THE EFFECTS OF HIGH-FRUCTOSE CORN SYRUP ON WEIGHT GAIN AND SPATIAL MEMORY

by

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THE EFFECTS OF HIGH-FRUCTOSE CORN

SYRUP ON WEIGHT GAIN AND SPATIAL MEMORY

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INTRODUCTION

In the past fifty years, the prevalence of high-fructose corn syrup (HFCS) as a dietary sweetener has increased exponentially. Before the introduction of sugar, the primary sweetener was honey, a relatively rare commodity, so the majority of people's diets contained no sweeteners (Johnson, 2007). Therefore, fructose, as well as other refined sugars like sucrose, has not appeared in diets until very recently in human history (Lenoir, 2007). Between the years 1970 and 1990, the consumption of HFCS increased more than 1000% (Bray, 2004). High-fructose corn syrup is produced from an enzymatic isomerization of glucose to fructose with either 42% fructose (HFCS-42) or 55% fructose (HFCS-55) and the remainder consists of glucose (Bray, 2004). In the United States, an estimated average of 7% of daily caloric consumption comes from HFCS (Bray, 2004). HFCS has become so widely used due, in part, to its contributions of sweetness, color, flavor, ability to retain moisture, osmotic stability and freezing-point depression, making it a convenient easy way to enhance flavor in prepared foods and beverages (Hanover, 1993). Alhough HFCS is manufactured and used in many countries throughout the world, the United States is the major consumer of foods and beverages containing HFCS (Bray, 2004). In fact, fructose represents over 40% of sweeteners added to prepared foods in the American diet (Bray, 2004). For example, it is used extensively in carbonated soft drinks, fruit drinks, baked goods, canned fruits, jams and jellies, and dairy products (Bray, 2004).

Despite its growing use in modern society, research into the long-term effects of refined sugars such as HFCS has been limited, and the results from studies that have been conducted are alarming. When rats were allowed to choose between water sweetened with the artificial sweetener saccharin and intravenous cocaine, 94% of rats preferred

saccharin, indicating a potential addictive quality to artificial sweeteners (Lenoir, 2007). Further, fructose metabolism is very different than glucose metabolism in the human body – fructose fails to induce the production of leptin, an important hormone for appetite suppression, increases uric acid levels, and favors de novo lipogenesis (Truax, 2011; Bray, 2004). High uric acid levels have been linked with obesity and hypertension (Truax, 2011). This coincides with research supporting the idea that fructose is a major cause of obesity (Bray, 2004). In addition, studies show that fructose also fails to stimulate insulin secretion, which leads to hyperglycemia and weight gain (Bray, 2004; Truax, 2011).

Animals fed a high-fructose diet gained significantly more weight than animals with equivalent high-sucrose diets in both the short-term and long-term (Bocarsly, 2010). Also in comparison with a sucrose diet, fructose-fed rats had significantly higher blood glucose and triglyceride levels (Jurdak, 2008; Bocarsly, 2010). A diet high in fructose has also been shown to cause numerous pathological changes, including increases in oxidative stress, glucose intolerance, insulin resistance, type-2 diabetes, liver disease, hepatomegaly, hypertension and cardiovascular disease (Ross, 2009). Diets high in fat have also been shown to produce insulin resistance (Banas, 2009). Rats fed a high-fat, high-glucose diet supplemented with HFCS showed metabolism similar to clinical diabetes with elevated fasting glucose, cholesterol and triglycerides (Stranahan, 2008). Fructose's unique ability to increase uric acid levels is hypothesized to be the major mechanism by which fructose contributes to cardiorenal disease – uric acid stimulates vascular smooth muscle as well as the release of inflammatory substances (Johnson, 2007). In fact, uric acid is an independent predictor of obesity, hypertension,

hyperinsulinemia, and renal disease, all of which are associated with HFCS (Johnson, 2007). Another study indicated that the damaging effects of a high fructose diet extend directly to the brain (Mielke, 2005). Indeed, a growing body of research strongly suggests that factors leading to cardiovascular disease, obesity, and diabetes may significantly increase one's odds for getting neurodegenerative disease and the resultant cognitive decline (Cao, 2007).

The hippocampus is a brain structure in the medial temporal lobe critical for learning and memory. This brain region is important for recalling facts, called semantic memory, and information specific to a time or place, called episodic memory (Manns, 2003b). In addition to recall, the hippocampus is also important for recognition (Manns, 2003a). Further, spatial working memory is also critically dependent upon the hippocampus. High-calorie diets have been shown to impair both the structure and function of hippocampus in rats (Molteni, 2002). Similarly, rats fed high fat, refined carbohydrate diet experienced spatial learning deficits associated with learning and memory dependent upon the hippocampus (Goldbart, 2006).

Several experiments have examined the relationship between HFCS and the brain. In one study, fructose consumption among the United States population has been linked to a greater risk for dementia either directly or in combination with the concomitant increases in obesity (Stephan, 2010). Even in non-aged animals without neurodegenerative diseases, HFCS has been shown to lead to problems with key aspects of learning. Consuming a diet high in fructose is similar to a diet high in saturated fatty acids, which has been linked to impaired performance on many hippocampus-dependent tasks (Ross, 2009). Interestingly, high fructose diets did not affect acquisition of the task

itself, but did impair retention, indicating that HFCS might allow animals to initially learn the task but not convert it to long-term storage and thus not be able to retrieve the memory (Ross, 2009). In a spatial water maze task commonly utilized in rodents, high fructose consumption caused elevated plasma triglycerides that positively correlated with latencies to reach the target and negatively correlated with target approaches (Ross, 2009). Injecting triglycerides directly into the brain has also been shown to impair hippocampal-dependent memory (Farr, 2008). In another study, rats fed HFCS-supplemented diets experienced more insulin resistance, cognitive impairment on spatial learning tasks, and reduction of long-term potentiation, dendritic spine density, and brain derived neurotrophic factor (BDNF) in the hippocampus than the regular rodent diet group (Stranahan, 2008). Neural insulin signaling facilitates hippocampal-dependent memory (Park, 2001), and direct infusions of insulin into the hippocampus enhance performance on memory tasks (Moosavi, 2006; Zhao, 1999). This indicates that HFCS-induced insulin resistance may contribute to the neural mechanisms that impair cognition (Mielke, 2005). It may be more complex that this, as findings show spatial learning and memory were actually impaired by lower doses of insulin, not affected by intermediate doses and improved by higher doses of insulin; it was also shown that the impairing effect of low doses of insulin was weaker than the improving effects of high doses, indicating a dose-dependent effect of insulin on learning and memory (Moosavi, 2006; Zhao, 1999). In addition to insulin's modulatory effects on learning and memory, animals undergoing spatial water maze tasks increased insulin signaling in the hippocampus (Zhao, 1999; Dou, 2005), indicating learning itself increases insulin. After a spatial learning experience, gene expression and protein levels of insulin receptors were up-

regulated within the CA1 region of hippocampus important in short-term memory formation (Zhao, 1999). In a similar experiment, animals had elevated BDNF in the hippocampus after a spatial memory task; a HFCS diet reduced levels of BDNF as well as performance on the spatial learning task (Molteni, 2002). However, these results are not universal. In another study by Messier (2007), fructose consumption was shown to enhance performance in mice in an operant learning task. This study also found that unlike high-fat diets, high-fructose diets did not lead to increased weight nor did it impair glucose tolerance (Messier, 2007). Due to these conflicting results, the effects of HFCS on learning and memory remain unclear.

Although it appears that high-fructose corn syrup has negative effects on learning and memory in the hippocampus as well as linked to several neuropathologies, many questions remain. For example, what kinds of learning and memory are harmed? All kinds? Can one see HFCS-induced memory impairment in the absence of HFCS-induced weight gain? In the current experiment, mice were fed either a normal mouse chow supplemented with HFCS, or standard chow not supplemented with sweetener for two months. The animal's weight and performance in a water radial arm maze, a commonly utilized spatial learning task that gives an investigator several key measures of different kinds of learning and memory, were then assessed. Based upon the findings of the majority of the studies published, we hypothesized that a diet rich in HFCS (but equivalent to that frequently consumed by humans) would produce significant elevations in weight gain, and would also produce select cognitive deficits. More specifically, we hypothesized that hippocampus-dependent reference memory would remain fully

functional in the experimental mice, but that our measures of spatial working memory would show clear impairment.

METHODS

Experimental subjects and housing

The effects of high fructose corn syrup diet on working and reference memory were assessed utilizing a water radial-arm maze (WRAM). Subjects were 4-month-old C57BL/6J mice bred in the Texas Christian University vivarium. Care of these mice was consistent with the *Guide for the Care and Use of Laboratory Animals*. The Institutional Animal Care and Use Committee at Texas Christian University approved all experimental protocols.

Subjects were housed in groups of three to four animals within standard cages (12.5cm x 15cm x 25cm). All mice were housed with identical light schedules and *ad libitum* access to food. The high-fructose corn syrup condition was engineered to be comparable to the amount ingested by humans, and was integrated into the normal rodent chow. Mice were given access to the HFCS-laced rodent chow or standard rodent chow over the course of 8 weeks, after which behavioral testing began.

Cognitive measures:

Water radial arm maze

Subjects were tested for 17 days to assess spatial working and reference memory. WRAM consists of 8 arms, and is used to assess hippocampus-dependent learning and memory. Four of these arms contain escape platforms hidden beneath the water surface. See Figure 1. Each subject had 4 trials per day and was given 3 minutes to locate the platform during each trial. After successful location of a platform, that platform was removed from the maze, and the subject was returned to a heated cage for a 30-second inter-trial interval. For each subject, during a daily session, the number of arms

containing a platform was reduced by one for each sequential trial. This procedure requires more effort from the subject's working memory as trials progress. Three different variables were analyzed – Working Memory Correct (WMC) errors, Reference Memory (RM) errors, and Working Memory Incorrect (WMI) errors. WMC errors are the number of first and repeated entries into any arm from which a platform has been removed during the daily session. RM errors refer to the number of first entries into an arm that never contained a platform. WMI errors are equal to the number of repeated entries into any RM arm (i.e., an arm that never has had a platform).

Statistics

To compare differences in the number of errors during each day, the data obtained from behavioral testing were analyzed utilizing repeated measures analysis of variance, with Condition [regular diet and HFCS diet] as a between-subjects variable and Timepoint [Day 2 – Day 17] as a within subjects variable. Data were analyzed using StatView 5.0.1 software. The alpha level used for all statistical analyses was 0.05.

RESULTS

Weight

Statistical analysis revealed a significant main effect of weight ($F(1,196) = 7.863$, $p<0.05$), in which animals gained weight over the course of free feeding. Analysis also evidenced significant interaction of Weight x Condition ($F(28,196) = 3.124$, $p<0.0001$) such that animals in the high fructose corn syrup condition gained more weight than those in the control condition. See Figure 2.

Reference Memory Errors

As hypothesized, animals significantly diminished their reference memory errors across days (indicating learning; $F(7,49)=2.307$, $p<0.05$), but the results revealed no main effect of Food Condition ($F(1,7)=2.307$, $p>0.05$), nor significant Food Condition x Day interaction ($F(7,49)=0.651$, $p>0.05$, not significant). See Figure 3.

Working Memory Incorrect Errors

For working memory incorrect errors, the data showed a significant main effect of Food Condition ($F(1,7)=9.286$, $p<0.025$), in which animals given high fructose corn syrup demonstrated significantly more errors. Further, the data revealed a significant Food Condition x Day interaction effect ($F(7,49)=3.076$, $p<0.01$), indicating that the largest differences between the treatment groups occurred at the beginning of testing. See Figure 4.

Working Memory Correct Errors

For working memory correct errors, the data showed a significant main effect of Food Condition ($F(1,7)=6.420$, $p<0.05$), indicating that the animals fed HFCS made significantly more working memory correct errors. In addition, the data revealed a

marginally significant Food Condition x Day interaction effect ($F(7,49)=2.126$, $p=0.0581$), in which the only significant difference between treatment groups occurred during the first two days of testing. See Figure 5.

DISCUSSION

High-fructose corn syrup (HFCS) consumption continues to rise, especially in the United States. Although its production offers a number of conveniences to the food industry, and cheap food options to the consumer, numerous scientific studies indicate problems looming on the horizon for consumers of this product. Indeed, numerous studies have linked high-fructose corn syrup with obesity, hypertension, insulin resistance, glucose intolerance, type-2 diabetes, liver disease, cardiovascular disease, and impairment of the central nervous system. Our experiment examined the effects of a high-fructose corn syrup supplemented diet on weight gain, reference memory, and spatial working memory. We hypothesized that animals fed a consistent diet (2 months) of high-fructose corn syrup would not only gain weight, but would demonstrate distinct spatial working memory deficits with no effects on longer-term spatial reference memory.

As hypothesized, in the present study, the high-fructose corn syrup diet caused significant weight gain. This result is consistent with most of the previous studies published, and this effect may help explain some of the reasons behind the increasing rates of obesity in the United States. Our results also suggest that high-fructose corn syrup affects hippocampus-dependent spatial memory but not spatial reference memory, as predicted. Analysis of reference memory errors revealed no significant difference between the high-fructose corn syrup and control conditions. It should be noted, however, that this may be due to a ceiling effect, as the maximum amount of reference errors is three. This is because four arms have platforms and one is the start arm. Therefore three arms are available for the mouse to make reference errors. If the mouse were to reenter

any of these three arms, it would then be considered a working memory incorrect error, so a ceiling effect may account for reference memory errors. Alternately, as the Ns for our study are rather small, our study may lack the requisite statistical power to uncover an effect that is really there. Only further study can test this possibility.

Conversely, analysis of both working memory incorrect and working memory correct errors revealed the hypothesized differences between food condition groups. Those fed a steady diet of high-fructose corn syrup showed diminished working memory function on two separate dependent measures of the phenomenon. These effects were driven mostly by a drastic difference in errors within the first two to three days. After the first few days, the high-fructose corn syrup condition seemed to even out with the control condition (i.e., it took the animals fed HFCS longer to "catch on"). One possible explanation for this phenomenon is that subjects were allowed to eat as much rodent chow as they desired, and the subjects in the HFCS Food Condition appeared to eat more chow in general, particularly within the first few days. Perhaps the added feeding in animals fed HFCS caused some type of behavioral alteration unrelated to learning/memory that might inadvertently affect their scores in this behavioral task. Another possible explanation for the initial impairment of working memory in the HFCS condition may be related to a physiological adaptation to the high-fructose corn syrup over time, although any such alteration should have occurred long before the animals were tested (given that they consumed the food for quite a while before testing began). Future studies are needed to answer these important questions, and to identify the mechanisms causing these adverse effects on cognition. In the meantime, these data,

along with large numbers of other publications, strongly suggest that current consumption levels of HFCS may lead to numerous health problems in our population.

FIGURES

Figure 1

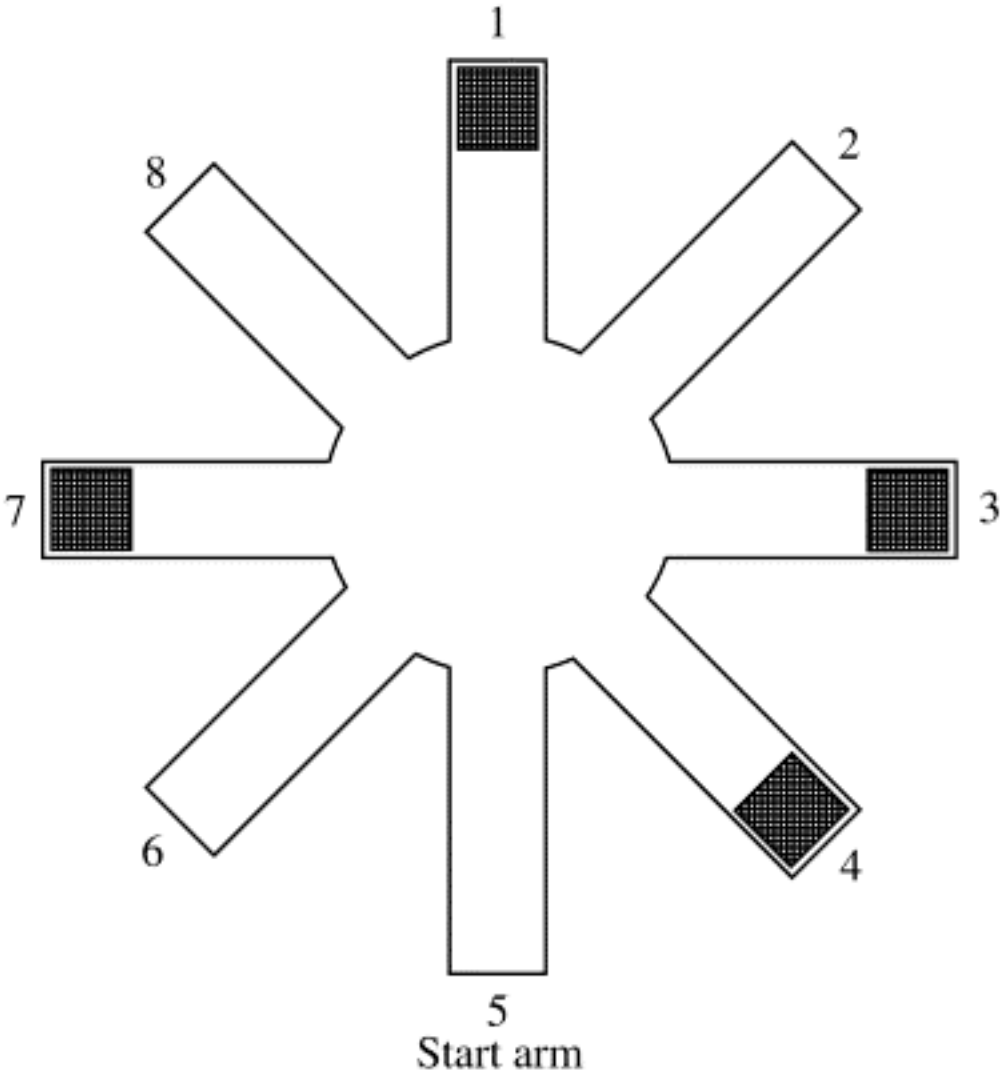

Figure 1. Diagram of water radial arm maze (WRAM). WRAM consists of 8 arms, 4 of which have hidden platforms beneath the water's surface at the start of a daily session. Each daily session includes 4 trials per subject. For each sequential trial, the number of arms containing a platform is reduced by one.

Figure 2

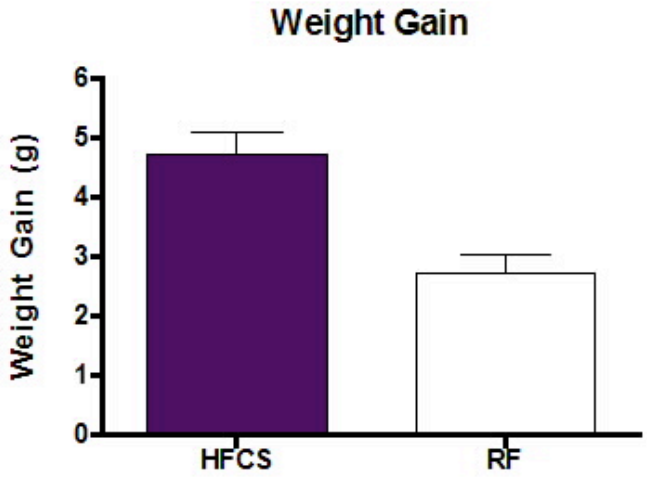

Figure 2. Weight gain between Food Conditions. This figure compares the amount of weight gained in grams between animals that consumed a diet supplemented with high-fructose corn syrup and animals with a normal diet. A significant interaction of Weight x Condition ($F(28,196) = 3.124$, $p<0.0001$) was revealed, such that animals in the high fructose corn syrup condition gained more weight than the control condition.

Figure 3

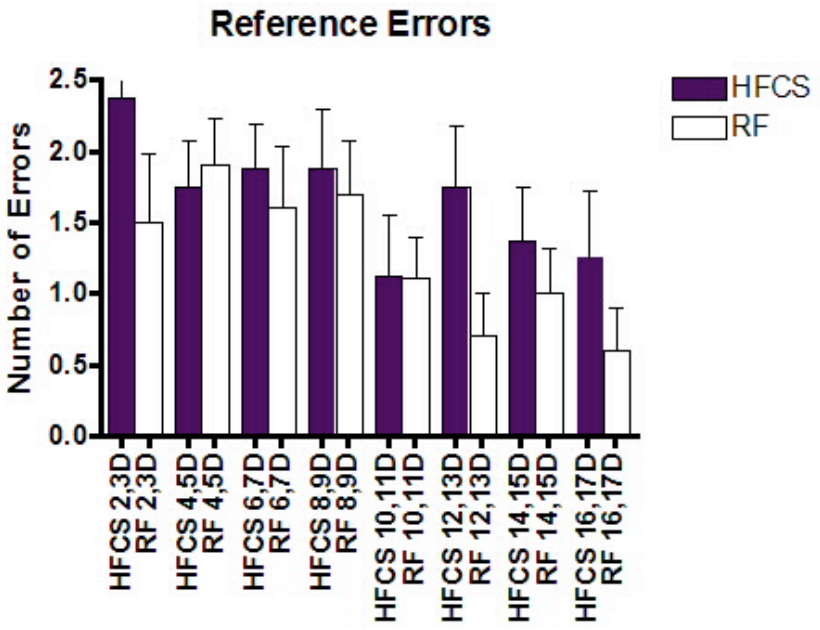

Figure 3. Reference memory errors. Reference memory errors diminished across days (indicating learning; $F(7,49)=2.307$, $p<0.05$), but the results revealed no main effect of Food Condition ($F(1,7)=2.307$, $p>0.05$), nor significant Food Condition x Day interaction ($F(7,49)=0.651$, $p>0.05$, not significant).

Figure 4

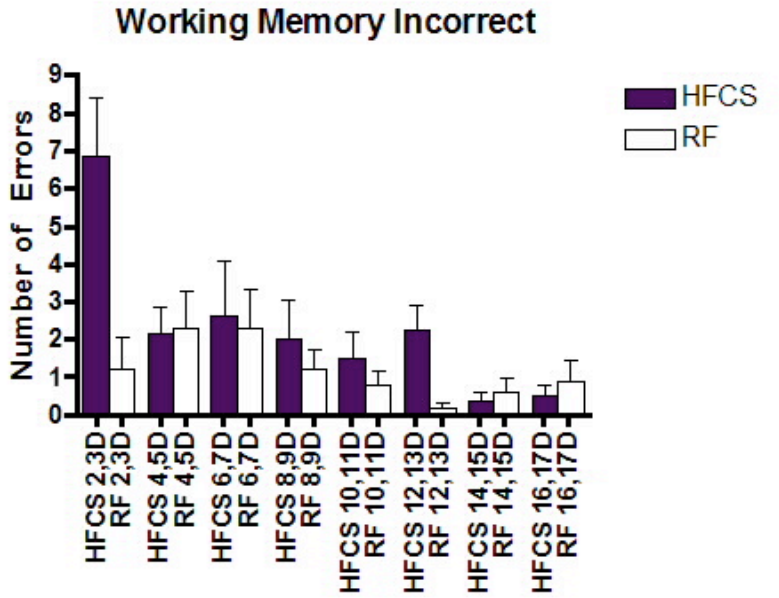

Figure 4. Working memory incorrect errors. Results show a significant main effect of Food Condition ($F(1,7)=9.286$, $p<0.025$), in which animals given high fructose corn syrup demonstrated significantly more errors. The data revealed a significant Food Condition x Day interaction effect ($F(7,49)=3.076$, $p<0.01$), indicating that the largest differences between the treatment groups occurred at the beginning of testing.

Figure 5

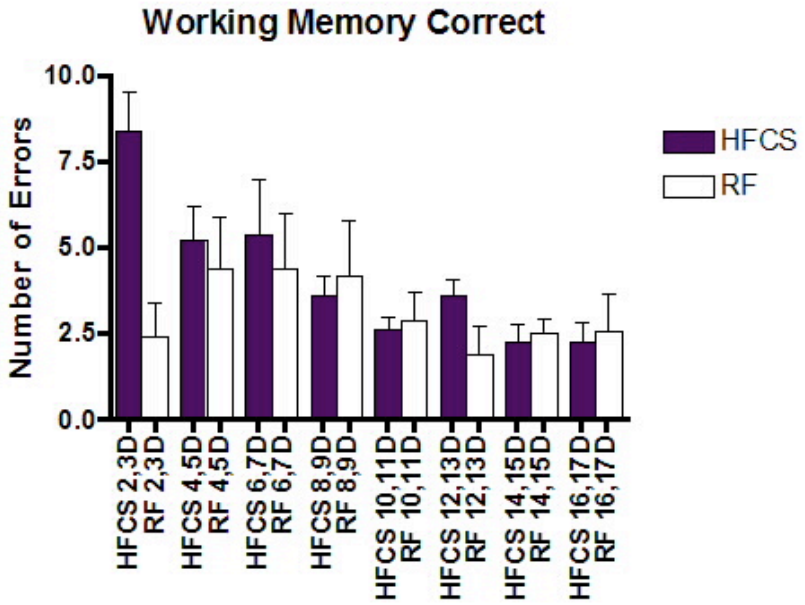

Figure 5. Working memory correct errors. Data shows a significant main effect of Food Condition ($F(1,7)=6.420$, $p<0.05$), indicating that the animals given HFCS made significantly more working memory correct errors. In addition, the data reveals a marginally significant Food Condition x Day interaction effect ($F(7,49)=2.126$, $p=0.0581$), in which the only significant difference between treatment groups occurred during the first two days of testing.

REFERENCES

Banas, S.M., Rouch, C., Kassis, N., Markaki, E.M., Gerozissiz, K., 2009. A dietary fat excess alters metabolic and neuroendocrine responses before the onset of metabolic diseases. Cellular and Molecular Neurobiology 29, 157-168.

Bocarsly, M.E., Powell, E.S., Avena, N.M., Hoebel, B.G., 2010. High-fructose corn syrup causes characteristics of obesity in rats: increased body weight, body fat and triglyceride levels. Pharmacology, Biochemistry and Behavior 97, 101-106.

Bray, G.A., Nielson, S.J., Popkin, B.M., 2004. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. American Journal of Clinical Nutrition 79, 537-543.

Cao, D., Lu, H., Lewis, T.L., Li, L., 2007. Intake of sucrose-sweetened water induces insulin resistance and exacerbates memory deficits and amyloidosis in a transgenic mouse model of Alzheimer disease. Journal of Biological Chemistry 282, 36275-36282.

Dou, J., Chen, M., Dufour, F., Alkon, D.L., Zhao, W., 2005. Insulin receptor signaling in long-term memory consolidation following spatial learning. Learning and Memory 12, 646-655.

Farr, S.A., Yamada, K.A., Butterfield, D.A., Abdul, H.M., Xu, L., Miller, N.E., Banks, W.A., Morley, J.E., 2008. Obesity and hypertriglyceridemia produce cognitive impairment. Endocrinology 149, 2628-2636.

Goldbart, A.D., Row, B.W., Kheirandish-Gozal, L., Cheng, Y., Brittain, K.R., Gozal, D., 2006. High fat/refined carbohydrate diet enhances the susceptibility to spatial

learning deficits in rats exposed to intermittent hypoxia. Brain Research 1090, 190-196.

Hanover, L.M., White, J.S., 1993. Manufacturing, composition, and applications of fructose. American Journal of Clinical Nutrition 58, 724S-732S.

Johnson, R.J., Segal, M.S., Sautin, Y., Nakagawa, T., Feig, D.I., Kang, D., Gersch, M.S., Benner, S., Sanchez-Lozada, L.G., 2007. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. American Society for Nutrition 86, 899-906.

Jurdak, N., Lichtenstein, A.H., Kanarek, R.B., 2008. Diet-induced obesity and spatial cognition in young male rats. Nutritional Neuroscience 11, 48-54.

Lenoir, M., Serre, F., Cantin, L., Ahmed, S.H., 2007. Intense sweetness surpasses cocaine reward. PLoS ONE 2, e698.

Manns, J.R., Hopkins, R.O., Reed, J.M., Kitchener, E.G., Squire, L.R., 2003a. Recognition memory and the human hippocampus. Neuron 37, 171-180.

Manns, J.R., Hopkins, R.O., Squire, L.R., 2003b. Semantic memory and the human hippocampus. Neuron 38, 127-133.

Messier, C., Whately, K., Liang, J., Du, L., Puissant, D., 2007. The effects of a high-fat, high-fructose, and combination diet on learning, weight, and glucose regulation in C57BL/6 mice. Behavioural Brain Reasearch 178, 139-145.

Mielke, J.G., Taghibiglou, C., Liu, L., Zhang, Y., Jia, Z., Adeli, K., Wang, Y.T., 2005. A biochemical and functional characterization of diet-induced brain insulin resistance. Journal of Neurochemistry 93, 1568-1578.

Molteni, R., Barnard, R.J., Ying, Z., Roberts, C.K., Gomez-Pinilla, F., 2002. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience 112, 803-814.

Moosavi, M., Naghdi, N., Maghsoudi, N., Asl, S.Z., 2006. The effect of intrahippocampal insulin microinjection on spatial learning and memory. Hormones and Behavior 50, 748-752.

Park, C.R., 2001. Cognitive effects of insulin in the central nervous system. Neuroscience and Biobehavioral Reviews 25, 311-323.

Ross, A.P., Bartness, T.J., Mielke, J.G., Parent, M.B., 2009. A high fructose diet impairs spatial memory in male rats. Neurobiology of Learning and Memory 92, 410-416.

Stephan, B.M., Wells, J.K., Brayne, C., Albanese, E., Siervo, M., 2010. Increased fructose intake as a risk factor for dementia. Journal of Gerontology: Biological Sciences 65, 809-814.

Stranahan, A.M., Norman, E.D., Lee, K., Cutler, R.G., Telljohann, R.S., Egan, J.M., Mattson, M.P., 2008. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus 18, 1085-1088.

Truax, A., Bliss, A.K., Gupta, S., 2011. High fructose corn syrup. Annals of Clinical Psychiatry 23, 228-229.

Zhao, W., Chen, H., Xu, H., Moore, E., Meiri, N., Quon, M.J., Alkon, D.L., 1999. Brain insulin receptors and spatial memory: correlated changed in gene expression, tyrosine phosphorylation, and signaling molecules in the hippocampus of water maze trained rats. Journal of Biological Chemistry 274, 34893-34902.

ABSTRACT

High-fructose corn syrup (HFCS) has become an increasingly used substitute for sugar in people's diets worldwide. Despite its growing prevalence, HFCS is linked to many detrimental conditions such as obesity, hypertension, type 2 diabetes, liver disease and hypertension. HFCS has also been linked to insulin resistance. Because neural insulin signaling facilitates hippocampal-dependent memory, we hypothesized that a diet high in HFCS would impair hippocampal-dependent learning and memory. In this article, the effects of high-fructose corn syrup on spatial learning and memory are investigated using C57BL/6 mice and water radial arm maze (WRAM).