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Cerebral Cortex

Time-dependent cortical plasticity during moderate-intensity continuous training *versus* high-intensity interval training in rats

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Running title: Time course of cortical plasticity after training

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Abstract

The temporal pattern of cortical plasticity induced by high-intensity interval training (HIIT) and moderate-intensity continuous training (MICT) is required to clarify their relative benefits to prevent neurological disorders. The purpose of this study is to define the time-dependent effects of work-matched HIIT and MICT on cortical plasticity, endurance and sensorimotor performances over an 8-week training period in healthy rats. Adult healthy rats performed incremental exercise tests and sensorimotor tests before and at 2, 4 and 8 weeks of training. In parallel, cortical markers related to neurotrophic, angiogenic and metabolic activity were assessed. Results indicate that HIIT induced an early and superior endurance improvement compared to MICT. We found significant enhancement of speed associated with lactate threshold (S_{LT}) and maximal speed (S_{max}) in HIIT animals. MICT promoted an early increase in brain-derived neurotrophic factor (BDNF) and angiogenic/metabolic markers but showed less influence at 8 weeks. HIIT upregulated the insulin-like growth factor-1 (IGF-1) as well as neurotrophic, metabolic/angiogenic markers at 2 and 8 weeks and downregulated the neuronal K-Cl cotransporter KCC2 that regulates $GABA_A$ mediated transmission. HIIT and MICT are effective in a time-dependent manner suggesting a complementary effect that might be useful in physical exercise guidelines for maintain brain health.

Key words: BDNF; FNDC5; IGF-1; KCC2; VEGF.

1. Introduction

In addition to an important role in improving cardiovascular and muscular functions, chronic endurance exercise is now recognized to prevent neurological pathologies by promoting cerebral plasticity in the cortex and hippocampus, two of the brain areas highly sensitive to endurance training (Cabral et al. 2019; Hill and Polk 2019). However, exercise-induced plasticity has been less studied in the cortex than in the hippocampus, although cortical plasticity is strongly involved in sensorimotor tasks and cognitive functions.

Endurance training can be carried out by the two main types of exercise regimens: the moderate-intensity continuous training (MICT) and the high-intensity interval training (HIIT). MICT is defined by an exercise intensity ~50–70% of the maximal heart rate (HR_{max}) or the velocity at VO_2max (vVO_{2max}) or the maximal speed (S_{max}). This corresponds to an exercise intensity below the speed associated with lactate threshold (S_{LT}). HIIT is a safe and time-efficient alternative to MICT characterized by repeated bouts of intense exercise (above S_{LT}, ~85–95% of HR_{max} or vVO_{2max} / S_{max}) separated by active or passive recovery periods (Buchheit and Laursen 2013). These training regimens both improve aerobic capacities in healthy rats with higher effects of HIIT on skeletal muscle and liver glycogen content and anabolic processes (de Araujo et al. 2013, 2015, 2016). Also, HIIT induced a higher enhancement of both contractile and relaxation myocardial parameters and a greater cardiac hypertrophy than MICT (Jakovljevic et al. 2019; Verboven et al. 2019).

It was mainly demonstrated in the hippocampus that these two training regimens can stimulate neurogenesis, neuronal survival and synaptic plasticity (Voss et al. 2013; Hugues et al. 2021). To promote such plasticity processes, MICT and HIIT might specifically trigger the upregulation of several neurotrophic and angiogenic factors in brain, such as brain-derived neurotrophic factor (BDNF), insulin-like growth factor 1 (IGF-1) and vascular-endothelial growth factor (VEGF) (Voss et al. 2013). However, the relative impact of each exercise

regimen on the expression of neurotrophic factors remains controversial. Indeed, few studies in rodents observed a higher effectiveness following MICT (Nokia et al. 2016; So et al. 2017) while others found greater effects with HIIT (Afzalpour et al. 2015; Constans et al. 2021).

Importantly, the actions of BDNF are strongly driven by its binding to high-affinity Tropomyosin receptor kinase B (TrkB) and/or pan-neurotrophin receptor 75 ($p75^{NTR}$) (De Vincenti et al. 2019; Zanin et al. 2019). Likewise, IGF-binding proteins-2 (IGFBP-2) interacts with IGF-1 to stimulate or inhibit IGF actions in adult brain (Lewitt and Boyd 2019). In addition to VEGF, the neuronal Apelin receptor plays an important role in the regulation of angiogenesis (Huang et al. 2016). Surprisingly, no study has investigated the time course of specific receptors of key neurotrophic factors over MICT and HIIT protocols.

In addition, the above neurotrophic factors could be at the upstream or downstream of several brain signaling pathways by interfering with metabolic markers and/or exercise-induced myokines (Wrann et al. 2013), but such interactions remain unknown when comparing MICT with HIIT. It has been elsewhere reported that BDNF can also interact with the potassium–chloride cotransporter 2 (KCC2, a neuronal chloride extruder) in the brain, known to be involved in the excitation/inhibition interplay by regulating the GABAergic activity (Rivera et al. 2002; Pozzi et al. 2020). For instance, it was shown that the downregulation of KCC2 leads to an increase of neuronal activity that stimulates BDNF secretion (Rivera et al. 2002). Even though endurance training might influence KCC2 expression (Pin-Barre et al. 2017; Takahashi et al. 2017; Coxon et al. 2018; Pozzi et al. 2020), the effects of HIIT and MICT on KCC2 is completely unknown.

Beyond the lack of studies in this research area, the many controversial findings might be partially explained by the high heterogeneity of training protocols (Constans et al. 2021; Hugues et al. 2021). One of the major limitations found in most studies is related to the assessment of neuroplasticity at only one-time point of the training protocol, potentially

leading to miss out on relevant changes. Very few studies revealed a specific time-dependent effect of endurance training programs on the upregulation of neurotrophic factors and other cerebral plasticity processes (Kim et al. 2003; Berchtold et al. 2005). Indeed, late increases in hippocampal BDNF concentrations were reported after voluntary running-wheel in healthy rats without changes at earlier stages (Berchtold et al. 2005). However, the related time-dependent effects of exercise regimens on sensorimotor and endurance performances remain unclear (Berchtold et al. 2010; Takimoto and Hamada 2014; Cabral et al. 2019; Stevenson et al. 2020). The relationship between neurotrophic factors and functional outcomes was mainly established after motor skill training in which an increase in neurotrophic factor expression preceded cortical map reorganization and motor skill improvements (Adkins et al. 2006). Interestingly, the BDNF gene deficiency limited the cortical map reorganization after motor training associated with poor recovery in individuals with stroke (Stampanoni Bassi et al. 2018). In addition, gains in endurance performance were positively correlated with hippocampal plasticity markers in healthy rats after 8 weeks of HIIT while no gain in both performance and cerebral plasticity was observed with MICT at this time point (Constans et al. 2021). In this previous study, both HIIT and MICT induced early endurance improvements from 2 weeks, but no neuroplasticity markers have been measured at such early time points. Therefore, a more detailed investigation of the temporal pattern of expression in both HIIT and MICT could be timely and highly relevant in cerebral areas involved in many behavioral functions, such as the cortex.

Based on these findings, the purpose of this study was to define the respective time-depend effects of work-matched HIIT and MICT on cortical plasticity markers as well as endurance and sensorimotor performances over an 8-week training period in healthy rats. This study is thus intended to help define the exercise guidelines to maintain/improve brain plasticity and associated behavioral outcomes, which are nowadays inexistent. Also, the

translational relevance of rodent studies to humans is enhanced by using common physiological indicators of endurance performance, the S_{LT} and S_{max}. These physiological parameters also allow to individually determine exercise intensity and standardize MICT with HIIT sessions (Pin-Barre et al. 2017, 2021; Gronwald et al. 2019; Andreato et al. 2021; Constans et al. 2021). We assume that the cortical expression of neurotrophic/angiogenic factors might be specific to each endurance regimen in a time-dependent manner, thereby revealing their complementary to both stimulate cortical plasticity and improve functional outcomes.

2. Materials and Methods

2.1 Ethics approval statement

All experimental protocols were approved by the Animal Care Committees of Marseille n°14 (approval number: APAFiS #11558). The 3Rs principle was applied: i) Replacement – No substitution *in vitro* model exists to sufficiently mimic endurance training effects on brain functions and endurance performance at the same time; ii) Reduction – The same animals performed training, incremental and behavioral tests to reduce the number of animals. Moreover, they were used for molecular measurements; iii) Refinement – Experiments were either non-invasive or without awakening. Rats were housed by 3-4 per cage for preserving social interaction and were familiarized with experimenters during the week preceding experiments. Food and water were given *ad libitum* and rats maintained at 22°C with a 12-h light/dark cycle. Enriched environment was provided for all animals while avoiding a cognitive training.

Signs of animal suffering and welfare were monitored. Animals were excluded when one of the following points was observed: 1) animals unable to run on treadmill during the familiarization or the training protocol, 2) clinical signs of pain and discomfort: prostrated animals with absence of movement in the cage and/or hunched posture, ungroomed

appearance, weight loss higher than 20%, dehydration, decreased urine/fecal output, piloerection, chronic porphyrin staining around eyes, nose or forelimbs and rapid respirations, and 3) absence of social interaction in the cage and decrease of food and/or water intake.

2.2 Animals

Fifty-nine adult male Sprague-Dawley rats, sexually mature, (training protocol started from P60-P80; JANVIER®, France) were randomly assigned to one of the following groups:

1) Control (n=11), in which rats did not perform training program. These control rats were placed on the treadmill twice a week for 5 min to maintain effects of treadmill familiarization, thereby limiting stress during incremental exercise tests. Also, this Control group included 3 additional control rats used to assess the influence of repetitive blood samples at the tail level on maximal performance during incremental exercise test (Høydal et al. 2007). Indeed, these authors postulated that repetitive lactate sampling might decrease the oxygen transport, and thus, both maximal oxygen uptake (VO_{2max}) and endurance performance. This methodological point refers to the additional experimental 1 (effect of repetitive blood samples during an incremental exercise test on endurance performance).

2) MICT (n=24), in which rats carried out 2 weeks (MICT-2W, n=8), 4 weeks (MICT-4W, n=8) or 8 weeks of MICT program (MICT-8W, n=8) and the cerebral cortex was removed (30-40h after the last incremental test) at 2, 4 or 8 weeks of training for MICT-2W, MICT-4W and MICT-8W groups, respectively.

3) HIIT (n=24), in which rats carried out 2 weeks (HIIT-2W, n=8), 4 weeks (HIIT-4W, n=8) or 8 weeks of HIIT program (HIIT-8W, n=8). Cerebral cortex was also removed at 2, 4 or 8 weeks of training for HIIT-2W, HIIT-4W and HIIT-8W groups, respectively.

For the Control, MICT-8W and HIIT-8W groups, animals were subjected to incremental exercise tests on treadmill and sensorimotor tests at 4 time points: PRE (1 day

before training onset) and 2, 4 and 8 weeks **(Fig. 1)**. For MICT-2W and HIIT-2W, animals performed incremental exercise tests at PRE and 2 weeks of training before the cortex was sampled while incremental exercise tests were achieved at PRE, 2 and 4 weeks of training for MICT-4W and HIIT-4W following by cortex sampling. MICT-2W, HIIT-2W, MICT-4W and HIIT-4W did not perform sensorimotor tests. For all trained groups, only the last incremental exercise test was used to assess endurance performance (by taking into account the PRE test) with the same number of animals per time point (n=8). The intermediate tests were used to readjust running intensity. The body weight of rats at 2, 4 and 8 weeks was indicated in the **Supplementary Figure 1**.

All acquisitions and analyses were performed in a single-blind manner. To ensure that HIIT and MICT sessions induced different physiological response, blood lactate concentration ($mmol.L^{-1}$) was also measured after the third HIIT and MICT sessions (n=8 per group). It refers to the Additional experiment 2. We used adult males only as in accordance with many studies (Takimoto and Hamada 2014; Afzalpour et al. 2015; Nokia et al. 2016; Stevenson et al. 2020; Constans et al. 2021) because this aim was to avoid ovarian cycle influence on both performance and expression of neuroplasticity markers.

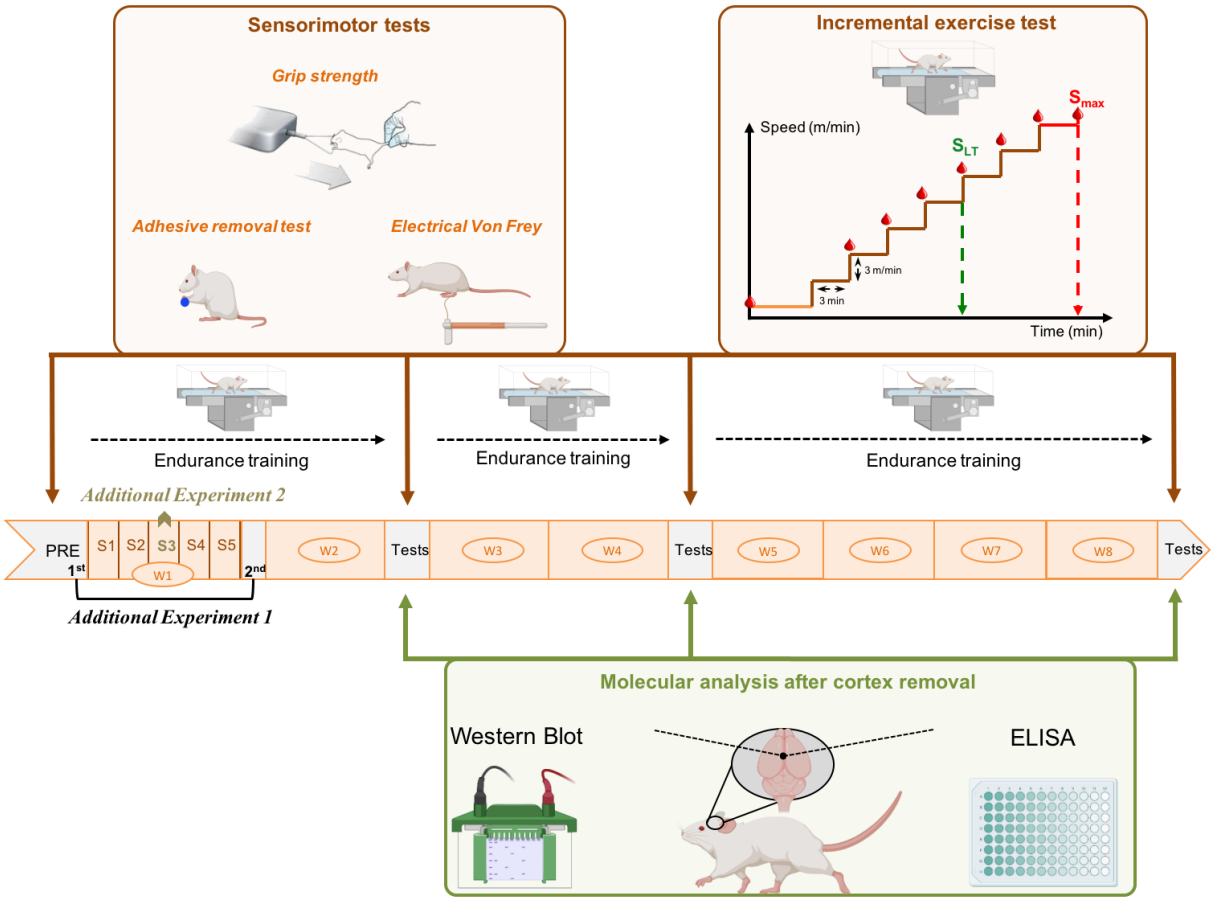

Fig. 1: Experimental protocol during 8 weeks of endurance training. W = week, D = day. Endurance training refers either to MICT or HIIT. Sensorimotor and incremental exercise tests were performed at PRE, 2W, 4W and 8W of training. Sensorimotor tests, including adhesive removal test, Electronic Von Frey and forelimb grip strength, always preceded incremental exercise tests on treadmill to avoid fatigue accumulation and stress. Moreover, these sensorimotor tests were randomly conducted with a 10-min rest interval. Running speed associated with lactate threshold (S_{LT}) and maximal running speed (speed to exhaustion or S_{max}) were obtained during incremental exercise tests and expressed in $m.min^{-1}$. At 2W, 4W and 8W, the cerebral cortex was removed for Western blot and ELISA analyses. The additional experimental 1 refers to the effect of repetitive blood samples during an incremental exercise test on endurance performance in 3 additional control animals. Additional experiment 2 refers to the measurement of blood lactate concentrations before

(baseline) and 2 min after the third session of rats from HIIT-2W and MICT-2W groups (n=8 per group).

2.3 Habituation to treadmill and behavioral arena

All rats were familiarized before the experiment with running on a treadmill (Decathlon® with the use of a power supply (the ELC – AL924A, Farnell France® for determining suitable running speed in rats) to limit stress of rats during training (5 min per day for 5 days, at low speeds, 10-15 $m.min^{-1}$). Stress was also reduced during behavioral tests by familiarizing each rat with the arena before starting tests (5 days, 5 min/day in the arena). Moreover, the same experimenter carried out behavioral tests in a quiet room throughout the protocol. Sensorimotor tests were randomly conducted (with a 10-min rest period between each test) before incremental exercise test to avoid fatigue effects on results.

2.4 Sensorimotor tests

2.4.1 Forelimb grip force. The grip force exerted by both forelimbs was quantified by using a grip force tester (Grip Strength Tester bio-GT3, Bioseb®, Vitrolles, France) (Pin-Barre et al. 2014, 2017). To summarize, when rat forelimbs grasped the middle of bar, the experimenter pulled it following the axis of sensor until grasp was released. The time interval between each trial was fixed to 1 min. Five to ten trials were performed per session. The top 2 maximal forces (in grams) were averaged and normalized by animal weight (grip strength ratio).

2.4.2 Adhesive removal test (ART). This test was used to independently assess tactile sensitivity and motor coordination of the right forepaw (Pin-Barre et al. 2018). Before each testing session, the rat was placed into a box (67x37x30) during 90 seconds for a new

familiarization period. Thereafter, a round adhesive tape (Ø 8 mm) was applied two times under the right forepaw separated by a 2-min rest. The time to detect and to remove adhesive tape for the right forepaw was measured in second (s) and averaged between the 2 trials per session. The maximal recording time was fixed to 120 s.

2.4.3 **Electronic Von Frey test.** Mechanical sensitivity was assessed with the Electronic Von Frey instrument (Bio-EVF, Bioseb®, Vitrolles, France) (Pin-Barre et al. 2018). The rat was acclimated 5 min before test with plastic contention box (26x20x14 cm) placed on an elevated mesh-bottomed platform to facilitate the surface access of forepaws. The threshold of forepaw withdrawal was obtained by applying the plastic tip perpendicular to the middle of the plantar surface of the forepaw at constant progressive pressure until withdrawal. The pressure value (in grams) was recorded. Four trials for both left and right forepaws were assessed and averaged. Each trial was followed by 15 s of resting period. The order of forepaw was randomized between trials.

2.5 Incremental exercise test on treadmill: S_{LT} and S_{max}

All incremental tests (and training sessions) were performed on 1° inclined treadmill (Pin-Barre et al. 2017; Constans et al. 2021). After a 5-min warm-up at 9 $m.min^{-1}$, the speed increment was 3 $m.min^{-1}$ every 3 min with a starting running speed set at 12 $m.min^{-1}$. A blood sample was collected from the tail vein during a 15-20 s rest period at the end of each speed level to measure lactate concentration ($mmol.L^{-1}$) with a portable device (Lactate Scout+, EKF diagnostics®, France). The lactate threshold was determined when 1) the inflection point of blood lactate concentration was observed and/or 2) an increase of 1 $mmol.L^{-1}$ between two blood lactate values was measured (Pin-Barre et al. 2017; Constans et al. 2021). The speed corresponding to lactate threshold was S_{LT} (in $m.min^{-1}$). It refers to the highest possible

equilibrium between lactate production and uptake. The test was stopped when the rat could not maintain the imposed running speed. The last completed speed level was considered as the S_{max} ($m.min^{-1}$). They are considered as powerful aerobic determinants of performance. Therefore, to avoid any influence of the inter-individual PRE value variation on progression in endurance performance, we compared the running speed difference (at S_{LT} or at S_{max}) between PRE and each time point for each group (post training at 2, 4 or 8 weeks).

Additional experiment 1 – Effect of repetitive blood samples during an incremental exercise test on endurance performance. Three control rats performed 2 incremental exercise tests separated by one week in a randomized order: one with blood sample at the end of each running level and the other without it. The S_{max} ($m.min^{-1}$) was compared between these 2 incremental exercise tests to assess if repetitive blood samples might reduce endurance performance.

2.6 Work-matched HIIT and MICT programs on treadmill

Experimenters individually trained each rat (5 days/week). Both training protocols started with a 5-min warm-up at speed 30% below S_{LT}. According to previous authors, training for rodents should be individualized and transferable to humans by using a physiological parameter (S_{LT} and S_{max} in our study) (Gronwald et al. 2019; Andreato et al. 2021). Moreover, intensity training should progressively increase over the training period from regular performance assessment (Høydal et al. 2007). Hence, concerning the first 2-running weeks, running speed was determined according to S_{LT} and S_{max} measured during the incremental exercise test at PRE. Then, running speeds were adjusted from S_{LT} and S_{max} obtained at 2 weeks for the following weeks for the MICT-4W, HIIT-4W, MICT-8W and HIIT-8W groups (**see Supplementary Table 1**).

2.6.1 HIIT. Training sessions were composed of 4x4 min of high intensity exercise (running speed fixed at 80-100% of the variation between S_{LT} and S_{max}) interrupted by a 3-min active recovery (30% below S_{LT}). Thus, one session lasted 28 min (exercise + active recovery). It ensured that running speed remained above S_{LT} and below S_{max}. Indeed, HIIT intensity need to be higher than lactate threshold to be considered as a HIIT regimen (Buchheit and Laursen 2013). Running speed progressively increased over the 8 weeks.

2.6.2 MICT. Running speed was fixed at 20% below S_{LT} to avoid fatigue accumulation. The total workload was the same between groups to match energy expenditure between exercise regimens. Therefore, session duration (sustained exercise without recovery period) depended on HIIT workload (exercise series and active recovery series) to match the total amount energy expenditure (isocaloric sessions) between groups from the following formula:

Mass (kg) x Intensity ($m.min^{-1}$) x Duration (min) x Treadmill inclination (°) x 9.8 (J/kg.m) (Pederson et al. 2005).

Additional experiment 2 - Blood lactate concentration before and after a single training session. To ensure that the different intensities related to HIIT and MICT sessions induced different physiological effects during sessions, we also measured blood lactate concentration ($mmol.L^{-1}$) from the tail vein before (baseline) and 2 min after the third session of HIIT-2W and MICT-2W groups (n=8 per group). Blood lactate was measured 2 min after the end of session because peak blood lactate concentration was frequently observed during this acute period of post exercise (Parker et al. 2017). An HIIT session should induce a higher blood lactate concentration (higher lactate production than uptake) in contrast with MICT session during which blood lactate concentration should be lower (higher lactate uptake than

production) as demonstrated in humans (Costill 1970; Spengler et al. 1999). We did not reproduce this measurement throughout the training protocol to avoid both additional rat stress and repetitive hemisections of tail. The third session was chosen because rats were well familiarized with these specific training sessions.

2.6 Cortex analyses at the molecular level

To assess the influence of training regimens on neurotrophin and associated markers, rats were anaesthetized with intraperitoneal ketamine/xylazine injection (120mg/kg-20mg/kg) 30-40h after their last incremental exercise test at 3 time points (2, 4 and 8 weeks for MICT-2W/HIIT-2W, MICT-4W/HIIT-4W and MICT-8W/HIIT-8W groups, respectively). After decapitation, the right entire cortex was removed from other brain tissues and was collected. Tissues were flash-frozen in liquid nitrogen and stored at -80°C until analyses. Then, the cortex tissues were homogenized in RIPA buffer (150 mM NaCl, 1% Triton X100, 0.1% SDS, 50 mM Tris HCl) containing protease inhibitors (Complete Mini; Roche). Lysates were centrifuged (10.000 g for 30 min at 4 °C) to keep the supernatant for ELISA and Western blotting.

2.8.1 mature BDNF (mBDNF) and IGF-1 Immunoassay

Quantification of mBDNF in the provided Quality Control, mBDNF standard and cortical samples was performed with mBDNF Rapid ELISA Kit (Biosensis®, BEK-2211-1P/2P – sandwich ELISA - Thebarton, SA, Australia) in the concentrated solutions following the manufacturer's protocol. As recommended, all samples (i.e., standards, controls, blanks and test animals) were performed in duplicate. Quality Control sample ranged between 175-325 $pg.ml^{-1}$ and the mBDNF standard ranged between 7.8-500 $pg.ml^{-1}$.

Quantification of IGF-1 standard and cortical samples was performed with IGF-1 ELISA Kit (Biosensis®, BEK-2150-2P – sandwich ELISA - Thebarton, SA, Australia) in the concentrated solutions following the manufacturer's protocol. All samples (i.e., standards, controls and test animals) were also performed in duplicate. The IGF-1 standard ranged between 62.5-4000 $pg.ml^{-1}$.

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 the mBDNF and IGF-1 protein concentrations of the sample through a 4-PL regression analysis. Concentrations were determined using the FLUOstar® OPTIMA microplate reader (BMG Labtech, France).

2.8.2 Western blot analysis in the cortex

Equal amount of proteins (40 µg) was separated in Bolt Bis-Tris Plus gel (4-12%; Invitrogen gel, ThermoFischer®) and transferred to nitrocellulose membrane (7 min). Membranes were blocked in Tris-buffered saline with Tween 20 (TBS-T) containing 5% of bovine serum albumin (BSA) for 1h. After washing with TBST (3x5 min), membranes were exposed overnight to a 4°C temperature with the primary antibodies diluted in blocking solution (TBS-T/2.5% BSA) indicated in **Supplementary Table 2**. After washing with TBST (3x5 min), membranes were incubated with HorseRadishPeroxidase-conjugated secondary antibodies diluted in TBST for 2h at room temperature before chemiluminescence detection. Several samples from each of the 7 groups are present on each nitrocellulose membrane to allow comparison between groups (x4 membranes per protein). Signal intensities were measured with the image analysis software G box (Syngene®) and normalized to either α-tubulin or GAPDH protein levels depending on the molecular weight of each protein of

interest. The pTrkB/TrkB ratio was also calculated. Quantification was performed using Gel Plot Analysis plugin (ImageJ®). Intensity values were expressed relative to Control group.

2.9 Statistical analysis

Results of sensorimotor tests were compared by means of two-way ANOVA (training regimens x time) from the HIIT-8W, MICT-8W and Control. For the incremental exercise test, we separately compared each time point by means of one-way ANOVA (training regimens) to have the same rat number per group (*i.e.,* HIIT-2W, MICT-2W and Control, then HIIT-4W, MICT-4W and Control, and finally, HIIT-8W, MICT-8W and Control). We also compared by means of one-way ANOVA the results between the different time points within each training regimen (HIIT-2W, HIIT-4W and HIIT-8W as well as MICT-2W, MICT-4W and MICT-8W).

Results of molecular measurements were compared by means of one-way ANOVA (training regimens) at each time point. Post-hoc comparisons were performed with Tukey multiple post-test comparisons. Paired t-test (two-tailed) was used to compare the blood lactate concentration before and after the HIIT and MICT sessions. Wilcoxon test was used to compare the S_{max} between the 2 incremental exercise tests of the 3 additional Control (additional experiment 1). Unpaired t-test (two-tailed) was used to compare the blood lactate concentration after the session between HIIT and MICT (additional experiment 2). Results were considered significant when $p<0.05$. All data were expressed in mean ± SD.

Pearson's correlation coefficient with two-tailed significance was used to analyze the relationship between relative levels of neuroplasticity markers as well as between mBDNF, pTrkB, IGF-1 and gains in both sensorimotor tests and S_{LT} and S_{max}.

3. Results

3.1 Sensorimotor tests

Both regimens improved the forelimb grip strength as well as the forepaw mechanical sensibility, as indicated by the adhesive removal test and the Von Frey **(Fig. 2)**. No difference was observed between Control, HIIT and MICT groups at PRE for sensorimotor tests.

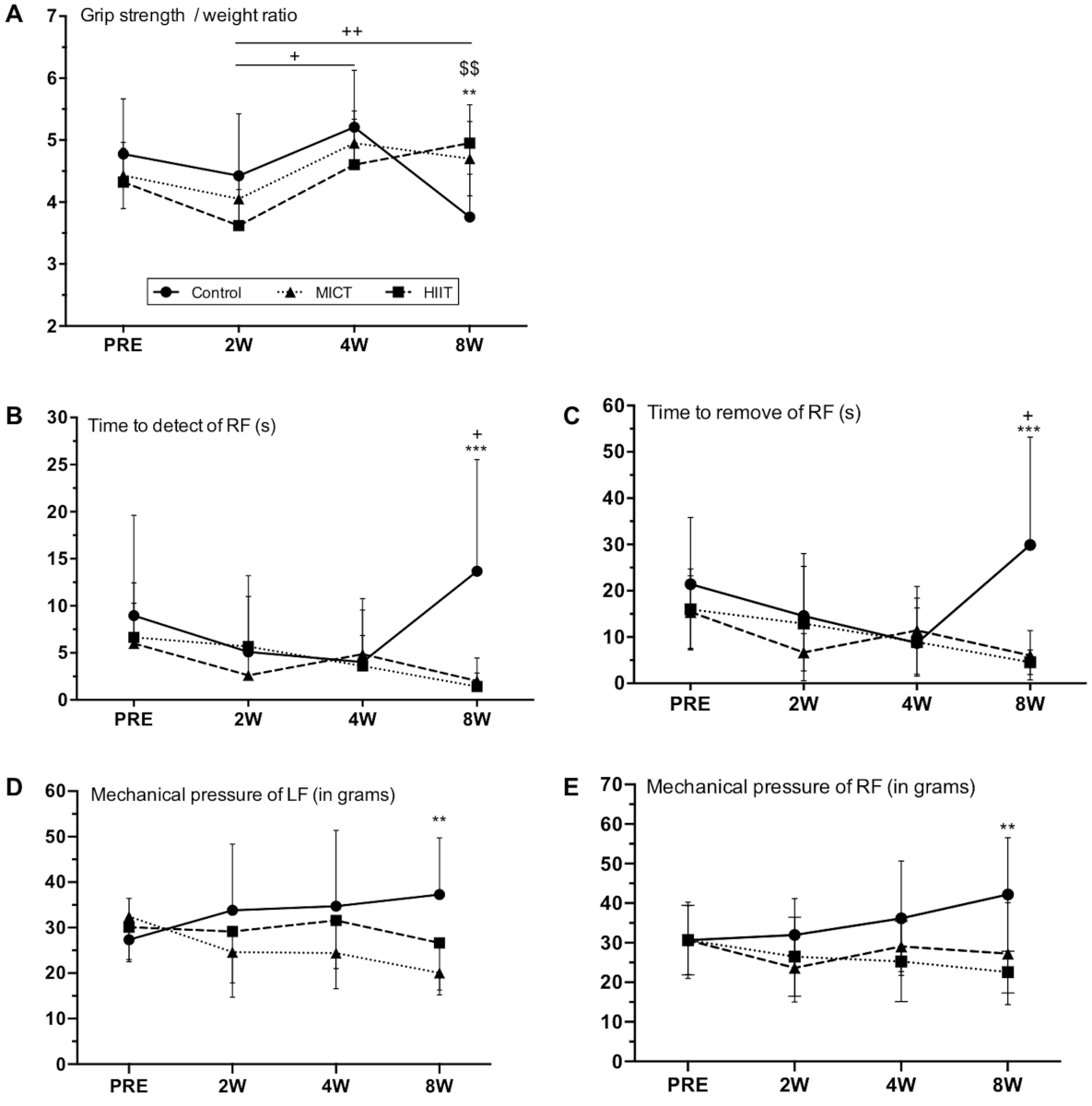

Fig. 2. Time course for forelimb grip strength, adhesive removal test and Electronic Von Frey. Results were obtained from Control and MICT-8W and HIIT-8W (n=8 per group). **A) Forelimb grip strength ratio. *** Higher in both HIIT ($p<0.01$) and MICT

($p>0.05$) than Control at 8 weeks. $^{+}$ Increase from 2 to both 4 and 8 weeks in HIIT ($p<0.01$) and from 2 to 4 weeks in MICT ($p>0.05$). $^{\$}$ Decrease from PRE to 8 weeks in Control ($p<0.01$). **(B) Adhesive removal test - time to detect**. ***** Lower in both HIIT and MICT than Control at 8 weeks ($p<0.001$ for both). $^{+}$ Increase at 8 weeks compared to 2 and 4 weeks in Control ($p<0.05$). **(C) Adhesive removal test - time to remove**. ***** Lower in both HIIT and MICT than Control at 8 weeks ($p<0.001$ for both). $^{+}$ Increase at 8 weeks compared to 2 weeks in Control ($p<0.05$). **(D) Electronic Von Frey - Left forepaw. *** Lower mechanical pressure in HIIT than Control at 8 weeks ($p<0.01$). **(E) Electronic Von Frey - Right forepaw. *** Lower mechanical pressure in HIIT ($p<0.001$) and MICT ($p<0.01$) than Control at 8 weeks.

3.1.1 Forelimb grip strength. Two-way ANOVA analysis showed significant difference between times ($F_{(3,88)} = 7.05$, $p<0.001$) and Training regimens x times ($F_{(6,88)} = 3.73$, $p<0.01$). At 8 weeks, the grip strength ratio was higher in HIIT ($p<0.01$) and MICT ($p<0.05$) groups compared to Control **(Fig. 2A)**. The grip strength ratio increased from 2 to both 4 ($p<0.05$) and 8 weeks ($p<0.01$) in HIIT. The grip strength ratio also increased between 2 to 4 weeks ($p<0.05$) in MICT. No difference was observed between MICT and HIIT groups. Interestingly, grip strength was reduced from PRE to 8 weeks in Control ($p<0.01$).

3.1.2 Adhesive removal test (ART). For the time to detect, two-way ANOVA analysis showed significant difference between Training regimens ($F_{(2,96)} = 4.34$, $p<0.05$) and Training regimens x times ($F_{(6,96)} = 2.49$, $p<0.05$). The time to detect of the right forepaw is significantly lower in HIIT and MICT groups than Control at 8 weeks ($p<0.001$ for both) **(Fig. 2B)**. In Control group, the time to detect increased from 2 weeks to 8 weeks ($p<0.05$).

For the time to remove, two-way ANOVA analysis showed significant difference between Training regimens ($F_{(2,96)}$ = 6.70, p<0.01), times ($F_{(3,96)}$ = 2.77, p<0.05) and Training regimens x times ($F_{(6,96)}$ = 3.33, p<0.01). The time to remove at 8 weeks was lower in HIIT and MICT groups than Control (p<0.001 for both) **(Fig. 2C)**. The time to remove increased from 2 weeks to 8 weeks in Control (p<0.05).

3.1.3 Electronic Von Frey test

Two-way ANOVA analysis showed significant difference between Training regimens for the left forepaw ($F_{(2,103)}$ = 5.06, p<0.01) and for the right forepaw ($F_{(2,102)}$ = 8.35, p<0.01). At 8 weeks, the mechanical pressures of left forepaw needed to induce forepaw withdraw were lower in HIIT than Control (p<0.01) **(Fig. 2D)**. At 8 weeks, the mechanical pressure of right forepaw was lower in HIIT (p<0.001) and MICT (p<0.01) than Control **(Fig. 2E)**.

3.2 Incremental exercise test

Overall, S_{LT} and S_{max} increased for the 2 trained groups as early as 2 weeks of training. However, these parameters continued to be enhanced by HIIT between 2 and 8 weeks (p=0.04 for S_{LT} and p=0.079 for S_{max}) while an early plateau of performance was observed in MICT. HIIT induced superior improvement of aerobic fitness compared to work-matched MICT following 8 weeks of training **(Fig. 3)**. It seems necessary to add that endurance performance decreased in sedentary/Control rats (p<0.05). In addition, the performance varied from a rat to another during the incremental exercise test at PRE that showed why it could be relevant to individualize the training workload. Including all groups, S_{LT} varied from 15 to 39 $m.min^{-1}$ (160%) and S_{max} from 22 to 48 $m.min^{-1}$ (118%) at PRE explaining why we compared the variation of running speed (at S_{LT} or S_{max}) between PRE and each time point for each group.

Nevertheless, no significant difference was observed between PRE values ($F_{(6,49)}$ = 1.86) with p=0.11. For S_{LT}, 27±3.9 m.min^{-1}, 28.5±6 m.min^{-1} and 26.1±4.9 m.min^{-1} were found for MICT-2W, MICT-4W and MICT-8W, respectively. Also, 23.2±4.3 m.min^{-1}, 27.4±3.7 m.min^{-1} and 24±3.2 m.min^{-1} were found for HIIT-2W, HIIT-4W and HIIT-8W, respectively. For S_{max}, 35.2±4.6 m.min^{-1}, 36.6±6 m.min^{-1} and 34.5±6.4 m.min^{-1} were found for MICT-2W, MICT-4W and MICT-8W, respectively. Also, 32.2±5.2 m.min^{-1}, 36.8±6.4 m.min^{-1} and 32.7±3 m.min^{-1} were found for HIIT-2W, HIIT-4W and HIIT-8W, respectively. S_{LT} and S_{max} PRE value for Control were 28±3 m.min^{-1} and 35±2 m.min^{-1}, respectively.

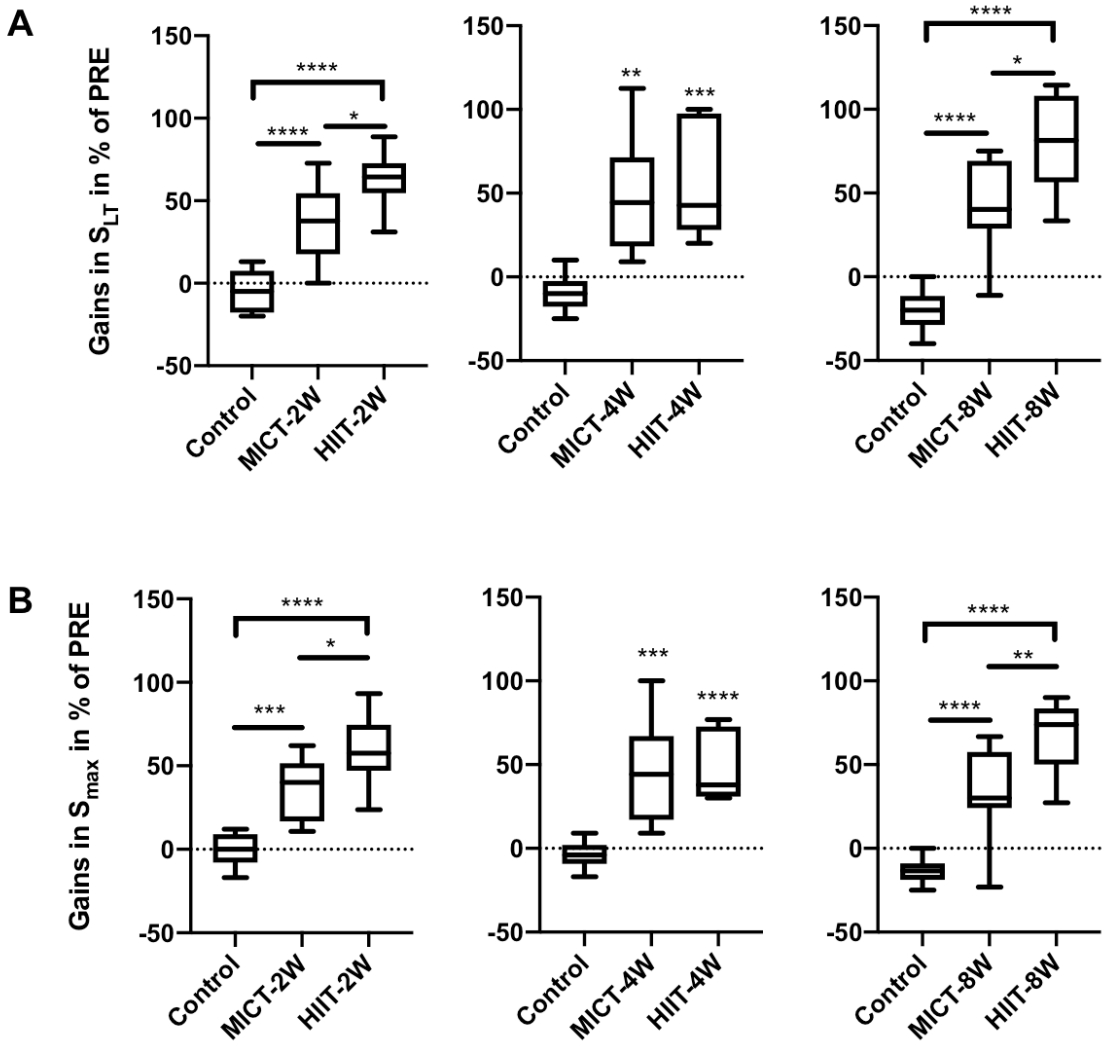

Fig. 3. Time course of endurance performance throughout 8 weeks of training expressed in % of PRE value. Results were obtained from Control, MICT-2W and HIIT-2W (n=8 per group),

MICT-4W and HIIT-4W (n=8 per group) and MICT-8W and HIIT-8W (n=8 per group). **(A) S_{LT}.** * Higher in both HIIT and MICT than Control at 2W, 4W and 8W ($p<0.001$ for all) and higher in HIIT than MICT at 2W and 8W ($p<0.01$). **(B) S_{max}.** * Higher in both HIIT and MICT than Control at 2W, 4W and 8W ($p<0.001$ for all) and higher in HIIT than MICT at 2W and 8W ($p<0.01$). W= week. All data were expressed in mean ± SD.

3.2.1 S_{LT}

One-way ANOVA analysis showed significant difference between training regimens at 2 weeks ($F_{(2,21)} = 16.71$), 4 weeks ($F_{(2,21)} = 13.29$) and 8 weeks ($F_{(2,21)} = 64.53$) with $p<0.001$ for all. S_{LT} of HIIT and MICT were higher than Control from 2 to 8 weeks (compared to PRE: +59% and +38% at 2 weeks; +57.2% and +42% at 4 weeks; +82.4% and +41.8% at 8 weeks, respectively with $p<0.001$ for all). Moreover, S_{LT} of HIIT was higher than MICT at 2 and 8 weeks ($p<0.05$) **(Fig. 3A)**.

3.2.2 S_{max}

One-way ANOVA analysis showed significant difference between training regimens at 2 weeks ($F_{(2,21)} = 30.11$), at 4 weeks ($F_{(2,21)} = 20.62$) and at 8 weeks ($F_{(2,21)} = 67.14$) with $p<0.001$ for all. S_{max} of HIIT and MICT were higher than Control (compared to PRE: +59% and +37% at 2 weeks; +48.8% and +45.4% at 4 weeks; +67% and +33% at 8 weeks respectively, $p<0.001$). Moreover, S_{max} of HIIT was higher than MICT at 2 and 8 weeks ($p<0.05$ and $p<0.01$, respectively) **(Fig. 3B)**.

Additional experiment 1 – Effect of blood samples during an incremental exercise test on endurance performance. There was no difference in the S_{max} between the 2 tests (with and

without blood sampling) for the 3 additional Control showing that repetitive blood samples had no effect on endurance performance (**see Supplementary Figure 2**).

3.3 Characteristics of endurance training protocols

All included animals were able to perform each session of MICT and HIIT protocols. Progression of exercise parameters over the 8 weeks was described in **Table 1**.

		Running speed ($m.min^{-1}$)	**Recovery speed** ($m.min^{-1}$)	**Session duration** (min)	**Total workload** (Joules)	**Distance** (meters per session)
HIIT	**Week 1**	30.2±6.0	19.5±3.4	28	2005±591	716±120
	Week 2	31.4±6.3	19.5±3.4	28	2252±533	737±123
	Week 3	46.7±8.4	28.4±4.2	28	3007±598	1088±174
	Week 4	47.6±8.6	28.4±4.2	28	3321±649	1103±178
	Week 5	44.1±4.2	29.6±7.6	28	3358±684	1061±141
	Week 6	44.7±4.2	29.6±7.6	28	3577±687	1069±143
	Week 7	44.5±3.4	25.8±2.4	28	3569±549	1021±82
	Week 8	44.9±3.5	25.8±2.4	28	3797±605	1026±83
MICT	**Week 1**	19.8±4.3	None	36.1±7.6	2005±591	697±122
	Week 2	19.8±4.3		36.6±6.9	2252±533	708±116
	Week 3	27.9±6.8		37.2±5.3	3007±598	1024±169
	Week 4	27.9±6.8		38.0±5.0	3321±649	1044±188
	Week 5	26.7±3.7		40.5±11.2	3358±684	1054±177
	Week 6	26.7±3.7		40.0±10.6	3577±687	1043±167
	Week 7	26.1±2.7		39.2±6.1	3569±549	1012±94
	Week 8	26.1±2.7		40.5±8.7	3797±605	1040±129

Table 1: Exercise intensity, duration, covered distance and energy expenditure during HIIT and MICT sessions (n = 24 for Week 1 to Week 2, n = 16 for Week 3 to Week 4 and n = 8 from Week 5

to Week 8 per trained group). The 5-min warm-up is not included in the session duration (expressed in min). No recovery period for MICT because it was a continuous exercise. Running and recovery speeds were indicated for each week of training in $m.min^{-1}$. Energy expenditure was indicated in Joules. All data were expressed in mean ± SD.

Additional experiment 2 - Blood lactate concentration before and after a single training session. Unpaired t-test revealed that blood lactate concentration after a HIIT session (2.4±0.7 $mmol.L^{-1}$) was higher than after a MICT session (1.4±0.2 $mmol.L^{-1}$) in our protocol ($t=3.870$, $df=14$, $p<0.01$). Moreover, compared to baseline values, paired t-test revealed that the blood lactate concentration was reduced after the MICT session (-19±21%, $t=2.505$, $df=7$, $p<0.05$) while it increased after the HIIT session (+45±42%, $t=3.055$, $df=7$, $p<0.05$) (**see Supplementary Figure 3**).

3.4 Cortex measurements

Overall, MICT and HIIT are both effective in promoting key neuroplasticity markers, but the time course varies according to the training regimen.

3.4.1 *mBDNF and IGF-1 immunoassay*

Our results indicated that mBDNF is early upregulated by MICT while IGF-1 protein levels only increased with HIIT at 2 and 8 weeks. One-way analysis showed significant difference between training regimens for mBDNF at 2 weeks ($F_{(2,21)} = 14.30$ with $p<0.01$) and at 4 weeks ($F_{(2,20)} = 4.23$ with $p<0.05$). One-way analysis showed significant difference between training regimens ($F_{(2,14)} = 4.80$ with $p<0.05$) at 8 weeks. One-way analysis also showed significant difference between times within the HIIT group for IGF-1 ($F_{(2,15)} = 4.081$ with $p<0.05$).

At 2 weeks, the mBDNF concentrations in MICT were higher than Control and HIIT groups ($p<0.01$). Then, the mBDNF concentrations in MICT seemed to progressively decrease through the protocol, but a difference remained between MICT and HIIT only at 4 weeks ($p<0.05$). No difference was observed between HIIT and Control (**Fig. 4A**). The IGF-1 concentrations in HIIT were higher compared with Control only at 8 weeks ($p<0.05$) although they tended to increase in HIIT at 2 weeks ($p=0.0755$). Interestingly, when comparing only training regimens at 2 weeks, IGF-1 concentrations were higher in HIIT than MICT ($t=2.326$, $df=10$, $p<0.05$, unpaired t-test). Moreover, IGF-1 concentrations increased between HIIT-4W and HIIT-8W ($p<0.05$). No difference was observed between MICT and Control although IGF-1 concentrations tended to be higher in MICT than in Control ($p=0.064$) at 4 weeks (**Fig. 4B**).

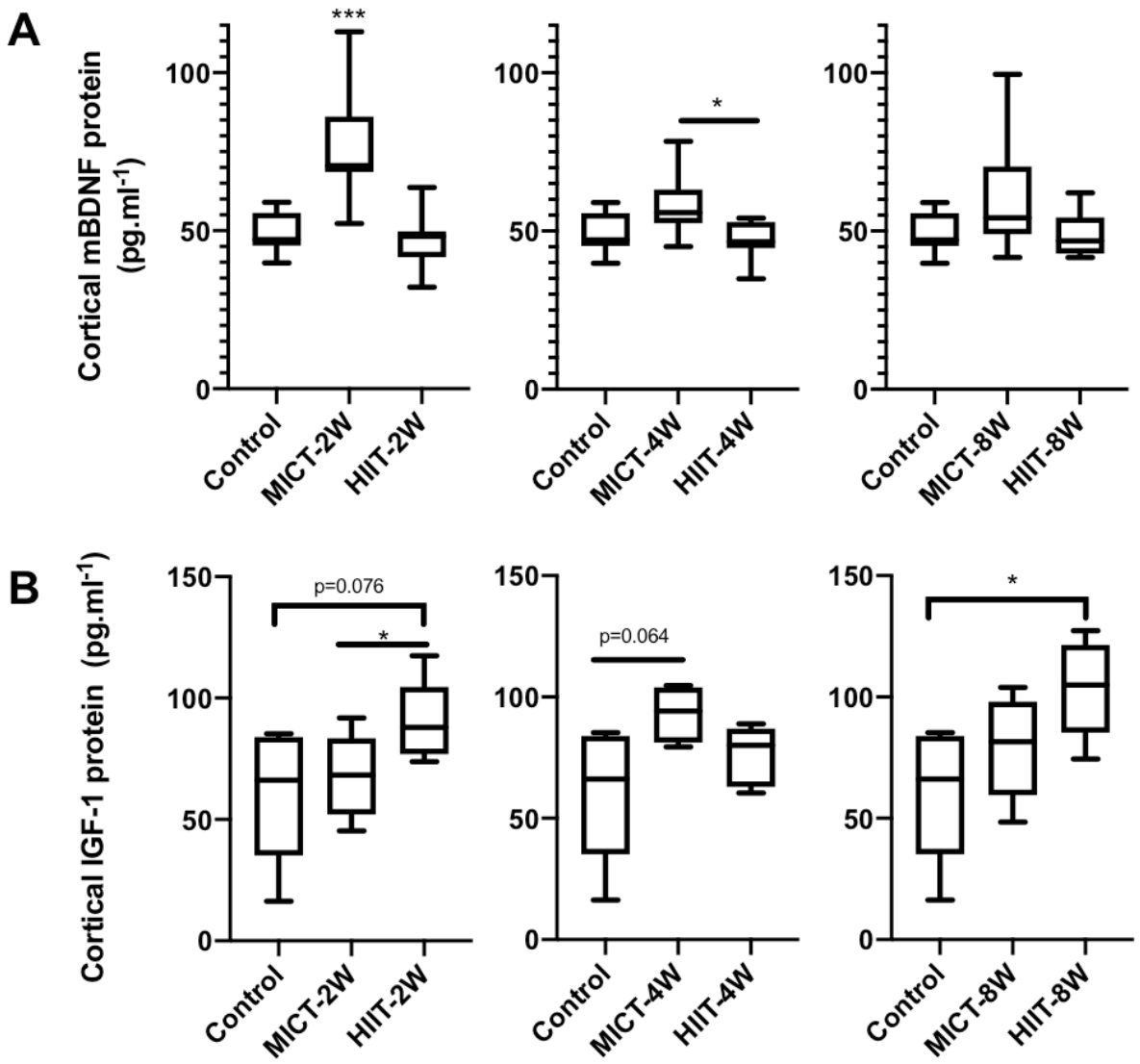

Fig. 4. Impact of training regimens on cortical mBDNF and IGF-1 protein levels by ELISA. Relative levels of **(A)** mBDNF and **(B)** IGF-1 at 2W, 4W and 8W for Control, HIIT and MICT groups. * Difference in protein levels between groups. All data were expressed in mean ± SD.

3.4.2 Western Blotting

Overall, an early VEGF overexpression was observed with MICT followed by a decrease expression while a more progressive upregulation was observed until 8 weeks with HIIT. MICT mainly promotes metabolic (PGC-1α and AMPKα), angiogenic (VEGF and Apelin receptor) and neurotrophic (TrkB and IGFBP2) markers at 2 weeks, while AMPKα is

upregulated from 2 to 8 weeks. As for HIIT, it promotes cortical plasticity at 2 and 8 weeks for IGF-1, COX4, $p75^{NTR}$ and FNDC5. TrkB, PGC-1α and VEGF are also upregulated at 8 weeks with HIIT. No difference was found for the pTrkB/TrkB ratio (*data not shown*).

At 2 weeks. One-way analysis showed significant difference between training regimens for pTrkB ($F_{(2,21)} = 3.74$, $p<0.05$), TrkB ($F_{(2,17)} = 9.16$, $p<0.01$), $p75^{NTR}$ ($F_{(2,21)} = 3.64$, $p<0.05$), IGFBP2 ($F_{(2,13)} = 4.21$, $p<0.05$), FNDC5 ($F_{(2,15)} = 7.60$, $p<0.05$), KCC2 ($F_{(2,21)} = 3.81$, $p<0.05$), VEGF ($F_{(2,15)} = 5.77$, $p<0.05$), Apelin receptor ($F_{(2,14)} = 6.92$, $p<0.05$), COX4 ($F_{(2,18)} = 4.82$, $p<0.05$), AMPKα ($F_{(2,15)} = 4.19$, $p<0.05$) and PGC-1α ($F_{(2,18)} = 5.60$, $p<0.05$). Concerning the neurotrophic markers, levels of pTrkB and $p75^{NTR}$ in HIIT were higher than Control ($p<0.05$) **(Fig. 5A.C)**. Levels of TrkB and IGFBP2 in MICT were higher than Control ($p<0.05$) **(Fig. 5 B.F)**. Levels of FNDC5 in HIIT and MICT were higher than Control ($p<0.05$) **(Fig. 5.D)**. Levels of KCC2 in HIIT were lower than Control ($p<0.05$) **(Fig. 5.E)**. Concerning the metabolic and angiogenic markers, levels of COX4 in HIIT was higher than Control while levels of PGC-1α were higher in MICT than HIIT and Control ($p<0.05$ for all) **(Fig. 6A.C)**. Levels of VEGF were higher in MICT than HIIT ($p<0.05$) **(Fig. 6.E)**. Levels of AMPKα and Apelin receptor were higher in MICT than Control ($p<0.05$ and $p<0.01$, respectively) **(Fig. 6B.D)**.

At 4 weeks. One-way analysis showed significant difference between training regimens for pTrkB ($F_{(2,21)} = 4.80$, $p<0.05$), $p75^{NTR}$ ($F_{(2,19)} = 3.94$, $p<0.05$), FNDC5 ($F_{(2,14)} = 6.79$, $p<0.01$), COX4 ($F_{(2,19)} = 3.37$, $p=0.056$), Apelin receptor ($F_{(2,13)} = 5.48$, $p<0.05$) and AMPKα ($F_{(2,15)} = 7.24$, $p<0.01$). Concerning the neurotrophic markers, levels of pTrkB and FNDC5 in HIIT were higher than Control ($p<0.01$ for both) **(Fig. 5A.D)**. Levels of $p75^{NTR}$ in HIIT were higher than Control ($p<0.05$) **(Fig. 5C)**. Concerning the metabolic and angiogenic markers, levels of COX4 were higher in MICT than Control ($p<0.05$) **(Fig. 6A)**. Levels of AMPKα and Apelin receptor were higher in MICT than HIIT and Control ($p<0.05$) **(Fig. 6B.D)**.

At 8 weeks. One-way analysis showed significant difference between training regimens for TrkB ($F_{(2,20)}$ = 6.61, p<0.01), $p75^{NTR}$ ($F_{(2,19)}$ = 4.49, p<0.05), IGFBP2 ($F_{(2,14)}$ = 7.41, p<0.05), FNDC5 ($F_{(2,14)}$ = 7.37, p<0.05), VEGF ($F_{(2,15)}$ = 6.38, p<0.01), COX4 ($F_{(2,19)}$ = 5.25, p<0.05), AMPKα ($F_{(2,15)}$ = 4.85, p<0.05) and PGC-1α ($F_{(2,16)}$ = 4.42, p<0.05). Concerning the neurotrophic markers, levels of TrkB, FNDC5 (p<0.01) and $p75^{NTR}$ (p<0.05) in HIIT were higher than Control **(Fig. 5B.C.D)**. Levels of IGFBP2 were higher in both HIIT and MICT than Control (p<0.05) **(Fig. 5.F)**. Interestingly, MICT strongly tended to decrease KCC2 levels (p = 0.065) without effect of HIIT **(Fig. 5.E)**. Concerning the metabolic and angiogenic markers, levels of PGC-1α were higher in HIIT than Control (p<0.05), but tended to be higher than MICT (p =0.07) **(Fig. 6.C)**. Levels of VEGF were higher in HIIT than MICT and Control groups (p<0.05 for both) **(Fig. 6.E)**. Levels of COX4 were higher in both HIIT and MICT than Control (p<0.05) **(Fig. 6.A)**. Levels of AMPKα were higher in MICT than Control (p<0.05) **(Fig. 6.B)**.

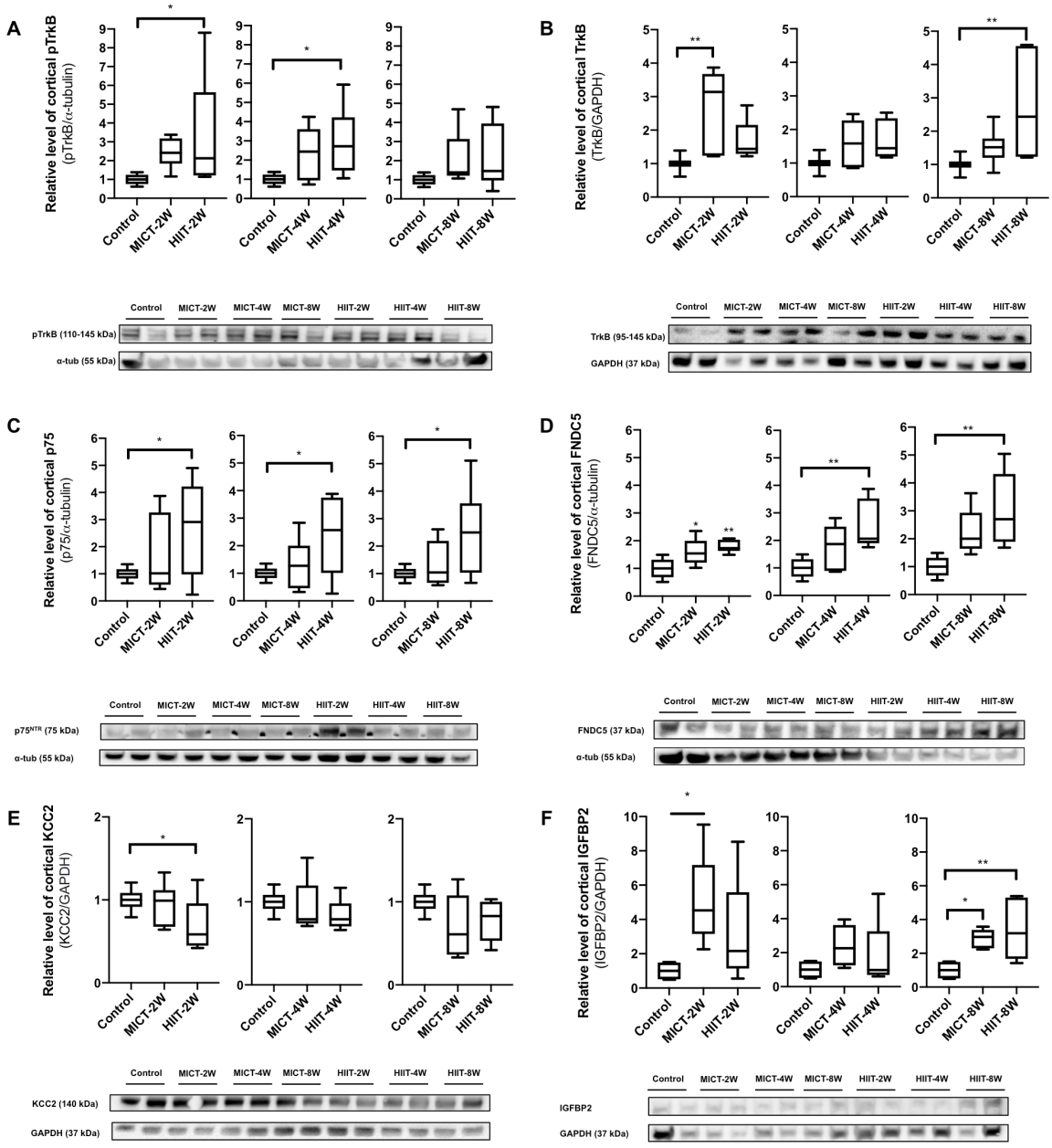

Fig. 5. Impact of training regimens on cortical neurotrophic markers at 2W, 4W and 8W by Western blot. Ratio protein/normalization protein (α-tubulin or GAPDH depending on the molecular weight of the proteins of interest) was calculated and expressed relative to Control. Representative cropped immunoblots from the same membrane are also shown at the bottom of each figure. Relative levels of **(A)** pTrkB, **(B)** TrkB, **(C)** $p75^{NTR}$, **(D)** FNDC5, **(E)** KCC2 and **(F)** IGFBP2. * Difference in protein levels between groups. All data were expressed in mean ± SD.

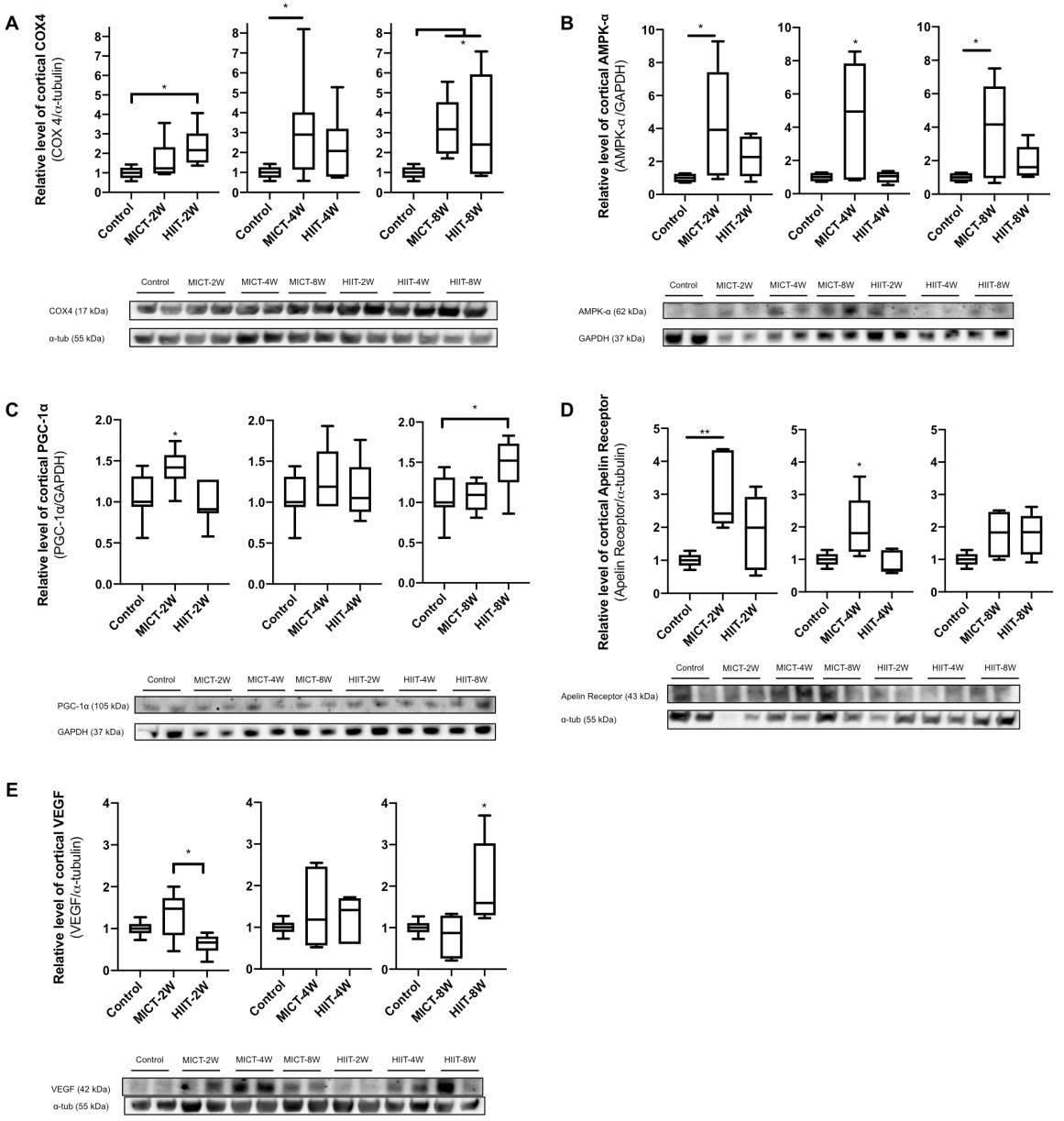

Fig. 6. Impact of training regimens on cortical angiogenic and metabolic markers at 2W, 4W and 8W by Western blot. Ratio protein/normalization protein (α-tubulin or GAPDH depending on the molecular weight of the proteins of interest) was calculated and expressed relative to Control. Representative cropped immunoblots from the same membrane are also shown at the bottom of each figure. Relative levels of **(A)** COX4, **(B)** AMPKα, **(C)** PGC-1α, **(D)** Apelin receptor and **(E)** VEGF. * Difference in protein levels between groups. All data were expressed in mean ± SD.

3.5 *Pearson's* correlation

3.5.1 *Correlation between cortical FNDC5 and cortical neurotrophic markers*

FNDC5 was positively correlated with both pTrkB and TrkB ($r = 0.35$, $p<0.05$ and $r = 0.55$, $p<0.01$ respectively, medium effect size for both) after 2 weeks **(Fig. 7A-B)**. Also, FNDC5 was positively correlated with pTrkB and IGF-1 ($r = 0.56$, $p<0.001$ and $r = 0.40$, $p<0.05$ respectively; medium effect size for both) after 4 weeks **(Fig. 7C-D)** and with mBDNF ($r = 0.25$, small effect size; $p<0.05$) and pTrkB ($r = 0.39$, medium effect size; $p<0.01$) at 8 weeks **(Fig. 7E-F)**.

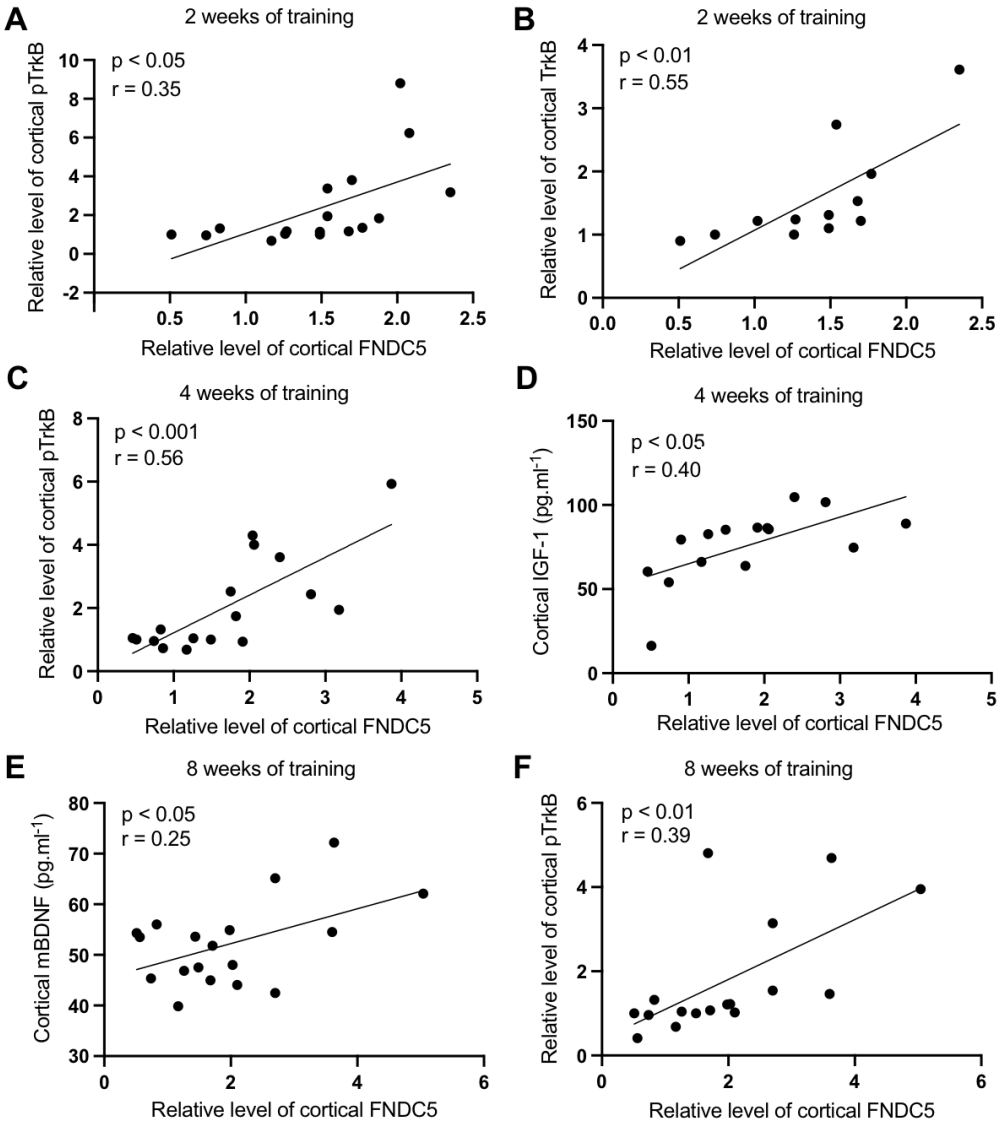

Fig. 7. Correlation between FNDC5 and neurotrophic markers after 2W, 4W and 8W of training. (A-B) Cortical FNDC5 was positively correlated with pTrkB and TrkB at 2 weeks, with **(C-**

D) pTrkB and IGF-1 at 4 weeks and with **(E-F)** mBDNF and pTrkB at 8 weeks. Pearson's correlation with two-tailed significance was conducted (n=15-18, pooled samples of Control, MICT and HIIT groups at each time).

3.5.2 *Correlation between cortical neuroplasticity markers and functional outcomes*

We found significant correlations between gains in endurance parameters (S_{LT} and S_{max}) and some neuroplasticity markers. At 2 weeks, FNDC5 was positively correlated with gains in S_{LT} ($r = 0.28$, small effect size; $p<0.05$) and S_{max} ($r = 0.41$, medium effect size; $p<0.01$) **(Fig. 8A)**. Also, pTrkB was positively correlated with gains in both S_{LT} and S_{max} ($r = 0.18$ and $r = 0.18$, small effect size; $p<0.05$ for both) **(Fig. 8B).** At 4 weeks, pTrkB was only positively correlated with gains in S_{LT} ($r = 0.21$, small effect size; $p<0.05$) **(Fig. 8C)**. At 8 weeks, gains in S_{LT} and S_{max} were positively correlated with both FNDC5 ($r = 0.25$ and $r = 0.27$ respectively, small effect size with $p<0.05$ for both) and IGF-1 ($r = 0.43$, and $r = 0.46$ respectively, medium effect size; $p<0.01$ for both) **(Fig. 8D-E)**. No significant correlation was found between neuroplasticity markers and grip strength, adhesive removal test and electrical Von Frey test *(data not shown)*.

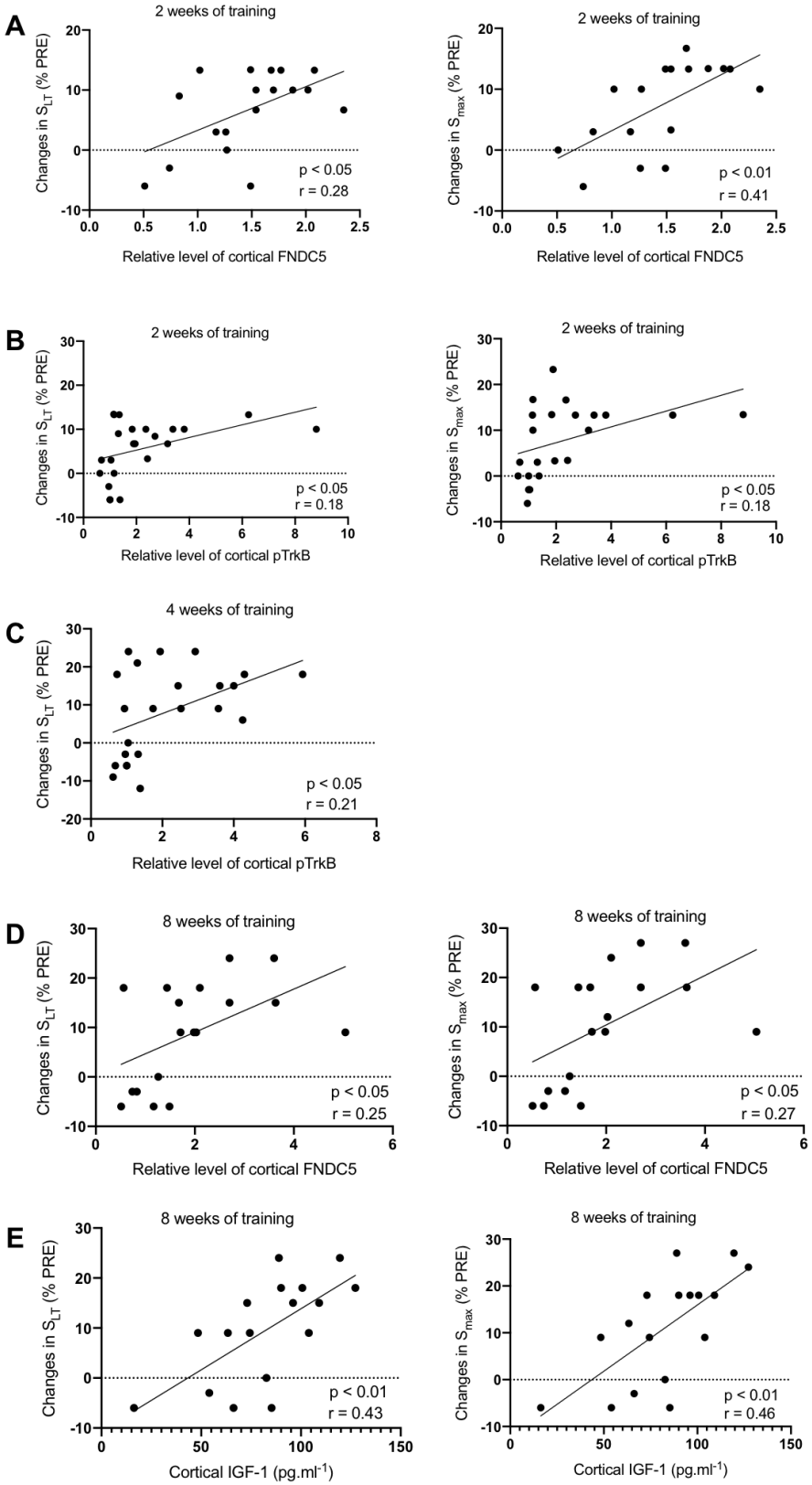

Fig. 8. Correlation between neurotrophic factors and endurance parameters after 2W, 4W and 8W of training. (A) At 2 weeks between FNDC5 and S_{LT} and S_{max} and **(B)** between pTrkB and S_{LT} and S_{max}. **(C)** At 4 weeks between pTrkB and S_{LT}. **(D)** At 8 weeks between FNDC5 and S_{LT}

and S_{max} and **(E)** between IGF-1 and S_{LT} and S_{max}. Pearson's correlation with two-tailed significance was conducted (n=15-18, pooled samples of Control, MICT and HIIT groups at each time).

4. Discussion

Methodological considerations

The individualized training programs performed in the present study can be easily reproduced in future preclinical experiments and extrapolated to humans for clinical/performance purpose by using S_{LT} to separate the high from low running speeds, as recommended in both human and rodent studies (Fabre et al. 1997; Gronwald et al. 2019; Constans et al. 2021). Nevertheless, such training protocol can be constraining for experimenters who need to train rats individually, which is a highly repetitive task. Also, incremental exercise tests are more expensive when measuring blood lactate with single use lactate reagents. To confirm that high and low intensities are physiologically distinct, we found a higher blood lactate concentration following a HIIT session reflecting a higher anaerobic contribution. In contrast, the decreased blood lactate concentration at the end of MICT session suggested a major contribution of aerobic metabolism as expected (Costill 1970; Spengler et al. 1999). Such different production/uptake lactate induced by endurance regimens might partially explain why neuroplasticity processes are different between HIIT and MICT (see below) (Morland et al. 2017; El Hayek et al. 2019). The need to perform repetitive venous blood samples to measure blood lactate are not considered a limitation in endurance performance because S_{max} is not altered in these conditions, in contradiction with what was suggested in a previous study (Høydal et al. 2007).

It is important to underline that rats should remain in the same range of age during long training protocols to match the comparison of cortical parameters between time points. To the best of our knowledge, no study indicated significant variations of neurotrophin expression or synaptic plasticity over the young adult period suggesting that comparison between Control

and trained groups at each time point can be acceptable in our study (Rex 2005). Hence, starting a program at the beginning of adult age seems suitable to avoid significant difference in neuroplasticity processes during several weeks of training. It also allows limiting the achievement of high body weight during the training protocol although an increase of weight is inevitable.

The lack of direct link between molecular measurements and sensorimotor functions is frequently observed in exercise training studies, including the present one in which no positive correlation was found between these parameters. Nevertheless, further studies should be focused on this relationship to define the potential influence of these neuroplasticity markers on sensorimotor functions after training.

HIIT induces higher endurance performance than work-matched MICT by being more effective between 2 and 8 weeks of training.

HIIT promotes earlier gains in endurance performance than MICT as demonstrated by a higher increase in S_{LT} and S_{max} at 2 weeks despite shorter duration of HIIT sessions than MICT (from -30 to -50%). Given that S_{max} is related to VO_{2max}, our findings suggest that HIIT might be considered a time-efficient strategy to improve VO_{2max} when compared to work-matched MICT (Høydal et al. 2007; Constans et al. 2021). Interestingly, similar results were found in humans with HIIT eliciting a greater response relative to MICT when workload is matched (Milanović et al. 2015).

Given that S_{LT} and S_{max} are higher until 8 weeks of training following HIIT, it is suggested that the use of HIIT in endurance programs could avoid a plateau of endurance performance as observed with MICT (Constans et al. 2021). HIIT also elicits a higher effectiveness to improve S_{LT} over a given training period indicating that animals trained with HIIT might run at higher speeds during a long period.

HIIT and MICT could prevent the decline in strength, motor coordination and mechanical sensitivity over time.

Only the HIIT regimen improves the grip strength from PRE to 8 weeks in contrast with Control in which grip strength decreases over time. It likely explains why grip strength is higher in HIIT group than Control. Our results seem in line with previous findings in healthy rats indicating a muscle hypertrophy (that might influence muscle strength) following a 8-week HIIT program (Biglari et al. 2020). However, a potential impact of hypertrophy in forelimb muscles on strength gains needs to be taken with caution because no study has assessed hypertrophy in forelimb muscles following HIIT. In humans, HIIT stimulates muscle protein synthesis, but it remains to be determined whether it is associated with muscle hypertrophy that might reveal a significant role of HIIT in limiting muscle atrophy (Callahan et al. 2021). The difference between MICT and Control seems to require another explanation. It could be mainly related to the strength decline in Control rather than the modest and early effect of MICT on muscle strength.

In addition, both training regimens seem to maintain motor coordination and tactile sensitivity by avoiding the decrease of mechanical sensitivity observed in sedentary rats. It suggests that both MICT and HIIT exhibit benefits on sensorimotor functions that should be considered in patients with sensitive deficits provided they are physically able to perform this type of session.

Levels of neuroplasticity markers are time-dependent over the 8-week training period.

We have identified four different patterns of neurotrophic factor expression over the training period. First, some neuroplasticity markers are upregulated from the first 2 weeks, and then, maintain a high level of expression throughout the protocol. Second, after having a

peak of expression at 2 weeks, neuroplasticity markers could also decrease from 4 to 8 weeks. The third pattern is first characterized by an early upregulation at 2 weeks followed by a downregulation at 4 weeks, and finally exhibited a bouncing effect at 8 weeks. For the last pattern, neurotrophic factors only displayed a late upregulation at 8 weeks.

Both training protocols promote earlier increase in cortical plasticity markers while only HIIT displays prolonged neurotrophic effects.

Neuroplasticity markers and endurance performance follow a similar time course. Indeed, both peak at 2 weeks with MICT and then decrease over time while HIIT mainly promotes cortical plasticity and endurance performance at 2 and 8 weeks. It is reinforced by the positive correlation observed between FNDC5, pTrkB as well as IGF-1 and aerobic parameters.

MICT is effective in stimulating early cortical angiogenic markers such as Apelin Receptor and VEGF, known to contribute to synaptic plasticity in the cortex. Also, angiogenic factors are strongly involved in metabolic activity of cells by providing them O_2 and nutrients. At the same time point, only MICT enhances AMPKα levels, a key regulator of energy metabolism triggered by ATP depletion. The maintained AMPKα upregulation over the whole MICT protocol reinforces the growing interest in its agonist (AICAR) to investigate specific role of AMPKα in mediating exercise-induced neuroplasticity and behavioral benefits (Kim and Leem 2016; Moon et al. 2019). AMPKα is also known to regulate PGC-1α that stimulates mitochondrial biogenesis and glucose metabolism at 2 weeks (Jager et al. 2007). Just as in the hippocampus, the upregulation of PGC-1α is accompanied by an increase in FNDC5 levels in the cortex at the same time point (2 weeks) (Wrann et al. 2013; Constans et al. 2021). FNDC5, originated partially from active skeletal muscles, might be involved in the early upregulation of mBDNF and TrkB that is supported by the positive correlation found in

our study. This enhancement of cortical mBDNF is in line with the increased hippocampal mBDNF that was recently found following a 4-week MICT (Okamoto et al. 2021). The decline in mBDNF concentrations between 4 and 8 weeks is not in accordance with previous studies showing later BDNF enhancement after voluntary running wheel (Berchtold et al. 2005). The lack of prolonged benefits of MICT on mBDNF levels in our study might be associated with the shorter distance of our MICT sessions (~1 km) compared to previous studies using running wheel (~4-5 km). Indeed, they showed a positive correlation between the running distance per day and both adult hippocampal neurogenesis and mBDNF concentrations (Cotman and Berchtold 2002; Nokia et al. 2016). In other words, duration of MICT session on treadmill might be insufficient to maintain mBDNF/TrkB upregulation in the present study. Interestingly, even though IGFBP-2 levels display an early and then a bouncing effect without increase in IGF-1 levels, IGFBP-2 enhancement might contribute to neuroprotection in addition to mBDNF/TrkB (Lewitt and Boyd 2019). Overall, it suggests early beneficial effects of MICT on neuronal survival and synaptic plasticity (Liu and Nusslock 2018). Finally, the overexpression of COX4 suggests a higher aerobic activity in the cortex until 8 weeks of MICT (Bikas et al. 2020).

HIIT induces a different combination between metabolic, angiogenic and neuroplasticity markers, mainly observed at 2 and 8 weeks. Surprisingly, the upregulation of FNDC5 at each time-point is not accompanied by cortical mBDNF changes. This absence of mBDNF overexpression is not in agreement with results observed in the whole brain and hippocampus with different high-intensity training protocols (Afzalpour et al. 2015; So et al. 2017; Freitas et al. 2018; Okamoto et al. 2021). Nevertheless, both pTrkB and $p75^{NTR}$ levels rise concomitantly during the first 4 weeks that might potentiate the neurotrophin actions to regulate neuronal survival, without significant mBDNF upregulation (Shulga et al. 2012; Zanin et al. 2019). Given that neurotrophin signaling pathways interact with KCC2 to

influence neuronal activity, it can be asked if there is an interplay between the early pTrkB and $p75^{NTR}$ upregulation and the KCC2 downregulation at the same time point (Bibel 1999; Rivera et al. 2002; Zanin et al. 2019). It is the first study showing an early decrease in KCC2 levels in the cortex following HIIT while MICT failed to induce any significant alteration over the 8 weeks of training. The decrease in KCC2 might lead to a reduced inhibitory signaling in the cortex, and thus potentially, enabling the enhancement of synaptic activity through neurotrophin actions (indicating here by the concomitant pTrkB and $p75^{NTR}$ upregulation) (Nicolini et al. 2020). This result is in line with those showing an enhancement of cortical excitability with a 2-week HIIT in humans (Andrews et al. 2020; Nicolini et al. 2020). To reinforce the neurotrophic effect of HIIT, we found higher levels of cortical IGF-1, as it was shown in skeletal muscles via growth hormone following intense exercises (Foster et al. 2012). Interestingly, IGF-1 enhancement might play a role in modulating TrkB expression, thus regulating BNDF activity without necessarily altering neurotrophin levels in cortical neurons (McCusker et al. 2006). Also, by interacting with VEGF, IGF-I is thought to mediate exercise-induced effects on neuroplasticity that is supported by the positive correlation between IGF-1 and gains in endurance performance at 8 weeks (Fabel et al. 2003; Llorens-Martín et al. 2010; Maass et al. 2016). Upregulation of IGFBP-2, which is required for optimal IGF-1 neurotrophic actions, reinforces effects of HIIT on IGF signaling at 2 and 8 weeks (Khan 2019). An early VEGF overexpression was observed with MICT while a more progressive upregulation was seen with HIIT indicating an opposite time course of this angiogenic factor between MICT and HIIT. Our results are in accordance with a previous study using the same HIIT protocol, in which VEGF as well as TrkB and PGC-1α are upregulated in the hippocampus following 8 weeks of HIIT (Constans et al. 2021). It thus confirms that HIIT is effective in enhancing these key neuroplasticity markers at 8 weeks in both cortex and hippocampus. As we found an increase in blood lactate concentrations during

HIIT sessions, it is suggested that lactate, which can cross the blood-brain barrier, might contribute to explain the neurotrophic effects of HIIT (Coxon et al. 2018). It is in accordance with previous studies showing an increase in cortical lactate concentrations after an acute endurance exercise on treadmill in healthy rats (Takimoto and Hamada 2014; Coxon et al. 2018). Other studies reported a direct influence of lactate originated from active skeletal muscles, on neurotrophic/angiogenic activity and on long-term potentiation that constitutes the basis of learning and memory (Suzuki et al. 2011; Morland et al. 2017; El Hayek et al. 2019; Huang et al. 2021). Finally, HIIT also upregulates cortical COX 4 levels, concomitantly with endurance improvements at 2 and 8 weeks.

Practical recommendations and perspectives

Our findings might be used to schedule endurance programs dedicated to general and brain health. Indeed, we suggest starting an endurance program based on ventilatory threshold or lactate threshold by a significant period of MICT, as frequently observed in runners, to promote both metabolic/angiogenic markers and neurotrophin (mBDNF). This period could be followed by a training block of HIIT to avoid the plateau of endurance performance, but also to diversify cortical neuroplasticity by maintaining an overexpression of angiogenic (VEGF is delayed with HIIT) and promoting complementary neurotrophic factors (IGF-1 is upregulated during HIIT in contrast to MICT).

Further studies should investigate the effectiveness of longer endurance training programs than 8 weeks on neuroplasticity processes so that we can plan more comprehensive programs for brain health. For instance, it remains to define whether neuroplasticity processes continue to be stimulated, or how they evolve over time, when using longer period of HIIT or MICT. It should be speculated that delayed neuroplasticity processes might be observed on

functional and structural processes such as long-term potentiation enhancement and increase in the number of dendritic spines or neuronal survival.

5. Conclusions

This study indicates that both MICT and HIIT should be planned in endurance programs dedicated to brain health and could thus contribute to schedule training programs according to the time-dependent effects of each training regimen on cortical plasticity and behavioral outcomes. These findings are also of clinical relevance because both exercise regimens might play a role in improving recovery of patients with neurological diseases such as Alzheimer disease and stroke. Given that we were unable to determine which cortical plasticity processes were specifically stimulated by HIIT and MICT, the molecular signaling pathways linking neuroplasticity markers should be investigated at suitable time points for each training regimen. However, it remains methodologically challenging to define brain mechanisms with such quite long training experiments. This study also opens new perspectives on determining which are the main cortical areas involved in neuroplasticity processes at the different time points for each endurance regimen. Indeed, if these regimens act on different cortical areas, it might also highlight their different roles on sensorimotor and/or cognitive functions.

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Conflict of Interest

Authors report no conflict of interest

References

Adkins DL, Boychuk J, Remple MS, Kleim JA. 2006. Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. J Appl Physiol. 101:1776–1782.

Afzalpour ME, Chadorneshin HT, Foadoddini M, Eivari HA. 2015. Comparing interval and continuous exercise training regimens on neurotrophic factors in rat brain. Physiol Behav. 147:78–83.

Andreato LV, Andrade A, Esteves JV. 2021. Why equalising HIIT and MICT is important: attention to methodological details. Trends Endocrinol Metab. 32:657–658.

Andrews SC, Curtin D, Hawi Z, Wongtrakun J, Stout JC, Coxon JP. 2020. Intensity Matters: High-intensity Interval Exercise Enhances Motor Cortex Plasticity More Than Moderate Exercise. Cereb Cortex. 30:101–112.

Berchtold NC, Castello N, Cotman CW. 2010. Exercise and time-dependent benefits to learning and memory. Neuroscience. 167:588–597.

Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW. 2005. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. Neuroscience. 133:853–861.

Bibel M. 1999. Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. EMBO J. 18:616–622.

Biglari S, Afousi AG, Mafi F, Shabkhiz F. 2020. High-intensity interval training-induced hypertrophy in gastrocnemius muscle via improved IGF-I/Akt/FoxO and myostatin/Smad signaling pathways in rats. Physiol Int.

Bikas A, Jensen K, Patel A, Costello J, Reynolds S, Mendonca-Torres M, Thakur S, Klubo-Gwiezdzinska J, Ylli D, Wartofsky L, Burman K, Vasko V. 2020. Cytochrome C Oxidase Subunit 4 (COX4): A Potential Therapeutic Target for the Treatment of Medullary Thyroid

Cancer. Cancers. 12:2548.

Buchheit M, Laursen PB. 2013. High-Intensity Interval Training, Solutions to the Programming Puzzle: Part I: Cardiopulmonary Emphasis. Sports Med. 43:313–338.

Cabral DF, Rice J, Morris TP, Rundek T, Pascual-Leone A, Gomes-Osman J. 2019. Exercise for Brain Health: An Investigation into the Underlying Mechanisms Guided by Dose. Neurotherapeutics. 16:580–599.

Callahan MJ, Parr EB, Hawley JA, Camera DM. 2021. Can High-Intensity Interval Training Promote Skeletal Muscle Anabolism? Sports Med. 51:405–421.

Constans A, Pin-Barre C, Molinari F, Temprado J-J, Brioche T, Pellegrino C, Laurin J. 2021. High-intensity interval training is superior to moderate intensity training on aerobic capacity in rats: Impact on hippocampal plasticity markers. Behav Brain Res. 398:112977.

Costill DL. 1970. Metabolic responses during distance running. J Appl Physiol. 28:251–255.

Cotman CW, Berchtold NC. 2002. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 25:295–301.

Coxon JP, Cash RFH, Hendrikse JJ, Rogasch NC, Stavrinos E, Suo C, Yücel M. 2018. GABA concentration in sensorimotor cortex following high-intensity exercise and relationship to lactate levels: GABA modulation in cortex following HIIT exercise. J Physiol. 596:691–702.

de Araujo GG, Gobatto CA, Marcos-Pereira M, Dos Reis IGM, Verlengia R. 2015. Interval versus continuous training with identical workload: physiological and aerobic capacity adaptations. Physiol Res. 64:209–219.

de Araujo GG, Papoti M, Delbin MA, Zanesco A, Gobatto CA. 2013. Physiological adaptations during endurance training below anaerobic threshold in rats. Eur J Appl Physiol. 113:1859–1870.

de Araujo GG, Papoti M, dos Reis IGM, de Mello MAR, Gobatto CA. 2016. Short and Long

Term Effects of High-Intensity Interval Training on Hormones, Metabolites, Antioxidant System, Glycogen Concentration, and Aerobic Performance Adaptations in Rats. Front Physiol. 7.

De Vincenti AP, Ríos AS, Paratcha G, Ledda F. 2019. Mechanisms That Modulate and Diversify BDNF Functions: Implications for Hippocampal Synaptic Plasticity. Front Cell Neurosci. 13:135.

El Hayek L, Khalifeh M, Zibara V, Abi Assaad R, Emmanuel N, Karnib N, El-Ghandour R, Nasrallah P, Bilen M, Ibrahim P, Younes J, Abou Haidar E, Barmo N, Jabre V, Stephan JS, Sleiman SF. 2019. Lactate mediates the effects of exercise on learning and memory through SIRT1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF). J Neurosci. 1661–18.

Fabel K, Fabel K, Tam B, Kaufer D, Baiker A, Simmons N, Kuo CJ, Palmer TD. 2003. VEGF is necessary for exercise-induced adult hippocampal neurogenesis. Eur J Neurosci. 18:2803–2812.

Fabre C, Masse-Biron J, Ahmaidi S, Adam B, Prefaut C. 1997. Effectiveness of Individualized Aerobic Training at the Ventilatory Threshold in the Elderly. J Gerontol A Biol Sci Med Sci. 52A:B260–B266.

Foster EB, Fisher G, Sartin JL, Elsasser TH, Wu G, Cowan W, Pascoe DD. 2012. Acute regulation of IGF-I by alterations in post-exercise macronutrients. Amino Acids. 42:1405–1416.

Freitas DA, Rocha-Vieira E, Soares BA, Nonato LF, Fonseca SR, Martins JB, Mendonça VA, Lacerda AC, Massensini AR, Poortamns JR, Meeusen R, Leite HR. 2018. High intensity interval training modulates hippocampal oxidative stress, BDNF and inflammatory mediators in rats. Physiol Behav. 184:6–11.

Gronwald T, de Bem Alves AC, Murillo-Rodríguez E, Latini A, Schuette J, Budde H. 2019.

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. 8:353–354.

Hill T, Polk JD. 2019. BDNF, endurance activity, and mechanisms underlying the evolution of hominin brains. Am J Phys Anthropol. 168:47–62.

Høydal MA, Wisløff U, Kemi OJ, Ellingsen Ø. 2007. Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training: Eur J Cardiovasc Prev Rehabil. 14:753–760.

Huang C, Dai C, Gong K, Zuo H, Chu H. 2016. Apelin-13 protects neurovascular unit against ischemic injuries through the effects of vascular endothelial growth factor. Neuropeptides. 60:67–74.

Huang Z, Zhang Y, Zhou R, Yang L, Pan H. 2021. Lactate as Potential Mediators for Exercise-Induced Positive Effects on Neuroplasticity and Cerebrovascular Plasticity. Front Physiol. 12:656455.

Hugues N, Pellegrino C, Rivera C, Berton E, Pin-Barre C, Laurin J. 2021. Is High-Intensity Interval Training Suitable to Promote Neuroplasticity and Cognitive Functions after Stroke? Int J Mol Sci. 22:3003.

Jager S, Handschin C, St.-Pierre J, Spiegelman BM. 2007. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1. Proc Natl Acad Sci. 104:12017–12022.

Jakovljevic B, Nikolic Turnic T, Jeremic N, Savic M, Jeremic J, Srejovic I, Belic B, Ponorac N, Jakovljevic V, Zivkovic V. 2019. The impact of high-intensity interval training and moderate-intensity continuous training regimes on cardiodynamic parameters in isolated heart of normotensive and hypertensive rats. Can J Physiol Pharmacol. 97:631–637.

Khan S. 2019. IGFBP-2 Signaling in the Brain: From Brain Development to Higher Order Brain Functions. Front Endocrinol. 10:822.

Kim D-M, Leem Y-H. 2016. Chronic stress-induced memory deficits are reversed by regular exercise via AMPK-mediated BDNF induction. Neuroscience. 324:271–285.

Kim Y-P, Kim H-B, Jang M-H, Lim B-V, Kim Y-J, Kim H, Kim S-S, Kim E-H, Kim C-J. 2003. Magnitude- and Time-Dependence of the Effect of Treadmill Exercise on Cell Proliferation in the Dentate Gyrus of Rats. Int J Sports Med. 24:114–117.

Lewitt MS, Boyd GW. 2019. The Role of Insulin-Like Growth Factors and Insulin-Like Growth Factor–Binding Proteins in the Nervous System. Biochem Insights. 12:117862641984217.

Liu PZ, Nusslock R. 2018. Exercise-Mediated Neurogenesis in the Hippocampus via BDNF. Front Neurosci. 12.

Llorens-Martín M, Torres-Alemán I, Trejo JL. 2010. Exercise modulates insulin-like growth factor 1-dependent and -independent effects on adult hippocampal neurogenesis and behaviour. Mol Cell Neurosci. 44:109–117.

Maass A, Düzel S, Brigadski T, Goerke M, Becke A, Sobieray U, Neumann K, Lövdén M, Lindenberger U, Bäckman L, Braun-Dullaeus R, Ahrens D, Heinze H-J, Müller NG, Lessmann V, Sendtner M, Düzel E. 2016. Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. NeuroImage. 131:142–154.

McCusker RH, McCrea K, Zunich S, Dantzer R, Broussard SR, Johnson RW, Kelley KW. 2006. Insulin-like growth factor-I enhances the biological activity of brain-derived neurotrophic factor on cerebrocortical neurons. J Neuroimmunol. 179:186–190.

Milanović Z, Sporiš G, Weston M. 2015. Effectiveness of High-Intensity Interval Training (HIT) and Continuous Endurance Training for VO2max Improvements: A Systematic Review

and Meta-Analysis of Controlled Trials. Sports Med. 45:1469–1481.

Moon HY, Javadi S, Stremlau M, Yoon KJ, Becker B, Kang S-U, Zhao X, van Praag H. 2019. Conditioned media from AICAR-treated skeletal muscle cells increases neuronal differentiation of adult neural progenitor cells. Neuropharmacology. 145:123–130.

Morland C, Andersson KA, Haugen ØP, Hadzic A, Kleppa L, Gille A, Rinholm JE, Palibrk V, Diget EH, Kennedy LH, Stølen T, Hennestad E, Moldestad O, Cai Y, Puchades M, Offermanns S, Vervaeke K, Bjørås M, Wisløff U, Storm-Mathisen J, Bergersen LH. 2017. Exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1. Nat Commun. 8:15557.

Nicolini C, Michalski B, Toepp SL, Turco CV, D'Hoine T, Harasym D, Gibala MJ, Fahnestock M, Nelson AJ. 2020. A Single Bout of High-intensity Interval Exercise Increases Corticospinal Excitability, Brain-derived Neurotrophic Factor, and Uncarboxylated Osteolcalcin in Sedentary, Healthy Males. Neuroscience. 437:242–255.

Nokia MS, Lensu S, Ahtiainen JP, Johansson PP, Koch LG, Britton SL, Kainulainen H. 2016. Physical exercise increases adult hippocampal neurogenesis in male rats provided it is aerobic and sustained: Aerobic exercise promotes adult neurogenesis. J Physiol. 594:1855–1873.

Okamoto M, Mizuuchi D, Omura K, Lee M, Oharazawa A, Yook JS, Inoue K, Soya H. 2021. High-intensity Intermittent Training Enhances Spatial Memory and Hippocampal Neurogenesis Associated with BDNF Signaling in Rats. Cereb Cortex. 31:4386–4397.

Parker L, Trewin A, Levinger I, Shaw CS, Stepto NK. 2017. The effect of exercise-intensity on skeletal muscle stress kinase and insulin protein signaling. PLOS ONE. 12:e0171613.

Pederson BA, Cope CR, Schroeder JM, Smith MW, Irimia JM, Thurberg BL, DePaoli-Roach AA, Roach PJ. 2005. Exercise Capacity of Mice Genetically Lacking Muscle Glycogen Synthase: IN MICE, MUSCLE GLYCOGEN IS NOT ESSENTIAL FOR

EXERCISE. J Biol Chem. 280:17260–17265.

Pin-Barre C, Constans A, Brisswalter J, Pellegrino C, Laurin J. 2017. Effects of High- Versus Moderate-Intensity Training on Neuroplasticity and Functional Recovery After Focal Ischemia. Stroke. 48:2855–2864.

Pin-Barre C, Hugues N, Constans A, Berton E, Pellegrino C, Laurin J. 2021. Effects of Different High-Intensity Interval Training Regimens on Endurance and Neuroplasticity After Cerebral Ischemia. Stroke. 52:1109–1114.

Pin-Barre C, Laurin J, Felix M-S, Pertici V, Kober F, Marqueste T, Matarazzo V, Muscatelli-Bossy F, Temprado J-J, Brisswalter J, Decherchi P. 2014. Acute neuromuscular adaptation at the spinal level following middle cerebral artery occlusion-reperfusion in the rat. PloS One. 9:e89953.

Pin-Barre C, Pellegrino C, Laurin F, Laurin J. 2018. Cerebral Ischemia Changed the Effect of Metabosensitive Muscle Afferents on Somatic Reflex Without Affecting Thalamic Activity. Front Physiol. 9.

Pozzi D, Rasile M, Corradini I, Matteoli M. 2020. Environmental regulation of the chloride transporter KCC2: switching inflammation off to switch the GABA on? Transl Psychiatry. 10:349.

Rex CS. 2005. Long-Term Potentiation Is Impaired in Middle-Aged Rats: Regional Specificity and Reversal by Adenosine Receptor Antagonists. J Neurosci. 25:5956–5966.

Rivera C, Li H, Thomas-Crusells J, Lahtinen H, Viitanen T, Nanobashvili A, Kokaia Z, Airaksinen MS, Voipio J, Kaila K, Saarma M. 2002. BDNF-induced TrkB activation down-regulates the K+–Cl− cotransporter KCC2 and impairs neuronal Cl− extrusion. J Cell Biol. 159:747–752.

Shulga A, Magalhaes AC, Autio H, Plantman S, di Lieto A, Nykjaer A, Carlstedt T, Risling M, Arumae U, Castren E, Rivera C. 2012. The Loop Diuretic Bumetanide Blocks

Posttraumatic p75NTR Upregulation and Rescues Injured Neurons. J Neurosci. 32:1757–1770.

So JH, Huang C, Ge M, Cai G, Zhang L, Lu Y, Mu Y. 2017. Intense Exercise Promotes Adult Hippocampal Neurogenesis But Not Spatial Discrimination. Front Cell Neurosci. 11.

Spengler CM, Roos M, Laube SM, Boutellier U. 1999. Decreased exercise blood lactate concentrations after respiratory endurance training in humans. Eur J Appl Physiol. 79:299–305.

Stampanoni Bassi M, Leocani L, Comi G, Iezzi E, Centonze D. 2018. Can pharmacological manipulation of LTP favor the effects of motor rehabilitation in multiple sclerosis? Mult Scler J. 24:902–907.

Stevenson ME, Miller CC, Owen HA, Swain RA. 2020. Aerobic exercise increases sprouting angiogenesis in the male rat motor cortex. Brain Struct Funct. 225:2301–2314.

Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, Alberini CM. 2011. Astrocyte-Neuron Lactate Transport Is Required for Long-Term Memory Formation. Cell. 144:810–823.

Takahashi K, Maejima H, Ikuta G, Mani H, Asaka T. 2017. Exercise combined with low-level GABAA receptor inhibition up-regulates the expression of neurotrophins in the motor cortex. Neurosci Lett. 636:101–107.

Takimoto M, Hamada T. 2014. Acute exercise increases brain region-specific expression of MCT1, MCT2, MCT4, GLUT1, and COX IV proteins. J Appl Physiol. 116:1238–1250.

Verboven M, Cuypers A, Deluyker D, Lambrichts I, Eijnde BO, Hansen D, Bito V. 2019. High intensity training improves cardiac function in healthy rats. Sci Rep. 9:5612.

Voss MW, Vivar C, Kramer AF, van Praag H. 2013. Bridging animal and human models of exercise-induced brain plasticity. Trends Cogn Sci. 17:525–544.

Wrann CD, White JP, Salogiannnis J, Laznik-Bogoslavski D, Wu J, Ma D, Lin JD,

Greenberg ME, Spiegelman BM. 2013. Exercise Induces Hippocampal BDNF through a PGC-1α/FNDC5 Pathway. Cell Metab. 18:649–659.

Zanin JP, Montroull LE, Volosin M, Friedman WJ. 2019. The p75 Neurotrophin Receptor Facilitates TrkB Signaling and Function in Rat Hippocampal Neurons. Front Cell Neurosci. 13:485.