nature neuroscience

Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons

Alexis M Stranahan[1,2], Thiruma V Arumugam[2,4], Roy G Cutler[2], Kim Lee[2], Josephine M Egan[3] & Mark P Mattson[2]

Many organ systems are adversely affected by diabetes, including the brain, which undergoes changes that may increase the risk of cognitive decline. Although diabetes influences the hypothalamic-pituitary-adrenal axis, the role of this neuroendocrine system in diabetes-induced cognitive dysfunction remains unexplored. Here we demonstrate that, in both insulin-deficient rats and insulin-resistant mice, diabetes impairs hippocampus-dependent memory, perforant path synaptic plasticity and adult neurogenesis, and the adrenal steroid corticosterone contributes to these adverse effects. Rats treated with streptozocin have reduced insulin and show hyperglycemia, increased corticosterone, and impairments in hippocampal neurogenesis, synaptic plasticity and learning. Similar deficits are observed in *db/db* mice, which are characterized by insulin resistance, elevated corticosterone and obesity. Changes in hippocampal plasticity and function in both models are reversed when normal physiological levels of corticosterone are maintained, suggesting that cognitive impairment in diabetes may result from glucocorticoid-mediated deficits in neurogenesis and synaptic plasticity.

As a result of high-calorie diets and sedentary lifestyles, diabetes is rapidly becoming more prevalent in Western societies[1]. In addition to its well known adverse effects on the cardiovascular and peripheral nervous systems, diabetes also appears to negatively affect the brain, increasing the risk of depression and dementia[2,3]. Human subjects with either type 1 (caused by insulin deficiency) or type 2 (mediated by insulin resistance) diabetes typically show impaired cognitive function compared to age-matched nondiabetic subjects[3,4]. Cognitive deficits have also been documented in studies of rodent models of diabetes. For example, rats rendered diabetic by treatment with the pancreatic β-cell toxin streptozocin (STZ) a model of type 1 diabetes, show impaired performance in tests of spatial learning ability[5,6]. Similar deficits have been reported in the *db/db* mouse[7], a model of type 2 diabetes in which obesity, hyperglycemia and elevations in circulating corticosterone arise from a mutation that inactivates the leptin receptor[8]. However, the mechanism(s) responsible for cognitive dysfunction in diabetes has not been established.

Within the hippocampus, changes in the strength of synapses between groups of neurons are critical in certain types of learning and memory. At the level of the dentate gyrus, regulation of synaptic connectivity extends beyond changes in the number and strength of synapses to the *de novo* addition of new neurons in adulthood[9]. Data from studies of animal models suggest impairment of both synaptic plasticity and adult neurogenesis in diabetes. Long-term potentiation (LTP) of synaptic transmission, believed to be a cellular mechanism of learning and memory, is impaired in the dentate gyrus of rats with streptozocin-induced diabetes[10]. Diabetic rodents also show lower rates of adult neurogenesis[11], whereas exercise and dietary energy restriction, which have antidiabetic effects, can enhance synaptic plasticity[12,13] and neurogenesis[12,14,15]. Because cognitive ability is impaired in subjects with either type 1 or type 2 diabetes and in animal models of both types of diabetes, it is unlikely that global changes in insulin concentrations are directly responsible for impaired neuronal plasticity.

Humans with poorly controlled diabetes show hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in elevated circulating cortisol[2,4]. Similarly, adrenal glucocorticoids are elevated in rodents with experimental diabetes[16–20]. The specific mechanism by which diabetes results in hyperactivation of the HPA axis is unknown, but it is apparently not the result of the hyperglycemia *per se*[17]. Although it is not known whether glucocorticoids are involved in cognitive dysfunction in diabetes, elevated cortisol has been associated with poor cognitive ability in humans subjected to psychosocial stress[21], during normal aging[22] and in Alzheimer's disease[23]. Studies of rodents have provided evidence that elevated adrenal glucocorticoids mediate deficits in cognitive function caused by chronic stress[24,25]. In addition, chronic stress and high corticosterone can impair synaptic

[1]Psychology Department, Princeton University, Green Hall Washington Road, Princeton, New Jersey 08544, USA. [2]Laboratory of Neurosciences, Cellular and Molecular Neurosciences Section and [3]Laboratory of Clinical Investigation, Diabetes Section, National Institute on Aging Intramural Research Program, 5600 Nathan Shock Drive, Baltimore, Maryland 21224, USA. [4]Present address: Department of Pharmaceutical Sciences, School of Pharmacy, Texas Tech University Health Sciences Center, Amarillo, Texas 79430, USA. Correspondence should be addressed to M.P.M. (mattsonm@grc.nia.nih.gov).

Received 13 December 2007; accepted 24 January 2008; published online 17 February 2008; doi:10.1038/nn2055

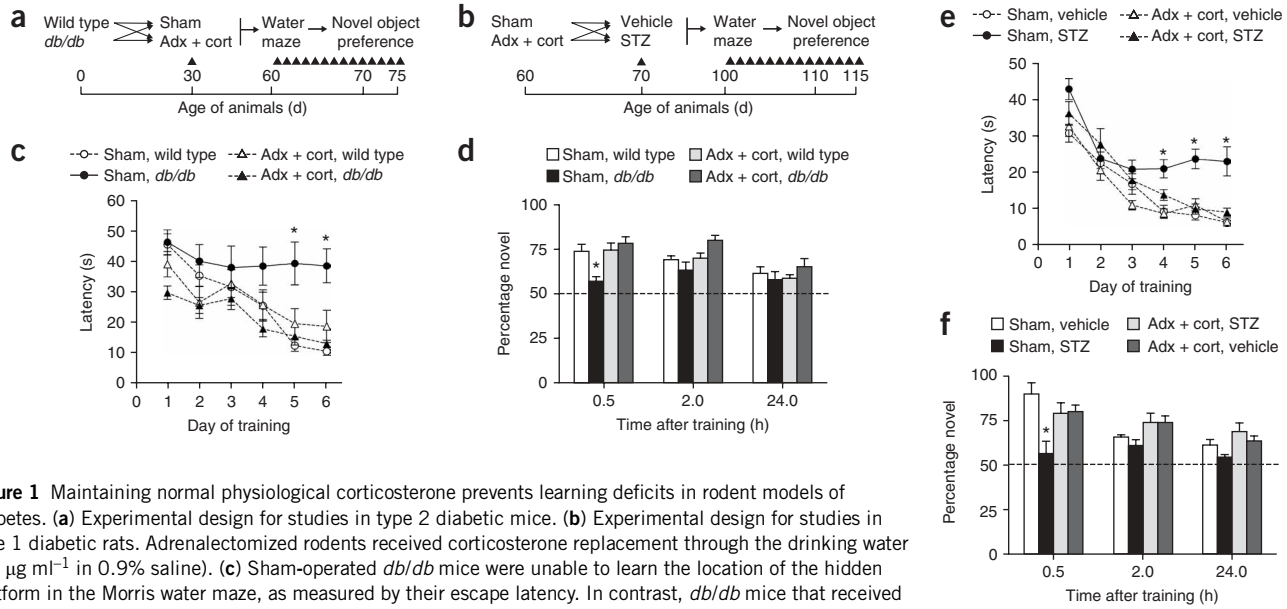

Figure 1 Maintaining normal physiological corticosterone prevents learning deficits in rodent models of diabetes. (**a**) Experimental design for studies in type 2 diabetic mice. (**b**) Experimental design for studies in type 1 diabetic rats. Adrenalectomized rodents received corticosterone replacement through the drinking water (25 μg ml^{-1} in 0.9% saline). (**c**) Sham-operated *db*/*db* mice were unable to learn the location of the hidden platform in the Morris water maze, as measured by their escape latency. In contrast, *db*/*db* mice that received adrenalectomy and corticosterone replacement learned the location of the platform as effectively as nondiabetic mice. Shorter latencies on the first day of training in adrenalectomized *db*/*db* mice were attributable to performance on successive trials, as escape latencies were similar during the first trial (see Results). (**d**) *db*/*db* mice with intact adrenal glands showed impaired object recognition, while *db*/*db* mice with corticosterone 'clamped' through adrenalectomy and corticosterone replacement showed preference for the novel object that was similar to wild-type controls. (**e**) Learning was impaired in insulin-deficient diabetic rats experiencing elevated corticosterone, but not in diabetic rats that received adrenalectomy and corticosterone replacement. (**f**) Novel object preference was reduced in sham-operated diabetic rats, but preserved in diabetic rats with normal levels of corticosterone. Adx + cort., adrenalectomized with 25 μg ml^{-1} corticosterone replacement. Error bars, s.e.m.

plasticity[26–29]. Moreover, concentrations of corticosterone characteristic of stress inhibit neurogenesis in the hippocampus of adult rats[30], and corticosterone concentrations during the course of aging are correlated with age-related declines in neurogenesis and memory[31]. It is therefore possible that elevated corticosterone in diabetes may mediate central impairment of neuronal structure and function.

Here we provide direct evidence that elevated glucocorticoids contribute to the impairment of synaptic plasticity and neurogenesis, and to associated learning and memory deficits, in rodent models of both insulin-resistant and insulin-deficient diabetes. These findings support a role for HPA axis hyperactivity in diabetes-induced cognitive impairment, and suggest new approaches for improving cognitive function in subjects with diabetes.

RESULTS

Lowering corticosterone reverses learning deficits

To evaluate whether elevated corticosterone is accompanied by alterations in hippocampus-dependent learning in diabetic animals, we tested cognitive function in diabetic and nondiabetic mice and rats that had been adrenalectomized and administered low-dose corticosterone replacement[30] or had been sham-operated (**Fig. 1a**,**b**). Adrenalectomized rodents received corticosterone replacement through the drinking water (25 mg ml^{-1} in 0.9% saline). This intervention has previously been used to both lower and normalize corticosterone after stress[32]. First we evaluated performance in the hippocampus-dependent version of the water maze task. In both *db*/*db* mice and STZ-treated rats, sham-operated diabetic animals had longer escape latencies and took a less direct route to the hidden platform (**Fig. 1**; **Supplementary Fig. 1a**,**b** online). These findings concur with previous reports[5–7]. Both of these deficits were reversed in diabetic mice and rats with normal physiological levels of corticosterone (escape latency in *db*/*db* mice, $F_{1,32} = 4.91$, $P = 0.03$; in STZ-treated rats, $F_{1,46} = 7.19$, $P = 0.01$; path length in *db*/*db* mice, $F_{1,33} = 4.52$, $P = 0.04$; path length in STZ-treated rats, $F_{1,31} = 13.62$, $P = 0.001$). There was no effect of adrenalectomy and corticosterone replacement in nondiabetic mice and rats. Additionally, there were no significant differences in swimming speed across diabetic and nondiabetic mice and rats with different levels of corticosterone (*db*/*db* mice, $F_{1,33} = 1.42$, $P = 0.24$; STZ-treated rats, $F_{1,43} = 0.14$, $P = 0.71$). Although *db*/*db* mice that had received adrenalectomy and corticosterone replacement had shorter escape latencies and path lengths on the first day of training (**Fig. 1c**, **Supplementary Fig. 1a**), this was primarily due to improvements during the successive trials, as latency and path length during trial 1 were not different from other groups (data not shown).

In a probe trial conducted 24 h after the last session of acquisition training, sham-operated STZ-diabetic rats spent less time searching in the target quadrant, relative to nondiabetic, sham-operated rats ($F_{1,27} = 5.07$, $P = 0.03$; **Supplementary Fig. 1d**). This contrasts with the results of the probe trial in the *db*/*db* mice, where we observed no significant effect of diabetes or adrenalectomy on the percentage of time spent searching in the target quadrant ($F_{1,33} = 0.85$, $P = 0.36$; **Supplementary Fig. 1c**). Performance in the visible-platform version of the Morris water maze, which is not hippocampus dependent, was similar across conditions in both models (*db*/*db* mice, $F_{1,33} = 0.001$, $P = 0.96$; STZ-treated rats, $F_{1,28} = 0.57$, $P = 0.45$; data not shown).

Next we tested recognition memory in the novel object preference test. This task takes advantage of the natural bias for novelty in rodents and, unlike the water maze, does not depend on aversive motivation. In both models, nondiabetic mice and rats showed robust preference for the novel object, particularly at the shortest post-training interval. However, sham-operated diabetic mice and rats showed less of a preference for the novel object (*db*/*db* mice, $F_{1,26} = 10.52$, $P = 0.003$; STZ-treated rats, $F_{1,11} = 11.68$, $P = 0.006$, **Fig. 1d**,**f**). In contrast, diabetic mice and rats that had received adrenalectomy and

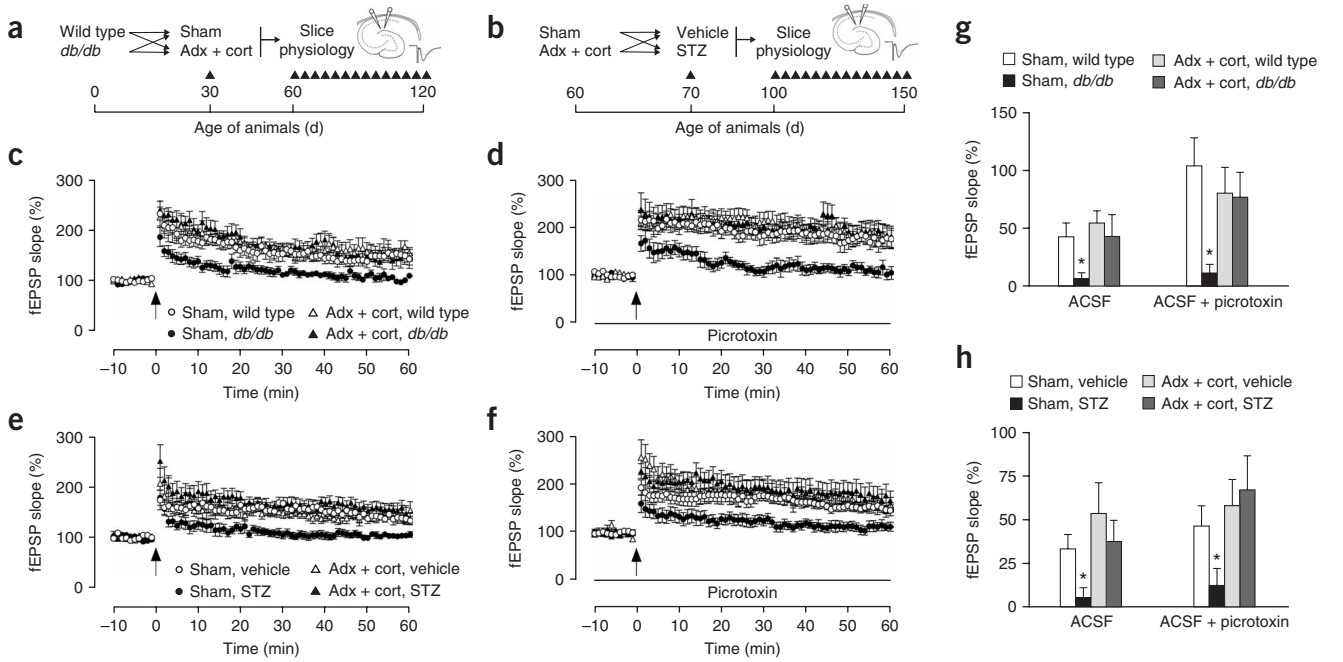

Figure 2 Lowering corticosterone regulates synaptic plasticity in diabetic rodents. (**a**) Design for studies in type 2 diabetic mice. (**b**) Design for studies in type 1 diabetic rats. (**c**) Sham-operated *db*/*db* mice showed reduced dentate gyrus LTP, but *db*/*db* mice with normal physiological corticosterone were not impaired. (**d**) Insulin-resistant mice that had been sham-operated also showed impaired LTP in the presence of picrotoxin, which decreases local inhibition and also blocks GABAergic excitation on new neurons[34,35]. In contrast, insulin-resistant mice with normal physiological corticosterone showed control levels of LTP under these conditions. (**e**) Sham-operated insulin-deficient rats showed reduced LTP; preventing elevation of corticosterone before induction of experimental diabetes restored LTP. (**f**) STZ-diabetic rats with intact adrenal glands showed reduced LTP in the presence of picrotoxin. Lowering corticosterone also reversed the effect of diabetes on LTP under these conditions. (**g**,**h**) Comparison of the amount of potentiation in slices from diabetic and nondiabetic mice (**g**) and rats (**h**) with different levels of corticosterone, recorded in ACSF and in ACSF with picrotoxin. Error bars, s.e.m.; Adx + cort., adrenalectomized with 25 μg ml^{-1} corticosterone replacement; fEPSP, field excitatory postsynaptic potential.

corticosterone replacement preferred to explore the novel object, with biases that were similar to those of nondiabetic rodents. We also recorded the latency to begin exploring and the total time spent exploring both objects during each trial (novel + familiar/duration of behavioral observation; see **Supplementary Methods** online). In the *db*/*db* mouse model, diabetic mice spent more time exploring the objects ($F_{1,28} = 22.78$, $P = 0.001$; **Supplementary Fig. 2a** online), and latency to approach either object was not different across groups (**Supplementary Fig. 2b**). In the STZ-treated rat model, there were no differences in the amount of time spent exploring the objects (**Supplementary Fig. 2c**), but sham-operated diabetic rats waited longer before approaching the objects, and adrenalectomized diabetic rats waited less ($F_{1,12} = 6.14$, $P = 0.001$; **Supplementary Fig. 2d**). The parameters surrounding object exploration are difficult to interpret, because neither total time exploring nor the latency to explore was significantly correlated with preference for the novel object (data not shown). However, together with the water maze results, these data suggest that untreated diabetes exerts pervasive negative effects on hippocampus-dependent memory, and that these effects can be reversed by lowering corticosterone.

Normalizing corticosterone restores LTP

We further examined the role of corticosterone in the diabetes-induced impairment of hippocampal learning by measuring synaptic plasticity at perforant path–dentate gyrus synapses in acute slices from another group of adrenalectomized or sham-operated diabetic and nondiabetic rodents (**Fig. 2a**,**b**). In agreement with previous studies[6,7], both *db*/*db* mice and STZ-diabetic rats showed reduced LTP at medial perforant path synapses in the dentate gyrus when recordings were made in the presence of the $GABA_A$ receptor antagonist picrotoxin (100 μM; **Fig. 2**). Adrenalectomy and corticosterone replacement prevented LTP impairment in both models (*db*/*db* mice, $F_{1,36} = 5.15$, $P = 0.03$; STZ-treated rats, $F_{1,35} = 5.90$, $P = 0.02$). Baseline synaptic transmission was not different in *db*/*db* mice and controls, irrespective of corticosterone manipulation (**Supplementary Fig. 3a** online). However, in rats, adrenalectomy and corticosterone replacement reduced baseline synaptic transmission, in both diabetic and nondiabetic rats ($F_{1,39} = 3.65$, $P = 0.03$; **Supplementary Fig. 3c**). No alterations in the paired-pulse depression that is characteristic of this pathway were observed in either model (**Supplementary Fig. 3b**,**d**). Taken together, these findings suggest that diabetes causes a primarily postsynaptic deficit in dentate gyrus plasticity that is reversible by lowering corticosterone.

Adult-generated neurons show a number of distinct electrophysiological properties. Among these is the transient capacity for GABAergic excitation[33,34]. It was recently demonstrated that changes in adult neurogenesis correlate with changes in medial perforant path LTP in the absence, but not in the presence, of picrotoxin[35,36]. We confirmed this in slices from wild-type mice that had been infused with the antimitotic drug cytosine arabinoside (AraC), which effectively reduced progenitor cell proliferation (BrdU-labeled cells with vehicle, 4,440 ± 822.6; with AraC, 696.0 ± 230.9; $t_{10} = 5.08$, $P = 0.0005$; **Figs. 3** and **4a**). We also counted pyknotic cell profiles to determine whether antimitotic treatment might influence cell death; there was no significant difference between vehicle- and AraC-treated mice in the number of pyknotic cells in the dentate gyrus (vehicle, 216 ± 54; AraC, 450 ± 160; mean ± s.e.m.; $t_7 = 1.58$, $P = 0.16$).

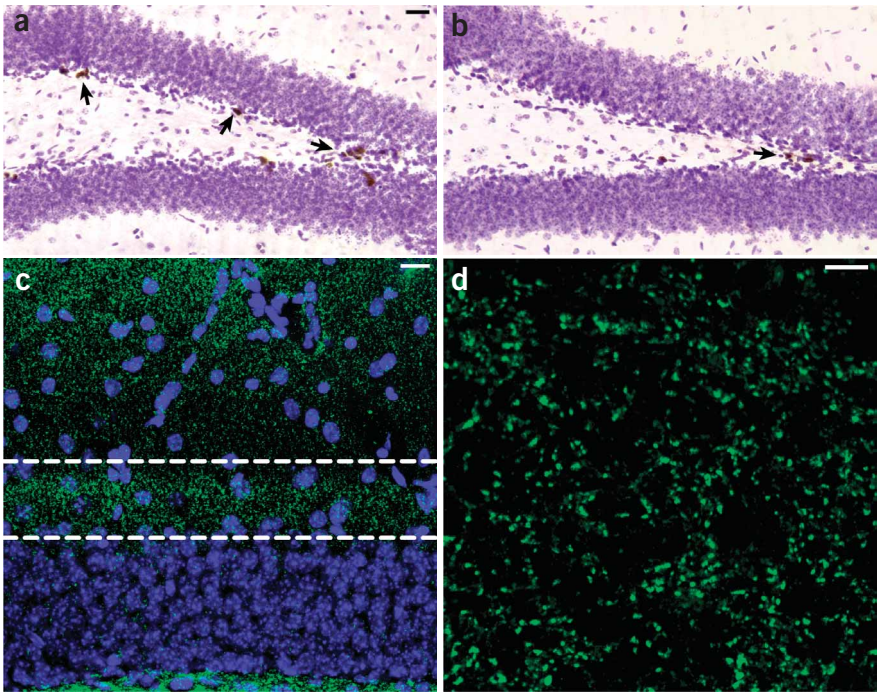

Figure 3 AraC treatment reduces cell proliferation, without altering synaptic marker immunoreactivity. (**a**,**b**) Dentate gyri after a single injection of 300 mg kg^{-1} BrdU with a 24 h survival period, from a mouse infused with vehicle (**a**) and a mouse infused with AraC (**b**). Arrows, BrdU-labeled cells. Scale bar (**a**,**b**), 30 μm. (**c**) Confocal micrograph showing synaptic marker expression in the inner third of the dentate molecular layer, where the medial perforant path synapses are located. Outline, anatomical region where scans were taken for analysis of synaptophysin labeling and where electrodes were positioned for electrophysiological recordings in slices. Scale bar, 20 μm. (**d**) Micrograph taken at the resolution and scale used for analysis of synaptophysin labeling (see **Supplementary Methods**). Scale bar, 10 μm.

To evaluate whether AraC treatment might alter synaptic marker expression in the anatomical region where medial perforant path synapses are located, we used immunofluorescence labeling for synaptophysin. There were no differences in the area or intensity of synaptophysin immunofluorescence in AraC- and vehicle-treated mice, suggesting no loss of synapses among the larger population of mature granule neurons (optical intensity index with vehicle, $1.1 \times 10^6 \pm 3.1 \times 10^5$; with AraC, $1.2 \times 10^6 \pm 2.1 \times 10^5$; $t_{10} = 0.21$, $P = 0.84$; **Fig. 3c**,**d**). In slices from AraC-treated mice, we observed selective deficits in medial perforant path LTP recorded in plain artificial cerebrospinal fluid (ACSF) (**Fig. 4b**,**c**). These deficits were not detected when recordings were made in the presence of picrotoxin (100 μM; **Fig. 4b**,**d**). Although there was a significant effect of picrotoxin on the input-output curve, there was no effect of AraC treatment (**Fig. 4e**). There was also no effect of AraC infusion on paired-pulse depression, recorded in plain ACSF (**Fig. 4f**).

To measure LTP in diabetic animals under conditions that would permit the activation of newly generated neurons, we induced LTP in the absence of picrotoxin. Diabetic rodents showed impaired LTP, and maintaining low corticosterone through adrenalectomy restored LTP to a level similar to that in controls (*db*/*db* mice, $F_{1,33} = 3.10$, $P = 0.04$; STZ-treated rats, $F_{1,39} = 5.24$, $P = 0.03$; **Fig. 2c**,**e**). These results suggest that diabetes alters synaptic plasticity through multiple mechanisms involving both changes in new neurons and changes in the mature neuronal population.

Corticosterone-mediated impairment of cell proliferation

To assess what role elevated corticosterone might play in the diabetes-induced suppression of hippocampal cell proliferation, we administered a single injection of the DNA synthesis marker bromodeoxyuridine (BrdU; 300 mg per kilogram body weight intraperitoneally) to adrenalectomized and sham-operated rodents with and without diabetes (**Fig. 5a**,**b**). In *db*/*db* mice, adrenalectomy and corticosterone replacement prevented the reduction in BrdU labeling in the dentate gyrus that we observed in sham-operated *db*/*db* mice 2 h after injection ($F_{1,31} = 5.82$, $P = 0.02$; **Figs. 5** and **6**). Similarly, in STZ-diabetic rats, adrenalectomy and corticosterone replacement before the induction of experimental diabetes prevented the decrease in BrdU-labeled cell number observed in sham-operated diabetic rats ($F_{1,41} = 10.76$,

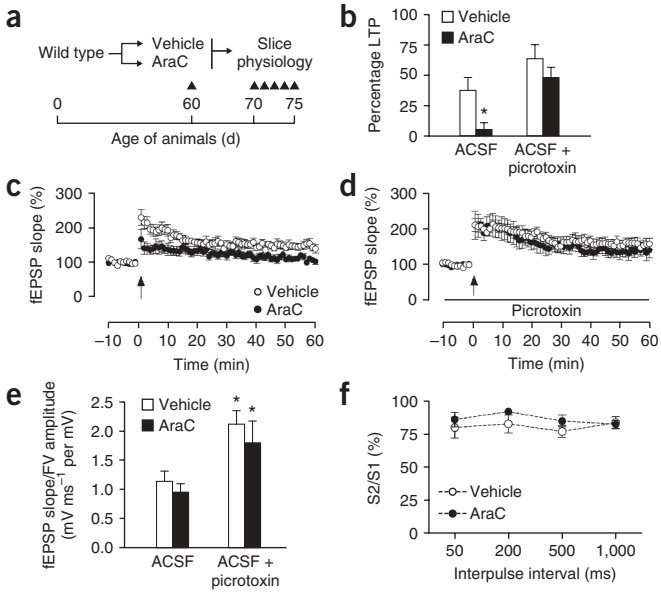

Figure 4 Antimitotic treatment selectively impairs dentate gyrus LTP recorded in the absence of picrotoxin. (**a**) Experimental design for studies using minipump delivery of antimitotic drugs. (**b**) Comparison of the amount of LTP in vehicle- and AraC-infused mice when recordings were made in the presence or absence of the $GABA_A$ antagonist picrotoxin (100 μM). $^*P < 0.05$, 2×2 ANOVA. (**c**) LTP at medial perforant path synapses in the dentate gyrus was impaired in AraC-infused mice. (**d**) LTP recorded in the presence of picrotoxin was not influenced by AraC infusion. (**e**) The relationship between the slope of the dendritic field potential and the amplitude of the axonal fiber volley was influenced by picrotoxin, but not by AraC treatment. (**f**) Presynaptic paired-pulse depression measured in plain ACSF was not altered by treatment with AraC. Error bars, s.e.m.; fEPSP, field excitatory postsynaptic potential; FV, fiber volley (the amplitude of the response among presynaptic axons); S1 and S2, slopes of the first and second fEPSPs, respectively.

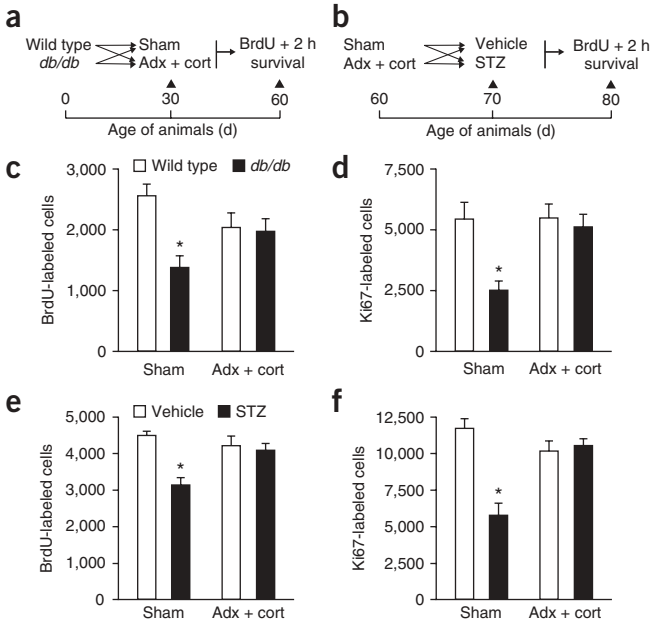

Figure 5 Elevated corticosterone contributes to the suppression of dentate gyrus cell proliferation in diabetic rodents. (**a**) Design for studies in type 2 diabetic mice. (**b**) Design for studies in type 1 diabetic rats. (**c**) Sham-operated *db*/*db* mice showed reduced BrdU labeling in the dentate gyrus, whereas *db*/*db* mice that received adrenalectomy and corticosterone replacement were not different from nondiabetic mice. (**d**) Labeling for the endogenous proliferation marker Ki67 was reduced in sham-operated type 2 diabetic mice, whereas type 2 diabetic mice that had been adrenalectomized and given corticosterone replacement were not different from nondiabetic mice. Legend in **c** applies to **d**. (**e**) Type 1 diabetic rats with intact adrenal glands had fewer BrdU-labeled cells in the dentate gyrus; this reduction was not observed in type 1 diabetic rats with normal levels of corticosterone. (**f**) Labeling for the endogenous proliferation marker Ki67 followed a similar pattern: diabetic rats with intact adrenal glands had significantly fewer Ki67-labeled cells than controls, whereas diabetic rats that received adrenalectomy and low-dose corticosterone replacement were not significantly different from nondiabetic rats. Legend in **e** applies to **f**. Error bars, s.e.m.; Adx + cort., adrenalectomized with 25 μg ml^{-1} corticosterone replacement.

$P = 0.002$; **Fig. 5e**, **Supplementary Fig. 4a**,**b** online). There was no effect of adrenalectomy and corticosterone replacement in vehicle-treated rats or in wild-type mice.

Diabetes has been associated with neurovascular pathologies, which could alter the availability of the exogenous marker BrdU. For an index of hippocampal cell proliferation that would not be influenced by availability, we used the endogenous proliferative marker Ki67. Labeling for Ki67 followed the same pattern as labeling for BrdU; sham-operated diabetic rodents had fewer Ki67-labeled cells, whereas diabetic rodents that had been adrenalectomized and given corticosterone replacement were not significantly different from nondiabetic controls (*db*/*db* mice, $F_{1,20} = 5.24$, $P = 0.03$; STZ-treated rats, $F_{1,35} = 5.89$, $P = 0.02$; **Figs. 5d**,**f** and **6c**,**d**; **Supplementary Fig. 4c**,**d**). There were no effects of adrenalectomy and corticosterone replacement on Ki67 labeling in nondiabetic wild-type mice or vehicle-treated rats. These results indicate that lowering corticosterone prevents the decrease in hippocampal progenitor cell proliferation in insulin-resistant and insulin-deficient diabetes.

Lasting suppression of adult neurogenesis with diabetes

To evaluate whether suppression of hippocampal cell proliferation in diabetic animals translates into a reduction in adult neurogenesis, we administered a single injection of BrdU (300 mg kg^{-1} intraperitoneally) to diabetic and nondiabetic rodents and killed them three weeks later. In *db*/*db* mice, we observed a reduction in the number of BrdU-labeled cells in the dentate gyrus (mean ± s.e.m. for wild type, 860 ± 69; for *db*/*db*, 416 ± 85; $t_8 = 4.05$, $P = 0.004$). There was no difference in the proportion of cells expressing the mature neuronal marker NeuN (wild type, 98 ± 1.22; *db*/*db*, 96 ± 1.87; $t_8 = 0.89$, $P = 0.39$; **Fig. 6e**) or the immature neuronal marker Tuj1 (wild type, 93 ± 0.96; *db*/*db*, 93 ± 1.26; $t_8 = 0.03$, $P = 0.97$; **Fig. 6f**). There was also no change in the percentage of BrdU-labeled cells expressing the astroglial marker GFAP (wild type, 7.35 ± 1.01; *db*/*db*, 8.92 ± 2.51; $t_8 = 0.58$, $P = 0.57$; **Fig. 6g**). Because the analyses were made separately, in adjacent series of stereological sections, values reflect relative expression of each marker among

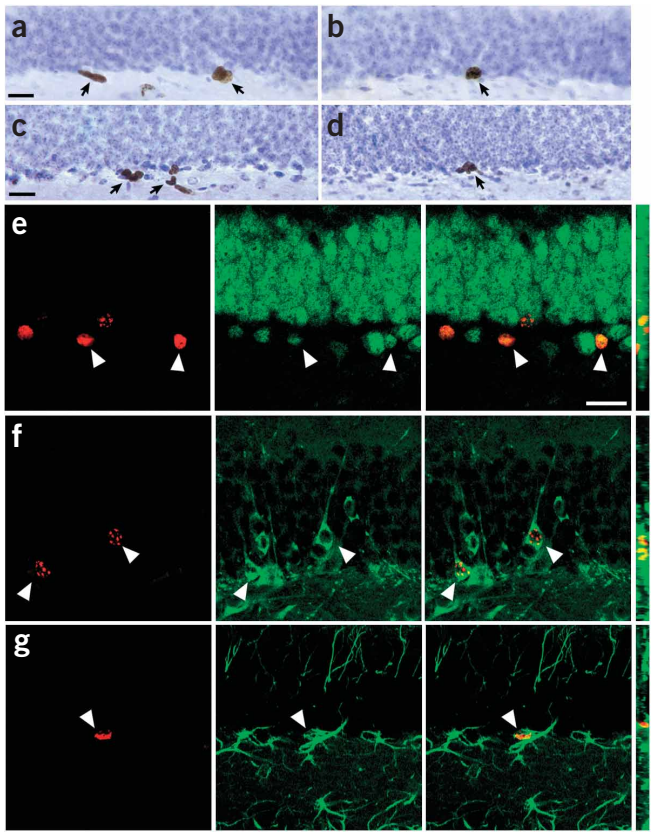

Figure 6 Hippocampal cell proliferation and neurogenesis is reduced in a mouse model of type 2 diabetes. (**a**) BrdU-labeled cells (arrows) in the proliferative dentate subgranular zone of a wild-type mouse 2 h after injection. (**b**) BrdU-labeled cells (arrows) in the subgranular zone of a *db*/*db* homozygous mouse. (**c**) Progenitor cells expressing the endogenous proliferation marker Ki67 (arrows) in the dentate gyrus of a wild-type mouse. (**d**) Cells labeled with antibodies to Ki67 (arrows) in the dentate gyrus of a *db*/*db* mouse. (**e**) Cells positive for both the proliferative marker BrdU (red, left) and the mature neuronal marker NeuN (green, middle) 3 weeks after injection (merged image shown to the right). (**f**) Double labeling with BrdU (red) and Tuj1 (green), also 3 weeks after injection (merged image shown to the right). (**g**) Cells double-labeled with antibodies to BrdU (red) and the astroglial marker GFAP (green) 3 weeks after injection (merged image shown to the right). For **e**–**g**, the far right panel shows the merged image across the *z* axis. Scale bars, 20 μm. Scale bars in **a** and **c** apply to **b** and **d**, respectively; scale bar in **e** applies to **f** and **g**.

the BrdU-labeled cell population. However, the absence of any proportionate difference in the expression of neuronal and glial markers suggests that differentiation of newly generated cells was not affected.

Similarly, in STZ-diabetic rats, there were fewer cells labeled with BrdU relative to the number in vehicle-treated controls (mean ± s.e.m. for vehicle, 3,728 ± 412; for STZ, 2,070 ± 466.6; $t_9 = 2.66$, $P = 0.02$). There was no change in the proportion of cells positive for both BrdU and the neuronal markers NeuN (vehicle, 91.33 ± 2.81; STZ, 84 ± 5.21; $t_9 = 1.30$, $P = 0.23$; **Supplementary Fig. 4e**) or Tuj1 (vehicle, 87 ± 2.40; STZ, 86.67 ± 1.33; $t_{10} = 0.24$, $P = 0.81$; **Supplementary Fig. 4f**). The percentage of BrdU-labeled cells expressing GFAP, a marker of astrocytes, was not altered in type 1 diabetic rats (vehicle, 7.33 ± 1.91; STZ, 9.33 ± 1.33; $t_{10} = 0.86$, $P = 0.41$; **Supplementary Fig. 4g**). However, coincident with the reduction in BrdU-labeled cell number, these data indicate a net reduction in the number of new neurons and astrocytes for both insulin-resistant mice and insulin-deficient rats.

Table 1 Endocrine characteristics of type 1 and type 2 diabetes in rodents with different levels of corticosterone

		Fasting glucose (mg dl^{-1})	Fed glucose (mg dl^{-1})	Insulin (ng ml^{-1})	Corticosterone (ng ml^{-1})
Wild type	Sham	71.77 (8.63)	140.65 (15.67)	1.41 (0.15)	46.16 (15.99)
	Adx + cort	64.80 (1.87)	95.95 (20.65)	1.41 (0.14)	18.85 (3.95)
db/db	Sham	330.27 (21.01)*	334.12 (41.09)*	3.16 (0.68)*	258.55 (43.11)*
	Adx + cort	98.40 (17.51)	328.88 (19.83)*	1.03 (0.17)	11.24 (2.21)
Vehicle	Sham	30.19 (5.77)	129.86 (21.72)	2.07 (0.27)	53.08 (17.72)
	Adx + cort	44.74 (2.23)	170.87 (11.74)	1.73 (0.27)	19.87 (4.56)
STZ	Sham	290.96 (35.94)*	318.46 (16.76)*	0.79 (0.09)*	418.24 (18.26)*
	Adx + cort	221.08 (14.80)*	263.06 (11.29)*	0.91 (0.16)*	30.14 (5.78)

Data were analyzed using 2 × 2 ANOVA; Values are means with s.e.m. in parentheses. Sham, sham-operated; Adx + cort, adrenalectomized with corticosterone replacement.
*$P < 0.05$ relative to sham-operated nondiabetic controls.

Corticosterone regulates glucose and insulin concentrations

To determine whether lowering corticosterone might prevent or alter the impact of experimental diabetes, we measured insulin and glucose in serum from diabetic and nondiabetic rodents that had been adrenalectomized or sham-operated. In both diabetes models, sham-operated diabetic rodents showed corticosterone concentrations that were comparable to those reported in nondiabetic rats after an acute stressor[32] (**Table 1**). In the type 1 diabetes model, preventing the elevation of corticosterone did not alter STZ-induced hyperglycemia (**Table 1**). Effects were similar in serum samples from fed and fasted rats (fed glucose, $F_{1,41} = 10.19$, $P = 0.002$; fasting glucose, $F_{1,13} = 90.36$, $P < 0.001$; **Table 1**). Likewise, adrenalectomy and corticosterone replacement had no impact on the ability of STZ to reduce insulin ($F_{1,33} = 25.57$, $P < 0.001$; **Table 1**). There was no long-term effect of STZ diabetes on feeding or body weight (**Supplementary Table 1** online). Because we administered corticosterone replacement through the drinking water, it is important to note that despite the higher volume of solution consumed by adrenalectomized rats in both the STZ-treated and vehicle-treated conditions ($F_{1,15} = 23.78$, $P < 0.001$; **Supplementary Table 1**), these animals maintained serum corticosterone concentrations that were similar to those of sham-operated nondiabetic controls (**Table 1**).

db/db mice respond differently than STZ-treated rats to adrenalectomy and corticosterone replacement. In this model, adrenalectomy and corticosterone replacement reversed the increase in fasting glucose in *db/db* mice ($F_{1,39} = 21.38$, $P < 0.001$; **Table 1**). However, postprandial glucose in adrenalectomized *db/db* mice remained higher than those of nondiabetic controls ($F_{1,33} = 47.46$, $P < 0.001$; **Table 1**). Lowering corticosterone also attenuated hyperinsulinemia ($F_{1,23} = 5.69$, $P = 0.03$; **Table 1**). Both sham-operated and adrenalectomized *db/db* mice weighed more than wild-type mice ($F_{1,31} = 32.43$, $P < 0.001$) and consumed more food ($F_{1,48} = 51.90$, $P < 0.001$) (**Supplementary Table 1**). *db/db* mice characteristically show polydipsia, and we observed this in sham-operated *db/db* mice but not in *db/db* mice that had received adrenalectomy and corticosterone replacement ($F_{1,42} = 14.77$, $P = 0.004$; **Supplementary Table 1**).

To evaluate whether levels of insulin and glucose in the hippocampus were altered in diabetic rodents, we measured them in whole-hippocampal homogenates from STZ-diabetic rats and *db/db* mice. We observed no effect of STZ diabetes on hippocampal glucose or insulin concentrations (glucose with vehicle, 23.34 ± 4.08 mmol mg^{-1}; with STZ, 24.24 ± 5.96 mmol mg^{-1}; $t_{10} = 0.12$, $P = 0.90$; insulin with vehicle, 1.55 ± 0.15 µmol mg^{-1}; with STZ, 1.81 ± 0.25 µmol mg^{-1}; $t_{10} = 0.86$, $P = 0.42$). Similarly, concentrations of glucose and insulin in the hippocampus of *db/db* mice were not different from those in wild-type mice (glucose in wild type, 10.73 ± 1.85 mmol mg^{-1}; in *db/db*, 13.48 ± 1.16 mmol mg^{-1}, $t_6 = 1.26$, $P = 0.25$; insulin in wild type, 1.26 ± 0.15 µmol mg^{-1}; in *db/db*, 1.60 ± 0.21 µmol mg^{-1}; $t_6 = 1.32$, $P = 0.25$). Although these results do not preclude a change in the availability or sensitivity to glucose and/or insulin at the level of individual cells, they do provide indirect support for the idea that another factor, namely corticosterone, contributes to the impairment of hippocampal plasticity in diabetic rodents.

Corticosterone mediates learning impairments in *db/db* mice

Because adrenalectomy removes not only endogenous corticosterone but also the primary source of peripheral epinephrine, we replicated our previous experimental design with an additional group of *db/db* mice that were adrenalectomized and given a high replacement dose of corticosterone through the drinking water (250 µg ml^{-1} in 0.9% saline; **Fig. 7a**). This regimen resulted in circulating corticosterone concentrations similar to those of sham-operated *db/db* mice (mean ± s.e.m., 333.28 ± 47.38 ng ml^{-1}). We tested these mice in the Morris water maze and object recognition tasks. In support of our earlier result, *db/db* mice that had received adrenalectomy and 25 µg ml^{-1} corticosterone replacement learned the location of the hidden platform more rapidly than sham-operated *db/db* mice and adrenalectomized *db/db* mice receiving 250 µg ml^{-1} corticosterone replacement ($F_{2,10} = 8.35$, $P < 0.001$; **Fig. 7b**, **Supplementary Fig. 5a** online). *db/db* mice that had been adrenalectomized and given low-dose corticosterone replacement also showed greater improvement over successive trials on day 1, but performance on the first trial was not different from that of sham-operated *db/db* mice or that of *db/db* mice that had been adrenalectomized and given a higher dose of corticosterone (data not shown). There were no effects of any of the treatments on swimming speed (**Supplementary Fig. 5b**).

Higher doses of corticosterone also reinstated deficits in object-recognition memory. *db/db* mice that had been adrenalectomized and administered a low dose of corticosterone spent more time exploring the novel object than did sham-operated *db/db* mice. In contrast, in

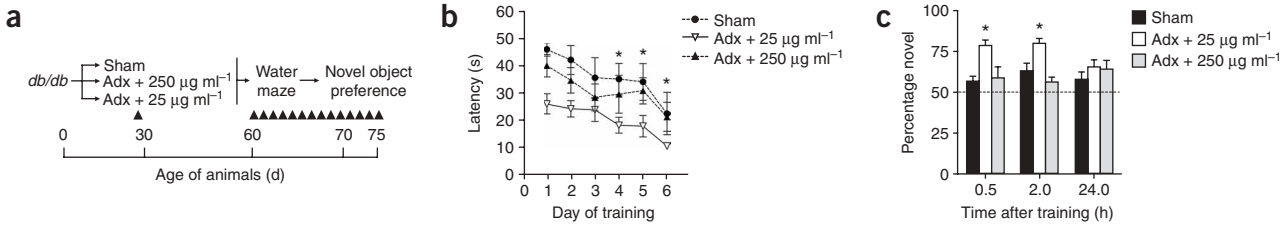

Figure 7 A high replacement dose of corticosterone reinstates learning deficits in adrenalectomized *db*/*db* mice. (**a**) Experimental design: *db*/*db* mice were sham-operated or adrenalectomized (Adx); adrenalectomized mice received corticosterone replacement at 25 or 250 μg ml^{-1} in their drinking water. One month after surgery, the mice were tested in the Morris water maze and object recognition tasks. (**b**) Lowering corticosterone restored hippocampal learning in insulin resistant mice, whereas a high replacement dose of corticosterone was associated with learning impairments comparable to those in sham-operated diabetic mice. Differences in performance on the first day of training in adrenalectomized *db*/*db* mice receiving low-dose corticosterone replacement were due to improvements over successive trials, as no differences were observed during the first trial (see Results). (**c**) *db*/*db* mice that had been adrenalectomized and given a low replacement dose of corticosterone spent more time exploring the novel object, relative to sham-operated *db*/*db* mice and to *db*/*db* mice administered a high dose of corticosterone. $^*P < 0.05$, one-way repeated-measures ANOVA. Error bars, s.e.m.

adrenalectomized *db*/*db* mice that received a higher dose of corticosterone, the reduction in novel object preference was identical to that seen in sham-operated *db*/*db* mice ($F_{2,8} = 11.05$, $P = 0.03$; **Fig. 7c**). Again, *db*/*db* mice that had been adrenalectomized and administered a low dose of corticosterone showed a preference for the novel object that was similar to nondiabetic mice.

Administration of a high dose of corticosterone had complex effects on the endocrine parameters of adrenalectomized *db*/*db* mice. These mice showed hyperglycemia, at a level similar to sham-operated *db*/*db* mice (mean ± s.e.m. fasting, 339.30 ± 34.10 mg dl^{-1}; fed, 450.59 ± 46.04 mg dl^{-1}). Serum insulin was also elevated (mean ± s.e.m., 1.98 ± 0.57 ng ml^{-1}). *db*/*db* mice receiving a high dose of corticosterone showed increased water intake, similarly to sham-operated *db*/*db* mice (mean ± s.e.m., 52.73 ± 7.24 ml d^{-1}). However, their food intake and body weights were similar to those of wild-type mice (food intake, 5.97 ± 0.65 g d^{-1}; body weight, 41.07 ± 4.34 g). These results suggest that elevated corticosterone contributes centrally to learning deficits and peripherally to the endocrine characteristics of diabetes.

DISCUSSION

Diabetes is associated with several adverse effects on the brain, some of which may result primarily from direct consequences of chronic hyperglycemia. However, our findings demonstrate a pivotal role for the adrenal steroid corticosterone as a mediator of diabetes-induced impairments in hippocampal synaptic plasticity and neurogenesis, and associated cognitive deficits. Lowering corticosterone prevented the diabetes-induced impairment of learning and memory in insulin-deficient rats and-insulin resistant mice. Maintaining normal physiological corticosterone also restored LTP at perforant path–dentate gyrus synapses and prevented the impairment of adult neurogenesis in the dentate gyrus. The restorative effect of lowering corticosterone was observed when recordings were made under conditions that either permitted or excluded the contribution of newly generated neurons. Enhancement of hippocampal function by normalizing corticosterone in diabetic mice or rats was completely reversed by administration of high levels of corticosterone, demonstrating that corticosterone (rather than some other adrenal-derived factor) was responsible for the adverse effects of diabetes on hippocampal plasticity. These findings strongly support a role for elevated corticosterone in impaired hippocampal plasticity and cognition induced by diabetes.

It is well established that chronic exposure to high levels of corticosterone is detrimental for learning and synaptic plasticity in nondiabetic animals[24–29]. The corticosterone-mediated adverse effects of diabetes were not determined by changes in insulin production, because they occurred both in *db*/*db* mice with elevated insulin and in insulin-deficient rats. We also observed no change in hippocampal insulin levels under baseline conditions in diabetic mice and rats. However, this does not rule out the possibility that insulin signaling pathways might be impaired in diabetes. The effects of insulin on learning and memory oppose those of glucocorticoids at several levels. Specifically, intrahippocampal insulin[37] or activation of insulin signaling pathways[38] can block the effects of stress on learning and memory. Exposure to elevated corticosterone reduces insulin receptor signaling in many somatic tissues, including the brain[39]. Therefore, it is possible that the negative effect of diabetes on hippocampal plasticity may be attributable to an interaction between elevated glucocorticoids and insulin receptor signaling.

Local cerebral glucose usage is tightly linked with neural activity and cognition. In contrast, glucocorticoids inhibit glucose usage in neurons[40]. In normal (that is, nondiabetic) rats, hippocampus-dependent learning is correlated with a decrease in extracellular glucose, and intrahippocampal injection of glucose improves performance[41]. No studies so far have reported an effect of diabetes on learning-induced changes in hippocampal glucose metabolism, but alterations in basal hippocampal glucose transporter expression have been demonstrated in diabetic rats[42]. Although we observed no difference in glucose concentrations in whole hippocampal homogenates from insulin-resistant mice or insulin-deficient rats, our results do not preclude a role for corticosterone in modulating the diabetes-induced alterations in hippocampal glucose metabolism.

Lowering corticosterone in diabetes can restore behavioral function on tasks that recruit both new and mature neurons. While the Morris water maze task is not influenced by antimitotic treatment[35,43], newly generated neurons are activated after this task at a higher rate than mature neurons[44]. Similar distinctions have been reported with respect to the role of adult-generated neurons in recognition memory: systemic treatment with an antimitotic reversed enhancement of performance on the novel-object preference task after environmental enrichment[45], but focal cranial irradiation did not affect spontaneous alternation in the Y-maze, which also involves recognition memory[35]. Although it remains to be determined whether adult-generated granule neurons make a meaningful contribution to performance on these tasks under baseline conditions, the therapeutically relevant question is whether new neurons can enhance performance after neurodegeneration or injury.

The absence of an effect of corticosterone reduction on postprandial serum glucose in *db*/*db* mice is in line with previous studies. Adrenalectomy and corticosterone replacement do not normalize fed glucose values in the *ob*/*ob* mouse[18]. Similar results occur upon treatment with

the glucocorticoid receptor antagonist RU486 in the Zucker (*fa/fa*) rat, with no effect of antiglucocorticoid treatment on fed glucose levels[19]. In contrast, treatment with antisense oligonucleotides directed against the glucocorticoid receptor restores normal fasting glucose in Zucker diabetic rats[20]. Taken together, these results suggest that inhibiting the actions of corticosterone by various methods will influence fasting but not fed glucose in rodent models of type 2 diabetes.

Studies of human subjects have provided evidence that diabetes adversely affects learning and memory, but they also suggest that not all cognitive domains are equally affected. Diabetic humans show accelerated decline on tasks that require episodic memory and rapid information processing, whereas attention and language abilities are unaffected[2]. Because episodic memory places a greater demand on temporal lobe structures, and language and attention primarily recruit other cortical and prefrontal regions, these data have been interpreted to suggest that the hippocampus is particularly susceptible to the negative consequences of diabetes. Other studies have begun to explore the role of cortisol in diabetes-induced cognitive deficits in humans. For example, inhibition of the enzyme 11-β-hydroxysteroid dehydrogenase 1, which locally modulates the actions of glucocorticoids in the brain by reactivating cortisol from its inactive form, was shown to ameliorate cognitive deficits in humans with type 2 diabetes[46]. Overall, the task-specific cognitive impairments induced by diabetes and the demonstration of improved cognitive performance in diabetic humans after treatments that alter the availability of cortisol suggest that elevated cortisol in human diabetics may also contribute to deficits in hippocampal function.

METHODS

Animals and surgery. Animal care and experimental procedures followed US National Institutes of Health guidelines and were approved by the US National Institute on Aging Animal Care and Use Committee. Adult male Sprague-Dawley rats were purchased from Charles River Laboratories and housed individually for a minimum of 2 weeks before the start of experiments. We administered streptozocin through the femoral vein at a dose of 70 mg kg^{-1} as described[16]. In order to be included in the study, STZ-treated rats were required to have serum glucose $\geq$ 200 mg dl^{-1}. Male mice mutant for the leptin receptor (*db/db* mice), bred on a C57BL/6 background, were purchased from Jackson Laboratories. Age-matched male C57BL/6 mice were used as controls. Rats and mice were subjected to bilateral adrenalectomy or sham operation. Adrenalectomized rats and mice received corticosterone replacement (25 μg ml^{-1} or 250 μg ml^{-1} in 0.9% saline; Sigma) in the drinking water to manipulate glucocorticoid levels[30]. Corticosterone replacement was available to the animals immediately after surgery. Mice were adrenalectomized at postnatal day 30; rats were adrenalectomized at postnatal day 60. All rats and mice were administered a single injection of the DNA synthetic marker BrdU (300 mg kg^{-1}; $n = 6$–8 rats or mice per group). This dosage was based on previous studies[47] demonstrating maximal labeling at 300 mg kg^{-1}. Animals were put to death 2 h or 3 weeks after BrdU. In a separate experiment, 2-month-old wild-type mice were implanted with Alzet minipumps to deliver the antimitotic drug AraC into the right lateral ventricle (2.2 mg ml^{-1}, 0.25 μl h^{-1}, pump model 1002; bregma coordinates anterior-posterior –0.3 mm, medial-lateral –1.0 mm). These mice were injected once with BrdU (300 mg kg^{-1}; $n = 6$–8 mice per group) and put to death 24 h later. All mice and rats had *ad libitum* access to food and water, and the room was maintained on a 12 h light-dark schedule (lights on at 06:00). For some experiments, the animals were weighed once weekly, and their food and water were weighed on two successive days per week for 4 weeks. Food consumption was measured in grams per day, and water bottle weights were converted to volumes. The techniques for quantifying glucose, insulin and corticosterone are described in **Supplementary Methods**.

Electrophysiology and behavioral testing. The procedures used for slice preparation and recording are available in **Supplementary Methods**. Procedures for water maze training and novel object preference testing are also included in **Supplementary Methods**.

Immunohistochemistry and microscopy. Immunolabeling for BrdU and Ki67 was carried out as described[15]. Full description of the methods for brightfield and fluorescence tissue labeling are available in **Supplementary Methods**. We quantified single- and double-labeled cells using standard protocols[15]. Detailed cell-counting criteria are available in **Supplementary Methods**. We also quantified the optical intensity of fluorescence staining for synaptophysin[48]; full description available in **Supplementary Methods**.

Statistics. Statistical analyses were made using SPSS version 11.0, with significance set at $P < 0.05$; graphs were generated using Graphpad Prism 4 software. Cell counts, hormone profiles, feeding, drinking, animal weights and the amount of LTP were compared using separate 2 × 2 analysis of variance (ANOVA) designs (diabetes × surgery). Behavioral data from the Morris water maze and novel object preference task were analyzed using 2 × 2 repeated-measures ANOVA. The number of BrdU-labeled cells 3 weeks after injection was compared across diabetic and nondiabetic animals using bidirectional, unpaired *t*-tests. Percentages of cells double-positive for BrdU and a cell type–specific marker were also analyzed using *t*-tests. In experiments where we administered a high dose of corticosterone to adrenalectomized *db/db* mice, we analyzed behavioral data from the Morris water maze and novel object preference tasks using one-way repeated measures ANOVA with Tukey's post hoc test.

Note: Supplementary information is available on the Nature Neuroscience website.

ACKNOWLEDGMENTS

This research was supported by US National Institutes of Health National Research Service Award Predoctoral fellowship F31AG024690-03 to A.M.S. through Princeton University, and by the Intramural Research Program of the US National Institute on Aging. We thank D.L. Longo for suggestions and T. Lamb, O. Carlson, J.S. Villareal and R. Telljohann for technical assistance. We are also grateful to E. Gould and H. van Praag for comments on the manuscript.

AUTHOR CONTRIBUTIONS

A.M.S., M.P.M. and J.M.E. contributed to the conceptual design and development of the experiments. A.M.S., K.L., T.V.A. and R.G.C. performed surgeries, ran experiments and contributed data. All authors assisted with writing and revising the manuscript.

Published online at http://www.nature.com/natureneuroscience
Reprints and permissions information is available online at http://npg.nature.com/reprintsandpermissions

1. Reaven, G.M. The insulin resistance syndrome: definition and dietary approaches to treatment. *Annu. Rev. Nutr.* **25**, 391–406 (2005).
2. Messier, C. Impact of impaired glucose tolerance and type 2 diabetes on cognitive aging. *Neurobiol. Aging* **26** (suppl. 1), S26–S30 (2005).
3. Greenwood, C.E. & Winocur, G. High-fat diets, insulin resistance and declining cognitive function. *Neurobiol. Aging* **26** (suppl. 1), 45 (2005).
4. Desrocher, M. & Rovet, J. Neurocognitive correlates of type 1 diabetes mellitus in childhood. *Child Neuropsychol.* **10**, 36–52 (2004).
5. Biessels, G.J. *et al.* Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. *Diabetes* **45**, 1259–1266 (1996).
6. Biessels, G.J. *et al.* Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. *Brain Res.* **800**, 125–135 (1998).
7. Li, X.L. *et al.* Impairment of long-term potentiation and spatial memory in leptin receptor–deficient rodents. *Neuroscience* **113**, 607–615 (2002).
8. Hummel, K.P., Dickie, M.M. & Coleman, D.L. Diabetes, a new mutation in the mouse. *Science* **153**, 1127–1128 (1966).
9. Leuner, B., Gould, E. & Shors, T.J. Is there a link between adult neurogenesis and learning? *Hippocampus* **16**, 216–224 (2006).
10. Kamal, A., Biessels, G.J., Urban, I.J. & Gispen, W.H. Hippocampal synaptic plasticity in streptozotocin-diabetic rats: impairment of long-term potentiation and facilitation of long-term depression. *Neuroscience* **90**, 737–745 (1999).
11. Zhang, W.J., Tan, Y.F., Yue, J.T., Vranic, M. & Wojtowicz, J.M. Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. *Acta Neurol. Scand.*, published online 14 September 2007 (doi:10.1111/j.1600-0404.2007.00928.x).
12. van Praag, H., Christie, B.R., Sejnowski, T.J. & Gage, F.H. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc. Natl. Acad. Sci. USA* **96**, 13427–13431 (1999).

13. Fontan-Lozano, A. *et al.* Caloric restriction increases learning consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. *J. Neurosci.* **27**, 10185–10195 (2007).
14. Lee, J., Duan, W. & Mattson, M.P. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J. Neurochem.* **82**, 1367–1375 (2002).
15. Stranahan, A.M., Khalil, D. & Gould, E. Social isolation delays the positive effects of running on adult neurogenesis. *Nat. Neurosci.* **9**, 526–533 (2006).
16. Magarinos, A.M. & McEwen, B.S. Experimental diabetes in rats causes hippocampal dendritic and synaptic reorganization and increased glucocorticoid reactivity to stress. *Proc. Natl. Acad. Sci. USA* **97**, 11056–11061 (2000).
17. Chan, O. *et al.* Hyperglycemia does not increase basal hypothalamo-pituitary-adrenal activity in diabetes but it does impair the HPA response to insulin-induced hypoglycemia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **289**, R235–R246 (2005).
18. Tokuyama, K. & Himms-Hagen, J. Increased sensitivity of the genetically obese mouse to corticosterone. *Am. J. Physiol.* **252**, 202–208 (1987).
19. Langley, S.C. & York, D.A. Effects of antiglucocorticoid RU486 on development of obesity in obese *fa/fa* Zucker rats. *Am. J. Physiol.* **259**, 539–544 (1990).
20. Watts, L.M. *et al.* Reduction of hepatic and adipose tissue glucocorticoid receptor expression with antisense oligonucleotides improves hyperglycemia and hyperlipidemia in diabetic rodents without causing systemic glucocorticoid antagonism. *Diabetes* **54**, 1846–1853 (2005).
21. Oei, N.Y., Everaerd, W.T., Elzinga, B.M., van Well, S. & Bermond, B. Psychosocial stress impairs working memory at high loads: an association with cortisol levels and memory retrieval. *Stress* **9**, 133–141 (2006).
22. MacLullich, A.M. *et al.* Plasma cortisol levels, brain volumes and cognition in healthy elderly men. *Psychoneuroendocrinology* **30**, 505–515 (2005).
23. Elgh, E. *et al.* Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer's disease. *Biol. Psychiatry* **59**, 155–161 (2006).
24. Oitzl, M.S., Fluttert, M., Sutanto, W. & de Kloet, E.R. Continuous blockade of brain glucocorticoid receptors facilitates spatial learning and memory in rats. *Eur. J. Neurosci.* **10**, 3759–3766 (1998).
25. Wright, R.L., Lightner, E.N., Harman, J.S., Meijer, O.C. & Conrad, C.D. Attenuating corticosterone levels on the day of memory assessment prevents chronic stress–induced impairments in spatial memory. *Eur. J. Neurosci.* **24**, 595–605 (2006).
26. Alfarez, D.N., Joels, M. & Krugers, H.J. Chronic unpredictable stress impairs long-term potentiation in rat hippocampal CA1 area and dentate gyrus in vitro. *Eur. J. Neurosci.* **17**, 1928–1934 (2003).
27. Kerr, D.S., Campbell, L.W., Hao, S.Y. & Landfield, P.W. Corticosteroid modulation of hippocampal potentials: increased effect with aging. *Science* **245**, 1505–1509 (1989).
28. Korz, V. & Frey, J.U. Stress-related modulation of hippocampal long-term potentiation in rats: Involvement of adrenal steroid receptors. *J. Neurosci.* **23**, 7281–7287 (2003).
29. Pavlides, C., Watanabe, Y. & McEwen, B.S. Effects of glucocorticoids on hippocampal long-term potentiation. *Hippocampus* **3**, 183–192 (1993).
30. Gould, E., Cameron, H.A., Daniels, D.C., Woolley, C.S. & McEwen, B.S. Adrenal hormones suppress cell division in the adult rat dentate gyrus. *J. Neurosci.* **12**, 3642–3650 (1992).
31. Montaron, M.F. *et al.* Lifelong corticosterone level determines age-related decline in neurogenesis and memory. *Neurobiol. Aging* **27**, 645–654 (2006).
32. Tanapat, P., Hastings, N.B., Rydel, T.A., Galea, L.A. & Gould, E. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J. Comp. Neurol.* **437**, 496–504 (2001).
33. Karten, Y.J., Jones, M.A., Jeurling, S.I. & Cameron, H.A. GABAergic signaling in young granule cells in the adult rat and mouse dentate gyrus. *Hippocampus* **16**, 312–320 (2006).
34. Ge, S. *et al.* GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* **439**, 589–593 (2006).
35. Saxe, M.D. *et al.* Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. *Proc. Natl. Acad. Sci. USA* **103**, 17501–17506 (2006).
36. Snyder, J.S., Kee, N. & Wojtowicz, J.M. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. *J. Neurophysiol.* **85**, 2423–2431 (2001).
37. Moosavi, M., Naghdi, N., Maghsoudi, N. & Zahedi Asl, S. Insulin protects against stress-induced impairments in water maze performance. *Behav. Brain Res.* **176**, 230–236 (2007).
38. Revest, J.M. *et al.* The MAPK pathway and Egr-1 mediate stress-related behavioral effects of glucocorticoids. *Nat. Neurosci.* **8**, 664–672 (2005).
39. Piroli, G.G. *et al.* Corticosterone impairs insulin-stimulated translocation of GLUT4 in the rat hippocampus. *Neuroendocrinology* **85**, 71–80 (2007).
40. Sapolsky, R.M. Glucocorticoid toxicity in the hippocampus: reversal by supplementation with brain fuels. *J. Neurosci.* **6**, 2240–2244 (1986).
41. McNay, E.C., Fries, T.M. & Gold, P.E. Decreases in rat extracellular hippocampal glucose concentration associated with cognitive demand during a spatial task. *Proc. Natl. Acad. Sci. USA* **97**, 2881–2885 (2000).
42. Reagan, L.P. *et al.* Localization and regulation of GLUTx1 glucose transporter in the hippocampus of streptozotocin diabetic rats. *Proc. Natl. Acad. Sci. USA* **98**, 2820–2825 (2001).
43. Shors, T.J., Townsend, D.A., Zhao, M., Kozorovitskiy, Y. & Gould, E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* **12**, 578–584 (2002).
44. Kee, N., Teixeira, C.M., Wang, A.H. & Frankland, P.W. Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat. Neurosci.* **10**, 355–362 (2007).
45. Bruel-Jungerman, E., Laroche, S. & Rampon, C. New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. *Eur. J. Neurosci.* **21**, 513–521 (2005).
46. Sandeep, T.C. *et al.* 11β-Hydroxysteroid dehydrogenase inhibition improves cognitive function in healthy elderly men and type 2 diabetics. *Proc. Natl. Acad. Sci. USA* **101**, 6734–6739 (2004).
47. Cameron, H.A. & McKay, R.D. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J. Comp. Neurol.* **435**, 406–417 (2001).
48. Kozorovitskiy, Y. *et al.* Experience induces structural and biochemical changes in the adult primate brain. *Proc. Natl. Acad. Sci. USA* **102**, 17478–17482 (2005).