SUPPLEMENTAL MATERIAL

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 on7 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 (mm^2) were calculated by tracing the area on the computer screen. The lesion volume (mm^3) 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 I: Statistical tests and results on key behavioral data.

Variable	Parameter	Level	DF	Chi Square	P value	Estimate	Standard Error	Figure
Water maze	Day		4	246.44	<.0001			
Water maze	Group		1	24.64	<.0001			
Water maze	Group x Day		4	46.99	<.0001			
Water maze	Contracts	Non-MCAO VS MCAO in DM rats at D61	1	0.1	0.7509	-0.8333	2.6253	
Water maze	Contracts	Non-MCAO VS MCAO in DM rats at D62	1	9.5	0.0021	6.75	2.1896	
Water maze	Contracts	Non-MCAO VS MCAO in DM rats at D63	1	17.37	<.0001	8	1.9196	
Water maze	Contracts	Non-MCAO VS MCAO in DM rats at D64	1	39.33	<.0001	9.5833	1.5281	
Water maze	Contracts	Non-MCAO VS MCAO in DM rats at D65	1	51.89	<.0001	10	1.3882	Fig. 1C
Water maze	Contracts	Non-DM VS DM after MCAO at D61	1	0.01	0.9292	0.25	2.8131	
Water maze	Contracts	Non-DM VS DM after MCAO at D62	1	3.8	0.0512	5.1667	2.6499	
Water maze	Contracts	Non-DM VS DM after MCAO at D63	1	4.86	0.0276	5.5833	2.5339	
Water maze	Contracts	Non-DM VS DM after MCAO at D64	1	6.23	0.0126	6.25	2.505	
Water maze	Contracts	Non-DM VS DM after MCAO at D65	1	7.73	0.0054	6.8333	2.4577	Fig. 1C
Water maze	Group		1	7.21	0.0072			
Water maze	Day		4	150.19	<.0001			
Water maze	Group x Day		4	5.77	0.2171			
Water maze	Contracts	Non-DM VS DM (with/without MCAO) at D61	1	0.1	0.752	1.15	3.6396	
Water maze	Contracts	Non-DM VS DM (with/without MCAO) at D62	1	6.62	0.0101	8.75	3.3999	
Water maze	Contracts	Non-DM VS DM (with/without MCAO) at D63	1	4.12	0.0424	7.05	3.473	
Water maze	Contracts	Non-DM VS DM (with/without MCAO) at D64	1	4.79	0.0287	7.55	3.4503	
Water maze	Contracts	Non-DM VS DM (with/without MCAO) at D65	1	4.86	0.0275	7.6	3.4469	Fig. 1C

Adhesive	Group		1	3.14	0.0762			
Adhesive	Day		5	877.9	<.0001			
Adhesive	Group x Day		5	44.28	<.0001			
Adhesive	Contracts	Non-DM VS DM at D1	1	0.03	0.8565	*	*	
Adhesive	Contracts	Non-DM VS DM at D7	1	1.86	0.1722	*	*	
Adhesive	Contracts	Non-DM VS DM at D14	1	0.11	0.7425	*	*	
Adhesive	Contracts	Non-DM VS DM at D21	1	2.57	0.1092	*	*	
Adhesive	Contracts	Non-DM VS DM at D28	1	5.59	0.0181	*	*	
Adhesive	Contracts	Non-DM VS DM at D35	1	12.4	0.0004	*	*	Fig. 3B
mNSS	Group		1	5.68	0.0172			
mNSS	Day		5	279.36	<.0001			
mNSS	Group x Day		5	12.91	0.0242			
mNSS	Contracts	Non-DM VS DM at D1	1	0.28	0.5994	*	*	
mNSS	Contracts	Non-DM VS DM at D7	1	0.26	0.6116	*	*	
mNSS	Contracts	Non-DM VS DM at D14	1	3.5	0.0615	*	*	
mNSS	Contracts	Non-DM VS DM at D21	1	5.71	0.0168	*	*	
mNSS	Contracts	Non-DM VS DM at D28	1	9.64	0.0019	*	*	
mNSS	Contracts	Non-DM VS DM at D35	1	8.96	0.0028	*	*	Fig. 3C

*Ranked data