



**HAL**  
open science

## High-intensity training with short and long intervals regulate cortical neurotrophic factors, apoptosis markers and chloride homeostasis in rats with stroke

Nicolas Hugues, Caroline Pin-Barre, Thomas Brioche, Christophe Pellegrino, Eric Berton, Claudio Rivera, Jérôme Laurin

### ► To cite this version:

Nicolas Hugues, Caroline Pin-Barre, Thomas Brioche, Christophe Pellegrino, Eric Berton, et al.. High-intensity training with short and long intervals regulate cortical neurotrophic factors, apoptosis markers and chloride homeostasis in rats with stroke. *Physiology & behavior*, 2023, 266, pp.114190. 10.1016/j.physbeh.2023.114190 . hal-04069357

**HAL Id: hal-04069357**

**<https://amu.hal.science/hal-04069357>**

Submitted on 18 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



# High-intensity training with short and long intervals regulate cortical neurotrophic factors, apoptosis markers and chloride homeostasis in rats with stroke

Nicolas Hugues<sup>a,b</sup>, Caroline Pin-Barre<sup>a</sup>, Thomas Brioché<sup>c</sup>, Christophe Pellegrino<sup>a</sup>, Eric Berton<sup>b</sup>, Claudio Rivera<sup>a</sup>, Jérôme Laurin<sup>a,\*</sup>

<sup>a</sup> Aix Marseille Univ, INSERM, INMED, Marseille, France

<sup>b</sup> Aix Marseille Univ, CNRS, ISM, Marseille, France

<sup>c</sup> Université de Montpellier, INRAE, DMEM, Montpellier, France

## ARTICLE INFO

### Keywords:

Endurance  
NKCC1/KCC2  
BAX/Bcl-2  
Neurotrophin  
Sensorimotor function

## ABSTRACT

**Background/purpose:** The optimal endurance exercise parameters remain to be defined to potentiate long-term functional recovery after stroke. We aim to assess the effects of individualized high-intensity interval training (HIIT) with either long or short intervals on neurotrophic factors and their receptors, apoptosis markers and the two-main cation-chloride cotransporters in the ipsi- and contralesional cerebral cortices in rats with cerebral ischemia. Endurance performance and sensorimotor functions were also assessed

**Methods:** Rats with a 2 h transient middle cerebral artery occlusion (tMCAO) performed work-matched HIIT4 (intervals: 4 min) or HIIT1 (intervals: 1 min) on treadmill for 2 weeks. Incremental exercises and sensorimotor tests were performed at day 1 (D1), D8, and D15 after tMCAO. Molecular analyses were achieved in both the paretic and non-paretic *triceps brachii* muscles and the ipsi- and contralesional cortices at D17

**Results:** Gains in endurance performance are in a time-dependent manner from the first week of training. This enhancement is supported by the upregulation of metabolic markers in both *triceps brachii* muscles. Both regimens alter the expression of neurotrophic markers and chloride homeostasis in a specific manner in the ipsi- and contralesional cortices. HIIT acts on apoptosis markers by promoting anti-apoptotic proteins in the ipsilesional cortex

**Conclusion:** HIIT regimens seem to be of clinical relevance in the critical period of stroke rehabilitation by strongly improving aerobic performance. Also, the observed cortical changes suggest an influence of HIIT on neuroplasticity in both ipsi- and contralesional hemispheres. Such neurotrophic markers might be considered as biomarkers of functional recovery in individuals with stroke.

## 1. Introduction

Endurance training represents a crucial part of stroke rehabilitation although optimal exercise parameters, such as intensity and duration, remain to be defined to improve physical activity guidelines [1,2]. Interestingly, preclinical experiments in rodents help define the effectiveness and/or shortcomings of exercise regimens, provided that training protocols are easily extrapolated to humans [3,4]. To reach this purpose, the use of common physiological parameters of exercise intensity in humans and rodents, such as the speed associated with lactate threshold ( $S_{LT}$ ) and maximal speed ( $S_{max}$ ), allows both distinguishing

low- from high-intensities and individualizing running speed [5–8] as recommended in humans [1].

Moreover, both humans and rodents studies suggest to initiate training protocols during the critical period of stroke rehabilitation, corresponding to the first weeks in rodents and first months in humans [6,7,9–11]. Indeed, it has been found that the effectiveness of rehabilitation strategies is potentiated when implemented during this period by enhancing the upregulation of key neurotrophic factors in the brain, such as the mature brain-derived neurotrophic factor (mBDNF) and/or the insulin-growth factor-1 (IGF-1) [10,12,13]. The direct link between neurotrophic factors and functional recovery has been demonstrated in

\* Corresponding author at: UMR 1249 - Institut de Neurobiologie de la Méditerranée, 163, avenue de Luminy – BP13, 13273 Marseille cedex 09 France.

E-mail address: [jerome.laurin@univ-amu.fr](mailto:jerome.laurin@univ-amu.fr) (J. Laurin).

<https://doi.org/10.1016/j.physbeh.2023.114190>

Received 21 January 2023; Received in revised form 29 March 2023; Accepted 4 April 2023

Available online 11 April 2023

0031-9384/© 2023 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

rodents with cerebral ischemia by an improvement of the tactile sensitivity and sensorimotor performance after BDNF or IGF-1 treatment [14–17].

Neurotrophic factors expression are endogenously upregulated by individualizing endurance training protocols, including the increasingly investigated high-intensity interval training (HIIT) regimens [18–27]. However, different types of HIIT regimens can be used in both patients and healthy people. The most frequent are the HIIT with long (>1–4 min) and short intervals ( $\leq 1$  min) [28]. These 2 regimens differ in terms of muscle power, peak oxygen uptake ( $VO_{2peak}$ ) and blood lactate accumulation [29,30]. For instance, higher blood lactate concentrations and peak heart rate were observed during an HIIT session with long intervals as compared with short ones [29]. Also, a meta-analysis of randomized controlled trials found a higher effectiveness of HIIT with long intervals to promote  $VO_{2peak}$  [30]. Interestingly, these physiological parameters can contribute to stimulate the expression of neurotrophic factors and seem to indicate that HIIT with long intervals is the most suitable [31,32]. However, other authors suggested that HIIT with short intervals are more recommended to improve aerobic capacity and functional recovery in chronic stroke patients [33]. Therefore, the effects of HIIT regimens on neurotrophic factors, functional recovery and aerobic capacity after stroke remain controversial and poorly investigated.

The endurance training-induced functional recovery might be partially associated with the major roles of these neurotrophic factors in neuronal survival and synaptic plasticity. Indeed, treadmill training can improve the neurological score by suppressing ischemia-induced neuronal apoptosis [34]. Also, among the effects at the synaptic level, endurance training can influence GABAergic activity by interacting with the two-main cation-chloride cotransporters in the brain, i.e., potassium-chloride-cotransporter 2 (KCC2), a neuronal chloride extruder, and sodium-potassium-chloride-cotransporter 1 (NKCC1), a chloride intruder [35–37], both of which are affected by stroke [38,39]. It was previously found that a 2-week HIIT alters cortical KCC2 expression in healthy rats [27] and can also restore the NKCC1/KCC2 ratio in the contralesional hemisphere following cerebral ischemia [6]. Nevertheless, the specific influence of HIIT regimens on these key markers remain unknown in both ipsi- and contralesional cortices. The cortex is yet one of the main areas affected by ischemic stroke, strongly involved in sensorimotor functions and considered a highly sensitive area to endurance training [40]. Moreover, significant concentrations of neurotrophic factors can be found [41].

The objective of this study was therefore to evaluate the effects of individualized HIIT programs with either short or long intervals over a 2-week training period on the expression of neurotrophic factors and their receptors, key markers of apoptosis and cation-chloride cotransporters in both ipsi- and contralesional cerebral cortices in rats with cerebral ischemia. Sensorimotor functions, cardiorespiratory capacities and metabolic changes in paretic and non-paretic *triceps brachii* muscles, involved in the locomotor activity in rats, are also assessed.

## 2. Materials and methods

Supplemental methodological information is found in the supplemental files.

### 2.1. Animals and ethics approval

All experimental protocols were approved by the Animal Care Committees of Marseille n°14 (APAFis #11,519) and complied with the ARRIVE guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. The 3Rs principle was applied: i) Replacement – No substitution in vitro model exists to mimic endurance training; ii) Reduction – The same animals performed training, incremental and behavioural tests to reduce the number of

animals; iii) Refinement – Experiments were either non-invasive or without awakening, except for the tMCAO surgery.

Fifty-five male Sprague-Dawley rats, sexually mature (starting protocol after P60; JANVIER®, France), were included. Each animal was randomly assigned to one of the following groups: SHAM ( $n = 10$ ), surgery without artery occlusion and training; transient middle cerebral artery occlusion (tMCAO;  $n = 17$ ), tMCAO without training including 3 rats for the TTC staining at D1 confirming the infarct size; HIIT4 ( $n = 14$ ) and HIIT1 ( $n = 14$ ), rats with tMCAO performed HIIT with long or short intervals, respectively (Fig. 1). As in clinical trial, all acquisitions and analyses were performed in a single-blind manner as no sign indicating the belonging to an experimental group was added on animals (just the animal number on the tail by using permanent marker). Experimenters performing behavioral and incremental tests could only see the scar on neck. The method of randomization was related to coin toss after the familiarization session to treadmill.

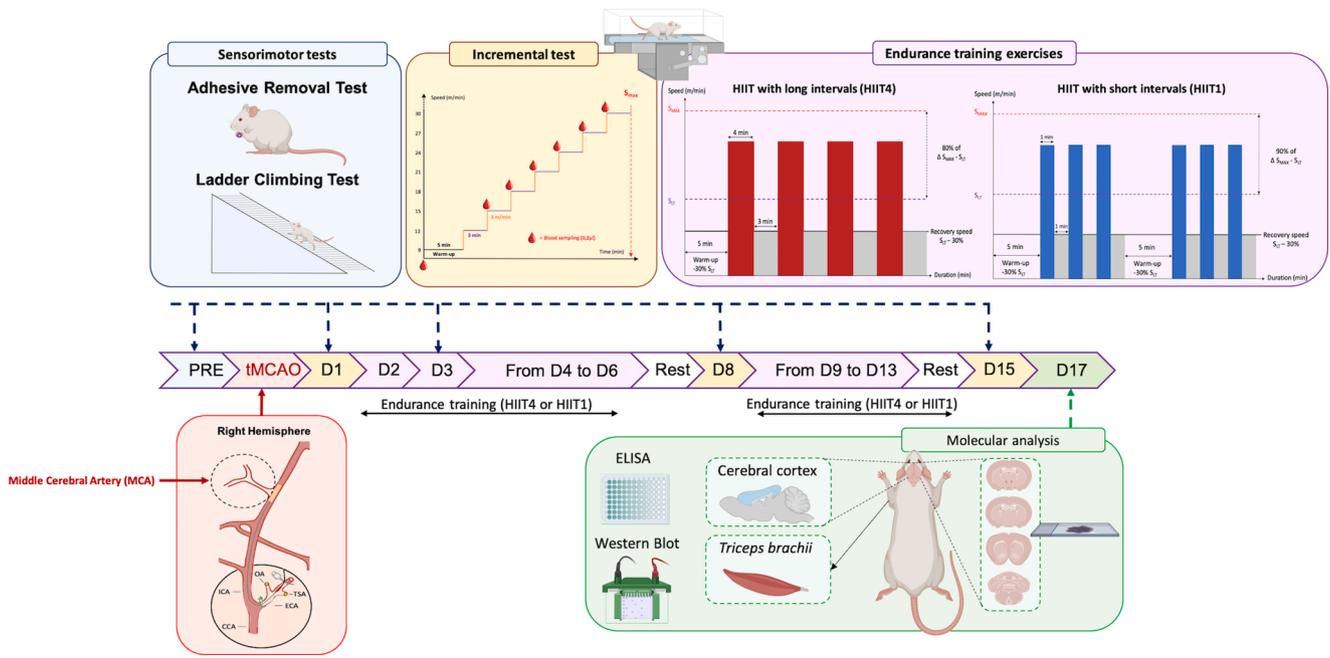
**Familiarization procedures.** To both limit stress of rats during training and behavioral tests, rats were familiarized with both running on treadmill (Decathlon, Lille, France); 5 min per day for 5 days, at 10–15  $m \cdot min^{-1}$ , thereby detecting which rats were able to run and with arenas for behavioral tests (for 5 days, 5 min/day). Stress was also reduced because the same researcher performed behavioral tests in a quiet room. A longer familiarization period increases the risk of improving endurance performance before the experimental protocol begins.

### 2.2. tMCAO

A 2 h right tMCAO was performed by the same experimenter, as described elsewhere<sup>7</sup>. Central temperature (maintained at 37–38 °C), age, weight and ischemia duration were rigorously controlled to improve reproducibility of neurological deficits during the cerebral ischemia procedure and the reperfusion surgery. A single-use 0–4 monofilament nylon suture with silicon-coated tip (diameter: 0.39 mm; tip length: 7 mm) was used for each surgery. Also, the exclusion criteria help to improve the lesion reproducibility. Anesthesia was induced and maintained with 5% and 2–3% of isoflurane respectively (Anesteo, France). A 0.2 ml of 0.5% bupivacaine was injected subcutaneously along the prospective incision site. Although the blood pressure, oxygenation and cerebral blood flow were not monitored during surgery, we previously found that the infarct size is reproducible at D1 between animals through 2,3,5-Triphenyltetrazolium chloride (TTC) and Cresyl violet stainings following the same protocol [6,42]. Three anesthetized tMCAO rats were decapitated at D1 to confirm the infarct size [42] with TTC staining. For that, fresh brain tissues were quickly removed and placed at  $-80$  °C during 4 min before performing a 2 mm-thick coronal sections using a brain slicer device. Coronal sections were then dropped in 5 ml of 1% TTC solution (Sigma-Aldrich®) during 30 min at 37 °C and washed 3 times with PBS-1X and fixed with antigenfix during 24 h. Infarcted tissues (white-colored area) were delimited with ImageJ® software and infarct size was expressed both in percentage of right hemisphere and total brain volume.

### 2.3. Incremental exercise test (IET)

In both humans and rats, 3-minute increments make it possible to distinguish more accurately the lactate threshold [6,7,43]. Endurance performance from IET was assessed on 1° inclined treadmill at day 1 (D1), D8 (corresponding to the intermediate IET), and D15 after tMCAO<sup>7</sup>. The warm-up running speed was set at 9  $m \cdot min^{-1}$  (3 min). The speed increment was set at 3  $m \cdot min^{-1}$  every 3 min with a starting speed at 12  $m \cdot min^{-1}$ . The lactate concentration (mmol/L) was measured between each speed level (15–20 s of rest) to determine the lactate threshold by collecting blood samples from the tail vein (0.2  $\mu L$ ; Lactate Scout+, France). As in humans, the lactate threshold was observed when 1) the inflection point of blood lactate concentration was reached



**Fig. 1.** Timeline of the experimental protocol. The experimental protocol includes the training sessions, behavioral tests, incremental tests, but also the cortex and muscles samples for Western blot, ELISA and immunohistochemical measurements. Recovery days are fixed at D7 and D14. The 10 training sessions were performed on treadmill from day 2 to day 13 after tMCAO. Sensorimotor tests included ladder climbing and adhesive removal tests. Abbreviation: ART=Adhesive removal test, D = day, HIIT1 = High-intensity interval training with short intervals, i.e., 1 min, HIIT4 = High-intensity interval training with long intervals, i.e., 4 min, LCT = Ladder-climbing test, SLT = speed associated with lactate threshold, Smax = maximal speed, tMCAO = transient middle cerebral artery occlusion.

and/or 2) an increase of  $1 \text{ mmol.L}^{-1}$  between two blood lactate values was detected. The speed corresponding to lactate threshold was  $S_{LT}$ . The test was stopped when rat could not maintain the imposed speed. The last completed speed level was  $S_{max}$ .

#### 2.4. Work-matched HIIT4 and HIIT1 programs on treadmill

HIIT4 (long intervals) and HIIT1 (short intervals) included 10 sessions (5 sessions per week) from D2 to D13, including two rest days at D7 and D14 for reducing fatigue accumulation before incremental tests. The warm-up lasted 5 min. To improve clinical relevance of this study,  $S_{LT}$  and  $S_{max}$  were used to individualize both intense series and recovery speeds over the 2-week HIIT programs. Each rat was thus trained individually. When necessary, rats were forced to run by stimulating the tail with a soft bristle brush to reduce stress compared to electrical shocks. Hence, no electrical shocks were used to motivate the rats in this study. In addition, running speed, session duration and the total workload progressively increased over the protocol in a work-matched manner (see supplemental files for more information) to allow comparable exercise doses between regimens [44] with the formula:  $Mass \text{ (kg)} \times Intensity \text{ (m/min)} \times Duration \text{ (min)} \times Treadmill \text{ inclination } (1^\circ) \times 9.8 \text{ (J/kg.m)}$

**HIIT4.** Sessions with long intervals included  $4 \times 4$  min of running on treadmill separated by 3 min of active recovery (warm-up and recovery speed: 30% below  $S_{LT}$ ). Running speed was set at 80% of the difference between  $S_{max}$  and  $S_{LT}$  both for the first training week following the first IET (at D1) and for the last training week following the intermediate IET (at D8).

**HIIT1.** Short intervals lasted 1 min separated by 1 min of active recovery (30% below  $S_{LT}$ ). Running speed of short series corresponded to 90% of the difference between  $S_{max}$  and  $S_{LT}$  both for the first training week following the first IET (at D1) and for the last training week following the intermediate IET (at D8).

It is worthy to notice that some of the major physical exercise recommendations were followed in the present study in rats to reinforce the translational relevance of these training regimens to individuals with

stroke in accordance with the STAIR study [3]: 1) Training started during the critical period of stroke rehabilitation, which can potentiate the incomplete spontaneous recovery by upregulating levels of neurotrophic factors, as recommended for both animals and humans [13]. 2) Running speed was progressively increased over the 2 weeks of training to optimize the training speed by using an intermediate incremental test at D8 to physiologically reassess the speed above the new  $S_{LT}$  and below the new  $S_{max}$ . 3) The running speeds are comprised between  $S_{max}$  and  $S_{LT}$  to ensure that running speed remained above  $S_{LT}$  and below  $S_{max}$ . Therefore, intensity is always in the “aerobic range” that can be reached by animals, and thus, below the maximum tolerated. It seems that such intensities in rats could be applied to moderate stroke patients who can reach these intensities because most of them could exceed the speed associated with ventilatory threshold [45]. Also, HIIT intensity needs to be higher than lactate threshold to be considered as a HIIT regimen. It is an important physiological characteristic of HIT [46]. Also, a higher running speed is better tolerated during HIIT with short intervals. It is why the individualized running speed is closer to  $S_{max}$  in HIIT1 than HIIT4.

4) Training lasted 15 days after ischemia that is the minimum required [3] to assess the effectiveness of treatments. 5) For HIIT1, short duration intervals were fixed to 1 min, and not to 30–45 s, to reduce the number of series per session while reaching a similar workload to that reported for HIIT4. In other words, the number of short series would have been too high with 30-s intervals, which could be less clinically relevant.

#### 2.5. Sensorimotor tests

Both ladder-climbing test and adhesive removal test were performed before (PRE), at D1, D3, D8 and D15, always preceding IET to avoid the influence of fatigue and stress.

**Ladder-climbing test.** Rats climbed up an inclined ladder (inclination:  $45^\circ$ ; rungs of equal 10 mm spacing) [42]. Depending on number of performed steps (20 steps minimum), the successful score was normalized by maximal score.

**Adhesive removal test (ART).** This test was used to independently assess tactile sensitivity and motor coordination of both left and right forepaws (LF and RF, respectively) [42]. The time to detect the adhesive tape (TD) for each forepaw was averaged (in seconds) between the 2 trials per session as well as for time to remove (TR). The maximal recording time was fixed to 120 s.

## 2.6. Western blot

At D17, rats were anaesthetized with intraperitoneal ketamine/xylazine (120 mg/kg–20 mg/kg) injection ( $n = 6–8$  per group). Both *triceps brachii* muscles were first removed. Then, ipsi- and contralesional cortices were dissected out and separated, flash-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until processed [7]. The Western blot for muscles and brain was performed in different laboratories (Marseille-INMED and Montpellier-DMEM). Although Western blot methods were different, it is not a methodological limitation because no comparison was performed between muscles and cortex proteins.

### 2.6.1. Triceps brachii muscles

The *triceps brachii* muscles are strongly involved during running in rats [47]. Also, previous articles found significant muscle activation disturbances following tMCAO [42,48]. The total proteins were extracted from paretic and non-paretic *triceps brachii* muscles to detect Citrate Synthase (CS), Vascular endothelial growth factor (VEGF) and Fibronectin type III domain-containing protein 5 (FNDC5; **Table S1**). Intensity values were expressed relative to SHAM. Several samples of paretic and non-paretic *triceps brachii* muscles in each group were represented on each nitrocellulose membrane (x2 membranes per protein).

### 2.6.2. Cortex

Cortex tissue was homogenized in RIPA lysis buffer (50 mM Tris–HCl pH 8; 150 mM NaCl; SDS 0.1%; Deoxycholic Acid 0.5%; 1% Triton X-100), containing complete Protease/Phosphatase Inhibitor Tablet (ThermoFisher®) and loaded with Laemmli X-3 loading buffer. The sample proteins (50  $\mu\text{g}$ ) were separated in SDS–PAGE gel (4–12%; Criterion gel, Bio-Rad®) by electrophoresis under constant voltage (175 V in stacking gel and 200 V in revolving gel). The total proteins were extracted from both ipsi- and contralesional cortices to detect KCC2, NKCC1, mBDNF, its high-affinity tropomyosin receptor kinase B (TrkB), its phosphorylated form (pTrkB), B-cell lymphoma 2 (Bcl-2), Bcl-2 associated X protein (BAX), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 $\alpha$ ) and Cytochrome c oxidase subunit 4 (COX4, (**Table S1**). Several ipsi- and contralesional samples of each group were represented on each nitrocellulose membrane to allow comparison between groups (2 membranes per protein). The pTrkB/TrkB ratio, BAX/Bcl-2 ratio and NKCC1/KCC2 ratio were calculated.

## 2.7. Immunohistochemistry

At D17, animals were perfused transcardially with cold phosphate buffer saline (PBS 1X) after intraperitoneal ketamine-xylazine injection (120 mg/kg–20 mg/kg), followed by paraformaldehyde 3% (Antigenfix). Brain tissue was removed, post-fixed for 2 h at  $4^{\circ}\text{C}$  in antigenfix, cryoprotected 24 h at  $4^{\circ}\text{C}$  in 30% sucrose and then snap-frozen with isopentane and stored at  $-80^{\circ}\text{C}$ . Coronal sections of rat brain (thickness: 80  $\mu\text{m}$ ) were performed. We choose fields at the peri-lesional site both from ipsi- and contralesional hemispheres and acquired 25  $\mu\text{m}$  stack at 2.5  $\mu\text{m}$  step images (primary motor cortex, stereotaxic coordinates: Interaural: 6.72 mm; Bregma:  $-2.28$  mm) [49]. The Caspase-3 and Hoechst staining were manually quantified using the ImageJ software after making images projection. Acquisition parameters were the same for all experimental conditions. Images were then acquired consecutively for Caspase-3 and Hoechst staining and 25  $\mu\text{m}$  stack were acquired at 2.5  $\mu\text{m}$  step images. The Pearson correlation coefficient of colocalization between Caspase-3 and Hoechst staining was measured in ipsi-

and contralesional cortex (x20).

## 2.8. Immunoassay (ELISA)

Quantification of cortical mBDNF (dilution 1/10) and IGF-1 (dilution 1/5) was performed in duplicate with mBDNF Rapid ELISA Kit and IGF-1 ELISA Kit (Biosensis®, BEK-2211–1P/2P and BEK-2150–2P, respectively) following the manufacturer's protocol. After having detected mBDNF and IGF-1 with streptavidin-HRP conjugate, the concentration of mBDNF and IGF-1 were allowed by the addition of a substrate (3,3',5,5'-tetramethylbenzidine, TMB). The standard curve was plotted allowing the interpolation of mBDNF and IGF-1 protein concentrations through a 4-PL regression analysis. Concentrations were determined using the FLUOstar® OPTIMA microplate reader (BMG Labtech, France).

## 2.9. Statistical analysis

Statistical analysis was performed using Prism software program (data expressed in mean $\pm$ SD; Significant results when  $p < 0.05$ ). Results of sensorimotor and incremental tests were compared by means of two-way ANOVA (groups x time) while unpaired *t*-test (two-tailed) was used to compare HIIT sessions duration and speed. Western blotting analyses, mBDNF and IGF-1 ELISA and immunostaining were compared by means of one-way ANOVA (groups). Post-hoc comparisons were performed by means of Tukey's multiple post-test comparisons. Pearson's correlation coefficient with two-tailed significance was used to analyze the relationship between relative levels of neuroplasticity and muscular markers and gains in both  $S_{LT}$  and  $S_{max}$ .

## 3. Results

### 3.1. Work-matched HIIT4 and HIIT1 programs on treadmill

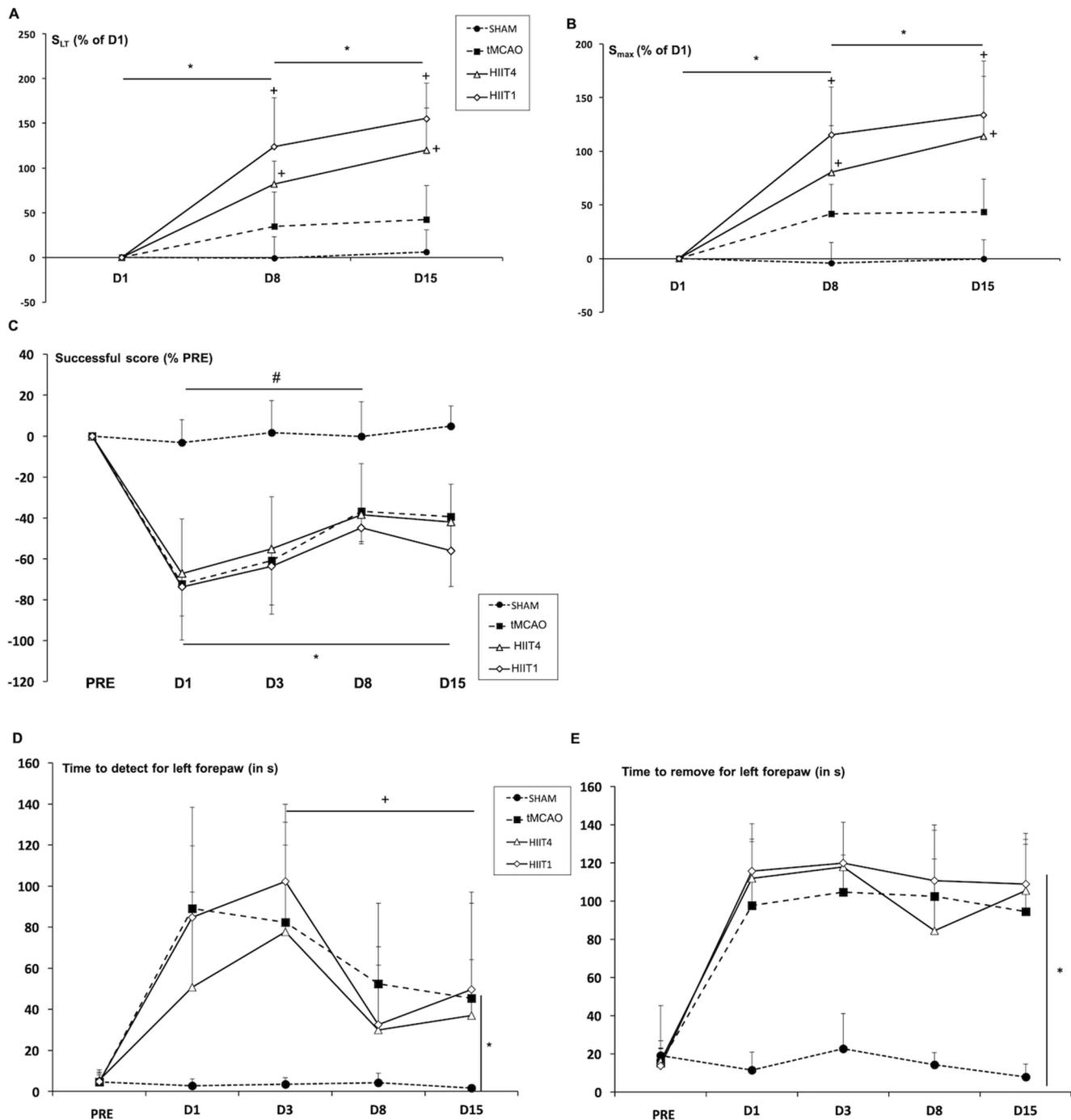
Session duration for HIIT1 was 22.1% longer than for HIIT4 during the first week ( $p < 0.005$ ,  $t = 3.460$ ,  $df = 27$ ). Series and recovery speeds during HIIT1 sessions were higher during the week 2 compared to the week 1 (14.6%,  $p < 0.05$ ,  $t = 2.151$ ,  $df = 28$  and 29.8%,  $p < 0.005$ ,  $t = 3.4$ ,  $df = 28$  respectively). Running speed increased to 69.6% for HIIT4 and 111.7% for HIIT1 and the total workload increased to 76.6% between the 2 weeks (**Table S2**).

### 3.2. IET and sensorimotor tests

For  $S_{LT}$ , two-way ANOVA analysis showed significant difference between training regimens ( $F_{(3,48)} = 16.53$ ), times ( $F_{(1,91,91.72)} = 123.9$ ) and training regimens x times ( $F_{(6,96)} = 28.06$ ;  $p < 0.001$ ) (**Fig. 2A**). For  $S_{max}$ , two-way ANOVA analysis showed significant difference between training regimens ( $F_{(3,48)} = 17.84$ ), times ( $F_{(21,90,91.33)} = 153.9$ ) and training regimens x times ( $F_{(6,96)} = 29.51$ ;  $p < 0.001$ ). See raw values in **Table S3**.

$S_{LT}$  and  $S_{max}$  of HIIT4, HIIT1 and tMCAO groups were lower at D1 than SHAM ( $p < 0.01$  and  $p < 0.001$ , respectively).  $S_{LT}$  of HIIT4 and HIIT1 were higher than tMCAO ( $p < 0.05$ ) at D8.  $S_{LT}$  and  $S_{max}$  increased from D1 to D8 for HIIT4 and HIIT1 ( $p < 0.001$ ).  $S_{max}$  of HIIT4 and HIIT1 were higher than SHAM ( $p < 0.05$  and  $p < 0.001$ , respectively) and tMCAO at D8 and D15 ( $p < 0.001$ ). Then,  $S_{LT}$  of HIIT1 was higher than SHAM and tMCAO ( $p < 0.05$ ) while  $S_{LT}$  of HIIT4 was only higher than tMCAO at D15. However, a continuous increase for  $S_{LT}$  and  $S_{max}$  remained from D8 to D15 only for HIIT4 ( $p < 0.01$ ; **Fig. 2B**). See examples of  $S_{LT}$  and  $S_{max}$  for tMCAO, HIIT4 and HIIT1 rats over time in **Fig. S1**

For the ladder-climbing test, two-way ANOVA analysis showed significant difference between training regimens ( $F_{(3,48)} = 86.54$ ), times ( $F_{(2,87,137.5)} = 101.6$ ) and training regimens x times ( $F_{(12,192)} = 9.63$ ;  $p < 0.001$ ). For the ladder-climbing test, the successful scores remained



**Fig. 2.** Effects of HIIT regimens on endurance performance and sensorimotor tests. A) SLT. +Higher in HIIT4 and HIIT1 groups than tMCAO at D8 and D15 ( $p < 0.01$ ). \*D8 higher than D1 for HIIT4 and HIIT1 ( $p < 0.001$ ). \*D15 higher than D8 for HIIT4 ( $p < 0.01$ ). B) Smax. +Higher in HIIT groups than tMCAO and SHAM at D8 and D15 ( $p < 0.05$ ). \*Higher at D8 and D15 than D1 in HIIT groups ( $p < 0.001$ ). \*Higher Smax at D15 than D8 for HIIT4. C) Ladder climbing test - Successful score (% of PRE). \*Significant decrease at D1, D3, D8 and D15 compared to PRE for injured groups and significant higher score for SHAM compared to lesioned groups from D1 to D15. #Significant higher score for tMCAO, HIIT1 and HIIT4 groups at D8 and D15 compared to D1. D) Adhesive removal test of paretic forepaw: Time to detect/Tactile sensitivity. \*Significant faster time to detect in SHAM than in lesioned groups from D1 to D15. +Significant faster time to detect at D15 than D3 only for HIIT1 and HIIT4 groups. E) Time to remove/Motor coordination. \*Significant faster time to remove in SHAM compared to lesioned groups from D1 to D15. Abbreviation: D = day, HIIT1 = High-intensity interval training with short intervals, i.e., 1 min, HIIT4 = High-intensity interval training with long intervals, i.e., 4 min, SLT = speed associated with lactate threshold, Smax = maximal speed, TD = time to detect the adhesive tape for each forepaw and TR = time to remove, tMCAO = transient middle cerebral artery occlusion.

higher for SHAM ( $p < 0.001$ ) despite a spontaneous incomplete recovery observed in lesioned groups ( $p < 0.001$ ; Fig. 2C).

For the adhesive removal test, two-way ANOVA analysis showed a significant difference between training regimens ( $F_{(3,48)} = 48.04$ ), times ( $F_{(2,715,130.3)} = 45.22$ ) and training regimens x times ( $F_{(12,192)} = 5.575$ ;  $p$

$< 0.001$ ) for the time to detect. HIIT regimens induce an incomplete recovery from D3 to D15 ( $p < 0.05$ ). For the time to remove, no difference was found except between SHAM and the three lesioned groups at each time point (Fig. 2D and E).

3.3. TTC staining

The reproducibility of infarct size was controlled on 3 animals 24 h after cerebral ischemia onset. Brain tissues were removed before being sectioned and stained with TTC. Infarct size represents  $32 \pm 6\%$  of the whole brain and  $61 \pm 11\%$  of the right hemisphere at D1. (Fig. S2). TTC staining at D1 confirms the reproducibility of the 2-hour tMCAO [6,7,42,48,50].

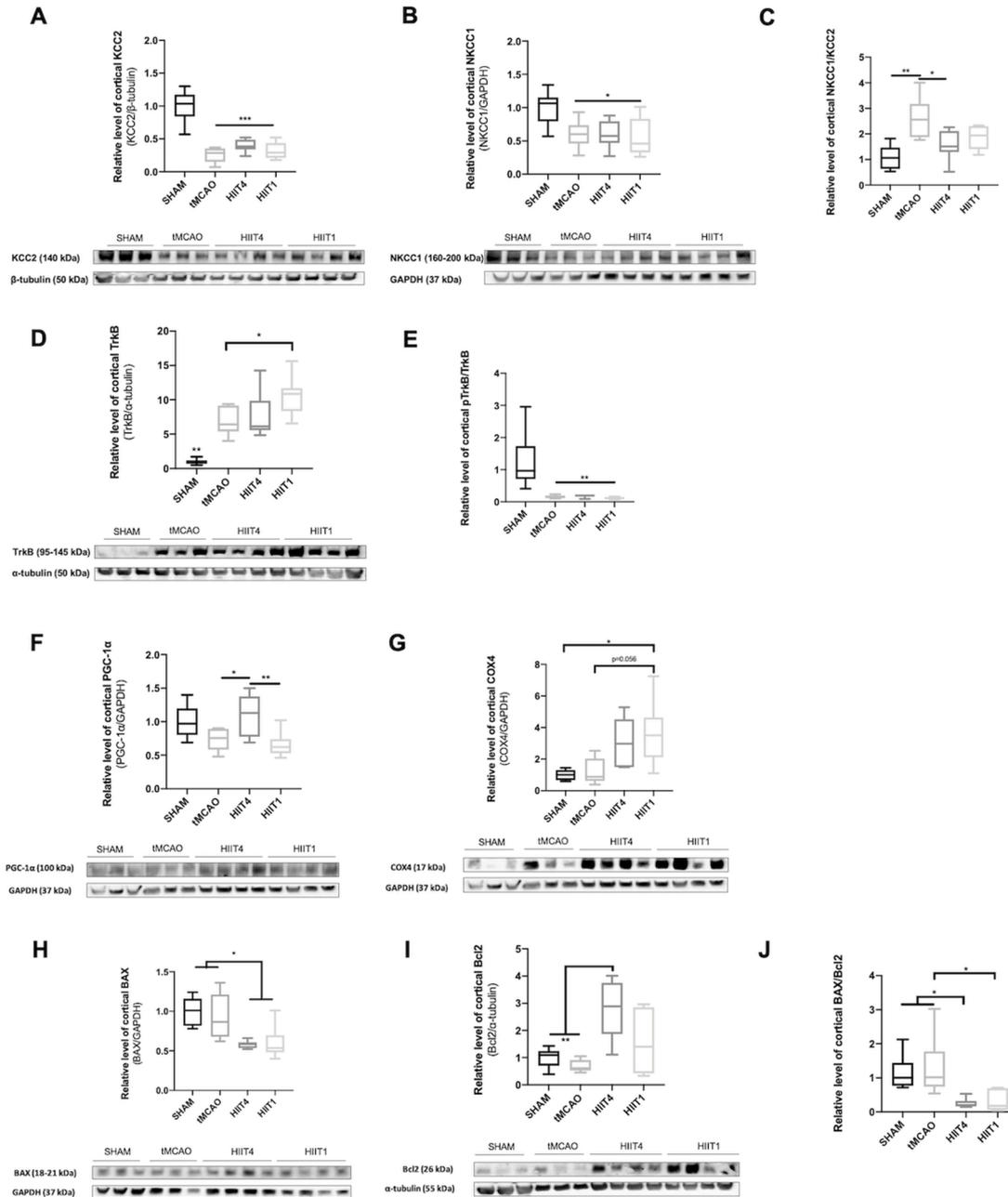
3.4. Western blot

3.4.1. Paretic triceps brachii muscles

One-way analysis showed significant difference between training regimens for CS ( $F_{(2, 17)} = 5.08$ ;  $p < 0.05$ ). Levels of CS were higher in HIIT1 than tMCAO ( $p < 0.05$ ). Levels of FNDC5 in tMCAO tended to be higher than SHAM ( $p < 0.057$ ; Fig. S3A and B). No significant difference was observed for VEGF.

3.4.2. Non-paretic triceps brachii muscles

One-way analysis showed significant difference between regimens for CS ( $F_{(3,23)} = 3.01$ ;  $p < 0.05$ ), FNDC5 ( $F_{(3,21)} = 3.52$ ;  $p < 0.05$ ) and



**Fig. 3.** Impact of HIIT regimens on protein levels within the ipsilesional cortex. A) KCC2, B) NKCC1, C) NKCC1/KCC2, D) TrkB, E) pTrkB/TrkB, F) PGC-1 $\alpha$ , G) COX4, H) BAX, I) Bcl-2 and J) BAX/Bcl-2. Representative cropped immunoblots from the same membrane are shown at the bottom. \*Difference between groups. Abbreviation: Bcl-2 = B-cell lymphoma 2, BAX = Bcl-2 Associated X protein, COX4 = Cytochrome c oxidase subunit 4, D = day, HIIT1 = High-intensity interval training with short intervals, i.e., 1 min, HIIT4 = High-intensity interval training with long intervals, i.e., 4 min, KCC2 = potassium chloride cotransporter 2, NKCC1 = sodium-potassium-chloride transporter, PGC-1 $\alpha$  = Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, pTrkB = phosphorylated form of tropomyosin receptor kinase B, TrkB = tropomyosin receptor kinase B, tMCAO = transient middle cerebral artery occlusion.

VEGF ( $F_{(3,24)} = 3.46; p < 0.05$ ). Levels of FNDC5 in HIIT4 were higher than SHAM and HIIT1 ( $p < 0.05$ ). Levels of CS in HIIT1 were higher than tMCAO ( $p < 0.05$ ). Levels of VEGF in tMCAO were lower than SHAM ( $p < 0.05$ ) without difference between SHAM and HIIT groups (Fig. S3C-E).

3.4.3. Ipsilesional cortex

One-way analysis showed significant difference between regimens for KCC2 ( $F_{(3,24)} = 32.18; p < 0.001$ ), NKCC1 ( $F_{(3,22)} = 4.65; p < 0.05$ ), NKCC1/KCC2 ratio ( $F_{(3,23)} = 7.31; p < 0.01$ ), TrkB ( $F_{(3,24)} = 17.60; p < 0.001$ ), pTrkB/TrkB ratio ( $F_{(3,22)} = 9.91; p < 0.001$ ), BAX ( $F_{(3,20)} = 7.30$ ) and Bcl-2 ( $F_{(3,19)} = 6.78; p < 0.01$ ) for both, BAX/Bcl-2 ratio ( $F_{(3,20)} = 6.03; p < 0.01$ ), COX4 ( $F_{(3,19)} = 4.93; p < 0.05$ ) and PGC-1 $\alpha$  ( $F_{(3,23)} = 5.57; p < 0.001$ ). Levels of KCC2 and NKCC1 were lower in HIIT4, HIIT1 and tMCAO groups than SHAM ( $p < 0.001$  and  $p < 0.05$ , respectively). Nevertheless, KCC2 levels appeared to be higher in HIIT4 than tMCAO when analyzed with *t*-test ( $p < 0.05$ ). Finally, NKCC1/KCC2 ratio was higher in tMCAO compared to SHAM and HIIT4 ( $p < 0.05$ ). Levels of TrkB were higher in HIIT4, HIIT1 and tMCAO groups ( $p < 0.05$ ) than SHAM. Moreover, levels of TrkB in HIIT1 were higher than tMCAO ( $p < 0.05$ ). The pTrkB/TrkB ratio was downregulated in HIIT4, HIIT1 and tMCAO groups. Levels of PGC-1 $\alpha$  were higher in HIIT4 than both tMCAO and HIIT1 ( $p < 0.05$  and  $p < 0.01$ , respectively) and levels of COX4 in HIIT1 were only higher than SHAM ( $p < 0.05$ ). Levels of COX4 tended to be higher in HIIT1 than tMCAO ( $p = 0.056$ ). Levels of BAX were lower in HIIT4 and HIIT1 than tMCAO and SHAM ( $p < 0.05$ ) while levels of Bcl-2 were higher in HIIT4 than tMCAO and SHAM ( $p < 0.01$ ). The BAX/Bcl-2 ratio was lower in both HIIT groups than tMCAO ( $p < 0.05$ ; Fig. 3).

3.4.4. Contralateral cortex

One-way analysis showed significant difference between regimens for KCC2 ( $F_{(3,20)} = 3.6; p < 0.05$ ), NKCC1/KCC2 ratio ( $F_{(3,21)} = 3.63; p < 0.05$ ), pTrkB/TrkB ratio ( $F_{(2,16)} = 13.69; p < 0.01$ ), PGC-1 $\alpha$  ( $F_{(3,22)} = 4.75; p < 0.05$ ) and COX4 ( $F_{(3,23)} = 4.10; p < 0.05$ ). Levels of KCC2 were

only higher in HIIT4 than tMCAO ( $p < 0.05$ ; Fig. 4) without effects on NKCC1. Nevertheless, NKCC1/KCC2 ratio was downregulated in HIIT4 compared to SHAM ( $p < 0.05$ ). PGC-1 $\alpha$  levels were higher in HIIT4 than SHAM ( $p < 0.01$ ). Moreover, pTrkB/TrkB ratio was upregulated in HIIT4 compared to SHAM ( $p < 0.05$ ) and tMCAO ( $p < 0.001$ ). COX4 levels in HIIT1 were higher than SHAM and tMCAO ( $p < 0.05$ ).

3.5. Immunoassay

3.5.1. Ipsilesional (right) cortex versus contralateral (left) cortex

The mBDNF concentrations were higher in the ipsilesional cortex than in the contralateral cortex in tMCAO ( $p < 0.01$ ) and HIIT4 ( $p < 0.001$ ) groups. IGF-1 concentrations in the ipsilesional cortex were higher than in the contralateral cortex for tMCAO, HIIT4 and HIIT1 groups ( $p < 0.05$ ; Fig. 5).

3.5.2. Ipsilesional and contralateral cortex

One-way analysis showed significant difference between groups for mBDNF in the ipsilesional cortex ( $F_{(3,25)} = 4.94; p < 0.05$ ) and in the contralateral cortex ( $F_{(2,18)} = 4.03; p < 0.05$ ).

In the ipsilesional cortex, mBDNF concentrations were higher in tMCAO and HIIT4 groups than in SHAM ( $p < 0.05$ ) without difference between HIIT1 and SHAM. The IGF-1 concentrations in tMCAO were higher than SHAM ( $p < 0.05$ ) without difference between SHAM and HIIT regimens. In the contralateral cortex, mBDNF concentrations in HIIT1 were higher than tMCAO ( $p < 0.05$ ) but tended to decrease in tMCAO compared to SHAM ( $p = 0.063$ ; Fig. 5). No difference was found for IGF-1 in the contralateral cortex ( $F_{(3,20)} = 0.35; p > 0.05$ ).

3.6. Immunohistochemistry

One-way analysis showed significant difference between training regimens and tMCAO for the Pearson correlation coefficient of colocalization between Caspase-3 and Hoechst ( $F_{(2,24)} = 16.64; p < 0.001$ ). In

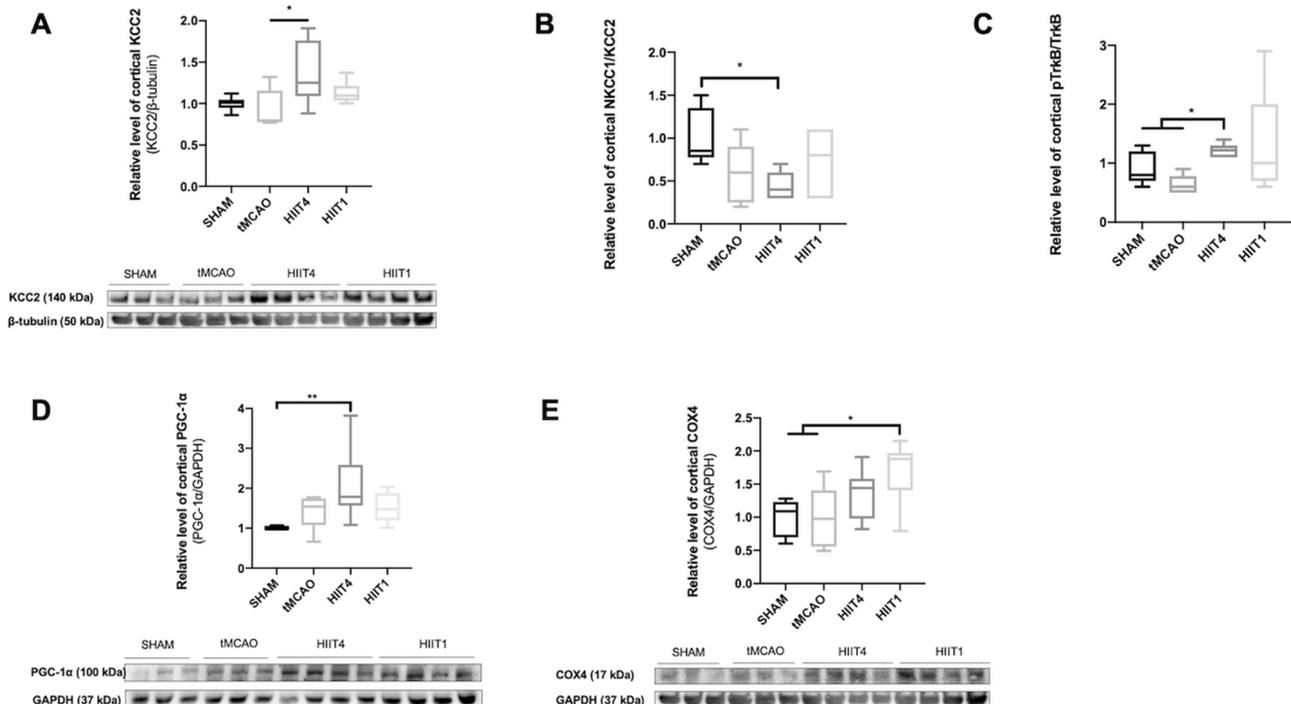
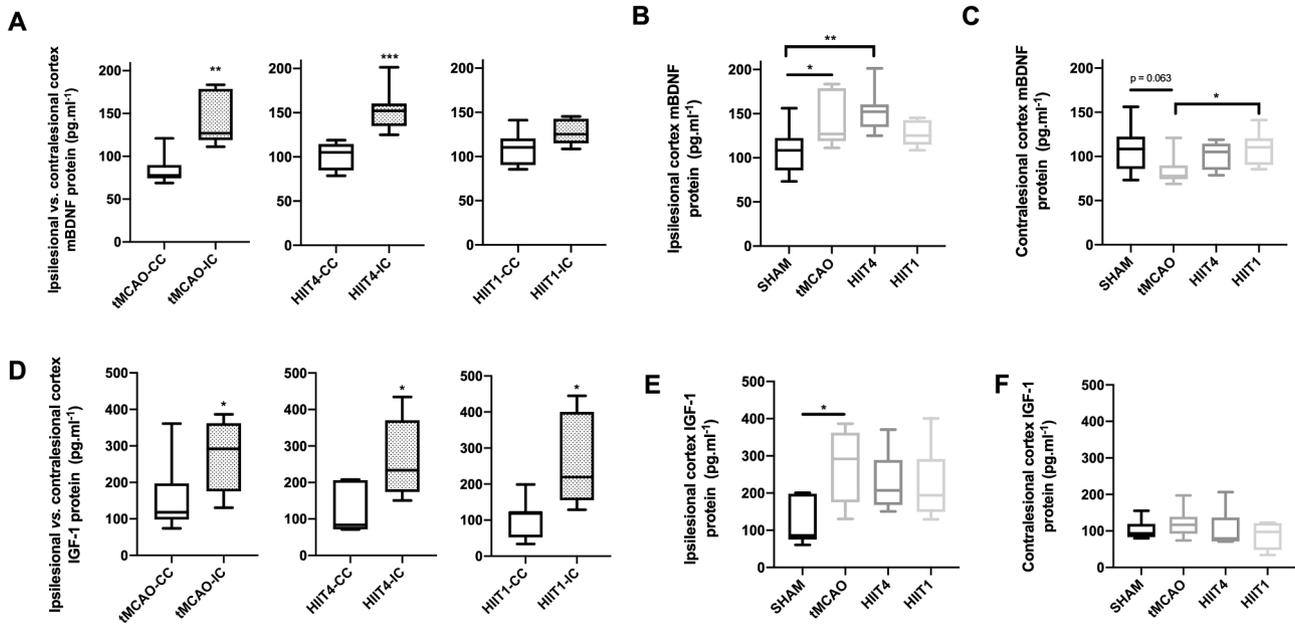


Fig. 4. Impact of HIIT regimens on protein levels within the contralateral cortex. A) KCC2, B) NKCC1/KCC2, C) pTrkB/TrkB, D) PGC-1 $\alpha$  and E) COX4. Representative cropped immunoblots from a same membrane are shown. \*Difference between groups. Abbreviation: COX4 = Cytochrome c oxidase subunit 4, D = day, HIIT1 = High-intensity interval training with short intervals, i.e., 1 min, HIIT4 = High-intensity interval training with long intervals, i.e., 4 min, KCC2 = potassium chloride cotransporter 2, NKCC1 = sodium-potassium-chloride transporter, PGC-1 $\alpha$  = Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, pTrkB = phosphorylated form of tropomyosin receptor kinase B, tMCAO = transient middle cerebral artery occlusion, TrkB = tropomyosin receptor kinase B.



**Fig. 5.** Impact of HIIT regimens on cortical mBDNF and IGF-1. mBDNF, A) ipsi- versus contralesional cortex, B) ipsilesional cortex (IC), C) contralesional cortex (CC); IGF-1, D) ipsi- versus contralesional cortex, E) ipsilesional cortex and F) contralesional cortex. \*Difference between groups. Abbreviation: D = day, HIIT1 = High-intensity interval training with short intervals, i.e., 1 min, HIIT4 = High-intensity interval training with long intervals, i.e., 4 min, IGF-1 = insulin-like growth factor-1, KCC2 = potassium chloride cotransporter 2, mBDNF = mature brain-derived neurotrophic factor, tMCAO = transient middle cerebral artery occlusion.

HIIT4 and HIIT1, there was a lower colocalization between Caspase-3 and Hoechst than tMCAO in the ipsilesional cortex ( $p < 0.001$ ), without difference in the contralesional cortex (Fig. 6).

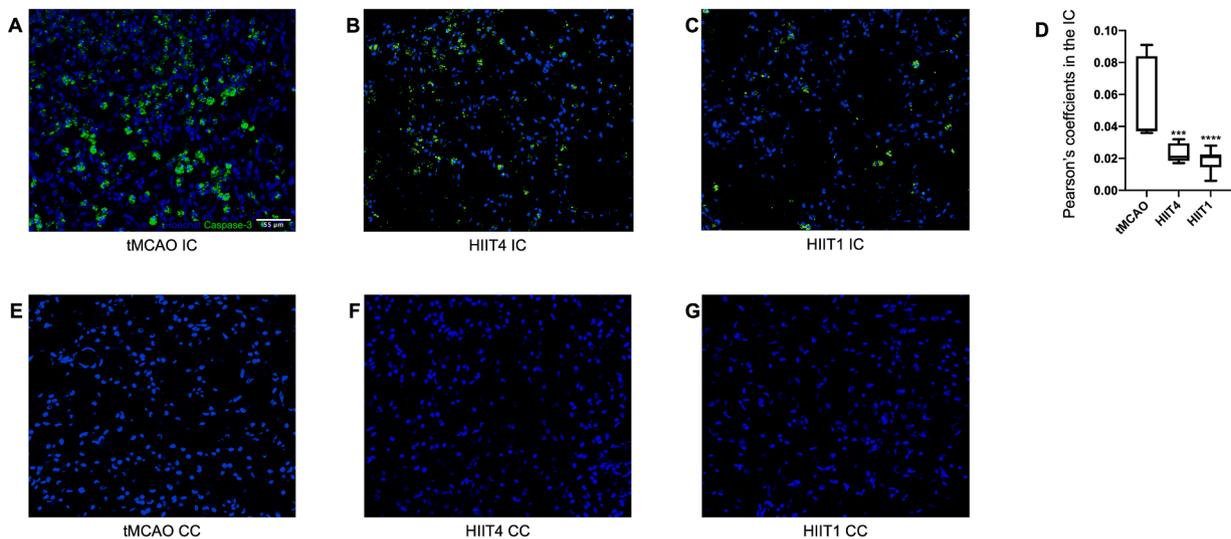
**3.7. Correlation between neurotrophic markers in the ipsilesional cortex and aerobic parameters**

Levels of TrkB, COX4 and Bcl-2 were positively correlated with gains in  $S_{LT}$  ( $r = 0.74, r = 0.75, r = 0.54$  respectively, medium effect size;  $p < 0.01$ ). Also, levels of BAX, BAX/Bcl2, KCC2 and NKCC1 were negatively correlated with gains in  $S_{LT}$  ( $r = -0.47, r = -0.59, r = -0.52, r = -0.43$  respectively, small effect size;  $p < 0.05$ ; Fig. S4) and  $S_{max}$  ( $r = -0.47, r$

$= -0.61, r = -0.52, r = -0.46$  respectively, small effect size;  $p < 0.05$ ).

**3.8. Correlation between neurotrophic markers in the contralesional cortex and aerobic parameters**

Levels of PGC-1 $\alpha$ , COX4 and KCC2 were positively correlated with gains in  $S_{LT}$  ( $r = 0.45, r = 0.48, r = 0.58$  respectively, small effect size;  $p < 0.05$ ) and  $S_{max}$  ( $r = 0.48, r = 0.37, r = 0.60$  respectively, small effect size;  $p < 0.05$ ). TrkB was positively correlated with gains in  $S_{LT}$  ( $r = 0.39$ , small effect size;  $p < 0.05$ ; Fig. S5).



**Fig. 6.** Impact of HIIT1 and HIIT4 regimens on Caspase-3 in ipsi- and contralesional primary motor cortices by immunohistostaining (x20). In the ipsilesional cortex (IC), A) tMCAO, B) HIIT4, C) HIIT1, D) the Pearson correlation coefficient of colocalization between Caspase-3 (green) and Hoechst (Blue) staining in the IC ( $n = 3$  per group; 1 slice with 3 stacks per rat). \*Difference between groups. In the contralesional cortex (CC), no Caspase-3 staining was found for E) tMCAO, F) HIIT4, and G) HIIT1. Abbreviation: D = day, HIIT1 = High-intensity interval training with short intervals, i.e., 1 min, HIIT4 = High-intensity interval training with long intervals, i.e., 4 min, tMCAO = transient middle cerebral artery occlusion.

### 3.9. Correlation between markers in both triceps brachii muscles and aerobic parameters

In the non-paretic forelimb, levels of VEGF were negatively correlated with gains in  $S_{\max}$  ( $r = -0.43$ , small effect size;  $p < 0.05$ ). Levels of FNDC5 were positively correlated with gains in  $S_{\max}$  ( $r = 0.50$ , small effect size;  $p < 0.05$ ; Fig. S6). Also, a positive correlation is noticed between non-paretic forelimb and PGC-1 $\alpha$  in the contralesional cortex ( $r = 0.5694$ ;  $p = 0.0138$ , Fig. S7) and no significant correlation was found between mBDNF and PGC-1 $\alpha$  neither in the contralesional nor in the ipsilesional cortex (Fig. S8).

## 4. Discussion

By enhancing  $S_{LT}$ , HIIT regimens might improve the ability to sustain higher submaximal speeds during a long period after stroke [5]. Also,  $S_{\max}$  improvements suggest higher maximal aerobic abilities, i.e., maximal oxygen uptake ( $VO_{2\max}$ ) [51]. Such improvement in cardio-respiratory fitness increases submaximal exercise tolerance, involved in the quality of life [52]. Gains in endurance for lesioned animals even outperform the SHAM aerobic abilities while we previously found that training is not effective to reduce infarct size [6] and seems to act rather in spared and remoted areas from the lesion [12].

The time course of gains in performance varies between HIIT regimens in our study as HIIT4 enhances  $S_{LT}$  and  $S_{\max}$  between D8 and D15 in contrast with HIIT1. Although these results suggest a higher effectiveness of HIIT4 at this period, both regimens appear to be equally effective at the end of the 2 training weeks. Our results support the view that these HIIT regimens might be used during the critical period of stroke rehabilitation depending on the exercise preference of patients, without reducing the effectiveness of endurance program. Interestingly, a recent clinical trial found a higher effectiveness of an intensive task-specific motor training within the first 2 – 3 months after stroke compared to delayed training (> 3 months), similarly to what was found during the first weeks in rodents [10]. It is important to add that individuals with stroke should perform an IET with electrocardiogram monitoring before starting a HIIT program to ensure their safety and define individualized intensities, as we performed in rats.

We show for the first time that gains in endurance performance seem to be supported by the upregulation of CS, a strong indicator of mitochondrial biogenesis, in both *triceps brachii* muscles following HIIT1. Moreover, FNDC5, correlated with aerobic parameters, is upregulated in the non-paretic muscles after HIIT4 while no difference was found after HIIT1. It might suggest that the duration of intense series could have an impact on this myokine. It remains to define if the time spend close to  $VO_{2\text{peak}}$  during a HIIT session could regulate the FNDC5 expression known to be released by the active muscles to the brain [53]. Consequently, HIIT regimens seem to be complementary in upregulating aerobic markers. In addition, FNDC5 might promote neurotrophic factors because it stimulates the mBDNF/TrkB signaling pathways in the brain [53,54].

An upregulation of cortical mBDNF and IGF-1 and/or their receptors are frequently associated with neuronal survival by reducing apoptosis, promoting synaptic plasticity through chloride homeostasis (among others) and functional recovery after stroke [55,56]. Concentrations in both cortical mBDNF and IGF-1 are higher in the ipsilesional than in the contralesional cortex. However, although an increase in neurotrophic factors is classically associated with beneficial effects, the mBDNF and/or IGF-1 overexpression could also be deleterious for cell repair and functional recovery [57–60]. Besides, in the ipsilesional cortex, HIIT regimens seem to reduce the increase in IGF-1 and mBDNF (only for HIIT1) concentrations compared to untrained tMCAO animals in contrast with those showing an upregulation of mBDNF in the ipsilesional hippocampus after HIIT [22]. It suggests that HIIT regimens regulate neurotrophic factor concentrations in the affected and surrounding areas by avoiding an overexpression associated with

deleterious effects [61]. As we observed, the stroke-induced increase in mBDNF is accompanied by a reduction of KCC2 expression and a concomitant increase in NKCC1/KCC2 ratio [60]. However, after HIIT4, KCC2 expression is enhanced without NKCC1 alteration, thereby decreasing the NKCC1/KCC2 ratio that suggests a partial restoration of chloride homeostasis [62]. To complete these results, HIIT regimens promote the upregulation of COX4 and PGC-1 $\alpha$ , which might be neuroprotective [63,64].

In addition, the decrease in pro-apoptotic BAX expression and the concomitant increase in anti-apoptotic Bcl-2 expression induced by HIIT are demonstrated for the first time in the ipsilesional cortex. These 2 proteins are involved in the activation of caspase-3 signaling pathway, a key effector enzyme inducing apoptosis [65]. In our study, the decrease in BAX/Bcl-2 ratio might be associated with the observed decline in cortical caspase-3 suggesting an anti-apoptotic effect of HIIT [66].

Although events in the ipsilesional hemisphere remain of primary importance, other findings demonstrated the clinical relevance of studying contralesional events [7,67]. The upregulation of neurotrophic factors in the contralesional side is associated with improvements in functional recovery [68]. In our study, HIIT1 and HIIT4 seem to act differently on the BDNF/TrkB signaling pathways in the contralesional cortex because HIIT1 raises mBDNF concentrations while HIIT4 enhances pTrkB/TrkB ratio without influencing mBDNF concentrations. However, TrkB could also be phosphorylated by other molecules such as the serine/threonine kinase cyclin-dependent kinase 5 (Cdk5) [69], which is involved in neuroprotection after stroke [70]. It reveals that the relation between BDNF and TrkB is not necessary linear after training. An enhancement of BDNF/TrkB signaling pathways is in accordance with previous studies, in which an upregulation of pTrkB and FNDC5 was found in the contralesional cortex after HIIT4 [7] or after a moderate exercise (for TrkB) [68]. Our results suggest that HIIT regimens act more on BDNF and IGF-1 than on the vascular endothelial growth factor (VEGF) during the critical rehabilitation period [7]. It is not surprising because we previously showed in healthy rats that 8 weeks of HIIT are required to promote VEGF contrary to moderate intensity exercise (2 weeks are sufficient) [27]. Moreover, the increase in PGC-1 $\alpha$  expression in both cortices following HIIT4 might be related to the neurotrophin upregulation. Indeed, a previous study found a strong link between endurance exercise, PGC-1 $\alpha$  and FNDC5, with BDNF expression in the brain, even if a very different training protocol (a 30-day running wheel) was used [53]. As in the ipsilesional cortex, the increased COX4 expression mainly in HIIT1 reinforces its metabolic effect in the contralesional cortex. In addition, HIIT4 up-regulates KCC2 in parallel with the increase in the pTrkB/TrkB ratio, which might be involved in functional recovery. Interestingly, this KCC2 upregulation following HIIT4 seems to be concomitant in both cortices. Besides, this result is supported by the correlation between aerobic parameters and KCC2 upregulation. Similar results in rats with spinal cord injury indicated baseline levels of KCC2 expression after a 2-week training while KCC2 remained downregulated in untrained animals [71].

HIIT regimens modestly accelerate the recovery of tactile sensitivity, which may be relevant for long-term recovery. This result is in agreement with a previous study that postulated that endurance training reduces sensorimotor disorders by increasing neurotrophic factors [72]. Interestingly, the sensorimotor recovery remains incomplete at this period, suggesting that upregulation of neurotrophic markers is not sufficient. It was suggested elsewhere that endurance training might act as a powerful neurotrophic stimulus potentiating the effectiveness of other strategies such as sensorimotor or cognitive tasks [73,74]. Hence, other rehabilitation strategies should be combined with HIIT to potentiate their benefits.

Some methodological limitations should be mentioned. First, young rats were used for several reasons (less mortality rate, focus on cerebral ischemia effects, lower cost and to be closer with most preclinical studies on training) while cerebral ischemia in older rats, with or without comorbidities (hypertension, heart failure, systemic atherosclerosis,

metabolic disorders...) could be closer to human stroke conditions [75]. Comorbidities also influence patient recovery and their abilities to perform relative intense exercises. Then, we used only males as observed in most studies. Given that the training adaptations might be different between males and females, further studies should be focused on this question. In addition, there is no direct evidence on the interaction between ipsi- and contralesional cortices as it was previously demonstrated [76]. Unfortunately, although mechanisms related to the link between neurotrophic factors with chloride homeostasis and/or apoptosis could not be defined with transgenic rodents in this study, we have shown the relevance to study them after HIIT in rodent models of stroke. Such mechanisms are strongly required to confirm the role of HIIT and/or other rehabilitation strategies in an injured brain, but it remains a significant experimental challenge in training conditions.

## 5. Conclusion

This study shows for the first time that HIIT regimens act differently in both hemispheres on neurotrophic factors, chloride homeostasis and apoptosis markers. Both of them seem to be of clinical relevance during the critical period of stroke rehabilitation as observed with gains in endurance performance in a time-dependent manner. Such results are encouraging for optimizing exercise guidelines after stroke by pre-norming HIIT with short and/or long intervals in addition with other types of endurance sessions. These HIIT regimens might be complementary during the stroke rehabilitation. Also, this study could be a useful step in defining the most suitable biomarkers that are required for the patient follow-up. Further studies should now focus on the mechanisms explaining the link between neurotrophic factors and chloride homeostasis and/or apoptosis events.

## Author's contributions

NH performed research / analyzed data (behavior, muscle-brain parts). CPB performed research / analyzed data (behavior, exercise, and brain parts). TB designed and performed research (muscle part). CP designed molecular measurements. EB designed research. CR designed brain molecular measurements. JL designed and performed research (behavior, exercise, and muscle-brain parts). All authors contributed to the manuscript writing. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

## Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

## Declaration of Competing Interest

The authors declare that they have no competing interests. All the authors disclose professional relationships with companies or manufacturers who will benefit from the results of the present study.

## Data availability

Data will be made available on request.

## Acknowledgments

Public Aix-Marseille Université (AMU), STAR CARNOT INSTITUTE and the Eranet Neuron III program to CR through the Acrobat grant supported this work.

## References

- [1] S.A. Billinger, R. Arena, J. Bernhardt, J.J. Eng, B.A. Franklin, C.M. Johnson, et al., Physical activity and exercise recommendations for stroke survivors: a statement for healthcare professionals from the American Heart Association/American Stroke Association, *Stroke* 45 (2014) 2532–2553, <https://doi.org/10.1161/STR.0000000000000022>.
- [2] C. Pin-Barre, J. Laurin, Physical exercise as a diagnostic, rehabilitation, and preventive tool: influence on neuroplasticity and motor recovery after stroke, *Neural Plast.* 2015 (2015) 1–12, <https://doi.org/10.1155/2015/608581>.
- [3] M. Fisher, G. Feuerstein, D.W. Howells, P.D. Hurn, T.A. Kent, S.I. Savitz, et al., Update of the stroke therapy academic industry roundtable preclinical recommendations, *Stroke* 40 (2009) 2244–2250, <https://doi.org/10.1161/STROKEAHA.108.541128>.
- [4] F. Vahidy, W.-R. Schäbitz, M. Fisher, J. Aronowski, Reporting standards for preclinical studies of stroke therapy, *Stroke* 47 (2016) 2435–2438, <https://doi.org/10.1161/STROKEAHA.116.013643>.
- [5] P. Abreu, S.V.D. Mendes, J.H. Leal-Cardoso, V.M. Ceccatto, Anaerobic threshold employed on exercise training prescription and performance assessment for laboratory rodents: a short review, *Life Sci.* 151 (2016) 1–6, <https://doi.org/10.1016/j.lfs.2016.02.016>.
- [6] C. Pin-Barre, A. Constans, J. Brisswalter, C. Pellegrino, J. Laurin, Effects of high-versus moderate-intensity training on neuroplasticity and functional recovery after focal ischemia, *Stroke* 48 (2017) 2855–2864, <https://doi.org/10.1161/STROKEAHA.117.017962>.
- [7] C. Pin-Barre, N. Hugues, A. Constans, E. Berton, C. Pellegrino, J. Laurin, Effects of different high-intensity interval training regimens on endurance and neuroplasticity after cerebral ischemia, *Stroke* 52 (2021) 1109–1114, <https://doi.org/10.1161/STROKEAHA.120.031873>.
- [8] T. Gronwald, A.C. de Bem Alves, E. Murillo-Rodríguez, A. Latini, J. Schuette, H. Budde, Standardization of exercise intensity and consideration of a dose–response is essential. Commentary on “Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer’s models”, by Lourenco et al., published 2019 in nature medicine, *J. Sport Health Sci.* 8 (2019) 353–354, <https://doi.org/10.1016/j.jshs.2019.03.006>.
- [9] T.I. Gjellesvik, B. Brurok, J. Hoff, T. Tørhaug, J. Helgerud, Effect of high aerobic intensity interval treadmill walking in people with chronic stroke: a pilot study with one year follow-up, *Top. Stroke Rehabil.* 19 (2012) 353–360, <https://doi.org/10.1310/tsr1904-353>.
- [10] A.W. Dromerick, S. Geed, J. Barth, K. Brady, M.L. Giannetti, A. Mitchell, et al., Critical period after stroke study (CPASS): a phase II clinical trial testing an optimal time for motor recovery after stroke in humans, *Proc. Natl. Acad. Sci. U. S. A.* 118 (2021), e2026676118, <https://doi.org/10.1073/pnas.2026676118>.
- [11] Y.-R. Yang, R.-Y. Wang, P.S.-G. Wang, Early and late treadmill training after focal brain ischemia in rats, *Neurosci. Lett.* 339 (2003) 91–94.
- [12] J. Biernaskie, Efficacy of rehabilitative experience declines with time after focal ischemic brain injury, *J. Neurosci.* 24 (2004) 1245–1254, <https://doi.org/10.1523/JNEUROSCI.3834-03.2004>.
- [13] T.H. Murphy, D. Corbett, Plasticity during stroke recovery: from synapse to behaviour, *Nat. Rev. Neurosci.* 10 (2009) 861–872, <https://doi.org/10.1038/nrn2735>.
- [14] K. Ravina, D. Briggs, S. Kislal, Z. Warraich, T. Nguyen, R. Lam, et al., Intracerebral delivery of brain-derived neurotrophic factor using hyStem®-C hydrogel implants improves functional recovery and reduces neuroinflammation in a rat model of ischemic stroke, *LJMS* 19 (2018) 3782, <https://doi.org/10.3390/ljms19123782>.
- [15] W. Zhu, Y. Fan, T. Frenzel, M. Gasmí, R.T. Bartus, W.L. Young, et al., Insulin growth factor-1 gene transfer enhances neurovascular remodeling and improves long-term stroke outcome in mice, *Stroke* 39 (2008) 1254–1261, <https://doi.org/10.1161/STROKEAHA.107.500801>.
- [16] A. Serhan, E. Boddeke, R. Kooijman, Insulin-like growth factor-1 is neuroprotective in aged rats with ischemic stroke, *Front. Aging Neurosci.* 11 (2019) 349, <https://doi.org/10.3389/fnagi.2019.00349>.
- [17] A. Serhan, J.L. Aerts, E.W.G.M. Boddeke, R. Kooijman, Neuroprotection by insulin-like growth factor-1 in rats with ischemic stroke is associated with microglial changes and a reduction in neuroinflammation, *Neuroscience* 426 (2020) 101–114, <https://doi.org/10.1016/j.neuroscience.2019.11.035>.
- [18] P. Boyne, K. Dunning, D. Carl, M. Gerson, J. Khoury, B. Rockwell, et al., High-intensity interval training and moderate-intensity continuous training in ambulatory chronic stroke: a feasibility study, *Phys. Ther.* (2016), <https://doi.org/10.2522/ptj.20150277>.
- [19] P. Boyne, C. Meyrose, J. Westover, D. Whitesel, K. Hatter, D.S. Reisman, et al., Effects of exercise intensity on acute circulating molecular responses poststroke, *Neurorehabil. Neural Repair* 34 (2020) 222–234, <https://doi.org/10.1177/1545968319899915>.
- [20] D.L. Carl, P. Boyne, B. Rockwell, M. Gerson, J. Khoury, B. Kissela, et al., Preliminary safety analysis of high-intensity interval training (HIIT) in persons with chronic stroke, *Appl. Physiol. Nutr. Metab.* 42 (2017) 311–318, <https://doi.org/10.1139/apnm-2016-0369>.
- [21] J. Crozier, M. Roig, J.J. Eng, M. MacKay-Lyons, J. Fung, M. Ploughman, et al., High-intensity interval training after stroke: an opportunity to promote functional recovery, cardiovascular health, and neuroplasticity, *Neurorehabil. Neural Repair* 32 (2018) 543–556, <https://doi.org/10.1177/1545968318766663>.
- [22] L. Luo, C. Li, Y. Deng, Y. Wang, P. Meng, Q. Wang, High-intensity interval training on neuroplasticity, balance between brain-derived neurotrophic factor and precursor brain-derived neurotrophic factor in poststroke depression rats, *J. Stroke*

- Cerebrovascular Dis. (2018), <https://doi.org/10.1016/j.jstrokecerebrovasdis.2018.11.009>.
- [23] L. Luo, H. Meng, Z. Wang, S. Zhu, S. Yuan, Y. Wang, et al., Effect of high-intensity exercise on cardiorespiratory fitness in stroke survivors: a systematic review and meta-analysis, *Ann. Phys. Rehabil. Med.* 63 (2020) 59–68, <https://doi.org/10.1016/j.rehab.2019.07.006>.
- [24] A. Abou-Khzam, Is it time to start applying high-intensity interval training in stroke rehabilitation? *Eur. J. Phys. Rehabil. Med.* 55 (2019) <https://doi.org/10.23736/S1973-9087.18.05437-0>.
- [25] C.-C. Hsu, T.-C. Fu, S.-C. Huang, C.P.-C. Chen, J.-S. Wang, Increased serum brain-derived neurotrophic factor with high-intensity interval training in stroke patients: a randomized controlled trial, *Ann. Phys. Rehabil. Med.* (2020), <https://doi.org/10.1016/j.rehab.2020.03.010>. S1877065720300889.
- [26] N. Hugues, C. Pellegrino, C. Rivera, E. Berton, C. Pin-Barre, J. Laurin, Is high-intensity interval training suitable to promote neuroplasticity and cognitive functions after stroke? *IJMS* 22 (2021) 3003, <https://doi.org/10.3390/ijms22063003>.
- [27] N. Hugues, C. Pin-Barre, C. Pellegrino, C. Rivera, E. Berton, J. Laurin, Time-dependent cortical plasticity during moderate-intensity continuous training versus high-intensity interval training in rats, *Cereb. Cortex* (2022) bhab451, <https://doi.org/10.1093/cercor/bhab451>.
- [28] M. Buchheit, P.B. Laursen, High-intensity interval training, solutions to the programming puzzle: part I: cardiopulmonary emphasis, *Sports Med.* 43 (2013) 313–338, <https://doi.org/10.1007/s40279-013-0029-x>.
- [29] D. Warr-di Piero, T. Valverde-Estevé, J.C. Redondo-Castán, C. Pablos-Abella, J. V. Sánchez-Alarcos Díaz-Pintado, Effects of work-interval duration and sport specificity on blood lactate concentration, heart rate and perceptual responses during high intensity interval training. *Sandbakk Ø*, editor, *PLoS ONE* 13 (2018), e0200690, <https://doi.org/10.1371/journal.pone.0200690>.
- [30] D. Wen, T. Utesch, J. Wu, S. Robertson, J. Liu, G. Hu, et al., Effects of different protocols of high intensity interval training for VO2max improvements in adults: a meta-analysis of randomised controlled trials, *J. Sci. Med. Sport* 22 (2019) 941–947, <https://doi.org/10.1016/j.jsams.2019.01.013>.
- [31] L. El Hayek, M. Khalifeh, V. Zibara, R. Abi Assaad, N. Emmanuel, N. Karnib, et al., Lactate mediates the effects of exercise on learning and memory through SIRT1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF), *J. Neurosci.* (2019), <https://doi.org/10.1523/JNEUROSCI.1661-18.2019>, 1661–18.
- [32] E. Murawska-Ciałowicz, G.G. de Assis, F.M. Clemente, Y. Feito, P. Stastny, J. Zuwała-Jagiello, et al., Effect of four different forms of high intensity training on BDNF response to wingate and graded exercise test, *Sci. Rep.* 11 (2021) 8599, <https://doi.org/10.1038/s41598-021-88069-y>.
- [33] P. Boyne, K. Dunning, D. Carl, M. Gerson, J. Khoury, B. Kissela, Within-session responses to high-intensity interval training in chronic stroke, *Med. Sci. Sports Exercise* 47 (2015) 476–484, <https://doi.org/10.1249/MSS.0000000000000427>.
- [34] L.-M. Cao, Z.-Q. Dong, Q. Li, X. Chen, Treadmill training improves neurological deficits and suppresses neuronal apoptosis in cerebral ischemic stroke rats, *Neural Regen. Res.* 14 (2019) 1387, <https://doi.org/10.4103/1673-5374.253523>.
- [35] S.G. Kalinichenko, N.Y. Matveeva, A.V. Korobtsov, Brain-derived neurotrophic factor (BDNF) as a regulator of apoptosis under conditions of focal experimental stroke, *Bull. Exp. Biol. Med.* 169 (2020) 701–706, <https://doi.org/10.1007/s10517-020-04959-7>.
- [36] C. Rivera, H. Li, J. Thomas-Crusells, H. Lahtinen, T. Viitanen, A. Nanobashvili, et al., BDNF-induced TrkB activation down-regulates the K<sup>+</sup>-Cl<sup>-</sup> cotransporter KCC2 and impairs neuronal Cl<sup>-</sup> extrusion, *J. Cell Biol.* 159 (2002) 747–752, <https://doi.org/10.1083/jcb.200209011>.
- [37] L. Baroncelli, M.C. Cenni, R. Melani, G. Deidda, S. Landi, R. Narducci, et al., Early IGF-1 primes visual cortex maturation and accelerates developmental switch between NKCC1 and KCC2 chloride transporters in enriched animals, *Neuropharmacology* 113 (2017) 167–177, <https://doi.org/10.1016/j.neuropharm.2016.02.034>.
- [38] W. Xu, X. Mu, H. Wang, C. Song, W. Ma, J. Jolkkonen, et al., Chloride cotransporter NKCC1 inhibitor bumetanide enhances neurogenesis and behavioral recovery in rats after experimental stroke, *Mol. Neurobiol.* 54 (2017) 2406–2414, <https://doi.org/10.1007/s12035-016-9819-0>.
- [39] S. Khirug, S. Soni, M. Saez Garcia, M. Tessier, L. Zhou, N. Kuleshkaya, et al., Protective role of low ethanol administration following ischemic stroke via recovery of KCC2 and p75NTR expression, *Mol. Neurobiol.* (2020), <https://doi.org/10.1007/s12035-020-02176-x>.
- [40] M. Ploughman, S. Granter-Button, G. Chernenko, B.A. Tucker, K.M. Mearow, D. Corbett, Endurance exercise regimens induce differential effects on brain-derived neurotrophic factor, synapsin-I and insulin-like growth factor I after focal ischemia, *Neuroscience* 136 (2005) 991–1001, <https://doi.org/10.1016/j.neuroscience.2005.08.037>.
- [41] T. Hill, J.D. Polk, BDNF, endurance activity, and mechanisms underlying the evolution of hominin brains, *Am. J. Phys. Anthropol.* 168 (2019) 47–62, <https://doi.org/10.1002/ajpa.23762>.
- [42] C. Pin-Barre, C. Pellegrino, F. Laurin, J. Laurin, Cerebral ischemia changed the effect of metabosensitive muscle afferents on somatic reflex without affecting thalamic activity, *Front. Physiol.* 9 (2018), <https://doi.org/10.3389/fphys.2018.00638>.
- [43] D.J. Bentley, J. Newell, D. Bishop, Incremental exercise test design and analysis: implications for performance diagnostics in endurance athletes, *Sports Med.* 37 (2007) 575–586.
- [44] T. Vromen, J.J. Kraal, J. Kuiper, R.F. Spee, N. Peek, H.M. Kemps, The influence of training characteristics on the effect of aerobic exercise training in patients with chronic heart failure: a meta-regression analysis, *Int. J. Cardiol.* 208 (2016) 120–127, <https://doi.org/10.1016/j.ijcard.2016.01.207>.
- [45] S. Marzolini, P. Oh, W. McIlroy, D. Brooks, The feasibility of cardiopulmonary exercise testing for prescribing exercise to people after, *Stroke* 43 (2012) 1075–1081, <https://doi.org/10.1161/STROKEAHA.111.635128>.
- [46] P.B. Laursen, D.G. Jenkins, The scientific basis for high-intensity interval training: optimising training programmes and maximising performance in highly trained endurance athletes, *Sports Med.* 32 (2002) 53–73.
- [47] R.B. Armstrong, C.R. Taylor, Glycogen loss in rat muscles during locomotion on different inclines, *J. Exp. Biol.* 176 (1993) 135–144, <https://doi.org/10.1242/jeb.176.1.135>.
- [48] C. Pin-Barre, J. Laurin, M.-S. Felix, V. Pertierra, F. Kober, T. Marqueste, et al., Acute neuromuscular adaptation at the spinal level following middle cerebral artery occlusion-reperfusion in the rat, *PLoS ONE* 9 (2014) e89953, <https://doi.org/10.1371/journal.pone.0089953>.
- [49] Paxinos G., Watson C. *The Rat Brain in Stereotaxic Coordinates*. Elsevier/Academic, 2009.
- [50] V. Pertierra, C. Pin-Barre, M.-S. Felix, J. Laurin, J. Brisswalter, P. Decherchi, A new method to assess weight-bearing distribution after central nervous system lesions in rats, *Behav. Brain Res.* 259 (2014) 78–84, <https://doi.org/10.1016/j.bbr.2013.10.043>.
- [51] M.A. Hoydal, U. Wisløff, O.J. Kemi, Ø. Ellingsen, Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training, *Eur. J. Cardiovascular Prev. Rehabil.* 14 (2007) 753–760, <https://doi.org/10.1097/HJR.0b013e3281eacef1>.
- [52] R.F. Macko, F.M. Ivey, L.W. Forrester, D. Hanley, J.D. Sorokin, L.I. Katzel, et al., Treadmill exercise rehabilitation improves ambulatory function and cardiovascular fitness in patients with chronic stroke: a randomized, controlled trial, *Stroke* 36 (2005) 2206–2211, <https://doi.org/10.1161/01.STR.0000181076.91805.89>.
- [53] C.D. Wrann, J.P. White, J. Salogiannis, D. Laznik-Bogoslavski, J. Wu, D. Ma, et al., Exercise Induces Hippocampal BDNF through a PGC-1 $\alpha$ /FNDC5 Pathway, *Cell Metab.* 18 (2013) 649–659, <https://doi.org/10.1016/j.cmet.2013.09.008>.
- [54] P. Boström, J. Wu, M.P. Jedrychowski, A. Korde, L. Ye, J.C. Lo, et al., A PGC1- $\alpha$ -dependent myokine that drives brown-fat-like development of white fat and thermogenesis, *Nature* 481 (2012) 463–468, <https://doi.org/10.1038/nature10777>.
- [55] C.W. Cotman, N.C. Berchtold, L.A. Christie, Exercise builds brain health: key roles of growth factor cascades and inflammation, *Trends Neurosci.* 30 (2007) 464–472, <https://doi.org/10.1016/j.tins.2007.06.011>.
- [56] P.Z. Liu, R. Nusslock, Exercise-mediated neurogenesis in the hippocampus via BDNF, *Front. Neurosci.* 12 (2018), <https://doi.org/10.3389/fnins.2018.00052>.
- [57] C. Cunha, A. Angelucci, A. D'Antoni, M.D. Dobrossy, S.B. Dunnett, N. Berardi, et al., Brain-derived neurotrophic factor (BDNF) overexpression in the forebrain results in learning and memory impairments, *Neurobiol. Dis.* 33 (2009) 358–368, <https://doi.org/10.1016/j.nbd.2008.11.004>.
- [58] V.S. Boyce, J. Park, F.H. Gage, L.M. Mendell, Differential effects of brain-derived neurotrophic factor and neurotrophin-3 on hindlimb function in paraplegic rats, *Eur. J. Neurosci.* 35 (2012) 221–232, <https://doi.org/10.1111/j.1460-9568.2011.07950.x>.
- [59] C.D. De Magalhães Filho, L. Kappeler, J. Dupont, J. Solinc, S. Villapol, C. Denis, et al., Deleting IGF-1 receptor from forebrain neurons confers neuroprotection during stroke and upregulates endocrine somatotropin, *J. Cereb. Blood Flow Metab.* 37 (2017) 396–412, <https://doi.org/10.1177/0271678X15626718>.
- [60] S. Lee-Hotta, Y. Uchiyama, S. Kametaka, Role of the BDNF-TrkB pathway in KCC2 regulation and rehabilitation following neuronal injury: a mini review, *Neurochem. Int.* 128 (2019) 32–38, <https://doi.org/10.1016/j.neuint.2019.04.003>.
- [61] E. Ziemlińska, S. Kügler, M. Schachner, I. Wewiór, J. Czarkowska-Bauch, M. Skup, Overexpression of BDNF increases excitability of the lumbar spinal network and leads to robust early locomotor recovery in completely spinalized rats, Hochman S, editor, *PLoS ONE* 9 (2014) e88833, <https://doi.org/10.1371/journal.pone.0088833>.
- [62] X.P. Mu, H.B. Wang, X. Cheng, L. Yang, X.Y. Sun, H.L. Qu, et al., Inhibition of Nkcc1 promotes axonal growth and motor recovery in ischemic rats, *Neuroscience* 365 (2017) 83–93, <https://doi.org/10.1016/j.neuroscience.2017.09.036>.
- [63] J. Li, S.E. Benashski, V.R. Venna, L.D. McCullough, Effects of meformin in experimental stroke, *Stroke* 41 (2010) 2645–2652, <https://doi.org/10.1161/STROKEAHA.110.589697>.
- [64] B. Han, W. Jiang, H. Liu, J. Wang, K. Zheng, P. Cui, et al., Upregulation of neuronal PGC-1 $\alpha$  ameliorates cognitive impairment induced by chronic cerebral hypoperfusion, *Theranostics* 10 (2020) 2832–2848, <https://doi.org/10.7150/thno.37119>.
- [65] M. D'Amelio, V. Cavallucci, F. Cecconi, Neuronal caspase-3 signaling: not only cell death, *Cell Death Differ.* 17 (2010) 1104–1114, <https://doi.org/10.1038/cdd.2009.180>.
- [66] T. Okazaki, T. Magaki, M. Takeda, Y. Kajiwara, R. Hanaya, K. Sugiyama, et al., Intravenous administration of bone marrow stromal cells increases survivin and Bcl-2 protein expression and improves sensorimotor function following ischemia in rats, *Neurosci. Lett.* 430 (2008) 109–114, <https://doi.org/10.1016/j.neulet.2007.10.046>.
- [67] T.A. Jones, D.L. Adkins, Motor system reorganization after stroke: stimulating and training toward perfection, *Physiology* 30 (2015) 358–370, <https://doi.org/10.1152/physiol.00014.2015>.
- [68] M.-W. Kim, M.-S. Bang, T.-R. Han, Y.-J. Ko, B.-W. Yoon, J.-H. Kim, et al., Exercise increased BDNF and trkB in the contralateral hemisphere of the ischemic

- rat brain, *Brain Res.* 1052 (2005) 16–21, <https://doi.org/10.1016/j.brainres.2005.05.070>.
- [69] K.-O. Lai, A.S.L. Wong, M.-C. Cheung, P. Xu, Z. Liang, K.-C. Lok, et al., TrkB phosphorylation by Cdk5 is required for activity-dependent structural plasticity and spatial memory, *Nat. Neurosci.* 15 (2012) 1506–1515, <https://doi.org/10.1038/nn.3237>.
- [70] J.A. Gutiérrez-Vargas, H. Moreno, G.P. Cardona-Gómez, Targeting CDK5 post-stroke provides long-term neuroprotection and rescues synaptic plasticity, *J. Cereb. Blood Flow Metab.* 37 (2017) 2208–2223, <https://doi.org/10.1177/0271678X16662476>.
- [71] M.-P. Cote, S. Gandhi, M. Zambrotta, J.D. Houle, Exercise modulates chloride homeostasis after spinal cord injury, *J. Neurosci.* 34 (2014) 8976–8987, <https://doi.org/10.1523/JNEUROSCI.0678-14.2014>.
- [72] C.P. Mackay, S.G. Brauer, S.S. Kuys, M.A. Schaumberg, L.A. Leow, The acute effects of aerobic exercise on sensorimotor adaptation in chronic stroke, *RNN* 39 (2021) 367–377, <https://doi.org/10.3233/RNN-211175>.
- [73] Kobilio T., Liu Q.-R., Gandhi K., Mughal M., Shaham Y., van Praag H. Running is the neurogenic and neurotrophic stimulus in environmental enrichment. *Learn. Memory* 2011;18:605–9. <https://doi.org/wrann>.
- [74] D.M. Curlik, T.J. Shors, Training your brain: do mental and physical (MAP) training enhance cognition through the process of neurogenesis in the hippocampus? *Neuropharmacology* 64 (2013) 506–514, <https://doi.org/10.1016/j.neuropharm.2012.07.027>.
- [75] A.-M. Buga, M. Di Napoli, A. Popa-Wagner, Preclinical models of stroke in aged animals with or without comorbidities: role of neuroinflammation, *Biogerontology* 14 (2013) 651–662, <https://doi.org/10.1007/s10522-013-9465-0>.
- [76] A.L. Allegra Mascaro, E. Conti, S. Lai, A.P. Di Giovanna, C. Spalletti, C. Alia, et al., Combined rehabilitation promotes the recovery of structural and functional features of healthy neuronal networks after stroke, *Cell Rep.* 28 (2019) 3474–3485, <https://doi.org/10.1016/j.celrep.2019.08.062>, e6.