Supporting Information

Cheng et al. 10.1073/pnas.1404109111

SI Methods

Infarct Visualization and Quantitation. Mice were sacrificed on poststroke day 15. Mice were perfused transcardially with ice-cold 1× PBS followed by 3% paraformaldehyde. Brains were incubated overnight in a 20% sucrose/3% paraformaldehyde solution until they sank. Thirty-micrometer sections were collected using a cryostat and kept at −20 °C in an antifreeze solution (30% ethylene glycol + 30% glycerol in PBS). Sections were stained by the high-contrast silver stain method as described (1). Briefly, slides were vigorously shaken in a silver solution for 2 min, rinsed six times in water, then shaken vigorously for 3 min in a developing solution, rinsed, and dried. Images were scanned at 1,200 dots per inch, and infarct size was measured using ImageJ software (National Institutes of Health, NIH). Total ipsilesional, contralesional, and infarct areas were quantified, and lesion sizes were determined by this formula: lesion size = 100 × [total contralesional hemisphere area − (total ipsilesional hemisphere area − infarct area)]/(total contralesional hemisphere area).

Protein Extractions and Western Blots. At poststroke day 15, mice were sacrificed and perfused with cold sterile 1× PBS. Brain regions (iM1, cM1, iS1, and cS1) were dissected on ice with 1× PBS and stored at −80 °C until use. Proteins were extracted using a cell lysis buffer mixture (Cell Signaling; 9803) that contains protease phosphatase inhibitor mixture (Cell Signaling; 5872) and PMSF (Cell Signaling; 8553). Bradford assay was used to measure protein concentrations and proteins were diluted to equal concentrations using the cell lysis buffer mixture and 4× Laemmli sample buffer (Bio-Rad; 161-0747). SDS/PAGE was performed on 4–20% Mini-PROTEAN TGX precast gels (Bio-Rad; 456-1094) and proteins were subsequently transferred to PVDF membranes. Western blot was carried out by blocking the membranes in 5% milk for 30 min at room temperature, followed by incubation in primary antibodies overnight: GAP43 1:1,000 (Millipore; AB5220) or BDNF 1:800 (Santa Cruz; sc-546). Membranes were washed with 1× PBS + 0.1% Tween and incubated with anti-rabbit HRP secondary antibodies for 1 h at room temperature. Membranes were also blotted with GAPDH to control for equal loading. Bands were visualized using Amersham ECL Prime Western Blotting Detection Reagent (RPN2232) and imaged on the Bio-Rad Chemi-Doc MP Imager. Relative optical density was measured using NIH ImageJ and graphs were generated using Prism 5.

1. Vogel J, Möbius C, Kuschinsky W (1999) Early delineation of ischemic tissue in rat brain cryosections by high-contrast staining. *Stroke* 30(5):1134–1141.

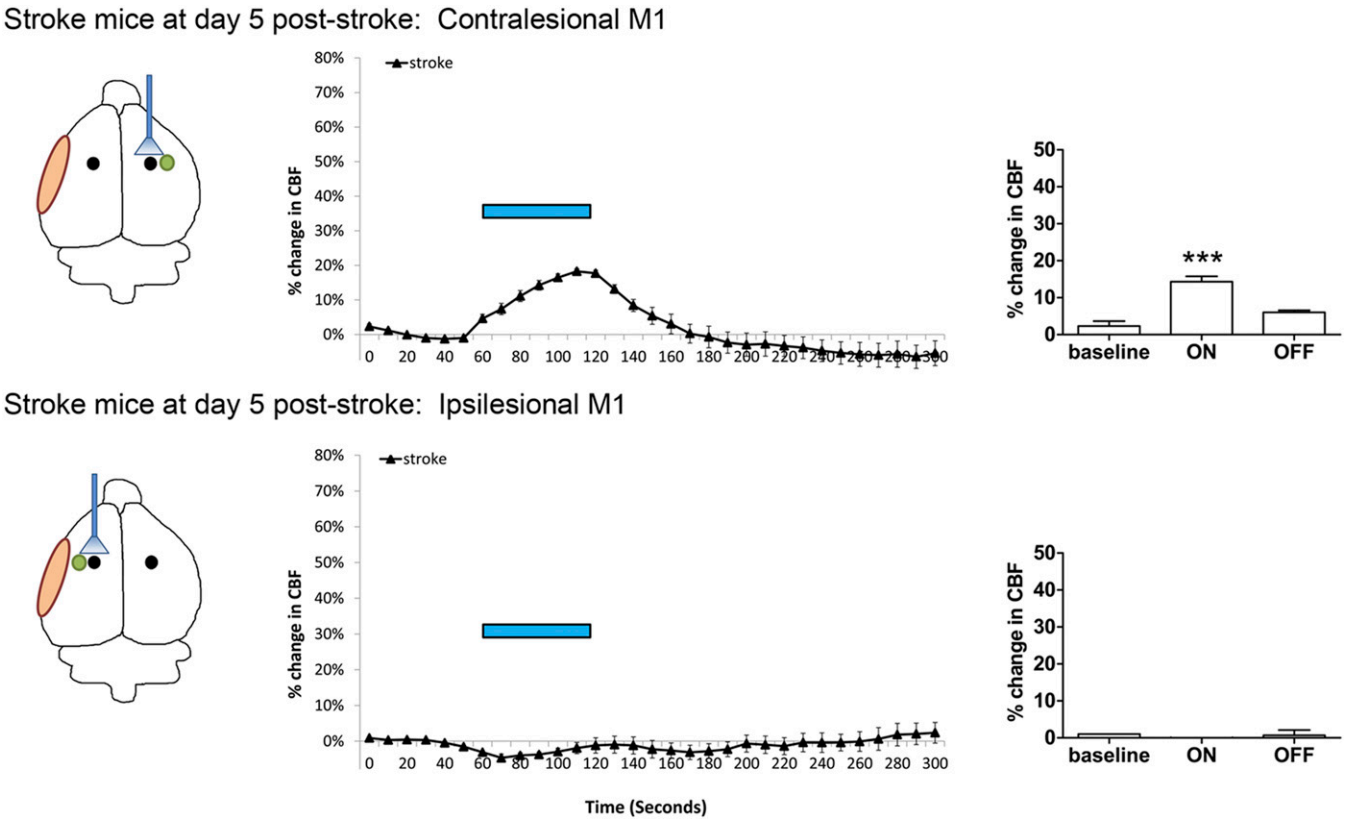


Fig. S1. Cerebral blood flow (CBF) measurements at poststroke day 5. CBF measured in contralesional primary motor cortex (cM1) and ipsilesional primary motor cortex (iM1) of stroke mice, without any treatment, at poststroke day 5. (*Left*) Illustration of the stimulation site (indicated by fiber), the ischemic area (orange) and the CBF measurement site (green). (*Middle*) Time lapse recordings of % change in CBF, consisting of 3 periods: baseline (1 min), laser-on stimulation (1 min) and a laser-off stimulation (3 min). (*Right*) The peak % CBF change in each period. Stroke mice exhibited a similar neurovascular coupling response in the cM1 during the laser-on period (indicated by the blue bar) but lacked a large response after the laser is turned off; however, this response was absent in iM1. ***$P < 0.001$; one-way ANOVA with Dunnet's post hoc test. $n = 4$.

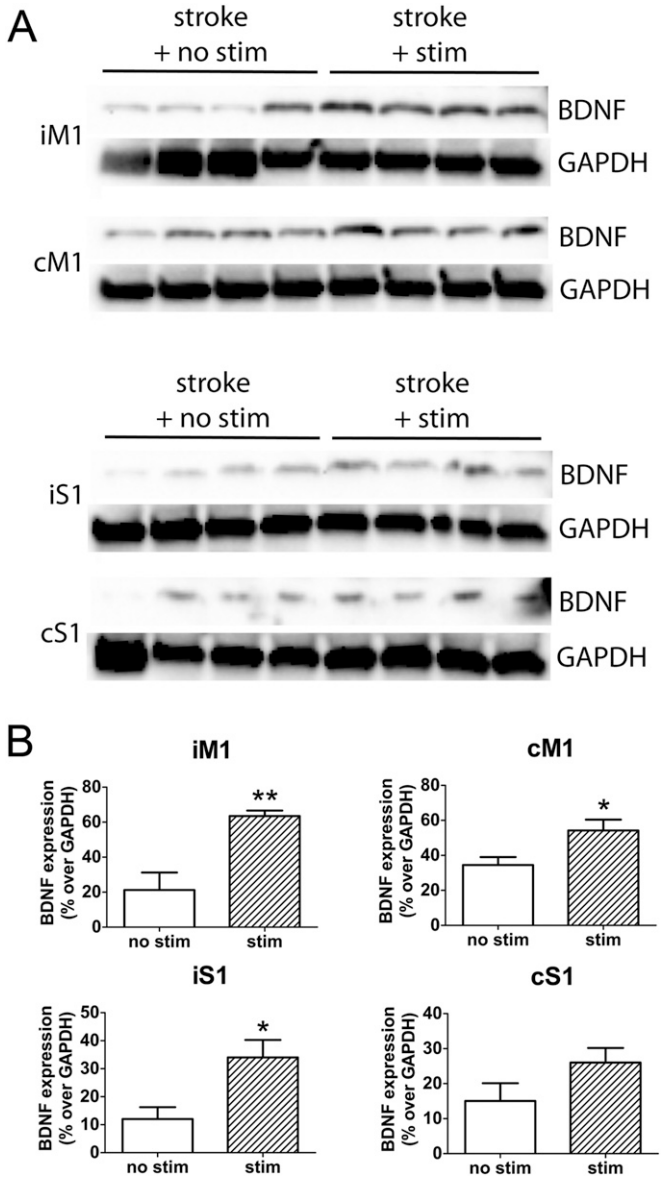


Fig. S2. Brain-derived neurotrophic factor (BDNF) protein levels are increased in stimulated mice. (*A*) Western blots of BDNF and GAPDH expression in iM1, cM1, ipsilesional somatosensory cortex (iS1), and contralesional somatosensory cortex (cS1) of nonstimulated and stimulated stroke mice (poststroke day 15). (*B*) Relative optical density measurements of BDNF expression expressed as percentage over GAPDH. $n = 4$ per group, $^*P < 0.05$, significant difference between stim and nonstim groups, Student *t* test.

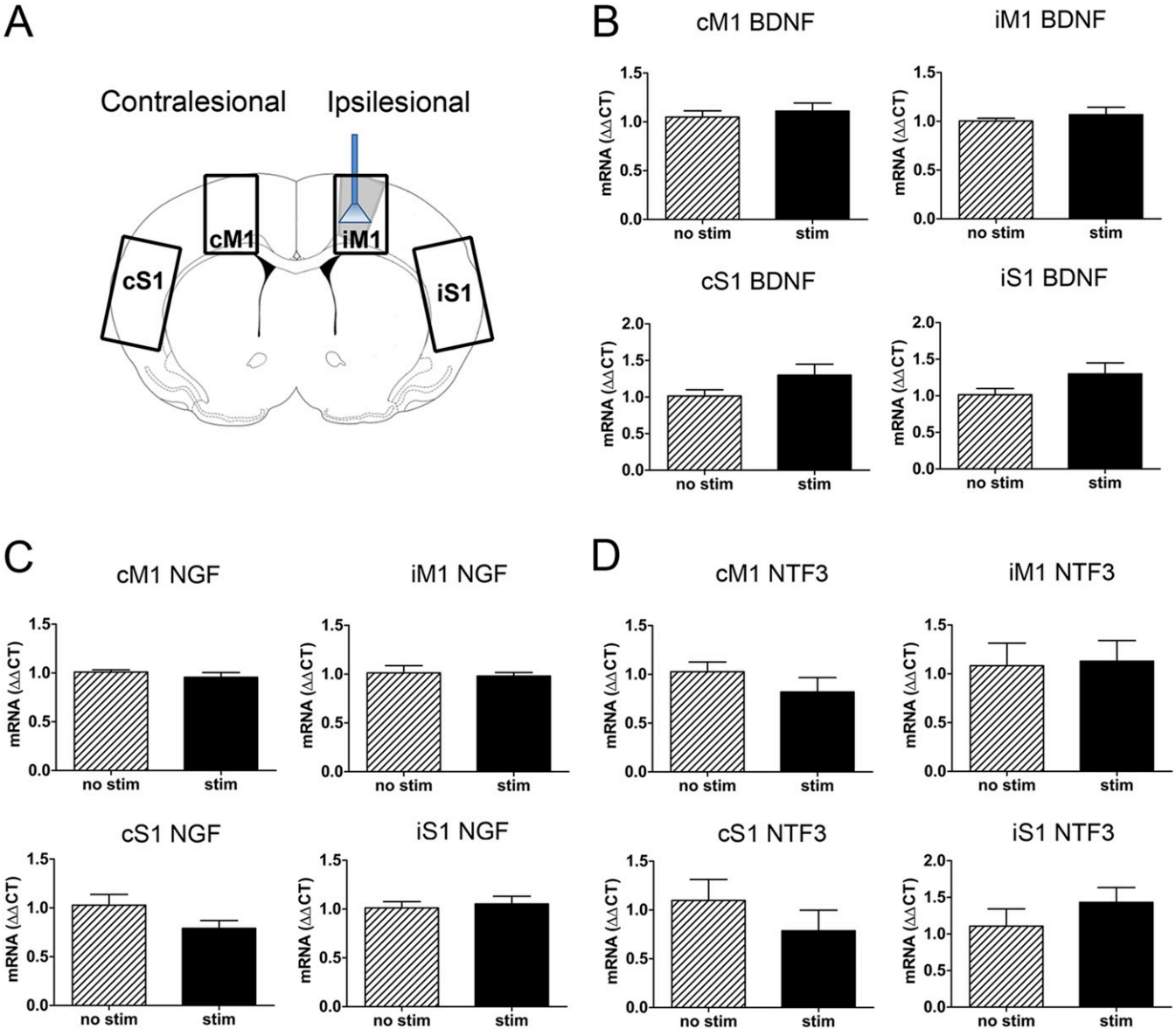


Fig. S3. Repeated iM1 neuronal stimulation in normal mice did not alter neurotrophin expression levels. (*A*) Neurotrophin expression in brains of stimulated and nonstimulated normal mice sacrificed on day 15. Diagram illustrates the stimulation site, infarct regions, and iM1, cM1, iS1, and cS1 dissected. qPCR was used to examine the expression of neurotrophins. No difference was detected in the expression of (*B*) BDNF, (*C*) nerve growth factor (NGF), and (*D*) neurotrophin 3 (NTF3) between treatment groups. Student *t* test, $n = 6$ per group.

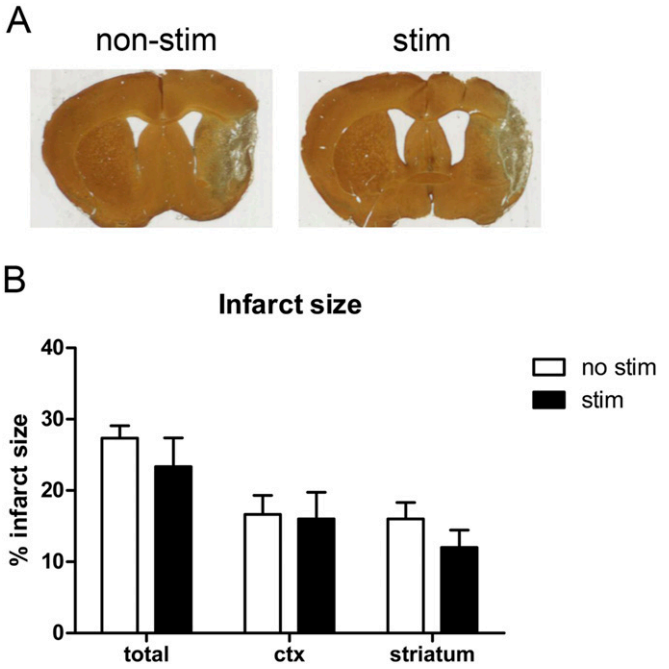


Fig. S4. Repeated iM1 neuronal stimulation did not alter infarct size. (*A*) Representative images of silver-stained brain sections illustrating the infarct size in stimulated and nonstimulated mice at poststroke day 15. (*B*) Quantitation of infarct size showed that there is no significant difference between the two groups. $n = 6$ per group.

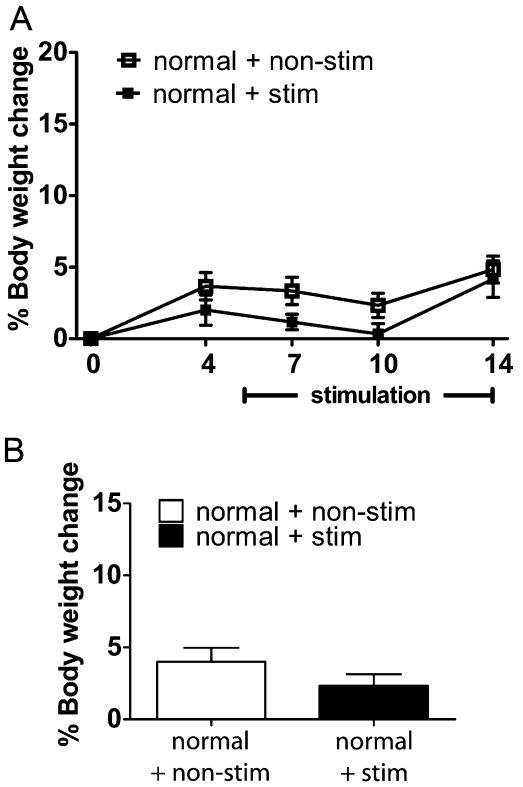


Fig. S5. iM1 neuronal stimulation did not affect body weight in normal mice. (*A*) Time course of body weight changes in normal mice (no stroke). Stimulations did not cause body weight change. (*B*) Average of percent body weight change during the stimulation period. $n = 6$ per group.

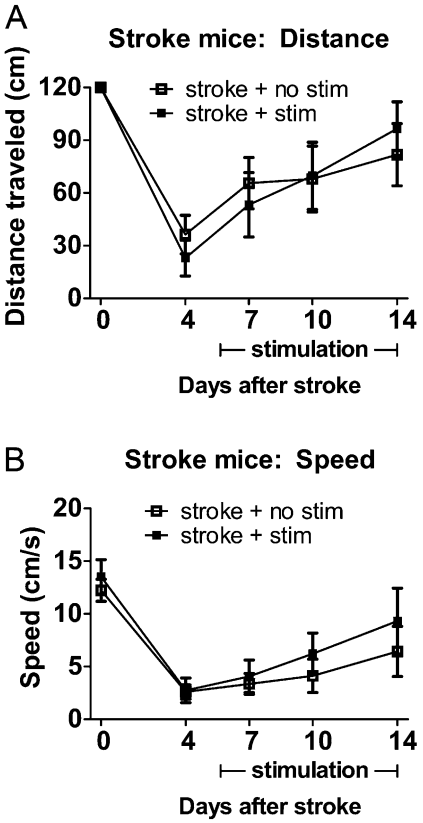
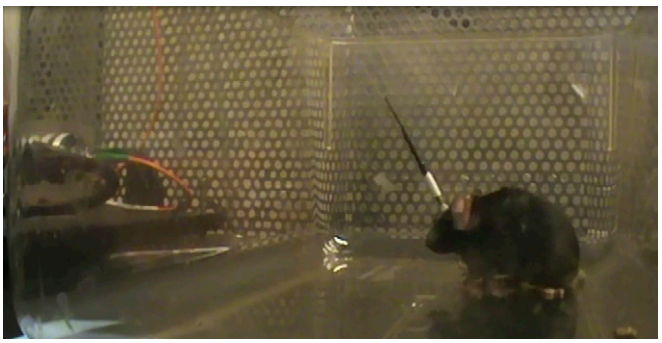


Fig. S6. Contralesional M1 (cM1) neuronal stimulations have no effect on functional recovery after stroke. Graph depicts the performance of contralesional nonstim and stim mice in rotating beam test, (*A*) distance, and (*B*) speed. No significant difference was observed between groups. Two-way repeated measures ANOVA with Fisher's least significant difference (LSD). Nonstim, $n = 8$; stim, $n = 7$.



Movie S1. Contralesional forelimb movements during iM1 neuronal stimulation. Movie demonstrates a representative Thy-1–ChR2 stroke mouse during iM1 neuronal stimulation. Visible movements in the affected forelimb were observed during the stimulation period.

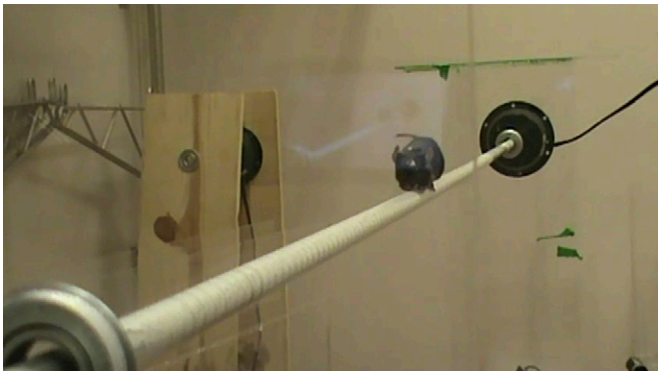
Movie S1

Movie S2. Rotating beam test for sham mouse. Movie demonstrates a representative Thy-1–ChR2 sham mouse walking on a rotating beam test. Normal mouse without stroke can walk and complete the beam test quickly.

Movie S2

Movie S3. Rotating beam test for nonstimulated stroke mouse. Movie demonstrates a representative Thy-1–ChR2 nonstimulated stroke mouse walking on a rotating beam test. Stroke mouse without stimulations recovered slowly and had difficulty balancing and walking on the beam.

Movie S3



Movie S4. Rotating beam test for stimulated stroke mouse. Movie demonstrates a representative Thy-1–ChR2 stimulated stroke mouse walking on a rotating beam test. Stroke mouse with repeated iM1 stimulations recovered faster by traveling with faster speed and longer distances.

Movie S4