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## Slow waves promote sleep-dependent plasticity and functional recovery after stroke

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1 **TITLE PAGE**

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3 **Title:** Slow waves promote sleep-dependent plasticity and functional recovery after stroke

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5 **Running title:** Slow waves promote functional stroke recovery

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26

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43

44 **Authors contribution:** LF, FP, SS, CB and AA designed the research; LF and CS performed  
45 the research; LF, CS, AM, MB analyzed the data; PAL developed the sleep scoring system.  
46 All authors wrote the paper.

47

48 **ABSTRACT**

49 Functional recovery after stroke is associated with a remapping of neural circuits. This  
50 reorganization is often associated with low frequency high amplitude oscillations in the peri-  
51 infarct zone in both rodents and humans. These oscillations are reminiscent of sleep slow  
52 waves (SW) and suggestive of a role for sleep in brain plasticity that occur during stroke  
53 recovery, however, direct evidence is missing. Using a stroke model in male mice, we  
54 showed that stroke was followed by a transient increase in NREM sleep accompanied by  
55 reduced amplitude and slope of ipsilateral NREM sleep SW. We next used 5 ms optical  
56 activation of Channelrhodopsin 2-expressing pyramidal neurons, or 200 ms silencing of  
57 Archeorhodopsin T-expressing pyramidal neurons, to generate local cortical UP, or DOWN,  
58 states, respectively, both sharing similarities with spontaneous NREM SW in freely-moving  
59 mice. Importantly, we found that single optogenetically-evoked SW (SW<sup>opto</sup>) in the peri-  
60 infarct zone, randomly distributed during sleep, significantly improved fine motor  
61 movements of the limb corresponding to the sensorimotor stroke lesion site, as compared to  
62 spontaneous recovery and control conditions, while motor strength remained unchanged. In  
63 contrast, SW<sup>opto</sup> during wakefulness had no effect. Furthermore, chronic SW<sup>opto</sup> during sleep  
64 were associated with local axonal sprouting as revealed by the increase of anatomical pre-  
65 and post-synaptic markers in the peri-infarct zone and corresponding contra-lesional areas to  
66 cortical circuit reorganization during stroke recovery. These results support a role for sleep  
67 SW in cortical circuit plasticity and sensorimotor recovery after stroke and provide a  
68 clinically-relevant framework for rehabilitation strategies using neuromodulation during  
69 sleep.

70

71

72 **SIGNIFICANCE STATEMENT**

73 Brain stroke is one of the leading causes of death and major disabilities in elderly worldwide.  
74 A better understanding of the pathophysiological mechanisms underlying spontaneous brain  
75 plasticity after stroke, together with an optimization of rehabilitative strategies, are essential  
76 to improve stroke treatments. Here, we investigate the role of optogenetically-induced sleep  
77 slow waves in an animal model of ischemic stroke and identify sleep as a window for post-  
78 stroke intervention that promotes neuroplasticity and facilitates sensorimotor recovery.

79

## 80 INTRODUCTION

81 Stroke is an acute brain injury caused by a sudden decrease in cerebral blood flow, followed  
82 by local inflammation (Huang et al., 2006), excitotoxicity (Lai et al., 2014) and cell death  
83 (Small et al., 1999). Changes in neuronal excitability after stroke are thought to promote  
84 long-term plasticity in surviving neurons that contributes to the reorganization of cortical  
85 maps and to the underlying level of axonal sprouting supporting brain functions (Carmichael,  
86 2012; van Meer et al., 2012; Silasi and Murphy, 2014), as observed in rodents (Nudo, 1997;  
87 Murphy and Corbett, 2009; Carmichael et al., 2017) and humans (Khedr et al., 2005;  
88 Lindenberg et al., 2010). To date, pharmacological treatments and non-invasive brain  
89 neuromodulation techniques hold promise in improving plasticity and functional recovery  
90 both in animal model (Zhang et al., 2007; Yoon et al., 2012) and human (Robinson et al.,  
91 2008; Ameli et al., 2009; Talelli et al., 2012), yet the underlying mechanisms remain unclear.

92  
93 Post-stroke hyperexcitability of surviving neurons contributes to the transient low-frequency  
94 (~ 1 Hz, 200-500 ms in duration), high amplitude, rhythmic waves (also coined ‘bistable  
95 state’) originating in the peri-infarct zone and propagating to contra-lesional brain areas. This  
96 distinctive 1-Hz slow and synchronous neural activity in the peri-infarct zone shares  
97 similarities with slow waves (SW) typically recorded during non-rapid eye movement  
98 (NREM) sleep in rodents and human. Indeed, SW reflect bistable states of thalamocortical  
99 neuron populations, described as a switch between UP states where depolarised membrane  
100 potentials are accompanied by high spiking activity, and DOWN states during which cells are  
101 hyperpolarized and show low spiking activities in cats (Steriade et al., 1993), rodents  
102 (Vyazovskiy et al., 2009; Zucca et al., 2017), non-human primates (Xu et al., 2019) and  
103 human (Csersca et al., 2010). These SW were hypothesized to guide axonal sprouting and

104 circuit rewiring through the formation of new connections after brain lesions (Carmichael and  
105 Chesselet, 2002) facilitating recovery, however this has not been directly demonstrated.

106

107 Extensive experimental evidence suggests a fundamental role for intact sleep, and SW in  
108 particular, in enhancing brain plasticity during spontaneous sleep (Tononi and Cirelli, 2016;  
109 Timofeev and Chauvette, 2017) and stroke recovery (Duss et al., 2017). The detrimental  
110 effects of sleep disturbances (Kaneko et al., 2003; Baglioni et al., 2016) and the beneficial  
111 effect of pharmacological NREM sleep enhancement after stroke support the hypothesis that  
112 SW contribute to brain plasticity underlying post-stroke functional and cognitive recovery  
113 both in animal models (Gao et al., 2008; Hodor et al., 2014) and patients (Vock et al., 2002;  
114 Siccoli et al., 2008; Sarasso et al., 2014).

115

116 Here, we used an optogenetic approach inspired by global and local SW changes after stroke  
117 to rescue SW-like activity in freely-moving mice. Optogenetic activation of pyramidal  
118 neurons in the peri-infarct zone during NREM sleep improved fine motor movements as  
119 compared to experimental control conditions. In contrast, optogenetically-evoked SW  
120 ( $SW^{opto}$ ) during wakefulness had no effect. Importantly,  $SW^{opto}$  evoked recovery after stroke  
121 was associated with axonal sprouting in the peri-infarct zone and corresponding contra-  
122 lesional areas.

123

124

## 125 **MATERIALS AND METHODS**

### 126 **Animals**

127 C57BL/6JRj male mice ([https://www.janvier-labs.com/en/fiche\\_produit/c57bl-6jtrj\\_mouse/](https://www.janvier-labs.com/en/fiche_produit/c57bl-6jtrj_mouse/))  
128 (5-6 weeks old, 23-30 grams) were used in the study. Animals were individually housed in

129 custom-designed polycarbonate cages (300 mm x 170 mm) under controlled conditions  
130 (regular circadian cycle of 12:12 h light:dark; light on at either 4 a.m. or 8 p.m. according to  
131 experimental design; constant temperature  $22 \pm 1$  °C and humidity 30-50%). Throughout the  
132 experiment animals were freely-moving with *ad libitum* food and water. Animals were kept  
133 in groups of 2-5 per IVC cage before instrumentation and after viral injection surgery.  
134 Following implantation mice were all housed individually. Animals were tethered, allowed to  
135 adapt to the EEG/EMG and optic stimulation cables in their home cage for at least 5-7 days,  
136 and remained plugged for the duration of the experiment. Animals were detached from all  
137 tethers for 4 days following stroke or sham surgery and for the duration of behavioural  
138 testing. Animals were randomly assigned to eight experimental groups: Channelrhodopsin  
139 (ChR2) transfected animals subjected to stroke (ChR2<sup>stroke</sup>), ChR2 transfected animals  
140 subjected to stroke and optogenetically stimulated mainly during wakefulness  
141 (ChR2<sup>stroke\_wake</sup>), Archaelhodopsin (ArchT) transfected animals subjected to stroke  
142 (ArchT<sup>stroke</sup>), mCherry transfected animals subjected to stroke (mCherry<sup>stroke</sup>), mCherry  
143 transfected animals subjected to sham surgery (mCherry<sup>sham</sup>), Naive, Sham and Stroke.  
144 Animals that displayed baseline asymmetry in limb usage or did not show a drop in cerebral  
145 blood flow (CBF) by ~ 80% during middle cerebral artery occlusion (MCAo) surgery were  
146 excluded from further experimental tests. Viral injections were performed when animals were  
147 5-6 weeks of age, instrumentation at 8 weeks of age and stroke/ sham surgery at 10 weeks of  
148 age. Between surgeries and before being tethered, animals were let recover undisturbed for at  
149 least 7 days. Naive mice did not undergo any surgical procedures. An additional set of  
150 heterozygous Tg(VGAT-Cre) mice, (5-6 weeks old, 23-30 grams) was used for an  
151 optogenetic screening of slow waves-like oscillations inducing protocols. All animals were  
152 treated according to animal care laws and experimental procedures were approved by local

153 authorities (Veterinary Office, Canton of Bern, Switzerland; licence numbers BE 113/13 and  
154 BE 41/17).

155

### 156 **Viral targeting**

157 For a detailed description of the surgical procedure refer to Herrera et al., 2016. Briefly, 5-6  
158 weeks old animals were anesthetized with isoflurane (4.0% induction; 1.0-1.5%  
159 maintenance). Body temperature was constantly monitored and kept at physiological range  
160 using a rectal thermoprobe and feedback-controlled heating system. Animals were fixed in a  
161 digital stereotaxic frame and analgesia was administered subcutaneously (Meloxicam,  
162 5mg/kg). Animals were randomly assigned to receive 0.6  $\mu$ L of recombinant AAV carrying  
163 either CaMKII-hChR2 (H134)-EYFP (activation), CaMKIIa-eArchT3.0-EYFP (silencing) or  
164 CaMKIIa-mCherry (control) respectively. Plasmids were stereotactically injected (0.1  
165  $\mu$ L/min infusion rate) through a 28 G needle (Plastic One), connected by a tubing to a 10  $\mu$ L  
166 Hamilton syringe in an infusion pump (Model 1200, Harvard Apparatus). Injections were  
167 performed within the left (prospective ipsilateral) primary somatosensory forelimb cortex  
168 (iS1FL, AP: -0.10 mm; ML: -2.00 mm; DV: -0.7 mm). Animals were given 7 days of  
169 recovery prior to instrumentation surgery. Tg(VGAT:Cre) mice underwent identical surgical  
170 procedures as wild type animals, randomly assigned to receive 0.6  $\mu$ L of recombinant AVV  
171 carrying Ef1 $\alpha$ -DIO-ChR2-EYFP (activation), Ef1 $\alpha$ -DIO-ArchT-EYFP (silencing) or Ef1 $\alpha$ -  
172 DIO-EYFP (control) respectively. All plasmids were obtained from the University of North  
173 Carolina Vector Core Facility. Mice belonging to Sham, Stroke and Naive groups did not  
174 received any AAV injection.

175

### 176 **Instrumentation**



177 Animals were chronically implanted with a unilateral optic fiber (200  $\mu\text{m}$  in diameter) within  
178 the iS1FL (AP: -0.10 mm; ML: -2.00 mm; DV: -0.5 mm) and an electroencephalography  
179 (EEG)/electromyography (EMG) connector. As previously reported (Gent et al., 2018),  
180 animals received analgesia (Meloxicam, 5mg/kg), were anaesthetised with isoflurane and  
181 anchored to a stereotaxic frame. Five stainless steel EEG electrode screws were inserted  
182 through each animal's skull; two screws over the frontal cortices (AP: +2 mm; ML:  $\pm 2$ mm),  
183 two screws over the posterior cortices (AP: -4 mm; ML:  $\pm 2$  mm) and one screw over the  
184 olfactory bulb as ground. For the stimulation recordings, the EEG signals from the frontal and  
185 posterior channels were referenced to each other directly, leaving only two EEG traces, one  
186 per hemisphere. Finally, two bare-ended EMG wires were sutured to the neck muscles to  
187 record postural tone. A subset of animals was additionally implanted with four tetrodes to  
188 record local field potentials (LFPs) and single unit activity during optogenetic stimulation, as  
189 well as EEG/EMG signals. Tetrodes were constructed by twisting four tungsten wires  
190 together (10  $\mu\text{m}$  in diameter, CFW0010954, California Fine Wire) and briefly heating them  
191 to favour the bond coating of each wire to another. Tetrodes were lowered within the iS1FL  
192 (AP: -0.10 mm; ML: -2.00 mm; DV: -0.5 mm), the ipsilateral primary motor cortex (iM1,  
193 AP: +1.10 mm; ML: -1.5 mm; DV: -1.20 mm), the contralateral S1FL (cS1FL, AP: -0.10  
194 mm; ML: +2.00 mm; DV: -0.5 mm) and the contralateral M1 (cM1, AP: +1.10 mm; ML:  
195 +1.5 mm; DV: -1.20 mm) respectively. The tetrode positioned in iS1FL was glued to the  
196 optic fiber, where the tip of the tetrode extended for ~0.2 mm beyond the end of the fiber  
197 (optrode). Optic fibers and implants were permanently secured to the skull with C&B  
198 Metabond (Patterson Dental) and methacrylate cement (Paladur). Animals were monitored  
199 post-operatively and left to recover undisturbed for at least 7 days. Animals were then  
200 plugged to the EEG/EMG/optic stimulation and tetrodes tethers (Neuralynx headstage).  
201 Black nail polish was applied at the connection point between optic fiber and patch cord to

202 limit laser light spreading during optogenetic stimulations. The implantation procedure for  
203 animals belonging to Sham and Stroke groups did not include either optic fiber or tetrodes  
204 placement.

205

#### 206 **Transient focal cerebral ischemic stroke**

207 Mice underwent MCAo via intraluminal filament model (Doeppner et al., 2010) at around 10  
208 weeks of age. To begin, mice were anaesthetised with isoflurane as previously described and  
209 placed in a prone position. Physiological temperature was maintained as mentioned above.  
210 The left common carotid artery (CCA) was dissected from the surrounding connective tissue.  
211 A monofilament suture (7-0 silicon rubber coated, coating length 5-6 mm, Doccol  
212 Corporation) was inserted in the CCA and introduced into the lumen of the MCA. The  
213 monofilament was left in place for 45 min to induce both striatal and cortical infarct and  
214 consequently withdrawn to allow the reperfusion of the territory targeted by the MCA. CBF  
215 was constantly monitored by a Laser Doppler probe (Moor Instrument, VMS-LDF2) glued to  
216 the skull above the MCA region. Ischemic stroke induction was considered successful when  
217 the CBF showed a ~ 80 % reduction from baseline values, as well as reperfusion of the MCA  
218 territory. Following surgery, mice were daily checked for pain and weight loss, received  
219 mashed, watered food, subcutaneous analgesia and 0.9% saline. Animals belonging to the  
220 Naive group did not undergo stroke or sham surgery. No filament was inserted into the MCA  
221 during sham surgery. Following MCAo, 40% of animals assigned to the Stroke group and  
222 33% of all animals allocated to optogenetic stimulations did not survive the post-operation  
223 phase.

224

#### 225 **Optogenetic stimulation**

226 Lasers (Laserglow Technologies) attached to the unilateral fiber via patch cord (Thorlabs)  
227 were triggered through TTL with a pulse stimulator (Master - 9, AMPI), this latter controlled  
228 by a function generator (Agilent, 33220A 20MHz Function/ Arbitrary Waveform Generator)  
229 to induce random pulse sequences. Animals received daily 2 h of randomly distributed single  
230 laser light pulses (inter-pulses interval 3-30 sec), from post-stroke day 5 until day 15. The  
231 random distribution of light pulses was selected to avoid hypersynchrony and entrainment of  
232 oscillatory activities which, *per se*, might influence the observed parameters. The optogenetic  
233 stimulation was semi-chronic: light pulses were distributed across sleep and wake states  
234 without simultaneous behavioural scoring by the experimenter and consequent state specific  
235 stimulation. Indeed, daily and chronic stimulation (11 days) of several animals (experimental  
236 and control were run in parallel) is not suited for a single experimenter. The specific time  
237 allocated for optogenetic intervention was therefore selected according to the natural  
238 distribution of the majority of NREM sleep and wakefulness episodes throughout the 12h  
239 light:dark cycle of the animals. Two stimulation protocols were employed: ChR2-expressing  
240 animals received 5 ms blue light pulses (473 nm wavelength), ArchT-expressing mice were  
241 stimulated with 200 ms green light pulses (532 nm wavelength) and mCherry-expressing  
242 animals were randomly subjected to either 200 ms or 5 ms light pulses. To assess whether the  
243 effect of SW<sup>opto</sup> on functional recovery was specific to brain activity occurring during sleep,  
244 in a separate group of animals (ChR2<sup>stroke\_wake</sup>) optogenetic stimulations were also delivered  
245 during the first part of the dark phase, when animals were mostly awake. Based on pre-  
246 instrumentation testing of both optic fiber and patch cord outputs, light power was set at 20-  
247 25 mW.

248

249 **Data acquisition**

250 EEG and EMG signals were amplified (Model 3500, AM System) and digitized at 512 Hz  
251 (NIDAQ 6363, National Instruments) using a sleep recording software (MATLAB written  
252 software, DaqReverse). A 24 h baseline of spontaneous sleep-wake behaviour was recorded  
253 for all animals. Stroke and Sham animals were recorded for 24 h at post-surgery days 1, 3, 5  
254 and 10. All optogenetic stimulations took place between 9 a.m. and 2 p.m., with light on at 4  
255 a.m. for ChR2<sup>stroke</sup>, ArchT<sup>stroke</sup>, mCherry<sup>stroke</sup> and mCherry<sup>sham</sup>. Since ChR2<sup>stroke</sup> and  
256 ArchT<sup>stroke</sup> animals showed similar functional outcomes upon neuronal manipulation during  
257 sleep, an additional ChR2-transfected set of animals received SW<sup>opto</sup> during animals' active  
258 phase (between 9 a.m. and 2 p.m., lights on at 8 p.m, ChR2<sup>stroke\_wake</sup>), from post-stroke day 5  
259 until day 15. Animals' spontaneous sleep was recorded for 18 h at post-stroke day 5, 6, 8, 12  
260 and 14 respectively. LFPs and EEG/EMG signals were amplified and digitized at 32 kHz  
261 (Cheetah 5 acquisition software, Neuralynx, [https://neuralynx.com/software/cheetah-5.0-](https://neuralynx.com/software/cheetah-5.0-legacy)  
262 legacy).

263

#### 264 **Behavioural tests**

265 All animals were trained in four behavioural tests and engaged in daily training sessions for  
266 three consecutive days. Behavioural baselines were acquired prior to stroke/ sham surgery.  
267 Functional outcomes were verified at post-stroke days 4, 7, 10 and 15. All behavioural tests  
268 were conducted at least 3 h apart from optogenetic stimulations and during animals' active  
269 phase (between 5 p.m. and 8 p.m.). Test sessions were recorded with a picamera (Raspberry  
270 Pi) and scored in slow motion (VideoPad software,  
271 <https://www.nchsoftware.com/videopad/index.html>).

272 **Balance beam test:** To assess motor balance and coordination (Brooks and Dunnett, 2009) a  
273 round wooden beam (12 mm in diameter, 80 cm long) was positioned at an angle so that one  
274 end of the beam was 60 cm elevated from the working table. At the beam's elevated end, the

275 animal's home cage served as motivation to complete the task. Soft fabric placed beneath the  
276 beam avoided possible falling injuries. The number of 'paw faults' (forelimb or hindlimb  
277 slipping off the beam) were counted during a maximal testing time of 60 sec. Each animal  
278 underwent three trials per time point and means were calculated.

#### 279 ***Tight rope test***

280 To measure grip strength and endurance (Balkaya et al., 2013) animals were suspended on a  
281 fine rope (60 cm above the working table) between two platforms (80 cm apart from one  
282 another). Mice were positioned at the middle point of the rope exclusively with their  
283 forepaws. The average time needed to reach one of the two platforms was calculated between  
284 two trials. The maximum testing time was 60 sec.

#### 285 ***Corner turn test***

286 To evaluate the presence of unilateral abnormalities (Park et al., 2014) mice were placed in  
287 between two vertical boards forming a 30° angle. Animals left- or right-turn decision was  
288 recorded for a total of 10 trials per testing session. Laterality index was calculated as (number  
289 of left turns – number of right turns)/10.

#### 290 ***Ladder walking rig test***

291 The test was chosen to measure paw accurate placement (Cummings et al., 2007). The  
292 apparatus consisted of a ladder (80 cm long), suspended between two platforms (60 cm above  
293 the working table) with randomly spaced rungs. Paw faults were recorded as animals walked  
294 to reach the home cage at one end of the ladder. Mice performances were scored in slow  
295 motion and the mean of three trials calculated. The position of the rungs was randomly  
296 changed across trials to avoid learning.

297

#### 298 **Signal processing**

299 As previously described (Jego et al., 2013), electrophysiological data were manually scored  
300 in 5 sec epochs and analysed using SlipAnalysis (custom written MATLAB program).  
301 Briefly, three vigilance state were identified based on EEG/EMG frequency and amplitude.  
302 Wakefulness was determined by low amplitude EEG and high activity EMG signals; NREM  
303 sleep as high amplitude and low-frequency EEG (0.5-4 Hz) paired with reduced EMG  
304 activity; REM was characterized by theta rhythm (6-9 Hz) EEG and flat EMG. Microarousals  
305 were defined and scored as cortical fast rhythm and EMG bursts of at least 1 sec. Sleep/  
306 wakefulness scoring was based on the visual characteristics of the contralateral EEG traces  
307 specifically. Electrophysiological analysis was completed using custom MATLAB scripts.

308

#### 309 **Automatic single SW detection**

310 Individual SW were detected during NREM sleep epochs during the first 7 h of the lights ON  
311 period in MATLAB using the SWA-MATLAB toolbox (Mensen et al., 2016), with detection  
312 parameters adjusted to rodents from settings described in Panagiotou et al., 2017. Briefly, in a  
313 first-pass of the data, the negative envelope across the 4 EEG channels was calculated,  
314 filtered between 0.5 and 4 Hz (Chebyshev Type II filter design), and consecutive zero-  
315 crossings were detected. If the duration between successive downward (negative going) zero-  
316 crossing and upward zero-crossing was between 100 msec and 1 sec, then the peak negative  
317 amplitude was examined and was required to be at least 3 deviations from the median  
318 amplitude of all negative peaks in the recording. The amplitude threshold eliminates the  
319 potential individual differences of electrodes reference type, distance to those reference, and  
320 electrode depth that would affect the record amplitude. In a second-pass, the activity over all  
321 4 channels was examined for each slow wave detected on the negative envelope to obtain  
322 individual channel data.

323

324 **Single unit analysis**

325 We performed spike detection and sorting as described previously (Gent et al., 2018). Briefly,  
326 we first extracted multiunit activity from band-pass filtered signals (600-4000 Hz, 4<sup>th</sup>-order  
327 elliptic filter, 0.1 dB passband ripple, -40 dB stopband attenuation), by applying a detection  
328 threshold of  $7.5 \times$  the median of the absolute values of the filtered signal. We then extracted  
329 wavelet coefficients from the detected multiunit activity using a four-level discrete wavelet  
330 transform (Harr wavelet, 'wavedec', MATLAB), and subsequently sorted the coefficients  
331 using the super-paramagnetic clustering. We visually inspected the sorted units and excluded  
332 the clusters with a symmetric shape or an average firing rate less than 0.2 Hz from our  
333 analyses.

334

335 **Optogenetic response analysis**

336 We assessed the optogenetic response analysis for each vigilance state separately. For unit  
337 activity, we calculated mean firing rates during optogenetic perturbations by averaging firing  
338 rates across trials using a non-overlapping moving window of 5 ms. For LFP analysis, we  
339 averaged raw LFP signals across trials of each vigilance state.

340

341 **Infarct volume evaluation and immunohistochemistry**

342 Animals were sacrificed at post-stroke day 15 with 15 mg pentobarbital intraperitoneal  
343 injection (Esconarkon ad us. vet., Streuli Pharma) and transcardially perfused with 1x  
344 phosphate buffered saline (PBS) followed by 4% formalin. Brains were post-fixed overnight,  
345 cryoprotected in 30% sucrose (24-48 h at 4°C), frozen in 2-methyl-butane on dry ice and cut  
346 into 40  $\mu$ m sections. Every third slice was mounted onto a glass slide, dried at room  
347 temperature (RT), rehydrated and processed for Nissl staining. Briefly, sections were  
348 immersed in Cresyl Violet (Klüver Barrera, Bio-Optica), washed in distilled water and

349 dehydrated in graded alcohols, cleared in Xylene (Sigma-Aldrich) and mounted (Eukitt  
350 mounting medium, Bio-Optica) on microscope slides. Stroke edges were delineated per  
351 section using Imagej software (<https://imagej.nih.gov/ij/>). The damaged area was measured in  
352 each brain slice and multiplied by the distance between brain sections. Stroke volume relative  
353 to the whole brain was calculated as follows:  $((\text{volume of contralesional hemisphere} - \text{volume}$   
354  $\text{of ipsilesional hemisphere})/2 * \text{volume of contralesional hemisphere}) * 100$  (Lin et al., 1993).  
355 Fluorescent immunohistochemical staining was performed with free-floating brain sections.  
356 Brain slices were washed in PBS-Triton (PBS-T) and incubated in blocking solution (1 h at  
357 RT; PBS-T with 4% of bovine serum albumin, SIGMA Life Science). Free-floating slices  
358 from ChR2- and ArchT-expressing animals were incubated in a primary antibody to GFP  
359 (chicken IgY fraction anti-GFP, 1:5000, Cat# A10262, RRID:AB\_2534023, Life  
360 Technologies) in blocking solution (24-48 h at 4°C). Following repeated washes in PBS-T,  
361 sections were incubated with the secondary antibody (1:500, Cat# ab96947,  
362 RRID:AB\_10681017, Abcam) in PBS-T (1 h at RT). Sections were then washed in PBS-T,  
363 mounted and covered on microscope slides.

364

#### 365 **Axonal sprouting quantification**

366 Four brains per experimental group were randomly chosen for axonal sprouting evaluation.  
367 Brains were fixed, frozen and cut as previously described. Several 40  $\mu\text{m}$  sections per brain  
368 were selected (approximately, from Bregma 1.10 mm to Bregma -0.70 mm) and stained for  
369 Vglut1, PSD-95 and DAPI. Floating sections were washed in PBS and blocked in PBS with  
370 0.5% Triton X-100 and 10% normal donkey serum (NDS, Jackson ImmunoResearch Code:  
371 017-000-121) (2 h at RT). Sections were then incubated with the following primary  
372 antibodies: chicken IgY fraction anti-GFP (ChR2<sup>stroke</sup> and ArchT<sup>stroke</sup>, Cat# A10262,  
373 RRID:AB\_2534023, Life Technologies), rabbit anti-Vglut1 (ChR2<sup>stroke</sup>, ArchT<sup>stroke</sup>, mCherry-



374 expressing animals, 1:1000, Cat# 135 303, RRID:AB\_887875, SYSY), goat anti-PSD-95  
375 (Chr2<sup>stroke</sup>, ArchT<sup>stroke</sup>, mCherry-expressing animals, 1:500, Cat# ab12093,  
376 RRID:AB\_298846, Abcam) and mCherry respectively (mCherry-expressing animals, 1:1000,  
377 Cat# M11217, RRID:AB\_2536611, Life Technologies) in PBS containing 3% NDS and  
378 0.5% Triton X-100 solution (overnight at 4°C). Brain slices were repeatedly washed in PBS  
379 and incubated with appropriate secondary antibodies (1:500, Alexa Fluor 488 Ab96947,  
380 Abcam; all others 1:1000, Invitrogen) in PBS containing 3% NDS and 0.5% Triton X-100  
381 solution (2 h at RT). A negative control (no addition of primary antibody) was carried out to  
382 confirm the antibody selectivity. Sections were further stained for DAPI (1:500 in PBS, 10  
383 minutes), washed in PBS, mounted on microscope slides and covered. Photomicrographs  
384 were acquired with Olympus Fluoview 1000-BX61 confocal microscope (Olympus, Tokyo)  
385 fitted with 60X oil-immersion objective (4x zoom, 0.5  $\mu\text{m}$  step size). Three fields of interest  
386 (52.172  $\mu\text{m}$  x 52.172  $\mu\text{m}$ ) within iS1FL and cS1FL were imaged in three sections per animal.  
387 Imaris software (Microscopy Image Analysis Software, Bitplane, <https://imaris.oxinst.com/>)  
388 was used to reconstruct the 3D view of the Z stacks and to evaluate pre- and post- synaptic  
389 compartments' density and volume. Briefly, background subtraction, image smoothing via  
390 gaussian filtering and channel intensity adjustment were applied and maintained identical for  
391 all the acquired confocal images. A preliminary stack selection was carried out to localize  
392 puncta distributed within two consecutive stacks. A puncta diameter threshold was specified at  
393 0.6  $\mu\text{m}$  and when this value was exceeded, puncta were separated upon visual confirmation  
394 by the experimenter.

395

### 396 **Statistical analysis**

397 For the analyses of the 24 h recordings of stroke and sham animals, a two-level analysis was  
398 performed using linear mixed models. A first-level analysis on each animal and recording

399 day, including temporal predictors of recording time, time since last wake epoch in order to  
400 estimate the homeostatic effect on individual slow wave characteristics across the lights on  
401 period as well as the potential differences between the ipsi- and contralateral hemisphere. At  
402 the second level, the parameter estimates from the first level data for each animal for each  
403 day were used to examine the overall effects of stroke over the course of 10 days after stroke.  
404 The potential effects of days, stroke, stimulation group, on sleep parameters and behavioural  
405 outcomes were tested using linear mixed models. Sleep and behavioural values from day 0  
406 was assigned as a baseline predictor, while those from day 4 were used as pre-stimulation  
407 baseline. Main effects and interactions were tested for significance using the log-likelihood  
408 ratio test between the full model and the model without the specific factor in question. The  
409 effects between the stimulation groups were examined by post-hoc t-tests within the linear  
410 mixed model. As an exploratory analysis, macro and micro sleep parameters during the  
411 stimulation time were included as a potential predictor of behavioural outcome: percentage of  
412 NREM, number of micro-arousals, NREM-to-wake transition ratio, wave incidence, wave  
413 amplitude, wave duration, positive and negative slope.

414 For the pre- and post-synaptic markers assessment, statistical comparisons were determined  
415 with Student's *t*-test, one-way ANOVA, where corrections for multiple comparisons were  
416 carried out using Bonferroni correction, if not otherwise indicated (Prism 6 GraphPad;  
417 <https://www.graphpad.com/scientific-software/prism/>). Data are presented as mean  $\pm$  standard  
418 error of the mean (s.e.m.) and levels of statistical significance were set at threshold  $p < 0.05$   
419 unless otherwise indicated. Sample sizes were defined based on previous studies (Gao et al.,  
420 2008; Jago et al., 2013; Herrera et al., 2016). For each experiment, sample numbers are  
421 indicated in the corresponding figure legends. Animals that did not perform behavioural  
422 testing were excluded from the analysis; as well as mice that lost EEG/EMG signals during  
423 longitudinal measurements. Data distribution was tested for normality using the Lilliefors test

424 on the residuals from each linear mixed model calculated and found to be normally  
425 distributed. Experiments were not conducted in blinded fashion.

426

#### 427 **Data availability**

428 Dataset and coding supporting the current study are available from the corresponding author  
429 upon request.

430

431

## 432 **RESULTS**

### 433 **Stroke alters sleep architecture and SW profile**

434 SWs-like oscillations are frequently observed in peri-infarct zone during NREM sleep and  
435 wakefulness (Yokoyama et al., 1996; Murri et al., 1998; Fernandez-Bouzas et al., 2002). To  
436 refine the characterization of brain activity after stroke, including SW features, we first  
437 quantified the changes of sleep-wake architecture and sleep quality from animals subjected to  
438 MCAo and sham surgeries (Fig. 1A, B). Animals were chronically implanted with  
439 EEG/EMG electrodes for longitudinal sleep recordings prior to, and at 1, 3, 5, and 10 days  
440 after MCAo (see Methods; Fig. 1C). To control for multiple comparisons between the 8 sleep  
441 metrics, the significance threshold was reduced to  $p < 0.0063$  (i.e.  $0.05/8$ ; Bonferroni  
442 correction). MCAo resulted in an initial increase of NREM sleep duration with group  
443 differences dampening over the days recorded (Figure 1E, *Day by Stroke* interaction:  $LR(1) =$   
444  $7.977$ ,  $p = 0.0047$ ). Significant main effects of stroke were found for total wake duration  
445 (Figure 1D;  $LR(2) = 22.385$ ,  $p < 0.0001$ ); and wake bout duration ( $LR(2) = 34.502$ ,  $p <$   
446  $0.0001$ ), but this general effect was not significantly different over the days after correction  
447 (*Day by Stroke* interaction:  $LR(1) = 4.328$ ,  $p = 0.0375$ ). No significant results were observed  
448 for REM sleep total duration (Fig. 1F). We further explored the potential effect of stroke size

449 within the MCAo group on all sleep architecture measures but found no main effects or  
450 interaction effects with the day of recording (all  $p$ -values  $> 0.0063$ ).

451 NREM sleep instability, describing the ratio between the animals' capacity of remaining  
452 asleep compared to waking up, showed that stroke animals were significantly more likely to  
453 wake up (Fig. 1J; LR(2) = 14.918,  $p = 0.0006$ ).

454 The number of microarousals, scored as single epoch of 1 sec (minimum) increased EMG  
455 signal within a NREM sleep episode, did not differ between Stroke and Sham (Fig. 1K;  
456 LR(2) = 4.651,  $p = 0.0977$ ).

457

458 To assess SW features and changes after MCAo stroke, animals were prepared for  
459 simultaneous recordings of EEG/EMG, LFPs or single/ multi-units activities in iS1FL,  
460 cS1FL, iM1 and cM1 layer V (see Methods and Fig. 2B for illustration). Clear periods of  
461 neuronal quiescence corresponding to cortical DOWN states confirmed the selectivity of our  
462 SW detection method (see Methods for detection criteria; Fig. 2A-D). Indeed, perilesional  
463 tetrodes recordings of unit activity in S1FL showed suppression, and subsequent increase, in  
464 neuronal activity (Fig. 2D, top), validating the average unit firing rate observed during the  
465 detected SW (Fig. 2D, bottom). Both local and global SW occurred across all recorded  
466 neocortical areas (Fig. 2A), consistent with previous reports in rodents and humans (Huber et  
467 al., 2004; Vyazovskiy et al., 2011). If individual waves are detected across the 24 h period,  
468 we observed a significant reduction of ipsilateral SW's amplitude by  $-13.2 \pm 7.3\%$  after  
469 stroke as compared to sham controls that persisted for up to 10 days after stroke (Fig. 2F; day  
470 10:  $-15.4 \pm 6.4\%$ ;  $F(2, 70) = 13.82$ ,  $p < 0.0001$ ; two-way ANOVA, followed by Bonferroni  
471 post-hoc test). These findings are consistent with hemispheric stroke in rodents and human  
472 subjects (Ahmed et al., 2011; Poryazova et al., 2015). Moreover, the SW positive slope was  
473 reduced within the ipsilateral area of Stroke animals (Fig. 2G;  $F(2, 76) = 13.02$ ,  $p < 0.0001$ ),

474 whereas the negative slope increased (Fig. 2H;  $F(2, 76) = 15.89$ ,  $p < 0.0001$ ). No significant  
475 changes were found in the number of detected SW (Fig. 2I;  $F(2, 87) = 0.693$ ,  $p = 0.503$ ) or  
476 their duration (Fig. 2J;  $F(82, 83) = 0.744$ ,  $p = 0.478$ ; two-way ANOVA, followed by Tukey  
477 post-hoc test). When exploring for a homeostatic effect, we did not find differences in  
478 amplitude between Stroke and Sham for ipsi- and contralateral EEG traces ( $LR(2) = 0.940$ ,  $p$   
479  $= 0.625$ ) nor for *time of night* ( $LR(2) = 3.791$ ,  $p = 0.150$ ).

480

#### 481 **SW<sup>opto</sup> revealed a critical window of intervention after stroke**

482 Here, we aimed at identifying the effect of optogenetically-induced SW (SW<sup>opto</sup>) on the  
483 recovery of motor function following MCAo stroke in mice. Thus, we genetically targeted  
484 the expression of opsins to pyramidal neurons in layer V of the neocortex, given their  
485 implication in the generation of slow oscillations (McCormick et al., 2015; Beltramo et al.,  
486 2013). To achieve this, we stereotactically infused AAV2 viruses carrying Chr2, ArchT or  
487 mCherry gene cassettes under CaMKII promoter in iS1FL (Fig. 3A, B) before animals were  
488 chronically implanted with EEG/EMG electrodes, tetrodes in cS1FL, iM1 and cM1 cortices  
489 (layer V) and a single optrode in iS1FL (see above and Methods, Fig. 3A). We first optimized  
490 the frequency and duration of optogenetic stimulations to mimic NREM sleep SW in both  
491 wild type and VGAT-Cre transgenic mice to modulate excitatory or inhibitory neurons in  
492 iS1FL with 5 Hz, 1 ms light pulses (activation protocol) or 100, 200, 500 ms single pulses  
493 (silencing protocol) (Fig. 4). We found that 5 ms optogenetic activation of iS1FL Chr2-  
494 expressing pyramidal neurons induced a short UP-like state followed by a DOWN-like state,  
495 indistinguishable from spontaneous NREM sleep SW (Fig. 3C-E). Similar SW<sup>opto</sup> waveform  
496 profiles were obtained upon 200 ms optogenetic silencing of iS1FL ArchT-expressing  
497 pyramidal neurons (Fig. 3F, I). In the latter condition, the duration of the optogenetic  
498 silencing of iS1FL ArchT-expressing pyramidal neurons corresponded to the average

499 duration of spontaneous NREM sleep DOWN states (Fig. 2C; duration:  $205.2 \pm 4.4$  ms; Fig.  
500 3H). Off-line analysis confirmed that  $SW^{opto}$  duration, negative amplitude and slope were  
501 indistinguishable from naturally occurring NREM sleep SW from the same animal (Fig. 3E,  
502 I).  $SW^{opto}$  propagated to contralateral recording sites, where  $SW^{opto}$  of variable amplitudes  
503 were recorded in EEG, LFP and single activity traces (Fig. 3D, H and Fig. 5). No changes in  
504 EEG features were observed in control conditions (Fig. 3J-L).

505

506 To determine the optimal window for optogenetic intervention after MCAo, we evaluated the  
507 effect of this stimulation parameters on the survival rates of stroke animals. Strikingly, we  
508 observed that  $ChR2^{stroke}$  animals had lower survival rate than  $ArchT^{stroke}$  and  $mCherry^{stroke}$   
509 mice when the optogenetic manipulation started on post-stroke day 1 (single 5 or 200 ms  
510 light pulses, at 473 nm or 532 nm, respectively, randomly distributed over 2 h, daily; Fig. 6A;  
511 Chi square(2) = 7.941,  $p = 0.018$ ;  $ChR2^{stroke}$ : 30% survival;  $ArchT^{stroke}$ : 75% survival;  
512  $mCherry^{stroke}$ : 77.7% survival; Log-rank Mantel-Cox test), as compared to day 5 (Fig. 6B;  
513 Chi square(4) = 6.383,  $P = 0.172$ ;  $ChR2^{stroke}$ : 60% survival;  $ArchT^{stroke}$ : 70% survival;  
514  $mCherry^{stroke}$ : 70% survival;  $mCherry^{sham}$ : 100 % survival; Naive: 100 % survival; Long-rank  
515 Mantel-Cox test). These findings are consistent with an increased excitotoxicity after stroke  
516 (Nudo, 2006; Allman et al., 2016), hence, all our optogenetic experiments started on day 5.

517

### 518 **$SW^{opto}$ during sleep improves functional recovery**

519 We next tested whether sleep-specific  $SW^{opto}$  improves functional recovery after MCAo in  
520 mice. The expression of  $ChR2$ ,  $ArchT$  and  $mCherry$  was genetically targeted to iS1FL  
521 pyramidal neurons as described above (Fig. 3A) before animals were chronically implanted  
522 with a unilateral optic fiber on iS1FL and EEG/EMG electrodes for simultaneous optogenetic  
523 control and polysomnographic recordings in freely moving mice (Fig. 7A; see Methods).

524 Sparse SW<sup>opto</sup> were randomly distributed during sleep starting 5 days after stroke until day 15  
525 (single 5 or 200 ms light pulses, at 473 nm or 532 nm, respectively, randomly distributed  
526 over 2 h, daily; n = ~300 optical stimuli; Fig. 7B, C).

527

528 Evaluation of the animals' fine motor movements, coordination, strength and asymmetry at  
529 post-stroke day 4 (Fig. 7B) showed severe behavioural deficits in all animals subjected to  
530 MCAo. Indeed, on post-stroke day 4 stroke-induced animals were no longer able to finely  
531 coordinate their grasping movements (Fig. 7D; LR(1) = 27.498,  $p < 0.0001$ ; Fig. 7F; LR(1) =  
532 32.205,  $p < 0.0001$ ). As expected, no behavioural impairments were found in mCherry<sup>sham</sup>  
533 and Naive animals (Fig. 7D; F, respectively,  $p > 0.05$ ).

534 In the ladder walking rig test (Fig. 7D), a significant interaction between the *stimulation*  
535 *group* and *days* was found (LR(5) = 11.976,  $p = 0.035$ ). Post-hoc analysis revealed that the  
536 ArchT<sup>stroke</sup> group recovered at a faster pace than ChR2<sup>stroke\_wake</sup> ( $t(101) = 2.842$ ,  $p = 0.005$ ).  
537 Generally all mice improved across days (LR(6) = 28.235,  $p < 0.001$ ). Main effects of  
538 stimulation group were also found (LR(10) = 42.949,  $p < 0.001$ ). ChR2<sup>stroke</sup>, ChR2<sup>stroke\_wake</sup>  
539 were significantly different from mCherry<sup>stroke</sup> ( $t(101) = -2.430$ ,  $p = 0.017$ ;  $t(101) = -3.137$ ,  $p$   
540  $= 0.002$ ). For the beam balance (Fig. 7F) we found a significant interaction effect of  
541 *stimulation group* and *day* (LR(5) = 14.171,  $p = 0.015$ ). mCherry<sup>stroke</sup> did not show a  
542 significant improvement across days ( $t(152) = -1.671$ ,  $p = 0.097$ ). However, compared to  
543 mCherry<sup>stroke</sup>, ArchT<sup>stroke</sup> mice showed significantly more improvement over the course of  
544 days ( $t(110) = -2.866$ ,  $p = 0.005$ ). ChR2<sup>stroke</sup> mice also significantly improved across days  
545 after stroke ( $t(152) = -4.168$ ,  $p < 0.001$ ), but this improvement was less than the ArchT<sup>stroke</sup>  
546 group ( $t(110) = -2.285$ ,  $p = 0.024$ ) and on par with the mCherry<sup>stroke</sup> group ( $t(110) = 0.580$ ,  $p$   
547  $= 0.563$ ). Comparisons of animal improvement between post-stroke day 4 and 15 confirmed  
548 the functional recovery of ChR2<sup>stroke</sup> (Fig. 7E;  $t(14) = 3.46$ ,  $p = 0.007$ ; Fig. 7G;  $t(18) = 2.372$ ,

549  $p = 0.029$ ) and ArchT<sup>stroke</sup> (Fig. 7E;  $t(14) = 3.083$ ,  $p = 0.008$ ; Fig. 7G;  $t(18) = 3.895$   $p =$   
550  $0.002$ ; one-way ANOVA), in comparison to mCherry<sup>stroke</sup> control. In contrast, optogenetic  
551 intervention after stroke did not lead to any improvement of motor endurance, strength (Fig.  
552 7H) or asymmetry (Fig. 7I).

553

#### 554 **SW<sup>opto</sup> increases axonal sprouting**

555 Stroke triggers a cascade of molecular and cellular changes including synaptogenesis,  
556 neurogenesis and axonal sprouting in peri-infarct zone and remote connected circuits (Nudo,  
557 1997; Carmichael et al., 2017).

558 To quantify the anatomical changes induced by chronic SW<sup>opto</sup>, we quantified the expression  
559 of pre-synaptic Vglut1 and post-synaptic PSD-95 proteins as a direct measurement of axonal  
560 sprouting in cortical layers V (Liu et al., 2007; Sun et al., 2017) and connected circuits in  
561 layers II (Binzegger et al., 2004; Adesnik and Naka, 2018) (Fig. 8A). Puncta density  
562 quantification in both iS1FL and cS1FL cortices revealed a significantly higher Vglut1  
563 protein levels in ipsilateral layer II (Fig. 8B;  $F(3, 19) = 10.49$ ,  $p = 0.0003$ ), and layer V (Fig.  
564 8C;  $F(3, 18) = 16.02$ ,  $p > 0.0001$ ; one-way ANOVA) from ChR2<sup>stroke</sup> and ArchT<sup>stroke</sup> animals,  
565 as compared to mCherry controls. Consistently, analysis of Vglut1 positive puncta volume  
566 distribution revealed a significant increase of smaller, newly-formed puncta within ipsilateral  
567 layer V of both ArchT<sup>stroke</sup> and ChR2<sup>stroke</sup> as compared to mCherry<sup>stroke</sup> animals (Fig. 8E;  $F(2,$   
568  $2111) = 75.13$ ,  $p < 0.0001$ ). This was also true for *ex novo* Vglut1 puncta in post-synaptic  
569 sites of layer II from ChR2<sup>stroke</sup> animals (Fig. 8D; mCherry<sup>stroke</sup> vs. ChR2<sup>stroke</sup>  $t(2070) = 4.181$ ,  
570  $p < 0.0001$ ), but not ArchT<sup>stroke</sup> animals (mCherry<sup>stroke</sup> vs. ArchT<sup>stroke</sup>  $t(2070) = 3.015$ ,  $p =$   
571  $0.0078$ , ChR2<sup>stroke</sup> vs. ArchT<sup>stroke</sup>  $t(2070) = 0.903$ ,  $p > 0.999$ ; one-way ANOVA, followed by  
572 Bonferroni correction).



573 These pre-synaptic changes were concomitant to a significant decrease of post-synaptic PSD-  
574 95 protein expression in iS1FL layer II of both ChR2 and ArchT expressing animals when  
575 compared to control group (Fig. 8F;  $F(3, 23) = 8.609$ ,  $p = 0.0005$ ; one-way ANOVA), with  
576 no differences in layer V (Fig. 8G;  $F(3, 24) = 1.095$ ,  $p = 0.370$ ; one-way ANOVA). PSD-95  
577 positive puncta volume were significantly larger iS1FL layer II (Fig. 8H;  $F(2, 625) = 85$ ,  $p <$   
578  $0.0001$ ) and layer V from ChR2<sup>stroke</sup> animal as compared to mCherry<sup>stroke</sup> or ArchT<sup>stroke</sup> (Fig.  
579 8I;  $F(2, 2111) = 75.13$ ,  $p < 0.0001$ ; one-way ANOVA).

580

581

## 582 **DISCUSSION**

583 Stroke is a debilitating neurological disorder and one of the worldwide leading causes of  
584 adult disability and death in aging population. A better understanding of the complex  
585 pathophysiological mechanisms underlying the stroke event and the following brain plasticity  
586 warrants the improvement of existing strategies and the development of alternative therapies  
587 for stroke recovery (Feigin et al., 2017).

588 Here, we showed that MCAo induced an ipsilateral reduction of spontaneous SW amplitude,  
589 associated with sleep fragmentation and increased NREM sleep after stroke onset (Giubilei et  
590 al., 1992; Vock et al., 2002; Baumann et al., 2006; Hermann et al., 2008). Our results further  
591 indicate that sleep-specific optogenetic neuromodulation of brain activity after stroke had no  
592 effects on the sleep-wake cycle architecture, but it improved fine skilled motor movements in  
593 comparison to wakefulness interventions. These manipulations were accompanied by axonal  
594 sprouting of local and connected circuits, suggesting a direct role for SW in promoting  
595 anatomical and functional plasticity of neural circuit during sleep (Carmichael and Chesselet,  
596 2002; Aeschbach et al., 2008; Tononi and Cirelli, 2014). Collectively, these findings  
597 emphasize a role for NREM sleep SW as a window of intervention during stroke recovery

598 and a possible mechanism underlying the improvement of rehabilitative strategies using  
599 repetitive transcranial magnetic stimulation (rTMS) (Kim et al., 2006; Brodie et al., 2014)  
600 and transcranial direct current stimulation (tDCS) (Boggio et al., 2006; Lindenberg et al.,  
601 2010).

602

603 Spontaneous sleep SW are associated with neuroplastic changes (Tononi and Cirelli, 2006;  
604 Puentes-Mestral and Aton, 2017), inflammatory and immunological adaptive response  
605 (Irwin and Cole, 2011), protective functions during infection (Irwin, 2019), metabolic  
606 clearance (Xie et al., 2013). Clinical studies reported significant improvement in stroke  
607 rehabilitation upon non-invasive brain stimulation during sleep (Niimi et al., 2018) and SW  
608 enhancement (Ebajemito et al., 2016). We used physiologically-relevant stimulation  
609 protocols to avoid neuronal hypersynchrony, un-natural firing activities and circuit adaptation  
610 by using single optogenetic stimuli randomly distributed across sleep in freely-moving  
611 animals. These sparse optogenetics interventions induced  $SW^{opto}$  without perturbing sleep-  
612 wake cycle architecture. Our strategy contrasts from other studies that use long-lasting  
613 hypersynchronous optogenetic activation independently of the animal behaviour, sleep-wake  
614 states, or delivered during anaesthesia (Cheng et al., 2014; Shah et al., 2017; Tennant et al.,  
615 2017; Lu et al., 2017). Our findings show that sparse  $SW^{opto}$  delivered during sleep improved  
616 behavioural outcomes whereas  $SW^{opto}$  during wakefulness did not. An explanation for this  
617 striking difference is that low frequency, high amplitude waves during wakefulness represent  
618 dysfunctional waves, typical of pathological conditions that are often associated with  
619 functional abnormalities, including deafferented or lesioned thalamo-cortical circuits  
620 (Steriade et al., 1993; Butz et al., 2004). These results further emphasize the importance of  
621 sleep as a window for optimal modulation of brain activity that potentiates the effect of  
622  $SW^{opto}$  on brain plasticity and behavioural outcomes (see below).

623

624 *Alteration of sleep-wake cycle and slow wave*

625 Our findings showed that stroke injury induces a dramatic increase in NREM sleep on the  
626 day following stroke. This effect is accompanied by transient perturbation of the circadian  
627 sleep distribution across the light/ dark cycle. Although the causes of these transient changes  
628 remain unclear, they may result from a functional adaptation to the strong fragmentation of  
629 both NREM sleep and wakefulness.

630 Our experimental results are consistent with the sleep fragmentation, the increase in NREM  
631 sleep stages 1-2 and the decreased REM sleep observed during the first days following stroke  
632 in human (Giubilei et al., 1992; Vock et al., 2002). Sleep fragmentation may result from a  
633 lack of consolidated synchrony of neuronal activity amongst thalamo-cortical circuitries, as  
634 suggested by the decreased amplitude and positive slope of spontaneous SW after stroke  
635 observed in our study. These SW profiles are indicative of low spiking synchrony of thalamic  
636 and cortical neurons (Huber et al., 2004; Vyazovskiy et al., 2009), which may facilitate  
637 arousal upon wake-promoting inputs of sub-cortical origins (Adamantidis et al., 2007; Carter  
638 et al., 2010; Herrera et al., 2016; Gent et al., 2018). Whether the SW remaining after stroke  
639 are generated by a similar mechanism and support similar cortical functions, as the  
640 naturalistic SW recorded from an intact brain remains to be examined in light of the different  
641 cells types potentially implicated in SW generation (Gerashchenko et al., 2008; Cardin et al.,  
642 2009; Stroh et al., 2013; Jackson et al., 2016; Niethard et al., 2016). An important  
643 characteristic of spontaneous sleep SW is their propagation pattern across the brain cortex,  
644 originating at anterior regions and travelling to posterior directions (Massimini et al., 2004,  
645 Gent et al., 2018). Investigating SW's traveling changes across the ipsi-lateral hemisphere  
646 and the peri-infarct zone specifically represents an interesting additional aspect to explore in

647 future work. The experimental preparation of the present study (single EEG trace per  
648 hemisphere) limited further SW analysis in this direction.

649

650 ***SW<sup>opto</sup> promotes behavioural recovery after stroke***

651 Chronic SW<sup>opto</sup> over 11 days after stroke facilitated spontaneous functional recovery, while  
652 earlier interventions exacerbated brain injury and decreased the survival rate of the animals,  
653 possibly due to excessive glutamate release (Lai et al., 2014) leading to increased  
654 excitotoxicity (Nudo, 2006; Allman et al., 2016). This window of spontaneous recovery is  
655 limited to a month in rodents, and three months in humans, during which molecular and  
656 structural changes potentiate the responsiveness to rehabilitative treatments (Murphy and  
657 Corbett, 2009; Ng et al., 2015) and emphasize a crucial intervention timeframe (Dromerick et  
658 al., 2009). Although post-stroke excitotoxicity might be an accurate explanation for the  
659 detrimental effect observed in animals' survival, additional studies are required to further  
660 scrutinize markers of excitotoxicity (e.g. levels of glutamate, NMDA receptors, AMPA  
661 receptors and their activation, caspases, reactive oxygen species) in combination with  
662 optogenetic intervention at several time points following stroke.

663

664 An interesting finding in our study is that SW<sup>opto</sup> had no direct effects on sleep architecture  
665 but induced a delayed increase of sleep duration. This result is in agreement with studies  
666 showing prolonged NREM sleep upon activation of somatostatin interneurons (Funk et al.,  
667 2017), and to a lesser extent pyramidal neurons (Rodriguez et al., 2016) in the neocortex.  
668 Noteworthy, increased NREM sleep following SW<sup>opto</sup> intervention was present only within  
669 the first two days of stimulation (not shown), presumably due to the brain recovery processes  
670 or the adaptation of the sleep-promoting circuits to the SW<sup>opto</sup>, or both. Although we cannot  
671 rule out a possible role of this transient NREM sleep increase on the sensorimotor

672 improvement of the animals, it is unlikely that these early and transient changes are  
673 responsible for the motor improvements observed at the end of the experiment.

674

675 ***Slow wave, plasticity and axonal sprouting***

676 In our experiments, sensorimotor improvement after stroke was achieved by either chronic  
677 optogenetic activation, or silencing, of iS1FL pyramidal neurons in freely-moving mice,  
678 supporting an essential role for UP-DOWN states, rather than neuronal activation or silencing  
679 alone, in brain plasticity (Puentes-Mestril and Aton, 2017). These bistable states during  
680 NREM sleep (here, mainly SW<sup>opto</sup>) are associated with synaptic plasticity in local circuits and  
681 their postsynaptic targets, as observed for the beneficial effect of sleep low frequency  
682 stimulation of motor or somatosensory cortical circuits on perceptual learning (Miyamoto et  
683 al., 2016) or the formation of new dendritic spines in motor cortex (layer V) pyramidal  
684 neurons in mice (Yang et al., 2014). Furthermore, our results are in agreement with the  
685 finding that experimental disruption of cortical SW following learning impairs consolidation  
686 of visuomotor learning in human (Landsness et al., 2009).

687

688 The early stages of stroke recovery are classically attributed to brain oedema resorption and  
689 penumbra reperfusion, while later stages are associated with structural reorganization through  
690 axonal sprouting, synaptogenesis and neurogenesis (Nudo, 2006). Here, both ChR2-, and to a  
691 lesser extent ArchT-induced SW<sup>opto</sup> promoted an increase of pre- and post-synaptic markers  
692 in S1FL layers V and II respectively. Decreased PSD-95 density after SW<sup>opto</sup> is consistent  
693 with similar findings upon rTMS stimulation in rodents (Etiévant et al., 2015) that correlate  
694 with improved functional outcomes in non-human primates treated with PSD-95 inhibitors  
695 (Cook et al., 2012). Larger PSD-95 puncta were found within both layers II and V of  
696 ChR2<sup>stroke</sup> animals, suggestive of a stabilization of the functional synapse (Cane et al., 2014).

697 Thus, SW<sup>opto</sup> enhance UP/ DOWN state network synchronization (Gent et al., 2018) and  
698 facilitate the formation of new synapses which are not restricted to targeted cortical circuits  
699 (i.e. pyramidal neurons in the peri-infarct zone) but also anatomically connected circuits  
700 located in ipsi- and contra-lateral hemispheres (Liu et al., 2009; Cui et al., 2013).  
701 Noteworthy, brain activity in other cortical and sub-cortical networks and other sleep  
702 oscillations including spindles participate to synaptic plasticity during NREM sleep  
703 (Rosanova and Ulrich, 2005; Chauvette et al., 2012) and may contribute to the behavioral  
704 outcome reported here.

705

706 Collectively, our findings support a role for NREM sleep SW in neuronal circuit plasticity  
707 and provide a clinically-relevant framework for developing sparse, non-invasive  
708 neuromodulation, including acoustic brain stimulations (Ngo et al., 2013), TMS or tDCS  
709 (Ebajemito et al., 2016; Niimi et al., 2018) for optimal recovery after brain injury.

710

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- 995

996 **FIGURE LEGENDS**

997

998 **Figure 1.** Stroke alters sleep architecture. (A) Schematic of the Circle of Willis (CW) with  
999 highlighted common carotid artery (CCA), internal carotid artery (ICA), middle cerebral  
1000 artery (MCA) involved in MCAo procedure and filament placement. (B) Coronal sections (40  
1001  $\mu$ m) of a representative mouse 15 days after MCAo. Nissl staining. (C) Schematic  
1002 representation of EEG and EMG electrodes placements relative to stroke. 24 h recordings of  
1003 animals' sleep-wake cycles were performed before stroke (Baseline) and again at post-stroke  
1004 day 1, 3, 5 and 10 in Stroke (n = 11) and Sham (n = 9) animals. (D) Percentage changes of  
1005 wakefulness, NREM sleep (E) and REM sleep (F) total durations from each animal's baseline  
1006 values. (G) Comparison between bout durations of wakefulness, NREM sleep (H) and REM  
1007 sleep (I). (J) Ratio between NREM continuous episodes and transitions to wake. (K) Total  
1008 number of microarousals in 24 h recordings. (L) Percentage of epochs spent in wake or sleep  
1009 states for Stroke (blue table) and Sham (grey table) groups, respectively. Linear mixed model  
1010 of 8 matrices: Wake duration changes; NREM duration changes; REM duration changes;  
1011 Wake bout duration; NREM bout duration; REM bout duration; NREM stability;  
1012 Microarousals. Data are represented as means  $\pm$  s.e.m.; asterisks indicate significance \*p <  
1013 0.0063.

1014

1015 **Figure 2.** Stroke alters SW profile. (A) Automatic detection of single slow waves (SW) from  
1016 local field potential (LFP) recordings in ipsilateral primary somatosensory forelimb cortex  
1017 (iS1FL), ipsilateral primary motor cortex (iM1), contralateral M1 (cM1), contralateral S1FL  
1018 (cS1FL) and EEG traces from ipsilateral (iEEG) and contralateral (cEEG) hemispheres.  
1019 Representative traces in black and detected SW in colours (top). Magnification of one  
1020 episode of NREM sleep and detected SW (bottom). (B) Schematic of tetrodes and EEG/EMG  
1021 electrode implantation. (C) Representative average SW from 24 h baseline EEG recording.

1022 (D) Unit activity heat map of neurons recorded during detected SW; the graph shows  
1023 neuronal activity suppression corresponding to the silent SW DOWN state (top). Average  
1024 firing rate of single units recorded during the detected SW (bottom). (E) Schematic of EEG  
1025 electrodes position. (F) SW peak to peak amplitude prior to (Baseline) and following MCAo  
1026 or sham surgery (post stroke day 1, 3, 5 and 10). (G) SW positive slope. (H) SW negative  
1027 slope. (I) Number of single SW detected. (J) SW duration. Stroke n = 11, Sham n = 9; two-  
1028 way ANOVA, followed by Bonferroni post-hoc test. Data are represented as means  $\pm$  s.e.m.  
1029 Asterisks indicate significance \* $p < 0.05$ .

1030

1031 **Figure 3.** Optogenetic induction of SW-like bistable oscillations. (A) Scheme of a coronal  
1032 brain section with AAV injection site (left), AAV structure (top) and optrode/EEG/EMG  
1033 implantation representation (right). (B) Opsin distribution within the peri-infarct ipsilateral  
1034 primary somatosensory forelimb cortex (iS1FL) following AAV injection of CaMKII-ChR2-  
1035 EYFP. (C) Local field potential (LFP) traces, single unit activity and correspondent raster  
1036 plot and mean spike rate upon optogenetic stimulation during wakefulness (left), NREM  
1037 sleep (middle) and REM sleep (right) from one representative stimulation session. (D)  
1038 Average ipsilateral (iEEG) and contralateral EEG (cEEG) traces response to activation  
1039 (ChR2) of pyramidal neurons with 5 ms of single laser light pulses (473 nm). (E) Comparison  
1040 between spontaneous and optogenetically-evoked SW ( $SW^{opto}$ ) duration (left), negative  
1041 amplitude (middle) and slope (right) during NREM sleep for ChR2 stimulated animals;  
1042 Wilcoxon rank sum test, statistically significant if  $p < 0.05$ . (F) ArchT distribution within  
1043 iS1FL. (G) Representative EEG/EMG traces upon silencing of pyramidal neurons with 200  
1044 ms of single laser light pulses (532 nm) during NREM sleep. (H) Average iEEG and cEEG  
1045 responses to ArchT stimulation. (I) Comparison between  $SW^{opto}$  duration (left), negative  
1046 amplitude (middle) and slope (right) during NREM sleep for ArchT stimulated animals;

1047 Wilcoxon rank sum test, statistically significant if  $p < 0.05$ . (J) mCherry (control) expression  
1048 in iS1FL; representative EEG/EMG responses during 5ms light pulse stimulation (top) and  
1049 average EEGs (bottom). (L) Representative EEG/EMG traces response to 200ms pulse  
1050 stimulation in one mCherry transfected mouse (top) and its average EEGs response.

1051

1052 **Figure 4.** Optogenetic screening for slow waves induction in the peri-infarct zone. (A)  
1053 Coronal section indicating the AAV injection site (left primary somatosensory forelimb  
1054 cortex, S1FL) (top) and schematic of EEG/EMG/optic fiber/tetrodes implantation (bottom).  
1055 (B) LFP recordings from S1FL upon laser light stimulation (500 ms, 5 Hz and 200 ms) of  
1056 inhibitory (top) or pyramidal neurons (bottom) respectively.

1057

1058 **Figure 5.**  $SW^{opto}$  oscillations travel across hemispheres. (A) Local field potential (LFP)  
1059 traces, multi-unit activity from tetrodes placed in ipsilateral primary somatosensory forelimb  
1060 cortex (iS1FL), contralateral S1FL (cS1FL), ipsilateral primary motor cortex (iM1) and  
1061 contralateral M1 (cM1) and EEG/EMG traces recorded during one stimulating session  
1062 showing the travelling characteristic of the evoked waves ( $SW^{opto}$ ). (B) Raster plots  
1063 corresponding to one single light pulse stimulation event during wakefulness, NREM and  
1064 REM sleep as well as relative mean spike rate for iS1FL, cS1FL, cM1 respectively. No unit  
1065 was found for iM1. (C) Average LFP traces during the stimulation events for the four  
1066 recorded cortical areas respectively.

1067

1068 **Figure 6.**  $SW^{opto}$  defines a critical window of intervention for stroke recovery. (A) When the  
1069 stimulation protocol started at post-stroke day 1,  $Chr2^{stroke}$  animals in particular showed  
1070 lower survival percentage. (B) When the stimulation instead began at post-stroke day 5,

1071 ChR2<sup>stroke</sup> animal showed an improvement in survival percentage. Asterisks indicate  
1072 significance \* $p < 0.05$ .

1073

1074 **Figure 7.** SW<sup>opto</sup> during sleep improves functional recovery after stroke. (A) Schematics of  
1075 optic fiber/EEG/EMG implantation with opsin expression site. (B) Experimental timeline. (C)  
1076 Average number of single light pulses within sleep stages during the stimulation sessions of  
1077 ArchT<sup>stroke</sup>, ChR2<sup>stroke</sup>, mCherry<sup>stroke</sup>, mCherry<sup>sham</sup> and ChR2<sup>stroke\_wake</sup> respectively. (D)  
1078 Stimulated animals showed better performances in the ladder walking rig test compared to  
1079 mCherry-control animals (Naive  $n = 8$ , mCherry<sup>sham</sup>  $n = 8$ , ArchT<sup>stroke</sup>  $n = 4$ , ChR2<sup>stroke</sup>  $n = 7$ ,  
1080 mCherry<sup>stroke</sup>  $n = 6$ ). Induction of slow waves mainly during wakefulness (ChR2<sup>stroke\_wake</sup>  $n =$   
1081 6) did not result in faster improvement of performance compared to ChR2<sup>stroke</sup> stimulated  
1082 during NREM sleep. Linear mixed model. (E) Percentage of improvement from post-stroke  
1083 day 4 to post-stroke day 15 for ChR2<sup>stroke</sup> groups and ArchT<sup>stroke</sup> compared to mCherry<sup>stroke</sup>  
1084 control (one-way ANOVA). (F) Similar results was observed for performances in the balance  
1085 test (Naive  $n = 8$ , mCherry<sup>sham</sup>  $n = 6$ , ArchT<sup>stroke</sup>  $n = 7$ , ChR2<sup>stroke</sup>  $n = 7$ , mCherry<sup>stroke</sup>  $n = 7$ ,  
1086 ChR2<sup>stroke\_wake</sup>  $n = 6$ ). (G) Balance beam percentage of improvement from post-stroke day 4  
1087 to post-stroke day 15 for ChR2<sup>stroke</sup> groups and ArchT<sup>stroke</sup> compared to mCherry<sup>stroke</sup> control  
1088 (one-way ANOVA). (H) Tight rope test and corner test (I) did not show differences between  
1089 stimulated and control groups (Naive  $n = 8$ , mCherry<sup>sham</sup>  $n = 6$ , ArchT<sup>stroke</sup>  $n = 7$ , ChR2<sup>stroke</sup>  $n$   
1090  $= 6$ , mCherry<sup>stroke</sup>  $n = 8$ , ChR2<sup>stroke\_wake</sup>  $n = 6$ ). Asterisks indicate significances \* $p < 0.05$ .

1091

1092 **Figure 8.** SW<sup>opto</sup> increases axonal sprouting during stroke recovery. (A) Scheme of a brain  
1093 coronal section 15 days after stroke (end point of experiment) representing tissue atrophy  
1094 corresponding to the stroke area. Confocal micrography of iS1FL with 3D reconstruction of  
1095 pre- and post-synaptic markers contact (right). Blue: DAPI staining; red: Vglut1 pre-synaptic

1096 marker; aquamarine: PSD-95 post-synaptic marker. (B) Comparison of Vglut1 puncta  
1097 density between iS1FL and cS1FL cortical areas (mCherry<sup>stroke</sup> n = 4, ChR2<sup>stroke</sup> n = 4,  
1098 ArchT<sup>stroke</sup> n = 4, mCherry<sup>sham</sup> n = 4) in ipsilateral (F (3, 19) = 10.49, p = 0.0003) and  
1099 contralateral layers II (F (3, 19) = 1.069, p = 0.385), as well as in ipsilateral (F (3, 18) =  
1100 16.02, p < 0.0001) and contralateral layers V (F (3, 21) = 11.05, p = 0.0001) (C). (D) Vglut1  
1101 puncta volume distribution in iS1FL layer II (F (2, 1630) = 34.85, p < 0.0001) and layer V  
1102 (E) summarized in bar graph (F (2, 1617) = 155, p < 0.0001). (F) Comparison of PSD-95  
1103 puncta density in ipsilateral (F (3, 23) = 8.609, p = 0.0005) and contralateral (F (3, 21) =  
1104 1.105, p = 0.369) layers II, as well as in ipsilateral (F (3, 24) = 1.095, p = 0.370) and  
1105 contralateral (F (3, 24) = 2.498, p = 0.083) layers V (G). (H) PSD-95 puncta volume  
1106 distribution in iS1FL layer II summarized in bar graph (F (2, 2070) = 9.164, p = 0.0001); (I)  
1107 iS1FL layer V (F (2, 2111) = 75.13, p < 0.0001). One way-ANOVA. Data are represented as  
1108 means ± s.e.m. Asterisks indicate significance \*p < 0.05. n.s. = not significant.

Figure 1

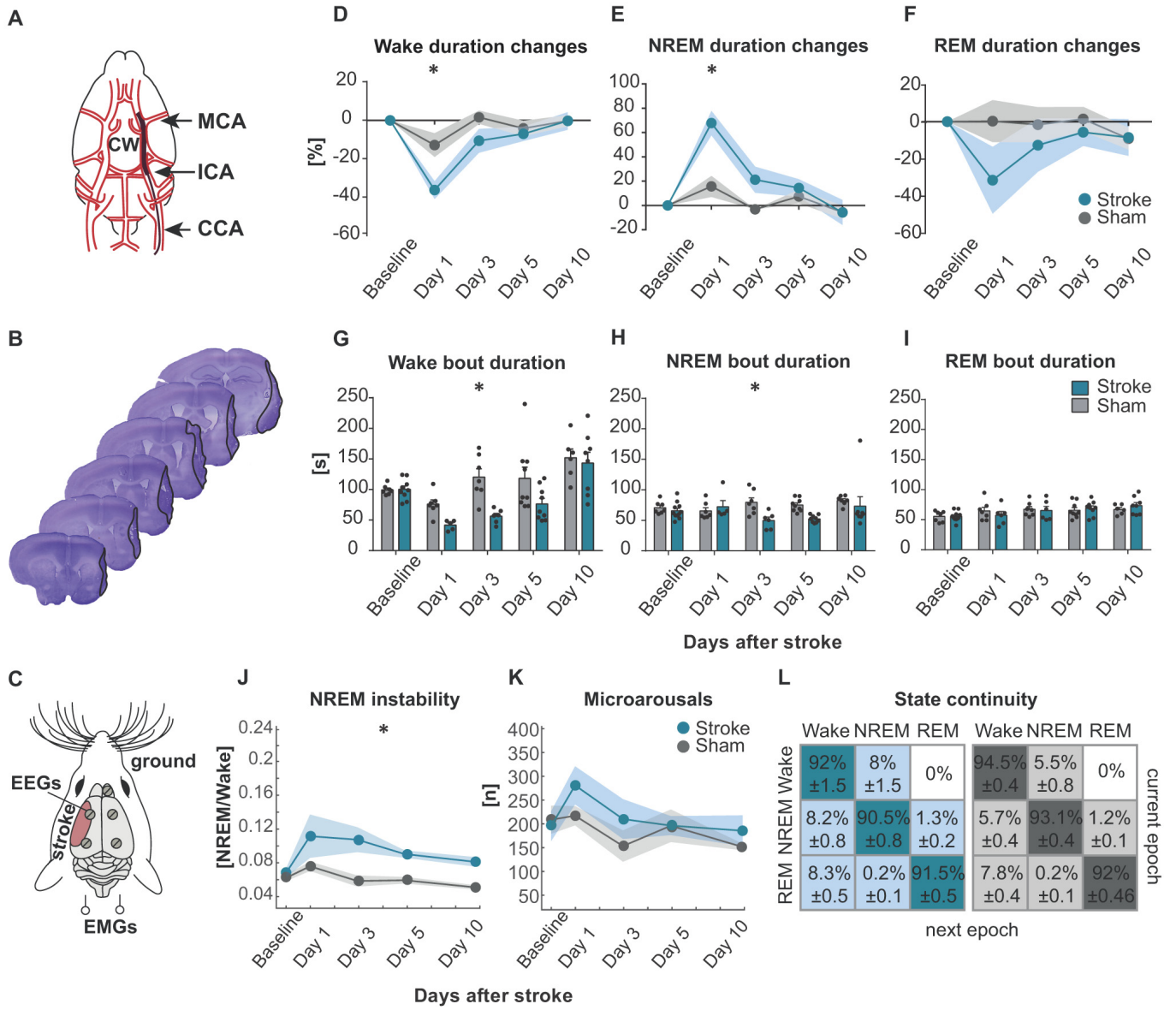




Figure 2

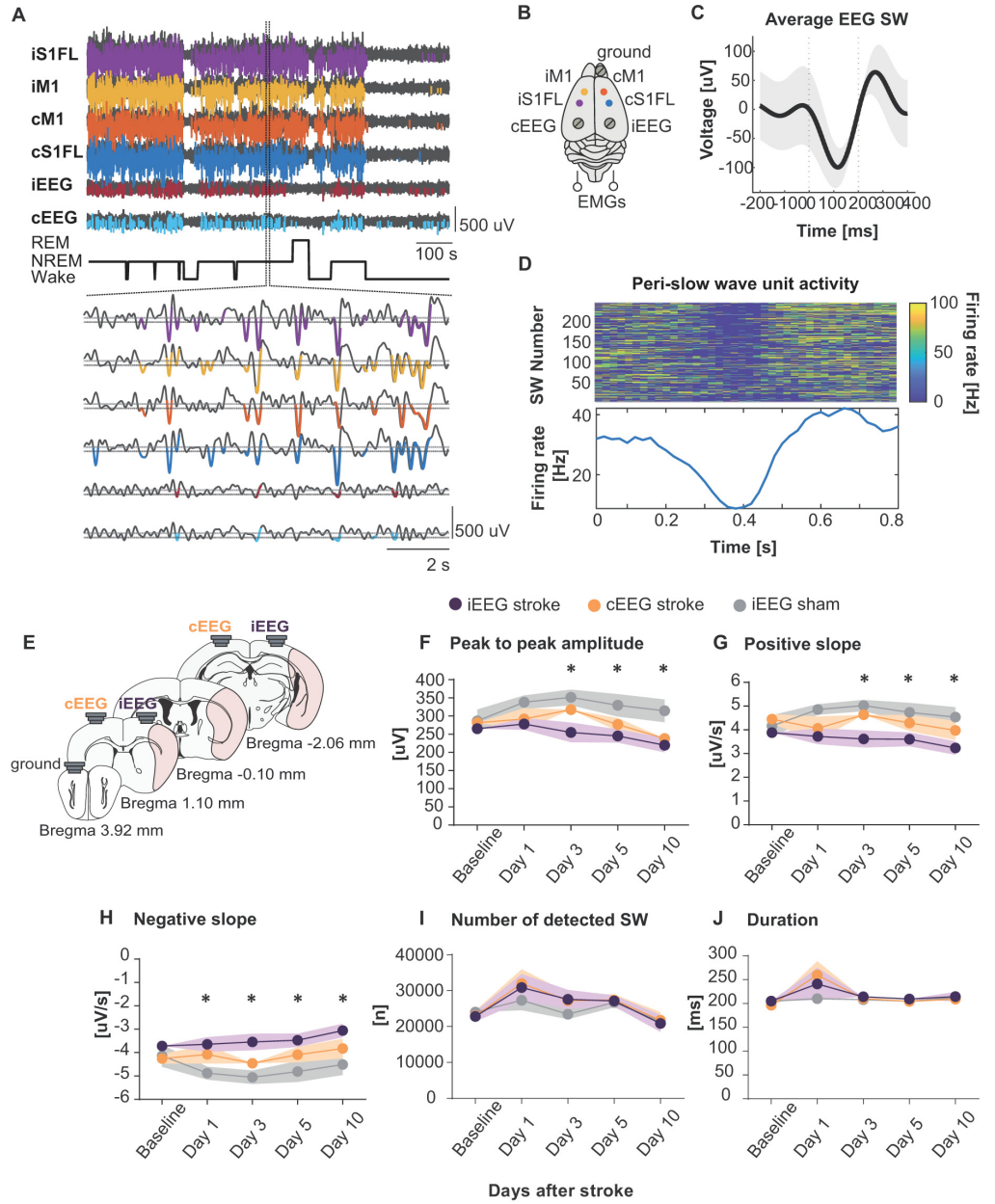
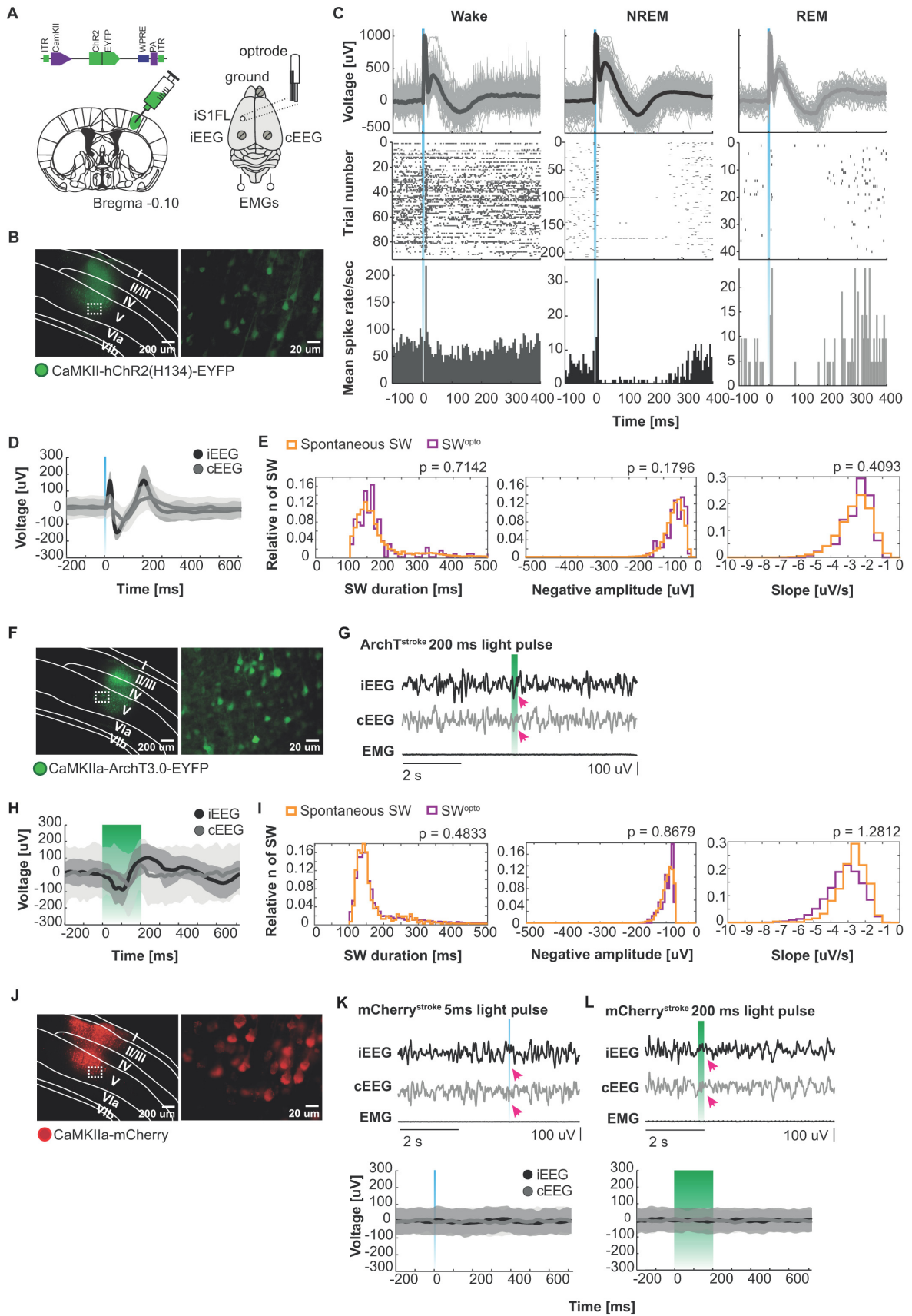


Figure 3



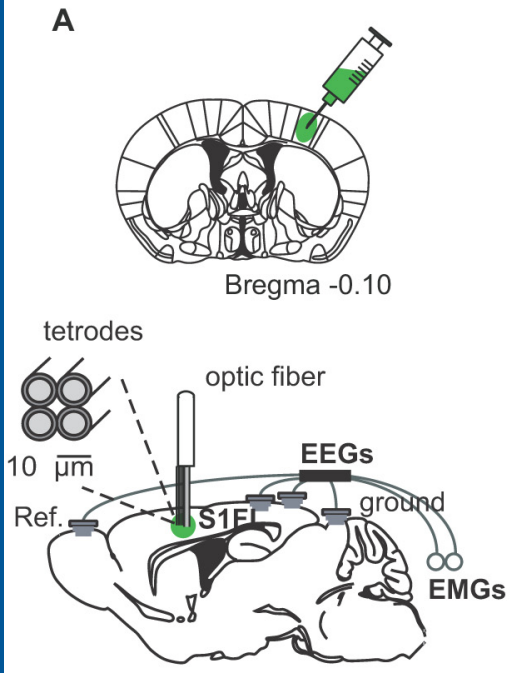


Figure 4

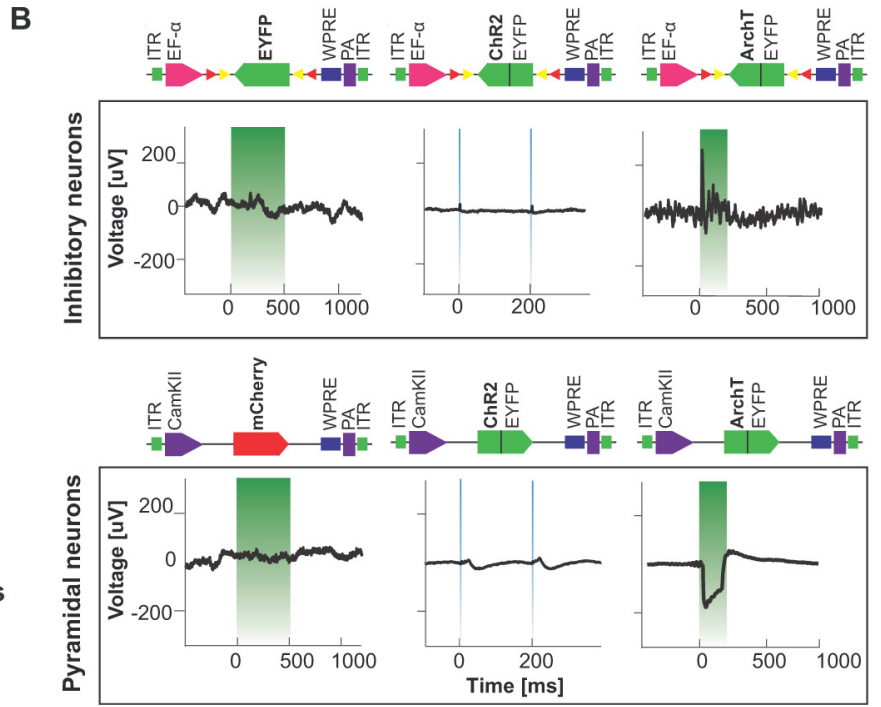


Figure 5

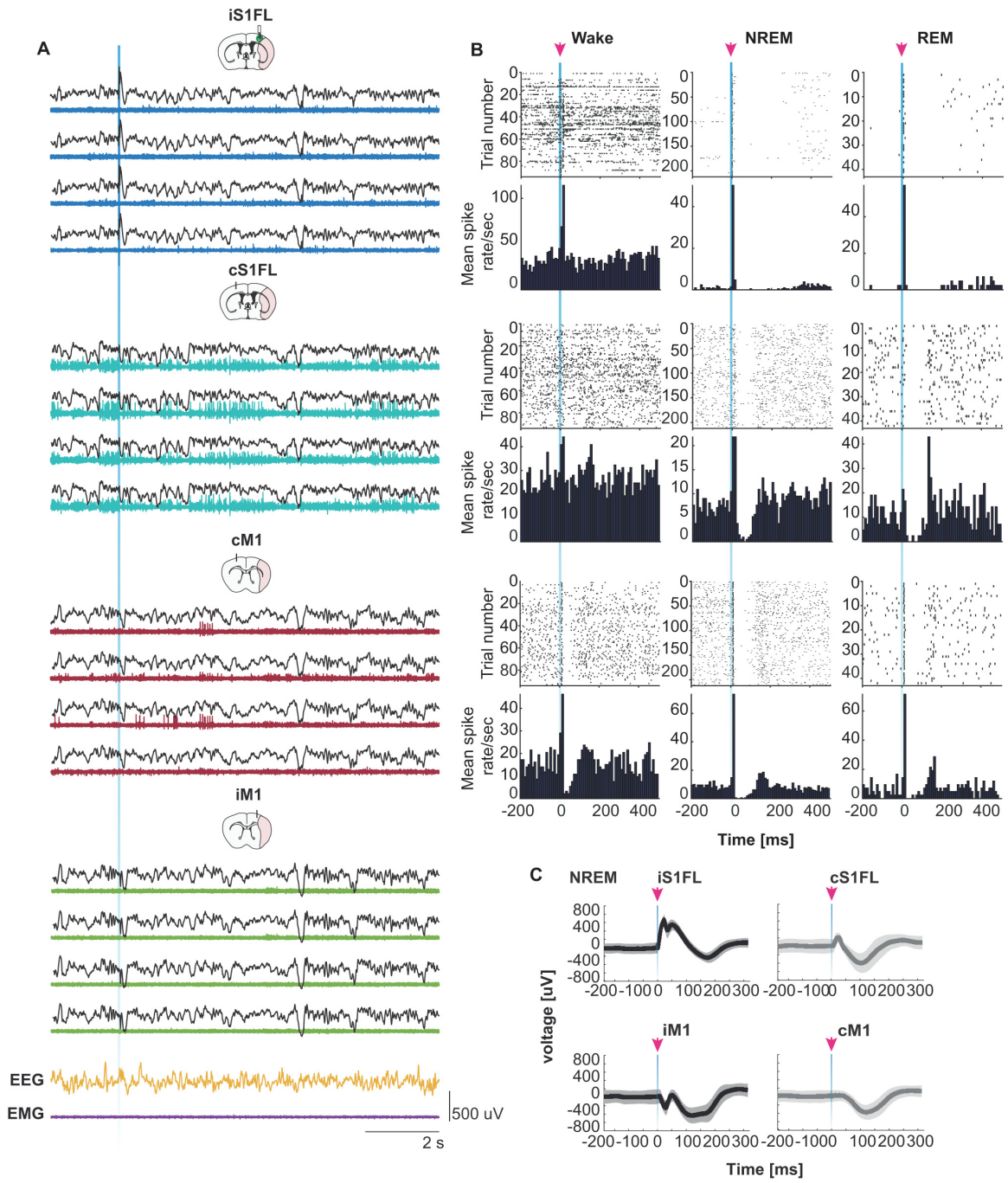


Figure 6

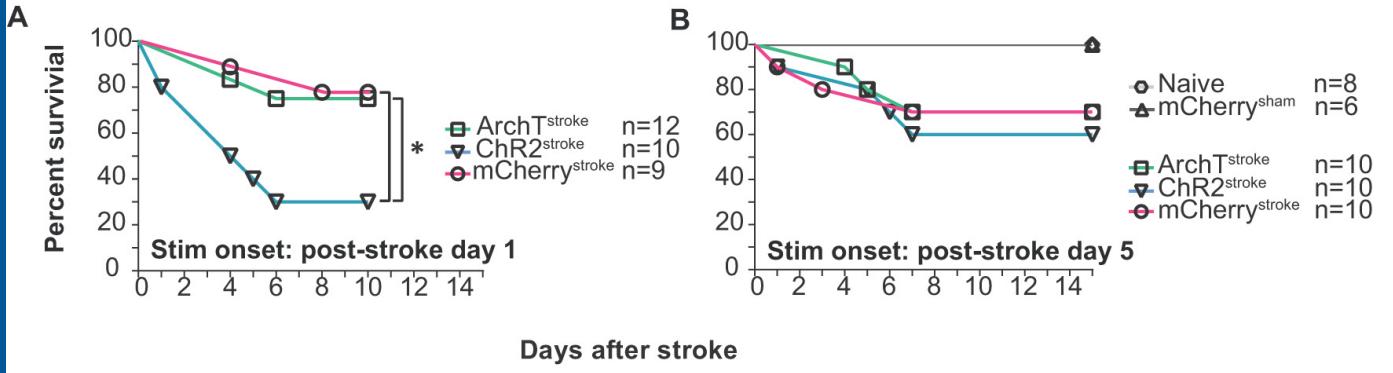


Figure 7

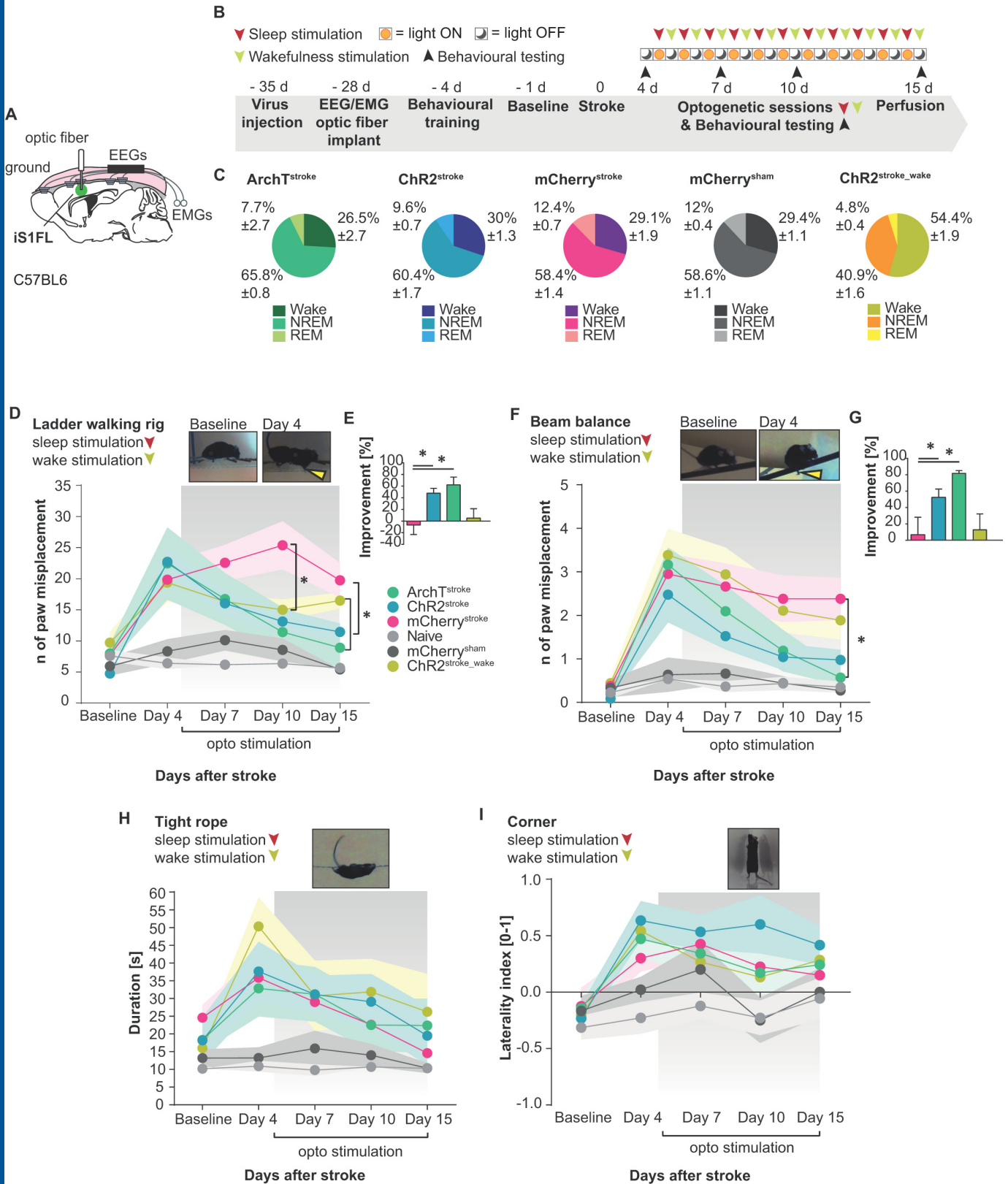


Figure 8

