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Diabetes Disrupts Copulatory Behavior and Neuroendocrine Responses of Male Rats to Female Conspecifics

ROBERT HAILS, CLARE FADDEN AND RICHARD W. STEGER[1]

Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL 62901

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HAILS, R., C. FADDEN AND R. W. STEGER. *Diabetes disrupts copulatory behavior and neuroendocrine responses of male rats to female conspecifics.* PHARMACOL BIOCHEM BEHAV 44(4) 837–842, 1993.—Streptozotocin-induced diabetes disrupts copulatory behavior in the male rat. The increase in serum luteinizing hormone (LH) that occurs in the male rat in response to the presence of a receptive female is absent in most diabetic rats. A female-induced testosterone rise is not seen in diabetic male rats, including those showing an increase in LH. The female-induced LH rise appears to be secondary to increased hypothalamic norepinephrine metabolism, which is severely attenuated in diabetic rats not exhibiting an LH rise in response to a female.

Diabetes Sex behavior Luteinizing hormone Norepinephrine Hypothalamus

SEXUAL dysfunction is frequently associated with diabetes in men and experimental animals (9,14,18). Many of these changes are due to diabetes-induced changes in the vascular and peripheral nervous system but increasing evidence suggests that CNS-related changes in endocrine function and sexual arousal may also contribute to sexual dysfunction (14). In a recent series of experiments, we demonstrated that male rats with streptozotocin (STZ)-induced diabetes exhibit severe deficits in copulatory behavior that are accompanied by marked changes in luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), and testosterone secretion (14). Although insulin replacement can prevent or partially reverse the adverse effects of STZ-induced diabetes on copulatory function (17), testosterone replacement was not able to restore normal sexual function (13).

In the male of many species, exposure to a female results in a rapid increase of LH that is followed by an increase in circulating testosterone levels (7,10,11,15). The increase in LH is associated with changes in central norepinephrine metabolism in several brain areas (4,15). It has been suggested that this rise in LH is a measure of sexual motivation because it is absent or severely attenuated in animals that show no interest in the female (6,15). In this regard, we have reported that hyperprolactinemia in male rats is associated with severe deficits in sexual behavior and the absence of an LH response to a receptive female (15). Further, the loss of the LH response appears to be secondary to changes in hypothalamic catecholamine metabolism.

The present experiments were designed to further characterize the effect of STZ-induced diabetes on male sexual function and determine if diabetes interrupts neuroendocrine responses of male rats to exteroceptive stimuli originating from the female.

METHOD

Animals and Experiments

Adult, male Sprague Dawley rats (225–250 g) were purchased from Harlan Industries (Madison, WI). Rats were housed in a temperature-controlled (22°C) room on a 12 L : 12 D cycle (lights on at 0700 h). Food (TekLab Rat Diet, Madison, WI) and tapwater were provided ad lib. Two weeks after arrival, rats were injected with STZ (50 mg/kg, IP in 0.01 M citrate buffer, pH 4.5) or the injection vehicle (day 1). All rats were weighed at least weekly during the course of the following experiments:

Experiment 1. Sexually naive diabetic and control rats were tested for neuroendocrine response to female rats 3 weeks after STZ or vehicle injection. The methodology is described below.

Experiment 2. Diabetic and control rats were subject to analysis of sex behavior 1, 2, 3, and 4 weeks after STZ or vehicle injection. At 8 weeks following induction of diabetes, a subset of the diabetic rats (n = 18) was tested for endocrine responses to a receptive female.

[1] To whom requests for reprints should be addressed.

Experiment 3. Diabetic male rats were subjected to sex testing at 1, 2, and 3 weeks after STZ and the neuroendocrine responses to female rats were evaluated at 4 weeks post-STZ.

Behavioral Analysis

Behavioral testing was conducted between 1430 and 1730 h in a separate room under dim red illumination in bedding-lined, 10-gal glass aquaria. Ovariectomized Sprague-Dawley female rats implanted with a 5-mm length of 0.125 in. o.d. × 0.095 in i.d. Silastic (Dow Corning, Midland, MI) capsules filled with estradiol were used as stimulus animals. Behavioral estrus was produced with a SC injection of 0.5 mg progesterone in 0.1 ml corn oil approximately 5 h before the beginning of a test. Before each test, each subject was allowed 5 min to acclimate to the test chamber. The test was terminated at the first intromission after ejaculation or when 30 min had elapsed. Mount, intromission, and ejaculation behavior were observed. A more detailed definition of these components of copulatory behavior have been published by Dewsbury (3).

Response to Female Conspecifics

The effects of female exposure on endocrine and neurotransmitter function were tested 1-3 h into the dark phase under dim red illumination. Subjects were transferred from their home cage to a clean cage and after a 10-min adaptation period were injected with saline or an inhibitor of tyrosine hydroxylase, α-methyl-*p*-tyrosine (αMPT; 250 mg/kg, IP) for determination of catecholamine turnover rates (Experiments 1 and 2). Ten minutes after this injection, an ovariectomized female rat made sexually receptive as described in the previous section was introduced into the cage. After 20 min of exposure to the female, male rats were removed and immediately sacrificed by decapitation (Experiments 1 and 3) or anesthetized with ether and a blood sample taken via the retroorbital sinus (Experiment 2). Controls remained in the cage for a corresponding length of time but were not exposed to a female.

Neurotransmitter Turnover and Content

At the time of sacrifice, trunk blood was collected and the brain was rapidly removed and the median eminence (ME) separated with irridectomy scissors. The brain and ME were then frozen on dry ice. Prior to assay, brains were partially thawed and medial basal hypothalamic (MBH) and olfactory bulbs (OLFB) dissected free as previously described (16). The MBH and OLFB were weighed and then sonicated in 0.1 M $HClO_4$ containing the internal standards for the catecholamine assay (dihydroxybenzylamine) and the 5-hydroxytryptamine (5-HT) assay (methyl 5-HT) and 1 mM sodium bisulfite. The ME was sonicated in the same solution but without the methyl 5-HT. Median eminence supernatants were separated by high-performance liquid chromatography (HPLC) and quantitated by electrochemical detection as previously described (16). The MBH and OLFB supernatants were subjected to alumina extraction prior to HPLC separation (16). Catecholamine turnover rates were estimated using the formula $K = k[CA]_0$, where $[CA]_0$ equals the mean catecholamine concentration at zero time (saline controls), and the rate constant, k, represents the $-\log$ of the slope of the line describing the decline of norepinephrine (NE) or dopamine (DA) concentration during the 1 h following the blockade of tyrosine hydroxylase with αMPT (16).

Radioimmunoassay and Glucose Determinations

Plasma, media, and tissue levels of LH, FSH, and PRL were measured by radioimmunoassay (RIA) using reagents provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) as described previously (12). Hormone values are reported only for animals not receiving αMPT. Plasma glucose was assayed by a glucose oxidase procedure using a kit purchased from Sigma Chemical Co. (St. Louis, MO). Glucose values include those for control and αMPT-treated rats as no effect of αMPT on glucose levels were seen here or in previous studies (14).

Data Analysis

The effects of treatment on behavior, hormone levels, and neurotransmitter content and/or turnover were evaluated using analysis of variance (ANOVA) or Student's *t*-test. The effects of treatment on mount, intromission, or ejaculatory behavior was compared using Fisher's exact probability test. Mean values between groups were considered significantly different when the p value was <0.05.

RESULTS

Injection of STZ resulted in the expected elevation of blood glucose levels and loss of body weight in all three experiments (Table 1). Three of 90 rats treated with STZ did not show elevated blood glucose (<200 mg/dl) levels and were deleted from the data analysis.

Copulatory Behavior

Experiment 2. During the first sex testing session (1 weeks post-STZ or vehicle), only 5 of 12 controls and 7 of 24 diabetic rats showed mounting behavior ($p > 0.05$). One diabetic and no control rats ejaculated. By the fourth sex test (4 weeks after STZ or vehicle), 11 of 12 control rats exhibited mount and intromission behavior and 8 rats ejaculated during the 30-min test period. Only 3 of 24 diabetic animals mounted the female and none reached ejaculation at this time period ($p < 0.05$).

Experiment 3. Only 1 of 36 rats reached ejaculation and

TABLE 1

EFFECTS OF STZ-INDUCED DIABETES ON BODY WEIGHT AND PLASMA GLUCOSE LEVELS

	Pre-STZ Body Weight (g)	Body Weight 21 days post-STZ (g)	Plasma Glucose at Autopsy (mg/dl)
Experiment 1			
Control (n = 28)	333 ± 2	358 ± 3	165 ± 11
Diabetic (n = 32)	335 ± 3	277 ± 10*	520 ± 35*
Experiment 2			
Control (n = 12)	367 ± 6	400 ± 8	154 ± 9
Diabetic (n = 24)	360 ± 4	268 ± 7*	784 ± 61*
Experiment 3			
Diabetic (n = 36)	360 ± 3	318 ± 4	478 ± 5

Values are reported as mean ± SEM.
*$p < 0.001$ vs. the respective control value.

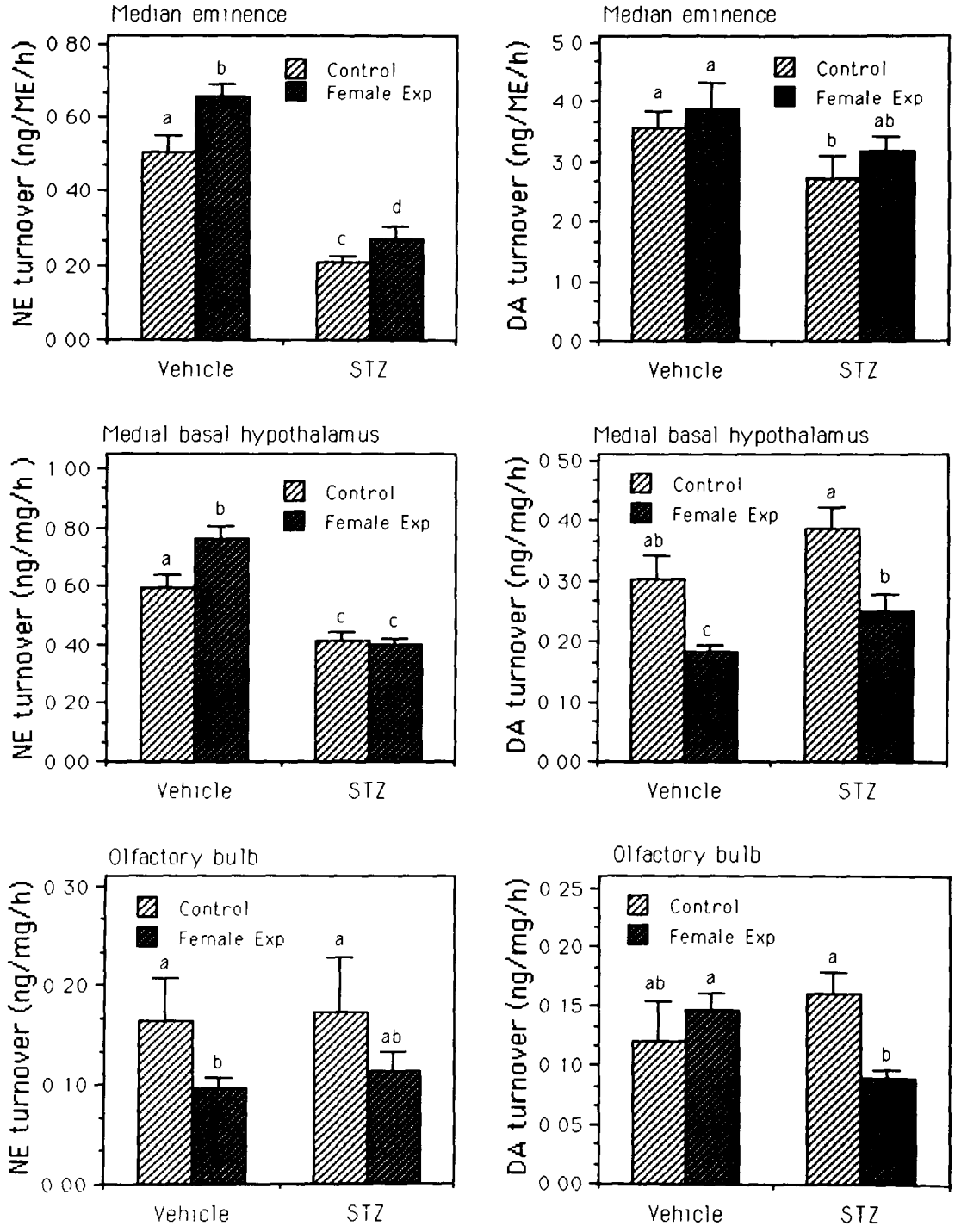

FIG. 1. Effects of a 20-min exposure to a sexually receptive female rat on concentrations of serum luteinizing hormone (LH), prolactin (PRL), and testosterone in vehicle- (VEH) or streptozotocin (STZ)-treated rats (mean ± SEM; n = 7–8 rats/group). Bars with different superscripts are significantly different ($p < 0.05$). Diabetes-induced changes in sexual behavior were not monitored in these animals.

only 11 rats even showed mount or intromission behavior when tested 3 weeks after STZ injection (data was not recorded for testing sessions at weeks 1 and 2). There were no nondiabetic rats included in this experiment but we usually see at least 90% of normal adult Sprague-Dawley rats exhibit mount and intromission behavior and greater than 70% of control rats reach ejaculation under similar testing conditions [Experiment 2 and (13,14)].

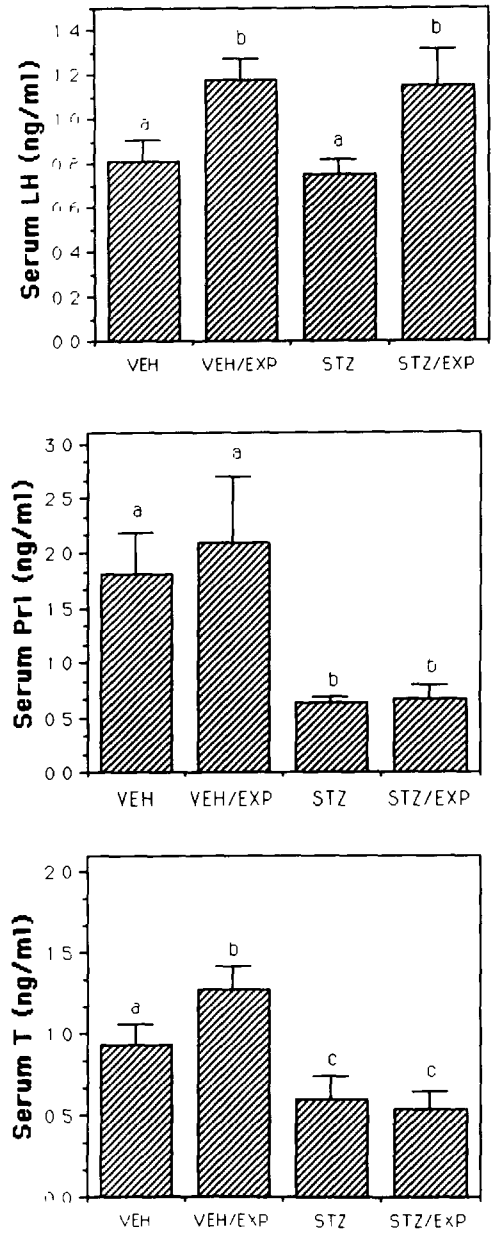

FIG. 2. Norepinephrine (NE) and dopamine (DA) turnover (mean ± SEM; n = 7–8) in defined brain regions of control (vehicle) and diabetic [streptozocin (STZ)] male rats exhibiting a luteinizing hormone (LH) response to a receptive female rat. NE or DA turnover, an index of neuronal activity, was calculated from the decline of NE or DA content after inhibition of tyrosine hydroxylase with αMPT. Bars with different superscripts are significantly different ($p < 0.05$).

Response to Female Conspecifics

In Experiment 1, both control and diabetic rats showed comparable basal LH levels and a similar LH rise in response to female exposure (Fig. 1). Serum PRL levels were significantly attenuated in STZ rats and female exposure had no effect on these levels in either group. Serum testosterone levels were lower in diabetic than in control rats and failed to rise after female exposure as they did in controls. Unfortunately, no behavioral observations were made.

Median eminence NE turnover was reduced in the STZ as compared to control rats but both groups of rats showed a slight rise of NE in response to female exposure (Fig. 2). The turnover of NE in the MBH was also reduced in STZ rats but did not increase after female exposure as it did in control rats. In the OLFB, NE turnover decreased in response to the female in both control and STZ rats but the apparent decrease in the latter group was not significant. There was no significant effect of STZ or female exposure on DA turnover in the ME but MBH DA turnover decreased in both groups of rats after female exposure.

In Experiment 2, no change in LH (0.45 ± 0.06 vs. 0.46 ± 0.07 ng/ml), PRL (8.1 ± 1.6 vs. 10.1 ± 2.8 ng/l), or testosterone (0.53 ± 0.23 vs. 0.60 ± 21) levels were seen in diabetic male rats in response to female exposure. During the course of the 30-min exposure to the female, diabetic male rats initially "investigated" the female but exhibited no attempts at even mounting behavior. Female rats were all clearly receptive.

In Experiment 3, LH levels also failed to increase in animals exposed to a receptive female (Fig. 3). Norepinephrine turnover in the ME and MBH also failed to increase after exposure to female rats (Table 2). Dopamine turnover in the MBH decreased after exposure to the female but ME DA was unaffected. As in Experiment 2, diabetic male rats initially "investigated" the female but exhibited no attempts to mount or intromit.

DISCUSSION

The results from these studies confirm previous observations concerning the adverse effects of STZ-induced diabetes on male sexual behavior (13,14,17). Further, these data also demonstrate that some diabetic male rats also fail to show a rise in LH when exposed to a receptive female rat.

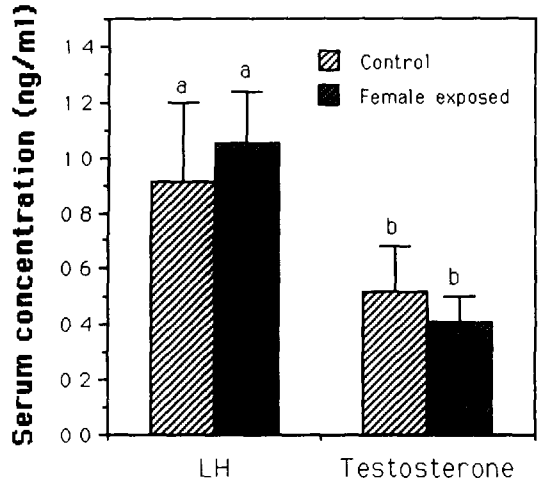

FIG. 3. Effects of a 20-min exposure to a sexually receptive female rat on concentrations of serum luteinizing hormone (LH) levels in streptozotocin (STZ)-treated rats (mean ± SEM; n = 8–9 rats/group). Bars with different superscripts are significantly different ($p < 0.05$). Rats in this experiment did not exhibit mount or intromission behavior.

TABLE 2

THE EFFECTS OF EXPOSURE OF DIABETIC MALE RATS TO SEXUALLY RECEPTIVE FEMALE RATS

	Control	Female Exposed
ME turnover (ng/ME/h)		
Norepinephrine	1.32 ± 0.13	0.97 ± 0.03
Dopamine	1.80 ± 0.14	2.13 ± 0.16
MBH turnover (ng/mg/h)		
Norepinephrine	0.42 ± 0.03	0.18 ± 0.06*
Dopamine	0.40 ± 0.03	0.24 ± 0.03*
AH turnover (ng/mg/h)		
Norepinephrine	0.52 ± 0.05	0.49 ± 0.03
Dopamine	0.31 ± 0.09	0.34 ± 0.07

Values are reported as mean ± SEM; n = 8-9 rats/group.

Despite the almost complete lack of copulatory behavior exhibited by most of the diabetic rats in Experiments 2 and 3, some diabetic rats continue to show fairly normal behavior. Similar findings were seen in previous experiments (13), and in one experiment we observed no decrement in sexual behavior in an entire group of diabetic animals (Steger and Hails, unpublished data). Likewise, rats in Experiment 1 responded with an LH increase when exposed to a receptive female despite the fact that they were clearly diabetic while those in Experiments 2 and 3 showed no LH rise. No behavioral observations were made on rats in Experiment 1 either preceding or during the period of female exposure; thus, we do not know if the LH rise seen in these animals was accompanied by copulatory activity. It is not clear why these differences in behavioral and endocrine responses to STZ occur, but they do not correlate with weight loss, serum glucose, or unstimulated testosterone, LH, or PRL levels. Previous studies have shown significant behavioral and endocrine changes within 1 week of STZ treatment [(13,14), unpublished observations]. It is possible that it takes longer for STZ to reduce female-induced LH rises than to affect other behavioral or endocrine parameters that could explain why an LH rise was seen at 3 weeks (Experiment 1) but not at 4 or 8 weeks (Experiments 2 and 3) after STZ treatment.

Despite the female-induced rise in LH levels seen in Experiment 1, diabetic animals did not show a female-induced increase in serum testosterone levels as seen in controls. Previously, we suggested that this lack of testosterone response to LH in diabetic male rats may be secondary to the low PRL levels (13) because it has been documented that PRL has an important stimulatory effect on testicular LH receptors (8,19). The low PRL and testosterone levels in STZ-treated rats in the present experiments are in agreement with previous studies (14). It should also be noted that although LH levels were not depressed below levels seen in nondiabetic control rats LH levels should be expected to be increased due to the significant depression of testosterone and the resulting attenuation of the negative feedback signal controlling LH release.

It is apparent from the first experiment that an increase in ME NE turnover accompanies the rise in LH and that no rise in ME NE is seen in diabetic animals not exhibiting a rise in LH (Experiment 3). This is similar to our previous report that hyperprolactinemic rats with deficits in sexual activity fail to show an increase in ME NE turnover and LH release when exposed to a sexually receptive female rat (15). In agreement with this same report is the rise in MBH NE turnover and the attenuation of NE turnover in the olfactory bulbs seen after exposure of nondiabetic control rats to receptive females. Even though diabetic rats in Experiment 1 showed an LH rise and an increase in ME NE turnover in response to a female, they failed to exhibit a rise in MBH NE turnover or a significant decline in OLFB NE turnover. In all likelihood, the increase in ME NE activity upon female exposure is responsible for the rise in LH levels, but it is unclear what these other changes in amine turnover might represent as far as behavior or neuroendocrine function.

A failure of the pituitary to respond to LH-releasing hormone (LHRH) release does not appear to be involved in decreased LH responses of diabetic rats because several studies have shown no differences in LH release after LHRH administration in vivo or in vitro (5,15). It has also been recently demonstrated that hypothalamic explants from diabetic male rats release LHRH normally in response to α-adrenergic or KCl stimulation (1). Depressed serum testosterone levels are also probably not involved in the behavioral and neuroendocrine changes seen in diabetic rats because testosterone levels were higher than the minimum needed for normal sexual behavior in the rat (2) and because testosterone replacement does not improve sexual behavior in the diabetic male rat (13).

In conclusion, the significant increase in LH and testosterone release that follows the exposure of a male rat to a sexually receptive female is attenuated in many diabetic animals. However, just as some clearly diabetic rats fail to develop deficits of copulatory behavior some diabetic rats continue to exhibit an LH response to a receptive female. Diabetes is associated with changes in neurotransmitter metabolism in response to female exposure in several areas of the brain, and these effects may explain many of the adverse effects of diabetes on LH release and copulatory behavior.

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