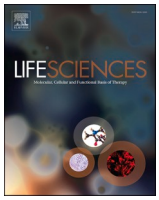
Life Sciences 374 (2025) 123710

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie



Physical exercise ameliorates pain, mood alterations and neuroinflammation in a murine model of osteoarthritis

G. Amodeo [1,*], G. Galimberti [1], S. Ceruti, B. Riboldi, S. Franchi [2], P. Sacerdote [2]

Department of Pharmacological and Biomolecular Sciences, Laboratory of Pain Therapy and Neuroimmunology, Università degli Studi di Milano, Milan, Italy

ARTICLE INFO

Keywords:
Anxiety
Depression
Murine model
Neuroinflammation
Pain
Knee joint

ABSTRACT

Osteoarthritis (OA) is largely associated with chronic pain, and it is a social and economic problem worldwide. OA pain can lead to psychiatric symptom onset, such as anxiety and depression. Emerging evidence suggest neuroinflammation as a common denominator between OA pain and mood disorders. OA pain pharmacological strategies are often ineffective or cause side effects, therefore, identifying novel non-pharmacological therapeutic approaches, like diet and exercise, could be important.

Here, using 8-month-old male C57BL/6 J mice, we evaluated the effect of regular physical exercise on OA pain and related comorbidities. OA was induced by monoiodoacetate-(MIA) administration in knee joint in mice that had undergone physical exercise-(PE) or a sedentary lifestyle-(SL) for two months. After OA induction, mice continued the exercise/not-exercise protocol for another month and subsequently all animals were sacrificed. An in-depth biochemical evaluation (RT-qPCR) was performed at the level of the knee joint, sciatic nerve, dorsal root ganglia, spinal cord and brain areas (brainstem, hypothalamus and hippocampus) and myenteric plexus to evaluate the effect of both OA and exercise on (neuro)inflammation in regions involved in pain and mood regulation. Throughout the experimental protocol, pain-like behavior and motor performance were evaluated, and before sacrifice, mood alterations and metabolic changes were also assessed.

Our results showed that OA_SL mice exhibit painful symptoms and mood disturbances. These alterations were also associated with (neuro)inflammation. PE mitigates pain-like behavior and mood alterations and reduces (neuro)inflammation, suggesting that a healthy and active lifestyle may have a positive impact in preventing and/or counteracting OA onset and related comorbidities.

1. Introduction

Osteoarthritis (OA) affects millions of people worldwide [1]. OA is more prevalent in older people (about 70 % are over the age of 55), even if the World Health Organization has stated that its onset is lowering, around the fourth decade of life. OA leads to bone changes, deterioration of connective tissue, and initial inflammation of the joint lining, which triggers a systemic inflammatory condition and chronic pain [2]. Recent studies highlight the critical role of synovial inflammation in OA progression and pain development [3–5]. This inflammation causes the release of chemokines, cytokines, and proteases, sensitizing primary afferent fibers in both the joint and adjacent tissues [4,6–8]. This, in turn, determines a central sensitization in the dorsal horns of the spinal cord, where neurons heightened responses to stimuli in both nearby and distant regions from the joint, expanding the receptive field. These changes represent the neuronal basis of primary (at the disease site) and secondary (in distant areas from the joint) nociceptive alterations [7]. Therefore, OA is a degenerative disease that worsens over time, and chronic pain can become severe enough to make patients' daily activities difficult. Moreover, anxiety, depression and sleep disturbances are also present [9–11], impacting the patient's quality of life and further aggravating pain perception, leading to the establishment of a self-perpetuating vicious circle [12,13]. The relationship between OA pain and mood disorders is reported not only in clinical, but also in some preclinical studies, including in OA murine models [14–20]. Chronic pain triggers neural changes likely responsible for affective and cognitive alterations [21–23], where the common denominator seems to be (neuro)inflammation [3,24,25]. Indeed, also in brain areas, increased

* Corresponding author.
E-mail address: giada.amodeo@unimi.it (G. Amodeo).
[1] These authors contributed equally as first author.
[2] These authors contributed equally as last author.

https://doi.org/10.1016/j.lfs.2025.123710
Received 7 January 2025; Received in revised form 30 April 2025; Accepted 9 May 2025
Available online 11 May 2025

levels of pro-inflammatory molecules, including cytokines [26–28], may participate in maladaptive neural reorganization, contributing to both the sensory and affective components of chronic pain [24,29]. Recent evidence indicates that OA progression may also be related to the establishment of chronic/persistent and low-grade systemic inflammation. This inflammatory condition could trigger generalized metabolic stress, which negatively may worsen OA itself and aggravating systemic inflammation, lead to the development of other pathologies [30,31]. Controlling inflammation may be a winning strategy to modulate both OA progression and neuroinflammation onset (thereby controlling pain and related mood disorders). Currently, OA treatment includes surgery, drug therapy and physical therapy [32]. In recent years, the treatment of some diseases by exercise has been receiving more and more attention among researchers. Exercise is a cost-effective and efficacious treatment [33]. In addition to the noted positive effect of regular physical exercise [34], it can also relieve pain [35–38] and could therefore counteract/block the onset of related psychiatric disorders [39,40]. However, the underlying mechanisms are not yet fully understood [41]. Although the effects of different types of exercise on OA have been widely studied through observational clinical studies [33], preclinical studies offering the possibility of understanding the molecular processes involved are still lacking.

In this study, we investigated the effect of physical exercise on pain, mood alterations and (neuro)inflammation in monosodium-iodoacetate (MIA)-induced OA model, the most widely used model to evaluate pain and possible therapies in rodents [42].

2. Material and methods

In this study, 8-month-old C57BL/6 J male mice (n = 32 - Charles River Laboratories, Italy) were used. Animals were housed 2 per cage (Macrolon type II cages, equipped with sawdust bedding, nesting and environmental enrichment), in standard conditions (i.e. 12 h of light/dark, temperature 22 ± 2 °C and humidity 55 ± 10 %) and with food and water ad libitum. All mice were acclimated for 2 weeks at the animal facility and accustomed to handling (few minutes/day). In addition, mice were subjected to periodic veterinary checks, to assess well-being and health conditions. In all experiments, ARRIVE guidelines and 3R principles were followed. All procedures were conducted in accordance with EU Directive 2010/63/EU and approved by the Committee for the Care and Use of Animals of the Italian Ministry of Health (authorization number 180/2020 to PS).

2.1. Experimental protocol

As reported in the schematic experimental protocol, Fig. 1, after two weeks of acclimatization to the animal facility, animals (n = 32) were randomized into two experimental groups (from the online source Graphpad - Randomly assign subjects to treatment groups). Half of the animals (n = 16) remained sedentary (sedentary lifestyle group, SL), while the remaining (n = 16) were subjected to active physical exercise (physical exercise group, PE). In detail, SL mice were not subjected to forced physical activity/exercise; they were only placed in the switched-off treadmill and movement within the home cage remained free/standard, without any limitation. Instead, PE animals were subjected to group running sessions 3 times a week for 8 weeks on a treadmill (see below for details). After this period, at the age of 10–11 months (i.e. transition from mature adult to middle age), OA was induced in half of the animals in each experimental group by intra-articular administration of monoiodoacetate (MIA, 1 mg in 10 μl saline) in the right knee (n = 8 OA_SL group; n = 8 OA_PE group); the other half of the animals in each experimental group were treated with vehicle (saline, 10 μl; n = 8 CTR_SL group; n = 8 CTR_PE group). After OA induction both OA_PE and CTR_PE experimental groups continued physical exercise for another 4 weeks, while SL groups (OA_SL and CTR_SL) remained sedentary. At the end of the experimental protocol, i.e. 12 weeks after the start of PE or SL and 4 weeks after OA induction (animal aged 11–12 months), all mice were sacrificed for biochemical evaluations. Furthermore, before (week 0) and at 4, 8, 9, 10, 11 and 12 weeks from the beginning of the experimental protocol, pain-like behavior (mechanical allodynia and thermal hyperalgesia) and motor performance (endurance, balance and muscle strength) were assessed in all mice. Additionally, the metabolic state and mood-like behavior (i.e., anxiety: open field test-OP, Elevated Plus Maze test-EPM and Light/Dark Box test-LDB; depression: Sucrose Preference test-SP, Tail Suspension test-TS and Force Swimming test-FS) were assessed, before sacrifice, in all mice.

2.2. Physical exercise protocol

Physical exercise (PE) was performed using a treadmill (47,300 - Ugo Basile, Italy). Three days before the start of the experimental protocol, all animals underwent a daily training session to accustom them to future PE sessions. Subsequently, the animals were subjected to PE for 12 weeks according to the following protocol: speed of the treadmill set to 10 m/min, 3 days/week for 20 min/day (corresponding to moderate activity in humans) [43–48]. PE on the treadmill was always voluntary (without stimulating the animals with shocks or air puffs). In order to

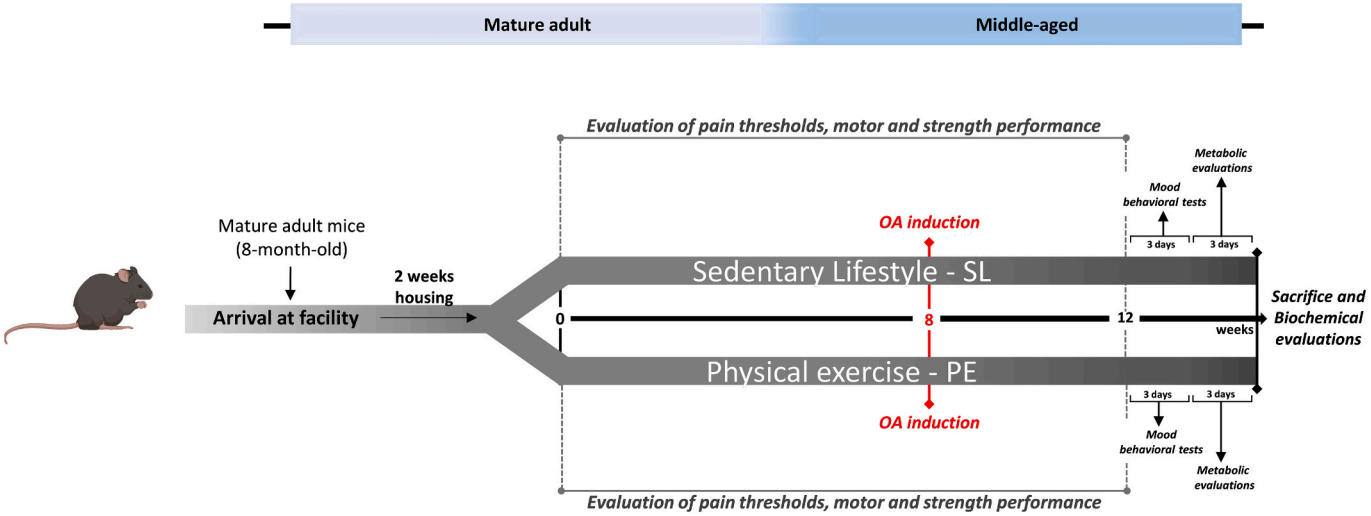


Fig. 1. Experimental protocol timeline.

encourage mice to complete the running session, the animals of the same experimental group were made to run in groups of 2 on a single running lane (each lane measuring w 45 x l 11 x h 15 cm, with transparent cover), since in previous experiments (*unpublished data*), we observed that running in a group, compared to running alone, stimulates animals to exercise. Additionally, SL mice were placed in the switched-off treadmill, for the same time (3 days per week, 20 min/day) as the PE animals.

2.3. Induction of osteoarthritis

After 8 weeks of physical activity (PE group) or sedentary lifestyle (SL group), mice were injected with a single intra-articular (IA) administration into the right knee with 1 mg of MIA (Sigma-Aldrich, Italy) in 10 μl of sterile saline (OA_PE group, $n = 8$; OA_SL group, n = 8) or with vehicle (CTR_PE group, n = 8; CTR_SL group, n = 8) under general anesthesia [15,16,49]. MIA was injected by expert researchers, using great attention to avoid substance leakage from the articular capsule, which would cause systemic toxicity with consequent animal death.

2.4. Behavioral tests

Before each behavioral test session, all animals were acclimated in the new room for 30 min and tests were performed from the least to the most impacting for the animal. Between one test and the next, the mice were given an adequate amount of time for rest. The tests were performed in the light phase, always in the morning, by blind researchers.

2.4.1. Pain-like behavior

For all nociceptive assessments, both ipsilateral (IA injection in the respective knee) and the contralateral (without IA injection) paw was tested (3 measurements for each paw). No nociceptive alterations were observed on the contralateral paw, therefore only the mean value obtained from the ipsilateral paw was calculated and used for statistical analysis.

2.4.1.1. Von Frey test. To assess mechanical allodynia, a dynamic plantar aesthesiometer (Model: 37550 – Ugo Basile, Italy) was used. Briefly, mice were accommodated in a single-chamber plexiglass box (w 85 x l 85 x h 100 mm) placed on a wire mesh. To assess sensitivity to mechanical touch (punctate stimulus), the stiff and blunt tip of a von Frey filament (Ø 0.5 mm) was applied, with increasing force (up to 10 g in 10 s), to the mid-plantar surface of the hind paws. The test ended when the cut-off (10 g) was reached or when the animal removed the paw. The measurement was recorded in grams (paw withdrawal threshold, PWT) [15,16,49].

2.4.1.2. Plantar test. Thermal hyperalgesia was tested using the Hargreaves apparatus (Model 37,570 – Ugo Basile, Italy). Briefly, mice were placed in a modular plexiglass enclosure (1 mouse per module, dimensions of each module in mm: w 96 x l 96 x h 140) positioned over an opaque glass plate. A constant thermal stimulus, generated by a focused light source (beam Ø 0.5 cm; intensity 20 I.R.), was applied through the glass pane to the plantar surface of the hind paw. The test ended when the cut-off (22 s) was reached or when the animal removed the paw. The measurement was recorded in seconds (paw withdrawal latency, PWL) [49].

2.4.2. Locomotion performance

2.4.2.1. Rotarod test. Before the start of the experimental protocol (before week 0), all mice were trained on the rotarod apparatus (47,650 - Ugo Basile, Italy), as previously described [49]. Subsequently, during the test days, two protocols were performed:

- Fixed rotation protocol (12 rpm, cut-off 300 s) for endurance assessments.
- Acceleration protocol (4–30 rpm in 300 s) for coordination assessments.

For both protocols, animals were tested three times with 15-min intervals between tests. The test ended when the cut-off (300 s) was reached or when the animal fell from the rod. Furthermore, if during the test the animal remained on the rod but performed 3 consecutive passive rotations, the test was considered terminated [49]. For each animal, the results of the three tests for both protocols, expressed in seconds, were averaged and used for statistical analysis.

2.4.2.2. Grip strength meter test. It was used to measure the muscle strength of the forelimbs, hindlimbs and all four limbs (47,200 - Ugo Basile, Italy). Briefly, the grip grid was mounted on a force sensor connected to the peak amplifier, which allowed the automatic detection of the mice response (detected force range: 0.1–1500 g). The test was repeated 3 consecutive times for each animal [49] and the values obtained were averaged and used for statistical analyses.

2.4.3. Mood-like behaviors

All depression- and anxiety-behavior tests were recorded and analyzed by ANY-maze video tracking software (v 7.1).

2.4.3.1. Anxiety-like behavior

2.4.3.1.1. Open field test – OF. The apparatus (47150-Ugo Basile, Italy) was composed of 4 arenas (w 46 x l 46 x h 40 cm) made of grey plastic. The mouse was placed in the middle of the arena (1 mouse per arena) and allowed to roam around freely for 10 min. The total distance travelled in the arena (in meters) and both the total time spent (in seconds) and the transitions (in number) in the center zone were used for statistical analysis [15].

2.4.3.1.2. Light/dark box test – LDB. The apparatus (47,445 – Ugo Basile, Italy) consisted of 4 plastic arenas (w 44 × l 44 x h 40 cm). Each arena was in turn divided in two chambers communicating through a door (5 × 5 cm). One chamber was black and not exposed to light (w 14.5 x l 44 x h 40 cm, 1/3 of the box), while another chamber was white and exposed to light (w 29 x l 44 x h 40 cm, 2/3 of the box). The mouse was put in the white chamber and allowed freely move between the two chambers for 5 min. The latency (i.e. the time spent in the light chamber before first entering the dark room, in seconds), the total time spent in the white chamber (in seconds) and the transitions between chambers (in number) were used for statistical analysis. Classically, a decrease in these parameters reflects anxiety-like behavior [15,50].

2.4.3.1.3. Elevated Plus Maze – EPM. The apparatus consisted of two arms without walls (open arms) and two with walls (closed arms) 40 cm long each and connected by a central zone. The structure was raised from the floor by about 60 cm (40,143 - Ugo Basile, Italy). During the test session, the mouse was positioned in the center zone and left free to explore the apparatus for 5 min. The total distance travelled (in meters), the total time spent in the open arms (in seconds) and the transitions in the open arms (in number) were analyzed [16].

2.4.3.2. Depression-like behavior

2.4.3.2.1. Sucrose preference test – SP. Mice were habituated for 48 h to the presence of two bottles filled with tap water (habituation period). Subsequently, mice were exposed for 24 h to two bottles, one filled with tap water and the other with 2 % sucrose solution (test period). The bottles were positioned at 7 a.m. and after approximately 12 h (7 p.m.) they were inverted to reduce any confusion produced by lateral bias. Tap water or sucrose solution consumption was measured the next morning (after 24 h). Both bottles were weighed before and after the test period.

For each cage (2 animals for cage), sucrose preference was calculated as a percentage of: volume of sucrose intake / (volume of sucrose intake + volume of water intake) [15,50].

2.4.3.2.2. Tail suspension test – TS. The apparatus consisted of 6 rectangular, three-walled, grey plastic chambers (l 20 x w 12 × h 55 cm), each equipped with a stainless-steel suspension bar (Ø 1 cm) positioned on top. Each mouse was attached by the tail to the suspension bar (with adhesive tape) in the center of the chamber (1 animal/chamber). The distance between the mouse's nose and the floor was about 20 cm. The duration of the test was 6 min, during which surrender-to-escape behaviors (immobility, in seconds) were scored and used for statistical analysis [15].

2.4.3.2.3. Forced swim test – FS. 3-l glass beakers (h 27 cm, Ø 14.5 cm) approximately 2/3 filled with water (23 ± 2 °C) were used. The animal was gently placed in the water and allowed to swim undisturbed for 6 min. After this period, it was removed, dried and returned to its home cage. The latency (i.e. the first moment of prolonged immobility >10 s) and the total immobility time during both the first 2 min (realization time) and the last 4 min (test time) of the test were used for statistical analysis [15,50].

2.5. Metabolic evaluations

Analyses were performed using metabolic cages through indirect calorimetry (Promethion Metabolic Screening, Sable Systems International, USA). The mice, placed one per metabolic cage, were left to acclimatize for 48 h before acquiring the experimental data, used for statistical analysis, for the following 24 h. During the entire session, the animals had ad libitum access to food and water. The processing of all parameters under study (i.e. food intake, locomotor activity, total energy expenditure and oxygen consumption) were recorded and analyzed (web software Indirect Calorimetry Experiments CalR17) [51].

2.6. Tissue sampling

At the end of the experimental protocol, all mice ($n = 8$/group) were sacrificed, and the following tissues were collected: ipsilateral tibialis, soleus, quadriceps and gastrocnemius muscles, ipsilateral knee, myenteric plexus, nervous tissues involved in OA pain transmission, i.e. the ipsilateral sciatic nerve, ipsilateral dorsal root ganglia (DRGs, L3-L5) and spinal cord (L3-L5) as well as supraspinal areas involved in pain/mood pathways, i.e. brainstem, hypothalamus and hippocampus. All tissue were immediately frozen in liquid nitrogen and stored at −80 °C until further biochemical analysis.

2.7. RT-qPCR

The RNA was extracted using TRIzol™ reagent (Invitrogen, USA) and subsequently reverse transcribed to cDNA with LunaScript® RT SuperMix Kit (BioLabs, UK), according to the respective manufacturers' instructions. Markers expression levels were analyzed by Real Time PCR (RT-qPCR, QuantStudio 5™ - Thermofisher Scientific, USA) using Luna® Universal qPCR Master Mix (BioLabs, UK) and TaqMan probes (Thermofisher Scientific, USA) specific for the genes under study (i.e. interleukin (IL)-1β - Mm00434228_m1; IL-6 -Mm00446190_m1; IL-10 - Mm00439616_m1; Tumor Necrosis factor (TNF)-α - Mm00443258_m1, Prokineticin (PK)2 - Mm01182450_g1, Cluster of Differentiation (CD)68 - Mm_03047343, CD11b - Mm00434455_m1, CD86 - Mm00444540_m1, CD206 - Mm01329359_m1, Toll-like Receptor (TLR)4 - Mm00445274_m1, Glial fibrillary acidic protein (GFAP) - Mm01253033_m1, activating transcription factor (ATF)3 - Mm00476033_m1, Brain-derived neurotrophic factor (BDNF) - Mm04230607_s1, Matrix metalloproteinase (MMP)13 - Mm00439491_m1 and Doublecortin (DCX) - Mm00438400_m1), according to the manufacturers' instructions. Each sample was run in triplicates alongside non-template controls (NTC). The mRNA levels of the interest genes were normalized to endogenous gene (GAPDH - Mm99999915_g1) and expressed as $2 - \Delta\Delta Ct$ relative to the sedentary lifestyle control group (CTR_SL).

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism 10 (v3, San Diego, CA). Data were analyzed using Three-way ANOVA for repeated measures followed by Tukey's post-test (Fig. 2; variables: time, pathology (CTR-healthy or OA-disease) and lifestyle (SL or PE)) or Two-Way ANOVA repeated measures followed by Tukey's post hoc test (Fig. 1S and Figs. 3– 11; variables: pathology (CTR-healthy or OA-disease) and lifestyle (SL or PE)). Data represent mean ± SEM of 8 animals/group. For all analyses, differences were considered significant at $p \leq 0.05$.

3. Results

3.1. Regular physical exercise positively modulates pain and motor alterations induced by osteoarthritis

Throughout the experimental protocol we monitored pain-like behaviors (Fig. 2). We observed that physical exercise did not alter either mechanical allodynia (Fig. 2 A) or thermal hyperalgesia (Fig. 2B) in healthy animals, since CTR_PE mice showed nociceptive thresholds like those of CTR_SL mice. However, although OA induction led to hypersensitivity in both pathological groups (OA_SL and OA_PE), OA_PE mice showed not only a lower reduction in nociceptive thresholds (OA_SL vs. OA_PE: mechanical allodynia, $p < 0.05$; thermal hyperalgesia, <0.0001), but also a significant recovery (9 vs. 12 weeks post-OA: OA_PE, $p < 0.05$ (von Frey test) and $p < 0.0001$ (plantar test); OA_SL, $p > 0.05$ (von Frey and plantar tests)). Additionally, body weight, locomotor performance and grip strength were also evaluated (Supplementary Fig. 1). Body weight variations were observed among the experimental groups, although none appeared statistically significant (Fig. S1A). Regarding motor (Fig. S1 B and C) and strength performance (Fig. S1 - D—F), only an increase in strength in the posterior limbs (Fig. S1E) and overall (Fig. S1F) between the SL and PE mice, regardless of OA induction was observed. Moreover, it can be observed that OA induced a reduction in total muscle mass of the ipsilateral limb in SL mice (Fig. S2A - vs. CTR_SL, $p < 0.0001$). In detail, no difference was observed in the tibialis and soleus muscles (Fig. S2B and S1C - vs. CTR_SL, $p > 0.05$), whereas both gastrocnemius and quadriceps muscle mass decreased (Fig. S2D and S2E - vs. CTR_SL, $p < 0.0001$ and $p < 0.01$ respectively). Physical exercise determined an increase in total muscle mass in CTR mice (Fig. S2A – vs. CTR_SL, $p < 0.05$), attributable to the increase in the tibialis and quadriceps muscles (Fig. S2B and S2E - vs. CTR_SL, $p < 0.05$). It is interesting to note that physical activity was able to counteract the OA-induced loss of muscle mass in OA_PE mice (vs. CTR_PE, $p > 0.05$).

3.2. Regular physical exercise counteracts the development of mood disorders

At the end of the experimental protocol, i.e. after 12 weeks of SL or PE and 4 weeks post-OA induction, anxiety- (Fig. 3) and depression- (Fig. 4) like behaviors were assessed.

3.2.1. Effect on anxiety like-behavior

Physical exercise in control mice led to an improvement in open field-(OF) locomotion activity (Fig. 3A – CTR_PE vs. CTR_SL, $p < 0.05$) and this probably also positively impacted on the other parameters evaluated through this test, i.e. the number of transitions and time spent in the center zone (Fig. 3B and C, $p < 0.01$). However, for the other anxiety tests (i.e. light/dark box-(LDB) and elevated plus maze-(EPM) tests) no difference was observed between CTR groups (Fig. 3D - I, $p > 0.05$). OA induction promoted an anxiety-like behavior in SL mice

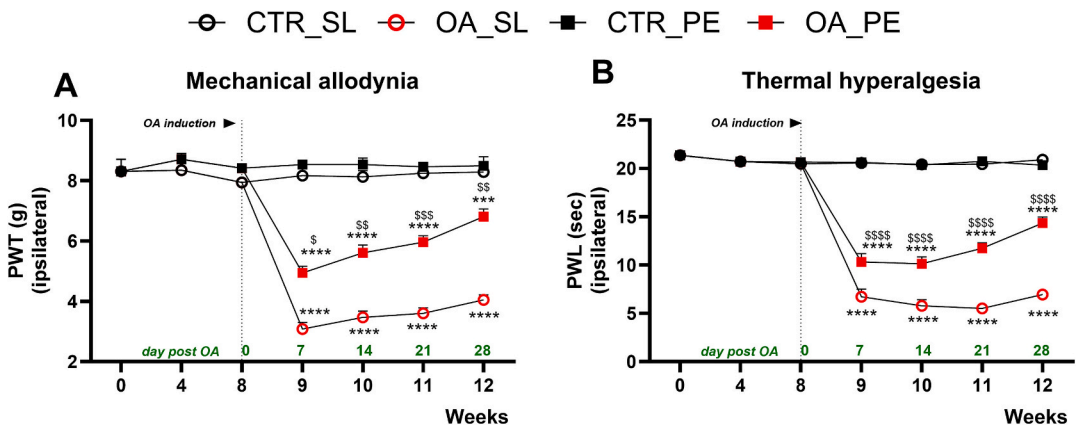


Fig. 2. Pain-like behavior. Control animals led either a sedentary lifestyle (CTR_SL, $n = 16$) or were subjected to physical exercise 3 times a week (CTR_PE, n = 16) for 8 weeks (week 0 to week 8). At week 8, osteoarthritis (OA) was induced in half of the animals of each experimental group by intra-articular administration of monoiodoacetate (MIA; 1 mg in 10 μl saline, right knee). Pathological animals that were sedentary continued to have the same lifestyle (OA_SL, $n = 8$), while exercised animals continued the activity 3 times/week until the end of the experimental protocol (OA_PE, n = 8). Throughout the study, in all experimental groups (**A**) mechanical allodynia and (**B**) thermal hyperalgesia were monitored. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by means of Three-way ANOVA followed by Tukey's post-test. ****$p < 0.0001$, ***$p < 0.001$ vs. respective CTR; $\$\$\$\$p < 0.0001$, $\$\$\$p < 0.001$, $\$\$p < 0.01$, $\$p < 0.05$ vs. respective SL group.

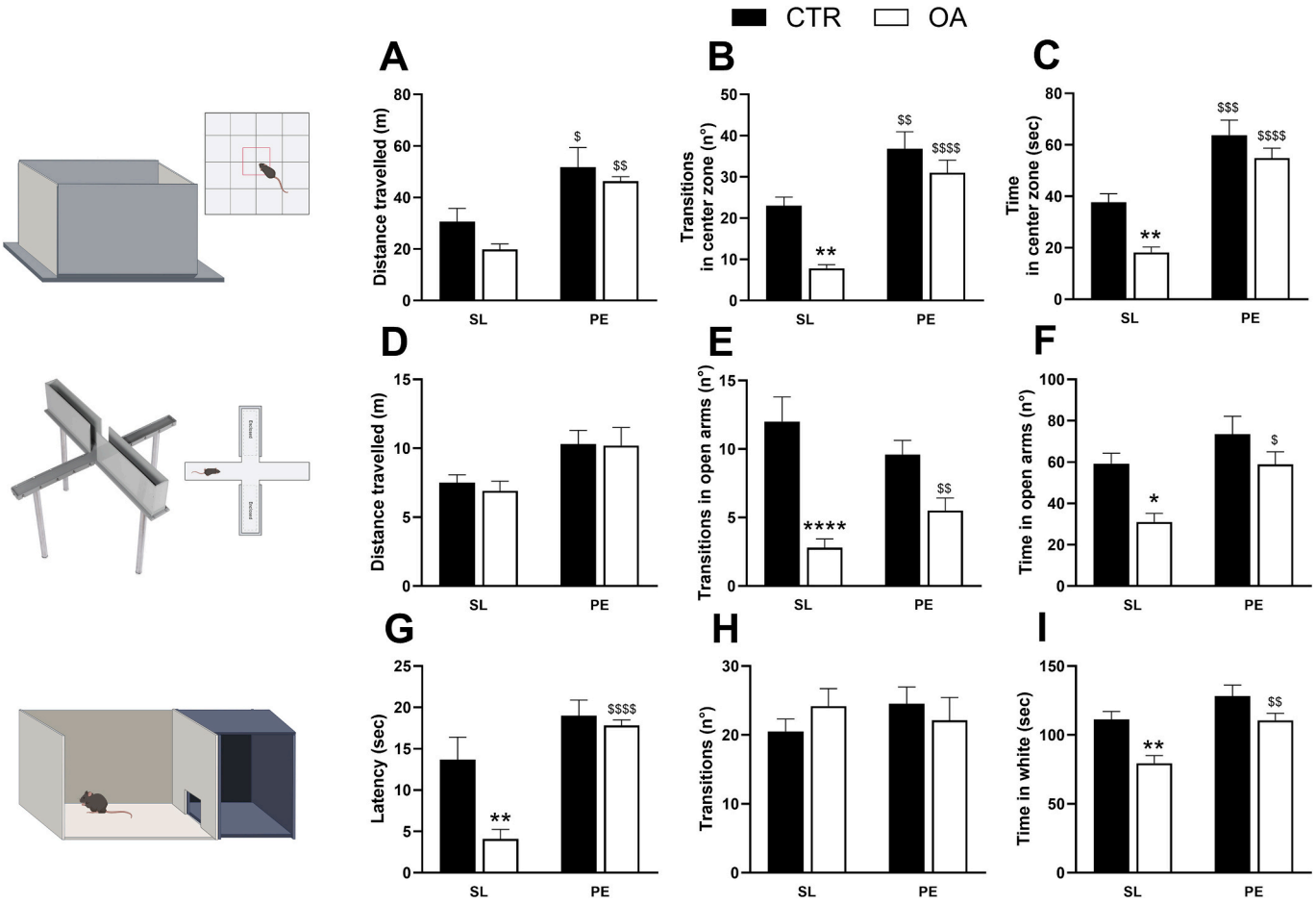


Fig. 3. Anxiety-like behavior. At the end of the experimental protocol, i.e. after 12 weeks of sedentary lifestyle (SL) or physical exercise (PE) and 4 weeks post-osteoarthritis (OA) induction (MIA, 1 mg in 10 μl saline, right knee), anxiety-like behaviors were assessed through the (**A-C**) open field test, (**D—F**) elevated plus maze test and (**G-I**) light/dark box test. In detail, for the open field test (**A**) the total distance travelled, (**B**) the number of transitions in center zone and (**C**) the time spent in center zone were evaluated; for the elevated plus maze (**D**) the total distance travelled, (**E**) the number of transitions in open arms and (**F**) the time spent in open arms were assessed; and for the light/dark box test (**G**) the latency, i.e. the first time needed to leave the light area to go into the dark area, (**H**) the transition between white and dark areas and (**I**) the time spent in white area were analyzed. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by means of Two-way ANOVA followed by Tukey's post-test. ****p < 0.0001, **p < 0.01, *p < 0.05 vs. respective CTR; $\$\$\$\$p < 0.0001$, $\$\$\$p < 0.001$, $\$\$p < 0.01$, $\$p < 0.05$ vs. respective SL group.

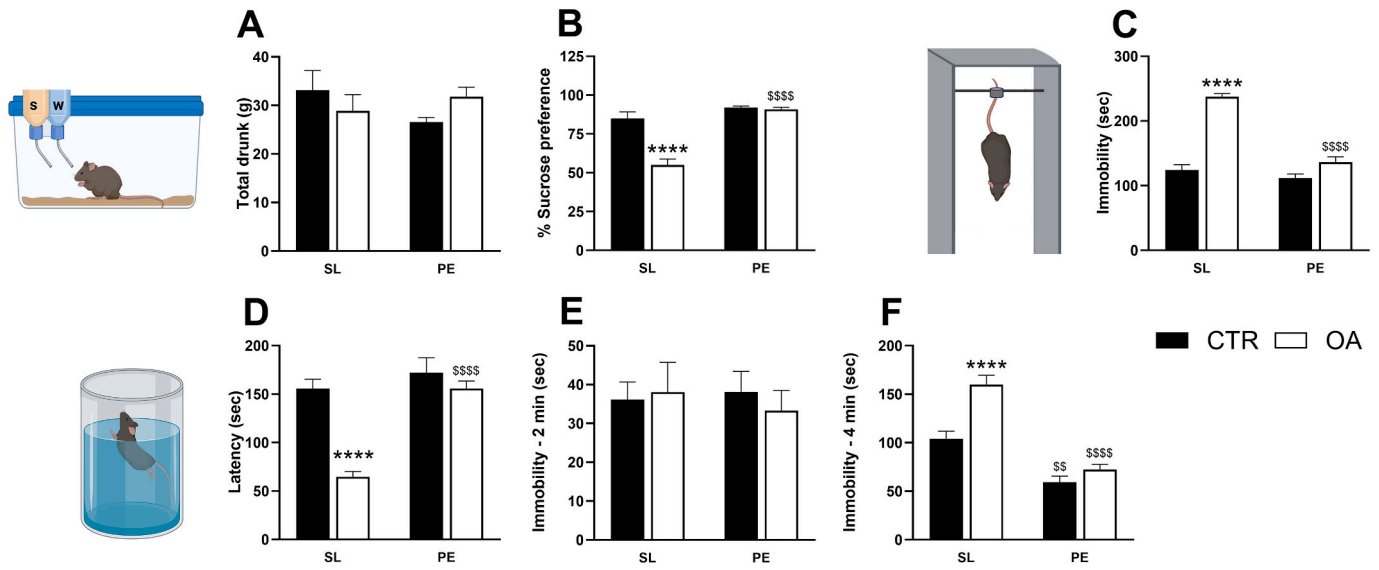


Fig. 4. Depression-like behavior. At the end of the experimental protocol, i.e. after 12 weeks of sedentary lifestyle (SL) or physical exercise (PE); 4 weeks post-osteoarthritis (OA) induction (MIA, 1 mg in 10 μl saline, right knee), the presence of depression-like behaviors was examined. In detail, (**A**) total fluid intake (water +2 % sucrose solution) and (**B**) sucrose preference were evaluated by sucrose preference test (S = sucrose, W = water); (**C**) total immobility time was assessed by tail suspension test; and through the forced swim test (**D**) the latency, i.e. the first time to stop swimming for 10 consecutive seconds, and the immobility both (**E**) in the first 2 min and (**F**) in the last 4 min of the test were detected. Data are expressed as mean ± SEM of 4 cage/group (**A-B**) or 8 animals per group (**C—F**). Statistical analysis was performed by means of Two-way ANOVA followed by Tukey's post-test. **** $p < 0.0001$ vs. respective CTR; $$$$$p < 0.0001$, $$p < 0.01$ vs. respective SL group.

compared to their CTR, with a significant decrease in the number of transitions and in the time spent both in the center zone of the OF (Fig. 3B and C; $p < 0.01$), and in the open arms of EPM tests (Fig. 3E - $p < 0.0001$ and 3F - $p < 0.05$). In LDB test, the first latency to move into the dark compartment (Fig. 3G – vs. CTR_SL, $p < 0.01$) as well as the time spent in the light area (Fig. 3I - $p < 0.01$) were significantly decreased in OA_SL mice, even if the number of transitions did not appear to be altered by pathology (Fig. 3H). Interestingly, in both tests in which it was possible to track the total mice locomotion, i.e. OF and EPM (Fig. 3A and D, respectively), no significant differences were highlighted between CTR_SL and OA_SL ($p > 0.05$), indicating that alterations found are attributable to anxiety condition and not to OA-induced reduced mobility. Conversely, it is interesting to note that no significant differences were detected between CTR_PE and OA_PE mice (Fig. 3A-I, $p > 0.05$), indicating that physical exercise was able to counteract the development of pain-related anxiety typical of OA mice.

3.2.2. *Effect on depressive-like behavior*

Sedentary animals affected by OA displayed depressive-like behavior. OA_SL mice compared to CTR_SL showed a decreased preference for sucrose solution intake (Fig. 4B, $p < 0.0001$), a longer immobility time in both tail suspension (Fig. 4C, $p < 0.0001$) and the forced swim test (FS) (Fig. 4F, $p < 0.0001$), as well as an early first arrest time (latency) in the latter test (Fig. 4D, $p < 0.0001$). Again, it should be highlighted that the alterations found are likely linked to a depressive-like condition onset and not to motor impairments induced by OA, since no significant alterations were observed between sedentary CTR and OA animals, neither in the total amount of liquids taken in the sucrose preference test (Fig. 4A) nor in the immobility time in the first 2 min of the FS test (habituation time/awareness of the new environment, Fig. 4E). However, it is very interesting to observe that exercise had a significantly beneficial impact on pathological animals, because it counteracted/prevented the onset of OA pain-related depressive condition (Fig. 4A-F - CTR-PE vs. OA_PE, $p > 0.05$).

3.3. *Effect of regular physical exercise on metabolism in OA*

Additionally, at the end of the experimental protocol (following mood tests), metabolic evaluations were conducted. Since they involved a significant change in environment and sociality for the animals (from multiple to single cages), the observation period was 3 days (Fig. 5A, C, E and G). To avoid any bias, in the first 48 h animals were allowed to get used to the new condition and only the data of the last 24 h of testing were used for statistical analyses to evaluate possible differences among groups (Fig. 5B, D, F and H). The assessment of locomotor activity (Fig. 5B) confirmed previous data: no significant differences were observed between CTR and OA mice or between SL and PE animals, both in the light phase and, above all, in the night phase, when mice activity increased. CTR_SL, CTR_PE and OA_PE groups showed similar food consumption, reduced in the light hours and increased in the dark hours (Fig. 5C and D). On the contrary, in the OA_SL group there was a significant reduction in food consumption in the dark hours (Fig. 5D - CTR_SL vs. OA_SL, $p < 0.01$), which is responsible for the lower overall daily food intake recorded. We also measured total energy expenditure-TEE (Fig. 5E and F) and oxygen consumption (Fig. 5G and H). Physical exercise, compared to a sedentary lifestyle, increased TEE in CTR mice (Fig. 5F - CTR_SL vs. CTR_PE: all day, $p < 0.0001$; dark, $p < 0.05$). Interestingly, OA_SL mice also showed a significant increase in TEE (CTR_SL vs. OA_SL, $p < 0.0001$), which was not detected in OA_PE animals. Furthermore, while physical exercise did not alter oxygen consumption in CTR mice (CTR_SL vs. CTR_PE, $p > 0.05$), it was significantly increased in OA mice compared to their CTRs (Fig. 5H - all day: CTR_SL vs. OA_SL, $p < 0.0001$; CTR_PE vs. OA_PE, $p < 0.05$), a condition that however appeared more significant in OA_SL animals than in OA_PE (light: CTR_SL vs. OA_SL, $p < 0,0001$; CTR_PE vs. OA_PE, $p > 0.05$; dark: CTR_SL vs. OA_SL, $p < 0,0001$; CTR_PE vs. OA_PE, $p > 0.05$).

3.4. *(neuro)inflammation in OA pain and the effect of regular physical exercise*

At the end of the in vivo evaluations, animals were sacrificed for

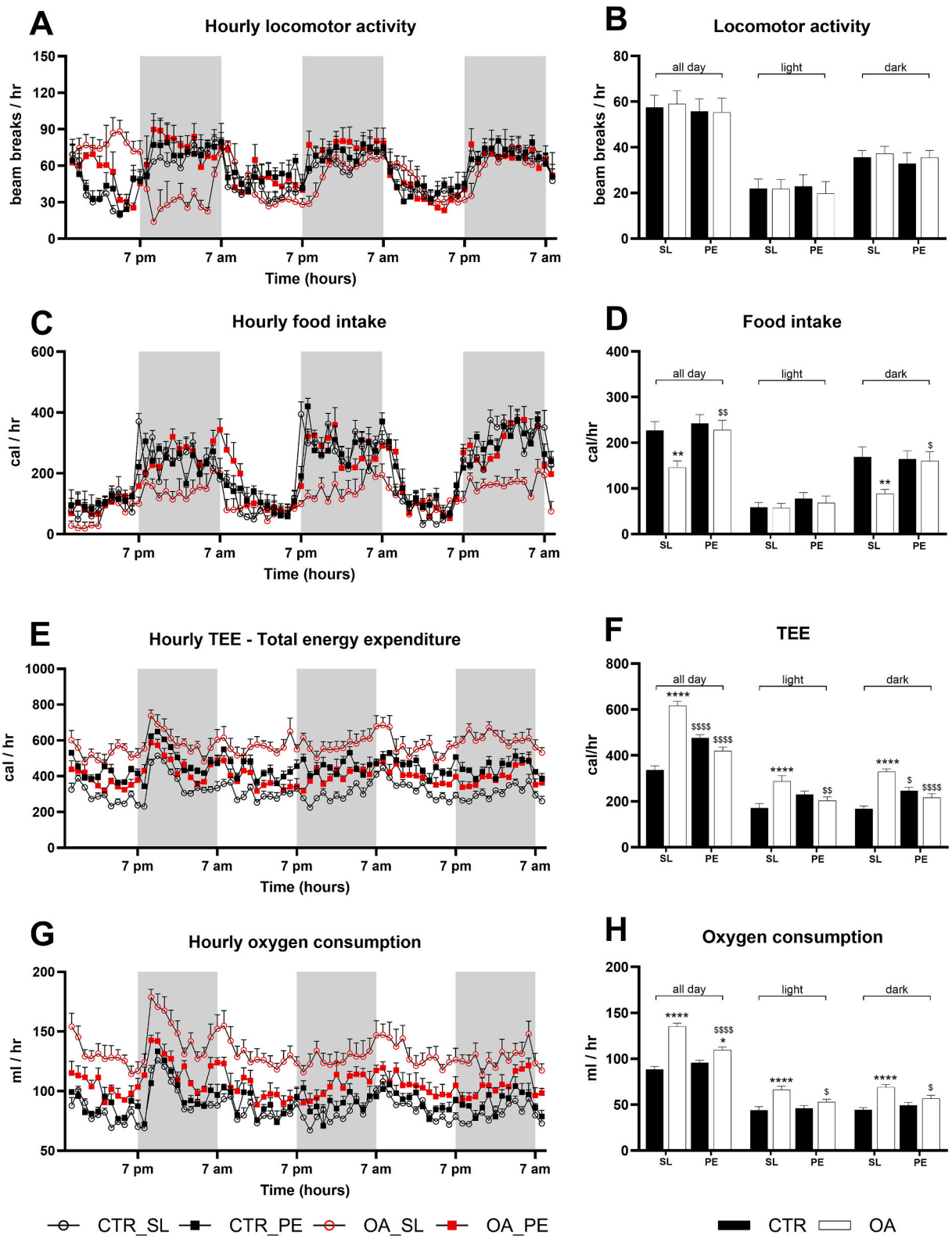


Fig. 5. Metabolic evaluations. Data were acquired at the end of the experimental protocol (i.e. after 12 weeks of sedentary life-SL or physical exercise-PE; 4 weeks after osteoarthritis-OA induction) using metabolic cages via indirect calorimetry for 3 consecutive days. Mice were placed one per metabolic cage and metabolic parameters, i.e. (**A-B**) locomotor activity, (**C—D**) food intake, (***E*-F**) TEE - total energy expenditure and (**G-H**) oxygen consumption, were recorded. Panels A, C, E and G show the total hourly time course, while panels B, D, F and G represent the assessments of the last 24 h. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by Two-way ANOVA followed by Tukey's post-test. ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$ vs respective CTR; $$$$$p < 0.0001$, $$$p < 0.01$, $p < 0.05$ vs. respective SL group.

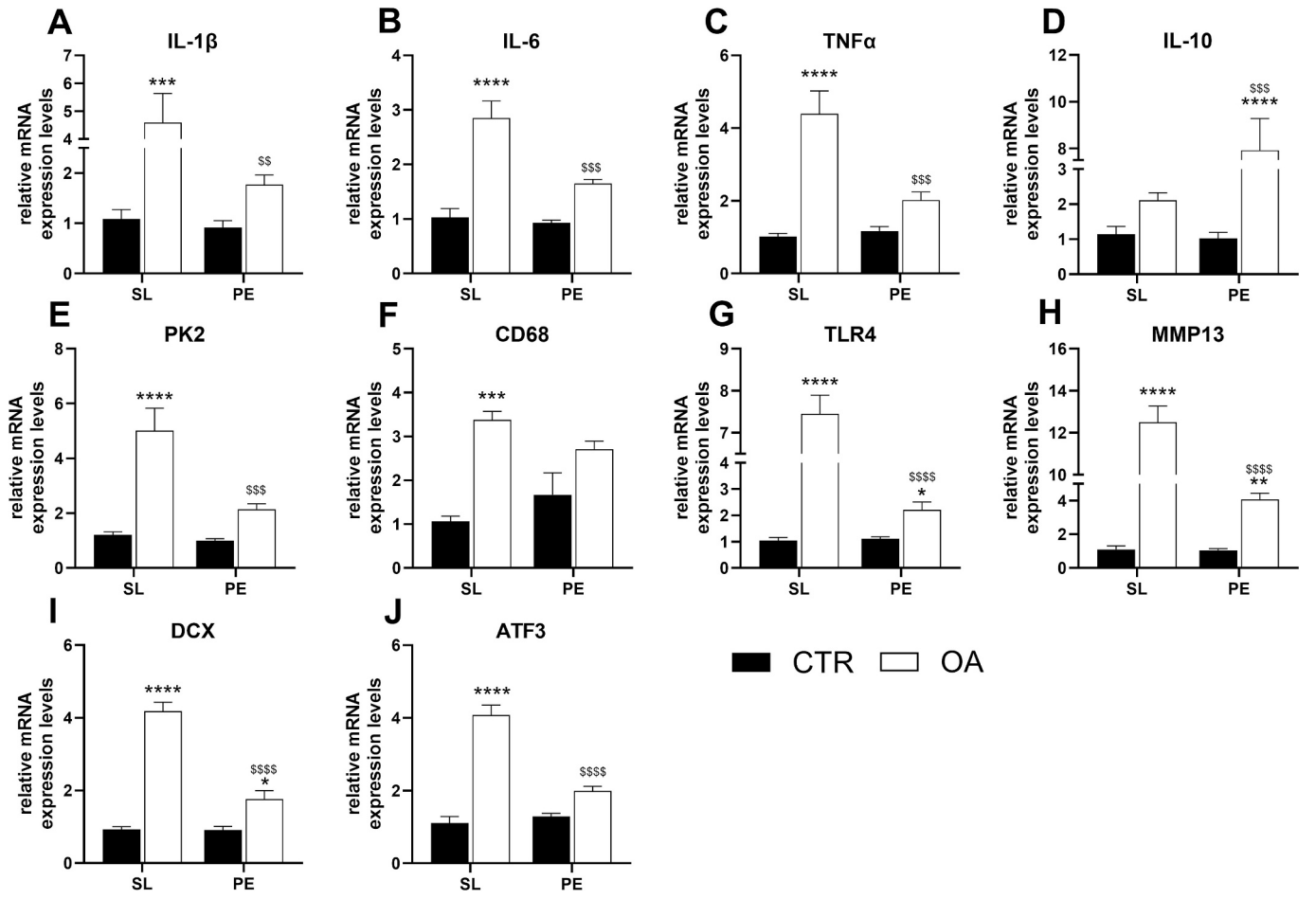


Fig. 6. Knee biochemical evaluations. Tissues were obtained at the end of the experimental protocol (i.e. after 12 weeks of sedentary life-SL or physical exercise-PE; 4 weeks after osteoarthritis-OA induction). mRNA levels of the pro-inflammatory cytokines (**A**) IL-1β, (**B**) IL-6 and (**C**) TNFα, (**D**) the anti-inflammatory cytokine IL-10, (**E**) the chemokine PK2, (**F**) the macrophage marker CD68, (**G**) toll-like receptor (TLR)-4, (**H**) the matrix metalloproteinase (MMP)-13, (**I**) neurogenesis marker (DCX) and (**J**) the cellular damage marker ATF3 were assessed by means of RT-qPCR. Data were normalized to GAPDH expression and presented as fold-changes over CTR_SL mice group. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by Two-way ANOVA followed by Tukey's post-test. ****p < 0.0001, ***p < 0.001, **p < 0.01, *p < 0.05 vs respective CTR; $$$$p < 0.0001, $$$p < 0.001, $$p < 0.01 vs. respective SL group.

tissue sampling and biochemical analyses.

3.4.1. *Knee joint*

In the right knee (Fig. 6), it is possible to observe that CTR_SL and CTR_PE did not show significant differences for any of the analyzed markers (Fig. 6A-J). Induction of OA led to significant inflammatory condition in SL mice with an increase in all analyzed parameters (Fig. 6A-C, E-J - $p < 0.001$), except for the anti-inflammatory cytokine IL-10 (Fig. 6D). Physical exercise in OA animals was able to mitigate this local inflammation. A statistically significant difference between OA_SL and OA_PE was observed for all the markers analyzed ($p < 0.01$), except for CD68 (Fig. 6F). Moreover, in OA_PE mice, the levels of both pro-inflammatory cytokines (Fig. 6A-C) and of the PK2 chemokine (Fig. 6E) as well as of markers of macrophage activation (CD68, Fig. 6F) and cell damage (ATF3, Fig. 6J) were comparable to CTR_PE mice. In OA mice, physical exercise also increased IL-10 levels (Fig. 6D, CTR_PE vs. OA_PE, $p < 0.0001$).

3.4.2. *Sciatic nerve*

Physical exercise in CTR animals did not modulate the expression levels of the analyzed parameters in the sciatic nerve (Fig. 7). In SL mice, OA induced a neuroinflammatory condition with a significant increase in all analyzed markers ($p < 0.001$). Physical exercise in OA animals positively modulated the neuroinflammatory condition in comparison to OA_SL mice. Therefore, the levels of all the markers analyzed in OA_PE mice appeared statistically lower than in OA_SL mice ($p < 0.01$). Additionally, in OA_PE mice, the mRNA levels of IL-1β, TNFα and IL-10 cytokines, as well as of TLR4, GFAP and ATF3 markers were comparable to CTR_PE animals.

3.4.3. *Dorsal root ganglia*

In the DRGs (Fig. 8) of CTR_SL and CTR_PE only the marker of anti-inflammatory macrophage CD206 was significantly increased in CTR_PE (Fig. 8H, $p < 0.05$). In OA_SL mice, a clear neuroinflammatory state was detected, with increased levels of pro-inflammatory cytokines (Fig. 8A-C), the chemokine PK2 (Fig. 8E), macrophage markers CD11b and CD86 (Fig. 8F and G), as well as the satellite glial cell marker GFAP (Fig. 8L). mRNA levels of CD206 were downregulated by the pathology (Fig. 8H - CTR_SL vs. OA_SL, $p < 0.01$), whereas the levels of anti-inflammatory cytokine IL-10 (Fig. 8D) and the damage marker ATF3 (Fig. 8M) were not altered by OA. Physical exercise positively modulated neuro-inflammation in the DRGs, as a significant difference was observed between the pathological SL and PE mice in the levels of TNFα, CD11b, CD86, CD206, TLR4, and GFAP ($p < 0.05$). No significant difference was found between OA_SL and OA_PE for the mRNA levels of IL-1β, IL-6, and IL-PK2. However, it is worth noting that for the pro-inflammatory cytokines, the levels in OA_PE animals were comparable to those of CTR_PE animals.

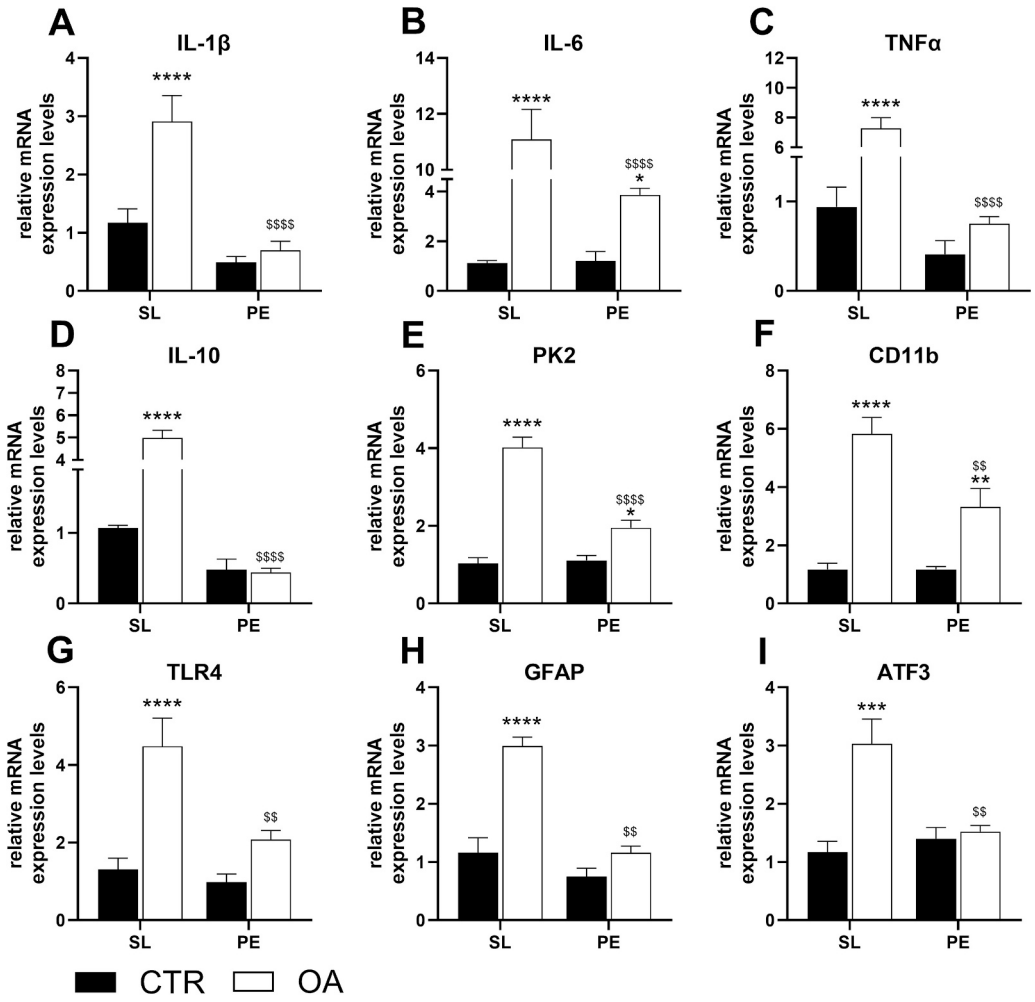


Fig. 7. Sciatic nerve biochemical evaluations. Tissues were obtained at the end of the experimental protocol (i.e. after 12 weeks of sedentary life-SL or physical exercise-PE; 4 weeks after osteoarthritis-OA induction). mRNA levels of the pro-inflammatory cytokines (**A**) IL-1β, (**B**) IL-6 and (**C**) TNFα, (**D**) the anti-inflammatory cytokine IL-10, (**E**) the chemokine PK2, (**F**) the macrophage marker CD11b, (**G**) TLR4, (**H**) the Schwann cell marker GFAP and the (**I**) cellular damage marker ATF3 were assessed by means of RT-qPCR. Data were normalized to GAPDH expression and presented as fold-changes over CTR_SL mice group. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by Two-way ANOVA followed by Tukey's post-test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs respective CTR; $$$$$p < 0.0001$, $$$p < 0.01$ vs. respective SL group.

3.4.4. Spinal cord

In the spinal cord, the CTR groups showed similar expression levels for all the markers analyzed, independent from the exercise (Fig. 9A-M). OA led to a neuroinflammatory condition in OA_SL animals, with a significant up-regulation of pro-inflammatory cytokines (Fig. 9A-C, $p < 0.01$), chemokine PK2 (Fig. 9E, $p < 0.001$), astrocytic marker GFAP (Fig. 9L, $p < 0.01$), as well as cellular damage marker ATF3 (Fig. 9M, $p < 0.01$). Interestingly, even if no microglia activation was detected since CD11b and TLR4 mRNA levels were not modulated by OA (Fig. 9F and I - CTR_SL vs. OA_SL, $p > 0.05$), it was clearly evident that an imbalance between pro- and anti-inflammatory microglia markers was present (Fig. 9G and H). Indeed, a significant increase in the microglia proinflammatory marker CD86 (Fig. 9G, $p < 0.01$) was associated with a decreasing trend, even if not significant, of the anti-inflammatory marker CD206 (Fig. 9H, $p = 0.0744$). Besides, a significant downregulation of IL-10 mRNA levels was detected in OA_SL mice (vs. CTR_SL, $p < 0.05$). Exercise counteracted OA-induced neuroinflammation, as a significant difference was found in OA_PE mice compared to OA_SL mice in the levels of pro−/anti-inflammatory cytokines, macrophage markers CD86 and CD206, GFAP, and ATF3 ($p < 0.05$). Furthermore, in OA_PE mice, the mRNA levels of all studied markers were comparable to those of CTR_PE animals.

3.4.5. Brain areas

The expression levels of selected markers were also evaluated in brain areas involved in pain and mood alterations pathways, i.e. brainstem (Fig. 10A-10H), hypothalamus (Fig. 10I-10P) and hippocampus (Fig. 10Q-10Z). In CTR animals, PE did not modify the expression levels of the analyzed markers compared to SL, except for an up-regulation of IL-10 in all brain areas (Fig. 10D, L and T, $p < 0.05$) and an increase in the brainstem BDNF levels (Fig. 10H, $p < 0.0001$). In OA_SL mice, high levels of the pro-inflammatory cytokines IL-1β (Fig. 10A, I and Q, $p < 0.05$) and TNFα (Fig. 10C, K and S, $p < 0.01$) were detected in all supraspinal areas, as well as an upregulation of IL-6 in both brainstem and hippocampus (Fig. 10B and R, $p < 0.05$) and of the chemokine PK2 in brainstem and hypothalamus (Fig. 10E and M, $p < 0.0001$). In the brainstem we observed a significant upregulation of the anti-inflammatory cytokine IL-10 (Fig. 10D, $p < 0.01$), while a significant downregulation was detected in the hypothalamus (Fig. 10L, $p < 0.05$). Only in the brainstem the mRNA levels of microglia activation marker CD11b were overexpressed (Fig. 10F, $p < 0.0001$), while in the other brain areas CD11b was not modulated by the pathology (Fig. 10N and V, $p > 0.05$). Moreover, astrocytic activation (GFAP) was observed in the brainstem and hippocampus (Fig. 10G and W, $p < 0.05$). The expression levels of BDNF were significantly downregulated only in the

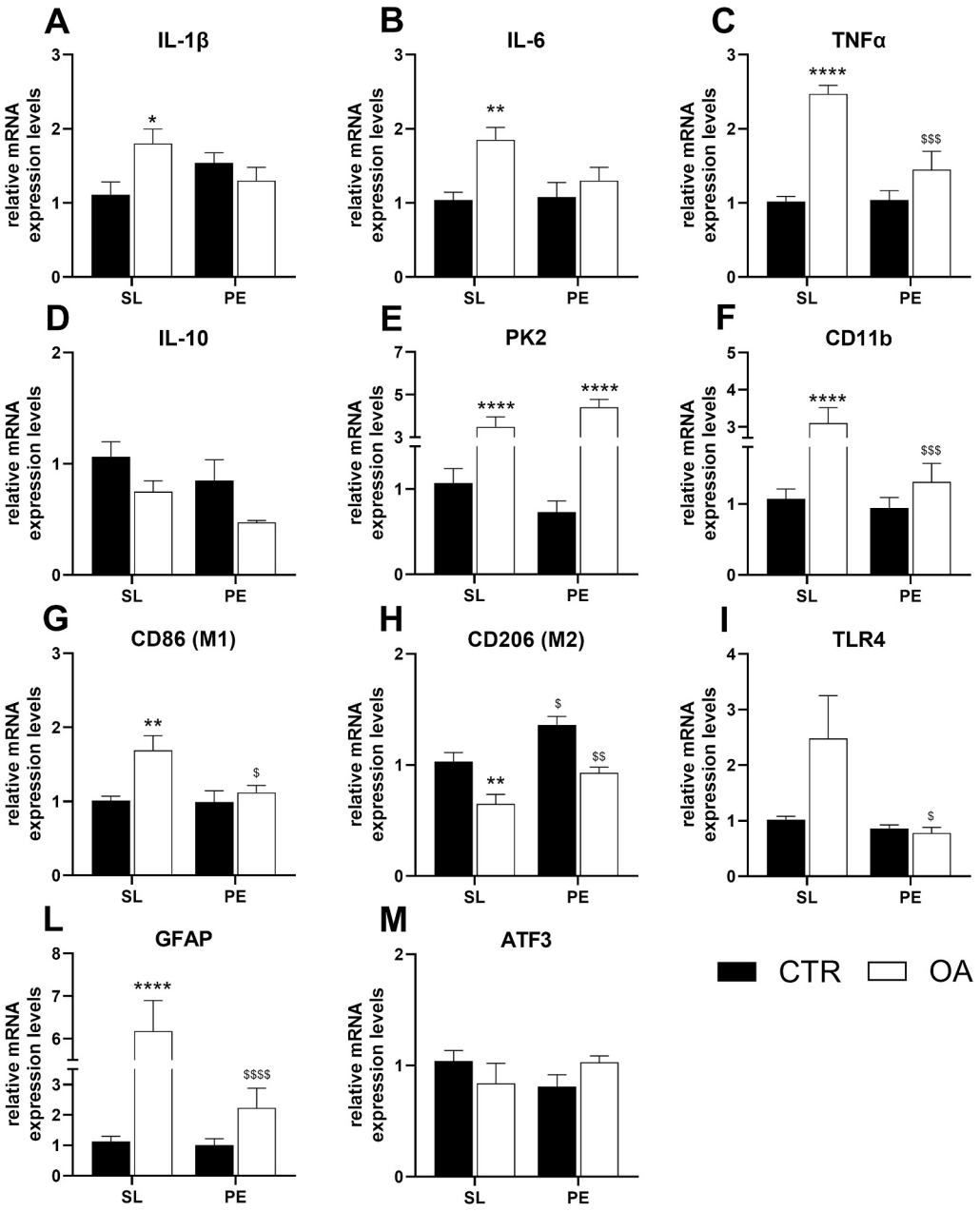


Fig. 8. Dorsal root ganglia biochemical evaluations. Tissues were obtained at the end of the experimental protocol (i.e. after 12 weeks of sedentary life-SL or physical exercise-PE; 4 weeks after osteoarthritis-OA induction). mRNA levels of the pro-inflammatory cytokines (**A**) IL-1β, (**B**) IL-6 and (**C**) TNFα, (**D**) the anti-inflammatory cytokine IL-10, (**E**) the chemokine PK2, (**F**—**H**) the macrophage markers CD11b, CD86, CD206, (**I**) TLR4, (**L**) satellite cell marker GFAP and (**M**) the cellular damage marker ATF3 were assessed by means of RT-qPCR. Data were normalized to GAPDH expression and presented as fold-changes over the levels of CTR_SL mice group. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by Two-way ANOVA followed by Tukey's post-test. ****$p < 0.0001$, **$p < 0.01$, *$p < 0.05$ vs respective CTR; $$$$$p < 0.0001$, $$$$p < 0.001$, $$$p < 0.01$, $$p < 0.05$ vs. respective SL group.

brainstem (Fig. 10H, $p < 0.05$), although a trend of reduction, although not statistically significant, was observed also in the hippocampus (Fig. 10Z, $p = 0.0595$). Therefore, all these alterations in OA_SL mice outline a clear neuroinflammatory condition also in brain areas, which was not present in OA_PE animals. Physical exercise in OA mice significantly counteracted supraspinal neuroinflammation. Only a reduction in BDNF mRNA levels in the brainstem (Fig. 10H, $p < 0.0001$) and in IL-10 in the hippocampus (Fig. 10L, $p < 0.05$) was detected. However, both BDNF and IL-10 levels appeared significantly higher than in OA_SL mice ($p < 0.0001$ and $p < 0.01$, respectively).

3.4.6. *Myenteric plexus*

Finally, to begin investigating whether also the intestinal alterations that may affect OA patients could be traced back to a neuroinflammatory condition, the myenteric plexus was analyzed. Sedentary OA mice were characterized by a relevant neuroinflammatory state. The levels of all pro-inflammatory cytokines were upregulated by the pathology (Fig. 11A-11C, $p < 0.05$), as well as those of the chemokine PK2 (Fig. 11E, $p < 0.001$), while the mRNA expression of the anti-inflammatory cytokine IL-10 appeared downregulated (Fig. 11 D, $p < 0.01$). It is interesting to note that although the expression levels of macrophage marker CD11b were not significantly modulated by the pathology (Fig. 11F), going into more detail and observing cell phenotype, it appears that pro-inflammatory macrophages were abundant (CD86 – Fig. 11G, $p < 0.0001$) while the anti-inflammatory macrophage marker was decreased (CD206 – Fig. 11H, $p < 0.05$) in OA_SL mice.

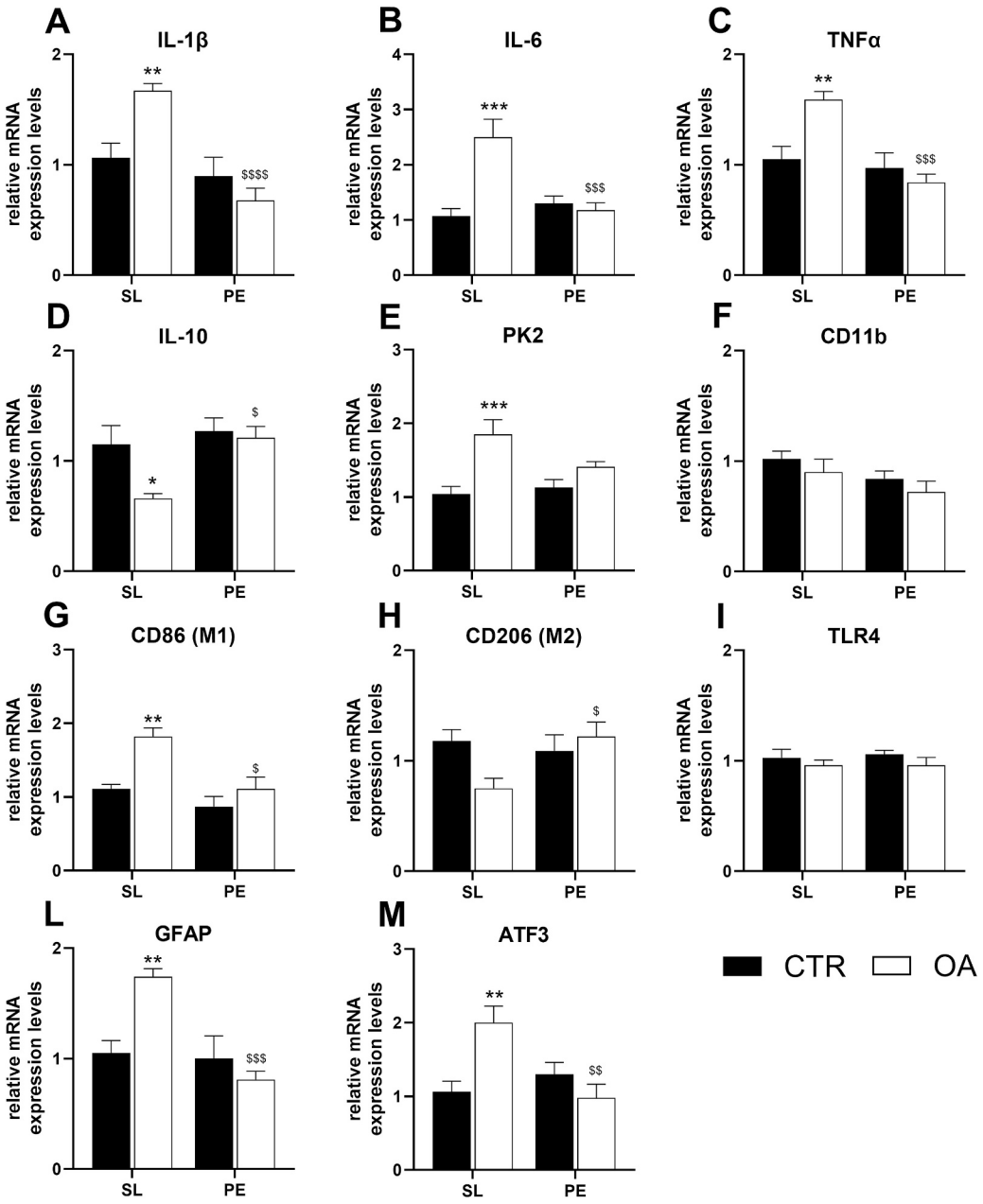


Fig. 9. Spinal cord biochemical evaluations. Tissues were obtained at the end of the experimental protocol (i.e. after 12 weeks of sedentary life-SL or physical exercise-PE; 4 weeks after osteoarthritis-OA induction). mRNA levels of the pro-inflammatory cytokines (**A**) IL-1β, (**B**) IL-6 and (**C**) TNFα, (**D**) the anti-inflammatory cytokine IL-10, (**E**) the chemokine PK2, (**F**—**H**) the macrophage markers CD11b, CD86, CD206, (**I**) TLR4, (**L**) the astrocytic cell marker GFAP and (**M**) the cellular damage marker ATF3 were assessed by RT-qPCR. Data were normalized to GAPDH expression and presented as fold-changes over the levels of CTR_SL mice group. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by Two-way ANOVA followed by Tukey's post-test. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs respective CTR; $$$$$p < 0.0001$, $$$p < 0.001$, $$p < 0.01$, $p < 0.05$ vs. respective SL group.

Furthermore, in OA_SL animals a significant increase in both TLR4 (Fig. 11I, vs. CTR_SL $p < 0.05$) and the enteric glia marker GFAP, was present (Fig. 11L, vs. CTR_SL $p < 0.0001$). Physical exercise in CTR animals did not significantly alter the expression levels of the studied markers, except for the upregulation of GFAP (Fig. 11L, vs. CTR_SL $p < 0.0001$). In OA animals, PE had a beneficial effect, as a significant difference between OA_SL and OA_PE was observed in the mRNA levels of pro-inflammatory cytokines (Fig. 11A and B), chemokine (Fig. 11E), all glial markers (Fig. 11F-11H and 11L), as well as TLR4 (Fig. 11I). Indeed, only the levels of the pro-inflammatory cytokine IL-6 were still up-regulated by the pathology (Fig. 11B, vs. CTR_PE, $p < 0.05$).

4. Discussion

Osteoarthritis (OA) is a chronic degenerative joint disease with a worldwide prevalence and with an increasingly higher rate of onset at younger age (around 4th life decade). In addition to experiencing motor disability, OA patients report chronic pain as a classic and highly debilitating symptom. Furthermore, these patients often report the presence of mood disorders, such as anxiety and depression [9–11], which can further aggravate pain perception, significantly affecting patient's quality of life. Low-grade systemic inflammation plays a key role in OA onset and propagation and a consequent development of a neuroinflammatory condition would drive the painful symptomatology and its comorbidities, like psychiatric disorders [14]. Moreover,

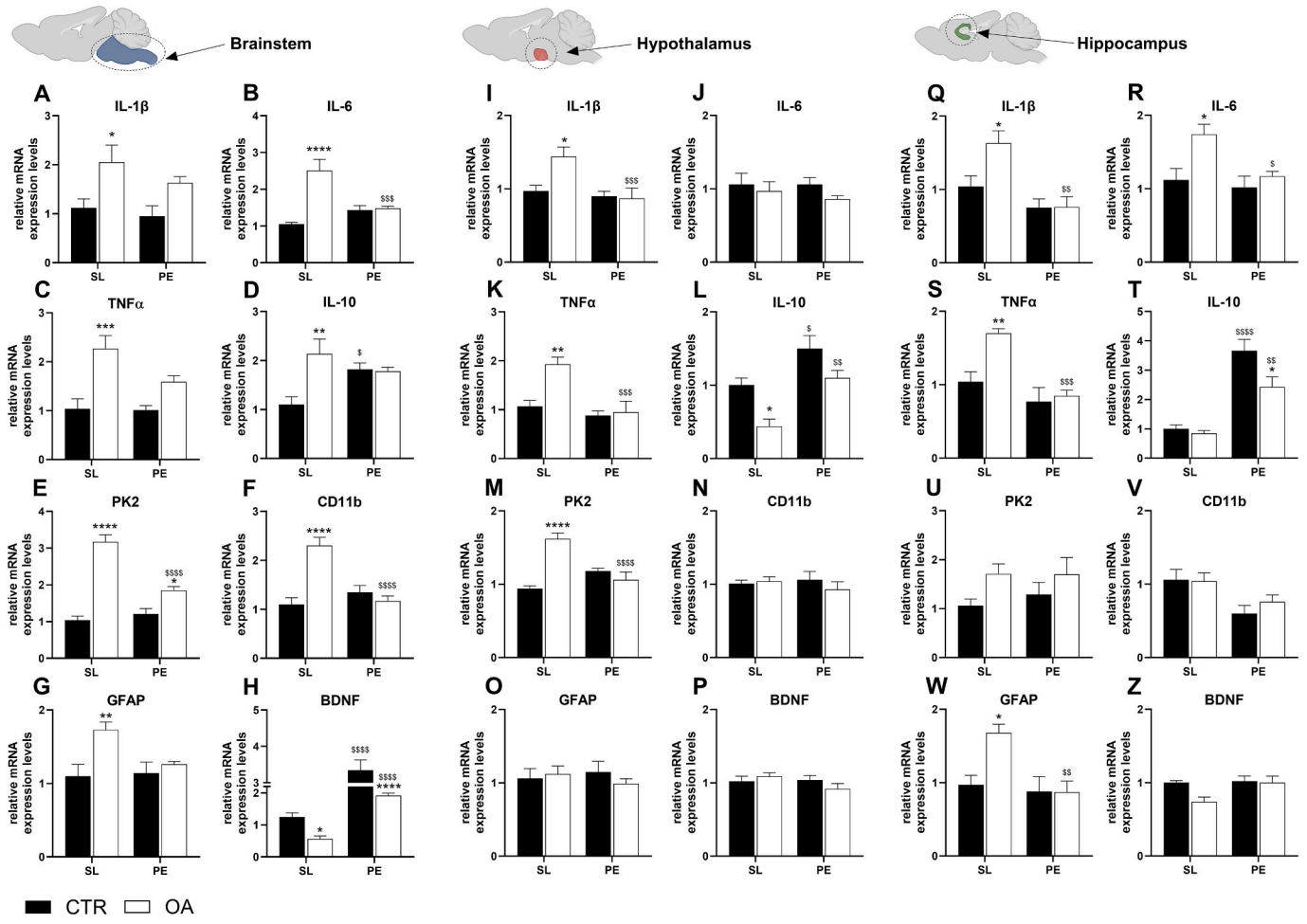


Fig. 10. Brain area biochemical evaluations. Tissues were obtained at the end of the experimental protocol (i.e. after 12 weeks of sedentary life-SL or physical exercise-PE; 4 weeks after osteoarthritis-OA induction). In (**A-H**) brainstem, (**I—P**) hypothalamus and (**Q-Z**) hippocampus the mRNA level of pro-inflammatory cytokines (**A, I, Q**) IL-1β, (**B, J, R**) IL-6 and (**C, K, S**) TNFα, (**D, L, T**) the anti-inflammatory cytokine IL-10, (**E, M, U**) the chemokine PK2, (**F, N, V**) the microglia marker CD11b, (**G, O, W**) the astrocytic marker GFAP and (**H, P, Z**) BDNF were assessed by means of RT-qPCR. Data were normalized to GAPDH expression and presented as fold-changes over the levels of CTR_SL mice group. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by Two-way ANOVA followed by Tukey's post-test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs respective CTR; $$$$$p < 0.0001$, $$$p < 0.001$, $$p < 0.01$, $p < 0.05$ vs. respective SL group.

pharmacological strategies for OA pain management are ineffective and are often associated with serious side effects, in addition to being unable to simultaneously control nociceptive and affective manifestations. Therefore, OA pain treatment remains an unmet clinical need. Recently, therefore, there has been a growing interest in understanding alternative and/or complementary treatments. Among these, a healthy lifestyle is at the forefront. In particular, recent evidence in clinical and preclinical research show that physical activity reduces pain [45,52], neuroinflammation [53–55], mood alterations [56,57] and even intestinal disturbances [58–60]. However, no study has been conducted to investigate the relationship among all these factors. For these reasons, here, we tested whether regular physical activity was able to prevent and/or counteract OA-induced pain and neuroinflammation and, therefore, put a brake on to the entire inflammatory cycle, also modulating psychiatric comorbidities. In this regard, we decided to consider a particularly sensitive age window in mice, corresponding to approximately 35–45-year-old in humans. Before OA induction, some mice were subjected to a regular physical exercise protocol for 2 months, to test whether maintaining a healthy lifestyle could counteract OA and prevent the development of its comorbidities with respect to animals with a sedentary lifestyle. As expected, MIA-induced OA caused significant nociceptive alterations associated with a modest reduction in motor performance [15,16,49]. For the first time in a MIA model, we also performed in vivo metabolic analyses. We observed that OA_SL mice showed a lower food consumption associated with higher energy expenditure. Pathological conditions, like chronic pain, can determine a variation in the basal metabolism in both patients and animal models, which commonly leads to a greater energy expenditure with consequences that further aggravate the clinical overall condition [61–63]. In addition, OA_SL animals were also characterized by a higher oxygen consumption during breathing at rest which could be related to a condition of hyperventilation, which in subjects with chronic pain is well documented [64–66]. Our data clearly show that physical exercise was able to reduce OA-induced pain and pain-related metabolic alterations. Indeed, exercised OA animals showed food consumption, energy expenditure and oxygen consumption similar to CTRs. Lifestyle is therefore a key element for controlling pain and we confirm that modifying physical inactivity through regular exercise was an effective approach to counteract OA pain and other OA comorbidities [67–70].

However, the metabolic assessments we conducted were limited to a relatively short observation period (3 days). Considering the interesting data obtained, long-term or multiple time-point metabolic assessments could provide a more comprehensive understanding of the effects of exercise on energy expenditure and metabolic profiles in OA mice over time. This opens a field of investigation that should be addressed in future studies. It should be emphasized, however, that with our

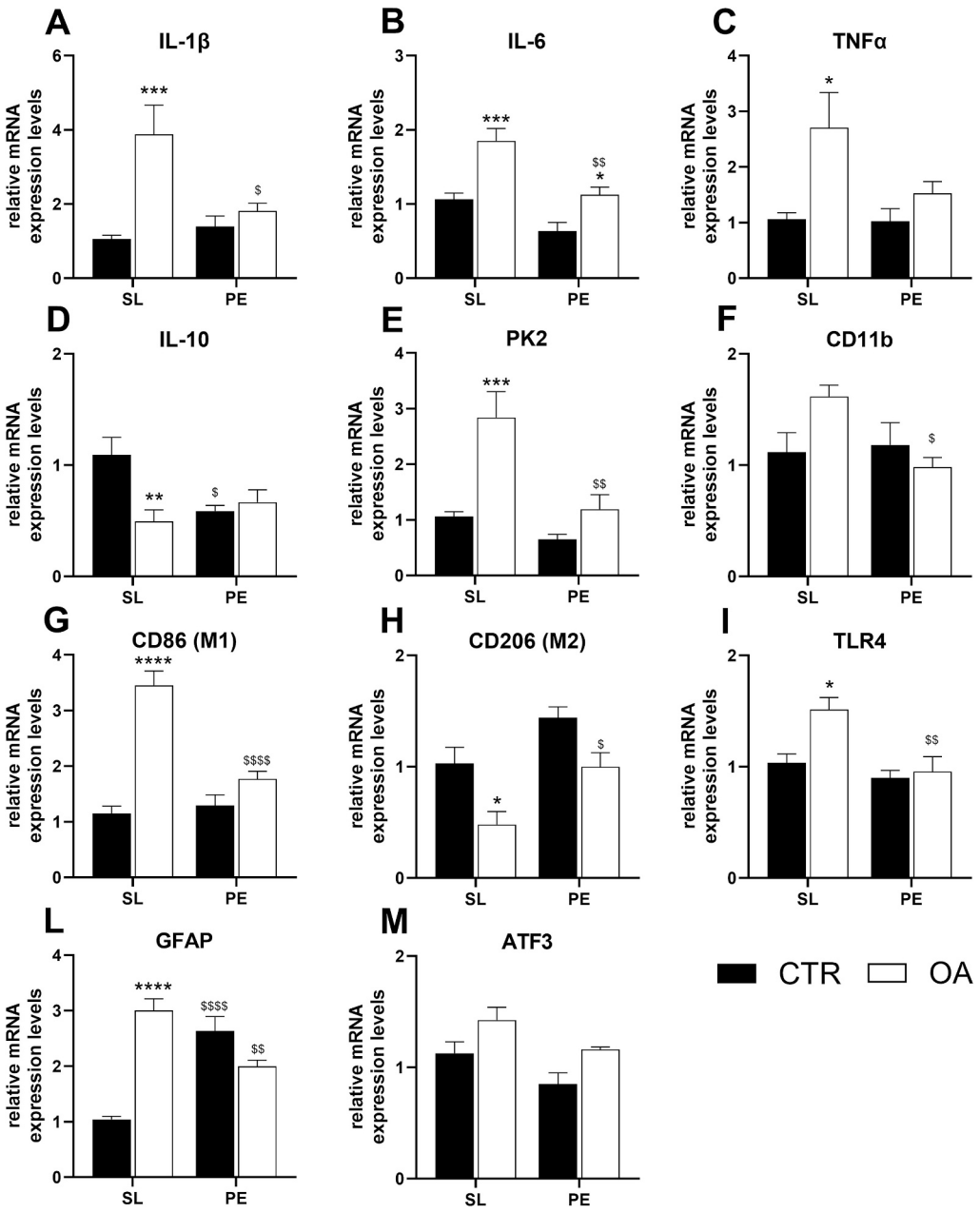


Fig. 11. Myenteric plexus biochemical evaluations. Tissues were obtained at the end of the experimental protocol (i.e. after 12 weeks of sedentary life-SL or physical exercise-PE; 4 weeks after osteoarthritis-OA induction). mRNA levels of the pro-inflammatory cytokines (**A**) IL-1β, (**B**) IL-6 and (**C**) TNFα, (**D**) the anti-inflammatory cytokine IL-10, (**E**) the chemokine PK2, (**F**—**H**) the macrophage markers CD11b, CD86, CD206 (**I**) TLR4, (**L**) the enteric glia marker GFAP and (**M**) the cellular damage marker ATF3 were assessed by means of RT-qPCR. Data were normalized to GAPDH and presented as fold-changes over the levels of CTR_SL mice group. Data are expressed as mean ± SEM of 8 animals per group. Statistical analysis was performed by Two-way ANOVA followed by Tukey's post-test. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ vs respective CTR; $$$$$p < 0.0001$, $$$p < 0.01$, $$p < 0.05$ vs. respective SL group.

experimental protocol, it is not possible to delineate whether the positive effects induced by physical exercise are preventive or therapeutic. Further studies should be conducted to distinguish between them. However, it is known from the literature that physical exercise in the context of pathological pain, both when implemented as a preventive and therapeutic strategy in murine models, is always effective in positively modulating the pathological symptoms [71–73]. Additionally, it is interesting to note that in patients with OA knee the leg muscles are compromised with a reduction in functionality and strength [74,75]. Our data demonstrated that a significant reduction in leg muscle mass with particular involvement of gastrocnemius and quadriceps muscles in OA and physical exercise was effective not only in reducing pain and improving motor function but also in significantly counteracting muscle wasting. However, in this study we limited ourselves to evaluating the weight of leg muscles, without analyzing the composition changes or molecular alterations. Thus, more detailed studies should be conducted. Furthermore, detailed analyses have been performed both at the knee joint level and at important stations in pain transmission. In the OA knee a significant inflammatory condition has been detected, with high levels of pro-inflammatory cytokines and chemokines associated with a significant macrophage infiltration. Our data indicate that the levels of MMP13 and ATF3, a key enzyme in cartilage degenerative processes and a cellular damage marker, respectively, are particularly high during OA and this up-regulation is also associated with an overexpression of the neurogenesis marker DCX. This confirms that during OA there is a continuous process of degradation and repair that leads to both new

vessel formation and nerve sprouting [76]. Consistent physical exercise, before and after OA induction, was able to effectively counteract local inflammation and joint damage. Our data are also supported by other recent studies in animal OA models, where therapeutic exercise effectively: *i.* increased muscle cross-sectional area; *ii.* inhibited osteocyte death and B-type synovial cell dysfunction; *iii.* alleviated cartilage degeneration and subchondral bone remodeling, thus reprogramming the cartilage-subchondral unit, stabilizing the bone structure, and *iv.* reduced the levels of caspase-3, MMP-13 and MMP-2, as well as regulated JNK/NF-KB signaling, resulting in a decrease in IL-1β, IL-6 and TNF-α expression levels and counteracting inflammation [38,77–83]. Furthermore, a recent experimental study indicated that early intervention by physical exercise, i.e. in the very early stages of cartilage damage, provides better effects than late intervention when post- traumatic OA is already under onset [84]. Both in the peripheral (i.e. sciatic nerve and DRGs) and central (i.e. spinal cord) nervous system, our data confirm what has already been observed in other studies [14]. Indeed, OA induced a neuroinflammatory condition in all nervous tissues, which moves from the periphery to the center. In sciatic nerve, significant cellular damage is observed associated with significant up-regulation of pro-inflammatory cytokines and of the chemokine PK2, as well as the anti-inflammatory cytokine IL-10. The increase in the latter could depend on a physiological mechanism of the organism to modulate neuroinflammation, that tries to rebalance the pro−/anti-inflammatory cytokine levels. Furthermore, it is possible to observe that there is a high recall of macrophages demonstrated by the increase in CD11b and TLR4, as well as a clear activation of Schwann cells characterized by higher GFAP expression.

In DRGs of OA_SL mice, the pro-inflammatory cytokines and PK2 levels were up-regulated along with both satellite cell and resident and/or recruited macrophage activation. Additionally, in DRGs, we further dissected macrophage phenotype, and we observed a significant overexpression of a pro-inflammatory marker (CD68) associated with a decrease in an anti-inflammatory marker (CD206). Recent evidence suggests a direct cross-talk between the joint and the spinal cord [76]. In this regard, a significant neuroinflammatory condition was present in the spinal cord of OA_SL mice. We observed not only an upregulation of mRNA levels of pro-inflammatory cytokines and PK2, but also significant cellular damage associated with astrogliosis. Furthermore, it is interesting to observe that microglia was shifted to a pro-inflammatory profile. Recent studies suggest that in addition to cytokines, chemokines are also reported as potential critical players [85]. Indeed, chemokines were consistently increased in several pain murine animal models [86,87] and among them we recently demonstrated that PK2 could play a key role in the onset and maintenance of neuroinflammation and OA pain [16]. In our experimental setting, we observed an extremely positive effect of physical exercise that was able to abolish neuroinflammation in all the peripheral and central nervous system areas that were evaluated. Additionally, our results indicate that apart from pain symptoms and neuroinflammation OA_SL mice also display anxiety- and depression-like behaviors. Previously published studies, including data from our group, have already shown that when persistent pain is present, including OA pain, alterations, including neuroinflammation, in several brain areas may be involved in affective and emotional responses [14–20,88–91]. Our present data show a neuroinflammatory condition at the level of the brainstem, hippocampus and hypothalamus in OA_SL mice. Although it has been demonstrated that an association between neuroinflammation and mood alterations, a causal relationship cannot yet be established [92–94]. Therefore, further studies will be necessary to confirm whether neuroinflammation determines the mood alterations observed in OA_SL animals. However, physical exercise was able to significantly ameliorate mood alterations and supraspinal neuroinflammation in OA mice, confirming that the positive effects of physical exercise on health also extend to brain areas. Several preclinical and clinical studies demonstrated that physical exercise has a beneficial repercussion on both young and old people's mood and in both healthy subjects and patients [39,40,95–99]. Physical activity increases the neuronal release of serotonin and dopamine [100,101] and enhances neurogenesis, as well as increases the expression of growth and neurotrophic factors, such as BDNF, VEGF, and GDNF [102,103]. Among the supraspinal areas that we evaluated, a central role is played by the brainstem, which is not only highly involved in nociception and pain processing [104] but also contains nuclei that directly project to the intestine and control gut function based on information coming from the cortex and the subcortical center [105]. Alterations at the brainstem level could therefore also have repercussions at the intestinal level, determining gut neuroinflammation onset. Emerging evidence suggests a close connection between the maintenance of OA inflammatory state and gastrointestinal alterations. In OA_SL mice, we observed consolidated intestinal neuroinflammation. The current understanding of gut alterations in OA patients is still unclear, but neuroinflammation may play an important role [106]. Interestingly, we demonstrated that physical exercise in OA animals was able to block gut neuroinflammation. Therefore, it is tempting to hypothesize that by neutralizing gut neuroinflammation, recurrent gastrointestinal problems in OA patients could also be counteracted.

In conclusion, our data show that in the presence of OA, there is a simultaneous development of pain, mood disorders, and neuroinflammation. Anyhow, we demonstrated that regular physical activity alone, before and during a pathological context, without any concomitant pharmacological support, was able to successfully modulate neuroinflammation, pain and mood alterations. Healthy lifestyle and active movement could be a winning strategy to mitigate both OA appearance and the sequelae that accompany this pathology.

Abbreviations

OA	osteoarthritis
MIA	monoiodoacetate
PE	physical exercise
SL	sedentary lifestyle
CTR_PE	control animals subjected to physical exercise
CTR_SL	control animals with sedentary lifestyle
OA_PE	osteoarthritic animals subjected to physical exercise
OA_SL	osteoarthritic animals with sedentary lifestyle
OP	open field test
EPM	Elevated Plus Maze test
LDB	Light/Dark Box test
SP	Sucrose Preference test-SP
TS	Tail Suspension test
FS	Force Swimming test
IA	intra-articular
DRG	dorsal root ganglia
IL-1β	Interleukin 1β
IL-6	Interleukin 6
IL-10	Interleukin 10
TNF-α	Tumor Necrosis factor α
PK2	Prokineticin (PK)2
CD68	Cluster of Differentiation 68
CD11b	Cluster of Differentiation 11b
CD86	Cluster of Differentiation 86
CD206	Cluster of Differentiation 206
TLR4	Toll-like Receptor 4
GFAP	Glial Fibrillary Acidic Protein
ATF3	Activating Transcription Factor 3
BDNF	Brain-Derived Neurotrophic Factor
MMP13	Matrix Metalloproteinase 13
DCX	Doublecortin (DCX)
GAPDH	GlycerAldehyde-3-Phosphate DeHydrogenase

CRediT authorship contribution statement

G. Amodeo: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **G. Galimberti:** Writing – review & editing, Methodology, Investigation, Conceptualization. **S. Ceruti:** Writing – review & editing. **B. Riboldi:** Writing – review & editing. **S. Franchi:** Writing – review & editing, Validation, Methodology, Investigation. **P. Sacerdote:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Funding sources

Amodeo G. is supported by Fondazione Umberto Veronesi ETS, Milan, Italy.

Galimberti G. is supported by the XXXVIII cycle of the Doctorate in Pharmacological Biomolecular Sciences, Experimental and Clinical, Università degli Studi di Milano, Milan, Italy.

Riboldi B. is supported by the XXXIX cycle of the Doctorate in Intersectoral Innovation, Università degli Studi di Milano, Milan, Italy.

Declaration of competing interest

There are no conflicts of interest to declare. All authors have read the journal's authorship declaration. The manuscript has been reviewed and approved by all named authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2025.123710.

Data availability

All data generated during the current study are available from the corresponding author on reasonable request.

References

[1] Q. Yao, X. Wu, C. Tao, W. Gong, M. Chen, M. Qu, Y. Zhong, T. He, S. Chen, G. Xiao, Osteoarthritis: pathogenic signaling pathways and therapeutic targets, Signal Transduct. Target. Ther. 8 (1) (2023) 56, https://doi.org/10.1038/s41392-023-01330-w.

[2] L. Tong, H. Yu, X. Huang, J. Shen, G. Xiao, L. Chen, H. Wang, L. Xing, D. Chen, Current understanding of osteoarthritis pathogenesis and relevant new approaches, Bone Res. 10 (1) (2022) 60, https://doi.org/10.1038/s41413-022-00226-9.

[3] F. Guida, M. Rocco, L. Luongo, P. Persiani, M.C. Vulpiani, S.M. Nusca, S. Maione, F. Coluzzi, Targeting Neuroinflammation in osteoarthritis with intra-articular adelmidrol, Biomolecules 12 (2022) 1453, https://doi.org/10.3390/biom12101453.

[4] E. Sanchez-Lopez, R. Coras, A. Torres, N.E. Lane, M. Guma, Synovial inflammation in osteoarthritis progression, Nat. Rev. Rheumatol. 18 (5) (2022) 258–275, https://doi.org/10.1038/s41584-022-00749-9.

[5] W.H. Robinson, C.M. Lepus, Q. Wang, H. Raghu, R. Mao, T.M. Lindstrom, J. Sokolove, Low-grade inflammation as a key mediator of the pathogenesis of osteoarthritis, Nat. Rev. Rheumatol. 12 (10) (2016) 580–592, https://doi.org/10.1038/nrrheum.2016.136.

[6] A. Mathiessen, P.G. Conaghan, Synovitis in osteoarthritis: current understanding with therapeutic implications, Arthritis Res. Ther. 19 (2017) 18, https://doi.org/10.1186/s13075-017-1229-9.

[7] H.G. Schaible, F. Richter, A. Ebersberger, M.K. Boettger, H. Vanegas, G. Natura, E. Vazquez, G. von Segond Banchet, Joint pain, Exp. Brain Res. 196 (1) (2009) 153–162, https://doi.org/10.1007/s00221-009-1782-9.

[8] S. Sutton, A. Clutterbuck, P. Harris, T. Gent, S. Freeman, N. Foster, R. Barrett-Jolley, A. Mobasheri, The contribution of the synovium, synovial derived inflammatory cytokines and neuropeptides to the pathogenesis of osteoarthritis, Vet. J. 179 (1) (2009) 10–24, https://doi.org/10.1016/j.tvjl.2007.08.013.

[9] A. Sharma, P. Kudesia, Q. Shi, R. Gandhi, Anxiety and depression in patients with osteoarthritis: impact and management challenges, Open Access Rheumatol. 8 (2016) 103–113, https://doi.org/10.2147/OARRR.S93516.

[10] J. Axford, A. Butt, C. Heron, J. Hammond, J. Morgan, A. Alavi, J. Bolton, M. Bland, Prevalence of anxiety and depression in osteoarthritis: use of the Hospital Anxiety and Depression Scale as a screening tool, Clin. Rheumatol. 29 (2010) 1277–1283, https://doi.org/10.1007/s10067-010-1547-7.

[11] D.L. Goldenberg, The interface of pain and mood disturbances in the rheumatic diseases, Semin. Arthritis Rheum. 40 (2010) 15–31, https://doi.org/10.1016/j.semarthrit.2008.11.005.

[12] O. Moriarty, D.P. Finn, Cognition and pain, Curr. Opin. Support. Palliat. Care 8 (2014) 130–136, https://doi.org/10.1097/SPC.0000000000000054.

[13] O. Moriarty, B.E. McGuire, D.P. Finn, The effect of pain on cognitive function: a review of clinical and preclinical research, Prog. Neurobiol. 93 (2011) 385–404, https://doi.org/10.1016/j.pneurobio.2011.01.002.

[14] G. Amodeo, G. Magni, G. Galimberti, B. Riboldi, S. Franchi, P. Sacerdote, S. Ceruti, Neuroinflammation in osteoarthritis: from pain to mood disorders, Biochem. Pharmacol. 30 (2024) 116182, https://doi.org/10.1016/j.bcp.2024.116182.

[15] G. Amodeo, S. Franchi, S. D'Agnelli, G. Galimberti, M. Baciarello, E.G. Bignami, P. Sacerdote, Supraspinal neuroinflammation and anxio-depressive-like behaviors in young- and older- adult mice with osteoarthritis pain: the effect of morphine, Psychopharmacology 240 (10) (2023) 2131–2146, https://doi.org/10.1007/s00213-023-06436-1.

[16] G. Galimberti, G. Amodeo, G. Magni, B. Riboldi, G. Balboni, V. Onnis, S. Ceruti, P. Sacerdote, S. Franchi, Prokineticin system is a pharmacological target to counteract pain and its comorbid mood alterations in an osteoarthritis murine model, Cells 12 (18) (2023) 2255, https://doi.org/10.3390/cells12182255.

[17] G. Batallé, X. Bai, E. Pouso-Vázquez, G. Roch, L. Rodríguez, O. Pol, The recovery of cognitive and affective deficiencies linked with chronic osteoarthritis pain and implicated pathways by slow-releasing hydrogen sulfide treatment, Antioxidants (Basel). 10 (10) (2021) 1632, https://doi.org/10.3390/antiox10101632.

[18] G. Batallé, L. Cabarga, O. Pol, The inhibitory effects of slow-releasing hydrogen sulfide donors in the mechanical allodynia, grip strength deficits, and depressive-like behaviors associated with chronic osteoarthritis pain, Antioxidants (Basel) 9 (1) (2019) 31, https://doi.org/10.3390/antiox9010031.

[19] M. Carcolé, D. Zamanillo, M. Merlos, B. Fernández-Pastor, D. Cabañero, R. Maldonado, Blockade of the Sigma-1 receptor relieves cognitive and emotional impairments associated to chronic osteoarthritis pain, Front. Pharmacol. 10 (2019) 468, https://doi.org/10.3389/fphar.2019.00468.

[20] C. La Porta, S.A. Bura, J. Llorente-Onaindia, A. Pastor, F. Navarrete, M.S. García-Gutiérrez, R. De la Torre, J. Manzanares, J. Monfort, R. Maldonado, Role of the endocannabinoid system in the emotional manifestations of osteoarthritis pain, Pain 156 (10) (2015) 2001–2012, https://doi.org/10.1097/j.pain.0000000000000260.

[21] L. Bravo, M. Llorca-Torralba, I. Suárez-Pereira, E. Berrocoso, Pain in neuropsychiatry: insights from animal models, Neurosci. Biobehav. Rev. 115 (2020) 96–115, https://doi.org/10.1016/j.neubiorev.2020.04.029.

[22] A. Dellarole, P. Morton, R. Brambilla, W. Walters, S. Summers, D. Bernardes, M. Grilli, J.R. Bethea, Neuropathic pain-induced depressive-like behavior and hippocampal neurogenesis and plasticity are dependent on TNFR1 signaling, Brain Behav. Immun. 41 (2014) 65–81, https://doi.org/10.1016/j.bbi.2014.04.003.

[23] M.J. Bair, R.L. Robinson, W. Katon, K. Kroenke, Depression and pain comorbidity: a literature review, Arch. Intern. Med. 163 (2003) 2433–2445, https://doi.org/10.1001/archinte.163.20.2433.

[24] F. Guida, M. Iannotta, G. Misso, F. Ricciardi, S. Boccella, V. Tirino, M. Falco, V. Desiderio, R. Infantino, G. Pieretti, V. de Novellis, G. Papaccio, L. Luongo, M. Caraglia, S. Maione, Long-term neuropathic pain behaviors correlate with synaptic plasticity and limbic circuit alteration: a comparative observational study in mice, Pain 163 (2022) 1590–1602, https://doi.org/10.1097/j.pain.0000000000002549.

[25] C. Ní Chasaide, M.A. Lynch, The role of the immune system in driving neuroinflammation, Brain Neurosci. Adv. 4 (2020) 2398212819901082, https://doi.org/10.1177/2398212819901082.

[26] R. Dantzer, Neuroimmune interactions: from the brain to the immune system and vice versa, Physiol. Rev. 98 (2018) 477–504, https://doi.org/10.1152/physrev.00039.2016.

[27] M.L. Pfau, C. Ménard, S.J. Russo, Inflammatory mediators in mood disorders: therapeutic opportunities, Annu. Rev. Pharmacol. Toxicol. 58 (2018) 411–428, https://doi.org/10.1146/annurev-pharmtox-010617-052823.

[28] A. Bhattacharya, D.N.C. Jones, Emerging role of the P2X7-NLRP3-IL1β pathway in mood disorders, Psychoneuroendocrinology 98 (2018) 95–100, https://doi.org/10.1016/j.psyneuen.2018.08.015.

[29] E.E. Barcelon, W.H. Cho, S.B. Jun, S.J. Lee, Brain microglial activation in chronic pain-associated affective disorder, Front. Neurosci. 13 (2019) 213, https://doi.org/10.3389/fnins.2019.00213.

[30] G. Wei, K. Lu, M. Umar, Z. Zhu, W.W. Lu, J.R. Speakman, Y. Chen, L. Tong, D. Chen, Risk of metabolic abnormalities in osteoarthritis: a new perspective to understand its pathological mechanisms, Bone Res. 11 (1) (2023) 63, https://doi.org/10.1038/s41413-023-00301-9.

[31] A. Courties, O. Gualillo, F. Berenbaum, J. Sellam, Metabolic stress-induced joint inflammation and osteoarthritis, Osteoarthr. Cartil. 23 (11) (2015) 1955–1965, https://doi.org/10.1016/j.joca.2015.05.016.

[32] G. Spahn, G.O. Hofmann, L.V. von Engelhardt, M. Li, H. Neubauer, H.M. Klinger, The impact of a high tibial valgus osteotomy and unicondylar medial arthroplasty on the treatment for knee osteoarthritis: a meta-analysis, Knee Surg. Sports Traumatol. Arthrosc. 21 (1) (2013) 96–112, https://doi.org/10.1007/s00167-011-1751-2.

[33] H. Kong, X.Q. Wang, X.A. Zhang, Exercise for osteoarthritis: a literature review of pathology and mechanism, Front. Aging Neurosci. 14 (2022) 854026, https://doi.org/10.3389/fnagi.2022.854026.

[34] M.G. Benedetti, G. Furlini, A. Zati, Mauro G. Letizia, The effectiveness of physical exercise on bone density in osteoporotic patients, Biomed. Res. Int. 2018 (2018) 4840531, https://doi.org/10.1155/2018/4840531.
[35] M.S. Peng, R. Wang, Y.Z. Wang, C.C. Chen, J. Wang, X.C. Liu, G. Song, J.B. Guo, P.J. Chen, X.Q. Wang, Efficacy of therapeutic aquatic exercise vs physical therapy modalities for patients with chronic low Back pain: a randomized clinical trial, JAMA Netw. Open 5 (1) (2022) e2142069, https://doi.org/10.1001/jamanetworkopen.2021.42069.
[36] B. Wu, L. Zhou, C. Chen, J. Wang, L.I. Hu, X. Wang, Effects of exercise-induced hypoalgesia and its neural mechanisms, Med. Sci. Sports Exerc. 54 (2) (2022) 220–231, 10.1249/MSS.0000000000002781.
[37] D.L. Belavy, J. Van Oosterwijck, M. Clarkson, E. Dhondt, N.L. Mundell, C. T. Miller, P.J. Owen, Pain sensitivity is reduced by exercise training: evidence from a systematic review and meta-analysis, Neurosci. Biobehav. Rev. 120 (2021) 100–108, https://doi.org/10.1016/j.neubiorev.2020.11.012.
[38] C.Y. Zeng, Z.R. Zhang, Z.M. Tang, F.Z. Hua, Benefits and mechanisms of exercise training for knee osteoarthritis, Front. Physiol. 12 (2021) 794062, https://doi.org/10.3389/fphys.2021.794062.
[39] V.S. Hooda, P.A. Muley, P.P. Muley, A. Anjankar, G. Bandre, Effect of exercise on depression, anxiety and mood: a narrative review, J. Clin. Diagn. Res. 18 (1) (2024) 1–4, https://doi.org/10.7860/JCDR/2024/65557.18929.
[40] S. Hu, L. Tucker, C. Wu, L. Yang, Beneficial effects of exercise on depression and anxiety during the Covid-19 pandemic: a narrative review, Front. Psychol. 11 (2020) 587557, https://doi.org/10.3389/fpsyt.2020.587557.
[41] P. Gonzalo-Encabo, G. Maldonado, D. Valadés, C. Ferragut, A. Pérez-López, The role of exercise training on low-grade systemic inflammation in adults with overweight and obesity: a systematic review, Int. J. Environ. Res. Public Health 18 (24) (2021) 13258, https://doi.org/10.3390/ijerph182413258.
[42] J. de Sousa Valente, The pharmacology of pain associated with the monoiodoacetate model of osteoarthritis, Front. Pharmacol. 10 (2019) 974, https://doi.org/10.3389/fphar.2019.00974.
[43] X. Zhou, H. Cao, M. Wang, J. Zou, W. Wu, Moderate-intensity treadmill running relieves motion-induced post-traumatic osteoarthritis mice by up-regulating the expression of lncRNA H19, Biomed. Eng. Online 20 (1) (2021) 111, https://doi.org/10.1186/s12938-021-00949-6.
[44] M.P. Massett, C. Matejka, H. Kim, Systematic review and meta-analysis of endurance exercise training protocols for mice, Front. Physiol. 12 (2021) 782695, https://doi.org/10.3389/fphys.2021.782695.
[45] B.N. Leitzelar, K.F. Koltyn, Exercise and neuropathic pain: a general overview of preclinical and clinical research, Sports Med Open. 7 (1) (2021) 21, https://doi.org/10.1186/s40798-021-00307-9.
[46] J.B. Guo, B.L. Chen, Y. Wang, Y. Zhu, G. Song, Z. Yang, Y.L. Zheng, X.Q. Wang, P. J. Chen, Meta-analysis of the effect of exercise on neuropathic pain induced by peripheral nerve injury in rat models, Front. Neurol. 10 (2019) 636, https://doi.org/10.3389/fneur.2019.00636.
[47] M.H. Pitcher, The impact of exercise in rodent models of chronic pain, Curr. Osteoporos. Rep. 16 (4) (2018) 344–359, https://doi.org/10.1007/s11914-018-0461-9.
[48] K. Flurkey, J.M. Currer, D.E. Harrison, The mouse in aging research, in: Book: The Mouse in Biomedical Research, 2nd ed. Volume 3, Elsevier, 2007 (eBook ISBN: 9780080469072).9780080469072.
[49] G. Amodeo, S. Franchi, G. Galimberti, L. Comi, S. D'Agnelli, M. Baciarello, E. G. Bignami, P. Sacerdote, Osteoarthritis pain in old mice aggravates Neuroinflammation and frailty: the positive effect of morphine treatment, Biomedicines 10 (11) (2022) 2847, https://doi.org/10.3390/biomedicines10112847.
[50] G. Amodeo, B. Verduci, P. Sartori, P. Procacci, V. Conte, G. Balboni, P. Sacerdote, S. Franchi, The antagonism of the prokineticin system counteracts bortezomib induced side effects: focus on mood alterations, Int. J. Mol. Sci. 22 (19) (2021) 10256, https://doi.org/10.3390/ijms221910256.
[51] L. Da Dalt, A. Moregola, M. Svecla, S. Pedretti, F. Fantini, M. Ronzio, P. Uboldi, D. Dolfini, E. Donetti, A. Baragetti, N. Mitro, L. Scorrano, G.D. Norata, The inhibition of inner mitochondrial fusion in hepatocytes reduces non-alcoholic fatty liver and improves metabolic profile during obesity by modulating bile acid conjugation, Cardiovasc. Res. 119 (18) (2024) 2917–2929, https://doi.org/10.1093/cvr/cvad169.
[52] M. Sumizono, Y. Yoshizato, R. Yamamoto, T. Imai, A. Tani, K. Nakanishi, T. Nakakogawa, T. Matsuoka, R. Matsuzaki, T. Tanaka, H. Sakakima, Mechanisms of neuropathic pain and pain-relieving effects of exercise therapy in a rat neuropathic pain model, J. Pain Res. 15 (2022) 1925–1938, https://doi.org/10.2147/JPR.S367818.
[53] R. Zhao, Exercise mimetics: a novel strategy to combat neuroinflammation and Alzheimer's disease, J. Neuroinflammation 21 (1) (2024) 40, https://doi.org/10.1186/s12974-024-03031-9.
[54] C.R. Sartori, M. Pagliusi Jr., I.J.M. Bonet, C.H. Tambeli, C.A. Parada, Running wheel exercise induces therapeutic and preventive effects on inflammatory stimulus-induced persistent hyperalgesia in mice, PLoS One 15 (10) (2020) e0240115, https://doi.org/10.1371/journal.pone.0240115.
[55] O. Mee-Inta, Z.W. Zhao, Y.M. Kuo, Physical exercise inhibits inflammation and microglial activation, Cells 8 (7) (2019) 691, https://doi.org/10.3390/cells8070691.
[56] F. El Gaamouch, H.Y. Lin, Q. Wang, W. Zhao, J. Pan, K. Liu, J. Wong, C. Wu, C. Yuan, H. Cheng, W. Qin, K. Hao, B. Zhang, J. Wang, Peripheral and cognitive benefits of physical exercise in a mouse model of midlife metabolic syndrome, Sci. Rep. 12 (1) (2022) 3260, https://doi.org/10.1038/s41598-022-07252-x.
[57] J.A. Morgan, G. Singhal, F. Corrigan, E.J. Jaehne, M.C. Jawahar, B.T. Baune, The effects of aerobic exercise on depression-like, anxiety-like, and cognition-like behaviours over the healthy adult lifespan of C57BL/6 mice, Behav. Brain Res. 337 (2018) 193–203, https://doi.org/10.1016/j.bbr.2017.09.022.
[58] K. Li, A. Liu, W. Zong, L. Dai, Y. Liu, R. Luo, S. Ge, G. Dong, Moderate exercise ameliorates osteoarthritis by reducing lipopolysaccharides from gut microbiota in mice, Saudi J Biol Sci. 28 (1) (2021) 40–49, https://doi.org/10.1016/j.sjbs.2020.08.027.
[59] W. Yang, Y. Liu, G. Yang, B. Meng, Z. Yi, G. Yang, M. Chen, P. Hou, H. Wang, X. Xu, Moderate-intensity physical exercise affects the exercise performance and gut microbiota of mice, Front. Cell. Infect. Microbiol. 11 (2021) 712381, https://doi.org/10.3389/fcimb.2021.712381.
[60] A. de Sire, R. de Sire, V. Petito, L. Masi, C. Cisari, A. Gasbarrini, F. Scaldaferri, M. Invernizzi, Gut-joint axis: the role of physical exercise on gut microbiota modulation in older people with osteoarthritis, Nutrients 12 (2) (2020) 574, https://doi.org/10.3390/nu12020574.
[61] R. Carvalho, Energy expenditure determined by indirect calorimetry during rest, pain and analgesia in a critically ill patient, Clin. Nutr. 63 (2024) 1039, https://doi.org/10.1186/s40560-021-00524-0.
[62] P.C. Coyle, J.A. Schrack, G.E. Hicks, Pain energy model of mobility limitation in the older adult, Pain Med. 19 (8) (2018) 1559–1569, https://doi.org/10.1093/pm/pnx089.
[63] P. Holland-Fischer, J. Greisen, T. Grøfte, T.S. Jensen, P.O. Hansen, H. Vilstrup, Increased energy expenditure and glucose oxidation during acute nontraumatic skin pain in humans, Eur. J. Anaesthesiol. 26 (4) (2009) 311–317, https://doi.org/10.1097/EJA.0b013e328324b5e9.
[64] S. Niwa, K. Fila-Pawłowska, O. Van den Bergh, J. Rymaszewska, Respiratory dysfunction in persistent somatic symptoms: a systematic review of observational studies, J. Psychosom. Res. 181 (2024) 111607, https://doi.org/10.1016/j.jpsychores.2024.111607.
[65] H. Jafari, I. Courtois, O. Van den Bergh, J.W.S. Vlaeyen, I. Van Diest, Pain and respiration: a systematic review, Pain 158 (6) (2017) 995–1006, https://doi.org/10.1097/j.pain.0000000000000865.
[66] P. Terekhin, C. Forster, Hypocapnia related changes in pain-induced brain activation as measured by functional MRI, Neurosci. Lett. 400 (1–2) (2006) 110–114, https://doi.org/10.1016/j.neulet.2006.02.040.
[67] N. Paans, I. van den Akker-Scheek, K. van der Meer, S.K. Bulstra, M. Stevens, The effects of exercise and weight loss in overweight patients with hip osteoarthritis: design of a prospective cohort study, BMC Musculoskelet. Disord. 10 (2009) 24, https://doi.org/10.1186/1471-2474-10-24.
[68] E. Roddy, M. Doherty, Changing life-styles and osteoarthritis: what is the evidence? Best Pract. Res. Clin. Rheumatol. 20 (1) (2006) 81–97, https://doi.org/10.1016/j.berh.2005.09.006.
[69] S.P. Messier, R.F. Loeser, G.D. Miller, T.M. Morgan, W.J. Rejeski, M.A. Sevick, W. H. Ettinger Jr., M. Pahor, J.D. Williamson, Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the arthritis, diet, and activity promotion trial, Arthritis Rheum. 50 (5) (2004) 1501–1510, https://doi.org/10.1002/art.20256.
[70] M.E. van Baar, W.J. Assendelft, J. Dekker, R.A. Oostendorp, J.W. Bijlsma, Effectiveness of exercise therapy in patients with osteoarthritis of the hip or knee: a systematic review of randomized clinical trials, Arthritis Rheum. 42 (7) (1999) 1361–1369, https://doi.org/10.1002/1529-0131(199907)42:7<1361::AID-ANR9>3.0.CO;2-9.
[71] Y.N. Zheng, Y.L. Zheng, X.Q. Wang, P.J. Chen, Role of exercise on inflammation cytokines of neuropathic pain in animal models, Mol. Neurobiol. 61 (12) (2024) 10288–10301, https://doi.org/10.1007/s12035-024-04214-4.
[72] J. Sakamoto, S. Miyahara, S. Motokawa, A. Takahashi, R. Sasaki, Y. Honda, M. Okita, Regular walking exercise prior to knee osteoarthritis reduces joint pain in an animal model, PLoS One 18 (8) (2023) e0289765, https://doi.org/10.1371/journal.pone.0289765.
[73] K. Townsend, I. Imbert, V. Eaton, G.W. Stevenson, T. King, Voluntary exercise blocks ongoing pain and diminishes bone remodeling while sparing protective mechanical pain in a rat model of advanced osteoarthritis pain, Pain 163 (3) (2022) e476–e487, https://doi.org/10.1097/j.pain.0000000000002392.
[74] A.M. Susko, G.K. Fitzgerald, The pain-relieving qualities of exercise in knee osteoarthritis, Open Access Rheumatol. 5 (2013) 81–91, https://doi.org/10.2147/OARRR.S53974.
[75] A.H. Alnahdi, J.A. Zeni, L. Snyder-Mackler, Muscle impairments in patients with knee osteoarthritis, Sports Health. 4 (4) (2012) 284–292, https://doi.org/10.1177/1941738112445726.
[76] M. Fusco, S.D. Skaper, S. Coaccioli, G. Varrassi, A. Paladini, Degenerative joint diseases and Neuroinflammation, Pain Pract. 17 (4) (2017) 522–532, https://doi.org/10.1111/papr.12551.
[77] X. Hao, S. Wang, J. Zhang, T. Xu, Effects of body weight-supported treadmill training on cartilage-subchondral bone unit in the rat model of posttraumatic osteoarthritis, J. Orthop. Res. 39 (6) (2021) 1227–1235, https://doi.org/10.1002/jor.24791.
[78] P. Castrogiovanni, M. Di Rosa, S. Ravalli, A. Castorina, C. Guglielmino, R. Imbesi, M. Vecchio, F. Drago, M.A. Szychlinska, G. Musumeci, Moderate physical activity as a prevention method for knee osteoarthritis and the role of synoviocytes as biological key, Int. J. Mol. Sci. 20 (3) (2019) 511, https://doi.org/10.3390/ijms20030511.
[79] J. Allen, I. Imbert, J. Havelin, T. Henderson, G. Stevenson, L. Liaw, T. King, Effects of treadmill exercise on advanced osteoarthritis pain in rats, Arthritis Rheum. 69 (7) (2017) 1407–1417, https://doi.org/10.1002/art.40101.

[80] L. Assis, L.P. Milares, T. Almeida, C. Tim, A. Magri, K.R. Fernandes, C. Medalha, A.C. Renno, Aerobic exercise training and low-level laser therapy modulate inflammatory response and degenerative process in an experimental model of knee osteoarthritis in rats, Osteoarthr. Cartil. 24 (1) (2016) 169–177, https://doi.org/10.1016/j.joca.2015.07.020.
[81] L. Assis, T. Almeida, L.P. Milares, N. dos Passos, B. Araújo, C. Bublitz, S. Veronez, A.C. Renno, Musculoskeletal atrophy in an experimental model of knee osteoarthritis: the effects of exercise training and low-level laser therapy, Am. J. Phys. Med. Rehabil. 94 (8) (2015) 609–616, https://doi.org/10.1097/PHM.0000000000000219.
[82] H. Iijima, T. Aoyama, A. Ito, S. Yamaguchi, M. Nagai, J. Tajino, X. Zhang, H. Kuroki, Effects of short-term gentle treadmill walking on subchondral bone in a rat model of instability-induced osteoarthritis, Osteoarthr. Cartil. 23 (9) (2015) 1563–1574, https://doi.org/10.1016/j.joca.2015.04.015.
[83] M. Fallah Mohammadi, A. Hajizadeh Moghaddam, H. Mirkarimpur, The effects of a moderate exercise program on knee osteoarthritis in male Wistar rats, Iran. J. Basic Med. Sci. 16 (5) (2013) 683–688, n.d.
[84] Y.L. Hsieh, C.C. Yang, Early intervention of swimming exercises attenuate articular cartilage destruction in a rat model of anterior cruciate ligament and meniscus knee injuries, Life Sci. 212 (2018) 267–274, https://doi.org/10.1016/j.lfs.2018.10.013.
[85] R.J. Miller, A.M. Malfait, R.E. Miller, The innate immune response as a mediator of osteoarthritis pain, Osteoarthr. Cartil. 28 (5) (2020) 562–571, https://doi.org/10.1016/j.joca.2019.11.006.
[86] B.C. Jiang, T. Liu, Y.J. Gao, Chemokines in chronic pain: cellular and molecular mechanisms and therapeutic potential, Pharmacol. Ther. 212 (2020) 107581, https://doi.org/10.1016/j.pharmthera.2020.107581.
[87] T.M. Griffin, J.L. Huebner, V.B. Kraus, Z. Yan, F. Guilak, Induction of osteoarthritis and metabolic inflammation by a very high-fat diet in mice: effects of short-term exercise, Arthritis Rheum. 64 (2) (2012) 443–453, https://doi.org/10.1002/art.33332.
[88] A.R. Khan, L. Geiger, O. Wiborg, B. Czéh, Stress-induced morphological, cellular and molecular changes in the brain-lessons learned from the chronic mild stress model of depression, Cells 9 (4) (2020) 1026, https://doi.org/10.3390/cells9041026.
[89] M.J.F. Levy, F. Boulle, H.W. Steinbusch, D.L.A. van den Hove, G. Kenis, L. Lanfumey, Neurotrophic factors and neuroplasticity pathways in the pathophysiology and treatment of depression, Psychopharmacology 235 (8) (2018) 2195–2220, https://doi.org/10.1007/s00213-018-4950-4.
[90] T. Suzuki, M. Amata, G. Sakaue, S. Nishimura, T. Inoue, M. Shibata, T. Mashimo, Experimental neuropathy in mice is associated with delayed behavioral changes related to anxiety and depression, Anesth. Analg. 104 (6) (2007) 1570–1577, https://doi.org/10.1213/01.ane.0000261514.19946.66.
[91] M. Narita, N. Kuzumaki, M. Narita, C. Kaneko, N. Hareyama, M. Miyatake, K. Shindo, K. Miyoshi, M. Nakajima, Y. Nagumo, F. Sato, H. Wachi, Y. Seyama, T. Suzuki, Chronic pain-induced emotional dysfunction is associated with astrogliosis due to cortical delta-opioid receptor dysfunction, J. Neurochem. 97 (5) (2006) 1369–1378, https://doi.org/10.1111/j.1471-4159.2006.03824.x.
[92] X.Y. Yang, H.Q. Wang, Z.Z. Wang, N.H. Chen, Linking depression and neuroinflammation: crosstalk between glial cells, Eur. J. Pharmacol. 995 (2025) 177408, https://doi.org/10.1016/j.ejphar.2025.177408 (Epub 2025 Feb 19. PMID: 39984011).
[93] Q. Li, Y. Xie, J. Lin, M. Li, Z. Gu, T. Xin, Y. Zhang, Q. Lu, Y. Guo, Y. Xing, W. Wang, Microglia sing the prelude of Neuroinflammation-associated depression, Mol. Neurobiol. 62 (4) (2025) 5311–5332, https://doi.org/10.1007/s12035-024-04575-w. Apr. Epub 2024 Nov 13. PMID: 39535682.
[94] A. Sălcudean, R.A. Popovici, D.E. Pitic, D. Sârbu, A. Boroghina, M. Jomaa, M. A. Salehi, A.A.M. Kher, M.M. Lica, C.R. Bodo, V.R. Enatescu, Unraveling the complex interplay between Neuroinflammation and depression: a comprehensive review, Int. J. Mol. Sci. 26 (4) (2025) 1645, https://doi.org/10.3390/ijms26041645. PMID: 40004109; PMCID: PMC11855341. Feb 14.
[95] S.S. Johnson, P.H. Castle, How do sleep, food, mood, and exercise relate to well-being? Am. J. Health Promot. 29 (4) (2015). TAHP4-5. n.d.
[96] M.B. Powers, G.J. Asmundson, J.A. Smits, Exercise for mood and anxiety disorders: the state-of-the-science, Cogn. Behav. Ther. 44 (4) (2015) 237–239, https://doi.org/10.1080/16506073.2015.1047286.
[97] B.C. Adamson, I. Ensari, R.W. Motl, Effect of exercise on depressive symptoms in adults with neurologic disorders: a systematic review and meta-analysis, Arch. Phys. Med. Rehabil. 96 (7) (2015) 1329–1338, https://doi.org/10.1016/j.apmr.2015.01.005.
[98] J.J. Annesi, G.A. Tennant, Exercise program-induced mood improvement and improved eating in severely obese adults, Int. Q. Community Health Educ. 33 (4) (2012) 391–402, https://doi.org/10.2190/IQ.33.4.f.
[99] J.M. Hoffman, K.R. Bell, J.M. Powell, J. Behr, E.C. Dunn, S. Dikmen, C. H. Bombardier, A randomized controlled trial of exercise to improve mood after traumatic brain injury, PM R 2 (10) (2010) 911–919, https://doi.org/10.1016/j.pmrj.2010.06.008.
[100] H. Alizadeh Pahlavani, Possible role of exercise therapy on depression: effector neurotransmitters as key players, Behav. Brain Res. 459 (2024) 114791, https://doi.org/10.1016/j.bbr.2023.114791.
[101] D. Dfarhud, M. Malmir, M. Khanahmadi, Happiness & health: the biological factors- systematic review article, Iran. J. Public Health 43 (11) (2014) 1468–1477, doi: n.d.
[102] L.J. Spielman, J.P. Little, A. Klegeris, Physical activity and exercise attenuate neuroinflammation in neurological diseases, Brain Res. Bull. 125 (2016) 19–29, https://doi.org/10.1016/j.brainresbull.2016.03.012.
[103] Q. Yu, X. Li, J. Wang, Y. Li, Effect of exercise training on long-term potentiation and NMDA receptor channels in rats with cerebral infarction, Exp. Ther. Med. 6 (6) (2013) 1431–1436, https://doi.org/10.3892/etm.2013.1319.
[104] V. Napadow, R. Sclocco, L.A. Henderson, Brainstem neuroimaging of nociception and pain circuitries, Pain Rep. 4 (4) (2019) e745, https://doi.org/10.1097/PR9.0000000000000745.
[105] M.G. Gwak, S.Y. Chang, Gut-brain connection: microbiome, gut barrier, and environmental sensors, Immune Netw. 21 (3) (2021) e20, https://doi.org/10.4110/in.2021.21.e20.
[106] L. Goudman, T. Demuyser, J.G. Pilitsis, M. Billot, M. Roulaud, P. Rigoard, M. Moens, Gut dysbiosis in patients with chronic pain: a systematic review and meta-analysis, Front. Immunol. 15 (2024) 1342833, https://doi.org/10.3389/fimmu.2024.1342833.