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Accelerated cognitive aging in diabetic rats is prevented by lowering corticosterone levels

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Abstract

Diabetes and normal aging are both characterized by increases in levels of glucocorticoids. Because long-term exposure to elevated glucocorticoids can be detrimental to hippocampal function, we evaluated the performance of young diabetic rats in the 14-unit T-maze, a task that is sensitive to hippocampal deficits. To assess the contribution of diabetes-induced elevations in corticosterone levels, we examined maze learning in diabetic rats that had levels of corticosterone ‘clamped’ through adrenalectomy and low dose corticosterone replacement. For comparison, we also tested a separate group of young and aged rats in the maze. Adrenally intact diabetic rats learned poorly in the 14-unit T-maze. Preventing the increases in corticosterone levels that accompanies the onset of experimental diabetes also prevented deficits in complex maze learning. The pattern of errors made by adrenally intact diabetic rats was similar to the pattern of errors made by aged rats, suggesting that the cognitive profiles of diabetic and aged rats share common features.

Keywords

stress; streptozocin; hippocampus; Stone maze; aging

The deleterious effects of stress on hippocampal structure and function are well-established. Levels of corticosterone, the primary stress-responsive hormone in rodents, are increased across multiple models of diabetes (Magarinos & McEwen, 2000; Watts, Mancham, Leedom, Rivard, McKay, Bao, Neroladakis, Monia, Bodenmiller, Cao, Zhang, Cox, Jacobs, Michael, Sloop, & Bhanot, 2005; Stranahan, Arumugam, Cutler, Lee, Egan, & Mattson, 2008). Chronic exposure to stress-induced elevations in corticosterone suppresses adult neurogenesis, reduces long-term potentiation, and impairs learning on hippocampus-dependent tasks (Conrad, Lupien, & McEwen 1999; Gould, Cameron, Daniels, Woolley, & McEwen, 1992; Korz & Frey, 2003).

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Previous studies have shown that diabetes impairs hippocampus-dependent learning, synaptic plasticity, and adult neurogenesis (Biessels, Kamal, Urban, Spruijt, Erkelens, & Gispen, 1998; Stranahan et al., 2008). However, the contribution of elevated corticosterone levels to diabetes-induced learning impairments remains largely unexplored. Here we report that lowering corticosterone levels attenuates learning deficits on a hippocampus-dependent, complex maze learning task in diabetic rats. Because adrenalectomized intact diabetic rats adopt inefficient navigation strategies that closely resemble those of aged animals, we conclude that diabetes induces a glucocorticoid dependent mechanism that results in aging-like alterations in cognition.

Adult male Sprague-Dawley rats (3–4 months old or 22–24 months old as indicated, N=7–10 animals per group; Charles River) were used in all of the studies. Rats were housed individually for a minimum of 2 weeks prior to beginning experiments, with food and water available ad libitum. The vivarium was maintained on a 12 hr light/12 hr dark cycle. Rats were bilaterally adrenalectomized under Isoflurane anesthesia and given corticosterone replacement via the drinking water (25µl/ml in 0.9% saline), as described (Stranahan et al., 2008). Ten days after adrenalectomy or sham operation, rats were injected with STZ (70mg/kg, IV) or vehicle (Fig. 2a). Glucose measurements were made using a commercially available Freestyle handheld glucose meter (Therasense, Alameda, CA) and STZ-treated rats were considered diabetic when serum glucose levels were >200 mg/dL. Corticosterone levels were quantified using a commercially available kit (Diagnostic Products Corp., San Diego, CA). Procedures were approved by the National Institute on Aging IACUC and followed the recommendations in the NIH *Guide for the Care and Use of Animals*.

Rats were tested in the 14-unit T-maze as described previously (Martin, Pearson, Kebejian, Golden, Keselman, Bender, Carlson, Egan, Ladenheim, Cadet, Becker, Wood, Duffy, Vinayakumar, Maudsley, & Mattson, 2007; Supplementary Movie 1). Briefly, all rats were handled daily for one week before pre-training on a one-way active avoidance task. The avoidance task required that the animal traverse a straight corridor (2 meters long) in less than ten seconds to avoid foot shock. The criterion for pre-training was thirteen out of fifteen successful avoidance runs. In the maze, the rat had ten seconds to navigate each section of the maze before administration of foot shock (0.5–0.8mA). Rats were trained in a series of fifteen massed trials during one day, with a ninety second inter-trial interval. Retention of spatial learning was tested over five trials conducted one week after acquisition training. Maze errors, run time, and time of beam breaks were recorded using custom software.

We also evaluated locomotor behavior and anxiety in the open field, using the same groups of diabetic and non-diabetic rats that were trained in the maze. The arena was a round chamber (120 cm diameter) and the time spent moving, as well as the time spent in the center and periphery of the maze, was compared across groups. Open field data were acquired using the HVS2020 video tracking system.

We evaluated shock reactivity after the open field testing. Rats were tested with 0.4, 0.6, and 0.8 mV shock intensities and responses were scored based on the presence or absence of 25 kHz ultrasonic vocalizations, which were detected using a bat detector (Batbox II, Ben Meadows Co., Janesville WI). The lowest stimulation intensity that evoked a vocalization was recorded as the animal's threshold for detection. In addition, the physiological reaction to the shock was categorized by a separate observer based on a scoring system where 0 indicated no reaction; 1, a paw lift; 2, alternate lifting of paws while stationary; and 3, escape-oriented locomotion. The minimum stimulation intensity required in order to evoke escape-oriented locomotion was recorded as the threshold.

The maze learning data were compared across diabetic and non-diabetic rats with different levels of corticosterone using 2×2 repeated measures ANOVA. Data from the aged animals were compared with young animals using one-way repeated measures ANOVA. Open field and shock reactivity data were analyzed using 2×2 ANOVA. All analyses were conducted using SPSS 11.0, with significance set at $p < 0.05$.

Fasting glucose levels were not significantly altered with age ($t_{14} = 1.73$, $p = 0.11$; mg/dL, young = 66.09 ± 5.64 , aged = 81.20 ± 2.97). STZ treatment, however, was associated with increased fasting glucose levels (mg/dL; sham/vehicle = 108.08 ± 6.41 , sham/STZ = 317.42 ± 13.31). Adrenalectomy and low-dose corticosterone replacement (ADX+CORT) did not influence the effect of STZ on glucose levels (mg/dL, ADX+CORT/vehicle = 110.92 ± 9.83 , ADX+CORT/STZ = 285.50 ± 10.97). We also confirmed that adrenalectomy and corticosterone replacement effectively normalized corticosterone levels (ng/ml, sham/vehicle = 12.69 ± 4.81 ; ADX + CORT/vehicle = 23.02 ± 4.77 , sham/STZ = 293.4 ± 53.44 , ADX+CORT/STZ = 28.54 ± 13.25).

Behaviorally, we observed no significant effects of age on pre-training performance in the straight run active avoidance task; latency to cross the straight runway and avoid the shock was similar across young and aged animals ($t_8 = 1.13$, $p = 0.28$, Fig. 1a). The number of trials to criterion was actually lower in the aged rats, mainly because young animals frequently engaged in non-productive escape strategies ($t_9 = 2.71$, $p = 0.024$, Fig. 1b). These results suggest that cognitive performance deficits in aged rats are not attributable to differences in their motivation to avoid and ability to detect the shocks.

Performance in the active avoidance task was also spared in young rats with diabetes (effect of diabetes, $F_{1,41} = 1.64$, $p = .21$; effect of adrenalectomy, $F_{1,41} = 0.52$, $p = .47$; Fig. 1c). We also observed no effect of diabetes or adrenalectomy in the number of trials to criterion during pre-training ($F_{1,30} = 1.82$, $p = 0.18$, Fig. 1d). The absence of any differences in pre-training performance suggests that STZ-diabetic rats do not exhibit gross motor impairments.

The 14-unit T-maze is a hippocampus-dependent, complex maze learning task in which animals make a series of right-left decisions (Fig. 2b; Supplementary Movie 1; Jucker, Kametani, Bresnahan, & Ingram, 1990). During maze training, inactivity (but not incorrect decisions) was associated with shocks delivered through the grid floor. This aversive motivation is sufficient to encourage forward taxis in the maze, and young rats typically reached asymptotic performance within the first six trials (Fig. 2c). Older rats, however, made more errors throughout acquisition training ($F_{1,8} = 20.56$, $p = 0.002$).

Naive rats typically make a specific pattern of errors in the maze during the initial training trials. This pattern is based on the “win-shift” strategy employed by foraging animals (Burke, Cieplucha, Cass, Russell, & Fry, 2002). In the maze, these errors are composed of sequential right-left-right or left-right-left turns that do not produce forward taxis along the correct path, termed ‘alternation errors’ (Ingram, 1985). With practice, rats learn to inhibit the alternation strategy and make a sequence of right-right-left or left-left-right turns, as appropriate to make forward progress along the most direct route through the maze. Aged animals are specifically impaired with respect to alternation errors (Fig. 2d; $F_{1,4} = 2.43$, $p = 0.04$). This suggests that older animals have greater difficulty switching from the win-shift strategy (right-left-right or left-right-left) to the win-stay strategy (right-left-left or left-right-right; Spangler, Chachich, Curtis, & Ingram, 1989).

As previously observed, young non-diabetic rats learned to navigate the maze within the first six trials ($F_{1,60} = 82.00$, $p < 0.0001$; Fig. 2). In contrast, adrenally intact, young STZ-diabetic rats made significantly more errors throughout the acquisition trials ($F_{4,68} = 3.41$, $p = .03$; Fig. 2e). This deficit was not observed in rats that had been adrenalectomized and given corticosterone replacement prior to the induction of experimental diabetes. Adrenalectomy and corticosterone

replacement did not alter the rapid acquisition of navigation skills in non-diabetic rats ($F_{1,15}=0.19$, $p=0.67$).

Adrenally intact, non-diabetic rats quickly learned to inhibit the win-shift strategy, and made no alteration errors after the first three trials ($F_{1,30}=2.70$, $p=0.03$; Fig. 2f). Adrenalectomized non-diabetic rats also abandoned the alternation strategy following the initial three trials. However, sham-operated diabetic rats persevered with the alternation strategy throughout maze training. This deficit was not observed in adrenalectomized diabetic rats that had received low dose corticosterone replacement. These patterns indicate that STZ diabetes is associated with an aged-like cognitive phenotype that can be reversed by lowering levels of corticosterone.

Our measure of retention consisted of five trials in the maze, with a ninety-second intertrial interval, conducted one week after the initial session of massed acquisition training trials. Both sham-operated and adrenalectomized non-diabetic rats were able to navigate quickly through the maze during retention testing. In contrast, sham-operated diabetic rats made significantly more errors (Fig. 2e; $F_{1,30}=15.16$, $p=.0005$). These deficits were not observed in adrenalectomized diabetic rats receiving corticosterone replacement. Overall, sham-operated diabetic rats performed poorly in the retention trial, although it remains to be determined whether this was due to a true retention deficit, or the impaired acquisition of efficient navigation skills.

The retention deficits that we observed in sham-operated, STZ-diabetic rats recapitulated our observations in aged rats. Twenty-four month old rats also made more errors during retention testing ($F_{1,4}=3.48$, $p=0.025$, Fig. 2c). These similarities further reinforce the idea that untreated diabetes is associated with aging-like cognitive deficits.

To test for differences in exploratory behavior, we measured open field activity in diabetic and non-diabetic rats with different levels of corticosterone. While STZ-diabetic rats exhibit higher levels of locomotor activity, this change occurred in both sham-operated and adrenalectomized diabetic rats (for the effect of diabetes on distance traveled, $F_{1,29}=6.21$, $p=.01$, Fig. 3a). Because increased locomotion was observed in both adrenally intact diabetic rats, which showed impaired spatial learning performance, and in diabetic rats that had received adrenalectomy and corticosterone replacement, which showed no learning impairments, it is unlikely that STZ-induced increases in locomotion could account for differences in maze performance. There was no effect of any of the treatments on the amount of time spent in the center of the open field ($F_{1,29}=0.66$, $p=0.4$; Fig. 3b).

Next we evaluated shock reactivity across diabetic and non-diabetic rats that had been adrenalectomized or sham-operated. There was no significant change in the threshold for emission of ultrasonic vocalizations in response to shock of different amplitudes ($F_{1,30}=1.47$, $p=0.23$, Fig. 3c). There was also no change in the minimum stimulation intensity required to elicit escape-oriented locomotion ($F_{1,30}=0.23$, $p=0.78$, Fig. 3d). These results suggest that performance deficits in the maze were not attributable to changes in the ability to detect the shocks.

Diabetes induces a number of neurological changes that may render the hippocampus more susceptible to age-related structural and functional deficits. In these studies, we have demonstrated a role for elevated corticosterone levels in the diabetes-induced impairment of hippocampus-dependent learning. A number of different groups have reported deficits in water maze learning following induction of diabetes with streptozocin (Biessels et al., 1998; Stranahan et al., 2008), but this study represents the first demonstration of impaired spatial-episodic memory in a task that requires egocentric rather than allocentric encoding. Because the water maze relies on visual cues, while the 14-unit T-maze can be done in the absence of visual cues (Ingram, 1985), impairment on this task may result from deficits in egocentric

encoding. Since reduced performance in both types of tasks exists, it is likely that untreated insulin-deficient diabetes results in deficits across multiple neural systems underlying hippocampus-dependent learning.

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Figure 1. Pretraining performance in an active avoidance task is not adversely affected by aging, diabetes, or corticosterone manipulation

(a), Twenty-four month old rats showed no change in the latency to cross a straight runway, motivated by avoidance of shock. (b), Old rats required fewer trials to reach criterion on the pretraining task compared to young rats; the criterion was thirteen out of fifteen successful trials. (c), Diabetes and adrenalectomy with low-dose corticosterone replacement did not influence pretraining latencies in the straight run task. (d), The number of trials to criterion were similar across diabetic and non-diabetic rats with different levels of corticosterone.

Figure 2. Diabetic young rats exhibit a navigation strategy similar to that of old control rats; this can be reversed by lowering levels of corticosterone

(a), Experimental design for studies in diabetic rats. (b), Diagram showing the correct path through the 14-unit T-maze. (c), Twenty-four month old rats made more errors in during acquisition training (Trials 1–15), and also exhibit retention deficits (Trials 16–20). (d), Aged rats made more frequent alternation errors during maze training. (e), Adrenally intact young diabetic rats made more errors throughout acquisition training (Trials 1–15) and during retention testing (Trials 16–20). In contrast, young diabetic rats with levels of corticosterone ‘clamped’ through adrenalectomy and corticosterone replacement performed at a level

identical to non-diabetic animals. There was no effect of adrenalectomy and low-dose corticosterone replacement on performance in non-diabetic rats.

Figure 3. Exploratory activity and shock reactivity in diabetic rats with different levels of corticosterone

(a), STZ-treated rats exhibit increased open field locomotion, irrespective of glucocorticoid status. However, because we observed increased activity in sham-operated STZ-diabetic rats, which show learning impairment, and in adrenalectomized diabetic rats, which had no such deficit, it is unlikely that changes in locomotor activity could account for our results in the maze. (b), There were no differences in the amount of time spent in the center of the open field arena across any of the groups. (c), There was no effect of diabetes or adrenalectomy and corticosterone replacement on the minimum stimulation intensity required to elicit ultrasonic vocalizations (USVs). (d), There were no group significant differences in the minimum stimulation intensity required to induce escape-oriented locomotion, suggesting that differences in learning were not attributable to differences in the ability to detect the shocks. For all graphs, error bars represent SEM, and asterisk (*) indicates significance following 2-way ANOVA.