**Supplemental Methods**:

*Construction of Cre-dependent AAV-EF1a-DIO-iLMO2 construct*

The iLMO2 cassette (previously described by Tung et al. 2015) was cloned into a double-floxed inverted orientation (DIO) AAV vector to make its expression Cre-dependent (Saunders et al. 2012). The eNpHR3.0-EYFP cassette was first excised out of the pAAV-Ef1α-DIO-eNpHR3.0-EYFP construct (provided by Karl Deisseroth, Stanford University) with NheI/AscI restriction enzymes. The iLMO2 cassette was then PCR amplified with flanking NheI/AscI restriction sites and ligated in an inverted orientation into the previously prepared vector. Correct ligation and orientation of the insert was confirmed by sequencing.

*AAV viral vector production*

The Emory Viral Vector Core prepared AAV2/9 tagged with construct Ef1a-DIO-iLMO2.

*Validation of the Cre-dependent expression of AAV-EF1a-DIO-iLMO2*

Cre-dependent expression of the AAV-EF1α-DIO-iLMO2 construct was confirmed by co-expressing the plasmid with Cre-recombinase (pAAV-Ef1α-Cre obtained from Addgene). HEK293 (Cat# 300192/p777_HEK293,

RRID:CVCL_0045) cells were transiently transfected using Lipofectamine 2000 following the manufacturer's recommendations. 24 hours after transfection, expression was confirmed by fluorescence microscopy. Functional expression of iLMO2 was confirmed by *in vitro* bioluminescence imaging of transfected HEK293 cells cultured in a 12-well plate. The functionality of the luciferase was confirmed by detecting bioluminescence with a plate imager (Fuji LAS-2000) after application of 15μM of h-CTZ to transfected HEK293 cells as previously described. Functional expression of iLMO2 was further confirmed by whole-cell patch clamp recordings of transfected HEK293 cells cultured on glass coverslips. Positive cells were identified by fluorescence microscopy, recorded under voltage clamp mode, and stimulated with green (TRITC) light as previously described (Tung et al. 2015).

| | Retrograde | iLMO2 + label | |
|---|---|---|---|
| | 173 | 17 | 0.0982659 |
| | 193 | 11 | 0.0569948 |
| | 377 | 30 | 0.0795756 |
| | 141 | 27 | 0.1914894 |
| **average** | **221** | **21.25** | **10.7%** |
| **sem** | **53.091117** | **4.4040701** | **3.0%** |

215x279mm (150 x 150 DPI)

## Lateral Gastrocnemius

| | Amplitude (mV) Pre CTZ | Percent (%) Pre CTZ | Amplitude (mV) 10-30mins Post CTZ | Percent (%) 10-30mins Post CTZ | Amplitude (mV) 60-90mins Post CTZ | Percent (%) 60-90mins Post CTZ |
|---|---|---|---|---|---|---|
| | 2.9 | 100% | 1.2 | 41% | 2.6 | 90% |
| | 0.62 | 100% | 0.063 | 10% | 0.5 | 81% |
| | 1.1 | 100% | 0.7 | 64% | *animal died* | |
| | 1.1 | 100% | 0.28 | 25% | 1.1 | 100% |
| | 0.5 | 100% | 0.08 | 16% | 0.08 | 16% |
| | 0.1 | 100% | 0.03 | 30% | 0.1 | 100% |
| **AVG** | **1.1** | **100%** | **0.4** | **31%** | **0.9** | **77%** |
| **STDEV** | **1.0** | **0%** | **0.5** | **19%** | **1.0** | **35%** |
| Paired T-test | | | | 0.00032 | | 0.2 |

## Tibialis Anterior

| | Amplitude (mV) Pre CTZ | Percent (%) Pre CTZ | Amplitude (mV) 10-30mins Post CTZ | Percent (%) 10-30mins Post CTZ | Amplitude (mV) 60-90mins Post CTZ | Percent (%) 60-90mins Post CTZ |
|---|---|---|---|---|---|---|
| | 0.8 | 100% | 0.4 | 50% | 0.65 | 81% |
| | 0.08 | 100% | 0.05 | 63% | 0.057 | 71% |
| | 0.04 | 100% | 0.01 | 25% | *animal died* | |
| | 0.2 | 100% | 0.15 | 75% | 0.2 | 100% |
| | 0.02 | 100% | 0.01 | 50% | 0.02 | 50% |
| | 0.04 | 100% | 0.02 | 50% | 0.04 | 50% |
| **AVG** | **0.2** | **100%** | **0.1** | **52%** | **0.2** | **71%** |
| **STDEV** | **0.3** | **0%** | **0.2** | **17%** | **0.3** | **21%** |
| Paired T-test | | | | 0.00088 | | 0.037 |

215x279mm (150 x 150 DPI)

Data

| Gast | TA | Group |
|---|---|---|
| 0.530 | 0.210 | IT |
| 0.202 | 0.298 | IT |
| 0.140 | 0.150 | IT |
| 0.120 | 0.230 | IT |
| 0.005 | 0.021 | IT&CTZ |
| 0.037 | 0.100 | IT&CTZ |
| 0.049 | 0.150 | IT&CTZ |
| | 0.030 | IT&CTZ |
| 0.160 | 0.060 | IT&CTZ |
| 0.013 | 0.008 | CTZ |
| 0.010 | 0.016 | CTZ |
| 0.050 | 0.040 | CTZ |
| 0.042 | 0.090 | CTZ |
| 0.030 | | CTZ |
| 0.033 | 0.090 | UT |
| 0.015 | 0.063 | UT |
| 0.014 | 0.021 | UT |
| 0.213 | 0.010 | UT |
| 0.008 | 0.116 | UT |

Statistics

Breakdown Table of Descriptive Statistics (LuminopsinANOVA.sta) Smallest N for any variable: 18

| Treatment | Gast | N | SD | TA | N | SD |
|---|---|---|---|---|---|---|
| IT | 0.248 | 4 | 0.191 | 0.222 | 4 | 0.061 |
| IT&CTZ | 0.063 | 4 | 0.067 | 0.072 | 5 | 0.053 |
| CTZ | 0.029 | 5 | 0.018 | 0.039 | 4 | 0.037 |
| UT | 0.057 | 5 | 0.088 | 0.060 | 5 | 0.045 |

Analysis of Variance (LuminopsinANOVA.sta) Marked effects are significant at p < .05000

| Variable | SS | df | MS | SS | df | MS | F | p |
|---|---|---|---|---|---|---|---|---|
| Gast | 0.127 | 3 | 0.042 | 0.156 | 14 | 0.011 | 3.807 | 0.034694 |
| TA | 0.086 | 3 | 0.029 | 0.035 | 14 | 0.002 | 11.562 | 0.000442 |

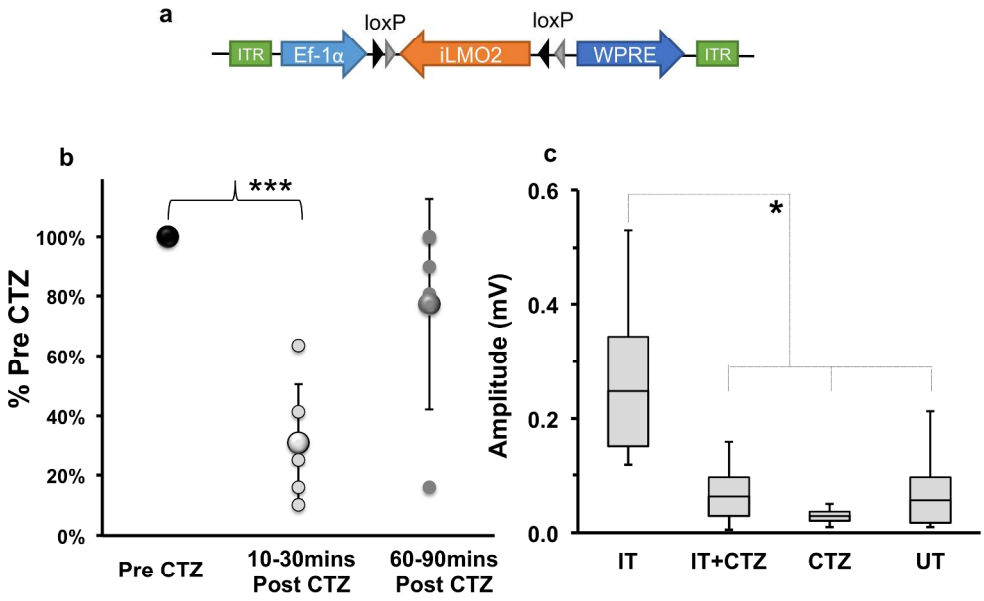
LSD Test; Variable: Gast (LuminopsinANOVA.sta)

| Treatment | IT | IT&CTZ | CTZ | UT |
|---|---|---|---|---|
| IT | | 0.026 | 0.008 | 0.017 |
| IT&CTZ | 0.026 | | 0.641 | 0.933 |
| CTZ | 0.008 | 0.641 | | 0.685 |
| UT | 0.017 | 0.933 | 0.685 | |

LSD Test; Variable: TA (LuminopsinANOVA.sta)

| Treatment | IT | IT&CTZ | CTZ | UT |
|---|---|---|---|---|
| IT | | 0.001 | 0.000 | 0.000 |
| IT&CTZ | 0.0005 | | 0.330 | 0.702 |
| CTZ | 0.0001 | 0.330 | | 0.531 |
| UT | 0.0003 | 0.702 | 0.531 | |

215x279mm (150 x 150 DPI)




(a) Schematic representation of Ef1a-DIO-iLMO2 construct. (b) Significant inhibition of epidural electrical stimulation evoked motor responses in intact animals 10-30mins after (Post CTZ) in the lateral gastrocnemius (LG) muscle and (c) Inhibition of motoneuronal activity blocks exercise-induced enhancements in functional recovery three weeks after a sciatic nerve injury. The amplitude of LG M-max responses in the exercise (IT) group was significantly greater than those mice exercised with luminopsin mediated inhibition (IT+CTZ), mice treated with h-CTZ (CTZ) and untreated mice (UT) groups.

1057x793mm (72 x 72 DPI)