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## Effects of D-Glucose on Acquisition of Implicit Mirror-Tracing and Explicit Word Recall in a Non-Diabetic Sample

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Glucose, a well-known memory modulator in humans and rodents, was examined for its potential to enhance acquisition of an implicit mirror-tracing task and recall of an explicit wordlist task. Participants consumed a lemon flavored beverage sweetened with either saccharin (23.7mg) or d-glucose (10, 100, or 500mg/kg or 50g). Ten minutes post-consumption, each subject studied a wordlist for 5-min followed immediately by 10 consecutive trials on the mirror-tracing task. Following the last mirror-tracing trial, subjects were given a free recall test for the wordlist. Results indicated that d-glucose did not have any differential effects on wordlist recall or acquisition of the mirror-tracing task. The use of different doses of d-glucose suggests that glucose may not modulate acquisition of sensorimotor implicit memory tasks and that wordlist recall tests are not sensitive to the memory modulating effects of glucose in non-diabetic young adults.

Memory modulation is a topic of intense interest, ranging from pharmacological studies of Alzheimer's patients in an effort to alleviate cognitive deficits, to basic laboratory research examining the neural substrates of drugs known to enhance memory. One such endogenous chemical that has received considerable attention is the simple sugar d-glucose (Messier, 2004), although study of its memory modulating effects in humans have been largely focused on explicit memory tasks. The purpose of this study was to examine the effects of different doses of glucose on acquisition of an implicit mirror-tracing task and an explicit word recall task in humans.

Studies of memory in humans have led to the designation of two broad categories of memory, explicit memories, commonly considered conscious and easily verbalized, and implicit memories, considered more difficult to verbalize and often involving 'unconscious' mnemonic processes (Gabrieli, 1998; Schacter & Tulving, 1994; Squire, 1994; Squire & Kandel, 1999). These memory classes may be further subdivided (Squire & Zola-Morgan, 1991), revealing that explicit memories include memory for basic facts and life events (semantic and episodic memory, respectively), while implicit memories include sensorimotor tasks (skills/habits), priming, and simple forms of associative and non-associative conditioning. Much of our understanding of these multiple memory systems comes from studies of traditional amnesic subjects. Research continues to shed light on the intricacies and nuances of these memory systems in the human brain, and attempts to develop memory enhancing compounds is ongoing.

Research has shown that the simple sugar d-glucose significantly improves memory in infants (Horne, Barr, Valiante, Zelazo, & Young, 2006), adolescents (Smith & Foster, 2008), young adults (Benton & Owens, 1993; Benton et al., 1994; Hall et al., 1989; Messier, Desrochers, & Gagnon, 1999; Messier, Pierre, Desrochers, & Gravel, 1998), elderly adults with age-related cognitive decline (Hall, Gonder-Frederick, Chewning, Silveira, & Gold, 1989; Manning et al., 1992; Parsons & Gold, 1992), rugby players with head trauma-induced cognitive deficits (Pettersen & Skelton, 2000), and in individuals with either senile dementia of the Alzheimer's type (Manning, Ragozzino, & Gold, 1993), schizophrenia (Newcomer et al., 1999; Stone & Seidman, 2008), or Down's Syndrome (Manning, Honn, Stone, Jane, & Gold, 1998). Results such as these, combined with meta-analytic reviews (Riby, 2004; Riby, Perfect, & Stollery, 2004) and the extensive non-human animal literature (Messier, 2004), suggests that d-glucose may be a reliable memory enhancer for some explicit tasks and that its memory modulatory properties generalize across species.

Two of the major differences between the human and non-human animal literature on glucose-induced memory modulation are the selection of treatment doses for empirical study and the route of administration. Non-human animal studies have traditionally injected glucose in bodyweight-dependent doses (e.g. 100 mg/kg) either subcutaneously or intraperitoneally (e.g. Flint & Riccio, 1996, 1997, & 1999) while studies with human participants traditionally require oral ingestion of 25g or 50 g of glucose mixed in an unsweetened beverage (e.g. Manning, Parsons, Cotter, & Gold; cf. Messier et al., 1998; Mohanty & Flint, 2001). While the selection of a route of administration is based both on simplicity and the control of invasive procedures in humans, it is not clear why research with human subjects has not adopted the use of controlled, bodyweight-dependent

treatment procedures. Human studies have shown that doses of 100 mg/kg, 500 mg/kg, and 50 g may produce significant elevations in blood glucose and alter cognition (Flint & Turek, 2003; Mohanty & Flint, 2001). One of the goals of the present study was to employ multiple bodyweight-dependent doses of glucose in an effort to provide a more controlled and accurate assessment of potential modulatory properties.

Glucose's modulatory effects across different subject populations and various task demands has led to the suggestion that it is a general memory modulator, being relatively non-specific with respect to its effects on mnemonic processes. However, very few studies have examined the memory modulating effects of glucose on implicit memory tasks. Research has demonstrated that glucose ingestion (50 g) significantly improved performance on an explicit free recall test, but not on an implicit word-stem completion task in elderly adults (Manning et al., 1997). In contrast, glucose did not affect performance on either task for young adults, adding to the mixed findings from prior research that indicate word recall tasks are not always sensitive to the modulatory effects of glucose in young adults (Azari, 1991; Benton & Owens, 1993; Benton et al., 1994, Messier et al., 1999; Messier et al., 1998). In another test of implicit performance, researchers reported that 50 g of glucose did not improve performance on a serial reaction time task of implicit motor memory (Craft, Murphy, Wemstrom, 1994).

The purpose of the present study was to examine the effects of a variety of doses (10 mg/kg, 100 mg/kg, 500 mg/kg and 50 g) of glucose on both implicit and explicit memory tasks. Prior research has used word-stem completion tasks to assess implicit priming (Manning et al., 1997) and a serial reaction time to assess implicit sensorimotor skill (Craft et al., 1994). In the present study, a mirror-tracing task was used in order to reassess the sensorimotor skills/habit category of implicit memory. As discussed above, the literature has produced inconsistent results with respect to the effects of glucose on explicit word recall. For this reason, a wordlist recall task was used to assess explicit memory and reexamine the effects of glucose on performance in young adults. If glucose enhances implicit tasks in young adults as it does for many explicit tasks, we would expect to see that 1) its presence during performance of the mirror-tracing task would lead to better performance (faster acquisition and fewer errors) in the glucose groups than in the saccharin control group. Based on prior literature, we predicted that 2) the 100-mg/kg and 50-g doses of glucose would have the greatest likelihood of modulating memory.

## Method

### *Participants*

Eighty (27 male, 53 female) college students age 17 to 33 ( $M = 19.83$ ,  $SD = 3.14$ ) with bodyweights ranging from 48.08 to 137.43 kg ( $M = 72.75$ ,  $SD = 19.26$ ) served as subjects (see Table 1). Participants were recruited from introductory psychology classes and were offered extra credit for participating in research. All participants were required to fast from midnight prior to the study until the testing session that occurred between 8:00 am and 11:00 am. The Institutional Review Board for research approved all procedures completed in this study prior to the implementation of research protocols with human subjects.

**Table 1.** *Sample descriptive statistics*

Group	Males	Females	Mean Age (SE)	Weight kg (SE)	Average Dose
Saccharin	3	16	19.79 (.76)	69.25 (5.48)	-----
Glucose 10 mg/kg	4	9	18.79 (.49)	74.58 (4.55)	.75 g
Glucose 100 mg/kg	7	8	20.06 (.92)	75.86 (4.73)	7.59 g
Glucose 500 mg/kg	6	10	20.57 (1.12)	73.61 (5.09)	36.81 g
Glucose 50 g	7	10	19.94 (.55)	71.51 (4.28)	50 g

*Apparatus & Materials*

A standard mirror-tracing apparatus (Lafayette Instrument Company; Lafayette, IN) was used for the implicit memory task. The mirror was attached to the base extending across the entire length of the top edge and was positioned at a 90° angle to the base. A six-point star with a diameter 17.5-cm was printed inside of another six-point star with a diameter of 21 cm. The stars were printed on a standard 21-cm by 28-cm piece of typing paper and were attached to the base of the apparatus. A 10-13 cm pencil was used to trace the star. An opaque obstruction platform was attached to the base and could be positioned so that each subject could not directly view their hand as they traced the star. Only the reflection of their hand in the mirror could be used to guide their performance. The wordlist was comprised of 20 common nouns (Paivio, Yuille, & Madigan, 1968) printed in 12 CPI font on a single sheet of typing paper (21 cm x 28 cm).

*Procedure*

Participants were randomly assigned to one of five groups: saccharin ( $n = 19$ ), 10 mg/kg of glucose ( $n = 14$ ), 100 mg/kg glucose ( $n = 16$ ), 500 mg/kg of glucose ( $n = 14$ ), and 50 g of glucose ( $n = 17$ ) and were asked to refrain from eating or drinking (except water) for at least 8-hrs prior to the start of the experiment. The doses of glucose used were selected based upon studies indicating that 50 g of glucose enhances declarative memory in humans (Craft et al., 1994; Craft, Zallen, & Baker, 1992; Manning, Hall, & Gold, 1990; Manning et al., 1992; Manning et al., 1997; Parsons & Gold, 1992) and research indicating that 100 mg/kg bodyweight enhances memory in humans (e.g., Mohanty & Flint, 2001) and animals (e.g., Flint & Riccio, 1996, 1997; Hughes & Neeson, 2003; Stone, Rudd, Ragozzino, & Gold, 1992). Participants were screened and those with diabetes, phenylketonuria, hypoglycemia, or an immediate family history of any of these disorders were excluded from the study. After being weighed each participant was asked to consume an 8 oz lemonade flavored beverage (Kool Aid), to increase palatability (e.g., Benton, 1990; Benton & Stevens, 2008; Flint & Turek, 2003; Hall et al., 1989; Kennedy & Scholey, 2000; Morris, 2008; Pettersen & Skelton, 2000; Sunram-Lea, Dewhurst, & Foster, 2008), sweetened with either saccharin (23.7 mg) or the appropriate amount of glucose (ADM Corn Processing), followed by a 15-min delay period. Prior studies have demonstrated that a 15-min delay is sufficient for significant increases in blood glucose levels (Hall et al., 1989; Manning et al., 1997;

Mohanty & Flint, 2001). During the first 10-min of the delay period subjects completed a questionnaire containing basic demographic information and questions regarding eating habits. During the remaining 5-min of the delay period participants were asked to study the list of 20 words and were instructed that they would be asked to recall them at a later time.

Immediately following the 15-min delay period subjects were asked to trace a double-star (staying in between the lines) using only the reflection of their hand in the mirror drawing apparatus to guide their performance. A blank star was attached to the base of the apparatus and the opaque obstruction platform was positioned so that the participant could not directly see their hand. The participant's dominant hand was placed with a pencil at the top corner of the star and he/she was instructed to trace the star as quickly and accurately as possible, without removing the pencil point from the paper, until they had completed the task. After a 1-min rest, the mirror drawing procedure was repeated again. This procedure continued until a total of 10 trials were completed. The time to complete the tracing and the number of errors (moving outside of the lines) were recorded for each trial as dependent measures. One minute following the 10th trial participants were given a free recall sheet and were instructed to write down as many of the words as possible from the wordlist they had studied prior to the mirror-tracing task. The total number of words correctly recalled during the 5-min test were recorded as a dependent measure of recall performance.

## Results

Data analyses were conducted with parametric statistics. Partial eta squared ( $\eta_p^2$ ) has been reported as a measure of effect size. Statistical power has also been reported for each omnibus ANOVA which failed to reach statistical significance.

A 5 (group) by 10 (trial) mixed analysis of variance (ANOVA) for the time to complete each trial revealed a significant effect of trial [ $\eta_p^2 = .47$ ; Huynh-Feldt Correction  $\epsilon = .17$ ;  $F(1.56, 116.81) = 65.14$ ,  $p = .000$ ] (see Table 2). There was no main effect of group [ $\eta_p^2 = .01$ ;  $F(4, 75) = .13$ ,  $p = .93$ ; power = .07] or group by trial interaction [ $\eta_p^2 = .03$ ; Huynh-Feldt Correction  $\epsilon = .17$ ;  $F(6.23, 116.81) = .72$ ,  $p = .44$ ; power = .25]. A 5 by 10 mixed ANOVA for the number of errors on each trial revealed a similar pattern of results. There was a main effect of trial [ $\eta_p^2 = .30$ ; Huynh-Feldt Correction  $\epsilon = .28$ ;  $F(2.50, 187.23) = 32.15$ ,  $p = .000$ ] (see Table 3), but no effect of group [ $\eta_p^2 = .04$ ;  $F(4, 75) = .68$ ,  $p > .05$ ; power = .21] or interaction [ $\eta_p^2 = .05$ ; Huynh-Feldt Correction  $\epsilon = .28$ ;  $F(9.99, 187.23) = 1.01$ ,  $p = .44$ ; power = .52].

A one-way ANOVA for the number of words recalled did not reveal any significant effects among the groups [ $\eta_p^2 = .02$ ;  $F(4, 75) = .34$ ,  $p > .05$ ; power = .12] (See Figure 1).

## Discussion

The purpose of this study was to examine the behavioral effects of glucose on acquisition of an implicit form of sensorimotor memory and an explicit memory word-recall test. Previous studies have revealed memory modulatory effects of glucose using numerous explicit memory tasks with a variety of subject populations, but tests of implicit performance have failed to reveal any reliable modulatory effect of glucose. In most of these studies, researchers have restricted their study to a single standard 50 g dose of glucose,

**Table 2.** Mean time (standard deviation) on each trial for the 10 mg/kg, 100 mg/kg, 500 mg/kg, and 50 g glucose groups and the saccharin control group.

Trial	Group				
	Saccharin	10 mg/kg	100 mg/kg	500 mg/kg	50 g
Trial 1	23.79 (22.67)	17.71 (21.19)	12.88 (12.44)	13.21 (10.09)	15.00 (15.51)
Trial 2	16.05 (14.71)	14.50 (13.69)	9.00 (9.56)	10.71 (9.02)	12.24 (17.64)
Trial 3	10.00 (7.20)	9.79 (9.59)	7.00 (5.49)	7.93 (8.47)	8.94 (11.29)
Trial 4	7.47 (5.14)	7.14 (5.08)	6.00 (4.47)	5.64 (4.29)	7.06 (8.43)
Trial 5	5.89 (4.79)	5.00 (3.68)	4.63 (3.76)	5.50 (4.78)	7.18 (8.22)
Trial 6	6.00 (3.46)	4.93 (4.57)	5.19 (4.12)	3.71 (3.52)	6.06 (6.82)
Trial 7	5.47 (4.07)	4.00 (3.06)	4.75 (3.61)	3.64 (2.59)	5.59 (6.02)
Trial 8	5.63 (3.88)	5.36 (5.92)	4.88 (3.56)	3.14 (3.08)	5.41 (5.99)
Trial 9	4.37 (3.79)	4.36 (4.09)	4.25 (3.07)	4.07 (3.41)	5.88 (6.69)
Trial 10	6.95 (7.58)	4.50 (4.11)	3.69 (2.80)	5.21 (6.38)	4.88 (6.02)

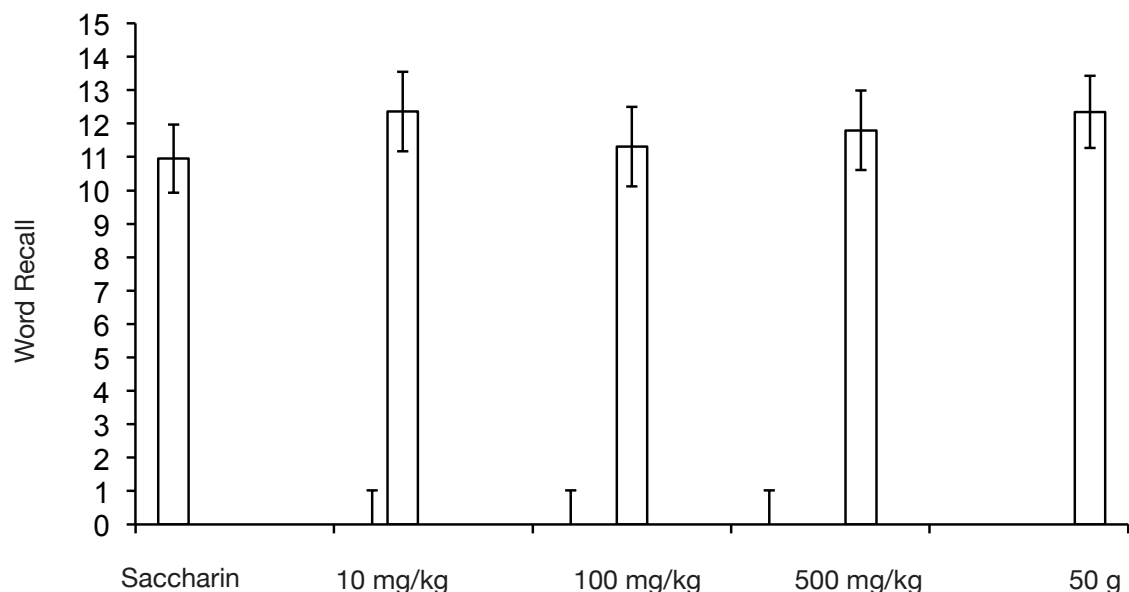
**Table 3.** Mean number of errors (standard deviation) on each trial for the 10 mg/kg, 100 mg/kg, 500 mg/kg, and 50 g glucose groups and the saccharin control group.

Trial	Group				
	Saccharin	10 mg/kg	100 mg/kg	500 mg/kg	50 g
Trial 1	152.53 (114.50)	124.64 (70.29)	155.25 (118.08)	126.00 (61.89)	173.29 (186.89)
Trial 2	86.58 (44.06)	87.57 (36.05)	78.81 (35.71)	106.21 (64.84)	92.35 (74.41)
Trial 3	59.16 (20.96)	65.93 (36.48)	67.44 (46.48)	74.43 (24.65)	65.12 (53.45)
Trial 4	48.53 (17.69)	56.29 (28.59)	55.13 (26.76)	54.07 (15.77)	56.24 (41.50)
Trial 5	43.42 (23.21)	47.29 (25.42)	51.69 (35.16)	50.57 (14.22)	48.00 (26.13)
Trial 6	38.32 (11.30)	43.43 (26.22)	42.44 (20.75)	46.86 (11.31)	43.71 (21.76)
Trial 7	34.26 (9.32)	40.57 (27.38)	36.75 (17.63)	40.64 (10.41)	39.53 (19.40)
Trial 8	33.58 (10.40)	40.29 (25.80)	34.88 (13.88)	37.71 (11.03)	35.76 (19.53)
Trial 9	32.00 (11.71)	36.71 (28.35)	32.31 (14.13)	35.64 (9.63)	33.76 (14.75)
Trial 10	29.53 (12.11)	33.64 (28.06)	29.13 (12.46)	37.14 (19.70)	31.53 (28.06)

independent of the participant's bodyweight. This procedure is inconsistent with the non-human animal literature that has repeatedly used multiple doses and indicated bodyweight-dependent memory modulatory effects. More specifically, low doses (10 mg/kg) and high doses (>400 mg/kg) have little or no effect on performance while moderate doses (100 mg/kg) produce optimal memory enhancement (cf. Rodriguez, Horne, Mondragon, & Phelps, 1994). For this reason, the present study employed a range of weight dependent doses plus a 50 g dose (utilized in prior studies) in order to maximize the likelihood of identifying any memory modulatory properties of glucose for these two tasks.

The results of this study indicate that glucose had no effect on either the implicit mirror-tracing task or the explicit word recall task. The first hypothesis was that the presence of glucose during acquisition and consolidation of the mirror-tracing task would lead to superior performance over the saccharin group. Results for all groups showed a general improvement in the time to complete the task and a decrease in the number of errors across the ten trials, however, no group differences were detected to support the hypothesis. These results are consistent with the failure to find memory modulatory effects of glucose for implicit priming (Manning et al., 1997) and an implicit serial reaction time task (Craft et al, 1994) in young and aged adults. The second hypothesis, that the 100 mg/kg and 50 g doses of glucose would reveal greater modulatory effects than the placebo and other glucose doses, was also unsupported. As indicated above, there were no group differences on the mirror-tracing task and the results of the explicit word recall task also failed to reveal any differences among the groups, consistent with the results of other studies with young adults. The mechanisms through which glucose modulates memory are not yet clear and may involve indirect effects on the peripheral nervous system, indirect effects on the central nervous system, and/or direct effects on specific anatomical substrates in the brain. It is

**Figure 1.** Mean number of words correctly recalled for the 10 mg/kg, 100 mg/kg, 500 mg/kg, and 50 g glucose groups and the saccharin control group. (Note: Error bars represent the standard error of the mean)





possible that the failure to find a memory enhancing effect of glucose on the explicit wordlist recall task in young adults is due to the simplicity of this task. Enhanced performance on wordlist recall is consistently seen in elderly adults who have normal age-related decline in cognitive ability and has been reported in other studies of young adults where there is likely greater cognitive demand (Flint, Zak, Kochan, & Papandrea, 2011). It is also possible that the failure to find an enhancing effect of glucose on the mirror-tracing task occurred for the same reason. In other words, the mirror-tracing task may have been too simple for the participants and made it difficult to empirically detect enhanced performance. Such a conclusion is supported by a number of studies that have indicated a relationship between cognitive demand and susceptibility to glucose-induced enhancement (Hoyland, Lawton, & Dye, 2008; Kennedy & Scholey, 2000; McNay, Fries, & Gold, 2000; Meikle, Riby, & Stollery, 2005; Scholey, Harper, & Kennedy, 2001).

A second explanation for the failure to find an enhancing effect of glucose on the mirror-tracing task may be associated with the neural substrates necessary for performance of this task. It is clear that there are multiple memory systems in the brain that are anatomically distinct. The implicit memory system required for the sensorimotor mirror-tracing task may not respond to the facilitating effects of glucose as well as or in the same manner as the anatomical systems required for explicit memory tasks, however, additional research will be necessary to confirm such a hypothesis.

The results of this study suggest that performance on the basic implicit mirror-tracing task in young adults is not sensitive to memory modulating properties of glucose, and indicate that a simple wordlist recall task may similarly lack sufficient sensitivity to detect memory enhancing characteristics of glucose in young adults. In order to better understand the potential memory modulating effects of glucose for implicit tasks, future research should focus on examining subjects that are impaired on implicit tasks and utilizing technology capable of assessing activity in the neural substrates important for implicit memory.



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