Diabetes Mellitus Impairs Cognitive Function in Middle-Aged Rats and Neurological Recovery in Middle-Aged Rats After Stroke

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Background and Purpose—Diabetes mellitus (DM) is a common metabolic disease among the middle-aged and older population, which leads to an increase of stroke incidence and poor stroke recovery. The present study was designed to investigate the impact of DM on brain damage and on ischemic brain repair after stroke in aging animals.

Methods—DM was induced in middle-aged rats (13 months) by administration of nicotinamide and streptozotocin. Rats with confirmed hyperglycemia status 30 days after nicotinamide–streptozotocin injection and age-matched non-DM rats were subjected to embolic middle cerebral artery occlusion.

Results—Middle-aged rats subjected to nicotinamide–streptozotocin injection became hyperglycemic and developed cognitive deficits 2 months after induction of DM. Histopathologic analysis revealed that there was sporadic vascular disruption, including cerebral microvascular thrombosis, blood–brain barrier leakage, and loss of paravascular aquaporin-4 in the hippocampi. Importantly, middle-aged DM rats subjected to stroke had exacerbated sensorimotor and cognitive deficits compared with age-matched non-DM ischemic rats during stroke recovery. Compared with age-matched non-DM ischemic rats, DM ischemic rats exhibited aggravated neurovascular disruption in the bilateral hippocampi and white matter, suppressed stroke-induced neurogenesis and oligodendrogenesis, and impaired dendritic/spine plasticity. However, DM did not enlarge infarct volume.

Conclusions—Our data suggest that DM exacerbates neurovascular damage and hinders brain repair processes, which likely contribute to the impairment of stroke recovery. (Stroke. 2016;47:2112-2118. DOI: 10.1161/STROKEAHA.115.012578.)

Key Words: blood-brain barrier ■ diabetes mellitus ■ hyperglycemia ■ recovery ■ stroke

Diabetes mellitus (DM), a common metabolic disease, affects >240 million people worldwide and is anticipated to become more prevalent as the population ages. Advanced age and DM are not only well-established risk factors for stroke, but also prominent predictors of poor recovery for stroke patients. Because of the risk of brain hemorrhage, patients with advanced age and DM are often excluded from tissue-type plasminogen activator treatment, the only US Food and Drug Administration (FDA)-approved treatment for acute ischemic stroke. Thus, it is imperative to understand the pathological manifestation of stroke in the aging DM brain and to develop therapies for this patient population.

DM-potentiated long-term neurological impairments including cognitive decline have been well described in stroke patients. Studies in young adult animals have shown that diabetes mellitus exacerbates stroke induced ischemic brain damage and impairs neural circuit plasticity, leading to impairment of functional recovery. However, because of the lack of a clinically relevant model of stroke with comorbid DM during aging, the effect of DM on ischemic brain repair processes in aged animals has not been extensively investigated. The present study was undertaken to develop a model of focal cerebral ischemia in the middle-aged rat with DM and then to investigate brain repair processes after stroke. We found that DM exacerbated sensorimotor and cognitive deficits in the middle-aged rat during stroke recovery. DM potentiated neurovascular disruption and suppressed stroke-induced neurogenesis and white matter remodeling processes in both hemispheres, which likely contribute to worse functional recovery.

Materials and Methods

All experimental procedures were approved by the Institutional Animal Care of Henry Ford Hospital. Rats were assigned randomly to DM induction and middle cerebral artery occlusion (MCAO) according to an online randomization tool (randomizer.org). All outcome
measurements were performed by observers blinded to the experimental groups.

Induction of DM
Male Wistar rats (Charles River Laboratories) at 13 months of age (n=26) were subjected to intraperitoneal injection of 210 mg/kg of nicotinamide (NTM) and streptozotocin (STZ, 60 mg/kg). This NTM-STZ protocol causes partial destruction of pancreatic β cells that leads to a decrease in blood insulin and a moderate increase in blood glucose in the rats.8 This method of inducing type 2 DM (T2DM) is contrast to the massive β cell destruction induced by STZ alone, which leads to severe hyperglycemia and mimics type 1 DM, resulting from insulin deficiency.10 These DM rats manifest metabolic defects, such as glucose intolerance and significant but inadequate glucose-stimulated insulin secretion by the β cells, which resemble the manifestations of insulin secretory dysfunction of human T2DM.8–11 Nonfasting blood glucose concentration was assessed 2 days after NTM-STZ injection and weekly thereafter with a portable blood glucose reader. Animals with glucose concentrations >250 mg/dL were considered diabetics. Age-matched rats without NTM-STZ injection (n=24/group) were used as controls.

Middle Cerebral Artery Occlusion
Rats with confirmed DM status at 1 month after DM induction (n=18) and age-matched Non-DM rats (n=16) were subjected to embolic MCAO, as previously described.12 Briefly, blood clots from a donor rat were obtained 24 hours before MCAO. A modified PE-50 catheter carrying a single blood clot was introduced from the right external carotid artery into the lumen of the internal carotid artery until its tip reached the origin of the MCA. The clot was then delivered via the catheter. After that, the catheter was immediately withdrawn. Six DM rats and 4 non-DM rats died during or shortly (within 3 days) after occlusion. Infarct volume was measured weekly starting 1 day after onset of MCAO.12 Morris water maze test13 and odor recognition test14 were performed in the non-diabetic rats and before killing in the ischemic rats. Please see online-only Data Supplement for details.

Functional Outcome
Modified neurological severity score and adhesive removal test were performed weekly starting 1 day after onset of MCAO.12 Morris water maze test13 and odor recognition test14 were performed in the non-diabetic rats and before killing in the ischemic rats. Please see online-only Data Supplement for details.

Histopathology and Immunohistochemistry
Rats were euthanized 35 days after MCAO. Infarct volume was measured on 7 hematoxylin and eosin–stained coronal brain sections as previously described.12 DM rats without MCAO were euthanized 65 days after NTM-STZ injection, whereas age-matched non-DM control rats were euthanized after the final behavioral tests. Immunofluorescent staining was performed on brain coronal sections according to our published protocols. The following primary antibodies were used: mouse anti-BrdU (Dako) for proliferating cells, goat anti-doublecortin (DCX; Santa Cruz) for neuroblasts, rabbit anti-GFAP (Millipore) for oligodendrocyte progenitor cells, mouse anti-myelin basic protein (anti-MBP; Abcam) for mature oligodendrocytes, mouse anti-phosphorylated neurofilament heavy chain (Affinity BioReagents) for axons and dendrites, rabbit anti-myelin basic protein (Millipore) for myelin, rabbit anti-glial fibrillary acidic protein (GFAP; Dako) for astrocytes, rabbit anti-aquaporin-4 (AQP4; Abcam), mouse anti-endothelial barrier antigen (Sternberger Monoclonals), and goat anti-fibrin/fibrinogen (Accurate Chemical & Scientific). The Golgi-Cox impregnation method along with the FD Rapid Golgi Stain kit (FD NeuroTechnologies) was used to identify Golgi-Cox impregnated dendrites and spines.16 Please see online-only Data Supplement for details.

Statistics
Data were evaluated for normality using the Shapiro–Wilk test. Ranked data were used for behavioral data (adhesive removal test and modified neurological severity score) because the data were not normally distributed. The analysis of covariance with repeated measure was used to test the DM effect on functional recovery after stroke by considering possible interactions between groups (with and without DM) and various time points. A group by time interaction indicates that DM group effects vary among times of assessments. If such interaction was detected, a subgroup analysis was conducted. A significant effect was set if P value is <0.05. Please see online-only Data Supplement for details on statistical tests and results on key behavioral data. This is a proof-of-concept study, and the analysis did not adjust for multiple end points. For histopathologic data, analysis of variance was used for analyzing differences between independent groups (eg, DM with or without MCAO), whereas analysis of covariance was used for analyzing the effect of DM and stroke on neurogenesis and oligodendrogenesis, which were obtained from multiple brain regions per rat. The paired t test or 2-sample t test was used if the main effect of DM or region was detected at a 0.05 level.

Results
DM Rats Exhibit Impairments of Cognitive Function and Neurovascular Damage in the Hippocampi
Significant (P<0.05) interactions were detected between the groups (eg, DM presence/absence and MCAO presence/absence) and times on all the repeated Morris water maze tests and glucose levels (Figure 1). However, the subgroup analysis did not show significant differences before the time of onset (eg, DM and MCAO), indicating that a proper randomization with no group selection bias was observed. The NTM-STZ–induced DM shares several features with human T2DM, including hyperglycemia, glucose intolerance, and abnormalities of insulin secretion, and has been widely used to induce experimental T2DM in the rat.10 To examine NTM-STZ–induced morphological changes in pancreatic β cells, insulin staining was performed 65 days after NTM-STZ administration. Middle-aged DM rats exhibited reduction of insulin-producing pancreatic β cells and disruptions in islet morphology compared with the age-matched rat without NTM-STZ injection (Figure 1A). These rats developed persistent hyperglycemia starting 1 week after administration of NTM-STZ (Figure 1B), which resembles the clinical manifestation of T2DM.

Analysis of the Morris water maze test revealed that DM rats spent significantly less time in the correct quadrant 2 months after administration of NTM-STZ compared with non-DM rats (Figure 1C). In addition, DM rats also spent much less time on a novel object measured by the odor recognition test 2 months after induction of DM (Figure 1D). These data indicate that DM induces impairment of cognitive function.

Immunofluorescent analysis of brain tissues showed that microvascular thrombosis measured by fibrin/fibrinogen immunoreactive microvessels and blood–brain barrier leakage assessed by extravascular fibrin deposition were localized to the hippocampi of DM rats (Figure 2A). Although GFAP+ astrocytes were activated, paravascular AQP4 immunoreactivity was substantially reduced (Figure 2B and 2C). These data indicate that DM induces neurovascular damage in the hippocampus.
DM Exacerbates Sensorimotor and Cognitive Function During Stroke Recovery

We then subjected DM rats to MCAO 1 month after the NTM-STZ injection. Significant (P<0.05) interactions were detected between the groups (eg, DM presence/absence and MCAO presence/absence) and the time on functional tests. The subgroup analysis showed that rats in the non-DM and DM groups exhibited severe neurological deficits measured by adhesive removal test and modified neurological severity score 24 hours after MCAO, and there were no significant differences between the 2 groups (Figure 3B and 3C), indicating that the initial sensorimotor deficits are comparable. Non-DM ischemic rats exhibited spontaneous functional improvement during stroke recovery (Figure 3B and 3C). However, DM ischemic rats exhibited significant impairment of spontaneous functional recovery measured by modified neurological severity score at 21, 28, and 35 days and by the adhesive removal test at 28 and 35 days after MCAO compared with non-DM rats (Figure 3B and 3C). Moreover, Morris water maze analysis showed that DM ischemic rats exhibited aggravated spatial learning deficits compared with age-matched non-DM ischemic rats 1 month after MCAO (Figure 1C). These data indicate that DM impairs spontaneous functional improvement during stroke recovery.

DM Exacerbates Microvascular Damage During Stroke Recovery

Infarct volume measured 35 days after MCAO revealed that DM rats had 27.7±2.4% infarct compared with the contralateral hemisphere (n=8), whereas non-DM rats had infarction of 27.1±2.6% (n=8), which was not statistically significant (P>0.05).

However, compared with non-DM ischemic rats, DM ischemic rats exhibited a significant increase in density of fibrin/fibrinogen immunoreactive vessels in the bilateral hippocampi, which was concurrent with aggravated astrocyte activation and reduction of paravascular AQP4 reactivity (Figure 2D). Additionally, DM ischemic rats exhibited

Figure 1. Pancreatic β cells islet morphology, blood glucose level, and cognitive deficits. Insulin immunoreactivity within the pancreas of middle-aged non-DM and DM rats (A). Insulin+ cells occupied most of the central region of the β cell islets (brown) in the pancreas of middle-aged non-DM rats, whereas decreased insulin+ cells and distortion of islet shape (A, arrow) were evident in middle-aged rats after NTM-STZ administration. B, Glucose levels in rats with or without induction of DM. DM rats exhibited significant cognitive deficits measured by the Morris water maze test (C) and odor recognition test (D) 2 months after induction of DM. *P<0.05 as compared with the non-DM rats. Analysis of covariance (ANCOVA) for panels B and C. Analysis of variance (ANOVA)/2-sample t test for panel D. ANOVA indicates analysis of variance; DM, diabetes mellitus; MCAO, middle cerebral artery occlusion; NTM, nicotinamide; and STZ, streptozotocin.

Figure 2. Vascular damage in the DM brain. A, The double immunofluorescent images of fibrin (green) with EBA (red) in the ipsilateral hippocampus in ischemic rats with and without induction of DM. B and C, The immunofluorescent images of GFAP+ astrocytes (green, B) and AQP4 immunoreactivity (green, C) within the ipsilateral hippocampus. Bar graphs in D show DM rats with and without MCAO exhibited significant increases of fibrin deposition and GFAP immunoreactivity and reductions of paravascular AQP4 immunoreactivity in the hippocampus, respectively (ANOVA). ANOVA indicates analysis of variance; AQP4, aquaporin-4; DM, diabetes mellitus; EBA, endothelial barrier antigen; GFAP, glial fibrillary acidic protein; and MCAO, middle cerebral artery occlusion.
significantly (P<0.05) increased density of fibrin/fibrinogen immunoreactive vessels (44.3±3.6/mm²) in the ipsilateral peri-infarct regions, including the corpus callosum and striatum, but did not exhibit increased density of fibrin/fibrinogen immunoreactive vessels in the corresponding contralateral regions (6.4±1.9/mm²) compared with the non-DM ischemic rats (17.5±1.7/mm² at ipsilateral and 4.9±1.4/mm² at contralateral). These data indicate that DM intensifies the neurovascular disruption after stroke.

DM Suppresses Stroke-Induced Neurogenesis

We and others have demonstrated that stroke induces neurogenesis in the subventricular zone (SVZ) of the lateral ventricle. Consistent with published studies, ischemic non-DM rats showed a significant increase in the number of BrdU+ neural progenitor cells (Figure 4B) and DCX+ neuroblasts in the ipsilateral SVZ (Figure 4D). In contrast, DM ischemic rats exhibited significant reductions of BrdU+ cells and DCX+ neuroblasts in the SVZ and the subgranular zone of the dentate gyrus of both hemispheres (Figure 4A–4D). Furthermore, Golgi-Cox staining showed that DM ischemic rats exhibited marked reductions of bilateral spine density (Figure 5A and 5B) and ipsilateral basal dendritic arborization (Figure 5C and 5D) of pyramidal neurons in the CA1 compared with that in non-DM ischemic rats. These data suggest that DM suppresses stroke-induced neurogenesis and exacerbates dendritic spine loss.

DM Suppresses Oligodendrogenesis and Aggravates White Matter Damage After Stroke

In addition to neurogenesis, we found that the DM ischemic rats exhibited profound bilateral reductions of NG2+ oligodendrocyte progenitor cells and APC+ oligodendrocytes in the hippocampus and subcortical regions compared with the non-DM ischemic rats (Figure 6A–6C). Double immunofluorescent staining revealed that DM ischemic rats exhibited significant reductions of BrdU+/NG2+ oligodendrocyte progenitor cells and BrdU+/APC+ oligodendrocytes in the hippocampus and subcortical regions (Figure 6A–6C). DM ischemic rats also exhibited significant bilateral reductions of phosphorylated neurofilament heavy chain and myelin basic protein immunoreactive density in the hippocampus and subcortical white matter structures compared with the non-DM ischemic rats (Figure 6D). However, the loss of phosphorylated neurofilament heavy chain and myelin basic protein immunoreactive density was more pronounced in the ipsilateral hippocampus and peri-infarct white matter, where there was blood–brain barrier leakage. Collectively, these data suggest that DM impairs oligodendrogenesis and aggravates white matter damage in the ischemic brain.

Discussion

The present study demonstrated that middle-aged rats with DM induced by NTM-STZ administration exhibited cognitive impairment, and DM rats subjected to stroke exhibited...
The prevalence of cognitive dysfunction is high in diabetic patients, especially among the elderly. Given the multifaceted nature of the metabolic disease, several structural and functional abnormalities present in the CNS after disturbances of glucose homeostasis, including micro/macrovacular complications, hyperglycemia-associated oxidative stress and neurotoxicity, defects in neural insulin and amyloid metabolism, and decreased neuroplasticity capacity, which have been implicated in the development of cognitive abnormalities. However, the causal neuropathological mechanisms underpinning cognitive impairment associated with DM remain to be investigated. In a mouse model of type 1 DM, a recent study investigated. In a mouse model of type 1 DM, a recent study demonstrated that middle-aged DM ischemic rats exhibited aggravated neurovascular disruption in the hippocampus and white matter and impaired brain repair processes, which likely contribute to exacerbated impairments of functional recovery after stroke induced by DM.

Figure 5. Dendritic spines and arborization. Golgi-Cox staining of dendritic spines (A, arrows) and dendrites (C) in the hippocampal CA1 of Non-DM and DM rats 35 days after MCAO. DM rats exhibited significant bilateral reduction of spine density (B) and ipsilateral basal dendritic arborization (D) compared with non-DM rats (ANCOVA). ANCOVA indicates analysis of covariance; DM, diabetes mellitus; and MCAO, middle cerebral artery occlusion.

The prevalence of cognitive dysfunction is high in diabetic patients, especially among the elderly. Given the multifaceted nature of the metabolic disease, several structural and functional abnormalities present in the CNS after disturbances of glucose homeostasis, including micro/macrovacular complications, hyperglycemia-associated oxidative stress and neurotoxicity, defects in neural insulin and amyloid metabolism, and decreased neuroplasticity capacity, which have been implicated in the development of cognitive abnormalities. However, the causal neuropathological mechanisms underpinning cognitive impairment associated with DM remain to be investigated. In a mouse model of type 1 DM, a recent study shows that DM downregulated vascular endothelial growth factor and its receptor VEGFR2 in the hippocampus where vascular disruption and reduction of dendritic spine density were detected, which were associated with cognitive impairment. Intraventricular infusion of vascular endothelial growth factor restored cognitive function, vascular integrity, and spine density in the hippocampus. These data suggest that the vascular endothelial growth factor signaling pathway plays an important role in DM-induced cognitive impairment. In addition, we have recently demonstrated that DM-induced cognitive deficits are highly correlated with the impairment of the glymphatic system, a brain-wide extracellular fluid exchange pathway driven by cerebrovascular pulsation and astrocyte AQP4 water channels that line paravascular space. The impairment of the glymphatic pathway after vascular disruption and loss of AQP4 water channels leads to the paravascular accumulation of waste metabolic products, including amyloid-beta, a characteristic feature that predicts the progression of cognitive decline and the development of Alzheimer's disease. Our current data reveal that DM-induced cognitive deficits were associated with hippocampi microvascular disruption, astrocyte activation, and loss of paravascular AQP4 immunoreactivity. The hippocampus is involved with learning and memory, which is highly susceptible to changes in oxygen and blood supply. Together with published studies, the present data suggest that DM-induced hippocampal neurovascular disruption along with an impairment of the glymphatic system may contribute to the development of cognitive deficits.

Comorbid DM in stroke aggravates ischemic brain damage and impairs neurological functional recovery in stroke patients. The present study suggests that brain repair processes exacerbated by DM likely lead to impairment of functional outcome because DM did not enlarge infarction. Stroke induces a cascade of interactive remodeling events, including neurogenesis, oligodendrogenesis, and rewiring of lost neuronal circuits, all of which contribute to stroke recovery. DM and hyperglycemia reduce neurogenesis in the adult brain and negatively impact cognitive function. However, the impact of DM on stroke-induced brain remodeling processes in aging brain has not been investigated. Our data demonstrated that DM suppressed the ipsilateral neurogenesis induced by stroke and also decreased contralateral neurogenesis in the SVZ and the subgranular zone of the dentate gyrus. Moreover, the spine number and dendritic arborization in the ipsilateral CA1 pyramidal neurons were drastically reduced in the DM rat subjected to MCAO, indicating an impairment of dendritic plasticity. Stroke-induced neuroblasts are required for brain repair processes and functional recovery. New neurons generated in the hippocampal regions of the adult rodent connect to resident neurons in the hippocampal regions and form neuronal circuitry, which mediates learning and memory function. Thus, neurogenesis suppressed by DM likely impairs hippocampal synaptic plasticity and dendritic remodeling in ischemic brain, leading to cognitive decline after stroke. However, additional experiments are warranted to determine whether stroke exacerbates DM-induced olfactory learning and memory deficits and the potential contribution of neurogenesis to olfactory function.

White matter damage, associated with vascular disruption, is an important brain structure substrate that contributes to neurobehavioral impairment in stroke patients. Experimental data from young adult animals have demonstrated that DM worsens stroke-induced vascular disruption and subsequent damage to peri-infarct neuronal circuits, which leads to impairment of stroke recovery. Here, we found that the observed microvascular disruption in the hippocampus and white matter structure in the ischemic DM brain were closely associated with myelinated axonal loss, which is consistent.
with previous studies and further validates our recent neuro-imaging observations showing that DM rats suffer persistent vascular disruption that hinders white matter reorganization after stroke. In addition to neurogenesis, neural progenitor cells within the SVZ and subgranular zone after brain injury generate oligodendrocyte progenitor cells that are recruited to the injured site where they differentiate into myelinating oligodendrocytes and contribute to neurological functional recovery. Thus, reduction of stroke-induced oligodendrogenesis in the ipsilateral hippocampus and peri-infarct white matter by DM may underlie DM-induced white matter damage after stroke.

A caveat of the present study is that the contralateral hemisphere was used as control, which could mask the effects of stroke-induced bilateral remodeling processes. Future experiments are warranted to include the naïve or sham-operated animals as control for investigating the relative contribution of DM and stroke to bilateral brain remodeling processes.

In conclusion, our data suggest that DM induces cognitive decline in middle-aged animals, which is associated with hippocampal neurovascular disruption. Furthermore, DM exacerbates neurovascular damage and inhibits brain interactive remodeling processes, leading to impairment of neurological functional recovery and cognitive decline in middle-aged rats after stroke, which mimics the pathological manifestations observed in stroke patients with DM.

Figure 6. Oligodendrogenesis. The double immunofluorescent images of BrdU+(green)/NG2+(red) oligodendrocyte progenitor cells (OPCs; A) and BrdU+(green)/APC+(red) oligodendrocytes (B) within the ipsilateral hippocampus. Bar graphs in C show that compared with ischemic non-DM rats, ischemic DM rats exhibited marked bilateral reductions of NG2+ OPCs and APC+oligodendrocytes, and the ipsilateral reduction of proliferating OPCs (BrdU+/NG2+ cells) and newly generated oligodendrocytes (BrdU+/APC+ cells). D, the double immunofluorescent images of MBP+(green)/pNFH+(red) myelinated axon within the ipsilateral hippocampus acquired from the adjacent coronal sections from B. The bar graph in D shows that ischemic DM rats exhibited significant bilateral reductions of myelinated axon density in the hippocampus and subcortical white matter structures (ANCOVA). ANCOVA indicates analysis of covariance; APC, antiadenomatous polyposis coli; BrdU, bromodeoxyuridine; DM, diabetes mellitus; MBP, myelin basic protein; and pNFH, phosphorylated neurofilament heavy chain.

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Disclosures
None.

References


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Supplemental Methods

Behavioral tests:

The modified neurological severity score (mNSS) was used to examine motor, sensory, reflex, and balance functions. Neurological function was graded on a scale of 0 to 18 (normal score, 0; maximal deficit score, 18).

Adhesive removal test was used to examine somatosensory function. Briefly, 2 pieces of adhesive-backed paper (113.1 mm$^2$) were used as bilateral tactile stimuli occupying the distal-radial region on the wrist of each forelimb. Each animal received 3 trials per testing day and the mean time (seconds) required to remove stimuli from the left forelimb was recorded.

The modified Morris water maze test was used for assessment of hippocampal-dependent spatial learning function. This test was performed daily for 5 days in non-ischemic rats at 2 months after NTM-STZ injection, and in ischemic rats before sacrifice. Briefly, the experimental apparatus consists of a circular water tank (140 cm in diameter) which is placed in a large test room with many external cues (e.g., pictures, lamps, etc.) that are visible to the rats. A transparent platform (15 cm in diameter) is submerged 1.5 cm below the surface of the water at a random location within the Northeast (correct) quadrant of the maze. For each trial, the rat was placed randomly at one of four fixed starting points (north, south, east, and west), and allowed to swim for a maximum of 90 sec. The latency to find the hidden platform and the time spent within the correct quadrant was recorded. Data are presented as the percentage of time spent within the correct quadrant relative to the total amount of time spent to find the platform.

Olfactory learning and memory were evaluated before sacrifice using a social odor recognition test. Briefly, identical round wooden beads (Woodworks Ltd.) were placed in the cages of two individually housed donor rats for 1 week without bedding change to acquire animal specific odors (designated as N1 and N2). During the initial familiarization period (2 days before testing), 4 unscented wooden beads (designated as F1–F4, Woodworks Ltd.) were introduced into the home cage of the testing rat for 24 hours. On the following day (1 day before testing), after removing the now-familiar beads (F1–F4) for 1 hour, 3 familiar beads (F1–F3) and a novel-rat-scented wooden bead (N1) were randomly placed back into the home cage for three 1-minute trials with 1-minute inter-trial intervals. This procedure produces habituation to N1 while minimizing olfactory adaptation. The odor-recognition memory test was performed 24 hours after the N1 habituation phase. Four beads including 2 familiarized home cage beads F1 and F2, a recently familiarized bead N1, and a novel-odor bead N2 were placed in the home cage, following the same procedure outlined for the habituation phase. Exploration time for each bead was recorded. The focus of this test was to assess the nonspatial social odor-based novelty recognition for N2 bead and the overnight memory for the N1 bead. Good odor recognition memory was indicated by more time spent exploring N2 than N1 and
other beads in the trial. Data are presented as the percentage of time spent on N2 relative to the total amount of time spent with all beads.

**Quantification for histology and immunohistochemistry:**

Infarct volume was measured on hematoxylin and eosin (H&E) stained coronal brain sections using the microcomputer imaging device (MCID) system (Imaging Research). Briefly, the area of the both hemispheres and the area containing the ischemic neuronal damage (mm²) were calculated by tracing the area on the computer screen. The lesion volume (mm³) was determined by multiplying the appropriate area by the section interval thickness. The ischemic volume is expressed as the percentage of infarct volume of the contralateral hemisphere (indirect volume calculation).

Immunoreactive cells were imaged under a fluorescent microscope by means of the MCID system. Briefly, eight coronal sections (8 µm in thickness), each of them spaced as 50 µm intervals, at the levels of lateral ventricle (bregma -0.4 to -1.4 mm) and dorsal hippocampus (bregma -2 to -4) per staining per rat were used. To examine neurogenesis, the density of BrdU⁺ and DCX⁺ in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus were measured. To examine axonal density and myelination, pNFH and MBP immunoreactive areas were measured throughout the peri-infarct corpus callosum, striatum, hippocampus, as well as in the corresponding areas in the contralateral hemisphere. To minimize structure associated variation in axonal density and myelination, data are presented as the percentage of immunoreactive area relative to the corresponding area obtained from the contralateral hemisphere of the non-DM rats. To examine OPCs and mature oligodendrocytes, the density of NG2⁺ cells and APC⁺ cells were measured throughout the peri-infarct corpus callosum, striatum, hippocampus, as well as in the corresponding areas in the contralateral hemisphere. Double immunofluorescently reactive BrdU⁺/NG2⁺ cells and BrdU⁺/APC⁺ cells were counted throughout the peri-infarct corpus callosum, striatum, hippocampus, as well as the corresponding areas in the contralateral hemisphere. Data are presented as percentage of each cell population within each hemisphere. To examine cerebrovascular disruption, the density of EBA⁺ vessels with microvascular fibrin deposition and extravascular fibrin leakage was measured in the peri-infarct corpus callosum, striatum, hippocampus, as well as the corresponding areas in the contralateral hemisphere. Data are presented as the density of fibrin/fibrinogen⁺ vessels within the hippocampus and peri-infarct regions. GFAP⁺ and perivascular AQP4 immunoreactive area within the hippocampus were measured and data are presented as percentage of area.

Dendritic spine density and dendritic arborization were measured on Golgi-Cox impregnated CA1 neurons at the level of dorsal hippocampus (Bregma -2 to -4). At least 20 neurons within the CA1 were assessed on each animal. Dendritic spines were measured from at least 4 segments of secondary or tertiary branches of each neuron using the MCID system under a 100x oil immersion objective. Basal dendritic arborization was measured by Sholl analysis (NIH ImageJ) under a 40x objective.
**Supplemental Table 1:** Statistical tests and results on key behavioral data.

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*Ranked data

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**Fig. 3B**

**Fig. 3C**