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Synergistic Exacerbation of Mitochondrial and Synaptic Dysfunction and Resultant Learning and Memory Deficit in a Mouse Model of Diabetic Alzheimer's Disease

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Abstract

Diabetes is considered to be a risk factor in Alzheimer's disease (AD) pathogenesis. Although recent evidence indicates that diabetes exaggerates pathologic features of AD, the underlying mechanisms are not well understood. To determine whether mitochondrial perturbation is associated with the contribution of diabetes to AD progression, we characterized mouse models of streptozotocin (STZ)-induced type 1 diabetes and transgenic AD mouse models with diabetes. Brains from mice with STZ-induced diabetes revealed a significant increase of cyclophilin D (CypD) expression, reduced respiratory function, and decreased hippocampal long-term potentiation (LTP); these animals had impaired spatial learning and memory. Hyperglycemia exacerbated the upregulation of CypD, mitochondrial defects, synaptic injury, and cognitive dysfunction in the brains of transgenic AD mice overexpressing amyloid- β as shown by decreased mitochondrial respiratory complex I and IV enzyme activity and greatly decreased mitochondrial respiratory rate. Concomitantly, hippocampal LTP reduction and spatial learning and memory decline, two early pathologic indicators of AD, were enhanced in the brains of diabetic AD mice. Our results suggest that the synergistic interaction between effects of diabetes and AD on mitochondria may be responsible for brain dysfunction that is in common in both diabetes and AD.

Keywords

Alzheimer's disease; cognitive impairment; diabetes; long-term potentiation; mitochondria; synaptic injury

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INTRODUCTION

Alzheimer's disease (AD) is an age-dependent neurodegenerative disorder functionally characterized by progressive cognitive decline. The primary pathological alterations in AD in brain parenchyma are progressive accumulation of amyloid- β peptide ($A\beta$) derived from amyloid- β protein precursor ($A\beta$ PP) and neurofibrillary tangles derived from abnormal phosphorylation of tau protein [1, 2]. $A\beta$ is formed after sequential cleavage of $A\beta$ PP and its most abundant isoforms are $A\beta_{40}$ and $A\beta_{42}$. Initially, $A\beta$ products are non-fibrillary and soluble, which can directly affect many intracellular components to induce neuronal malfunction. Among intracellular targets of $A\beta$, mitochondria play a central role in $A\beta$ -mediated toxicity in synaptic injury, cognitive decline, and neuronal loss [3–15]. Accumulation of $A\beta$ in AD patient brain and several transgenic mouse models of AD that overexpress $A\beta$ progressively induce the increase of cyclophilin D (CypD), a critical component of mitochondrial permeable transition pore (mPTP), mitochondrial respiratory chain impairment, and energy failure. Interaction of $A\beta$ with mitochondrial proteins, such as amyloid-binding alcohol dehydrogenase (ABAD) [4, 15–18] and CypD [11, 19, 20], exacerbates $A\beta$ -induced mitochondrial respiratory complex enzyme defects, synaptic plasticity impairment, and learning and memory decline. Thus, CypD and its associated mitochondrial function play a critical role in AD progression.

Both AD and diabetes are prevalent in elderly patients. Diabetes is a disorder of glucose metabolism characterized by perturbation of the insulin-signaling pathway, which results in hyperglycemia. These disturbances result from hypoinsulinemia diabetes due to impaired production of insulin in the case of type 1 diabetes, or from insulin resistance in type 2 diabetes. Diabetes is a chronic disease with multiple severe complications and affects an estimated 25.8 million patients in the United States, according to the report in 2011 National Diabetes Fact sheet of the United States Centers for Disease Control and Prevention. In addition to the well-known adverse effects of diabetes on the cardiovascular and peripheral nervous systems, diabetes can also causes complications in the central nervous system (CNS) leading to ischemic stroke, episodic memory impairment [21], and synaptic plasticity deficit [22–30]. Synaptic dysfunction and learning and memory deficit, common to AD, have been observed in diabetic patients; in addition, many other features of AD pathology are identified in diabetic subjects. For example, elevated oxidative stress and impaired mitochondrial respiratory enzyme activity is often seen in diabetes in a variety of cell types including islet cells [31], hepatocytes [32], skeletal muscle cells [33, 34], blood mononuclear cells [35], endothelial cells [36], and dorsal root ganglion neurons [37, 38]. Mitochondrial dysfunction is also suggested to be a causative factor in the development of insulin resistance in skeletal muscle cells and hyperglycemia in type 2 diabetes [39, 40]. Compared to other cells, neurons are morphologically quite complicated and more susceptible to mitochondrial dysfunction because the neuronal transmission is tightly regulated by energy homeostasis in synapses. Mitochondrial alterations in hypothalamus have recently been identified as a control mechanism for food intake and energy expenditure, which in turn causes diet-induced obesity and diabetes [41]. Regardless whether mitochondrial dysfunction is a cause or a consequence of diabetes, we believe mitochondria play a critical role in diabetes-induced brain dysfunction.

Recent epidemiological studies support a link between diabetes and AD in elderly patients [42]. A number of studies associate diabetes mellitus with AD pathogenesis [43–46]; indeed, it is considered a potential risk factor for developing AD [47]. Diabetes exacerbates features of AD pathology and learning and memory deficits in experimentally induced diabetes in mouse models of AD [46, 48–50]. However, the mechanisms underlying diabetes-induced synaptic dysfunction are not clear.

Mitochondrial dysfunction is critical to the development and progression of AD [3–15]. As the mitochondria are particularly affected by hyperglycemia in diabetes [31–38] and mitochondria play a key role in supporting synaptic plasticity, one of the mechanisms of learning and memory, [51, 52], we induced type 1 diabetes in transgenic AD mice over-expressing human A β (mA β PP mice) to determine whether mitochondrial perturbation is associated with the contribution of diabetes to AD progression.

MATERIALS AND METHODS

Animals

All studies were performed using the well-characterized transgenic mA β PP mouse model, which overexpresses hA β PP that encodes the hA β PP751-, and hA β PP770-bearing mutations of human AD (V717F, K670N, and M671L) and the control wild-type (WT) mice. Transgenic lines were maintained by crossing mA β PP transgenic mice with non-transgenic wild type (WT) C57BL/6 breeders. Animals were housed up to five per cage with free access to food and water and maintained in a vivarium proved by the American Association for the Accreditation of Laboratory Animal Care. All animal studies were approved by the Animal Care and Use Committee of the University of Kansas in accordance with the National Institutes of Health guidelines for animal care. Five to ten male mice were used per group.

Induction of type 1 diabetes

After being fasted for five hours, insulin-deficient diabetes was induced in 3 month-old mice by intraperitoneal (i.p.) injection of streptozotocin (STZ, Sigma, St. Louis, MO; dose 60 mg/kg dissolved in 100 mM citric acid, pH 4.5) on 5 consecutive days. Hyperglycemia was confirmed using a strip-operated reflectance meter on a blood sample from tail prick 2 weeks after STZ injection and in another sample collected after the study (2 to 3 months after STZ injection). Citric acid (pH 4.5) was injected as a vehicle control. 0.9% saline solution was administered subcutaneously to maintain animal health.

Morris water maze task

Two months following STZ treatment, mice were trained in the Morris water maze. The maze is a circular pool of water, maintained at $24 \pm 1^\circ\text{C}$, and divided into imaginary 4 quadrants. During daily training, an opaque (from white non-toxic paint) platform was placed in one of the quadrants and hidden 1 cm below the surface of water. Mice were trained to locate the hidden platform during four trials per day over 8 days. Starting points were changed every day. For each trial, mouse was allowed to search the platform for 60 s. Mice failed to find the platform within 60 s will be guided to the platform and rest for 15 s

before next trial. The time required for locating the submerged platform is termed “latency”. Twenty-four hours after the last training session on day 8, each animal was given a single 60 s probe trial. The submerged platform was removed and the animal was expected to enter the target quadrant and make more entries into the location that previously contained the platform (memory retention). Time spent in the target quadrant, swim speed, and the number of entries into the platform location were recorded by video tracking.

Electrophysiological studies

Electrophysiological recordings were performed on coronal hippocampal slices (400 μm in thickness), as described [11]. Briefly, the hippocampal slices were recovered at 37°C for at least 1 h and then maintained in an interface chamber at 29°C and perfused with artificial cerebrospinal fluid (124 mM NaCl, 4.4 mM KCl, 1 mM Na_2HPO_4 , 25 mM NaHCO_3 , 2 mM CaCl_2 , 2.0 mM MgSO_4 , and 10 mM glucose) continuously bubbled with 95% O_2 and 5% CO_2 . Field-excitatory post-synaptic potentials (fEPSPs) were recorded for the CA1 hippocampal region by placing both the stimulating and the recording electrodes in the CA1 stratum radiatum. Basal synaptic transmission (input-output curve) was assayed by plotting the stimulus voltage (V) against the slopes of fEPSPs to generate input-output relations. A 30 min baseline recording was established using low-frequency stimulation (0.033 Hz; 0.1 ms impulse duration) and the adjusted intensity that induced fEPSPs with ~30% of the maximal fEPSP amplitude. The LTP was induced using theta-burst stimulation (4 pulses at 100 Hz, with bursts repeated at 5 Hz, and each tetanus, including 3 10-burst trains separated by 15 s. Values of the fEPSP slope were expressed as mean \pm SEM percentage change relative to their mean baseline amplitude.

Immunoblotting analysis

We have performed western blot to determine CypD content in hippocampus homogenates. The hippocampi were lysed in cell lysis buffer (Cell signaling #9803; 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate) containing protease inhibitor cocktail (Calbiochem, set V, EDTA free). Equal amount of proteins were loaded and separated by SDS-PAGE (12% Bis-Tris gel, Invitrogen), and then transferred to nitrocellulose membrane (Amersham). Membrane was blocked in TBST buffer (20 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20) containing 5% non-fat dry milk (SantaCruz) for 1 h at room temperature, and then incubated and gently shaken overnight (at 4°C) with anti-CypD IgG (1:2000, generated in our laboratory) in TBST containing 5% non-fat dry milk; this was followed by incubation with corresponding secondary antibody for 1 h at room temperature. Chemiluminescence was detected by using ECL (GE). The same membrane was stripped and reprobed with antibody to tubulin (1:10,000, Sigma) as the protein loading control.

Mitochondrial isolation

Cortical mitochondria were isolated from whole brain cortex of mice as described previously [21]. Samples were placed in 9 ml of ice-cold mitochondria isolation buffer [225 mM mannitol, 75 mM sucrose, 2 mM K_2HPO_4 (pH 7.2)], and homogenized (10 strokes) using a Douce homogenizer (Kontes Glass Co.). Homogenate was centrifuged at 1300 g for

5 min at 4°C. The resultant supernatant was then centrifuged at 34,000 g for 10 min after layering on 15% Percoll. After centrifugation, the homogenate was resuspended and incubated for 5 min on ice in 20 ml of mitochondria isolation buffer with 0.02% digitonin, centrifuged at 8,000 g for 10 min. The pellet was washed twice in 1.5 ml mitochondria isolation buffer and centrifuges again at 8,000 g for 10 min. The final pellet were resuspended in 200ul mitochondria isolation buffer. Total protein concentration of isolated mitochondria fraction was determined by Bio-Rad DC protein assay (Bio-Rad Lab).

Mitochondrial oxygen consumption assay

Mitochondrial respiration rate was measured using a Clark oxygen electrode (Oxytherm, Hansatech) at with mitochondria kept at 30°C as previously described [21]. Three hundred µg mitochondria were added to 1 ml potassium buffer. State 3 respiration was triggered by the addition of 150 µM adenosine diphosphate (ADP) in the presence of 5 mM glutamate and 5 mM malate; state 4 respiration is defined as oxygen consumption after ADP has been consumed. The respiratory control ratio (RCR) of mitochondria is calculated as state 3/state 4.

Mitochondrial respiratory chain complex activity measurement

Mitochondrial respiratory chain complex activity was measured using the isolated mitochondrial fraction as described before [21, 53, 54]. Briefly, NADH: ubiquinone oxidoreductase (COX I) enzyme activity was determined in 25 mM potassium buffer containing KCl, Tris-HCl and EDTA (pH 7.4). The change in absorbance was monitored at 340 nm wavelength every 20 sec for 6 min using an Amersham Bio-sciences Ultrospect 3100 pro spectrophotometer. In the presence of mitochondria (50 µg protein), 2 µg/ml antimycin, 5 mM magnesium chloride, 2 mM potassium cyanine and 65 µM co-enzymes Q1 were added and the oxidation of NADH was recorded for 3 min; then 2 µg/ml rotenone was added and absorbance was measured for another 3 min. For measurement of cytochrome c oxidase (COX IV) enzyme activity, mitochondrial samples (50 µg protein) were gently added to a cuvette containing 0.95 mL of 1× assay buffer (10 mM Tris-HCl, and 120 mM potassium chloride, pH 7.0), and the reaction volume was brought to 1.05mL with the addition of 1× enzyme dilution buffer (10 mM Tris-HCl, pH 7.0). The reaction was then initiated by addition of 50 µL of ferrocy-tochrome substrate solution (0.22 mM). The change in absorbance of cytochrome c at 550 nm was measured using a kinetic program with a 5-s delay, 10-s interval and a total of 6 readings on an Amersham Biosciences Ultrospect 3100 pro spectrophotometer. Non-enzymatic background was measured in the sample without the isolated mitochondrial fraction.

Data analysis

Data are presented as mean ± SEM. Statistics analyses were performed using Statview software (SAS Institute). One-way ANOVA was followed by individual *post hoc* Fisher tests for statistical comparison. $p < 0.05$ was considered statistically significant.

RESULTS

Experimental induction of type 1 diabetes in mA β PP and nonTg mice

Mice, which were injected with STZ (60 mg/kg i.p.) after five hours of fasting for five successive days, exhibited hyperglycemia (blood sugar >250 mg/dl) 2 weeks after initiation of STZ injection (339.07 ± 50.9 mg/dl in nonTg/STZ mice and 342.88 ± 36.2 mg/dl in mA β PP/STZ mice, Fig. 1A). Two months later, blood glucose levels for diabetic mice remained significantly high in both mA β PP (498.09 ± 33.5 mg/dl) and nonTg (517.09 ± 27.6 mg/dl) mice (Fig. 1A). All mice maintained a healthy weight over the 2-month period (Fig. 1B). Mice injected with vehicle gained a small amount of weight over the 2-month period. Mice with induced diabetes lost weight, but the body weight loss was not significantly different between mA β PP (-6.30 ± 1.4 g) and nonTg (-5.79 ± 1.0 g) diabetic animals ($p > 0.05$, Fig. 1B). These results confirm that both nonTg and mA β PP mice developed type 1 diabetes with hyperglycemia as early as 2 weeks after STZ treatment and the resultant hyperglycemia was sustained more than 2 months post STZ injection.

Expression of CypD in diabetic and non-diabetic nonTg and mA β PP mice

CypD is a necessary component of mPTP formation, triggering the opening of mPTP by translocation of CypD to the inner membrane of mitochondria and causes oxidative stress. Previously, we observed up-regulation of CypD in mA β PP mice as compared to nonTg mice at the age of 6–12 months [11]. It is also demonstrated that CypD-mediated mPTP opening in face to high-fat diet treatment causes mitochondrial dysfunction and insulin resistance in skeletal muscle [55]. Therefore, it is possible that diabetes exaggerates CypD upregulation in the mA β PP mouse brain. In the present study, we compared CypD levels in mA β PP mice to nonTg mice when made diabetic. According to immunoblotting analysis, CypD levels in non-diabetic mA β PP or diabetic nonTg hippocampus were significantly increased as compared to non-diabetic nonTg controls (Fig. 2, $p < 0.05$). When mA β PP mice rendered to STZ-induced type 1 diabetes, the CypD expression was further increased significantly (Fig. 2, $p < 0.05$ versus mA β PP/Vehicle or nonTg/STZ mice).

Increased brain mitochondrial dysfunction in diabetic mA β PP mice

Since mitochondria are vulnerable to changes in glucose homeostasis in diabetes and play a critical role in AD progression, we investigated whether experimentally induced type 1 diabetes exacerbated mitochondrial dysfunction in relatively young transgenic AD mice (before onset of clinically observable symptoms) with otherwise normal mitochondrial function. Mitochondrial respiration is critical for energy metabolism, cell functioning and survival. Decreased mitochondrial respiratory function indicates severe damage to mitochondria. We first analyzed mitochondrial respiration by measuring ADP-stimulated RCR. During the consumption of 150 μ M ADP, STZ-treated nonTg mice (nonTg/STZ) cortical mitochondria showed decreased RCR as compared to that found in vehicle-treated nonTg (nonTg/Vehicle mitochondria (Fig. 3A). With vehicle treatment, mA β PP cortical mitochondria showed compromised RCR (4.92 ± 0.1 in mA β PP/Vehicle mice, $p < 0.05$ versus nonTg/Vehicle, Fig. 3A) compared to nonTg/Vehicle mitochondria. While the RCR was not significantly different between nonTg/STZ and mA β PP/Vehicle mitochondria ($p > 0.5$),

STZ-induced diabetic mA β PP mice had a significant reduction in RCR compared to vehicle-treated mA β PP mice ($p < 0.05$, Fig. 3A).

Cytochrome c oxidase (COX IV) activity was not significantly different when comparing nonTg/ Vehicle, nonTg/STZ and mA β PP/Vehicle mice (82.60 ± 2.9 nmol/mg protein/min in nonTg/Vehicle, 78.00 ± 4.7 nmol/mg protein/min in nonTg/STZ, 81.17 ± 3.5 nmol/mg protein/min in mA β PP/Vehicle mice, respectively, $p > 0.05$, Fig. 3B). However, mA β PP/STZ mitochondria showed a significantly decreased COX IV activity compared to non-diabetic mA β PP mitochondria (64.20 ± 8.0 nmol/mg protein/min in mA β PP/STZ mice, $p < 0.05$, Fig. 3B). Compared to vehicle-treated nonTg mice (202.32 ± 21.5 nmol/mg protein/min), COX I (NADH: ubiquinone oxidoreductase) activity was not significantly altered in nonTg/STZ (201.38 ± 31.1 nmol/mg protein/min) or mA β PP/Vehicle (197.42 ± 26.9 nmol/mg protein/min) mice ($p > 0.05$, Fig. 3C). In contrast, STZ-mA β PP mice had markedly decreased COX I activity (by 50–60%) compared to non-diabetic mA β PP mitochondria ($p < 0.01$, Fig. 3C).

Taken together, these data indicated that the mitochondrial respiration was impaired in STZ-induced diabetic nonTg mice (Fig. 3A) while COX I and COX IV activity remained unchanged (Fig. 3B–C). As reported previously [21], mA β PP mice at six to seven months of age had reduced RCR (Fig. 3A) but no detectable alterations in COX I or COX IV (Fig. 3B–C). Intriguingly, the young mA β PP mice with experimentally induced type 1 diabetes (STZ injection starting at three to four months of age) of two to three months duration showed early deficits in mitochondrial respiration and decline in both COX I and COX IV activity (Fig. 3).

Early deficit in synaptic plasticity in diabetic mA β PP mice

Given that mitochondria are vital for maintenance of synaptic function and transmission, we examined synaptic transmission under basal conditions, paired-pulse facilitation (PPF, a form of short-term synaptic plasticity), and during long-term potentiation (LTP, a form of synaptic plasticity that is widely studied as a cellular model for learning and memory). Consistent with previous observations [23, 29, 30], fEPSPs in the CA1 stratum radiatum revealed no alterations in basal synaptic transmission (BST) and PPF in nondiabetic and diabetic nonTg mice ($p > 0.05$ nonTg/Vehicle versus nonTg/STZ, Fig. 4A–B). Compared to vehicle-treated nonTg mice, mA β PP/Vehicle mice showed no significant difference in BST and PPF at 6–7 months of age ($p > 0.05$ mA β PP/Vehicle versus nonTg/Vehicle, Fig. 4A–B) until 10–12 month of age [11, 16, 56]. CA1 region hippocampal LTP was significantly decreased in diabetic nonTg mice ($143.07 \pm 5.1\%$) compared to non-diabetic nonTg mice ($209.41 \pm 12.5\%$, $p < 0.01$, Fig. 4C–D). However, diabetic mA β PP mice had significantly reduced hippocampal LTP compared to non-diabetic mA β PP mice ($112.22 \pm 5.9\%$, $p < 0.01$ versus mA β PP/Vehicle and $p < 0.05$ versus nonTg/STZ, respectively, Fig. 4C–D). Young (6 to 7 months of age) vehicle-treated mA β PP mice had normal LTP ($196.20 \pm 12.9\%$) ($p > 0.05$, mA β PP/Vehicle versus nonTg/Vehicle, Fig. 4C–D). Young diabetic mA β PP mice had no changes in BST or PPF ($p > 0.05$ mA β PP/Vehicle versus mA β PP/STZ, Fig. 4A, B). These data demonstrate an early deficit in synaptic dysfunction in diabetic mA β PP mice, suggesting that type 1 diabetes worsens synaptic damage in the early stage AD brain,

possibly due to deleterious effects of diabetes on mitochondrial respiratory function and enzyme activity.

Learning and memory impairments were further aggravated in diabetic mA β PP mice

Behavioral change is a functional manifestation of mitochondrial dysfunction and synaptic injury. Previous studies showed that hippocampus is particularly susceptible to diabetes-induced spatial learning and memory impairment [23, 29] due to synaptic plasticity deficit. Similarly, hippocampus-dependent cognitive deficits have been reported in transgenic mA β PP mice, which from the age of six months to 22–24 months [11]. We next assessed the synergistic effects of type 1 diabetes on learning and memory in young mA β PP mice. We evaluated spatial learning and memory in mice at age of six to seven months using a Morris water maze as previously described [11, 20]. Vehicle-treated nonTg mice showed good learning and memory during the training session (Fig. 5A). mA β PP mice treated with vehicle or STZ-treated nonTg mice required a longer time (higher latency) to locate the hidden platform during trials (Fig. 5A), indicating impaired spatial learning and memory. Notably, STZ-treated mA β PP mice had the longest latency to find the hidden platform (Fig. 5A).

In the memory retention test, both nonTg/STZ mice (33.16 \pm 2.6% time in the target quadrant, and 2–3 crosses to the platform location) and mA β PP/Vehicle mice (23.80 \pm 1.9% time in the target quadrant, and 1–2 crosses to the platform location) displayed memory deficit compared to nonTg/Vehicle mice (45.56 \pm 1.2% time in the target quadrant, and 4–5 crosses to the platform location) (p <0.01, Fig. 5B, C). Similarly, memory retention was further impaired (11.88 \pm 3.3% time in the target quadrant, and 0–1 crosses to the platform location) in diabetic mA β PP mice (p <0.01 mA β PP/Vehicle versus mA β PP/STZ, Fig. 5B, C). We did not find any significant difference in swimming speed in the four groups of mice (Fig. 5D). These data demonstrate that diabetes accelerates cognitive dysfunction in AD mice.

DISCUSSION

There is growing evidence of links between AD and diabetes [43–46]. Aged patients with diabetes are at increased risk for AD [47]. While experimental induction of diabetes in mouse models of AD exacerbates features of AD, including increased A β formation and accumulation [46, 48–50], tau phosphorylation [46, 48, 50], and learning and memory deficit [46, 48–50], the underlying mechanisms are unclear. Mitochondria are particularly affected by hyperglycemia in diabetes [31–38] and mitochondrial and synaptic dysfunction is an early pathological feature of AD-affected brain, playing a critical role in AD progression [3–15]. Therefore, the two diseases might share a common mitochondria-related mechanism that leads to brain dysfunction. In the present study, we induced type 1 diabetes in mA β PP transgenic mice to test whether mitochondrial perturbation secondary to diabetes contributes to AD progression. Indeed, experimental induction of type 1 diabetes accelerated mitochondrial defects and exacerbated impairments in synaptic plasticity and spatial learning and memory in mA β PP mice at 6–7 month old age.

CypD is a key component of mPTP. Oxidative and other cellular stresses promote CypD translocation to the inner membrane [57–62], which in turn triggers opening the mPTP leading to cell death. Low expression of CypD in brain mitochondria increases resistance of mPTP induced by calcium [63], whereas high levels of CypD in neuronal mitochondria result in their greater vulnerability to the opening of mPTP and require higher levels of cyclosporin A (an inhibitor of CypD) to inhibit mPTP opening [64]. Consistent with these observations, genetic deficiency in CypD protects cells against Ca^{2+} -, oxidative stress-, or $\text{A}\beta$ -induced cell death [61, 65–67]. CypD has been reported to play a vital role in both AD-induced brain dysfunction [11, 20, 68] and diabetes-induced peripheral organs damages such as heart [69, 70] and skeletal muscle [55]. In our present study, we found that CypD expression levels were significantly elevated in hippocampus of STZ-induced diabetic nonTg mice as compared to vehicle-nonTg mice (Fig. 2, $p < 0.05$). When mA β PP mice were subjected to STZ-induced type 1 diabetes, the CypD expression was further increased significantly in hippocampus of diabetic mA β PP mice as compared with non-diabetic mA β PP mice (Fig. 2, $p < 0.05$ versus mA β PP/Vehicle or nonTg/STZ mice). These data suggested that CypD expression level was increased in diabetes mellitus and further enhanced in an $\text{A}\beta$ -enriched environment. Increased amount of CypD in mitochondria would trigger/enhance the opening of mPTP, which leads to colloid osmotic swelling of the mitochondrial matrix, dissipation of the inner membrane potential (Ψ_m), accumulation/generation of reactive oxygen species, and the release of many pro-apoptogenic proteins and procaspases [71–75]. Indeed, our previous studies demonstrated an upregulation of CypD in non-diabetic mA β PP mice as compared to nonTg mice at the age of 6–12 months [11]. CypD-deficiency in AD mice overexpressing human $\text{A}\beta$ protected from $\text{A}\beta$ -induced mitochondrial oxidative stress, increased capacity of mitochondrial calcium uptake, improved synaptic function, and ameliorated cognitive deficits [11, 20]. Additionally, CypD-mediated mPTP opening in face to high-fat diet treatment causes mitochondrial dysfunction and insulin resistance in skeletal muscle [55]. Therefore, CypD-mediated mPTP opening may be one of the mechanisms for diabetes-accelerated mitochondrial dysfunction in AD brain, which could ultimately contribute to synaptic injury and cognitive deficits in diabetic AD mice. Other mPTP components such as the voltage dependent anion channel [76] in the outer membrane and the adenine nucleotide translocase in the inner membrane may also contribute importantly to mitochondrial dysfunction in diabetes mellitus. The detailed mechanistic studies relevant to mPTP in diabetes are required for the further investigation.

Mitochondrial dysfunction is known to play an important mechanistic role in both diabetes and AD. Mitochondrial respiration is the most important function of mitochondria and healthy respiratory function is required for normal mitochondrial function. While no significant alterations were observed in COX I or COX IV activity, decreased mitochondrial RCR in cortical mitochondria of diabetic nonTg and non-diabetic mA β PP littermates suggest impaired mitochondrial function. Early mitochondrial respiratory rate decline and impaired COX I and COX IV activity in cortical mitochondria from STZ-induced diabetic mA β PP mice suggest that type 1 diabetes exaggerates mitochondrial dysfunction at the early stage AD. Because respiratory chain reflects electron flux through complexes I, III, and IV, inactivation of enzyme activities associated with anyone/or all of these complexes in the

respiratory chain could disturb the respiratory chain function, leading to decreased oxygen consumption. Thus, reduced complex I and IV activity could be the mechanism for perturbing mitochondrial respiratory chain function observed in diabetic mA β PP mice.

Mitochondria are essential for synaptic function by providing energy and regulating intra-synaptic metabolic homeostasis [51, 52]. Damaged mitochondria in diabetic mA β PP mice are associated with synaptic injury. Consistent with previous reports [23, 29, 30], we observed LTP impairment in hippocampal CA1 region of STZ-treated animals. The LTP defect was significantly exacerbated in diabetic mA β PP mice though there was no significant synaptic plasticity deficit in young (6–7 month of age) mA β PP littermates, suggesting an accelerated AD progression with diabetes insult.

Decreased mitochondrial and synaptic function in diabetic young mA β PP mice is associated with a poor performance on the Morris water maze, reflecting impaired memory formation and retention. These differences in performance are not due to decreased swim speed, thereby excluding systemic consequences such as weight loss. We observed mildly impaired spatial learning and memory in diabetic nonTg mice compared to non-diabetic controls (Fig. 5). STZ-induced diabetic mA β PP mice experienced significantly enhanced cognitive decline compared to non-diabetic mA β PP mice. Thus, diabetes may be an exacerbating factor, at least in part, for AD-associated changes in learning and memory, especially in early stage AD.

Another potential mechanism underlying diabetes-induced mitochondrial and synaptic dysfunction in mA β PP mice may root in potentiated A β production, in parallel to the enhanced CypD expression. In our present study, A β ₄₀ and A β ₄₂ levels were significantly increased in mA β PP mice with and without STZ treatment compared to STZ-treated nonTg mice. However, no significant differences of A β levels were found between STZ-mA β PP and vehicle-mA β PP mice (data not shown). These results are consistent with previous studies showing no effect on A β production in either STZ-injected mA β PP/PS1 mice (A β PP/Ps1-STZ mice) [49] or leptin-deficient mA β PP subjects (mA β PP-ob/ob mice) [77]. Using high-fat diet-induced aging mouse model, A β levels were increased in brain of high-fat diet-induced senescence-accelerated SAMP8 mice (HF-SAMP8 mice) [50]. In addition, increasing evidence demonstrate that induction of chronic diabetes in transgenic mouse model of AD (in both HF-SAMP8 and mA β PP-ob/ob mice) increases A β accumulation in brain microvascular vessels [46, 77]. These studies suggest the close relationship of the chronic diabetes with A β accumulation. Our recent preliminary results showed a significant increased cerebellar A β level in brain of type 2 diabetes mouse model (DB/DB mice, data not shown). Nevertheless, up-regulation of CypD expression in diabetic AD mouse brain could be a link of mitochondrial perturbation to synaptic and cognitive dysfunction in an early stage of AD progression.

In summary, we provide evidence that diabetes-mediated mitochondrial perturbation is linked to synaptic dysfunction as well as learning and memory deficit, contributing to the early stage AD progression. Hyperglycemia may accelerate the onset of neurochemical indices of CNS damage in AD pathogenesis. Our results clearly revealed that CypD was elevated in hippocampus when mice were rendered to experimental induction of type 1

diabetes. Intriguingly, there is a synergistic exacerbation of hippocampal CypD upregulation in diabetes and diabetic AD mice. Such modulation of CypD expression may be one of the mechanisms for diabetes-accelerated mitochondrial dysfunction in AD brain, which could ultimately contribute to synaptic injury and cognitive deficits when superimposed with A β toxicity. Thus, preserving mitochondrial function may be beneficial in halting hyperglycemia-associated AD progression. Although many differences exist, some features such as hyperglycemia are common in both type 1 and type 2 diabetes. Given that type 2 diabetes is more common in patients, it will be highly interesting to understand the role and regulation of brain mitochondria in CNS complications of type 2 diabetes as well.

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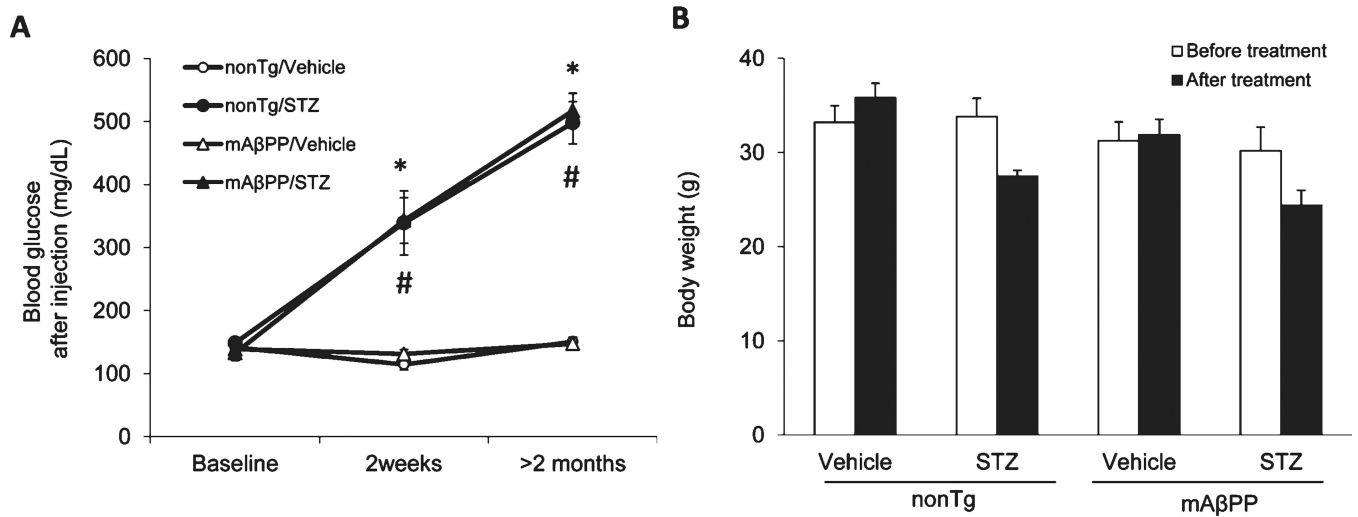
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**Fig. 1.**

Blood glucose and body weight of vehicle-treated nontransgenic (nonTg/Vehicle), diabetic nonTg (nonTg/STZ), vehicle-treated mAbetaPP (mAbetaPP/Vehicle), and diabetic mAbetaPP (mAbetaPP/STZ) mice before and after induction of diabetes. A) Blood glucose levels before treatment (baseline), 2 weeks after STZ injection (2 weeks) and >2 months after STZ injection in the indicated groups of mice. B) Body weight changes in the indicated groups of mice before (open bar) and after 2 months of STZ treatment (Black filled bar). * $p < 0.01$ nonTg/Vehicle versus nonTg/STZ, and # $p < 0.01$ mAbetaPP/Vehicle versus mAbetaPP/STZ. $n = 5-10$ per group.

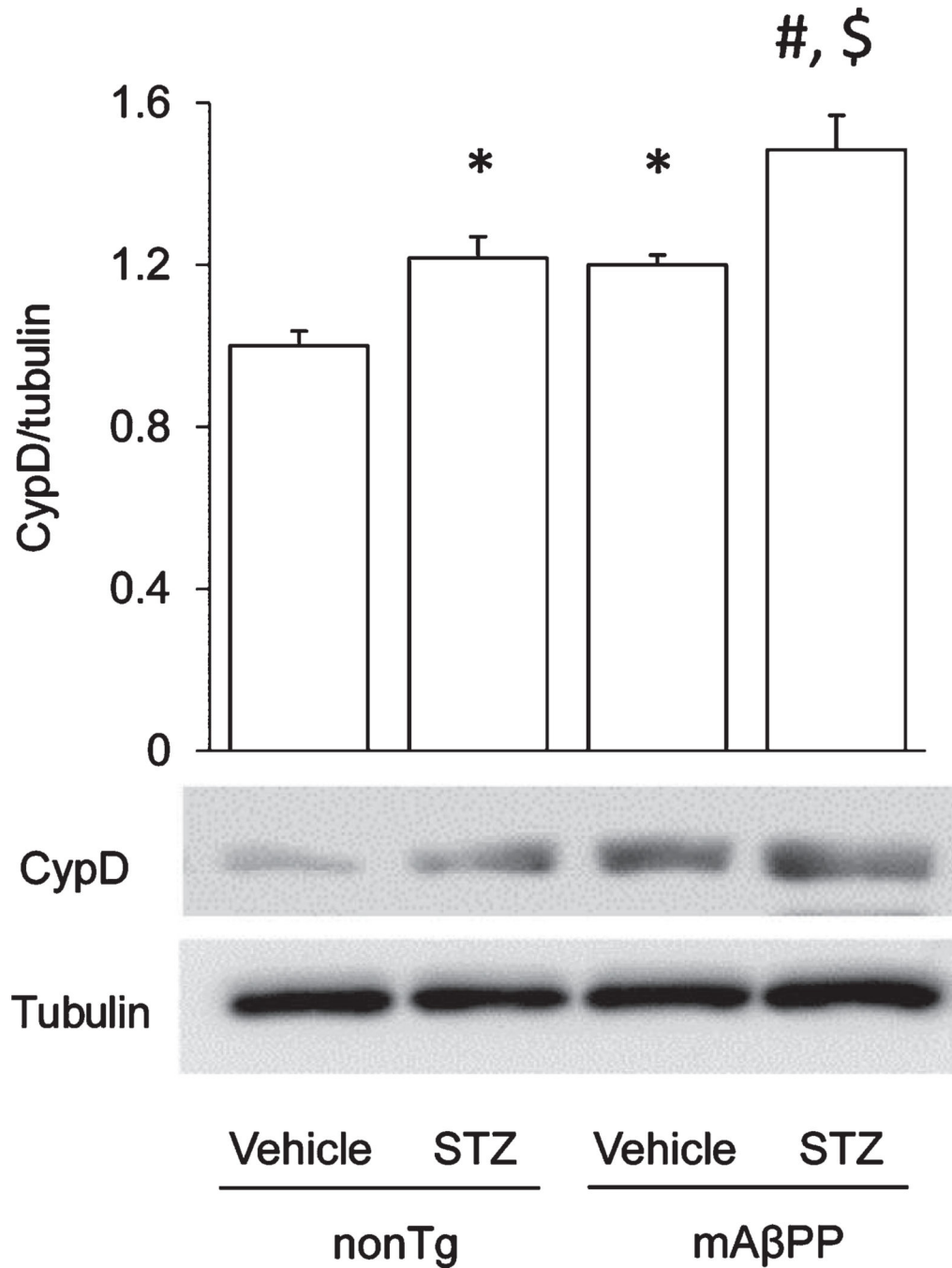


Fig. 2. Expression of CypD in diabetic and non-diabetic nonTg and mAbetaPP mice. Densitometry of CypD immunoreactive bands in the indicated groups of mice using NIH imageJ program (upper panel; $n = 5$ mice per group; $p < 0.05$). Lower panel shows representative images of immunoblots for CypD and tubulin (used as a protein loading control showing an equal amount of protein added to each lane). * $p < 0.05$ nonTg/STZ or mAbetaPP/Vehicle versus nonTg/Vehicle, \$ $p < 0.05$ mAbetaPP/STZ versus nonTg/STZ and # $p < 0.01$ mAbetaPP/Vehicle versus mAbetaPP/STZ. $n = 5$ mice per group.

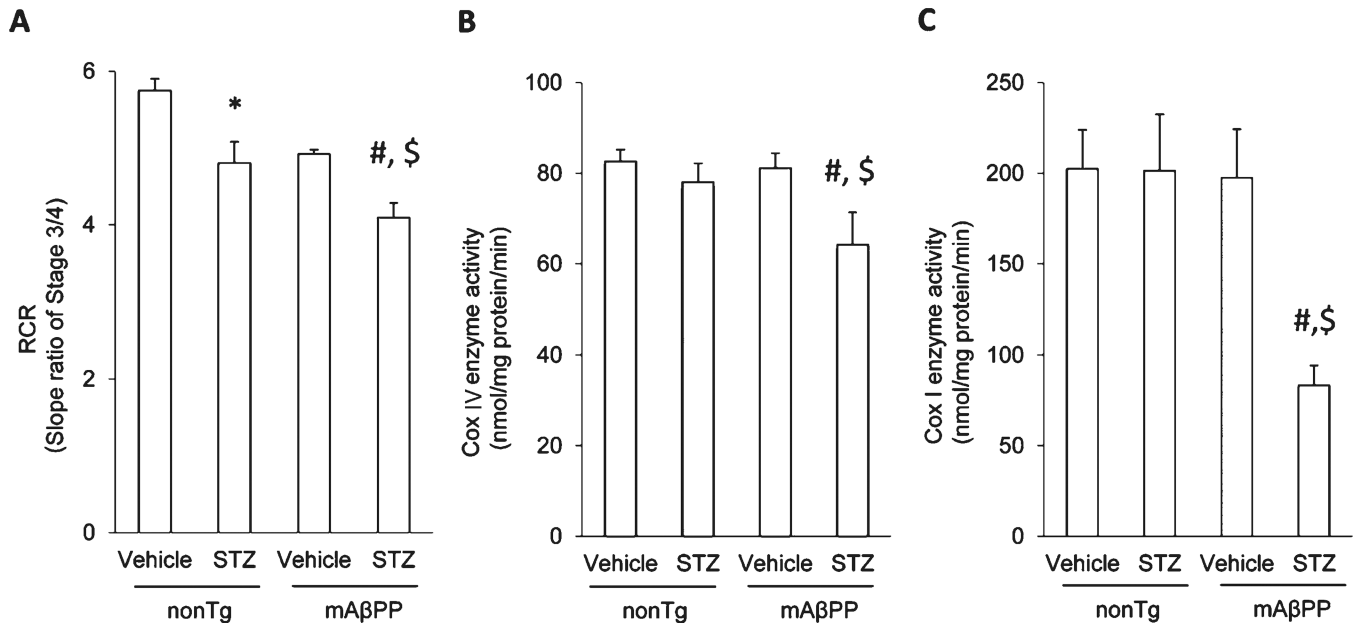


Fig. 3.

Effect of STZ-induced diabetes on mitochondrial respiratory function and complex I and IV enzyme activity in transgenic mAbetaPP mice and nonTg littermates. Respiration control ratio (RCR) (A), cytochrome c oxidase activity (B), and NADH: ubiquinone oxidoreductase (C) in cortical mitochondria from the indicated mice at 6–7 months of age. * $p < 0.01$ nonTg/Vehicle versus nonTg/STZ, # $p < 0.05$ mAbetaPP/Vehicle versus mAbetaPP/STZ, and \$ $p < 0.05$ mAbetaPP/STZ versus nonTg/STZ. $n = 4-5$ mice per group.

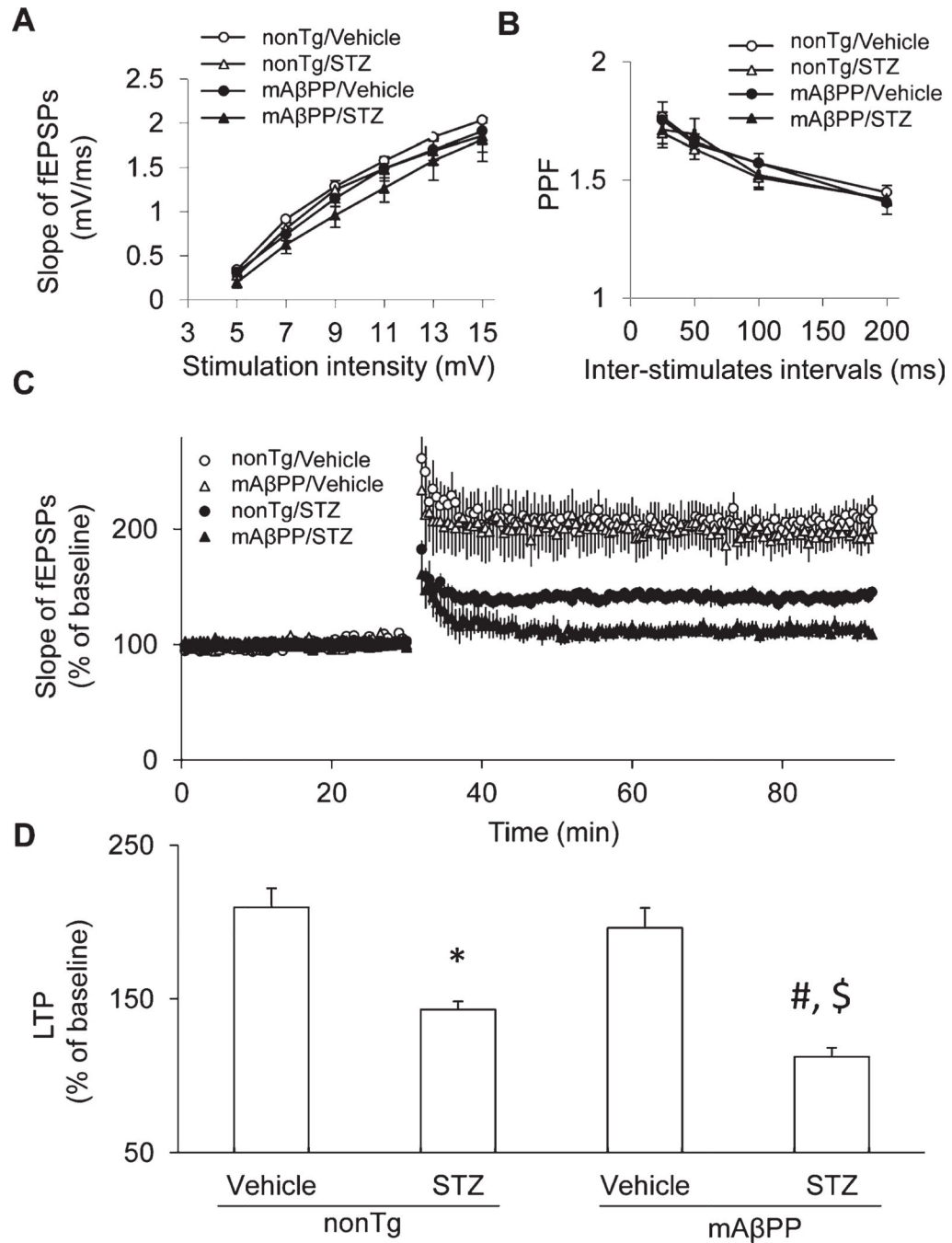


Fig. 4. Effect of STZ-induced diabetes on basal synaptic transmission (BST), paired-pulse facilitation (PPF) and long term potentiation (LTP) in transgenic mAbetaPP mice and nonTg littermates. A) BST, field-excitatory post-synaptic potentials (fEPSPs) plotted against stimulation intensity; and (B) PPF, the ratio of second pulse-induced fEPSPs to the first pulse-elicited fEPSPs, did not differ among indicated groups. C) Hippocampal LTP was recorded in the indicated groups for mice 6–7 months. Slices from 6–7 month-old mAbetaPP mice treated with vehicle showed no significant LTP reduction compared to nonTg/Vehicle slices.

However, experimental induction of type1 diabetes mA β PP and nonTg mice resulted in reduced impairment of hippocampal LTP. Note, LTP levels were almost completely abolished in mA β PP/STZ mice. D) LTP amplitudes among the indicated groups of mice were calculated by an average of fEPSP slopes during 50–60 minutes after theta burst stimulation. * $p < 0.01$ nonTg/Vehicle versus nonTg/STZ, # $p < 0.01$ mA β PP/Vehicle versus mA β PP/STZ, and \$ $p < 0.05$ mA β PP/STZ versus nonTg/STZ. $n = 9–14$ per group from 5–6 mice.

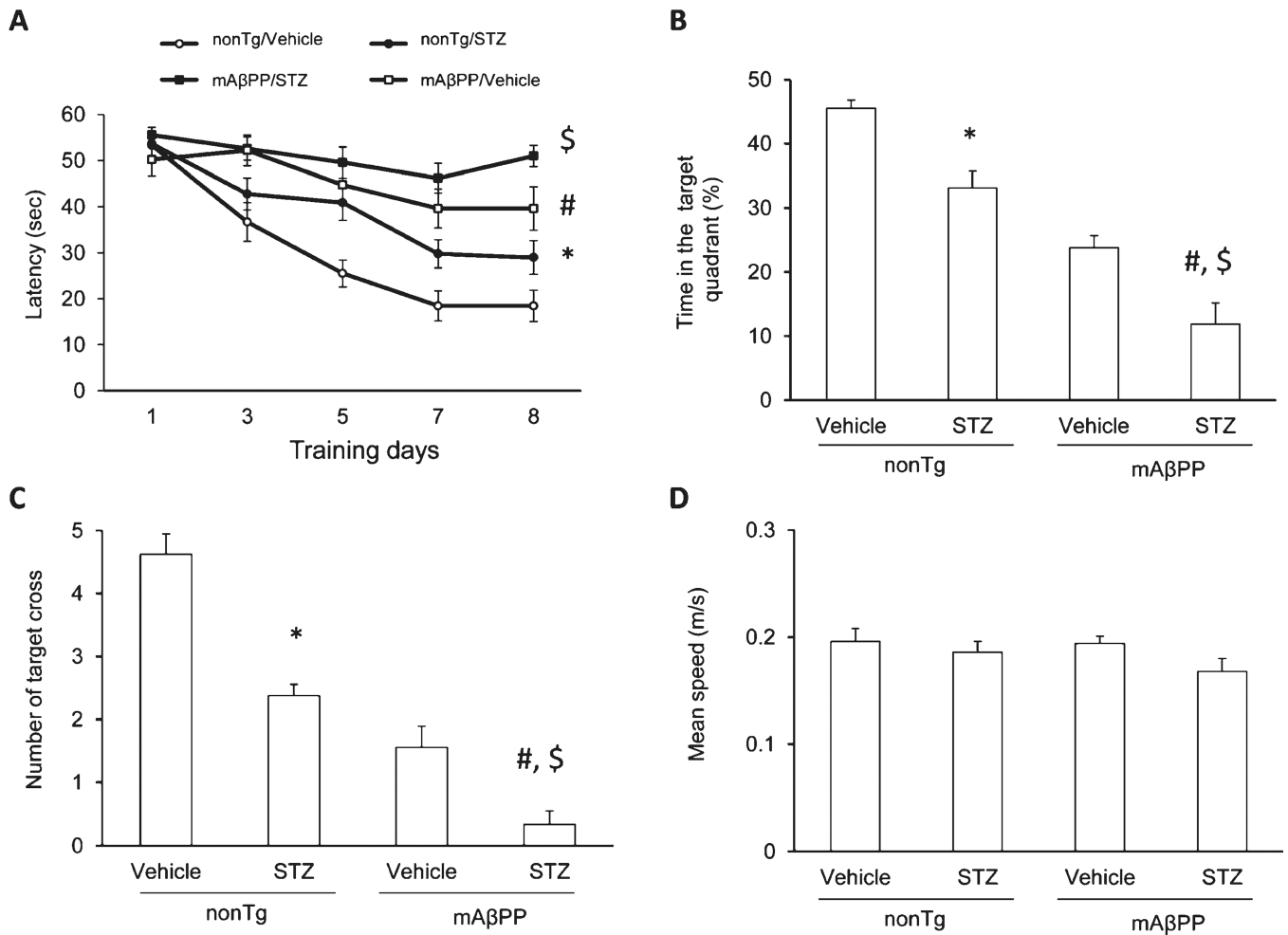


Fig. 5. Effect of STZ-induced diabetes on spatial learning and memory in transgenic mAbetaPP mice and nonTg littermates. Mice were tested in a Morris water maze at the age of 6–7 month-old. A) Escape latencies in hidden-platform during training in indicated groups. Time spent in target quadrant (B), number of annulus crossings (C), and swimming speed (D) are shown. Learning and memory are impaired in diabetic nonTg and nondiabetic mAbetaPP mice. Learning and memory deficits were exacerbated in mAbetaPP mice following induction of diabetes. “Target” indicates the area where the platform was located in the hidden-platform training session. * $p < 0.01$ nonTg/Vehicle versus nonTg/STZ, # $p < 0.01$ mAbetaPP/Vehicle versus mAbetaPP/STZ, and \$ $p < 0.05$ mAbetaPP/STZ versus nonTg/STZ. $n = 6–8$ per group.