Maternal Hyperglycemia Leads to Gender-Dependent Deficits in Learning and Memory in Offspring

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Pregnancy in the diabetic woman has long been associated with an increased risk of congenital malformation in the offspring. However, little is known about the effects of maternal diabetes on development of the central nervous system. To begin to gain an understanding of this problem, diabetes was induced in adult female Sprague-Dawley rats by injection with streptozotocin. Only animals with serum glucose levels greater than 200 mg/dl were used. Diabetic and control females were bred, and all newborn pups were cross-fostered to nondiabetic mothers. At 60 days of age, pups were tested in an elevated plus-maze to assess differences in emotionality and anxiety. There were no significant differences between offspring of diabetic dams and controls on this measure. All pups were then housed individually, put on food restriction, and maintained at 85% of their *ad libitum* weight. They were then trained in a Lashley III maze, which assesses learning and retention capability. The female offspring of diabetic dams performed poorer than controls, a finding that was supported by inhibitory avoidance data from a separate group of animals. All animals were then trained in a radial-arm maze. Results failed to find differences between experimental and control animals. It was concluded that the diabetic intrauterine environment has gender-specific effects on central nervous system development. Exp Biol Med 228:152–159, 2003

Key words: diabetes; maternal diabetes; offspring; gestational diabetes; learning and memory

In general, human studies attempting to assess intelligence or neurological function in the offspring of diabetic mothers have yielded contradictory results. Several studies reported no differences in intelligence and behavior between the infants of diabetic mothers and controls (1–3), whereas others reported a positive correlation between degree of maternal glycemic control and the intelligence and behavior in infants of diabetic mothers (4–12). Still, other reports suggest that intelligence does not differ between infants of diabetic mothers and controls, but there are differences in fine and gross motor skills, attention, and hyperactivity that may be the cause of poorer performance on intelligence tests (13, 14).

Studies reporting the presence of neurological delay in the offspring of diabetic mothers vary from 3.9% to 37% of the offspring tested (15). One review reported a number of central nervous system abnormalities thought to be caused by intrauterine exposure to maternal diabetes, including impaired motor function, low intelligence, Erb's palsy, seizure disorder, cerebral palsy, mental retardation, speech disturbances, reading difficulties, behavioral disturbances, psychosis, and deafness (16). In addition, neurophysiological studies done on infants of diabetic mothers have shown EEG (17) and REM sleep (18) patterns similar to those found in more immature infants. Such complications were reportedly minimized with good prenatal control of glucose levels, such that intelligence and behavior were not noticeably different from controls (19, 20). Furthermore, it was also reported that maternal diabetes was only weakly correlated with differences in intelligence and behavior, whereas maternal intelligence, emotional disorders, and conduct problems were much better predictors of low intelligence and behavioral disturbances in children (21).

Taken as a group, these studies and others have not yet yielded reliable conclusions as to whether neurodevelopmental differences exist between infants of diabetic and nondiabetic mothers. From this, it is clear that neurological and neurodevelopmental tests on the offspring of an animal model of maternal diabetes are needed to begin to resolve the controversy.

Although very few studies have been done that look at behavioral and cognitive development in rat pups born to diabetic dams, several studies support the notion that a diabetic intrauterine environment results in abnormal brain development. Similar to findings with human stillborn autop-

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sies (22), decreased brain weight was reported in rat pups and adult rats born to diabetic dams (23). Also, a study of genetically diabetic mice found that their offspring have lower brain weights as well as impaired development of myelin and neuronal membranes (24). In addition, the distance between synaptic terminals and the area of dendritic spines was increased in the Purkinje cells of the cerebellum in rat offspring of diabetics (23).

In vitro studies found that an increase in extracellular glucose results in an inhibitory effect on developing cranial neural crest cells in the rat (25). When taken from embryos of diabetic mothers, these cells showed decreased cell migration at all glucose levels, as well as decreased migratory expansion after culture in basal glucose concentrations. From this evidence, the authors suggested that maternal diabetes may permanently influence the future development of premigratory cranial neural cells (25).

To date, there are very few studies addressing neurodevelopment in terms of behavior and intelligence in the offspring of diabetic dams. One early study looked at ultrasound calls and exploratory behavior of rat pups exposed to maternal diabetes *in utero* (26). They found that at 4 and 6 days of age, offspring of diabetic mothers made more ultrasound calls than controls. The authors compared this behavior with that of pups experiencing cold stress, and this finding suggested that pups that have been subjected to maternal hyperglycemia *in utero* may have an exaggerated stress response to normal environmental factors or an increased need for maternal attention. Also, an exploratory test conducted at 20 days of age found that female offspring of diabetic dams displayed less rearing behavior than did controls (26). However, this difference did not persist when the test was repeated at 80 days of age. Therefore, it was concluded that there were no developmental differences between offspring of diabetic mothers and controls.

A study done in our laboratory looking at sex behavior in male offspring of diabetic dams and controls found that the offspring of diabetics showed a decrease in mounting, intromission, ejaculation, and postejaculatory intromission compared with controls (27). Follow-up studies suggested that these changes were caused by alterations in fetal testosterone levels on Days 18 and 19 of gestation. From this, it was concluded that alterations in prenatal testosterone levels in the offspring of diabetic dams might result in altered brain development, particularly in areas related to male sex behavior (27).

A recently published study looking at behavior in the offspring of diabetic dams found that these animals showed hyperactivity in the open-field test and anxious behavior in the elevated plus-maze (28). These studies failed to find differences in number of center squares crossed in the open-field test, implying that the offspring of diabetic mothers did not differ from controls in anxiety during this test. Moreover, the diabetic offspring showed hyperactivity by increased locomotion in the peripheral squares of the apparatus. This study also looked at anxiety using the elevated plus-maze, with which they found that the offspring of diabetic female rats made significantly fewer entries and spent less time in the open arms compared with controls, implying that these animals were anxious during this test (28).

To our knowledge, no human or animal studies have been done specifically comparing learning and memory in the offspring of diabetic mothers and controls. Therefore, we set out to assess learning and memory in the offspring of diabetic dams by using a battery of tests that measure learning and long- and short-term memory. Because previous studies on the human and rat offspring of diabetic mothers have shown hyperactivity, we also set out to assess differences in anxiety-related behavior using the elevated plus-maze.

Materials and Methods

Subjects. Female Sprague-Dawley rats (Harlan, Indianapolis, IN) were randomly divided into either a diabetic (streptozotocin [STZ]) group or a control group. They were housed individually in temperature-controlled rooms (20°C) with a 12:12-hr light:dark cycle and were provided food and water *ad libitum*. Protocols for animal use and procedures necessary for the experiments described here were approved through the Southern Illinois University Animal Resources Committee, which assures adherence to the standards established by the Animal Welfare Acts and to guidelines established by the National Institutes of Health.

Diabetes was induced by injection of STZ (Sigma Diagnostics, St. Louis, MO; 30–35 mg/kg i.p. in 100 m*M* citrate buffer, pH 4.5). STZ is an antibiotic with diabetogenic effects. It selectively destroys pancreatic β cells, resulting in a depletion of insulin, which causes hyperglycemia. Two days after the injection of STZ, a small amount of blood was collected under light ether anesthesia by retro-orbital sinus puncture to determine the glycemic state of the rats. Blood glucose levels were measured using a Diagnostics Glucose kit, procedure no. 510 (Sigma Chemical Company, St. Louis, MO). Only rats with glucose levels greater than 200 mg/dl were used in the diabetic groups. Compared with the average glucose levels for pregnant control rats (140 ± 20.9 mg/dl; see Ref. 38), diabetic mothers maintained a much higher glycemic status.

Females of diabetic and control groups were then mated to untreated male rats. Daily vaginal smears were collected to detect the presence of sperm (Day 0 of pregnancy). Rats were then observed daily and were weighed weekly. The date of birth and the number of pups per litter were recorded, and both STZ and control litters were culled to 12 and were cross-fostered (Experiments 1–3, and 5) to nondiabetic lactating rats. Diabetic mothers were then sacrificed by decapitation, and a final blood sample was collected and assayed for glucose. Although mean prepregnancy maternal glucose levels were 314.5 ± 25.39 mg/dl, glucose levels after parturition averaged 467.5 ± 22.41 mg/dl, suggesting that during the later stages of gestation, pups were exposed to relatively high levels of glucose *in utero*.

On Day 21, the pups were weaned and housed in same-sex pairs. Pups from four control and four diabetic mothers were used for behavioral studies. Four male and four female pups of three litters and two male and two female pups from the fourth litter were randomly chosen as experimental subjects.

Experiment 1: The Effects of Maternal Hyperglycemia on Emotionality and Anxiety. At 60 days of age, offspring were tested in an elevated-plus maze. The apparatus used in this experiment was modeled after that first described by Pellow *et al.* (1985) as a measure of anxiety and emotional response in the rat (29).

Subjects were placed one at a time in the central area of the apparatus facing an open arm, and a timer was started. The amount of time spent in the open versus closed arms was recorded for a period of 10 min. Once the test was completed, each rat was returned to its home cage, the apparatus was cleaned with 75% EtOH, and it was dried. The next rat was then tested.

Experiment 2: The Effects of Maternal Hyperglycemia on Lashley III Maze Learning and Retention in Offspring. Rats used in Experiment 1 were housed individually and were put on food restriction at 61 days of age. Rats were maintained at 85% of *ad libitum* weight for their age, as determined by a comparison group of age-matched *ad libitum*-fed controls for each gender. Once the animals reached body weight criteria, they were pretrained in the Lashley III maze.

The Lashley mazes were first developed as a test for neurological damage in rats (30). The Lashley III consists of a start box, four alleyways, and a goal box. The animal is required to make a series of alternating left and right turns to obtain a food reward.

Pretraining. Days 1 through 3 were considered exploration, during which the rats were allowed to become familiar with the apparatus. Six one-quarter-sized Fruit Loop (Kellogg USA Inc., Battle Creek, MI) pieces were scattered throughout the maze, and one at a time, the animals were placed in the middle of the maze and were given 3 min to explore. When this time elapsed, the animal was returned to its home cage and the maze was thoroughly cleaned with 75% EtOH. Each rat received one trial per day during this pretraining period.

Experimental training. Day 4 marked the first day of experimental training. Each rat was placed in the start box facing the rear. When it turned around to face the start box door, the manually operated door was opened, activating a silent digital timer. Turning errors were recorded until the animal passed by a photocell at the entrance of the goal box to stop the timer. The experimenter then closed the goal box door, confining the rat in the reward area.

Each rat was given a maximum of 5 min to get to the goal box, where it was rewarded with four one-quarter-sized Fruit Loop pieces. If a rat did not reach the goal box in the 5 min allotted, it was removed from the maze, placed in the goal box for 2 min, and a running time of 300 sec was recorded.

During each trial, the number of wrong turns between alleyways, as well as the amount of time required to negotiate the maze was recorded. Each rat received two trials per day, and all rats finished the first trial before any rat began the second. The running order was altered so that the order in which the rats were run changed daily.

The rate of learning was determined by comparing the rate at which STZ and control offspring reached the criteria of six consecutive errorless trials. Previous studies using this maze have shown that normal rats learn to traverse the maze with minimal to no errors within 40 trials (30). Therefore, once 20 training days (40 trials) elapsed, the test was terminated. All animals were tested again 2 and 4 weeks after the end of training to measure retention.

Experiment 3: Effects of Neonatal Exposure to Hyperglycemia on Short-Term/Working Memory. Food-restricted rats were tested in a wooden 12-arm radial arm maze, an apparatus commonly used to measure short-term/working memory (31, 32).

Pretraining. A 3-day shaping by successive approximation procedure was used to familiarize the rats with the maze arms and train them to go to the end of the arm for a reward. On Day 1, one-quarter-sized Fruit Loop pieces were placed in eight arms at one-quarter, approximately two-thirds the length of the arm from the central platform, and in the goal cup at the end of each arm. All doors were raised and kept open throughout the trial. One at a time, rats were placed on the center platform facing the control board, and the stopwatch was started. Each rat was given a total of 10 min in the maze. Once 10 min had elapsed, the rat was returned to its home cage, and the arms were rebaited. Days 2 and 3 were the same as Day 1, except that the piece of Fruit Loop closest to the center platform was removed each day. By Day 3, only the goal cup at the end of the arm was baited.

Experimental training. Day 4 marked the first day of training. Training began when the rat was placed in the center platform facing the experimenter. After 5 sec elapsed, all the doors were lifted, and the rat was allowed to choose an arm to traverse. Once its' tail had fully entered an arm, the experimenter closed all arm doors except the door for the arm being traversed. When the rat returned to the center platform, that door was also closed, and after a 5-sec period all doors were opened again, and the rat was allowed to enter another arm. The experimenter recorded all arms entered, and an error was recorded each time the rat entered an arm previously visited. This procedure continued until 10 min had elapsed or until all arms were entered, and all rewards were eaten. The rat was then returned to its home cage, and the maze arms were cleaned with 75% EtOH, dried, and rebaited.

Experiment 4: Determination of the Effects of Maternal Hyperglycemia on Inhibitory Avoidance Learning. Female Sprague-Dawley rats (60 days of age) were rendered diabetic in the same manner as previously

described experiments. However, for this experiment, STZ diabetic rats nursed their own pups and were given insulin replacement at 5 IU/kg/d during lactation. Insulin injections were given at 2.5 IU/kg 1 hr after lights on and 1 hr prior to lights off. Pups were trained at 90 days of age, between 0900 and 1200 hr, and a retention test was performed 24 hr later.

The inhibitory apparatus consists of a 16 × 19 × 29-cm illuminated trough-shaped alley with a sliding door leading to a darkened 16 × 19 × 57-cm compartment. The wall and floor of the dark compartment were made of two slanted stainless steel floor plates separated by a small gap. A shock generator (Lafayette Instruments, Lafayette, IN) was used to deliver a 1.0 mA shock through the floor plates, and a timer controlled the duration of the 1-sec shock.

Experimental training. Training began when a rat was placed in the illuminated compartment of the apparatus facing opposite the dark compartment and toward a 75-watt incandescent lamp. Once the rat turned to face the dark compartment, the sliding door was lowered to give the rat access to the dark compartment. When the rat entered the dark compartment, the dividing door was raised to confine the rat, and foot shock was administered. The amount of time taken for the rat to enter the dark compartment (training latency) and the response to shock were recorded.

Retention. Twenty-four hours after training, each rat was given a retention test that was conducted identical to the training trial but without shock. Again, the amount of time taken to enter the dark compartment was recorded. If the rat did not enter the shock compartment within 10 min, it was returned to its home cage and a time of 600 sec was recorded.

Experiment 5: Determination of the Effects of Maternal Hyperglycemia on Passive Avoidance Learning when Pups were Cross-Fostered at Birth

The protocol used in this experiment was similar to that used in Experiment 4. However, in this experiment, the offspring of STZ diabetic rats were cross-fostered to untreated lactating dams. The female offspring were ovariectomized 2 weeks prior to beginning the inhibitory avoidance test.

Results

Characteristics of Maternal Hyperglycemia and the Outcome of Gestation. A paired-sample *t* test comparing maternal glucose concentration in diabetic animals prior to and immediately after pregnancy showed a significant increase in glucose during gestation, $P = 0.019$. Mean prepregnancy maternal glucose levels were 314.5 ± 25.39 mg/dl, whereas glucose levels after parturition averaged 467.5 ± 22.41 mg/dl, suggesting that during the later stages of gestation, pups were exposed to relatively high levels of glucose *in utero*. Compared with the average glucose levels for pregnant control rats (140 ± 20.9 mg/dl; see Ref. 38), diabetic mothers maintained a much higher glycemic status. One-way analysis of variances (ANOVAs) comparing maternal weight gain during gestation did not differ between control and diabetic mothers from the first to second week or the second to third week, $P > 0.05$, of pregnancy (Fig. 1). Finally, an independent-sample *t* test comparing the number of pups born to diabetic ($n = 4$; mean 13.8 ± 0.75) and control ($n = 5$; mean 13.8 ± 0.49) mothers revealed no group differences.

Experiment 1: Elevated Plus-Maze. A two-way ANOVA revealed no differences in performance between control offspring and offspring of diabetic mothers in the elevated plus-maze, $P > 0.05$. This included comparisons of time spent in the closed arms and time spent in open arms. Similar tests comparing offspring gender also revealed no differences in the amount of time spent in the open or closed arms.

Experiment 2: Lashley III Maze. A two-way ANOVA comparing the number of trials necessary to reach the predetermined criteria of six errorless trials failed to find a significant main effect of gender, $P = 0.460$, whereas the main effect for treatment of mother was significant, $P = 0.011$. Additional *post hoc* comparisons found that the female offspring of control mothers required significantly fewer trials to reach criterion than did the female offspring of diabetic mothers, $P = 0.007$ (Fig. 2). Similar comparisons between the male offspring of controls and the male offspring of diabetics failed to find a significant difference, $P = 0.368$ (Fig. 2).

An ANOVA done on the mean speed at which the maze was completed for each animal over all trials failed to reveal a significant difference between female offspring of diabetic

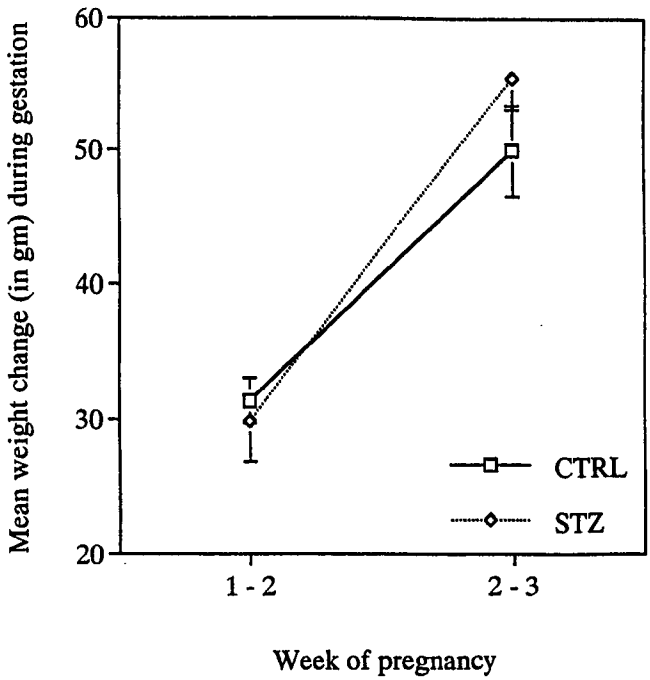

Figure 1. Mean maternal weight change during gestation. Values are expressed as means + SEM; $n = 12$/group.

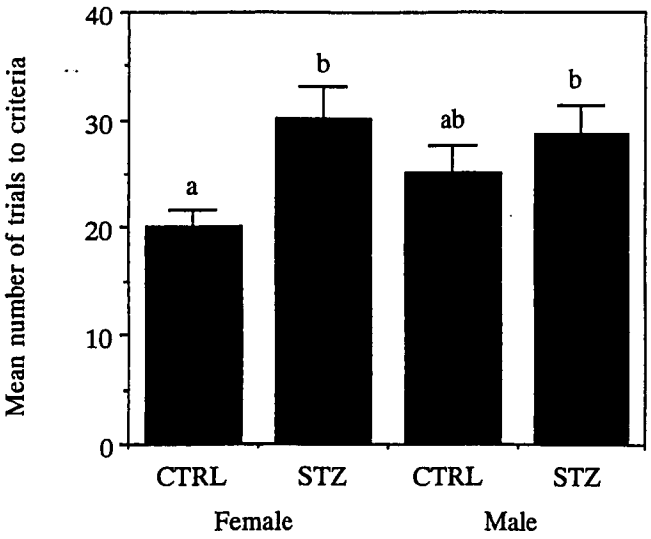

Figure 2. Number of trials necessary to reach a criterion of six errorless trials. Values are expressed as means + SEM; $n = 12$/group. Bars with differing letters are significantly different, $P \leq 0.05$.

mothers and female controls, $P = 0.078$, as well as between the male groups, $P = 0.834$.

Additional analysis looking at number of turning errors made during the 2- and 4-week retention tests were also performed. A chi-square analysis comparing offspring gender × treatment of the mother revealed a significant increase in the number of STZ females making errors during the 2-week retention test, $P < 0.005$. However, identical comparisons between male groups were not significant. Similar tests for the 4-week retention test revealed that neither the female or male groups differed in the number of errors (Table I).

Experiment 3: Radial-Arm Maze. A repeated-measures ANOVA comparing the number of arm entries by control offspring and offspring of diabetic mothers failed to find a significant difference in number of arms entered over all 20 trials in the radial-arm maze, $P = 0.798$. However, the main effect of offspring gender was highly significant at $P \leq 0.0001$, indicating that female animals entered more arms than males. Results were similar when the same test was performed looking at number of errors made over trials. The offspring did not differ by treatment of mother at $P = 0.663$, indicating that the offspring of control and diabetic mothers performed similarly in this test. Additionally, the main effect of trials was significant at $P < 0.001$, indicating that all groups learned the task.

Additional comparisons were made looking at the number of trials necessary to reach the predetermined criteria of three consecutive trials with one or zero errors (Table II). The main effect for offspring gender was significant at $P = 0.039$, revealing that the male offspring made more errors than did females. Comparisons between female control offspring and female offspring of diabetic mothers revealed a nonsignificant difference in the number of trials to reach criteria at $P > 0.05$. Similarly, identical comparisons between the male groups also revealed a nonsignificant difference in number of trials necessary to reach criteria at $P > 0.05$.

Experiment 4: Inhibitory Avoidance Learning in STZ Offspring Not Cross-Fostered at Birth. A comparison of median step-through latency for female offspring of control and diabetic dams revealed no differences during the training trial at $P > 0.05$. However, the median step through latency of female offspring of diabetic dams during the retention trial was significantly shorter than that of female control offspring at $P < 0.05$ (Table III). The median retention latency of female offspring of diabetic dams was 33.8 sec compared with 600 sec (the test ceiling) in the female control offspring (Table III). Identical comparisons for male offspring failed to reveal significant differences in median step-through training and retention latencies between control and STZ offspring (Table III).

Table I. Number of Animals Making at Least One Error During Retention Tests Conducted at 2 and 4 Weeks after Training

	n	2-week test	4-week test
CTRL female	12	2	3
STZ female	12	6*	6
CTRL male	12	4	5
STZ male	12	6	5

Note. Significant difference from controls is annotated with an asterisk and were calculated using a chi-square at $P < 0.05$.

Table II. Number of Animals Reaching the Predetermined Criteria of 1 or Less Errors or 0 Errors for 3 Consecutive Days in the Radial-Arm Maze. Also Shown Is the Mean Number of Days Needed to Reach the Criteria of 1 or Less Errors for 3 Consecutive Days ± SEM

	n	Criteria		Mean number of days to criteria (1 or less errors)
		1 or 0 error	0 error	
CTRL female	11	8	5	14.36, SEM ± 1.41
STZ female	12	8	5	14.58, SEM ± 1.38
CTRL male	12	4	2	18.33, SEM ± 0.84
STZ male	12	7	2	16.00, SEM ± 1.35

Table III. Mean Latency to Enter the Shock Compartment in an Inhibitory Avoidance Test Where the Offspring Were Not Cross-Fostered at Birth

	Training latency (sec) (median)	Retention latency (sec) (median)
CTRL female	6.4	600
STZ female	10	33.8*
CTRL male	8.1	600
STZ male	7.9	600

Note. Significant difference from controls is annotated with an asterisk and was calculated using the Mann Whitney *U* test at $P < 0.05$.

Experiment 5: Inhibitory Avoidance Learning in STZ Offspring Cross-Fostered at Birth. Similar to Experiment 4, the median step through training latencies did not significantly differ among female offspring, but the median step through retention latency of female offspring of diabetic dams was significantly shorter than female control offspring at $P < 0.05$ (Table IV). Similar to the fourth experiment, the median step through training and retention latencies among the male offspring were not significantly different.

Discussion

The most striking findings of the present studies are the learning deficits seen in the female offspring of diabetic mothers. Equally interesting are the gender differences observed in these studies, suggesting that intrauterine exposure to hyperglycemia may affect neurodevelopment in male and female progeny differently.

Glucose was not monitored throughout gestation in STZ-treated dams due to the potential confound of elevated glucocorticoid levels, which is known to have long-term behavioral consequences in the offspring (33, 34). However, the significant increase in glucose levels between the first blood sample (preconception) and the second (postconception) implies that the dams experienced increasing levels of hyperglycemia throughout gestation. This finding is in concert with previously reported increases in maternal insulin resistance throughout gestation, particularly in the third trimester, in both normal and diabetic pregnancies (35). Therefore, it appears as though pregnancy itself had diabetogenic influences on metabolism in the present studies, and that the hyperglycemic state induced by a low dose of STZ was exacerbated by pregnancy.

Because the number of live pups born to control and STZ-treated dams did not differ significantly, the injection of STZ apparently did not adversely affect fertility and the ability to maintain pregnancy. Although previous studies have shown that higher doses (40–50 mg/kg) of STZ result in more severe diabetes and a greater percentage of perinatal mortality (36, 37), data from Rabe (1997) and the present study failed to find such complications when 35 mg/kg STZ was used.

Table IV. Mean Latency to Enter the Shock Compartment in an Inhibitory Avoidance Test Where the Offspring Were Cross-Fostered at Birth and the Female Offspring Were Ovariectomized

	Training latency (sec) (median)	Retention latency (sec) (median)
CTRL female	6.1	600
STZ female	4.2	22.3*
CTRL male	13.6	557.7
STZ male	6.7	600

Note. Significant difference from controls is annotated with an asterisk and was calculated using the Mann Whitney U test at $P < 0.05$.

Additionally, changes in body weight during gestation did not differ significantly between the control and STZ-treated dams. Taken together, these findings suggest that although the metabolic state in the STZ and control groups differed, the hyperglycemia experienced by the STZ-treated dams was not severe enough to result in pup reabsorption or maternal starvation.

Several studies looking at human offspring of diabetic mothers have reported an array of behavioral differences compared with controls (4–14). Although animal studies could help elucidate any deficits occurring in the offspring of diabetic mothers, few such studies have been reported. Ramanathan *et al.* (2000) showed that offspring of diabetic female rats displayed hyperactivity in the open field test and anxiety in the elevated plus-maze. However, these studies did not take into account potential gender differences in the offspring's performance. Studies from our laboratory using activity meters have shown such gender differences, finding hyperactivity in the male but not female offspring of diabetic rats (28).

Although gender differences were shown in activity levels, no such differences were found in elevated plus-maze performance in the present studies. Additionally, offspring of control and diabetic dams did not differ in the amount of time spent in the open or closed arms. This finding indicates that offspring of diabetic rats may not behave differently from controls when subjected to a novel environment (29). These results are in contrast to those reported by Ramanathan *et al.* (2000). Possible explanations for discrepancies between these studies could include strain differences or differences in STZ dose used. Ramanathan *et al.* (2000) used a dosage of 50 mg/kg STZ, which could have resulted in higher gestational levels of diabetes than was observed in the present studies using 35 mg/kg. However, it is difficult to know whether this was a factor because Ramanathan *et al.* (2000) did not measure glucose levels in the diabetic dams after parturition. Taken together, it seems that the behavioral abnormalities in the offspring of diabetic rats resulting from intrauterine exposure to hyperglycemia may be dose dependent in nature.

To our knowledge, our laboratory is the first to look at learning and retention per se in the offspring of diabetic mothers from any species. The most striking findings presented here are the deficits observed in female offspring of diabetics in learning the Lashley III maze and the inhibitory avoidance task. Added interest comes from the findings that only female offspring of diabetic dams displayed learning deficits. The female offspring of diabetics required significantly more trials to reach the predetermined criteria of six errorless trials, whereas no such differences were seen in the males (Fig. 2).

Additional tests performed using the inhibitory avoidance task provided similar results. The failure to find significant differences in step-through training latency is important because this measure is important in assessing possible differences in motivation to enter the shock

compartment. However, the most interesting finding is that female offspring of diabetic dams performed poorer than controls, whereas the male groups did not differ. This finding is similar to the gender differences observed in the Lashley III maze.

The finding that female offspring of diabetic dams that were cross-fostered at birth performed similar to those that were nursed by their diabetic mother provides convincing evidence that the intrauterine glycemic state is more important for the neurodevelopment of rat offspring than the quality of milk produced during lactation. Furthermore, because there were no differences between the data from Experiment 4 where the females were not ovariectomized and Experiment 5, it seems that the presence of female sex hormones during testing also did not play a role in the observed learning deficits.

Although it is clear that learning deficits exist in the female offspring of diabetic mothers, it is difficult to distinguish possible mechanisms and neuroanatomical areas involved. Therefore, the radial-arm maze was employed to assess yet another learning parameter: short-term working memory. Results from this study suggest that there were no differences between the offspring of diabetic mothers and controls in locomotor activity and motivation in the maze, and that there are no differences in short-term working memory between the groups.

An alternative explanation for the learning deficits observed in the female offspring of diabetics is differences in motivation. Perhaps the female offspring of diabetics were not as motivated to learn the Lashley III maze as the female control group. One possible quantitative measure of motivation is the speed at which the animal traversed the maze. Comparisons of running speeds over all trials failed to find differences between offspring of diabetic mothers and controls or between just female or just male groups. Motivation in the inhibitory avoidance test can be measured by the preshock latency to enter the shock compartment. Findings from the present study failed to find differences in preshock step-through latency. Moreover, finding no differences in emotionality is important becasue anxiogenic or anxiolytic effects on behavior could potentially result in altered performance in learning and memory tests. Taken together, these findings imply that motivational level was not different for offspring of diabetic mothers and controls and may not account for the observed differences in learning.

Taken together, the studies presented here show that female offspring of diabetic dams perform poorer on two separate long-term learning and memory tests, but that they did not differ in performance in a working memory task or in measurements of anxious behavior. This implies that the alterations in neurodevelopment of the offspring of diabetic female rats is not a global effect, but instead is localized to specific areas of the brain, particularly those related to long-term memory.

Also, the observed gender differences in learning displayed by the offspring of diabetic dams suggest that the diabetic intrauterine environment exerts differential effects on neurodevelopment of offspring based on gender. Several studies have reported similar gender differences in brain neurodevelopment in rats exposed to an altered intrauterine environment (39, 40). Battaglia and Cabrera (1994) found gender differences in alterations of serotonin receptor-mediated function in the hypothalamus of offspring prenatally exposed to cocaine during the last trimester of pregnancy. Also, Poltyrey and Weinstock (1997) found that exploratory behavior was significantly reduced in female offspring of stressed mothers, whereas male offspring showed a lesser decrease. These studies suggest that the neurodevelopment in male and female offspring can be differentially affected by intrauterine exposure to various endogenous and exogenous compounds.

The precise mechanisms for the observed learning deficits in female offspring of diabetic mothers are unclear at present. Further studies will be required to elucidate how these neurodevelopmental alterations occur. There are many potential damaging factors involved in the exposure to intrauterine hyperglycemia. In response to elevated glucose levels, the fetus produces excess insulin, resulting in fetal hyperinsulinemia. Although previous studies reported evidence against the involvement of insulin in phenotypic abnormalities other than macrosomia (41, 42), it is not known whether fetal hyperinsulinemia can result in altered hippocampal development, one area known to be involved in learning and memory. Previous studies have provided evidence that fetal hyperinsulinemia present at birth may be related to alterations in the number of neurons in and hormonal output from the arcuate nucleus, paraventricular nucleus, and ventromedial hypothalamus, findings that were also apparent in the adult offspring of diabetic mothers (43, 44). Therefore, further studies should be done to look at the morphology of the hippocampus and other brain areas for possible alterations resulting from elevated levels of glucose and insulin in the offspring of diabetic female rats.

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