Supplementary Material

MATERIAL AND METHODS

1. Supplementary Table 1: 830 nm LED array lamp parameters for *in vitro* photobiomodulation.

Power (mW)	Exposure Time (minutes)	Energy Intensity (J/cm^2)
1	6	0.2
	12	0.4
	18	0.6
5	6	1
	12	2
	18	3
10	6	2
	12	4
	18	6

2. Sensorimotor test: Cylinder test

A cylinder test was used to evaluate the functional deficit and was performed 1 week prior to tMCAO (to assess the basal locomotor symmetry of the animals) and 24 hours (defined as 0), 1, 3, 5, 7 and 12 weeks after ischemia during the darkness cycle.

Functional outcome was evaluated using the cylinder test. Exploratory behaviour in each animal was recorded for 10 min in a 20 × 30 cm cylinder in a darkened room with an infrared video camera (Sony, Tokyo, Japan). All functional assessments were conducted during the animals' active periods (the first 6 h of the dark period). Scores were obtained from a total number of 10 full rears to control for differences in rearing between animals. Forelimb asymmetry was calculated using the formula: 100 × (ipsilateral forelimb use + 1/2 bilateral forelimb use)/total forelimb use observations.

3. Brain histological analysis

Animals were sacrificed 12 weeks after ischemic injury Animals were deeply anaesthetised with sevoflurane (6% in a mixture of 70% NO2 and 30% O2) and transcardially perfused with 100 mL of PBS (pH 7.4) followed by 150 mL of 4% (v/v) formaldehyde.

Brains were carefully removed from the skull and sectioned at 2 mm thick using a matrix. Slices were post-fixed by immersion in 4% (v/v) formaldehyde overnight, washed in PBS, and cryoprotected in a solution of 30% (v/v) sucrose in PBS with 0.05% sodium azide. Slices were embedded in OCT compound (Sakura Finetek, Torrance, CA, USA), flash-frozen with liquid nitrogen, and cut into 8 µm-thick slices using a cryostat (Sakura Finetek).

Neuronal nuclear protein (Fox3, Sigma-Aldrich) and astrocyte marker glial fibrillary acidic protein (GFAP, Sigma Aldrich) labeling was combined with DAPI stain (Thermo Fisher Scientific, Waltham, MA, USA). In addition, neurogenesis was examined in the striatal region by immunolabelling with a proliferating cell marker (Ki-67, Abcam) and doublecortin (DCX; Dako).

Slices were incubated overnight at RT with primary antibodies diluted in PBS with 0.2% (v/v) Triton X-100 and 15% (v/v) normal serum derived from the same species as the secondary antibody. The dilutions used for the primary antibodies were those specified in the data sheet. Slices were then washed with PBS and incubated for 1h at RT in the dark with the following secondary antibodies: biotinylated horse anti-rabbit (BA-1100; Vector Laboratories), biotinylated horse anti-mouse (BA-2001; Vector Laboratories, Peterborough, UK), DyLight 488 horse anti-mouse (IGGDY488H-OIMG-CUSTOM; Immunostep), or DyLight 488 goat anti-rabbit (DI-1488; Vector Laboratories. The secondary antibodies were diluted 1:200 in PBS with 0.2% (v/v) Triton X-100. Slices were washed and incubated for 30 min in the darkness with DyLight 594 streptavidin (SA-5594; Vector Laboratories) diluted 1:500 in PBS with 0.2% (v/v) Triton X-100. Finally, the slices were washed and incubated with DAPI stain diluted 1:6,000 in PBS for 10 min in the dark.

Slices were mounted with Aqua-Poly/Mount (Polysciences, Warrington, PA, USA) and photographed using a Leica DMI6000 B microscope with Leica Application Suite Advanced Fluorescence software, version 1.0.0 (LAS AF; Leica). A quantitative analysis of Doblecortin and Ki-67, GFAP and Fox3 immunoreactivity was performed using three animals from each group. Photomicrographs were obtained from the striatum and cortex in ischemic hemispheres. Replicate photomicrographs were obtained from two consecutive slices containing the central part of the injured region (between 0.7 mm anterior and 0.3 mm posterior to the bregma). Doblecortin and Ki-67 positive, GFAP-positive and Fox3-positive cells and nuclei were counted manually in ImageJ software.

The ARRIVE Guidelines Checklist

Animal Research: Reporting In Vivo Experiments

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	ITEM	RECOMMENDATION	Section/ Paragraph
Title	1	Provide as accurate and concise a description of the content of the article as possible.	Title
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.	Abstract
INTRODUCTION			
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.	Introduction
		b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.	Introduction
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.	Introduction
METHODS			
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.	Animal studies section
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.	Material and Methods sections
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).	Material and Methods sections
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.	Material and Methods sections

Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.	Animal studies section
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.	Animal studies section Light penetration and In vivo Led sections
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.	Material and Methods sections
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).	Material and Methods sections
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.	Statistical section
RESULTS			
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing. (This information can often be tabulated).	Results sections Figures1-4
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.	Material and Methods sections
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).	Results sections
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.	Results sections
DISCUSSION			
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.	Discussion section
Generalisability/ translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.	Discussion section
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.	Funding section

References:
1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.