unresponsive to a brightness change (as measured by first entries of the novel maze arm) compared with their younger counterparts, when treated with 50 or 100 mg/kg glucose, responsiveness was dramatically improved, but in females only. While the glucose effect might suggest a female-specific improvement in retention, other interpretations are possible. For example, because glucose was administered before each exposure trial, the treatment may have increased the females' ability to perceive and/or encode the brightness difference between the two arms. (Facilitation of attentional or encoding processes has been suggested by previous authors; Parkes and White, 2000.) Alternatively, the glucose treatment may have increased preferences for the novel arm through heightened curiosity or reduced neophobia, but there does not yet appear to be any evidence that acute treatment directly affects either of these processes in rats. It is also possible that males were unaffected by glucose because of their significantly higher baseline levels of responding following saline treatment. However, this possibility was not supported by a post hoc comparison of the two sexes' first entries of the novel arm in Experiment 1, which failed to reveal a significant difference between middle-aged males (mean±S.E.M.=0.8±0.1) and middle-aged females [0.9±0.1, $t(18)=0.33$].

To clarify any effects of glucose on memory and/or preferences for novelty vis à vis attention, it would be necessary to administer the compound after, instead of before, the rats' first exposure to the maze arms. A distinc-

tion could then be made between glucose effects on novelty preferences and memory processes by ensuring that responsiveness to change was determined only after sufficient time had elapsed for their blood levels to return to normal. This, of course, would also apply to other substances administered before exposure whose effects on responsiveness to change were believed to involve changes in memory (Łukaszewska and Dławichowska, 1985; Poucet and Buhot, 1989; Stewart, 1975).

Irrespective of precisely how glucose modified responsiveness to change in the present study, it is difficult to account for why only females were affected. It is equally difficult to account for why males alone showed increases in repeated entries of and time spent in both arms following glucose treatment. It is likely that this outcome was due to a male-specific increase in general activity resulting from arousing effects of the compound similar to its performance-enhancing effects in humans (Sugiura and Kobayashi, 1998). This would have resulted in a greater number of entries of each arm and thus inevitably a longer total time spent in them [as shown by a positive correlation between the two measures for all rats combined, $r(38)=.62$, $P<.001$]. Perhaps these glucose effects were examples of generally lower sensitivity of females to performance-enhancing influences (such as stimulant drug action; Hughes and Syme, 1972) which, in accord with the principle of rate dependency (Sanger and Blackman, 1976), are probably due to their higher baseline activity level (Steinberg et al., 1968). In support of this view is the fact that, as well as the sex difference observed following treatment with saline, middle-aged female rats in Experiment 1 entered both arms significantly more often (mean±S.E.M.=4.6±0.3) than males [3.4±0.4, $t(18)=2.41$, $P<.03$]. It is, therefore, possible that all of the sex-related effects of glucose observed in the present study were comparable to reports of the substance's enhancement of memory in humans and rodents being greatest when it is weakly established or impaired because of influences such as type of training (Sansone et al., 2000), aging (Hall et al., 1989) or treatment with amnesic drugs (Stone et al., 1992).

Unfortunately, no data were collected in the present study relating to the estrus cycle and estrogen status of the female rats. It is, therefore, not possible to determine if the sex differences in glucose's effects on processes underlying the behavioral changes observed arose from hormonal influences such as facilitation by estrogen of insulin-controlled glucose metabolism (Renard et al., 1993). If indeed improved memory were involved in the glucose-induced increase in responsiveness to change in females only, it is possible that sex differences in either hippocampal organization (Juraska, 1989) or facilitation of central glucose availability via hippocampal glucocorticoid receptors (Turner and Weaver, 1985) was responsible.

To clarify the effects of glucose on processes underlying responsiveness to change, further research that involves procedures for distinguishing between changes in attention, preference and memory is required. Also, if sex differences are to be further investigated within this context, measures of the estrogen status of female rats must also be provided.

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