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## Depressive Phenotypes Evoked By Experimental Diabetes are Reversed by Insulin

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### Abstract

Clinical studies suggest a bidirectional relationship between diabetes and depression, where diabetes may increase risk for depressive symptoms and depression may increase risk for diabetes. Preclinical models examining the effects of diabetes on brain and behavior can provide insights to the pathophysiology underlying this relationship. The current study comprehensively examined, in C57BL/6 mice, the development of depressive phenotypes evoked by diabetes induced by streptozotocin (STZ) and determined if insulin treatment was able to reverse the diabetes-related changes on brain and affective behavior. Since anxiety is often comorbid with mood disturbances, behavioral tests for both anxiety and depression were administered. Possible physiological correlates of behavioral changes, including hippocampal cell proliferation, brain derived neurotrophic factor, and plasma corticosterone, were also measured. STZ-induced diabetes resulted in increased immobility in the tail suspension test, increased intracranial self-stimulation thresholds, decreased hippocampal cell proliferation, and increased corticosterone levels. Insulin treatment, on the other hand, reduced hyperglycemia, reversed the behavioral effects, and returned hippocampal cell proliferation and corticosterone to levels comparable to the control group. Anxiety-related behaviors were unaffected. This study showed that experimental diabetes in the mouse produced depressive phenotypes that were reversed by insulin therapy. Changes in reward-related behaviors and hippocampal cell proliferation may be useful markers to identify therapeutic interventions for comorbid diabetes and depression.

### Keywords

diabetes; streptozotocin; insulin; insulin glargine; depression; BDNF; corticosterone; tail suspension; ICSS; neurogenesis; behavior

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## 1. Introduction

The relationship between comorbid diabetes and depression is likely bidirectional. In clinical studies, people with depression have higher fasting glucose levels [1] and adults with diabetes are twice as likely to have depression than adults without diabetes [2]. Diabetes with comorbid depression correlates with poorer management of plasma glucose and resultant increase in diabetes-related complications and healthcare expenses [3–5]. The mechanisms underlying comorbid diabetes and depression remain unclear. The development of preclinical diabetes models, with tests of neurobehavioral complications similar to those experienced by humans, can help elucidate the pathophysiology underlying comorbid depressive symptoms and identify specific targets for therapy.

Studies in this manuscript use the well-established streptozotocin (STZ)-induced diabetes model, a glucosamine-nitrosourea compound that produces a type 1-like diabetes. After uptake into the insulin-secreting pancreatic beta cells by highly expressed GLUT-2 transporters, STZ produces cell death through deoxyribonucleic acid (DNA) methylation [6]. This results in insulin-dependent diabetes with chronic hyperglycemia and hypoinsulinemia.

STZ-induced diabetes has demonstrated consequences for depressive behavior, such as increased immobility in the mouse tail suspension test (TST) [7–9]. Depressive behavior in rodents could also be studied by measuring reward processes directly in the brain using intracranial self-stimulation (ICSS), a procedure that measures performance to obtain stimulation of electrodes implanted in the medial forebrain bundle of the hypothalamus [10, 11]. Further, STZ-induced diabetes is known to decrease hippocampal neurogenesis [12–17]. Earlier studies have linked the consequence of decreased hippocampal neurogenesis in diabetes to cognitive impairment, and restoration of hippocampal neurogenesis, via regulation of glucocorticoids, prevented cognitive impairment [16, 18].

This study attempted to link the consequence of STZ-induced diabetes in the mouse to measures associated with depressive behavior. Behavioral tests associated with depression and anxiety were used, such as the TST, ICSS, and elevated zero maze (EZM). In addition, decreased hippocampal cell proliferation, one aspect of the neurogenesis process was measured along with possible mediators of hippocampal cell proliferation, such as brain derived neurotrophic factor (BDNF) and plasma corticosterone (CORT) levels. The association of changes in measures of depressive behavior with diabetes was examined by determining if insulin, the primary treatment for type 1 diabetes, could ameliorate complications of altered hippocampal cell proliferation and/or behavior in C57BL/6 mice treated with STZ. This study is the first study in mice to assess insulin's ability to restore deficits in hippocampal cell proliferation and affective behavior associated with depression.

## 2. Materials and methods

### 2.1. Animals

Mice were housed in a temperature- and humidity- controlled facility with a 12-hour light-dark cycle (lights on at 07:00) at the University of Pennsylvania Translational Research Laboratories (Philadelphia, PA) with standard laboratory pellet food and water freely available. All experimental procedures were conducted in accordance with the guidelines published in the NIH Guide for Care and Use of Laboratory Animals and approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

## 2.2. Procedure

Two separate cohorts of mice were used. Eight-week-old, male, C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME), housed in groups of 4–5 per cage, were used for the hippocampal cell proliferation experiment. Behavioral testing for mice began 2–3 weeks after treatment with STZ and 1–2 weeks after initiation of insulin treatment. Mice (n = 8–10 per group) underwent a series of behavioral tests in the following order: EZM, TST, and locomotor activity. Consecutive tests were administered at least 3 days apart. At the completion of behavioral testing, the effects of diabetes and insulin treatment were measured on hippocampal cell proliferation, BDNF, and CORT levels.

For the ICSS study, 4 month old, male C57BL/6J mice, weighing 23–31 g at the beginning of the experiment, were used that had been exposed once to the effects of acute nicotine treatment on ICSS performance 4–5 weeks prior to the start of the experiment. Mice in the ICSS experiment were housed singly because of their cranial apparatus. Mice were trained for ICSS prior to treatment with vehicle (n = 4) or STZ (n = 6). Then, after STZ administration, ICSS performance was assessed again, with and without insulin treatment, for a total of 4 weeks.

## 2.3. Induction of diabetes and glucose monitoring

To induce diabetes, mice received a single intraperitoneal (i.p.) dose of 195 mg/kg STZ (Sigma-Aldrich, St. Louis, MO) prepared in 5M sodium citrate, pH 4.5, or vehicle. Blood glucose levels were measured periodically, starting 2 days (in the ICSS experiment) or 7 days (in the hippocampal cell proliferation experiment) after STZ or vehicle injection, using a portable Freestyle glucometer (Abbott Laboratories, Abbott Park, IL). Blood was obtained via tail snip. Mice with blood glucose values > 250 mg/dl were included in the STZ groups. Glucose levels were then measured on a weekly basis, in the morning between 0800 – 1000, until the completion of the study.

## 2.4. Insulin treatment

For the hippocampal cell proliferation study, insulin was delivered to diabetic mice with sustained release LinBit insulin pellets (LinShin Canada, Toronto, ON, Canada). Pellets were implanted subcutaneously under isoflurane anesthesia and dosed according to manufacturer instructions. Insulin therapy began 7 days post STZ injection and continued for the duration of the study. Mice in the vehicle groups (Vehicle-Vehicle and STZ-Vehicle) underwent pellet insertion procedures with anesthesia but did not receive any implants. There were a total of 3 experimental groups in the hippocampal cell proliferation study: Vehicle-Vehicle (Veh-Veh; n = 8), STZ-Vehicle (STZ-Veh; n = 8), STZ-Insulin (STZ-Ins; n = 10).

For the ICSS study, injections of long-acting insulin glargine (Sanofi-Aventis, Bridgewater, NJ) were chosen over insulin pellets so that insulin therapy could be monitored and adjusted if needed during the study. During Week 2, 4 of the 6 STZ-treated mice that had developed earlier onset hyperglycemia were treated with insulin glargine at 100 mg/kg, s.c., twice daily. Vehicle treated mice received saline injections twice daily. By Week 3, all 6 STZ-treated mice had developed hyperglycemia and were untreated. During Week 4, all STZ-diabetic mice (n = 6) received insulin glargine, 100 mg/kg, s.q., once daily, while all mice in the vehicle group (n = 4) continued to receive saline once daily.

## 2.5. Behavioral tests

**2.5.1. Elevated Zero Maze (EZM)**—The EZM apparatus (Stoelting, Wood Dale, IL) was shaped in a circular form with a platform that had an outer diameter of 60 cm, a width of 5

cm, and was elevated 61 cm above the floor, as previously described [19]. The maze consisted of four quadrants: two closed arms with walls that extended 30.5 cm above the maze's surface and two open arms with lips that extended 1.3 cm above the maze's surface. Each mouse was placed in one of the closed arm quadrants and was allowed unlimited exploration of all 4 quadrants for 5 minutes under dim lighting (100 lux). Viewpoint Tracking System (Viewpoint, Champagne au Mont d'Or, France) was used to record and calculate the percent time each mouse spent in the open arms of the maze.

**2.5.2. Tail suspension test (TST)**—The duration of TST immobility was quantified using an automated TST device (Med Associates, St. Albans, VT), as described previously [20]. The base of each mouse's tail was aligned with and taped to the bottom of a centrally located, vertical, flat, metal bar that was connected to a force transducer that recorded the time that the mouse spent in an immobile state. Mice were suspended for a total of 6 minutes. Two mice (one vehicle and one STZ) were excluded because they climbed their tails during the test.

**2.5.3. Locomotor activity**—Each mouse was placed in an empty plastic cage (28.5 × 17.5 × 13.0 cm) in a room with dim lighting (140 lux). Spontaneous locomotor activity was recorded on videotape for a 30-minute period using a camera, suspended overhead analyzed using SMART (Spontaneous Motor Activity Recording & Tracking) computer software (Panlab, Barcelona, Spain). This system tracked horizontal movements and calculated the distance (in centimeters) each mouse traveled during the test.

**2.5.4. Intracranial self-stimulation (ICSS)**—A separate group of mice (n = 10) were used to measure ICSS performance during diabetes and after insulin treatment. Procedures were adapted from an earlier study [11]. Mice were trained and tested in one of six operant chambers (Med Associates, St. Albans, VT) with access to a response wheel (5.1 cm diameter). Bipolar electrodes were implanted into the medial forebrain bundle (1.9 mm posterior to bregma, 0.8 mm lateral to midline, 4.8 mm below dura) [21] of mice under isoflurane anesthesia. Electrodes were secured with a stainless steel screw threaded into the skull and fastened with dental cement. One week following the surgery, mice were first exposed to the operant chamber and allowed free access to the manipulandum for one hour. A one-quarter turn of the wheel initiated a 500-ms train of rectangular cathodal pulses (100 ms pulse duration) at a rate of 158 Hz followed by a 500-ms timeout period in which subsequent responses were not reinforced by stimulation. During an ICSS session, a mouse received 6 passes. Each pass contained 15 one-minute trials of different stimulation frequencies, ranging from 126 Hz to 24 Hz, presented in descending order. Higher frequencies maintained high levels of responding while the lower frequencies failed to elicit responses. The currents were adjusted such that the first half of the frequencies (between 7 or 8 trials) maintained stable response rates. The number of responses made at each frequency was recorded.

The response counts were sigmoidal in response to reward frequency, showing 0 or near 0 counts at low frequencies, and climbed upward to plateau at higher frequencies. The threshold, defined as the lowest frequency for which a mouse would theoretically respond, was identified using a non-linear regression fit to a logistic model. The threshold for stimulation was determined by drawing a tangent line from the inflexion point of the sigmoidal curve back to the x-axis. The baseline ICSS threshold was determined as the mean response counts for each trial in passes 2, 3, and 4 during the last 5 days of baseline training prior to diabetes induction. Daily threshold values (derived from passes 2, 3, 4) were then expressed as a percent of the baseline for each individual animal and were normalized to each animal's individual baseline threshold. Each mouse received one ICSS session per day

(from Monday to Friday) for Weeks 1–4 of the ICSS study. The weekly percent of baseline threshold means were then determined for each animal and used for data analysis.

**2.6. 5-Bromo-2'-deoxyuridine administration and procedures**—To measure the effect of diabetes and insulin treatment on hippocampal cell proliferation, all mice in the hippocampal cell proliferation study were injected with 5-bromo-2'-deoxyuridine (BrdU) (100 mg/kg, i.p.; Roche Applied Sciences, Indianapolis, IN), dissolved in 0.9% saline, for 4 consecutive days prior to sacrifice. These mice were euthanized 24 hours after the last BrdU injection. Flow cytometry was used to quickly and objectively quantify the number of positively labeled BrdU cells using a procedure that was described and validated previously [22]. In brief, mice were decapitated and hippocampal lobes were dissected on ice. The samples underwent mechanical trituration to make a single-cell suspension and were spun at  $300 \times g$  for 5 min in a centrifuge. The supernatant was removed and the resultant cells were stained using the fluorescence in isothiocyanate (FITC) BrdU Flow Kit (BD Biosciences, San Jose, CA). To visualize the cells, they were also labeled with the nuclear marker 7-AAD and analyzed on a BD FACS Canto system at the University of Pennsylvania Flow Cytometry Core Facility using BD FACSDiva software. Background signal was controlled by using stained tissue of animals that did not receive BrdU injections.

### 2.7. Brain derived neurotrophic factor (BDNF) protein quantification

The frontal cortex and contralateral hippocampus (that was not being used for flow cytometry analysis), of mice in the hippocampal cell proliferation study, were dissected and analyzed for BDNF levels. After sacrifice and dissection, the hippocampus was flash-frozen with isopentane and stored in  $-80^{\circ}\text{C}$  until analysis. Tissue homogenates were centrifuged at  $14,000 \times g$  for 30 minutes at  $4^{\circ}\text{C}$ , the supernatant was removed and analyzed for BDNF protein levels using a commercially available sandwich enzyme-linked immunosorbent assay kit (Promega, Madison, WI). Samples were analyzed in duplicate and BDNF levels were normalized to wet tissue weight.

### 2.8. Corticosterone (CORT) measurements

Blood was collected from the trunk after decapitation, between 0800 – 1000, and 0.5 ml heparin was added to each sample to prevent coagulation. Specimens were centrifuged at  $14,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . Plasma was separated and stored at  $-20^{\circ}\text{C}$  until analysis. CORT levels were quantified using a commercially available enzyme immunoassay kit (Immunodiagnostic Systems Inc., Scottsdale, AZ). Each sample was analyzed in duplicate according to manufacturer's instructions. The mean intra-assay coefficient of variability was 7%.

### 2.9. Data analysis

Data were analyzed using Prism GraphPad, Version 5.0b (La Jolla, CA). One-way analysis of variance (ANOVA) was used to evaluate time spent in open arms of the EZM, time spent immobile in the TST, distance traveled in the locomotor activity test, BrdU incorporation for proliferation of hippocampal cells, BDNF protein levels, and plasma CORT levels. The Newman-Keuls multiple comparison post hoc test was used to compare the means of the STZ-Veh and STZ-Ins groups to the Veh-Veh control group.

The ICSS performance was analyzed using repeated measures ANOVA from Weeks 1, 3 and 4. Effects of induction of diabetes and subsequent insulin treatment were tested within subjects and diabetic mice were also compared to control mice that did not receive STZ. Since not all STZ mice were treated the same during Week 2 of the ICSS study, the data collected during Week 2 were not included in the analysis. For the analysis of STZ and insulin effects, we used data collected during Weeks 3 and 4 where all mice were treated

exactly the same. These data were then analyzed with two-way ANOVAs (diabetes X insulin treatment) and Bonferroni post hoc tests. For all results,  $p < 0.05$  was considered statistically significant.

### 3. Results

#### 3.1. Effects of diabetes and insulin treatment on behavior

**3.1.1. Elevated zero maze**—The EZM was used to evaluate anxiety. STZ-induced diabetes, with and without insulin treatment, had no effect on the time mice spent in the open arms of the EZM (mean  $\pm$  SEM; Veh-Veh:  $66 \text{ s} \pm 7$ ; STZ-Veh:  $70 \text{ s} \pm 11$ ; STZ-Ins:  $80 \text{ s} \pm 10$ ;  $F(2, 24) = 1.404$ ,  $p = 0.557$ ). Additionally, there was no difference in number of entries into the open arms detected between groups (mean  $\pm$  SEM; Veh-Veh:  $24 \pm 1$ ; STZ-Veh:  $26 \pm 3$ ; STZ-Ins:  $30 \pm 3$ ;  $F(2, 24) = 1.358$ ,  $p = 0.278$ )

**3.1.2. Tail suspension test**—The TST was used to assess vulnerability to stress-induced depressive behavior (see figure 1a). STZ-induced diabetic mice had a 30% increase in TST immobility compared to controls ( $F(2, 23) = 6.949$ ,  $p < 0.01$ ). In contrast, immobility values of insulin-treated mice did not differ from controls. Locomotor activity in the same mice was also assessed to determine if changes in TST immobility was associated with reduced locomotor activity (see figure 1b). Untreated diabetic mice had significantly decreased locomotor activity compared to controls ( $F(2, 25) = 7.534$ ,  $p < 0.01$ ). However, insulin-treated mice did not show reversal of the reduction of locomotor activity ( $p < 0.01$ ), even though TST immobility was reversed in this group ( $p > 0.05$ ).

**3.1.3. Intracranial self-stimulation**—ICSS performance, used to examine reward sensitivity, was measured repeatedly in the same mouse during the development of diabetes (Weeks 1 and 3) and after insulin treatment (Week 4).

**3.1.3.1 Glucose levels for the intracranial self-stimulation study:** An overall ANOVA indicated significant effects on glucose between Groups ( $F_{1,24} = 30.96$ ,  $p < 0.001$ ), Weeks ( $F_{3,24} = 10.56$ ,  $p < 0.001$ ), and a significant interaction between Groups over Weeks ( $F_{3,24} = 10.88$ ,  $p < 0.001$ ). During Week 1, STZ-treated mice began to show hyperglycemia ( $t = 5.488$ ,  $p < 0.001$ , figure 2a) as 4 out of 6 mice developed diabetes. By Week 3, all mice had developed diabetes and demonstrated hyperglycemia ( $t = 6.063$ ,  $p < 0.001$ , figure 2a). Glucose levels of STZ-diabetic mice were reduced to normal levels during Week 4 when they were treated with long-acting insulin glargine ( $t = 0.639$ ,  $p > 0.05$ , figure 2a).

**3.1.3.2 Behavioral results for the intracranial self-stimulation study:** There were no significant changes in mean ICSS maximal response rates (see figure 2b) in STZ-treated mice compared to vehicle-treated mice, according to overall ANOVA ( $p > 0.05$  for main effects and interaction).

An overall ANOVA indicated significant effects on ICSS threshold between Weeks ( $F_{3,24} = 5.86$ ,  $p < 0.01$ ), no main effect between Groups and a significant interaction between Groups over Weeks ( $F_{3,24} = 6.82$ ,  $p < 0.01$ ). Mean ICSS thresholds did not differ between Groups during Week 1 ( $t = 0.599$ ,  $p > 0.05$ ). However, STZ-induced diabetes significantly increased the mean ICSS threshold during Week 3 compared to the control group ( $t = 3.688$ ,  $p < 0.01$ ). Mean ICSS thresholds returned to levels similar to that of the control, vehicle-treated group during Week 4, when STZ-diabetic mice were treated with insulin ( $t = 0.236$ ,  $p > 0.05$ ). These alterations in ICSS threshold levels during diabetes and insulin treatment were evident in both the grouped mean data (see figure 2c) and in data from a representative individual mouse (see figure 3).

### 3.2. Effects of STZ-induced diabetes on glucose levels and body weight for the hippocampal cell proliferation study

Diabetic mice maintained a 3.5-fold elevation in non-fasting glucose levels, when compared to controls ( $F(2, 25)= 254.4, p < 0.001$ , see table 1). Treatment with insulin significantly lowered glucose levels ( $p < 0.001$ ), although glucose remained significantly higher than controls ( $p < 0.001$ ). Diabetic mice decreased body weight compared to controls ( $F(2, 25)= 30.23, p < 0.001$ , see table 1), but the body weight of mice treated with insulin did not differ from controls.

### 3.3. Insulin treatment normalized hippocampal cell proliferation

STZ-induced diabetes significantly reduced the number of positively labeled BrdU cells incorporated into the hippocampus by more than 25% ( $F(2, 25)= 4.185, p < 0.05$ , see figure 4). This reduction in hippocampal cell proliferation was restored in the insulin-treated group to levels comparable to the control group. Despite difference in glucose levels between the insulin treated group and the control group, levels of hippocampal cell proliferation between these two groups did not differ.

### 3.4. Effects of diabetes and insulin treatment on brain derived neurotrophic factor levels

BDNF levels were measured in two brain regions: frontal cortex and hippocampus. STZ-induced diabetes decreased frontal cortex BDNF levels by 30% ( $F(2, 24)= 7.292, p < 0.01$ , see figure 5a) but this decrease was not restored by treatment with insulin. In the hippocampus, there was no effect of diabetes on BDNF levels. However, insulin treatment decreased hippocampal BDNF levels by 20% ( $F(2, 25)= 4.525, p < 0.05$ , see figure 5b).

### 3.5. Effects of diabetes and insulin treatment on corticosterone levels

Plasma levels of CORT were measured at the completion of the hippocampal cell proliferation study (see figure 6). CORT was significantly increased in the STZ-induced diabetic group compared to the control group ( $F(2, 20)= 5.698, p < 0.05$  and  $F(2, 27)= 9.723, p < 0.001$ ). Insulin treatment completely reversed the diabetes-induced increase in CORT levels.

## 4. Discussion

The current study provides supporting evidence in an animal model that experimental type 1-like diabetes produces a number of depressive phenotypes such as increased immobility in the TST, decreased hedonic state as measured by ICSS, and reduced hippocampal cell proliferation. Furthermore, these phenotypes were normalized by treatment with insulin. Reductions of reward-related behaviors and hippocampal cell proliferation may be markers for identifying pathophysiological mechanisms of depression associated with diabetes and markers for successful intervention. Since STZ-induced diabetes models type 1 diabetes, additional experiments are necessary to determine whether the results from this study might generalize to animal models for other types of diabetes.

The TST has mostly been used to measure the acute effects of antidepressant drugs [23], as shown previously under these testing procedures. However, increased baseline TST immobility after exposure to infection, hormones, or stress may reflect induction of depressive-like behavior because these conditions also cause depression in humans [7, 24, 25]. Similar to previous studies [7–9], mice in the current study developed increased TST immobility after the onset of diabetes suggesting that diabetes increased depressive-like behavior. The present study further showed that insulin treatment reversed the augmentation of TST immobility. Diabetes also decreased locomotor activity, which could indicate that increased TST immobility resulted from nonspecific effects of diabetes on motor behavior.

However, insulin treatment also decreased locomotor activity yet still restored TST behavior indicating that the changes in these measures produced by diabetes were independent. The general literature also shows that the effects of antidepressant drug testing on TST performance is independent of locomotor activity [23].

The ICSS testing paradigm measures affective behavior related to the motivation for obtaining reward. ICSS can be used as a measure of anhedonia, or diminished capacity to experience pleasure or reward, which is a cardinal symptom of major depressive disorder [26]. This is the first study to examine the effects of diabetes on ICSS performance in mice. By 3 weeks post STZ injection, diabetes resulted in shifting the ICSS curve to the right. This indicated the development of a decreased hedonic state, as the mouse no longer responded to stimulation frequencies that were previously reinforcing. One week of insulin treatment reduced hyperglycemia and shifted the curve back to baseline ICSS thresholds. The changes in reward sensitivity (i.e. during Week 3 of untreated diabetes and back to baseline during Week 4 with insulin treatment) without altering mean maximal response rate values suggest that rightward shifts in ICSS threshold are likely due to the effects of diabetes on hedonic state rather than on task performance.

The effects of diabetes were also measured on hippocampal cell proliferation and BDNF levels, two measures of hippocampal cellular plasticity. A number of rodent models have shown that experimental diabetes leads to decreased hippocampal cell proliferation and neurogenesis [13, 16, 17]. Hippocampal neurogenesis has been associated with the anhedonic effects of stress in recent studies [27]. Because hippocampal neurogenesis is also reduced in rodents by exposure to stress and models of depression and is reversed by antidepressant treatments [28], this process and related deficits of neuroplasticity could mediate the interface between diabetes and affective behavior. In the present study, exposure to insulin reduced hyperglycemia and increased hippocampal cell proliferation back to control values in STZ-treated mice. This important finding suggests that reversing the deficits in hippocampal cell proliferation could prevent some of the progressive complications in diabetes. However, additional studies showing that increases in cell proliferation/neurogenesis can restore ICSS performance or another behavioral measure of depression independent of restoring plasma glucose and corticosterone levels are needed to test this hypothesis.

Changes in hippocampal cell proliferation in diabetes, and its reversal by insulin, could have involved changes in BDNF levels because BDNF levels have been associated with diabetes, insulin and stress. Previous studies in rats have reported that STZ lowers BDNF levels [29–31] and BDNF gene expression was decreased in the hippocampus of the nonobese diabetic mouse [32]. Exogenous BDNF augmented the hypoglycemic effects of insulin in STZ-treated mice, although the treatment was ineffective when given alone [33]. Stress decreases hippocampal and cortical BDNF levels and gene expression and lowers neurogenesis, while antidepressant medications appear to increase neurogenesis by increasing BDNF levels [28]. In the present study, BDNF levels were reduced by STZ-induced diabetes in the frontal cortex, but not the hippocampus. Although insulin treatment restored hippocampal cell proliferation, this effect was not due to increases in BDNF levels in the cortex and hippocampus. Thus, endogenous BDNF may contribute to the pathology of diabetes, based on the correlation between diabetes and lower BDNF levels, but the restoration of hippocampal cell proliferation in diabetes by insulin treatment may involve other mechanisms.

Plasma CORT levels, on the other hand, increase with diabetes and likely contribute to the diabetes-associated decrease in hippocampal cell proliferation and neurogenesis [16]. Results from the current study provide corroborating evidence for the involvement of CORT



in regulating hippocampal cellular plasticity in diabetes. Treatment of diabetic mice with insulin reduced plasma CORT levels and produced a corresponding restoration of hippocampal cell proliferation. The involvement of CORT acting directly at glucocorticoid receptors provides the best explanation for how insulin restored hippocampal cell proliferation in diabetes [16], although other mechanisms associated with stress and may yet be shown to be involved in regulating hippocampal neurogenesis in diabetes.

Although anxiety is a common problem in depression, STZ-induced diabetes evoked depressive more than anxious phenotypes in C57BL/6 mice. Anxiety tests that rely on hunger and satiety signals, like the novelty induced hypophagia test or punished operant responding, would not be suitable for measuring anxiety in diabetic animals since diabetes increases appetite. Hyperphagia in diabetes can make it difficult to interpret the data from these tests as representing changes only in emotional behavior (data not shown). Thus, we used the EZM, which does not depend on hunger and satiety signals, to examine the effects of diabetes on anxiety. Results from the EZM indicated that experimental diabetes in C57BL/6 mice did not affect anxiety. These results do not corroborate with results from previous studies where STZ-induced diabetes led to anxiety-like behavior [34, 35]. Differences in results may be due to differences in species used, mouse versus rat, and choice of test used, EZM versus elevated plus maze or open field test, to measure anxiety. Future studies need to determine if certain tests of anxiety are more sensitive or appropriate for measuring anxiety in diabetic mice.

## 5. Conclusions

Diabetes likely increases risk for developing depressive behavior, however, the specific pathophysiological mechanisms underlying this relationship remain unclear [36]. Diabetic patients with comorbid depression have significantly increased risks for complications compared to their non-depressed counterparts [37]. Thus, treatment of depression in diabetes would not only lead to improved mood in patients but likely minimize complications and decrease healthcare expenses associated with maintenance of the disease. This study demonstrated that STZ-induced diabetes produced a number of depressive phenotypes, increased immobility in the TST, increased intracranial self-stimulation thresholds, decreased hippocampal cell proliferation, and increased corticosterone levels, that were reversed by exposure to insulin. The results of the current study apply to type 1 diabetes, but future studies will evaluate animal models for other types of diabetes. Nevertheless, further understanding of the neurobiological processes underlying the relationship between diabetes and depression may be possible through animal models and would provide targeted and more effective treatment of depression that is specific to the diabetic population.

### Highlights

- STZ-induced diabetes increased tail suspension test immobility, increased intracranial self-stimulation thresholds, decreased hippocampal cell proliferation, and increased corticosterone levels.
- Insulin treatment reduced hyperglycemia, reversed behavioral effects, and normalized hippocampal cell proliferation and corticosterone.
- Changes in reward-related behaviors and hippocampal cell proliferation may be useful markers to identify therapeutic interventions for comorbid diabetes and depression.

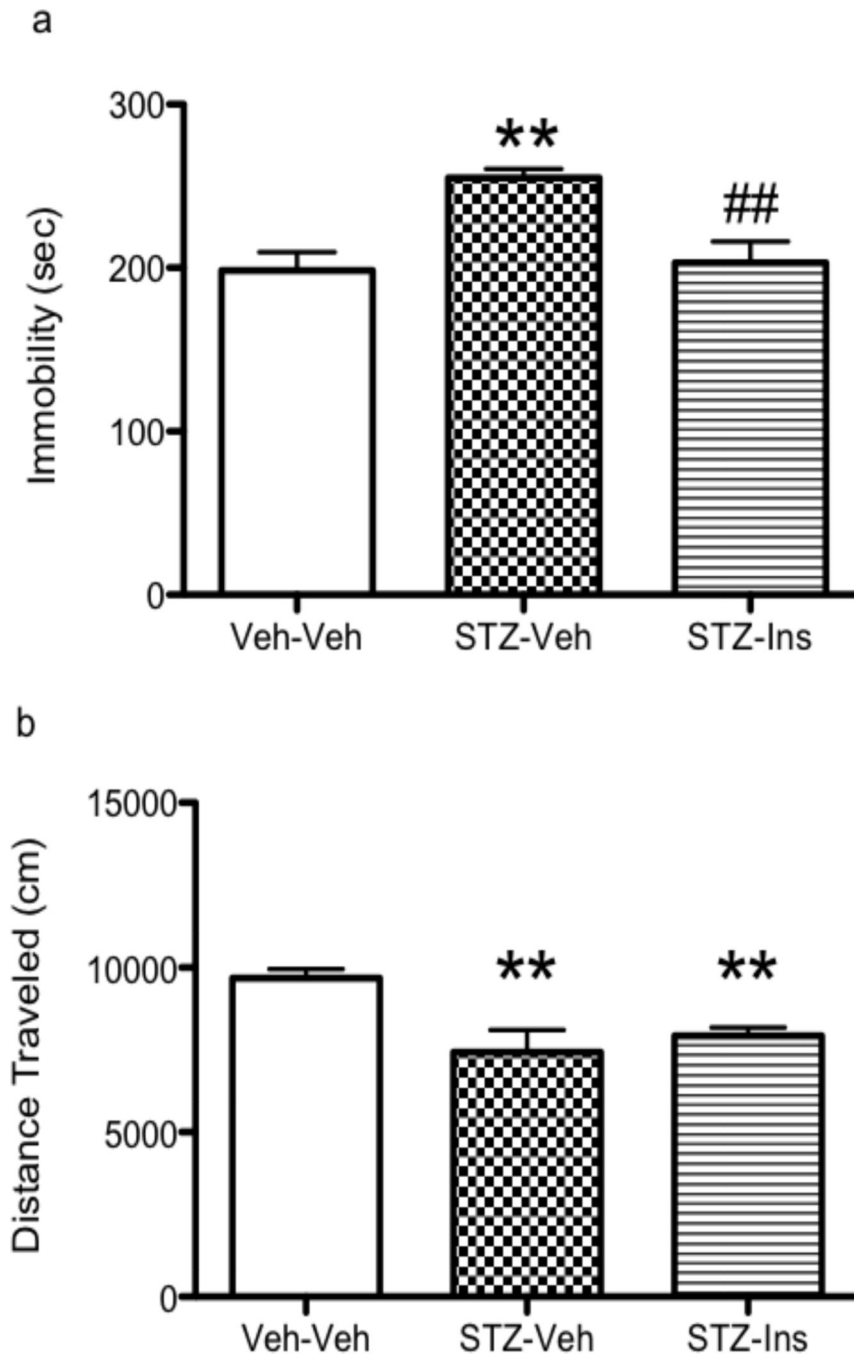
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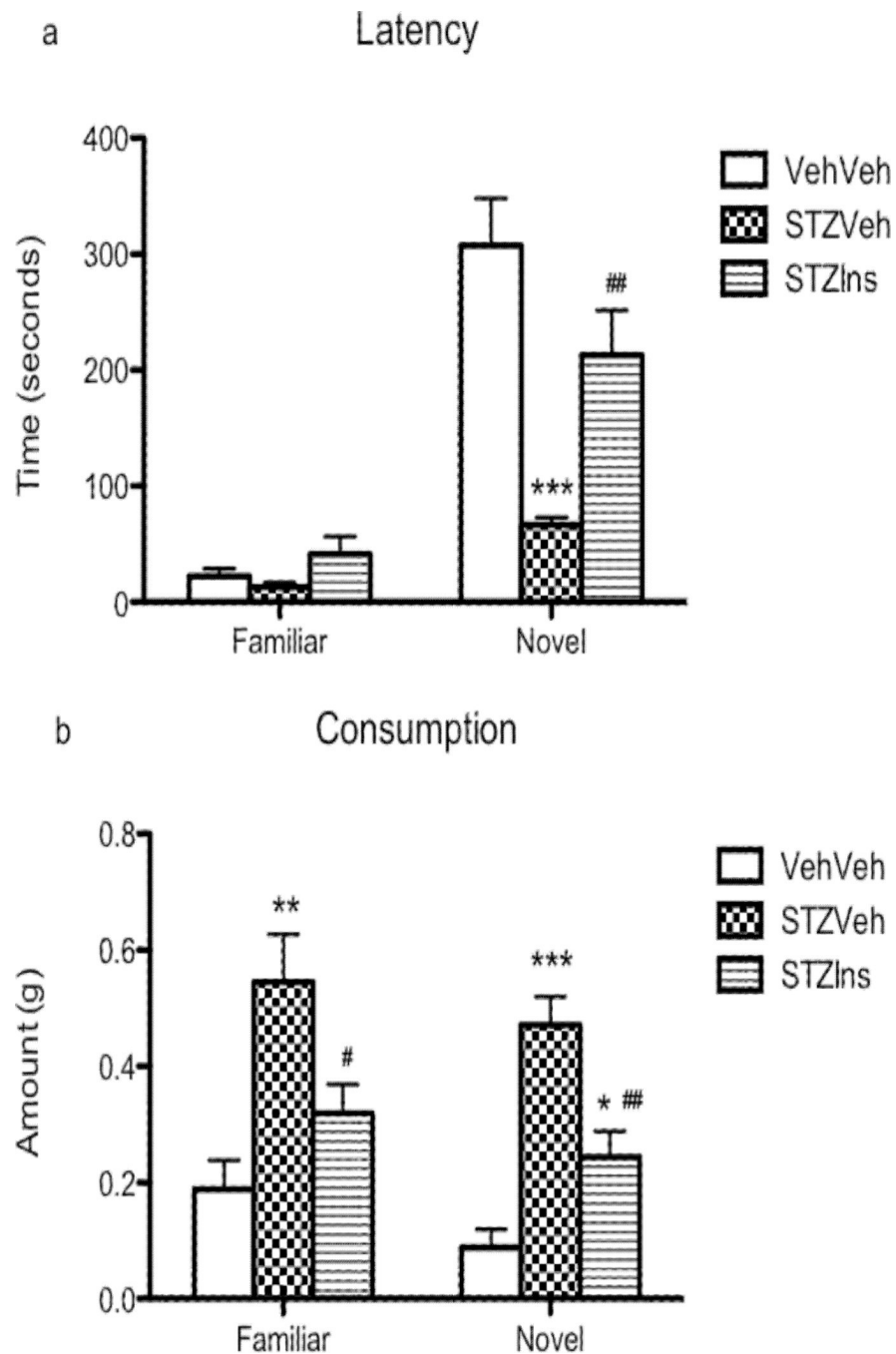
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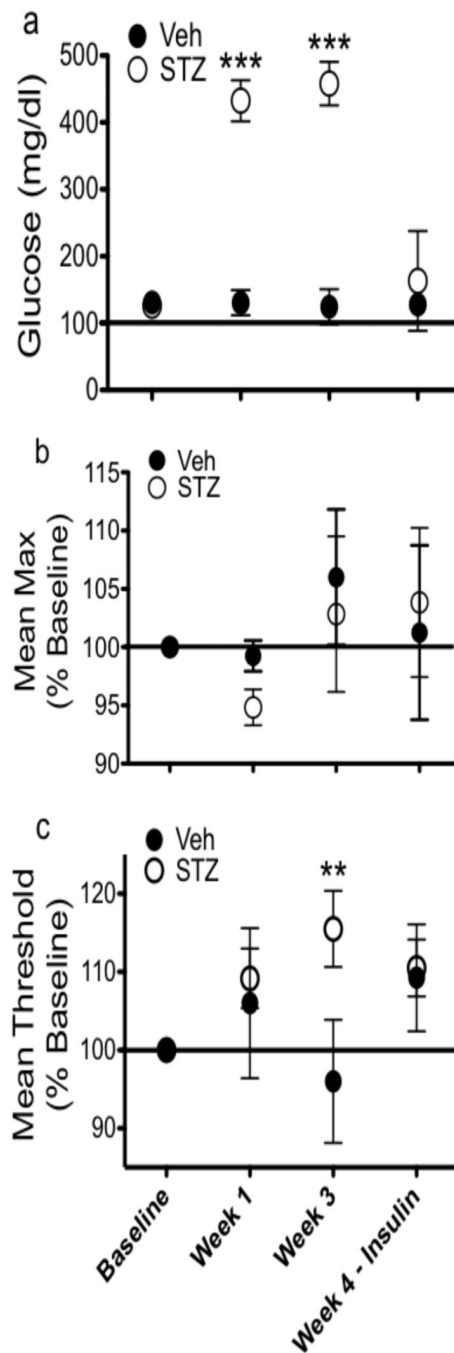
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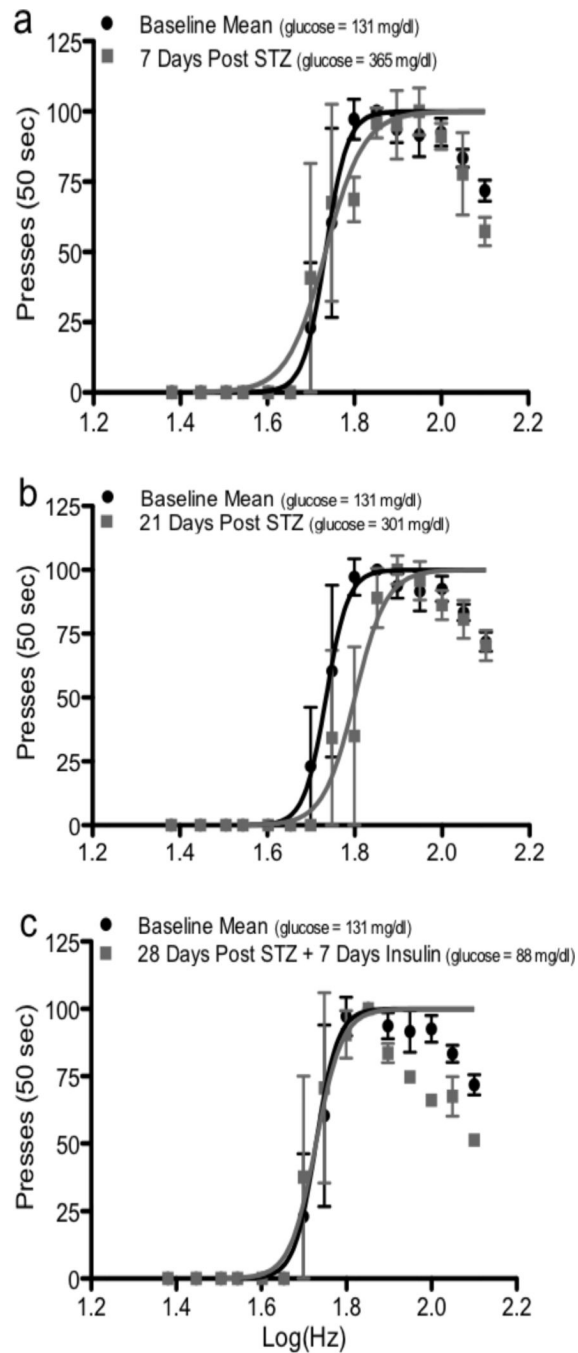
**Fig. 1.** Effect of diabetes and insulin treatment on behavior in the TST (a) and locomotor activity (b). (a) TST immobility was significantly increased in diabetic mice (STZ-Veh) compared to controls, while the insulin treatment (STZ-Ins) completely reversed the TST changes induced by diabetes. (b) Both diabetic and insulin-treated groups showed significantly decreased locomotor activity compared to controls. Symbols represent mean values  $\pm$  S.E.M. Asterisks (\*\*) denote significant difference compared to Veh-Veh ( $p < 0.01$ ). Number signs (##) denote significant difference compared to STZ-Veh ( $p < 0.01$ ).



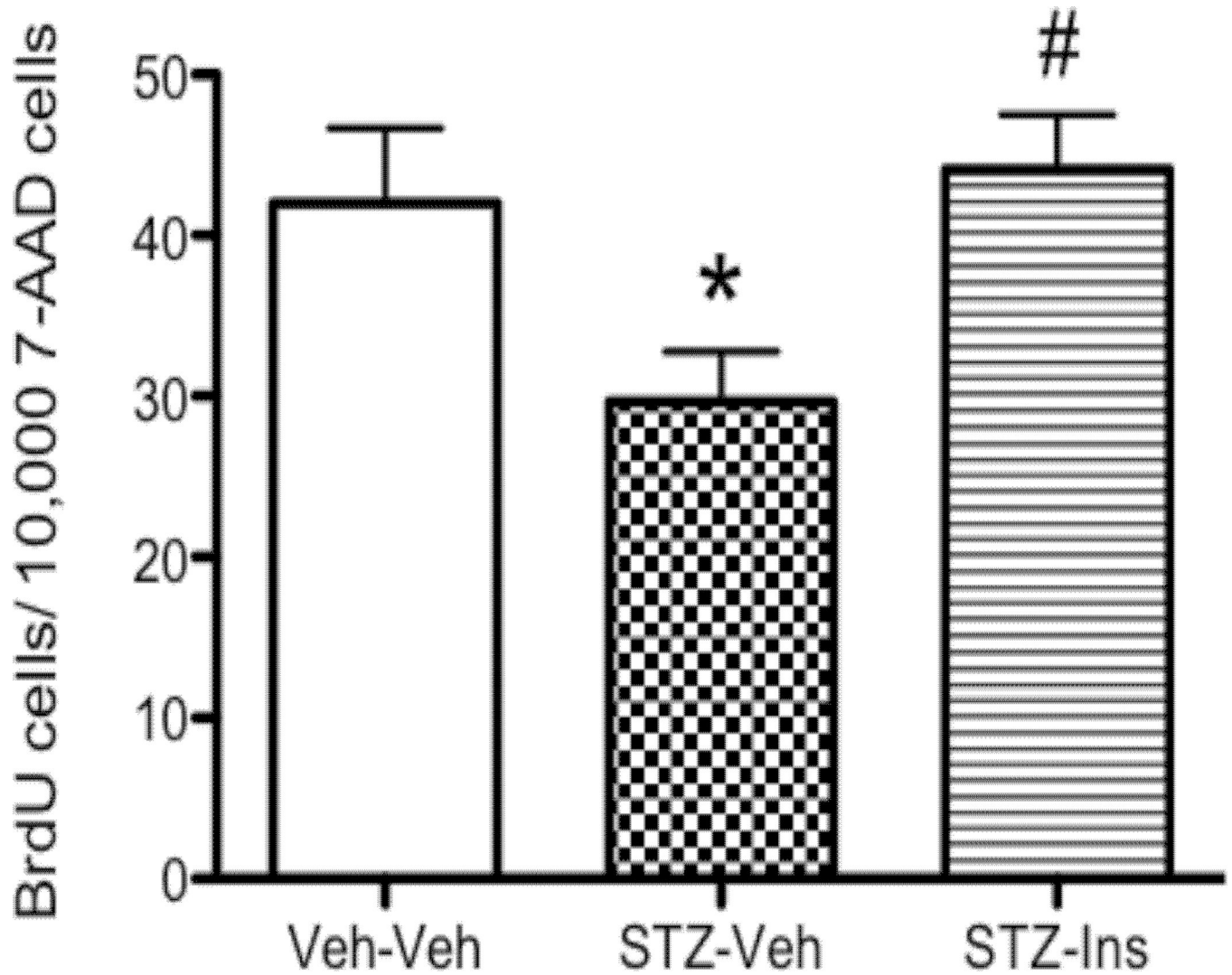
**Fig. 2.** Effect of diabetes and insulin treatment on ICSS performance. Graphs show data organized by treatment, vehicle (Veh,  $n = 4$ ) and streptozotocin (STZ,  $n = 6$ ). Baseline values were determined during the week prior to STZ treatment. Weeks 1 and 3 correspond with untreated diabetes. During Week 4, STZ animals were treated with glargine, 100 mg/kg, s.q., once daily, while vehicle animals received saline. (a) Blood glucose levels (mg/dl) were measured at the end of each week. (b) Weekly mean maximum response values are expressed as a percent of the baseline. (c) Weekly mean thresholds are expressed as a percent of the baseline. Symbols represent mean values  $\pm$  S.E.M. Asterisk (\*) denotes significant difference compared to vehicle (\*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ).



**Fig. 3.** Longitudinal ICSS data from a single, representative STZ-diabetic mouse. Graphs show response values (presses per 50 seconds) as a function of stimulation frequencies for (a) during the development of diabetes at the end of Week 1, 7 days post STZ injection, (b) during the expression of diabetes at the end of Week 3, 21 days post STZ injection, and (c) during treatment for diabetes with glargine, 100 mg/kg, s.c. at the end of Week 4, the 7<sup>th</sup> day of consecutive glargine administration, and 28 days post STZ injection. Symbols represent mean response counts for passes 2, 3, and 4  $\pm$  S.E.M.

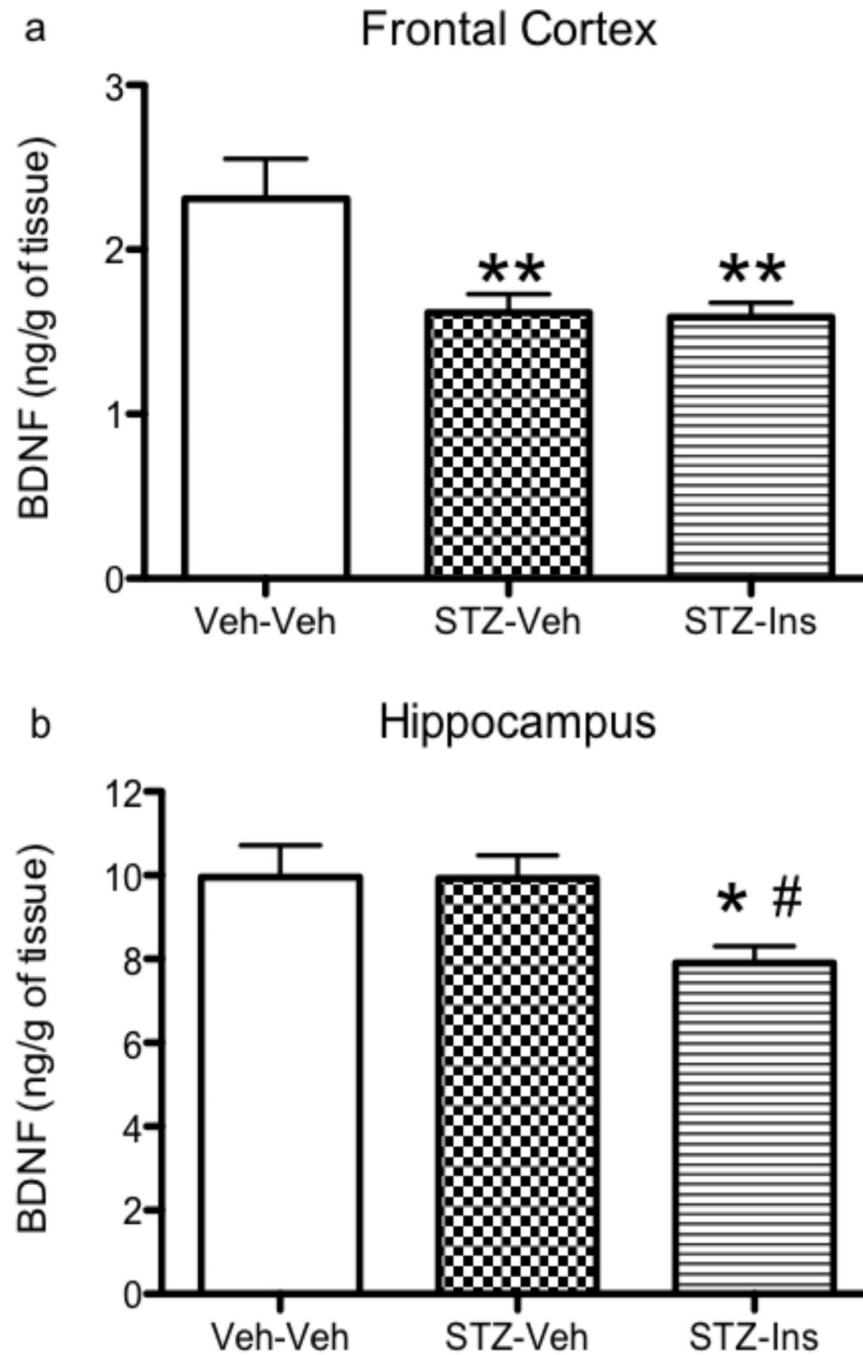


**Fig. 4.** Effect of diabetes and insulin treatment on hippocampal cell proliferation. To study cell proliferation ( $n = 8-10$  per group), BrdU ( $100 \text{ mg/kg} \times 4$ ) was injected 24 hours prior to sacrifice in mice 6 weeks after administration of STZ. Insulin treatment was administered by implanted s.c. pellet during the last 5 weeks of the study. Symbols represent mean values  $\pm$  S.E.M. Asterisk (\*) denotes significant difference compared to Veh-Veh ( $p < 0.05$ ). Number sign (#) indicates significant difference compared to STZ-Veh ( $p < 0.05$ ).



**Fig. 5.** Effect of diabetes and insulin treatment on hippocampal and cortical BDNF levels. The contralateral hippocampus that was not being used for flow cytometry measurement in the hippocampal cell proliferation study was used for BDNF analysis. Insulin pellets were implanted s.c. 1 week after STZ administration. Insulin treatment lasted for 5 weeks and frontal cortex (a) and hippocampal (b) BDNF levels were measured at study completion. Symbols represent mean values  $\pm$  S.E.M. Asterisk (\*) denotes significant difference compared to Veh-Veh (\*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ). Number sign (#) indicates significant difference compared to STZ-Veh ( $p < 0.05$ ).





**Fig. 6.** Effect of diabetes and insulin treatment on plasma CORT levels. CORT levels were measured in mice in the hippocampal cell proliferation study. Insulin pellets were implanted s.c. 1 week after STZ administration. Insulin treatment lasted for 5 weeks and CORT levels were measured at study completion. Symbols represent mean values  $\pm$  S.E.M. Asterisk (\*) denotes significant difference compared to Veh-Veh ( $p < 0.05$ ). Number sign (#) indicates significant difference compared to STZ-Veh ( $p < 0.05$ ).

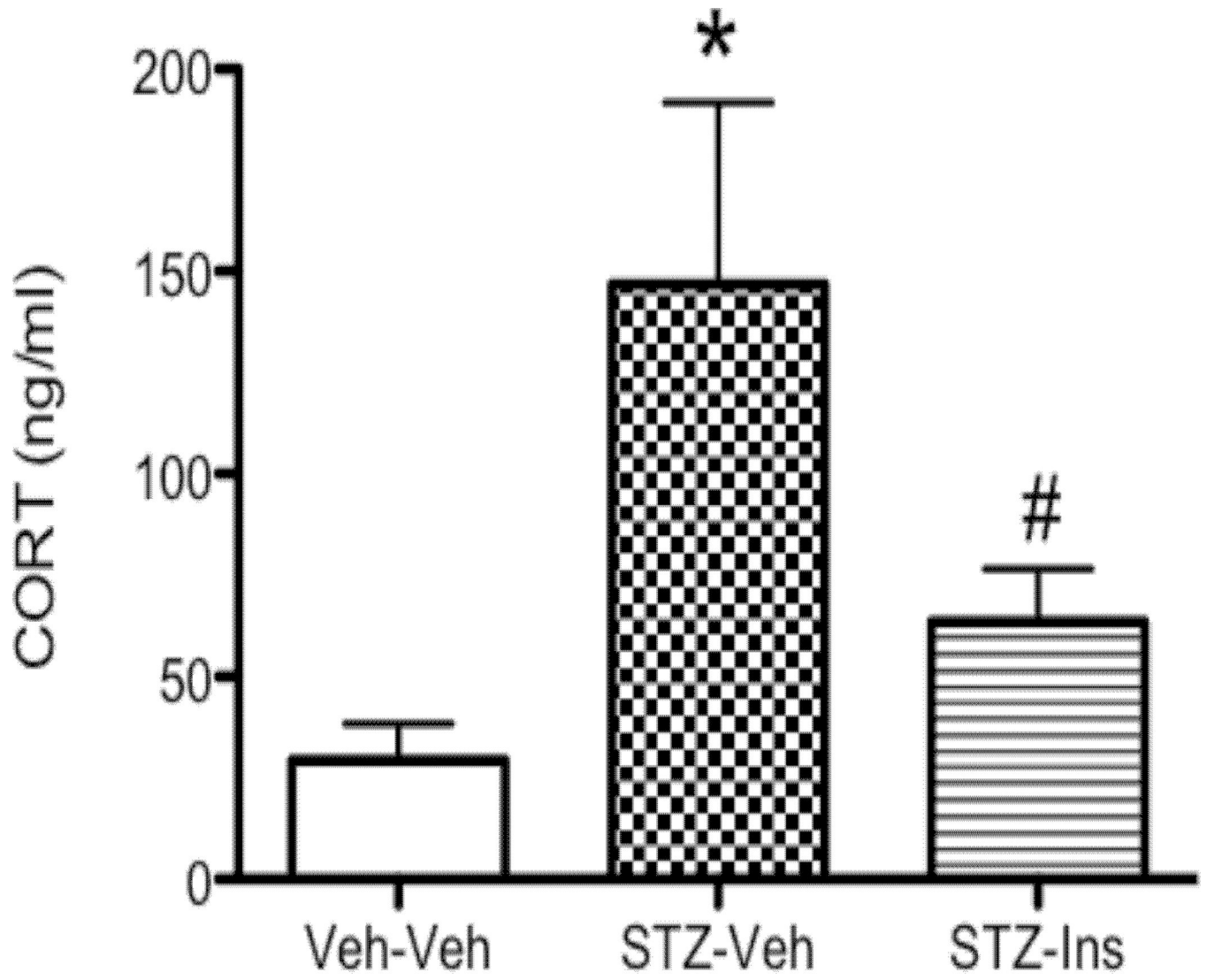


Fig. 7.

**Table 1**

Blood glucose levels and body weights for cell proliferation experiment.

	n	Glucose (mg/dl)	$\Delta$ Weight (g)
Veh/Veh	8	127 $\pm$ 4	2.14 $\pm$ 0.60
STZ/Veh	8	444 $\pm$ 8 <sup>***</sup>	-2.88 $\pm$ 0.30 <sup>***</sup>
STZ/Ins	10	181 $\pm$ 13 <sup>***</sup>	1.70 $\pm$ 0.54

Blood glucose levels (mg/dl) were measured weekly, with the last measurement taken on the day of sacrifice. Numbers expressed as mean  $\pm$  SEM.  $\Delta$  Weight is the change in weight from the beginning of the experiment, prior to STZ or saline injection, to the end of the experiment in grams (g).

Asterisks (\*\*\*) denote significant differences compared to Veh-Veh ( $p < 0.001$ ).