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Electrical Muscle Stimulation as an Alternative to Exercise: Improvements in Executive Function, Anxiety, and Hippocampal BDNF via Lactate Signaling

Peter H. Lawson[1]*, Olivia S. Brown[1], Daniel K. Moore[1]

[1]Department of Medical Sciences, University of Glasgow, Glasgow, United Kingdom.

Abstract

Exercise (EX) is widely acknowledged for supporting brain function, but traditional physical activity may not be accessible to all populations. This research investigates electromyostimulation (EMS) as a potential substitute for improving cognitive performance. Both human volunteers and rats underwent two EMS sessions targeting the quadriceps, each lasting 30 minutes and separated by one week. Cognitive and mood evaluations were performed in humans, while rats were analyzed histologically and biochemically in the prefrontal cortex, hippocampus, and quadriceps. EMS improved executive functioning and lowered anxiety in human participants. In animals, hippocampal levels of brain-derived neurotrophic factor (BDNF) were elevated following EMS. Interestingly, this effect did not coincide with increased neuronal activity or cerebral blood flow, indicating a humoral signal from muscle to brain. No detectable changes occurred in muscle-derived or circulating BDNF or in the FNDC5/irisin pathway, whereas lactate emerged as a key mediator linking muscle activity to brain responses. These results support EMS as a promising alternative to conventional exercise for promoting brain health and cognitive enhancement.

Keywords: Electrical muscle stimulation, Brain-derived neurotrophic factor, Lactate, FNDC5/Irisin, Muscle-brain communication, Cognition

Corresponding author: Peter H. Lawson
E-mail: peter.lawson@outlook.com

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Introduction

Physical exercise (EX) exerts robust effects on brain health, enhancing memory, executive function, and overall mood by reducing stress [1, 2]. Rodent studies highlight the critical role of brain-derived neurotrophic factor (BDNF), a protein essential for neurogenesis, synaptic plasticity, and neuronal survival, in mediating the cognitive benefits of EX [3–5]. In humans, the BDNF val66met polymorphism, which disrupts activity-dependent BDNF secretion, diminishes cognitive gains from exercise [6–8]. Several mechanisms have been proposed for exercise-induced BDNF increases: upregulation in neurons through activity, enhanced cerebral blood flow, and secretion of myokines from contracting muscles [9–11]. Certain myokines are capable of crossing the blood–brain barrier (BBB), promoting brain BDNF production.

Irisin and lactate have emerged as prominent myokines influencing brain health. Irisin, produced from FNDC5 via the PGC-1α pathway, was originally identified for its role in converting white fat to brown fat and enters systemic circulation [12]. Evidence suggests that skeletal muscle activation of the PGC-1α/FNDC5/irisin axis contributes to exercise-driven neuroplasticity [13, 14]. Irisin can cross the BBB and/or interact with integrin receptors to initiate neuroprotective intracellular pathways [14, 15]. Lactate, generated during anaerobic glycolysis in muscles, crosses

the BBB through monocarboxylate transporters (MCTs) [16]. In rodent models, lactate supports neurogenesis [17], neuronal excitability [18], and long-term potentiation (LTP) [19]. Blocking lactate transport during voluntary exercise prevents hippocampal BDNF upregulation [20]. In humans, cerebral lactate correlates with improved executive function as assessed by Stroop testing [21].

Despite the cognitive benefits of exercise, not all individuals can engage in regular physical activity. EMS (or NMES), which induces involuntary muscle contractions via electrical currents, offers a passive approach [22]. EMS targeting the quadriceps has been shown to prevent muscle wasting, improve cardiorespiratory fitness, and enhance carbohydrate metabolism [23–26]. However, its influence on the brain has been underexplored. This study examined the effects of EMS on cognitive performance in humans and on hippocampal and prefrontal BDNF expression in rats, investigating mechanisms including neuronal activity, cerebral hemodynamics, and muscle-derived BDNF, irisin, and lactate.

Both Wistar rats and human participants underwent two EMS sessions spaced one week apart, each lasting 30 minutes on the quadriceps. Stimulation parameters were 100 Hz frequency, 400 µs pulse duration, 7 s/14 s ON/OFF cycles, with intensity ramped from 6 mA to 20 mA in rats, and from 6 mA to the maximum tolerable intensity in humans (48.9 ± 22.4 mA). Human cognitive and mood assessments were conducted immediately and 24 hours after the second session using the Stroop task, the Rey figure, a 15-word memory test, and the Profile of Mood States (POMS). In rats, tissue samples from the prefrontal cortex, hippocampus, and quadriceps were collected 4 or 24 hours post-EMS for histology and biochemical analyses, including Western blot and RT-qPCR. Circulating BDNF and irisin levels were measured via ELISA immediately, 4 h, and 24 h post-EMS, while lactate was assessed immediately after the second session in both species.

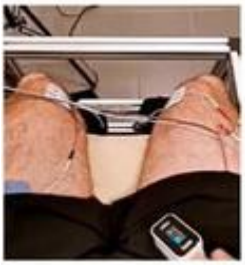
Results and Discussion

EMS protocol assessment in human participants

Forty healthy volunteers participated in this study. Electrical stimulation was applied to the quadriceps using three electrodes placed on the right femoris, vastus lateralis, and vastus medialis **(Figure 1a)**. Each session lasted 30 minutes and consisted of 7-second contractions followed by 14 seconds of rest. The current intensity was gradually increased from 6 mA up to the participant's maximum tolerable intensity (48.9 ± 22.4 mA). Participants were randomly divided into a control group (CTRL, n = 20) and an EMS group (n = 20).

Analysis of maximal voluntary contraction (MVC) revealed a decrease immediately following the first familiarization session (EMS 1, −32.4 N·m, t-test, p = 0.0011) and the second session (EMS 2, −49.6 N·m, t-test, p < 0.0001) **(Figure 1b)**. The magnitude of MVC reduction was similar between the two sessions, showing no statistically significant difference.

Muscle soreness, rated on a 0–10 scale, was significantly elevated during both sessions (ANOVA, time effect $F(6,168) = 359.8$, $p < 0.0001$; session effect $F(1,168) = 427.3$, $p < 0.0001$; interaction $F(6,168) = 54.02$, $p < 0.0001$) **(Figure 1c)**. Peak soreness occurred two days after EMS 1 and one day after EMS 2. While soreness persisted above 1 for five days following the first session, it decreased to below 1 within four days after EMS 2. Overall, discomfort was significantly lower during EMS 2 compared to EMS 1 ($p < 0.0001$).



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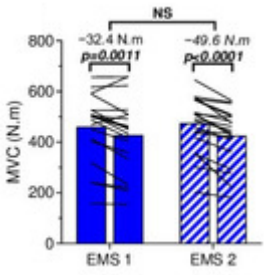

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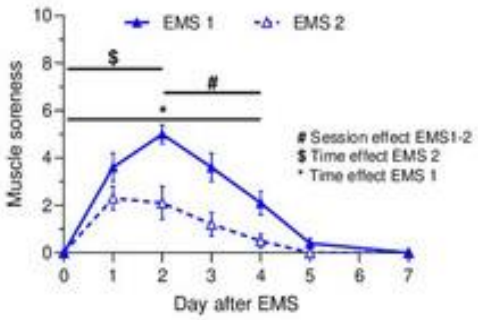

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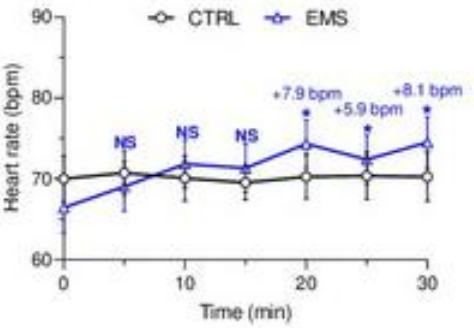

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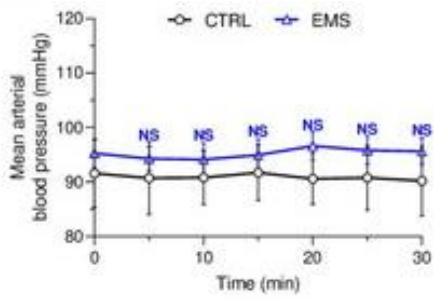

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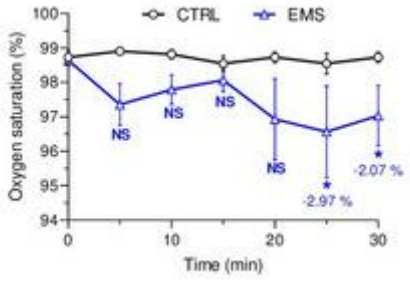

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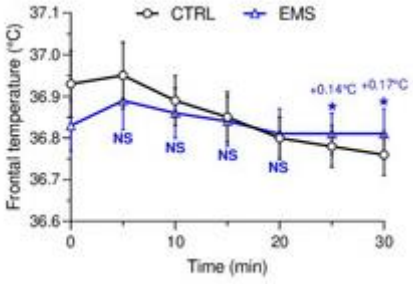

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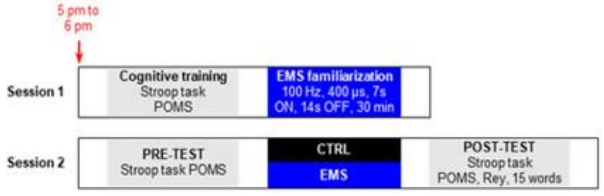
Figure 1. Human physiological responses to EMS.
(a) Electrode placement.
(b) MVC before (left) and after (right) EMS sessions; EMS 1 (solid bars), EMS 2 (hatched bars).
(c) Muscle soreness progression after EMS 1 (solid line) and EMS 2 (dashed line).
(d–g) HR (d), mean arterial BP (e), SaO2 (f), and frontal temperature (g) in CTRL and EMS groups; *p < 0.05 vs. baseline; NS = not significant.

During EMS sessions, heart rate (HR), blood pressure (BP), oxygen saturation (SaO2), and frontal temperature (T°) were continuously recorded **(Figures 1d–1g)**. CTRL participants showed no meaningful changes. In the EMS group, HR increased slightly in the final 10 minutes by 5.9–8.1 bpm (ANOVA, time effect $F(6,266) = 10.06$, $p < 0.0001$; group effect $F(1,266) = 12.57$, $p = 0.0005$; interaction $F(6,266) = 9.802$, $p < 0.0001$). BP remained steady, while SaO2 showed a minor reduction from −2.07% to −2.97% (ANOVA, time effect $F(6,266) = 16.13$, $p < 0.0001$; group effect $F(1,266) = 305.5$, $p < 0.0001$; interaction $F(6,266) = 14.90$, $p < 0.0001$). Frontal temperature remained stable for the EMS group, whereas the CTRL group experienced a slight decline of −0.15 °C during the final 10 minutes (ANOVA, time effect $F(6,266) = 27.23$, $p < 0.0001$; group effect $F(1,266) = 4.575$, $p = 0.0334$; interaction $F(6,266) = 7.221$, $p < 0.0001$).

Cognitive and mood effects of EMS in humans

To investigate potential cognitive and emotional effects of EMS, participants completed two experimental sessions between 5:00 p.m. and 6:00 p.m. **(Figure 2a)**. During the first session, participants performed the Stroop task and completed the POMS questionnaire to familiarize themselves with both the testing and EMS procedure; only the EMS group underwent this familiarization session. Seven days later, participants completed a PRE-TEST assessment (Stroop task and POMS), followed by either a 30-minute EMS session or a rest period, and concluded with a POST-TEST, which included the Stroop task, POMS, a 15-word memory list, and the Rey figure. Recall of the 15-word list was reassessed 24 hours post-EMS.



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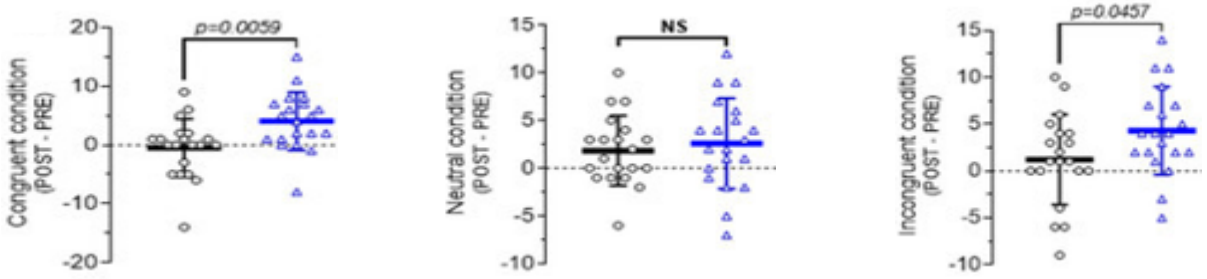

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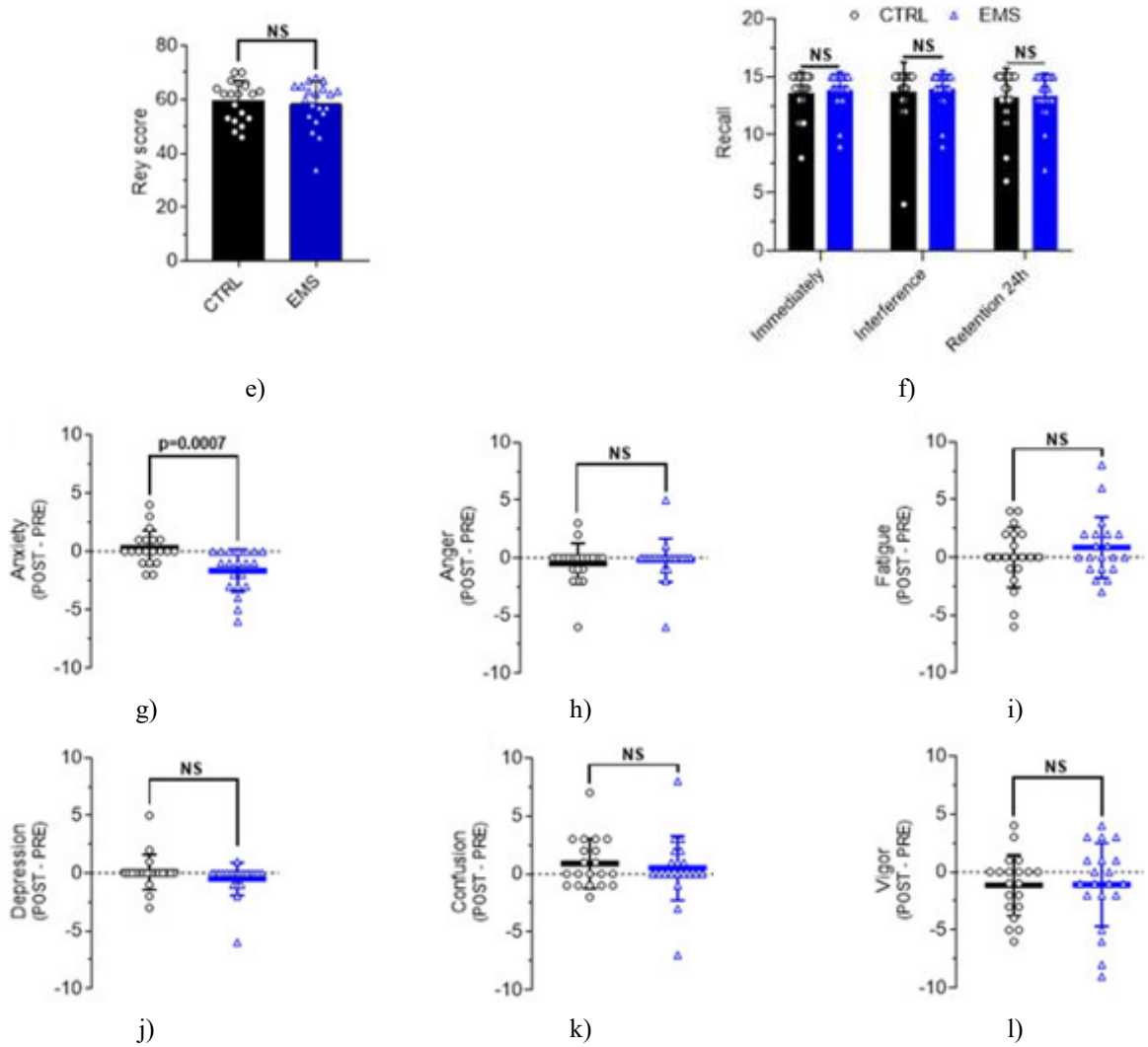


Figure 2. Cognitive and mood outcomes following EMS in humans.
(a) Layout of the human experiment. (b–d) Variation in word counts from POST- to PRE-assessment during the congruent (b), neutral (c), and incongruent (d) Stroop conditions after performing the CTRL task (black) and the EMS intervention (blue). (e) Rey figure scores obtained under double-blind conditions for both CTRL and EMS groups. (f) Total words recalled from the 15-item list immediately after CTRL and EMS sessions, after an interference list, and again 24 h later. (G–L) Shifts in mood-state measures—anxiety (g), anger (h), fatigue (i), depression (j), confusion (k), and vigor (l)—for CTRL (black) and EMS (blue). NS indicates non-significant changes.

EMS produced greater gains than CTRL in both the congruent (+4.55 words, t-test, p = 0.0059; **(Figure 2b)**) and incongruent conditions (+3.1 words, t-test, p = 0.0457; **(Figure 2d)**). The CTRL task produced minimal effects in these conditions (−0.5 word in congruent; +1.2 word in incongruent). In contrast, in the neutral condition, EMS and CTRL yielded similar outcomes (+2.6 vs. +1.8 words; **(Figure 2c)**). Error rates showed no group differences. Memory performance—Rey figure reproduction **(Figure 2e)** and list-learning recall **(Figure 2f)**—did not differ between groups.

For POMS, EMS reduced anxiety compared with CTRL (t-test, p = 0.0007; **(Figure 2g)**). A comparable reduction also occurred during the familiarization session. Other mood domains—anger, fatigue, depression, confusion, vigor—showed no detectable changes **(Figures 2h–2l)**. These findings indicate that brief EMS can enhance executive functioning and decrease anxiety in humans.

Characterization of the EMS protocol in rats

An EMS procedure was developed in rats to mirror the quadriceps-targeted stimulation used in humans. Under isoflurane anesthesia, the hind limbs were shaved, and two electrodes were fixed over the quadriceps **(Figure 3a)**. Current intensity started at 6 mA and was raised progressively to 20 mA. After stimulation, animals were awakened and returned to their cages. Remarkably, electro-stimulated rats exhibited a shorter recovery period compared to SHAM animals (−224 s, t-test, p < 0.0001; **(Figure 3b)**). To habituate the animals to the protocol, a

familiarization EMS session was performed 7 days after arrival. Another EMS session occurred 7 days later, and animals were euthanized either 4 or 24 h afterward **(Figure 3c)**.

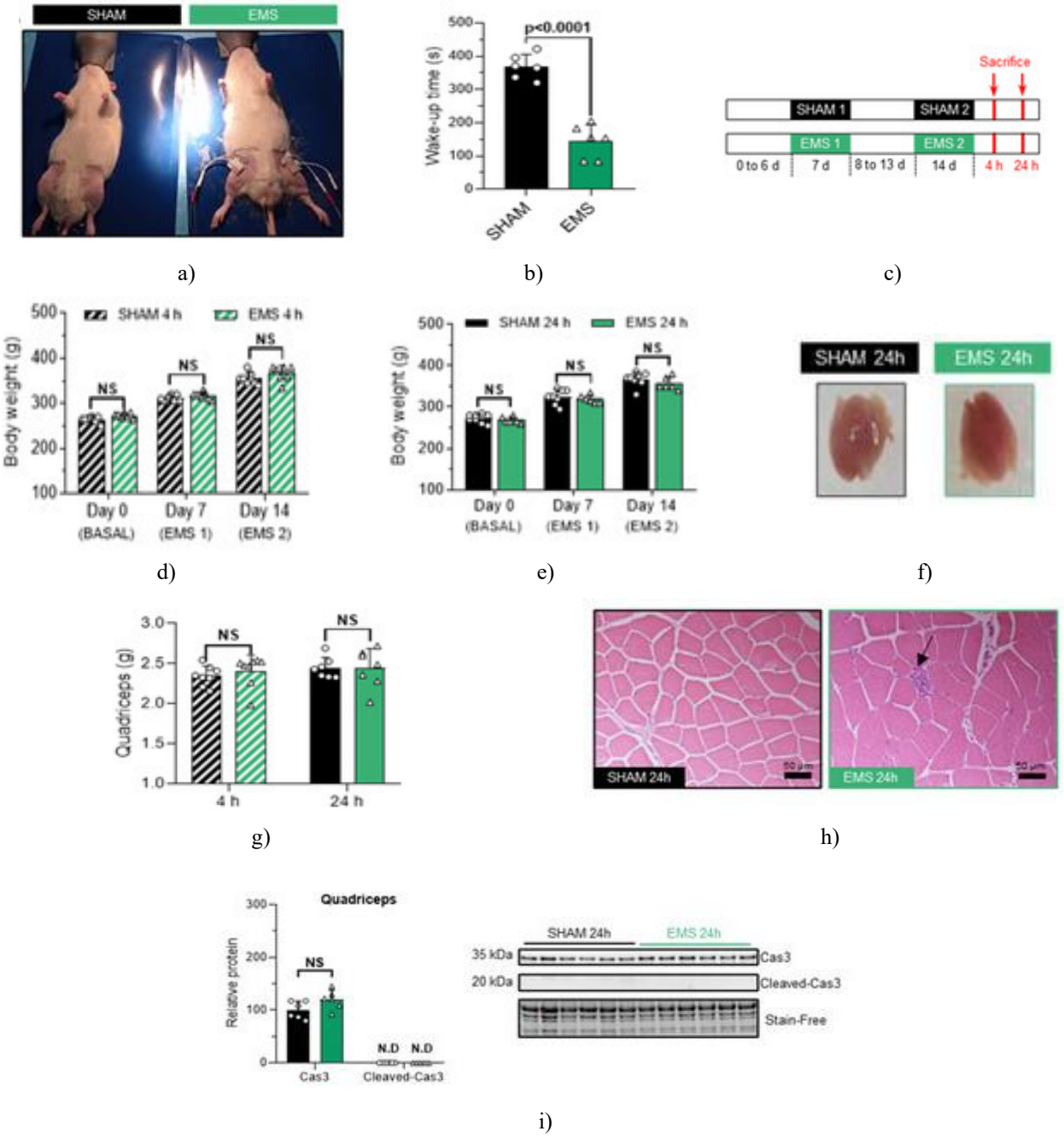


Figure 3. EMS protocol in rats. (a) Example of electrode placement. (b) Wake-up duration for SHAM (black) and EMS (green). (c) Experimental timeline; "d" = day, "h" = hour. (d,e) Body-weight profiles for SHAM and EMS animals, evaluated at 4 h ((E), hatched) or 24 h ((f), full) following EMS or SHAM treatment. (f) Representative quadriceps images 24 h after SHAM or EMS. (g) Quadriceps muscle mass for both groups. (h) H&E staining of quadriceps muscle 24 h after EMS or SHAM. (I) Relative abundance of caspase-3 and cleaved caspase-3 in quadriceps muscle at 24 h. NS = non-significant; N.D. = not detected.

Body weight was recorded from day 0 to 14, with no meaningful differences between SHAM and EMS groups at either the 4 h **(Figure 3e)** or 24 h points **(Figure 3e)**, indicating that the protocol did not cause stress or alter feeding or locomotion. Quadriceps mass and appearance were also unchanged **(Figures 3f and 3g)**. Since EMS has previously been linked to muscle alterations [27]—including sarcomere disruption, increased membrane permeability, and occasional fiber loss—we performed H&E staining to examine inflammatory-cell infiltration. Consistent with reports in human tissue, and in line with processes that clear damaged cells and promote satellite-

cell activity, only minimal inflammatory infiltration was detected **(Figure 3h)**.

Because apoptosis contributes to muscle pathology, we also measured caspase-3 and cleaved caspase-3 levels [28]. Modest tissue effects were confirmed by stable caspase-3 expression and the absence of detectable activated caspase-3 at 24 h **(Figure 3i)**.

Influence of EMS on BDNF and synaptic markers in rats

We evaluated whether EMS modified BDNF expression in the prefrontal cortex and hippocampus. In the prefrontal cortex, RT-qPCR detected no variation in bdnf mRNA 4 h after stimulation **(Figure 4a)**. Western blotting revealed that BDNF protein levels in this region also remained unchanged at both 4 and 24 h **(Figure 4b)**. In contrast, the hippocampus showed a marked rise in bdnf transcript levels 4 h after EMS (+116%, p = 0.0079, t-test; **(Figure 4c)**). Follow-up protein analysis confirmed a substantial increase in hippocampal BDNF 24 h after stimulation (+240%, t-test p < 0.0001; **(Figure 4d)**), while protein levels at 4 h were unaltered.

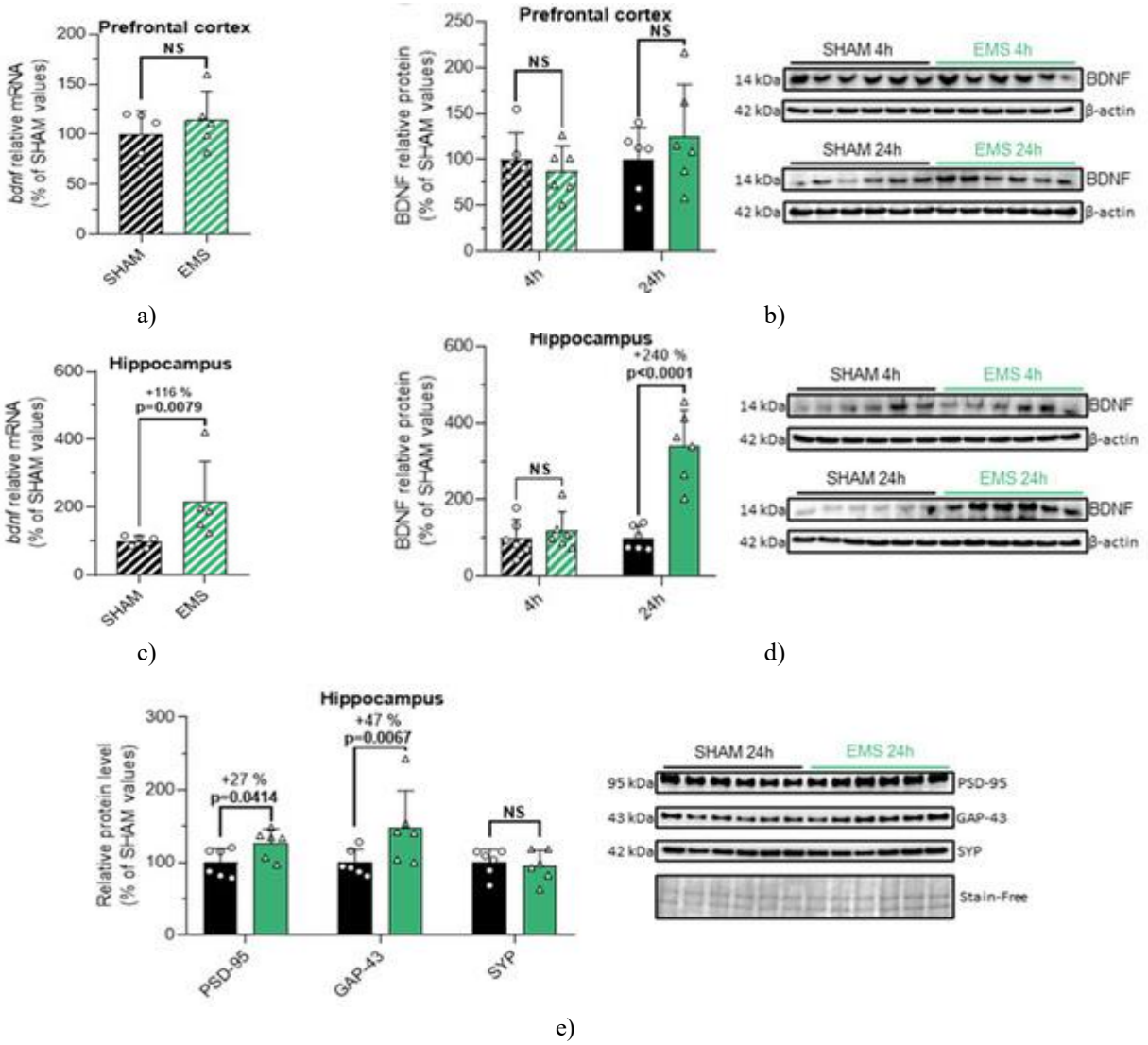


Figure 4. EMS-related shifts in BDNF and synaptic proteins. (a–d) Relative bdnf mRNA in prefrontal cortex (a) and hippocampus (c) 4 h after treatment (hatched bars), and BDNF protein in both regions (b,d) at 4 h (hatched) and 24 h (solid). SHAM = black, EMS = green. (e) PSD-95, GAP-43, and SYP protein levels at 24 h with associated immunoblots. NS = non-significant.

Because BDNF regulates synaptic function, we examined several synaptic components at 24 h, coinciding with the BDNF upregulation. In the hippocampus, PSD-95 rose by +27% (t-test, p = 0.0414) and GAP-43 by +47% (t-test, p = 0.0067), whereas SYP remained unchanged **(Figure 4e)**.

EMS effects on neuronal signaling and vascular-related activity in rats

After confirming the increase in hippocampal BDNF and the behavioral improvements seen in humans, we

investigated possible mechanistic pathways. We quantified c-fos and p-eNOSSer1177 in the hippocampus as indicators of neuronal activation and hemodynamic signaling. Neither marker differed from SHAM at 4 h or 24 h following EMS **(Figures 5a and 5b)**. These results suggest that the BDNF elevation is not driven by direct neuronal or vascular activation, implying involvement of alternative processes such as muscle-to-brain communication.

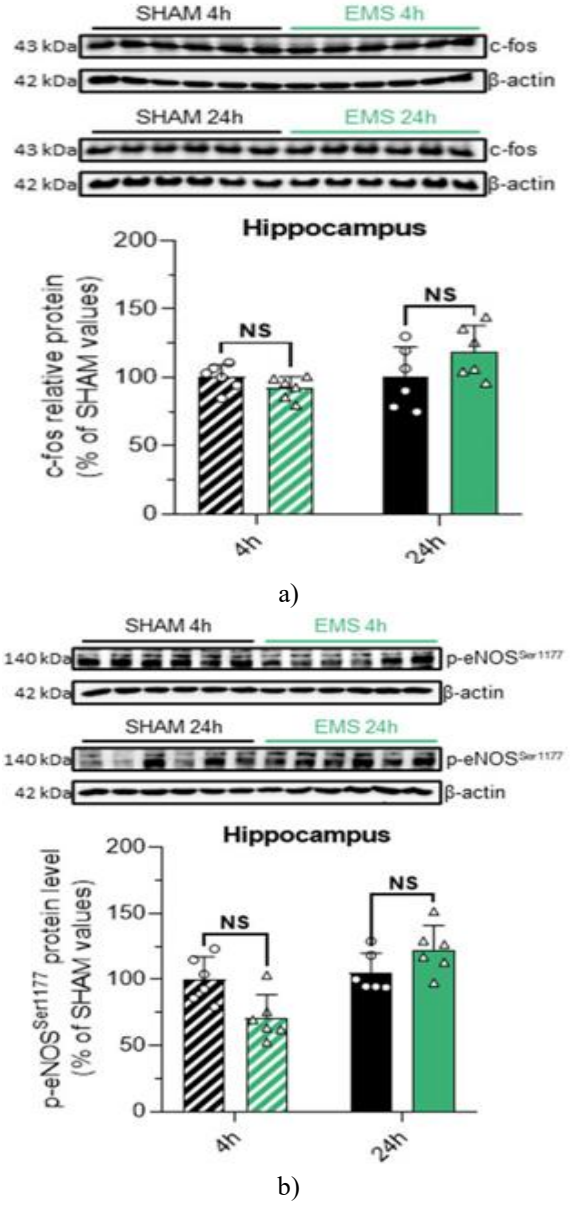


Figure 5. EMS influence on neuronal and vascular markers. (a,b) Protein levels of c-fos (a) and p-eNOSSer1177 (b) in hippocampus at 4 h (hatched) and 24 h (solid) in SHAM (black) and EMS (green). Immunoblots appear above the graphs. NS = non-significant.

EMS effects on muscle-derived and circulating BDNF in rats

Since skeletal muscle has been suggested as a source of BDNF [29, 30], we assessed whether EMS prompted BDNF production in muscle or release into the bloodstream. Quadriceps BDNF protein remained unchanged at both 4 h and 24 h after stimulation **(Figure 6a)**. Circulating BDNF levels also showed no differences—neither immediately after EMS (tail sample; **(Figure 6b)**) nor at 4 h or 24 h (intracardiac sampling; **(Figure 6c)**). Altogether, these data indicate that, in this model, muscle does not contribute to circulating BDNF or to the hippocampal increase.

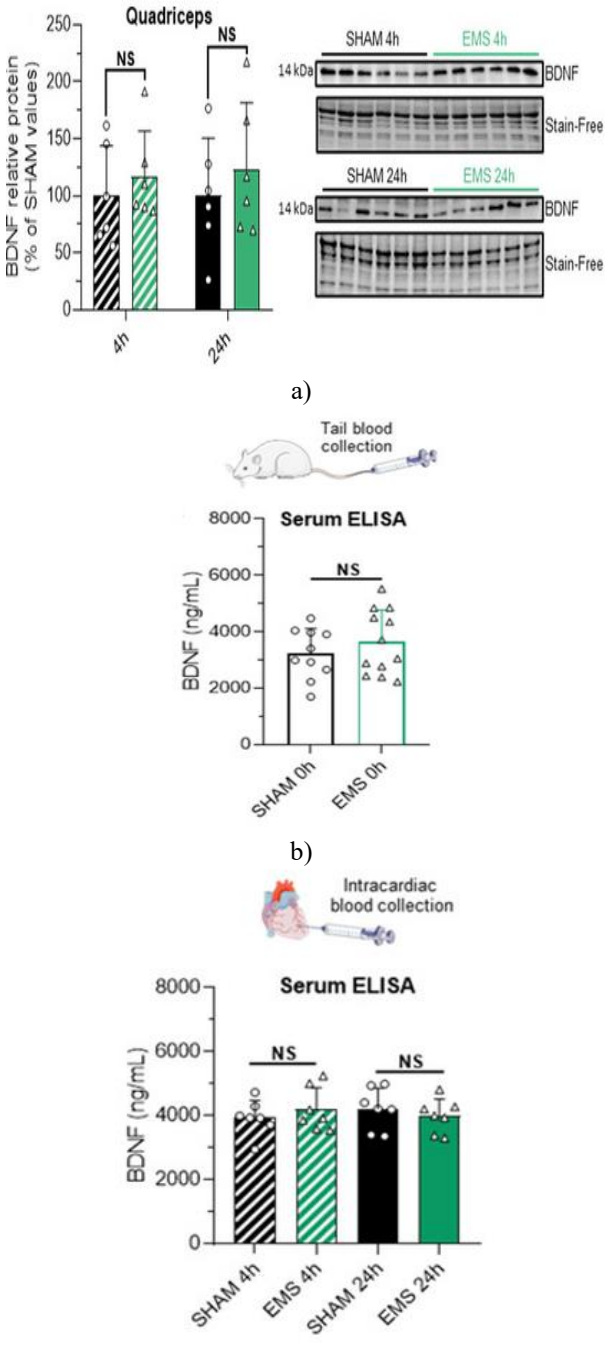


Figure 6. EMS impact on muscle and serum BDNF. (a) Quadriceps BDNF at 4 h (hatched) and 24 h (solid). (b,c) Serum BDNF immediately after EMS (b) and at 4

and 24 h (c). SHAM in black, EMS in green. NS = no significance.

EMS effects on the FNDC5/Irisin pathway in rats

We next focused on irisin, a key myokine involved in muscle–brain interactions. Because irisin originates from the cleavage of FNDC5 and standard immunoblotting detects both, we refer to the measured band (~24 kDa) as FNDC5/irisin, consistent with the literature [13, 31]. In quadriceps and hippocampus homogenates, a single band around 24 kDa was observed. Since FNDC5 is membrane-bound and should not circulate, measurements in serum reflect irisin specifically.

EMS produced a significant rise in quadriceps FNDC5/irisin 24 h after stimulation (+110%, t-test, p = 0.0466; **(Figure 7a)**), with no measurable difference at 4 h. Notably, this muscle increase did not correspond to the hippocampal BDNF elevation measured at the same time point **(Figure 7b)**.

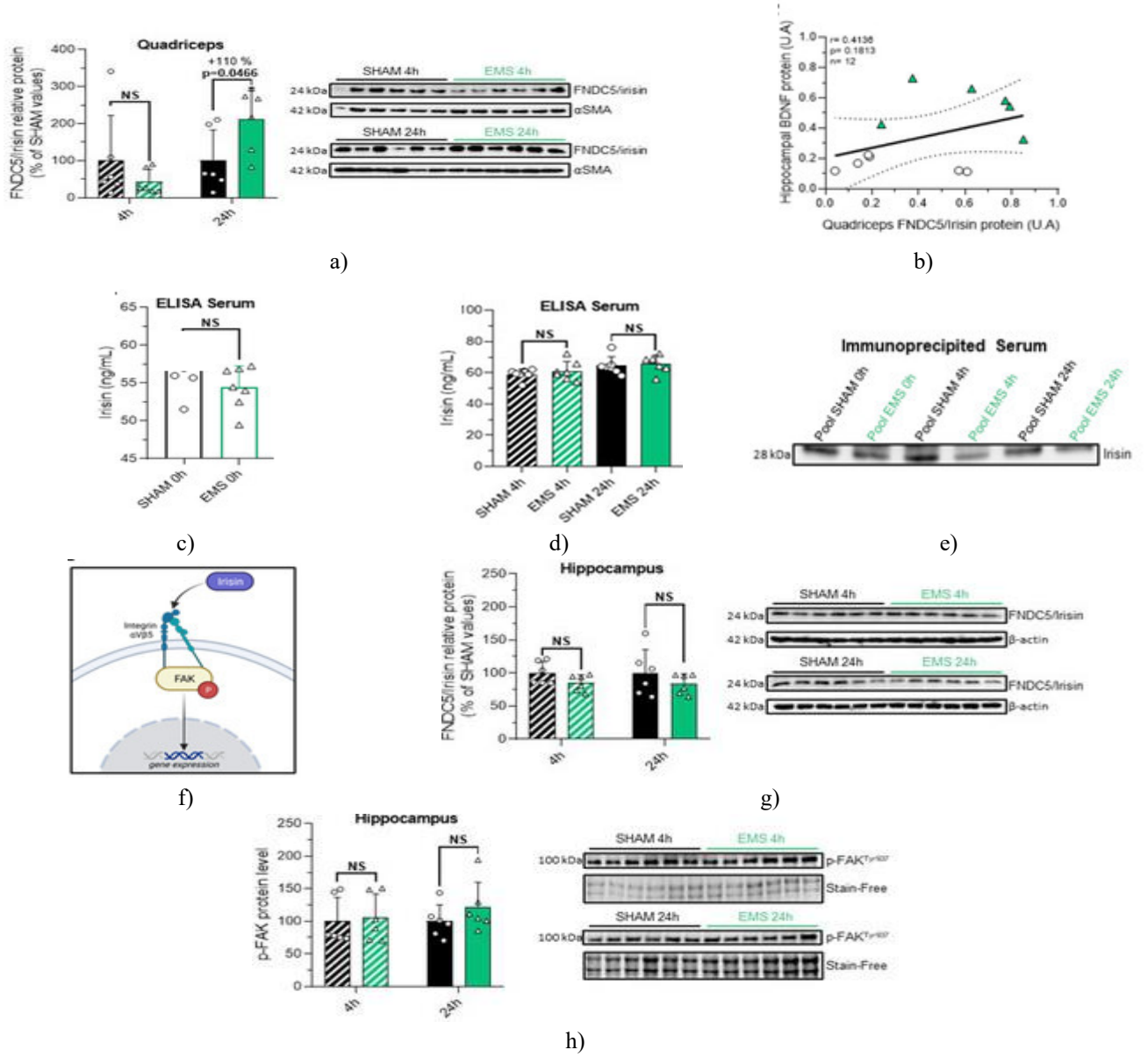


Figure 7. Effects of EMS on the FNDC5/Irisin System. (a) Relative FNDC5/irisin protein abundance in quadriceps muscle at 4 h (hatched) and 24 h (solid) for SHAM (black) and EMS (green).
(b) Spearman correlation comparing hippocampal BDNF and quadriceps FNDC5/irisin at 24 h for SHAM (black circles) and EMS (green triangles).
(c,d) Circulating irisin quantified by ELISA immediately after the procedure via tail sampling (c), and at 4 and 24 h using intracardiac blood collection (d).
(e) Immunoblots of immunoprecipitated pooled serum obtained immediately, 4 h, and 24 h post-procedure.
(f) Illustration of intracellular signaling triggered when irisin binds to its integrin receptor.
(g) FNDC5/irisin protein in the hippocampus at 4 and 24 h.

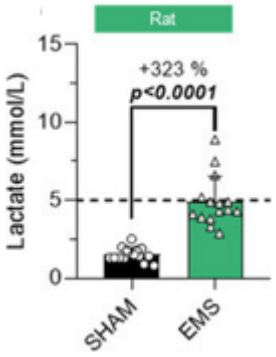
(h) Hippocampal p-FAKTyr397 levels at the same time points. Immunoblots are displayed alongside each graph. NS = non-significant.

Although EMS elevated FNDC5/irisin expression in muscle, circulating irisin did not rise at 0, 4, or 24 h based on ELISA measurements (Phoenix Pharmaceuticals EK-067-29; **(Figure 7c and 7d)**). Because several commercial irisin kits have been questioned for cross-reactivity, we additionally performed immunoprecipitation followed by Western blotting on pooled serum samples. A ~28 kDa band—likely representing glycosylated irisin—was detected **(Figure 7e)**, but its signal intensity did not differ between EMS and SHAM animals, and values immediately and 4 h after EMS even tended to decline.

Since irisin is known to pass the blood–brain barrier and interact with αVβ5 integrins to activate FAK-related signaling [14, 32] **(Figure 7f)**, we also measured hippocampal FNDC5/irisin **(Figure 7g)** and p-FAKTyr397 **(Figure 7h)** at 4 and 24 h. Neither marker changed following EMS. Altogether, these findings indicate that the EMS-induced increase in brain BDNF does not rely on irisin from peripheral tissues or from within the brain.

EMS-driven lactate release in rats and humans

Our quadriceps-focused EMS protocol produced a marked rise in blood lactate in both species—rats showed a +323% increase (t-test, $p < 0.0001$; **(Figure 8a)**), while humans exhibited a +339% rise (t-test, $p = 0.0108$; **(Figure 8b)**). Prior studies have shown that exercise-generated lactate can promote hippocampal BDNF production through a SIRT1-dependent mechanism, where SIRT1 (a NAD^+-dependent deacetylase) modulates multiple transcriptional regulators [33, 34]. In line with this, EMS elevated SIRT1 protein in the hippocampus 4 h after stimulation (+42%, t-test, $p = 0.0410$; **(Figure 8c)**), supporting a role for the lactate/SIRT1 pathway in the BDNF response to EMS.



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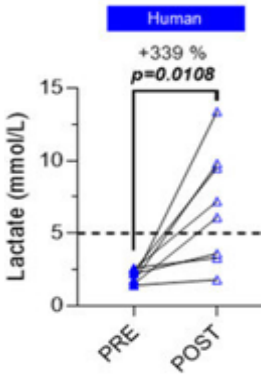

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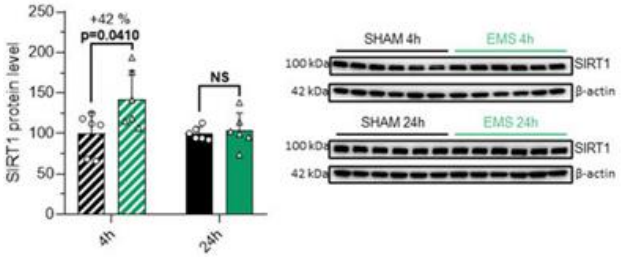

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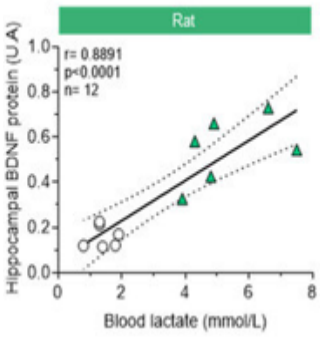

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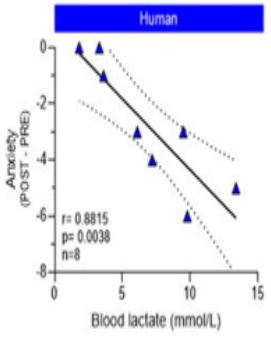

e)

f) g)

Figure 8. Relationship between EMS-induced lactate, hippocampal BDNF in rats, and cognitive/mood outcomes in humans.
(a,b) Blood lactate immediately after EMS in SHAM (black) and EMS (green) rats (a), and before (solid blue triangles) and after (open blue triangles) EMS in humans (b).
(c) Hippocampal SIRT1 levels at 4 h (hatched) and 24 h (solid) in SHAM and EMS groups.
(d) Spearman correlation between hippocampal BDNF at 24 h and immediate post-procedure lactate in rats.
(e–g) Spearman correlations between human lactate values and anxiety (e), congruent Stroop scores (f), and incongruent Stroop scores (g). NS = non-significant.

Supporting the proposed pathway, rat lactate levels strongly correlated with hippocampal BDNF expression at 24 h (Pearson $r = 0.8891$, $p < 0.0001$; **(Figure 8d)**). In humans, lactate production showed an inverse association with EMS-related reductions in anxiety (Pearson $r = 0.8815$, $p < 0.0038$; **(Figure 8e)**). Although not statistically significant, elevated lactate also tended to align with better performance in the congruent ($r = 0.6398$, $p < 0.0875$; **(Figure 8f)**) and incongruent ($r = 0.6468$, $p < 0.0830$; **(Figure 8g)**) Stroop conditions.

The study had two main objectives. The first was to assess whether an EMS protocol could influence human cognitive outcomes and modulate BDNF-related neural plasticity in animals in a way comparable to traditional EX. The second was to clarify contributors to muscle–brain communication. By applying EMS specifically to the quadriceps, we were able to separate the direct effects of muscle contraction from broader physiological reactions—such as elevated neuronal firing, changes in cerebral perfusion, and secretion of exerkines from non-muscular tissues—that typically accompany standard EX protocols [5, 35].

Our results show that EMS in people can improve executive processing, measured through the Stroop test, while leaving memory performance on the Rey complex figure task and a 15-word list unaffected. Emotional state was also influenced, with anxiety scores declining after EMS. Although the cognitive impact in humans was weaker than that produced by conventional exercise, it aligned with findings in rats, which displayed increased BDNF and synaptic markers in the hippocampus. Importantly, the rise in hippocampal BDNF in animals occurred without corresponding increases in neural activation, cerebral circulation, or muscle-derived BDNF and FNDC5/irisin. Instead, both the behavioral gains in humans and the BDNF elevation in rats were positively associated with lactate levels, suggesting that lactate may be a plausible mediator of hippocampal BDNF regulation under EMS.

To date, this is the first report showing that EMS can reduce anxiety and enhance executive control in humans, reflected by significant improvements in both congruent and incongruent Stroop conditions. Earlier work using 30-min EMS sessions failed to show such effects across several tasks (Stroop, Go/No Go, Wisconsin Card Sorting Test) [36, 37]. The inconsistency may stem from the very low stimulation frequency used previously (4 Hz), which produces only light, sensory-level twitches. Our approach employed 100 Hz high-frequency stimulation to evoke tetanic contractions, consistent with evidence supporting its effectiveness for boosting muscle strength and hypertrophy [38, 39]. Although participants reported soreness after the initial session, this diminished after the second treatment, and the protocol ensured strong muscle engagement. Sensory differences between low- and high-frequency EMS likely influence arousal differently—mild twitching may feel calming, whereas sustained tetanic activity may increase alertness. This aligns with the idea that acute EX can modulate cognition via arousal in a manner dependent on exercise intensity [40, 41].

Compared with conventional EX, EMS reproduced only part of its overall impact. For instance, high-intensity EX has been shown to improve Stroop performance across all conditions and to influence more emotional domains [42], reducing not only anxiety (as we observed with EMS) but also depression, confusion, and anger after a single EX session [2]. Such differences likely reflect disparities in the amount of muscle involved and the physiological

pathways activated. Heart rate (HR) rose only minimally with EMS, whereas conventional EX—depending on workload—can cause a substantial HR increase [43]. Blood pressure (BP) remained unchanged with EMS, whereas EX can raise BP by up to 30% [42]. Likewise, EX may reduce SaO_2 by more than 5% [44], while EMS in our study produced only a 2% drop. Further research using within-subject designs will be essential to refine stimulation parameters and determine how EMS can best support cognitive outcomes, especially for long-term protocols.

As noted earlier, elevated brain BDNF is widely considered a reliable indicator of cognitive performance, given its well-established importance in both humans and animal models during EX. By applying an identical EMS protocol across species, our objective was to clarify the underlying molecular pathways, focusing specifically on this neurotrophin in two cognition-related regions: the prefrontal cortex and the hippocampus. We observed that EMS did not influence the prefrontal area, but it did produce an increase in bdnf mRNA at 4 h and in BDNF protein at 24 h within the hippocampus. A similar regional dissociation in BDNF responsiveness has been described following low-intensity EX in rodents [45]. Another explanation may lie in the isoflurane anesthesia regimen, which can suppress cutaneous sensory feedback and nociceptive input [46, 47]—signals known to engage the prefrontal cortex during EMS in humans [48]. In contrast, our hippocampal results align with previous studies using functional electrical stimulation that mimicked gait [49], sciatic nerve activation [50], or stimulation of biceps and triceps brachii [51], all demonstrating enhanced hippocampal BDNF. These findings are also consistent with acute EX reports showing increased bdnf mRNA at 2 h and 6 h after a single treadmill bout [52]. Additionally, hippocampal BDNF elevation coincided with increased levels of PSD-95 and GAP-43, while SYP remained unchanged. Collectively, our results and existing literature suggest that an acute EMS stimulus can reproduce the hippocampal BDNF response typically seen after acute EX.

To determine how EMS triggers hippocampal BDNF elevation, we examined the three major routes implicated in BDNF upregulation after EX. We first assessed neuronal activation and cerebral perfusion, both of which rise during classical EX [5, 45, 53]. Using c-fos as a surrogate for neuronal activity [54] and phosphorylated eNOS Ser1177 as an indicator of increased blood flow [55], we detected no EMS-related variations in these markers at either 4 h or 24 h. Although not included in our human protocol, neuroimaging studies document sensorimotor cortical activation during EMS [56, 57] but do not show engagement of cognitive regions such as the hippocampus, whereas EX in humans activates the hippocampus for as long as 20 min post-exercise [58].

We next tested whether the hippocampal response might be driven by myokines released due to localized muscle contraction. We first examined BDNF and FNDC5/irisin expression in the quadriceps. BDNF can be synthesized in muscle by several cell types [30, 59] and contributes to regeneration and metabolic regulation [60–62]. More recent evidence suggests that muscle-derived BDNF can act hormonally to influence pancreatic glucose regulation [29], while peripheral BDNF delivery has been shown to promote neurogenesis and elevate hippocampal BDNF [63]. Exercise is known to increase bdnf mRNA and/or BDNF protein in skeletal muscle [64], and EMS has been associated with increased systemic BDNF in both humans [65, 66] and animals [51]. However, in our experimental context, neither quadriceps expression nor circulating BDNF levels changed after EMS, discounting a muscle-origin BDNF contribution to hippocampal BDNF elevation.

We then turned to FNDC5/irisin, since its activation in skeletal muscle has been strongly linked to EX-induced changes in brain plasticity [13]. Although quadriceps FNDC5/irisin protein levels rose significantly at 24 h post-EMS, measurements from ELISA and immunoprecipitation at 0 h, 4 h, and 24 h did not show any corresponding increase in serum irisin. This highlights that FNDC5 synthesis and its cleavage into irisin are distinct processes, with the latter depending on an unidentified protease. Thus, some forms of EX may increase FNDC5 production without activating the enzyme required to release irisin into circulation. Because FNDC5/irisin is also produced centrally, we assessed hippocampal expression but detected no EMS-related changes. To further explore this pathway, and given that irisin acts through αVβ5 integrin receptors and downstream FAK signaling [32], we analyzed FAK phosphorylation. No alterations were detected at 4 h or 24 h. Taking these findings together—and given the absence of any link between muscle FNDC5/irisin and hippocampal BDNF—our results suggest that neither peripheral nor central irisin explains the increased hippocampal BDNF observed following EMS.

Finally, we focused on lactate—a muscle-derived metabolite—because prior work has linked it to BDNF production in the brain [20]. Similar to observations in humans, our data showed that the EMS protocol caused a pronounced and comparable elevation in blood lactate in rats. Notably, Hayek and colleagues demonstrated that blocking lactate transport into the brain with the monocarboxylate transporter inhibitor AR-C155858 completely prevented the EX-induced rise in hippocampal bdnf transcription, whereas injecting lactate intraperitoneally produced a hippocampal BDNF increase

equivalent to that seen in trained animals. Mechanistically, lactate has been shown to mediate the influence of EX on learning and memory through SIRT1-dependent enhancement of hippocampal BDNF [20]. In line with this model, we observed a rise in SIRT1 expression 4 h after EMS. Beyond SIRT1 activation, lactate may also favor BDNF upregulation through modulation of NMDA receptor activity [67] or via histone lactylation, a recently identified epigenetic mechanism influencing transcription [68], although this has not yet been demonstrated for the BDNF promoter specifically. Importantly, in our work, hippocampal BDNF levels in rats showed a positive association with blood lactate. In humans, a significant inverse relationship emerged between lactate and anxiety scores, and a positive tendency was detected for both congruent and incongruent Stroop performance. These findings parallel earlier results linking EX-related cognitive enhancement to the arteriovenous lactate gradient in the brain [21], highlighting lactate as a strong candidate underlying the behavioral and molecular benefits seen with EMS. Although correlational analyses cannot establish causality, our data collectively support the idea that circulating lactate may act as an intermediary between EMS and BDNF-driven neuroplasticity in rats, while also contributing to EMS-related cognitive advantages in humans.

Materials and Methods

Human cohort and experimental overview

Research involving human participants received approval from the scientific committee of the Faculty of Sports Sciences at the University of Burgundy (CPP EST, approval A00064-49) and adhered to the principles of the Helsinki Declaration. All individuals were fully briefed about procedures, risks, and potential discomfort, and written informed consent was obtained before inclusion.

For the EMS application, participants sat in a chair equipped with ankle restraints to maintain an isometric contraction of the quadriceps. Two 5 × 5 cm electrodes (Fyzéa, BAS95050, La Roche-sur-Yon, France) were placed on the vastus lateralis and vastus medialis, and a 5 × 10 cm electrode was positioned over the rectus femoris to elicit quadriceps activation. These were connected to a stimulator (Cefar Rehab X2, 111126) programmed at 100 Hz, 0.4 ms pulse width, and cycles of 7 s ON / 14 s OFF for 30 min. Stimulation intensity was gradually increased from 6 mA up to each participant's maximal tolerable level (ranging from 26 to 99 mA across individuals).

Participant characteristics are summarized in **Table 1**. Individuals with neurological, psychiatric, cardiovascular, or metabolic disorders, or taking medications that could influence physiological measurements, were excluded. Eligible participants had a BMI between 18 and 25. In the first study (cohort 1; n = 40), we assessed acute cognitive responses to EMS. Each subject completed two laboratory visits spaced 7 days apart: one for familiarization with EMS and cognitive tasks, and a second to evaluate EMS-induced cognitive effects. Physiological variables were recorded during EMS. In a second experiment involving 8 participants (cohort 2), the same protocol was repeated with the addition of lactate measurements.

Table 1. Participant information.

Cohort	Cohort 1 (Control)	Cohort 2 (EMS)	EMS Subgroup
Treatment Group	CTRL	EMS	EMS
Total participants	20	20	8
Male	14	14	6
Female	6	6	2
Age (years)	22.2 ± 3.4	24.8 ± 2.5	24.2 ± 1.7
Body Mass Index	21.7 ± 2.2	21.6 ± 1.9	22.5 ± 3.0
Caffeine consumers	8	8	3
Smokers	2	2	1

BMI: Body mass index.
Caffeine: number consuming caffeine on test day.
Smoke: number reporting habitual tobacco use.

Physiological monitoring in humans

Throughout the 30-min EMS session, we continuously recorded heart rate (HR) and blood pressure (BP) using a BP monitor (BP 3NZ1-3P, Torm Copenhagen, Denmark), oxygen saturation (SpO_2) via a pulse oximeter (SaO_2, YK-81CEU, Braun, Kronberg-im-Taunus, Germany), and body temperature changes using a forehead infrared thermometer (T°, FH2—Thomson, Thermo, Issy-les-Moulineaux, France).

Neuropsychological assessment in humans

Cognitive outcomes following EMS were examined through tasks measuring executive control (Stroop), episodic learning (15-word list), and visuospatial memory (Rey figure). Effects on mood were also evaluated using the POMS questionnaire.

For the Stroop assessment, participants completed three separate sheets, each containing 100 items laid out in 10 × 10 grids.

- Sheet 1 displayed the color words bleu, rouge, jaune, and vert in black.
- Sheet 2 used XXXX printed in blue, red, yellow, or green.
- Sheet 3 showed color words printed in incongruent ink colors (e.g., bleu in yellow), requiring the participant to identify the ink color.

Performance was quantified as the number of items read in 45 s and the number of mistakes. During a familiarization session 7 days earlier, participants performed three 45-s trials for each sheet to limit practice effects. In the test session, they completed one block per sheet both before and after EMS.

In the Rey–Osterrieth complex figure, subjects copied the design prior to stimulation and reproduced it from memory immediately afterward. The score, ranging up to 72 points, was assigned under double-blind conditions.

The 15-word list task was administered directly after EMS. After hearing 15 items (list A) for 3 min, participants attempted free recall. They then received 15 different words (list B) for another 3 min, followed by recall of B and then A to assess interference effects. A delayed recall of list A occurred 24 h later by telephone. All participants received identical A and B lists.

For the POMS, individuals rated their recent feelings for 35 adjectives on a scale from 0 to 4, capturing six emotional domains: anxiety, depression, anger, vigor, fatigue, and confusion. The form was completed before and after EMS.

Animals and experimental design

All procedures followed regulations from the French Ministry of Agriculture (license 21-CAE-102) and of Research (APAFIS #333000), with approval from the Dijon ethics committee (105). Protocols were designed to minimize animal distress.

Experiments used adult male Wistar rats obtained from Janvier Labs. Animals were housed five per cage on a 12-h light/dark cycle, with free access to food and water. Environmental conditions were controlled: temperature 20–24 °C and humidity 45–65%, within ventilated racks.

EMS procedures began under isoflurane (5% induction; 2.5% maintenance). After shaving the thighs, electrodes—adapted versions of those used in humans—were fixed to the upper and lower quadriceps. The stimulator (same model as in humans: Cefar Rehab X2, 111126) delivered 100 Hz, 0.4 ms pulses in cycles of 7 s on / 14 s off for a 30-min session. Current intensity was progressively adjusted between 6 and 20 mA to maintain consistent contractions.

After 7 days of handling habituation, rats received an initial EMS familiarization session, followed by a second session 7 days later. Following the second session, animals were euthanized either 4 h (n = 16) or 24 h (n = 14) afterward. SHAM rats underwent identical procedures without stimulation.

Euthanasia occurred under 5% isoflurane, followed by intracardiac infusion of 0.9% NaCl. The quadriceps, prefrontal cortex, and hippocampus were dissected. Right quadriceps were fixed in 4% PFA; left quadriceps and left brain regions were snap-frozen in liquid nitrogen and stored at −80 °C. Blood was obtained via the heart or tail cut, centrifuged at 2000× g for 15 min at 4 °C, and serum was stored at −80 °C.

Quantitative real-time PCR

Total RNA from the prefrontal cortex and hippocampus was isolated using NucleoSpin RNA Set for NucleoZol (740406.50). cDNA synthesis used the iScript kit. qPCR was performed with PowerUp SYBR Green on a StepOnePlus™ system. Relative gene levels were calculated via ΔΔCt, normalizing to β-actin and 18S mRNA. Primers **(Table 2)** were sourced from Thermo Fisher Scientific.

Table 2. Primers employed.

Gene Name	Forward	Reverse
Bdnf total	TACCTGGATGCCGCAAACAT	TGGCCTTTTGATACCGGGAC
β-actin	ATGGAGGGGAATACAGCCC	TTCTTTGCAGCTCCTTCGTT
18S	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Western blot analysis

Proteins were extracted using the Precellys system at 4 °C (Berlin Technologies) with 7 volumes of lysis buffer (100 mM Tris base, 150 mM NaCl, 1 mM EGTA, 1% Triton X100, protease and phosphatase inhibitors, pH 7.4). Samples were sonicated for 15 s and centrifuged at 15,000×g for 20 min at 4 °C. Protein concentration in the supernatant was determined via the Lowry assay (Lowry

Pierce™, Thermo Fisher Scientific). Aliquots were mixed with 2× Laemmli buffer (125 mM Tris, 4% SDS, 20% glycerol, 0.01% bromophenol blue) and stored at −80 °C. Proteins were separated on stain-free SDS-PAGE gels (TGX Stain-Free FastCast Acrylamide kit, Biorad) and transferred to PVDF or nitrocellulose membranes (Biorad) using the TransBlot system (Biorad). Total protein imaging was acquired with the ChemiDoc system (Biorad). Membranes were blocked in 5% non-fat milk for 1 h at room temperature (RT) and incubated overnight at 4 °C with primary antibodies **(Table 3)**. Secondary HRP-conjugated antibodies, anti-rabbit (111-035-144) or anti-mouse (115-035-166, Jackson ImmunoResearch), were applied for 1 h at RT. Signal detection was performed using Clarity ECL substrate (Biorad) and imaged with ChemiDoc. Band intensities were quantified using ImageLab software v6.0.1 (Biorad), normalizing to stain-free total protein, β-actin, or αSMA.

Table 3. Primary antibodies used.

Protein Target	Antibody Details	Dilution
BDNF	Abcam recombinant anti-BDNF rabbit monoclonal [EPR1292] (ab108319)	1/3000 TBST-Milk (5%)
Cleaved Caspase-3	Cell Signaling Technology, D3R6Y rabbit mAb #14220	1/3000 TBST-Milk (5%)
c-fos	GeneTex rabbit polyclonal anti-c-fos (GTX129846)	1/3000 TBST-Milk (5%)
FNDC5 / Irisin	Abcam recombinant anti-FNDC5 rabbit monoclonal [EPR12209] (ab174833)	1/3000 TBST-Milk (5%)
GAP-43	Cell Signaling Technology GAP-43 (D9C8) rabbit mAb #8945	1/3000 TBST-Milk (5%)
phospho-eNOS (Ser1177)	BD Transduction Laboratories™ mouse anti-phospho-eNOS (pS1177) (612392)	1/3000 TBST-BSA (7.5%)
phospho-FAK (Tyr397)	Cell Signaling Technology phospho-FAK (Tyr397) rabbit polyclonal #3283	1/3000 TBST-BSA (7.5%)
PSD-95	Cell Signaling Technology PSD-95 (D27E11) XP® rabbit mAb #3450	1/3000 TBST-Milk (5%)
SIRT1	Cell Signaling Technology SIRT1 (D1D7) rabbit mAb #9475	1/3000 TBST-Milk (5%)
Synaptophysin (SYP)	Interchim rabbit polyclonal anti-synaptophysin (RB-1461-P1)	1/3000 TBST-Milk (5%)
α-Smooth Muscle Actin (αSMA)	Abcam mouse monoclonal anti-α-smooth muscle actin [Alpha Sr-1] (ab28052)	1/3000 TBST-Milk (5%)
β-actin	Sigma-Aldrich mouse monoclonal anti-β-actin clone A5441	1/3000 TBST-Milk (5%)

ELISA

Serum BDNF (BEK-2211-2P, Biosensis) and irisin (EK-067-29, Phoenix Pharmaceuticals) levels were quantified according to manufacturer instructions. Measurements were done in duplicate; results were valid if the coefficient of variation was below 10% and positive controls fell within the provided range.

Immunoprecipitation

Irisin in rat serum was enriched by immunoprecipitation. Protein A magnetic beads (Dynabeads, 10002D, Thermo Fisher Scientific) were incubated with anti-FNDC5 antibodies for 10 min at RT on a HulaMixer™ (15920D). Bead–antibody complexes were separated using DynaMag™-2 magnets (12321D) and incubated with pooled rat serum for 20 min at RT. After three PBS washes, complexes were eluted with 20 µL of 2× Laemmli buffer at 95 °C for 10 min. The supernatant was applied to SDS-PAGE.

Muscle histology

Quadriceps fixed in 4% PFA were paraffin-embedded and cut into 5 µm sections. Sections were deparaffinized in xylene, rehydrated through ethanol gradients, and stained with Harris hematoxylin (Leica 3801562E) and eosin Y (Leica 3801601E).

Blood lactate measurement

Blood lactate was assessed immediately after EMS (0 h) using Lactate Pro II. Samples were collected via fingertip lancet in humans or tail incision in rats.

Data analysis and statistics

GraphPad Prism v8.0.1 was used for statistical analyses. Data are expressed as mean ± SD. Normality was tested with the Shapiro-Wilk. Single-variable comparisons between SHAM and EMS rats or CTRL and EMS humans were done using Student's t-test or Mann–Whitney test, depending on normality. Multiple time-point comparisons were analyzed via one-way ANOVA. p-values <0.05 were deemed significant.

Conclusion

Our findings indicate that EMS is an effective alternative to traditional exercise for enhancing cognitive

performance. This is particularly relevant for patients unable to engage in conventional exercise, such as bedridden individuals or those with obesity, chronic heart failure, COPD, or stroke-related motor deficits. These results provide a basis for future studies aiming to optimize EMS parameters for chronic use in patients with cognitive impairments. Moreover, while additional studies are needed, including lactate transporter inhibition or manipulation of blood lactate under EMS conditions, our data support lactate as a key signaling molecule in the muscle–brain communication mediated by EMS. Lactate has the advantage of being more easily modifiable compared to other myokines.

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Conflict of interest: None

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Ethics statement: This study was conducted in accordance with the Declaration of Helsinki and approved by the ethic committee of the Faculty of Sports Sciences of the University of Burgundy in Dijon (CPP EST: approval number A00064-49).
Informed consent was obtained from all subjects involved in the study

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