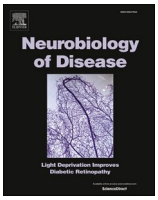
Neurobiology of Disease 172 (2022) 105817

Contents lists available at ScienceDirect

Neurobiology of Disease

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



Preventive exercise attenuates IL-2-driven mood disorders in multiple sclerosis

Luana Gilio [a,1], Diego Fresegna [b,1], Antonietta Gentile [b,2], Livia Guadalupi [b,c], Krizia Sanna [b,c], Francesca De Vito [a], Sara Balletta [a,c], Silvia Caioli [a], Francesca Romana Rizzo [a], Alessandra Musella [b,d], Ennio Iezzi [a], Alessandro Moscatelli [c,e], Giovanni Galifi [a], Roberta Fantozzi [a], Paolo Bellantonio [a], Roberto Furlan [f], Annamaria Finardi [f], Valentina Vanni [b], Ettore Dolcetti [a], Antonio Bruno [a], Fabio Buttari [a], Georgia Mandolesi [b,d], Diego Centonze [a,c,*], Mario Stampanoni Bassi [a]

[a] Unit of Neurology, IRCCS Neuromed, Pozzilli (IS), Italy
[b] Synaptic Immunopathology Lab, IRCCS San Raffaele Roma, Rome, Italy
[c] Department of Systems Medicine, Tor Vergata University, Rome, Italy
[d] Department of Human Sciences and Quality of Life Promotion, San Raffaele University, Rome, Italy
[e] Laboratory of Neuromotor Physiology, IRCCS Fondazione Santa Lucia, Rome, Italy
[f] Clinical Neuroimmunology Unit, Institute of Experimental Neurology (INSpe), Division of Neuroscience, San Raffaele Scientific Institute, Milan, Italy

ARTICLE INFO

Keywords:
Multiple sclerosis
Exercise
Inflammation
Depression
Anxiety

ABSTRACT

Background: Elevated levels of specific proinflammatory molecules in the cerebrospinal fluid (CSF) have been associated with disability progression, enhanced neurodegeneration and higher incidence of mood disorders in people with multiple sclerosis (MS). Studies in animal models of MS suggest that preventive exercise may play an immunomodulatory activity, with beneficial effects on both motor deficits and behavioral alterations. Here we explored the impact of lifestyle physical activity on clinical presentation and associated central inflammation in a large group of newly diagnosed patients with MS. Furthermore, we addressed the causal link between exercise-mediated immunomodulation and mood symptoms in the animal setting.

Methods: A cross-sectional study was conducted on 235 relapsing-remitting MS patients at the time of the diagnosis. Patients were divided into 3 groups ("sedentary", "lifestyle physical activity" and "exercise") according to the level of physical activity in the six months preceding the evaluation. Patients underwent clinical, neuropsychological and psychiatric evaluation, magnetic resonance imaging and lumbar puncture for diagnostic purposes. The CSF levels of proinflammatory and anti-inflammatory cytokines were analyzed and compared with a group of 80 individuals with non-inflammatory and non-degenerative diseases. Behavioral and electrophysiological studies were carried out in control mice receiving intracerebral injection of IL-2 or vehicle. Behavior was also assessed in mice with experimental autoimmune encephalomyelitis (EAE), animal model of MS, reared in standard (sedentary group) or running wheel-equipped (exercise group) cages.

Abbreviations: ACSF, artificial cerebrospinal fluid; Actb, beta-actin; BDI-II, Beck Depression Inventory–Second Edition; BMI, body mass index; BVMT-R, Brief Visuospatial Memory Test-Revised; CB1R, cannabinoid type 1 receptor; cDNA, complementary DNA; CFA, Complete Freund's Adjuvant; dpi 0, day of immunization; dpi, day post immunization; EAE, experimental autoimmune encephalomyelitis; EDSS, Expanded Disability Status Scale; FAB, Frontal Assessment Battery; FLAIR, fluid-attenuated inversion recovery; FSS, fatigue severity scale; FST, forced swimming test; Gd, Gadolinium; icv, intracerebroventricular; IL, interleukin; IQR, interquartile range; LDT, light-dark test; MMSE, Mini Mental State Examination; mRNAs, messenger RNA; MSNs, medium spiny neurons; MOG35–55, myelin oligodendrocyte glycoprotein 35–55; N, numbers; OCB, oligoclonal bands; OFT, open Field Test; PNS, peripheral nervous system; RA, receptor antagonist; RAVLT, Rey Auditory Verbal Learning Test; RR, relapsing-remitting; SD, standard deviation; SE, spin-echo; SDMT, Symbol Digit Modalities Test; SEM, standard mean error; sIPSCs, spontaneous inhibitory postsynaptic currents; STAI-Y, State-Trait Anxiety Inventory form Y; Th1, type 1 T helper; VH, Vehicle.

* Corresponding author at: Department of Systems Medicine, Tor Vergata University, Via Montpellier 1, 00133 Rome, Italy.
E-mail addresses: centonze@uniroma2.it, diego.centonze@neuromed.it (D. Centonze).

[1] These authors contributed equally to this work.

[2] Present address: Department of Biomedicine and Prevention, Tor Vergata University, Rome, Italy.

https://doi.org/10.1016/j.nbd.2022.105817
Received 28 March 2022; Received in revised form 5 July 2022; Accepted 8 July 2022
Available online 11 July 2022

Results: In exercising MS patients, depression and anxiety were reduced compared to sedentary patients. The CSF levels of the interleukin-2 and 6 (IL-2, IL-6) were increased in MS patients compared with control individuals. In MS subjects exercise was associated with normalized CSF levels of IL-2. In EAE mice exercise started before disease onset reduced both behavioral alterations and striatal IL-2 expression. Notably, a causal role of IL-2 in mood disorders was shown. IL-2 administration in control healthy mice induced anxious- and depressive-like behaviors and impaired type-1 cannabinoid (CB1) receptor-mediated neurotransmission at GABAergic synapses, mimicking EAE-induced synaptic dysfunction.
Conclusions: Our results indicate an immunomodulatory effect of exercise in MS patients, associated with reduced CSF expression of IL-2, which might result in reduced mood disorders. These data suggest that exercise in the early stages may act as a disease-modifying therapy in MS although further longitudinal studies are needed to clarify this issue.

1. Background

Neuroinflammation is a recognized trigger and enhancer of the pathological mechanisms underlying multiple sclerosis (MS), negatively influencing the disease course (Dendrou et al., 2015). Exacerbated CSF inflammation promotes disability progression and enhances neurodegeneration in MS (Romme Christensen et al., 2013; Rossi et al., 2014; Stampanoni Bassi et al., 2019; Magliozzi et al., 2021). Notably, CSF inflammation has also been associated with mood and cognitive disorders in patients with MS (Rossi et al., 2017). The role of inflammation in mood disorders has been clearly proved in the animal model of MS, the experimental autoimmune encephalomyelitis (EAE) (Gentile et al., 2015a, 2015b; Bruno et al., 2020). In EAE mice, inflammation was shown to cause anxiety- and depression-like behavior independently of motor deficits (Gentile et al., 2015a, 2015b; Haji et al., 2012; Peruga et al., 2011), suggesting that a specific inflammatory milieu in MS brain may contribute to the development of mood disorders. Moreover, experimental studies have shown that proinflammatory cytokines may directly promote the emotional disturbances observed after chronic stress (Rossi et al., 2012) and in EAE (Gentile et al., 2016), by interfering with cannabinoid-mediated control of GABA synapses in the striatum.

Physical exercise represents a promising therapeutic approach in MS, with beneficial effects on functional outcomes such as muscular strength and mobility, cardiovascular fitness, fatigue and overall quality of life (Amatya et al., 2019; Farrell 3rd et al., 2020). In addition, there is some evidence indicating positive effects of exercise on non-motor symptoms, such as mood and cognitive alterations, in MS patients (Motl and Pilutti, 2016; Motl et al., 2017). Clinical data suggest that exercise can slow down disease progression (Motl and Pilutti, 2016), as indicated by studies reporting reduced relapse rate (Langeskov-Christensen et al., 2021) and attenuated grey matter atrophy (Kjølhede et al., 2018) in exercising MS patients. However, clear evidence supporting a possible disease-modifying action mediated by the immunomodulatory effects of exercise in patients with MS is still lacking (Negaresh et al., 2018; Dalgas et al., 2019). Indeed, human studies exploring the impact of exercise on inflammatory biomarkers are limited by the fact that: 1. only peripheral inflammatory markers have been analyzed, 2. they have been conducted at relatively late disease stages, and with interventions of short-term duration (Dalgas et al., 2019; Riemenschneider et al., 2018).

In this regard, preclinical studies have shown that different types of exercise have immunomodulatory properties, by reducing the release of proinflammatory molecules, by decreasing microglial activation, and by promoting the expression of anti-inflammatory cytokines and regulatory T cells (Mandolesi et al., 2019; Rizzo et al., 2021; Souza et al., 2017; Xie et al., 2019; Gentile et al., 2019). Noteworthy, in most of the MS animal studies exercise was started in a preventive manner, either before or the day of disease induction, suggesting that exercise can modify the immune response exerting a preconditioning effect (Rizzo et al., 2021; Einstein et al., 2018). This raises the outstanding question as to whether in MS patients physical activity in the early phases of the disease can shape the brain inflammatory milieu and clinical expression of the disease at the time of diagnosis. To this aim, in the present study we explored the impact of different levels of physical activity in the six months before the diagnosis on the clinical presentation of the disease, including cognitive and psychiatric symptoms, and on the associated central inflammation, in a large group of newly diagnosed relapsing-remitting (RR) MS patients. Moreover, we addressed the causal link between exercise-mediated immunomodulation and mood symptoms in the animal setting.

2. Methods

2.1. Multiple sclerosis patients

235 consecutive RR MS patients admitted to the Neurology Unit of Neuromed Research Institute (Pozzilli, IS) between 2017 and 2020 were evaluated at the time of diagnosis. This study was approved by the Ethics Committee of Neuromed hospital, and all patients gave a written informed consent. All procedures were carried out in accordance with approved guidelines.

MS diagnosis was based on clinical, laboratory and MRI data (Thompson et al., 2018). The following clinical and demographical parameters were considered: disease duration was estimated as the interval between the first episode of focal neurological dysfunction suggestive of MS and the time of diagnosis. Clinical disability was evaluated with the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983). Clinical disease activity was defined as the presence of clinical symptoms at the time of evaluation. Smoking habit was investigated by asking all patients for information on current and previous smoking status. Body height and weight were measured, and body mass index (BMI) was calculated as weight (kg)/height (m^2). Missing data: disease duration in 23/235 patients, 9.8%; clinical activity in 10/243 patients, 4.2%; smoking in 24/235 patients, 10.2%; BMI in 1/235 patient, 0.4%.

2.2. Physical activity

At the time of diagnosis, patients were interviewed on the physical activity levels performed in the previous 6 months. Following a recent classification (Kalb et al., 2020), patients have been divided into three groups, according to the type of activity and the number of hours per week practiced: “sedentary”, “lifestyle physical activity”, “exercise”. The “exercise” group included patients who performed>150 min/week of repetitive physical activity (e.g. jogging, swimming) before diagnosis. Patients who performed at least 150 min per week of daily activities of moderate intensity were included in the “lifestyle physical activity” group. Patients who did not meet the criteria for “lifestyle physical activity” or “exercise” groups were included in the “sedentary” group.

2.3. MRI protocol

MRI examination consisted of 1.5- or 3.0-T scan, including dual-echo proton density sequences, fluid-attenuated inversion recovery (FLAIR), T1-weighted spin-echo (SE), T2-weighted fast SE, and contrast-enhanced T1-weighted SE after intravenous gadolinium (Gd) infusion

(0.2 mL/kg). A Gd-enhancing (Gd+) lesion was defined as an area of hyperintense signaling on contrast-enhanced T1-weighted images. Radiological activity at LP (lumbar puncture) was defined as the presence of a Gd + lesion at brain, and spine MRI scan performed at the time of LP. Missing data: radiological activity in 13/235 patients 5.5%.

2.4. CSF collection and analysis

LP was performed at the time of diagnosis. Corticosteroids or immunoactive therapies were initiated later, if indicated.

The presence of oligoclonal bands (OCB) was assessed (missing data in 14/235 patients, 5.96%).

In 227 patients the levels of several proinflammatory and anti-inflammatory molecules have been assessed. Missing data for 8/235 patients, 3.4%. CSF was collected by LP, centrifuged (1300 rpm, 10 min) to remove cellular elements, and stored at −80 °C until being analyzed using a Bio-Plex multiplex cytokine assay (Bio-Rad Laboratories, Hercules, CA), according to the manufacturer's instructions. Concentrations were calculated according to a standard curve generated for the specific target and expressed as picograms/ml. All samples were analyzed in triplicate. The CSF molecules examined included: interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-1 receptor antagonist (ra). Values were compared with those obtained in a group of 80 patients with non-inflammatory/non-degenerative CNS and peripheral nervous system disorders, such as: vascular leukoencephalopathy, metabolic and hereditary polyneuropathies, normal pressure hydrocephalus, functional neurological disorder, spondylotic myelopathy.

2.5. Neuropsychological evaluation

Neuropsychological test and fatigue scales were evaluated in 186 patients. Beck Depression Inventory–Second Edition (BDI-II) was used to assess the presence of depressive symptoms (Sica and Ghisi, 2007). This scale includes 21 items and the score range is from 0 to 63. A cutoff of 13 was used to detect depression (Goldman Consensus Group, 2005).

Levels of anxiety were assessed using the State-Trait Anxiety Inventory form Y (STAI-Y), a 40-item self-administered questionnaire exploring both the levels of situational anxiety (STAI-Y state) and the tendency to anxious situations (STAI-Y trait). A cutoff for high anxiety was derived according to the normative data (Pedrabissi and Santinello, 1989).

Neuropsychological examination consisted of Mini Mental State Examination (MMSE), Symbol Digit Modalities Test (SDMT) that measures cognitive processing speed, the Brief Visuospatial Memory Test-Revised (BVMT-R) that measures visuospatial memory, the Rey Auditory Verbal Learning Test (RAVLT) that measures verbal memory, and the Frontal Assessment Battery (FAB) assessing executive functions.

Fatigue was assessed using the fatigue severity scale (FSS), a nine-item questionnaire that measures the severity of fatigue symptoms on a scale from one to seven (Krupp et al., 1989).

All tests were administered during a single psychological examination conducted in a hospital setting by a certified neuropsychologist. Missing data: FSS in 13/186 patients, 7%; BDI-II/STAI-Y in 12/186 patients, 6.4%; MMSE in 10/186 patients, 5.4%; SDMT in 14/186 patients, 7.5%; RAVLT in 16/186 patients, 8.6%; BVMT-R in 15/186 patients, 8.1%; FAB in 15/186 patients, 8.1%).

2.6. Animal studies

Female C57BL/6 mice (5–6 weeks of age) were purchased from Charles-River (Italy) and kept under standard housing conditions with a light/dark cycle of 12 h, in a temperature-controlled environment (22 °C, 50–60% humidity) and free access to food and water. The day of immunization (see next paragraph) mice were randomly assigned to either standard or running wheel-equipped cages (throughout the text called "exercise"). Animal experiments were conducted according to the guidelines set by the Internal Institutional Review Committee, the European Directive 2010/63/EU and the European Recommendations 526/2007 and the Italian D.Lgs 26/2014. All efforts were made to minimize the number of animals used and their suffering.

2.7. EAE induction and exercise protocol

EAE was induced in 9-week-old female C57BL/6 mice, as previously described (Rizzo et al., 2021). Briefly, mice were injected subcutaneously with an emulsion containing 200 μg of myelin oligodendrocyte glycoprotein 35–55 (MOG35–55) in Complete Freund's Adjuvant (CFA), followed by intravenous administration of pertussis toxin (500 ng) twice (at days 0 and 2). Control mice, hereafter referred to as CFA, received the same treatment as EAE mice without the MOG peptide, including complete CFA and Pertussis toxin. Mice were daily scored for clinical symptoms of EAE according to the following 0–5 scale: 0 no clinical signs; 1 flaccid tail; 2 hindlimb weakness; 3 hindlimb paresis; 4 tetraparalysis; and 5 death due to EAE; intermediate clinical signs were scored by adding 0.5. For each animal, the onset day was recorded as the day post immunization (dpi) when it showed the first clinical manifestations (score > 0). $N = 5$–14 mice per group. Running activity was monitored through a revolution counter provided with each cage, which counts whole revolutions of the activity wheel. Four experimental groups were included in the study: CFA sedentary (CFA), CFA-exercise (CFA EX), EAE sedentary (EAE) and EAE-exercise (EAE EX). Running activity of EAE exercise mice (EAE EX) reduced soon after disease onset, as shown in Rizzo et al., 2021.

2.8. Stereotaxic surgery and intracerebroventricular (icv) injection

Mice were surgically implanted with a 22-gauge guide cannula (model C313G; Plastics One, Inc., Roanoke, VA) under isofluorane (3%) anesthesia. Cannulae were placed into the right lateral ventricle using the following coordinates: anterior-posterior: −0.4 mm; −1 mm lateral and 2 mm below the horizontal plane of bregma. To study direct effects of IL-2 in the brain of mice with uninjured blood brain barrier (BBB), mice were given icv injection of IL-2 (50 ng/mouse, $n = 12$) or vehicle ($n = 12$) and tested 24 h later for anxiety- and depression-like behavior and electrophysiological experiments.

2.9. Behavioral assessment

Anxiety- and depression-like behaviors were tested in: 1- presymptomatic EAE mice (7–8 dpi) ($n = 5$–14 per group) through the light-dark test (LDT) and the forced swimming test (FST), respectively; 2- VH and IL-2 icv injected mice ($n = 12$ per group) through the open Field Test (OFT) the FST, respectively. Behavioral tests were conducted during the light period (9:00–12:00 am) in a dedicated room with a constant temperature (26 ± 1 °C). Each session was preceded by at least 1 h habituation in the behavioral room.

2.10. OFT

OFT was carried out as previously reported (Haji et al., 2012; Gentile et al., 2016). Mice were placed in the center of the arena (40 cm × 30 cm) and video-recorded during exploration of the apparatus. The apparatus was cleaned with 10% ethanol between trials. Motor activity was measured as the total distance travelled in the arena during the 10 min-test session. The time spent in the center of the arena was measured by a dedicated software (Ethovision, Noldus and VideoTrack, ViewPoint). A trained researcher analyzed rearing activity of the mice over the course of the test.

2.11. LDT

The LDT was performed as in Gentile et al., 2016. Briefly, the LDT

apparatus consisted of an open white compartment (30 × 20 × 20 cm, 300 lx) joined by a 3 × 3 cm opening to a dark compartment (15 × 20 × 20 cm, 0 lx) which was painted black and covered with a lid. The anxiogenic nature of the white compartment was increased by additional illumination from a 60-W angle poise lamp placed 45 cm above the center of the apparatus. Mice were placed into the dark side and the door was opened 2 min later. After door opening, the latency of the mice to enter the light chamber may serve as an index of anxiety-like behavior. Mice were allowed to move freely between the two chambers with door open for 10 min. The score for the transition was assigned from the analysis of the video recordings when the animal came out of the dark chamber with all 4 paws. The apparatus was cleaned with 10% ethanol after each trial to effectively remove the scent of the previously tested animal. The time and distance spend in the light arena were recorded by Ethovision, Noldus tracking software.

2.12. FST

Each mouse was placed into a 5-l glass beaker (height 23.5 cm; diameter 16.5 cm) containing water up to a height of 15 cm at 25 ± 1 °C for 6 min. The water was exchanged after each trial. Floating (immobility) and struggling time were scored over the entire 6 min exposure by pressing pre-set keys on a computer keyboard using ViewPoint video tracking software. Latency to first episode of immobility was estimated during the first 2 min of the test. A mouse was judged floating when it stopped any movements except those that are necessary to keep its head above water.

2.13. Electrophysiology

Mice were killed by cervical dislocation, and corticostriatal coronal slices (200 μm) were prepared from fresh tissue blocks of the brain with the use of a vibratome (Musella et al., 2014). A single slice was transferred to a recording chamber and submerged in a continuously flowing artificial cerebrospinal fluid (ACSF) (34 °C, 2–3 ml/min) gassed with 95% O2–5% CO2. ACSF composition was (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl2, 1.2 NaH2PO4, 2.4 CaCl2, 11 Glucose, and 25 NaHCO3. The striatum could be readily identified under low power magnification, whereas individual neurons were visualized in situ using a differential interference contrast (Nomarski) optical system. This employed an Olympus BX50WI (Japan) noninverted microscope with 40× water immersion objective combined with an infra-red filter, a monochrome CCD camera (COHU 4912), and a PC compatible system for analysis of images and contrast enhancement (WinVision 2000, Delta Sistem, Italy). Recording pipettes were advanced towards individual striatal cells in the slice under positive pressure and visual control (WinVision 2000, Delta Sistemi, Italy) and, on contact, tight GΩ seals were made by applying negative pressure. The membrane patch was then ruptured by suction and membrane current and potential monitored using an Axopatch 1D patch clamp amplifier (Molecular Devices, Foster City, CA, USA). Whole-cell access resistances measured in voltage clamp were in the range of 5–20 MΩ. Whole-cell patch clamp recordings were made with borosilicate glass pipettes (1.8 mm o.d.; 2–3 MΩ), in voltage-clamp mode, at the holding potential of −80 mV. To study GABA-mediated spontaneous inhibitory postsynaptic currents (sIPSCs), the recording pipettes were filled with internal solution of the following composition (mM): 110 CsCl, 30 K+ gluconate, 1.1 EGTA, 10 HEPES, 0.1 CaCl2, 4 Mg-ATP, 0.3 Na-GTP. MK-801, and CNQX were added to the external solution to block, respectively, NMDA and non-NMDA glutamate receptors. Drugs were first dissolved in water or in DMSO (HU-210) and then in the ACSF to the desired final concentration. The concentrations of the various drugs were chosen according to previous in vitro studies on corticostriatal brain slices (Rossi et al., 2012; Musella et al., 2014) and were as follows (in μM): 10 CNQX, 25 MK-801, and 1 HU-210 (Tocris Bioscience). For ex vivo experiments slices taken from control mice were pre-incubated with IL-2 (Peprotech, 50 ng/ml for 10 min).

Synaptic events were stored by using P-CLAMP 9 (Axon Instruments) and analyzed offline on a personal computer with Mini Analysis 5.1 (Synaptosoft, Leonia, NJ, USA) software. The detection threshold of sIPSCs was set at twice the baseline noise. The fact that no false events would be identified was confirmed by visual inspection for each experiment. Offline analysis was performed on spontaneous synaptic events recorded during fixed time epochs (1–2 min), sampled every 2–3 min (5–12 samplings) (Centonze et al., 2009). Only data from putative GABAergic medium spiny projection neurons (MSNs) were included in the present study and identified immediately after rupture of the GΩ seal, by evaluating their firing response to the injecting of depolarizing current (typically tonic, with little or no adaptation).

One to five cells per animal were recorded. For each type of experiment and time-point, at least three mice per group were employed. Electrophysiological results from neurons recorded from the same animal were treated as a separate sample and averaged before calculating statistics. One animal per day was used for the electrophysiological experiment.

2.14. RNA extraction and qPCR

Striata of mice sacrificed at 21 dpi ($n = 4$–8 per group) were dissected in RNAse-free conditions and total RNA was extracted according to the standard miRNeasy Micro kit protocol (Qiagen). Next, 600 ng of total RNA were reverse-transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), and 6,43–19,8 ng of complementary DNA (cDNA) were amplified in triplicate using the Applied Biosystem 7900HT Fast Real-Time PCR system. SensiMix II Probe Hi-Rox Kit (Bioline; Meridian Life Science) and the following TaqMan gene expression assays were used for the quantification of messenger RNA (mRNAs) coding for IL-2 and beta-actin (Actb):

Il2 ID: Mm00434256_m1.

Actb ID: Mm00607939_s1mRNA relative quantification was performed using the comparative cycle threshold ($2 - \Delta\Delta Ct$) method. β-actin was used as endogenous control. All data are expressed relative to CFA-SED.

2.15. Statistical analysis

The normality distribution of continuous variables was tested using the Kolmogorov–Smirnov test. Data were expressed as mean (standard deviation, SD or standard mean error, SEM) or, when not normally distributed, as median (interquartile range, IQR). Categorical variables were presented as absolute numbers (n) proportion (%).

To determine the significance of the different proportions a chi square test was used, and the mean values compared by using ANOVA. In case of not normal distribution, non-parametric tests were used for testing statistical significance. Differences in continuous variables among two groups were evaluated by parametric *t*-test or, if necessary, nonparametric Mann–Whitney test. Bonferroni correction has been applied for post hoc comparisons. Kruskal-Wallis test was used to evaluate differences between exercise groups in CSF cytokines concentrations, demographic, clinical or MRI variables. Spearman's nonparametric correlation was used to test possible associations between nonparametric variables.

To recognize associations between physical activity and different variables, after adjustment for age, disease duration, OCB presence, sex, EDSS, clinical and radiological disease activity, linear regression models were used. Box plot was used to depict statistically significant differences between groups. A p value ≤ 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, NY, USA) and GraphPad Prism 6.0.

Multiple comparisons were performed by ANOVA followed by Tukey HSD or unpaired t-test. Paired *t*-test was used for electrophysiological data. Two-way ANOVA was used to analyze clinical score with disease and exercise as variables.

3. Results

3.1. Clinical characteristics

The clinical characteristics of MS patients and control patients are shown in Table 1. No significant differences emerged between MS and controls in age (MS, median = 35.2; controls, median = 41.7; $p = 0.057$) and sex distribution (MS, female = 68.9%; controls, female = 66.3%; $p = 0.656$).

MS patients were divided into three groups according to the levels of physical activity: "sedentary", "lifestyle physical activity", and "exercise" group (Table 2). Significant differences were found in age ($p = 0.004$) and sex ($p = 0.032$) among the three groups. No significant differences emerged between the exercise groups in EDSS score ($p = 0.222$), disease duration ($p = 0.171$), OCB presence ($p = 0.667$), radiological disease activity (p = 0.05), clinical disease activity ($p = 0.655$), BMI ($p = 0.196$), smoking ($p = 0.238$).

3.2. Mood alterations are reduced in exercising multiple sclerosis patients compared with sedentary patients

A significant difference was detected in FSS among the three groups ($p = 0.010$). In particular, FSS was significantly lower in the "exercise" group (median [IQR] = 1.33 [1.05–2.37]), compared with the "sedentary" group (median [IQR] = 2.55 [1.33–4.11]. However, linear regression showed that the association between FSS and physical activity was not statistically significant (β = −0.071, 95% CI -0.488, 0.195, $p = 0.396$) after adjustment by all potential confounders (age, sex, disease duration, EDSS, OCB presence, clinical and radiological disease activity).

Clinically significant depression has been identified in 25.9% of MS patients. In addition, STAI-Y state and trait scores suggestive of anxiety were found in 28.2% and 13.2% of MS patients, respectively.

The distribution of depression was significantly different among the three groups ("sedentary" 34%, "lifestyle physical activity" 20.9%, "exercise" 10.8%; $p = 0.017$); in particular, prevalence of depression was lower in the "exercise" group compared with "sedentary" group ($p = 0.021$) (Fig. 1A). The analysis of BDI-II score highlighted significant differences among the three subpopulations ($p = 0.005$), with a remarkable reduction in "exercise" group compared with "sedentary" subjects (Fig. 1B). Noteworthy, a significant association between lower BDI-II scores and physical activity (β = −0.176, 95% CI -2.891, −0.122, $p = 0.033$) was demonstrated by linear regression, after adjusting for age, sex, disease duration, EDSS, OCB presence, clinical and radiological disease activity, and FSS.

The presence of state and trait anxiety also significantly differed among groups: state anxiety ("sedentary" 39.4% vs "lifestyle physical activity" 9.3% vs "exercise" 21.6%; $p = 0.001$), trait anxiety ("sedentary" 19.1% vs "lifestyle physical activity" 4.7% vs "exercise" 8.1%; $p = 0.039$) (Fig. 1C and E). In particular, prevalence of state anxiety was reduced in the "lifestyle physical activity" group compared with the "sedentary" group.

When analyzing STAI-Y state and trait scores, significant differences were also found comparing "sedentary", "lifestyle physical activity" and "exercise" groups (STAI-Y state: p = 0.001, STAI-Y trait: $p = 0.007$). In particular, STAI-Y state was lower both in the "exercise" group and in the "lifestyle physical activity" group, compared with the "sedentary" group (Fig. 1D). STAI-Y trait was lower in the "exercise" group compared with the "sedentary" group (Fig. 1F). Noteworthy, a significant association between lower BDI-II scores and physical activity (β = −0.176, 95% CI -2.891, −0.122, p = 0.033) was demonstrated by linear regression, after adjusting age, sex, disease duration, EDSS, OCB presence, clinical and radiological disease activity, and FSS. In addition, associations between STAI-Y trait and state were also significant after adjustment (STAI-Y state: β = −0.176, 95% CI -4.948, −0.067, $p = 0.044$; STAI-Y trait: β = −0.170, 95% CI -4.248, −0.167, $p = 0.034$).

Cognitive evaluation showed significant differences in BVMT-R when comparing the three groups ($p = 0.003$). In particular, higher scores were found in the "exercise" group (median [IQR] = 30.5 [26–34.75]), compared with the "sedentary" group (median [IQR] = 25.5 [20.25–30], $p < 0.001$). However, no statistically significant association was found between score of BVMT-R and physical activity, after adjusting for all potential confounders (β = 0.142, 95% CI -0.279, 2.529, $p = 0.115$).

No significant differences were detected in the other neuropsychological tests administered.

3.3. CSF inflammatory molecules in multiple sclerosis patients and in control patients

The levels of pro- and anti-inflammatory cytokines were measured in the CSF of MS patients and control individuals. Among the assessed cytokines, IL-2, IL-6 and IL-10 were found significantly increased in MS subjects compared to controls (IL-2: MS, median [IQR] = 0.04 [0–0.57]; controls, median [IQR] = 0 [0–0.13]; p < 0.001); IL-6: MS, median [IQR] = 0.87 [0.11–2.09]; controls, median [IQR] = 0.34 [0.01–1.24]; $p = 0.006$; IL-10: (MS, median [IQR] = 1.99 [1.05–2.7]; controls, median [IQR] = 1.62 [0.88–2.25]; $p = 0.018$). Moreover, differences in IL-2 and IL-6 CSF levels between MS and control subjects remained significant after controlling for multiple comparisons (IL-2, B–H adjusted p < 0.01; IL-6, B–H adjusted $p = 0.036$) (Fig. 2).

3.4. Physical exercise is associated with reduced CSF inflammation at the time of diagnosis

To explore the association between the levels of prior physical activity and central inflammation at the time of diagnosis, we compared the concentrations of proinflammatory and anti-inflammatory cytokines in the three exercise groups.

Significant differences were found in the CSF concentrations of IL-2 ($p = 0.007$), IL-5 ($p = 0.017$), IL-6 ($p = 0.002$), IL-15 ($p = 0.014$), and IL-1ra ($p = 0.045$) comparing the "sedentary", "lifestyle physical actvity" and "exercise" groups. After controlling for multiple comparisons, differences in IL-2 and IL-6 CSF levels between groups remained significant (IL-2, B–H adjusted $p = 0.042$; IL-6, B–H adjusted $p = 0.024$). In particular, the CSF levels of IL-2 and IL-6 were significantly lower in the "exercise" group compared with the "sedentary" group (IL-2: "sedentary" median [IQR] = 0.16 pg/ml [0–0.69], "exercise" median [IQR] = 0 pg/ml [0–0.31], $p = 0.009$; IL-6: "sedentary" median [IQR] = 1.18 pg/ml [0.33–2.72], "exercise" median [IQR] = 0.40 pg/ml [0–1.12], $p =$

Table 1
Clinical characteristics.

		MS	Controls
Patients	N	235	80
Sex, Female	N (%)	162 (68.9)	53 (66.3)
Age, years	Median (IQR)	35.21 (27.15–46.73)	41.76 (33.34–47.18)
MS phenotype: CIS, RR	N (%)	20 (8.5), 215 (91.5)	–
Disease duration, months	Median (IQR)	3.75 (1.51–12.58)	–
OCB, yes	N (%)	163 (73.8)	–
EDSS score	Median (IQR)	1.5 (1–2)	–
Clinical disease activity, yes	N (%)	75 (33.3)	–
Radiological disease activity, yes	N (%)	89 (40.1)	–
BMI	Median (IQR)	24.46 (22.25–29.1)	–
Smoking: no, ex, yes	N (%)	108 (51.2), 42 (19.9), 61 (28.9)	–

Abbreviation: MS = multiple sclerosis.

Table 2
Clinical characteristics according to exercise group.

		Sedentary	Lifestyle physical activity	Exercise	#
Patients	N (%)	**141 (60)**	**52 (22.1)**	**42 (17.9)**	
Sex, Female	N (%)	104 (73.8)	36 (69.2)	22 (52.4)	*
Age, years	Median (IQR)	36.96 (28.91–47.6)	37.66 (24.44–47.39)	30.28 (24.94–37.4)	*
Disease duration, months	Median (IQR)	4.4 (1.83–12.6)	2.83 (1.1–14.92)	2.43 (0.91–8.22)	
OCB, yes	N (%)	101 (75.4)	33 (68.8)	29 (74.4)	
EDSS score	Median (IQR)	1.5 (1–2.5)	1.5 (1–2)	1 (1–2)	
Clinical disease activity, yes	N (%)	42 (31.3%)	17 (34%)	16 (39%)	
Radiological disease activity, yes	N (%)	45 (34.1%)	22 (44%)	22 (55%)	
BMI	Median (IQR)	24.63 (22.33–29.75)	24.76 (22.4–28.38)	23.58 (21.45–26.74)	
Smoking: no, ex, yes	N (%)	61 (48.8), 29 (23.2), 35 (28)	31 (63.3), 6 (12.2), 12 (24.5)	16 (43.2), 7 (18.9), 14 (37.8)	

= differences between "sedentary", "lifestyle physical activity" and "exercise" groups.
* denote statistical significance.

0.006 (Fig. 3).

Linear regression analysis showed a significant association between physical activity and reduced IL-2 CSF concentrations after adjusting for age, sex, disease duration, EDSS, OCB presence, clinical and radiological disease activity (β = −0196, 95% CI -0.405, −0.053, $p = 0.011$). Associations between physical activity and IL-6 were not significant after adjusting for clinical characteristics (β = −0.111, 95% CI -0.892, 0.122, $p = 0.135$).

3.5. IL-2 and exercise modulate behavior in mice

Clinical data suggest that the CSF levels of IL-2 positively correlate with mood disorders in MS at the time of diagnosis. We addressed the causal role of IL-2 and mood disturbancies in rodents, taking advantage of the possibility to modulate central levels of IL-2 by single intracerebroventricular injection of the cytokine (25 ng/mouse) in comparison with mice receiving vehicle (VH). In line with previous findings (Karrenbauer et al., 2009), at OFT mice injected with IL-2 showed no significant changes in anxiety-like measures, such as the time spent in the center zone of the arena (VH: 4.236 ± 1.027, IL-2: 4.775 ± 0.6309; $p = 0.65$, unpaired Student's *t*-test), but a reduced exploratory behavior measured as rearing activity ($p < 0.05$, unpaired Student's *t*-test; Fig. 4A). However, IL-2-receiving mice showed significantly reduced latency to first immobility state in the FST, suggesting increased despair in these mice ($p < 0.05$ unpaired Student's t-test; Fig. 4B).

We next-tested the hypothesis that preventive exercise (voluntary running wheel) alleviates the behavioral sequelae affecting EAE mice. Mice were housed in running-wheel cages the day of immunization (dpi 0), which is followed by a disease onset after about 10 dpi (Rizzo et al., 2021). As already reported (Rossi et al., 2009; Rizzo et al., 2021), preventive voluntary running wheel ameliorates motor disturbance in EAE mice ($p < 0.05$ at each time points starting from 14 dpi; Fig. 4C). Hence, mice were tested for anxiety- and depression-like behaviors before disease onset, at 7–8 dpi (aka presymptomatic phase). Although the one-way ANOVA did not reach the statistical significance, in agreement with previous findings (Gentile et al., 2016), at the LDT EAE mice showed significant preference for the dark compartment ($p < 0.05$ unpaired Student's *t*-test) and reduced distance travelled in the lit compartment ($p < 0.05$ unpaired Student's *t*-test) compared to control CFA mice (Fig. 4 D–E). Exercise partially recovered the anxiety-like behavior of EAE mice respect to EAE (% in the light: $p = 0.09$ unpaired Student's *t*-test; distance in the light: $p = 0.14$ unpaired Student's *t*-test; Fig. 4 D–E). As shown in Fig. 4E, EAE mice showed increased latency to immobility at FST, whereas EAE-exercise mice performed like CFA control and CFA EX mice in this behavioral task (Fig. 4F; One way ANOVA $p = 0.0032$, Tukey post hoc comparisons EAE vs EAE EX $p < 0.05$; EAE vs CFA EX $p < 0.01$; $p > 0.05$ for other comparisons; unpaired Student's *t*-test EAE vs CFA $p < 0.05$). Thus, exercise reduced depressive- and, to a lesser extent, anxiety-like behavior observed in presymptomatic EAE mice.

Single icv injection of IL-2 (50 ng/mouse) reduces vertical exploration during OFT compared to vehicle injected mice (**A**), *p < 0.05 Student's *t*-test; IL-2 icv injection also induces despair behavior measured by latency to first immobility episode in FST (**B**), *p < 0.05 paired Student's t-test. Exercise attenuates EAE disability (**C**; disease x exercise effect p ⟨0,001) and, though not significantly, anxiety-like behavior in MOG-immunized mice during LDT in both time (**D**) and distance (**E**) to explore lit compartment *p < 0.05 Student's *t*-test. EAE exercise mice show reduced immobility latency in FST (expressed in seconds of duration) compared to EAE sedentary mice (**F**), #p < 0.05 one-way ANOVA with Tukey's post hoc test; ##$p < 0.01$. Data are expressed as mean ± SEM.

3.6. The sensitivity of the cannabinoid type 1 receptor (CB1R) on GABA synapses is modulated by IL-2 and exercise in the mouse striatum

The endocannabinoid system, known to be involved in mood control, is profoundly altered in both MS and EAE (Centonze et al., 2007). Of note, we have previously shown that the sensitivity of the cannabinoid type 1 receptor (CB1R) on GABA synapses is altered in mice with a depressive-anxious phenotype induced by chronic defeat stress (Rossi et al., 2008, 2012), and is also selectively lost in the striatum of both acute and presymptomatic EAE mice (Gentile et al., 2016; Rossi et al., 2010), in association with mood disorders (Gentile et al., 2016). In addition, voluntary exercise was shown to rescue CB1R sensitivity in EAE striatum (Rossi et al., 2009). Interestingly, a significant increase of IL-2 mRNA was detected in the striatum of EAE mice (6.62 ± 1.2 fold change) compared to CFA mice (1.06 ± 0.21 fold change; One-way ANOVA $p = 0.0034$; Tukey post-hoc comparisons: CFA vs EAE $p < 0.05$; CFA EX vs EAE $p < 0.01$). A trend in reduced IL-2 expression was observed in EAE EX striata (3.52 ± 1.27 fold change; EAE vs EAE EX $p = 0.104$ unpaired Student's *t*-test). Exercise did not alter per se IL-2 mRNA expression (CFA-EX 1.41 ± 0.46, $p > 0.05$ for all comparisons). These data suggest that IL-2 might contribute to EAE-associated behavioral syndrome and exercise-mediated beneficial effects on mood.

Next we investigated whether IL-2 could affect the sensitivity of CB1R. We measured the GABAergic response to the CB1R stimulation from mice treated in vivo with icv injection of VH or IL-2 (25 ng/mouse). Electrophysiological recordings of MSNs from striatal slices of IL-2-treated mice showed a fully blocking effect of CB1Rs, while in VH-treated mice the CB1R agonist HU210 caused the expected reduction of sIPSP frequency (Fig. 5 A-A', paired Student's t-test $p = 0.0014$ compared with pre-HU210 values of vehicle treated mice; paired Student's t-test $p = 0.25$ compared with pre-HU210 values of IL-2 treated mice).

Moreover, we performed ex vivo experiments by perfusing HU210 (1 μM) on control mouse brain slices pre-incubated with IL-2 (50 ng/ml). Compared with control slices, the pre-incubation with IL-2 was able to prevent the reduction of sIPSP frequency caused by HU210 (Fig. 5 B-B′ paired Student's t-test p = 0,004 compared with pre-HU210 values of

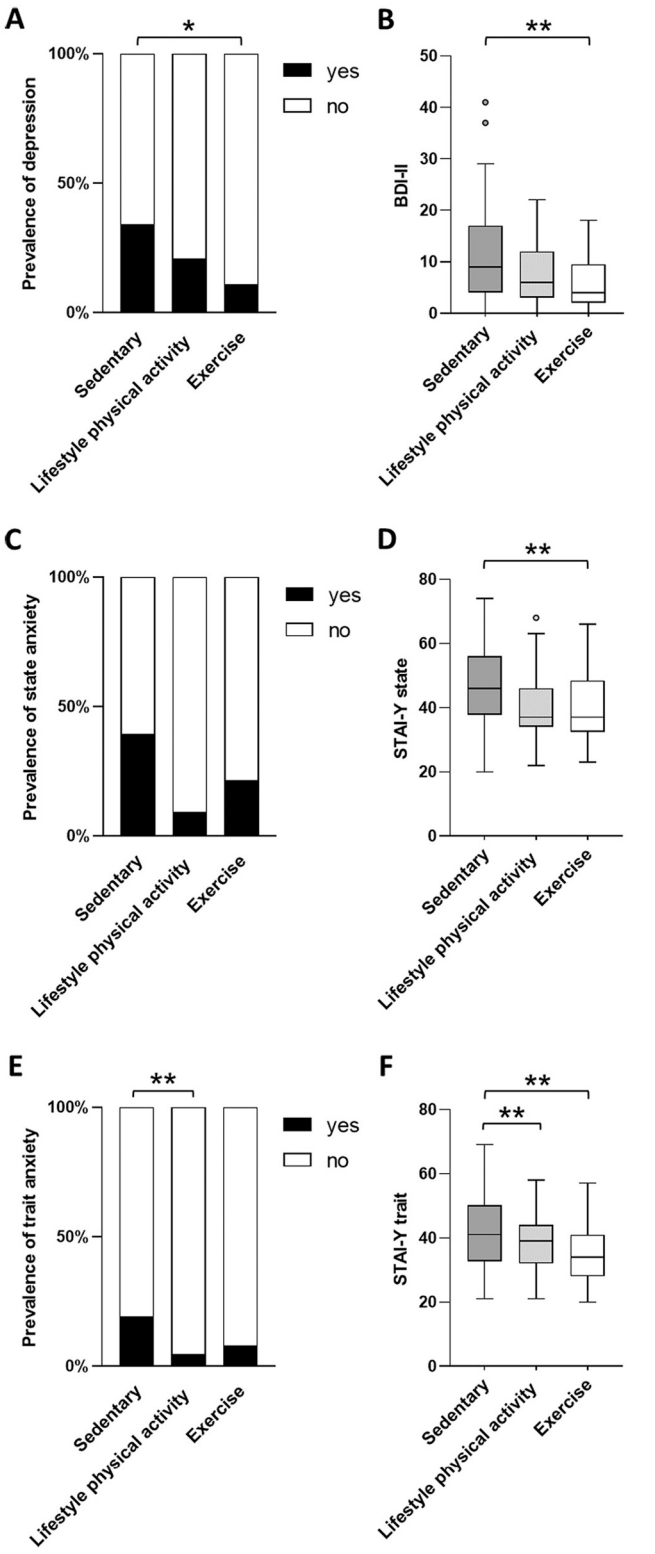


Fig. 1. Depression and anxiety in the exercise groups.
(**A**), (**C**), (**E**): Prevalence of depression, state and trait anxiety in "Sedentary", "Lifestyle physical activity" and "Exercise" groups.
(B), (D), (F): Tukey Box Plot showing the median and the 25–75 percentiles of BDI-II, STAI-Y state and trait scores in "Sedentary", "Lifestyle physical activity" and "Exercise" groups.
*Bonferroni-corrected $p < 0.05$; **Bonferroni-corrected $p < 0.01$.
Abbreviations: BDI-II = Beck Depression Inventory–Second Edition. STAI-Y = State-Trait Anxiety Inventory form Y

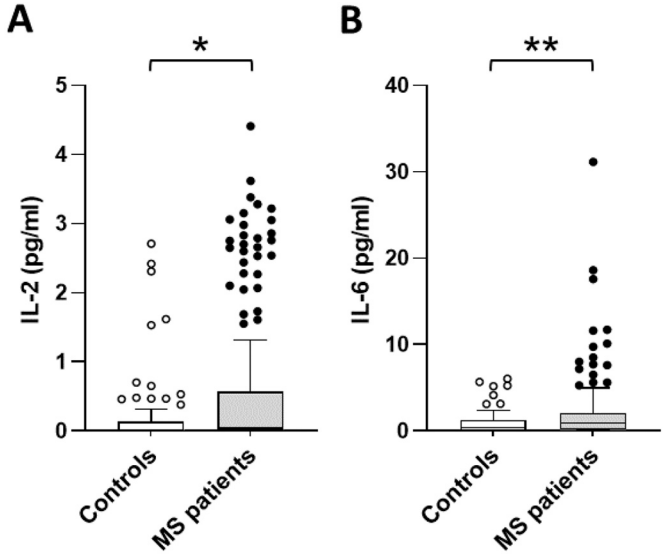


Fig. 2. IL-2 and IL-6 CSF levels in multiple sclerosis and control patients.
Tukey Box Plot showing the median and the 25–75 percentiles of CSF concentrations of IL-2 (**A**) and IL-6 (**B**) in MS and control patients. *Bonferroni-corrected $p < 0.05$; **Bonferroni-corrected $p < 0.01$.
Abbreviations: CSF = cerebrospinal fluid, IL = interleukin

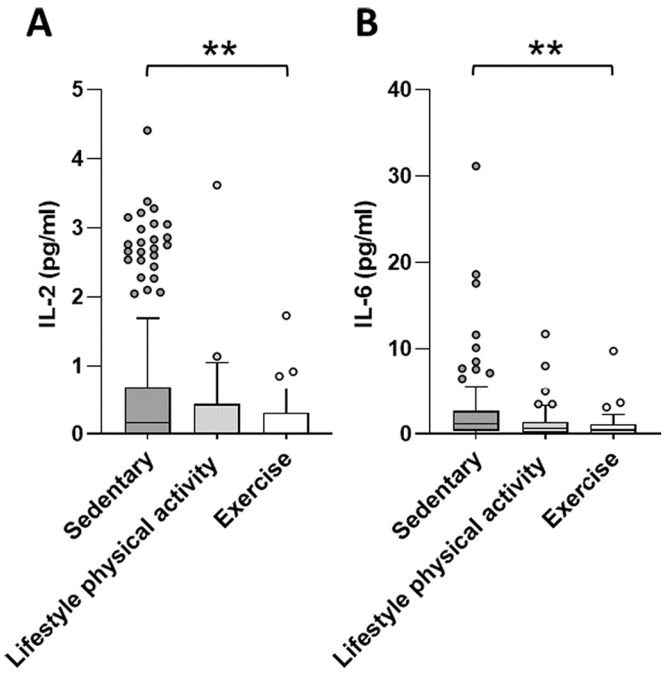


Fig. 3. Cerebrospinal fluid levels of IL-2 and IL-6 in the exercise groups.
Tukey Box Plot showing the median and the 25–75 percentiles of IL-2 (A) and IL-6 (B) CSF concentrations of in the exercise groups. **Bonferroni-corrected $p < 0.01$.
Abbreviations: CSF = cerebrospinal fluid, IL = interleukin

control mice; paired Student's t-test p = 0,72 compared with pre-HU210 values of control mice treated with IL-2). Thus, in vivo and ex vivo experiments show that IL-2 interferes with cannabinoid-mediated neurotransmission.

4. Discussion

In the present study, we found that exercise performed in the six

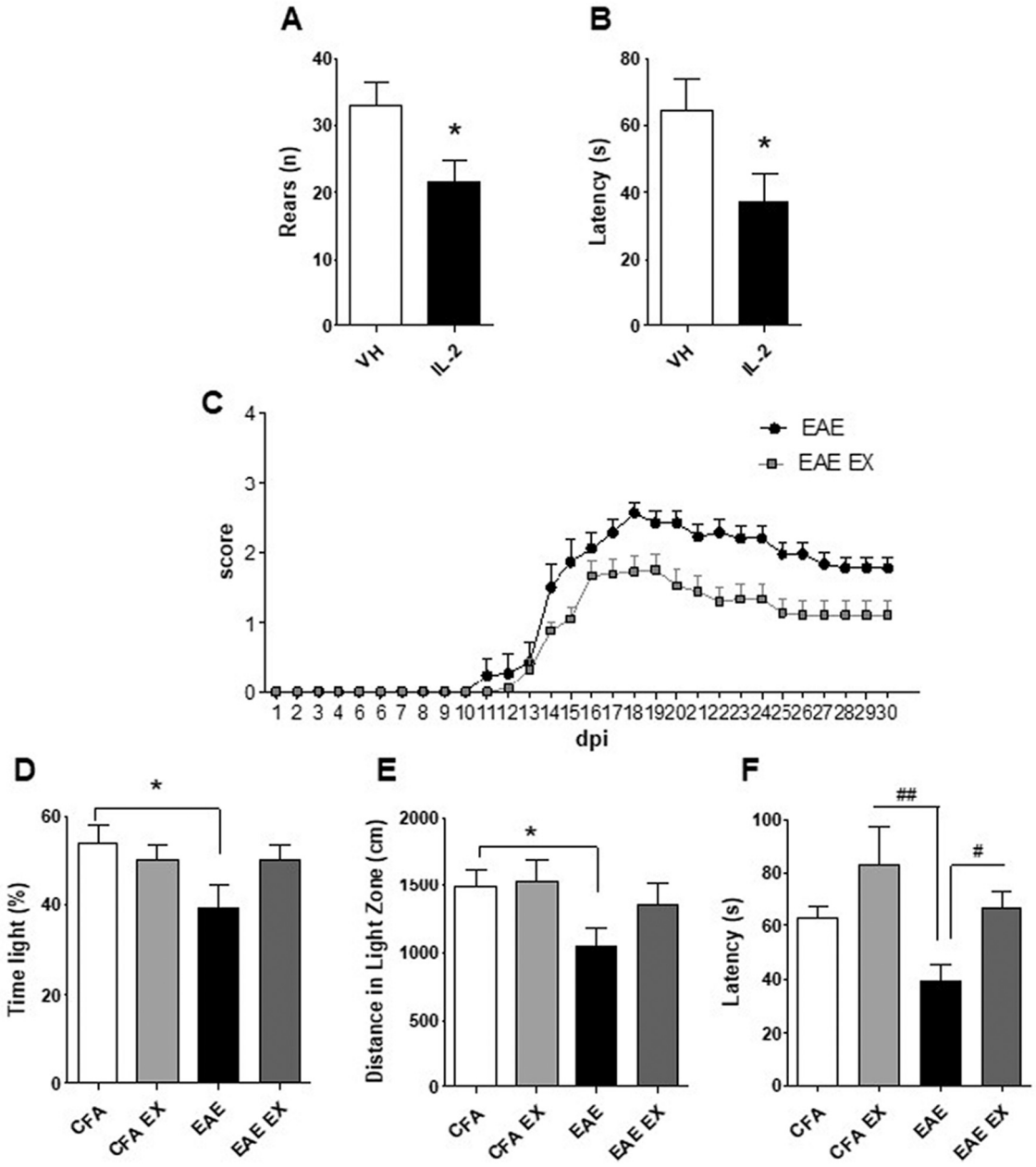


Fig. 4. IL-2 icv injection and exercise modulate behavior in mice.

months prior to MS diagnosis is associated with lower levels of anxiety and depression. In addition, preventive exercise has an immunomodulatory effect, being associated with lower concentrations of IL-6 and IL-2 in the CSF. IL-2 was the cytokine most strongly associated with exercise after controlling for several clinical parameters. Likewise, in the animal setting exercise started before disease onset reduced depressive- and, to a lesser extent, anxious-like behaviors in EAE mice and lowered striatal IL-2 expression. Furthermore, we provide evidence for a causal role of IL-2 in mood disorders. Specifically, in control healthy mice intracerebroventricular injection of IL-2 induced anxious- and depressive-like behavior and impaired CB1-mediated neurotransmission at GABAergic synapses, mimicking stress- and EAE-induced synaptic dysfunction, which we previously found to be restored by exercise (Rossi et al., 2009). Thus, preventive exercise in both human and experimental MS attenuated mood disturbances and reduced IL-2 increased brain levels, suggesting a protective effect against inflammation-induced mood disturbances.

MS is a multifactorial disease with recognized involvement of specific environmental/lifestyle risk factors (Olsson et al., 2017). Recent prospective studies suggested that low physical activity during adolescence is a risk factor for developing MS (Cortese et al., 2015; Gunnarsson et al., 2015; Wesnes et al., 2018). Exercise influences a wide range of biological functions with potential beneficial effects on MS (Dalgas et al., 2010). Physical activity regulates the hypothalamic-pituitary-adrenal axis and the response to stress, modulates neuronal activity and synaptic plasticity, and promotes the expression of neurotrophins (Cotman and Berchtold, 2002). Exercise sparks particular interest in the MS field due to its immunomodulatory properties, which might underlie the potential reduced risk of MS in people with increased physical activity level. We found reduced depression and anxiety in exercising MS patients compared with sedentary patients. Conversely, no significant differences were evidenced among the three groups in other clinical characteristics, including EDSS and cognitive profile. Thus, our data may indicate that preventive exercise exerts a beneficial effect

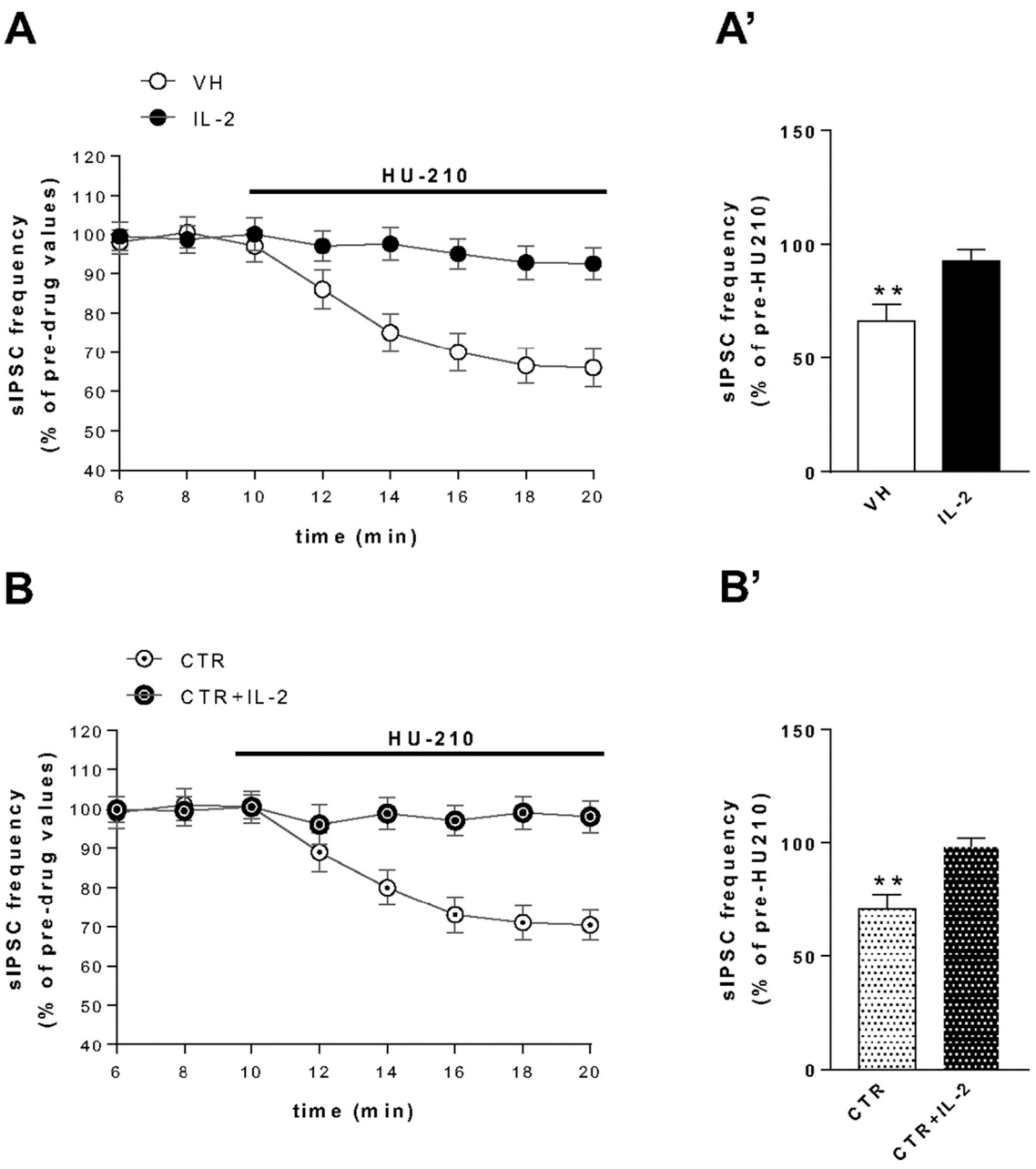


Fig. 5. Effects of IL-2 on CB1R-mediated GABAergic transmission in control mice.
(**A**) Single icv injection of IL-2 (50 ng/mouse) 24 h before sacrifice prevents the depressant action of HU210 on GABA-mediated IPSCs frequency in corticostriatal slices of control mice, compared to injection of VH. **p < 0.01 paired Student's t-test compared with pre-HU210 values.
(**B**) IL-2 acute perfusion (50 ng/ml) also impairs the CB1R-mediated reduced frequency of sIPSC from corticostriatal slices compared with control mice. **p < 0.01 paired Student's t-test compared with pre-HU210 values. Data are expressed as mean ± SEM.

specifically on mood disorders at the time of diagnosis.

Though underestimated, depression and anxiety are frequently observed in patients with MS and negatively influence quality of life (Marrie et al., 2015). Mood disorders can be present in the early stages of MS, regardless of clinical disability (Haussleiter et al., 2009; Lo Fermo et al., 2010; Rietberg et al., 2011). It has been demonstrated that neuroinflammation plays an important role in the pathogenesis of mood disorders in MS (Gentile et al., 2015a, 2015b), likely explaining the higher prevalence of depression and anxiety in MS compared with other neurological diseases (Patten et al., 2003). Proinflammatory molecules induce behavioral alterations, such as anxiety, anhedonia, fatigue, and social withdrawal in different inflammatory conditions in humans and in rodents Dantzer et al., 2008; Miller et al., 2009). Studies in animal models of MS contributed to elucidate the role of inflammation in the pathogenesis of mood alterations (Gentile et al., 2015a, 2015b; Pollak et al., 2000). In EAE mice, mood disorders detected in both presymptomatic mice and in mice with negligible motor defects, have been linked to increased CNS expression of proinflammatory cytokines, such as TNF and IL-1β, with detrimental effects on neurotransmission (Haji et al., 2012; Gentile et al., 2015a, 2015b, 2016). The role of CSF inflammation in mood alterations has also been demonstrated in human MS. A previous study in RR MS showed that, at the time of diagnosis, the CSF concentrations of IL-1β and TNF positively correlated with the levels of depression and a significant association between IL-2 and anxiety was also documented (Rossi et al., 2017). Notably, in the same study higher anxiety at diagnosis significantly predicted disease reactivation,

confirming that mood disorders in MS may reflect subclinical inflammation (Rossi et al., 2017). Here, we found that preventive exercise was associated with reduced expression of several proinflammatory molecules, including IL-2, IL-6, IL-15. Importantly, CSF levels of IL-2 and IL-6 were significantly higher in MS patients than in control subjects, but IL-2 CSF levels showed the strongest association with exercise after controlling for other possible confounding variables.

IL-6 and IL-2 are involved in both MS and major depressive disorder pathogenesis (Kothur et al., 2016; Primo de Carvalho Alves and Sica da Rocha, 2020). IL-6 is a prototypical pro-inflammatory cytokine released by both adaptive and innate immune cells and with broad physiological and pathological functions (Tanaka et al., 2014). From a neurophysiological point of view, the most consistent effect of increased levels of IL-6 is the impairment of synaptic plasticity (Stampanoni Bassi et al., 2019; Yirmiya and Goshen, 2011). Interestingly, IL-6 CSF levels have been associated with increased long-term disability (Stampanoni Bassi et al., 2019) and proposed as a useful biomarker in RR MS (Stampanoni Bassi et al., 2019, 2020). Moreover, increasing data support the causative role of IL-6 in the depressive phenotype (Hodes et al., 2014; Nasca et al., 2019) corroborating the idea that IL-6 might be part of the core cytokine signature promoting neuropsychiatric disturbances in MS. IL-2 is a homeostatic cytokine, known to stimulate T cell proliferation and differentiation (Ross and Cantrell, 2018). Increased CSF levels of IL-2 in MS may reflect aberrant type 1 T helper (Th1) cell expansion or increased production by brain-resident cells such as astrocytes (Eizenberg et al., 1995). IL-2 has been shown to interfere with the NMDA receptor functions (Ye et al., 2001) and to inhibit long term potentiation induction and maintenance (Tancredi et al., 1990). Moreover, IL-2 has also been shown to increase motor activity through an increase in dopaminergic activity (Petitto et al., 1997). Here, we show for the first time that IL-2 interferes with the cannabinoid-mediated neurotransmission on GABA synapses. In this study we found that the in vivo injection of IL-2 induces depressive- and anxiety-like behavior, closely resembling previous findings obtained with intrastriatal injection of IL-2 (Karrenbauer et al., 2009). In the pathological setting, exercise attenuated, though not significantly, IL-2 striatal expression (present study) and improved EAE-behavioral syndrome (Rossi et al., 2009). Our electrophysiological data broaden the role of IL-2 at synaptic level and add a new molecular player involved in CB1R dysfunction on GABA synapses during neuroinflammation. Considering that the dopaminergic system is an important regulator of emotional response, is dysregulated in MS (Cosentino et al., 2002) and implicated in symptom exacerbation and mood disorders in EAE (Bałkowiec-Iskra et al., 2007; Gentile et al., 2015a, 2015b), we cannot rule out an effect of IL-2 on striatal dopaminergic transmission and its regulation by exercise.

Although exact parallelism between exercise effects in mice and in humans cannot be drawn, these data corroborate the idea that preventive exercise can positively influence mood disorders and that IL-2 is among the target cytokines of exercise. Further studies are required to explore whether preventive exercise may induce protective effects on mood, cognitive and clinical disability during the disease course, which is suggested by animal studies (Rizzo et al., 2021; Gentile et al., 2019). A follow-up study on the present cohort or on a larger one will help clarifying this issue. In addition, lack of structural MRI measures of brain atrophy represents a limitation of the present study. Therefore, studies exploring the impact of preventive physical activity on MRI measures of neurodegeneration are needed to assess the protective effect of exercise in the early phases of MS.

In conclusion, our results provide evidence for an immunomodulatory effect of preventive exercise in patients with MS, associated with reduced CSF expression of the proinflammatory cytokine IL-2, which might result in reduced mood disorders. This finding supports the notion that exercise in the early stages may act as a disease-modifying therapy in MS, although further longitudinal studies are needed to clarify this issue.

Availability of data and materials

Anonymized datasets that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

The study was supported by:

Ministero della Salute (Italian Ministry of Health, Italy): Diego Centonze and Georgia Mandolesi RF-2018-12366144;

Ministero della Salute (Italian Ministery of Health, Italy): Antonietta Gentile and Fabio Buttari GR-2018-12366154;

Ministero della Salute (Italian Ministery of Health, Italy): Progetto Ricerca Corrente to IRCCS Neuromed;

Ministero della Salute (Italian Ministery of Health, Italy): Progetto Ricerca Corrente to IRCCS San Raffaele; Georgia Mandolesi;

Fondazione Italiana Sclerosi Multipla (FISM): Diego Centonze and Francesca Romana Rizzo cod. 2019/S/1 and financed or co-financed with the '5 per mille' public funding;

Fondazione Italiana Sclerosi Multipla (FISM): Francesca De Vito 2020/BS/003 and financed or co-financed with the '5 per mille' public funding;

Fondazione Italiana Sclerosi Multipla (FISM): Mario Stampanoni Bassi and Francesca Romana Rizzo cod. 2020/R-Multi/018 and financed or co-financed with the '5 per mille' public funding.

Private donation in memory Chiara Sardi to Diego Centonze;

Project 'Nuovi Biomarker Diagnostici e Terapeutici delle Malattie Neurodegenerative' - ADOPT co-funded by FOE 2020 - funding from CNR to Diego Centonze.

Declaration of Competing Interest

RFu received honoraria for serving on scientific advisory boards or as a speaker from Biogen, Novartis, Roche, and Merck and funding for research from Merck. FB acted as Advisory Board members of Teva and Roche and received honoraria for speaking or consultation fees from Merck Serono, Teva, Bio-gen Idec, Sanofi, and Novartis and non-financial support from Merck Serono, Teva, Biogen Idec, and Sanofi. DC is an Advisory Board member of Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novar-tis, Roche, Sanofi-Genzyme, and Teva and received honoraria for speaking or consultation fees from Almirall, Bayer Schering, Biogen, GW Pharmaceuticals, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva. He is also the principal investigator in clinical trials for Bayer Schering, Biogen, Merck Serono, Mitsubishi, Novartis, Roche, Sanofi-Genzyme, and Teva. His preclinical and clinical research was supported by grants from Bayer Schering, Biogen Idec, Celgene, Merck Serono, Novartis, Roche, Sanofi-Genzyme, and Teva. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results. All other authors report no competing interests.

References

Amatya, B., Khan, F., Galea, M., 2019. Rehabilitation for people with multiple sclerosis: an overview of Cochrane reviews. Cochrane Database Syst. Rev. 1 (1), CD012732 (Published 2019 Jan 14).

Bałkowiec-Iskra, E., Kurkowska-Jastrzebska, I., Joniec, I., Ciesielska, A., Muszynska, A., Przybyłkowski, A., et al., 2007 Aug. MPTP-induced central dopamine depletion exacerbates experimental autoimmune encephalomyelitis (EAE) in C57BL mice. Inflamm. Res. 56 (8), 311–317.

Bruno, A., Dolcetti, E., Rizzo, F.R., Fresegna, D., Musella, A., Gentile, A., et al., 2020. Inflammation-associated synaptic alterations as shared threads in depression and multiple sclerosis [published correction appears in Front Cell Neurosci. 2021 Jan 26; 14:647259]. Front. Cell. Neurosci. 14, 169. Published 2020 Jun 23.

Centonze, D., Bari, M., Rossi, S., Prosperetti, C., Furlan, R., Fezza, F., et al., 2007. The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. Brain. 130 (Pt 10), 2543–2553.

Centonze, D., Muzio, L., Rossi, S., Cavasinni, F., De Chiara, V., Bergami, A., et al., 2009. Inflammation triggers synaptic alteration and degeneration in experimental autoimmune encephalomyelitis. J. Neurosci. 29 (11), 3442–3452.
Cortese, M., Riise, T., Bjørnevik, K., Farbu, E., Grytten, N., Hogenesch, I., et al., 2015. Evidence of preclinical disease activity: a prospective study on cognitive and physical performance prior to first symptom of multiple sclerosis. Mult. Scler. 21 (Suppl. 11), 55.
Cosentino, M., Zaffaroni, M., Marino, F., Bombelli, R., Ferrari, M., Rasini, E., Lecchini, S., Ghezzi, A., Frigo, G., 2002 Dec. Catecholamine production and tyrosine hydroxylase expression in peripheral blood mononuclear cells from multiple sclerosis patients: effect of cell stimulation and possible relevance for activation-induced apoptosis. J. Neuroimmunol. 133 (1–2), 233–240.
Cotman, C.W., Berchtold, N.C., 2002. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 25 (6), 295–301.
Dalgas, U., Stenager, E., Jakobsen, J., Petersen, T., Hansen, H.J., Knudsen, C., et al., 2010. Fatigue, mood and quality of life improve in MS patients after progressive resistance training. Mult. Scler. 16 (4), 480–490.
Dalgas, U., Langeskov-Christensen, M., Stenager, E., Riemenschneider, M., Hvid, L.G., 2019. Exercise as medicine in multiple sclerosis-time for a paradigm shift: preventive, symptomatic, and disease-modifying aspects and perspectives. Curr. Neurol. Neurosci. Rep. 19 (11), 88. Published 2019 Nov 13.
Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9 (1), 46–56.
Dendrou, C.A., Fugger, L., Friese, M.A., 2015. Immunopathology of multiple sclerosis. Nat. Rev. Immunol. 15 (9), 545–558.
Einstein, O., Fainstein, N., Touloumi, O., Lagoudaki, R., Hanya, E., Grigoriadis, N., et al., 2018. Exercise training attenuates experimental autoimmune encephalomyelitis by peripheral immunomodulation rather than direct neuroprotection. Exp. Neurol. 299 (Pt A), 56–64.
Eizenberg, O., Faber-Elman, A., Lotan, M., Schwartz, M., 1995. Interleukin-2 transcripts in human and rodent brains: possible expression by astrocytes. J. Neurochem. 64 (5), 1928–1936.
Farrell 3rd, J.W., Merkas, J., Pilutti, L.A., 2020. The effect of exercise training on gait, balance, and physical fitness asymmetries in persons with chronic neurological conditions: a systematic review of randomized controlled trials. Front. Physiol. 11, 585765 (Published 2020 Nov 12).
Gentile, A., De Vito, F., Fresegna, D., Musella, A., Buttari, F., Bullitta, S., et al., 2015a. Exploring the role of microglia in mood disorders associated with experimental multiple sclerosis. Front. Cell. Neurosci. 9, 243 (Published 2015 Jun 25).
Gentile, A., Fresegna, D., Federici, M., Musella, A., Rizzo, F.R., Sepman, H., et al., 2015b. Dopaminergic dysfunction is associated with IL-1β-dependent mood alterations in experimental autoimmune encephalomyelitis. Neurobiol. Dis. 74, 347–358.
Gentile, A., Fresegna, D., Musella, A., Sepman, H., Bullitta, S., De Vito, F., et al., 2016. Interaction between interleukin-1β and type-1 cannabinoid receptor is involved in anxiety-like behavior in experimental autoimmune encephalomyelitis. J. Neuroinflammation 13 (1), 231. Published 2016 Sep 2.
Gentile, A., Musella, A., De Vito, F., Rizzo, F.R., Fresegna, D., Bullitta, S., et al., 2019. Immunomodulatory effects of exercise in experimental multiple sclerosis. Front. Immunol. 10, 2197. Published 2019 Sep 13.
Goldman Consensus Group, 2005. The Goldman consensus statement on depression in multiple sclerosis. Mult. Scler. 11 (3), 328–337.
Gunnarsson, M., Udumyan, R., Bahmanyar, S., Nilsagård, Y., Montgomery, S., 2015. Characteristics in childhood and adolescence associated with future multiple sclerosis risk in men: cohort study. Eur. J. Neurol. 22 (7), 1131–1137.
Haji, N., Mandolesi, G., Gentile, A., Sacchetti, L., Fresegna, D., Rossi, S., et al., 2012. TNF-α-mediated anxiety in a mouse model of multiple sclerosis. Exp. Neurol. 237 (2), 296–303.
Haussleiter, I.S., Brüne, M., Juckel, G., 2009. Psychopathology in multiple sclerosis: diagnosis, prevalence and treatment. Ther. Adv. Neurol. Disord. 2 (1), 13–29.
Hodes, G.E., Pfau, M.L., Leboeuf, M., Golden, S.A., Christoffel, D.J., Bregman, D., et al., 2014. Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc. Natl. Acad. Sci. U. S. A. 111 (45), 16136–16141.
Kalb, R., Brown, T.R., Coote, S., Costello, K., Dalgas, U., Garmon, E., et al., 2020. Exercise and lifestyle physical activity recommendations for people with multiple sclerosis throughout the disease course. Mult. Scler. 26 (12), 1459–1469.
Karrenbauer, B.D., Ho, Y.J., Ludwig, V., Löhn, J., Spanagel, R., Schwarting, R.K., et al., 2009. Time-dependent effects of striatal interleukin-2 on open field behaviour in rats. J. Neuroimmunol. 208 (1–2), 10–18.
Kjølhede, T., Siemonsen, S., Wenzel, D., Stellmann, J.P., Ringgaard, S., Pedersen, B.G., et al., 2018. Can resistance training impact MRI outcomes in relapsing-remitting multiple sclerosis? Mult. Scler. 24 (10), 1356–1365.
Kothur, K., Wienholt, L., Brilot, F., Dale, R.C., 2016. CSF cytokines/chemokines as biomarkers in neuroinflammatory CNS disorders: a systematic review. Cytokine. 77, 227–237.
Krupp, L.B., LaRocca, N.G., Muir-Nash, J., Steinberg, A.D., 1989. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch. Neurol. 46 (10), 1121–1123.
Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 33 (11), 1444–1452.
Langeskov-Christensen, M., Grøndahl Hvid, L., Nygaard, M.K.E., Ringgaard, S., Jensen, H.B., Nielsen, H.H., et al., 2021. Efficacy of high-intensity aerobic exercise on brain MRI measures in multiple sclerosis. Neurology. 96 (2), e203–e213.
Lo Fermo, S., Barone, R., Patti, F., Laisa, P., Cavallaro, T.L., Nicoletti, A., et al., 2010. Outcome of psychiatric symptoms presenting at onset of multiple sclerosis: a retrospective study. Mult. Scler. 16 (6), 742–748.
Magliozzi, R., Pitteri, M., Ziccardi, S., Pisani, A.I., Montibeller, L., Marastoni, D., et al., 2021. CSF parvalbumin levels reflect interneuron loss linked with cortical pathology in multiple sclerosis. Ann. Clin. Transl. Neurol. 8 (3), 534–547.
Mandolesi, G., Bullitta, S., Fresegna, D., De Vito, F., Rizzo, F.R., Musella, A., et al., 2019. Voluntary running wheel attenuates motor deterioration and brain damage in cuprizone-induced demyelination. Neurobiol. Dis. 129, 102–117.
Marrie, R.A., Reingold, S., Cohen, J., Stuve, O., Trojano, M., Sorensen, P.S., et al., 2015. The incidence and prevalence of psychiatric disorders in multiple sclerosis: a systematic review. Mult. Scler. 21 (3), 305–317.
Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65 (9), 732–741.
Motl, R.W., Pilutti, L.A., 2016. Is physical exercise a multiple sclerosis disease modifying treatment? Expert. Rev. Neurother. 16 (8), 951–960.
Motl, R.W., Sandroff, B.M., Kwakkel, G., Dalgas, U., Feinstein, A., Heesen, C., et al., 2017. Exercise in patients with multiple sclerosis. Lancet Neurol. 16 (10), 848–856.
Musella, A., Sepman, H., Mandolesi, G., Gentile, A., Fresegna, D., Haji, N., et al., 2014. Pre- and postsynaptic type-1 cannabinoid receptors control the alterations of glutamate transmission in experimental autoimmune encephalomyelitis. Neuropharmacology. 79, 567–572.
Nasca, C., Menard, C., Hodes, G., Bigio, B., Pena, C., Lorsch, Z., et al., 2019. Multidimensional predictors of susceptibility and resilience to social defeat stress. Biol. Psychiatry 86 (6), 483–491.
Negaresh, R., Motl, R.W., Mokhtarzade, M., Dalgas, U., Patel, D., Shamsi, M.M., et al., 2018. Effects of exercise training on cytokines and adipokines in multiple sclerosis: a systematic review. Mult. Scler. Relat. Disord. 24, 91–100.
Olsson, T., Barcellos, L.F., Alfredsson, L., 2017. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nat. Rev. Neurol. 13 (1), 25–36.
Patten, S.B., Beck, C.A., Williams, J.V., Barbui, C., Metz, L.M., 2003. Major depression in multiple sclerosis: a population-based perspective. Neurology. 61 (11), 1524–1527.
Pedrabissi, L., Santinello, M., 1989. Manuale dell'adattamento Italiano dello STAI di Spielberger, Forma Y. Organizzazioni Speciali, Firenze, Italy.
Peruga, I., Hartwig, S., Thöne, J., Hovemann, B., Gold, R., Juckel, G., et al., 2011. Inflammation modulates anxiety in an animal model of multiple sclerosis. Behav. Brain Res. 220 (1), 20–29.
Petitto, J.M., McCarthy, D.B., Rinker, C.M., Huang, Z., Getty, T., 1997 Mar. Modulation of behavioral and neurochemical measures of forebrain dopamine function in mice by species-specific interleukin-2. J. Neuroimmunol. 73 (1–2), 183–190.
Pollak, Y., Ovadia, H., Goshen, I., Gurevich, R., Monsa, K., Avitsur, R., et al., 2000. Behavioral aspects of experimental autoimmune encephalomyelitis. J. Neuroimmunol. 104 (1), 31–36.
Primo de Carvalho Alves, L., Sica da Rocha, N., 2020. Different cytokine patterns associate with melancholia severity among inpatients with major depressive disorder. Ther. Adv. Psychopharmacol. 10, 2045125320937921 (Published 2020 Sep 1).
Riemenschneider, M., Hvid, L.G., Stenager, E., Dalgas, U., 2018. Is there an overlooked "window of opportunity" in MS exercise therapy? Perspectives for early MS rehabilitation. Mult. Scler. 24 (7), 886–894.
Rietberg, M.B., van Wegen, E.E., Uitdehaag, B.M., Kwakkel, G., 2011. The association between perceived fatigue and actual level of physical activity in multiple sclerosis. Mult. Scler. 17 (10), 1231–1237.
Rizzo, F.R., Guadalupi, L., Sanna, K., Vanni, V., Fresegna, D., De Vito, F., et al., 2021. Exercise protects from hippocampal inflammation and neurodegeneration in experimental autoimmune encephalomyelitis. Brain Behav. Immun. 98, 13–27.
Romme Christensen, J., Börnsen, L., Khademi, M., Olsson, T., Jensen, P.E., Sørensen, P.S., et al., 2013. CSF inflammation and axonal damage are increased and correlate in progressive multiple sclerosis. Mult. Scler. 19 (7), 877–884.
Ross, S.H., Cantrell, D.A., 2018. Signaling and function of Interleukin-2 in T lymphocytes. Annu. Rev. Immunol. 36, 411–433.
Rossi, S., De Chiara, V., Musella, A., Kusayanagi, H., Mataluni, G., Bernardi, G., et al., 2008. Chronic psychoemotional stress impairs cannabinoid-receptor-mediated control of GABA transmission in the striatum. J. Neurosci. 28 (29), 7284–7292.
Rossi, S., Furlan, R., De Chiara, V., Musella, A., Lo Giudice, T., Mataluni, G., et al., 2009. Exercise attenuates the clinical, synaptic and dendritic abnormalities of experimental autoimmune encephalomyelitis. Neurobiol. Dis. 36 (1), 51–59.
Rossi, S., De Chiara, V., Musella, A., Sacchetti, L., Cantarella, C., Castelli, M., et al., 2010. Preservation of striatal cannabinoid CB1 receptor function correlates with the antianxiety effects of fatty acid amide hydrolase inhibition. Mol. Pharmacol. 78 (2), 260–268.
Rossi, S., Sacchetti, L., Napolitano, F., De Chiara, V., Motta, C., Studer, V., et al., 2012. Interleukin-1β causes anxiety by interacting with the endocannabinoid system. J. Neurosci. 32 (40), 13896–13905.
Rossi, S., Studer, V., Motta, C., Germani, G., Macchiarulo, G., Buttari, F., et al., 2014. Cerebrospinal fluid detection of interleukin-1β in phase of remission predicts disease progression in multiple sclerosis. J. Neuroinflammation 11, 32 (Published 2014 Feb 18).
Rossi, S., Studer, V., Motta, C., Polidoro, S., Perugini, J., Macchiarulo, G., et al., 2017. Neuroinflammation drives anxiety and depression in relapsing-remitting multiple sclerosis. Neurology. 89 (13), 1338–1347.
Sica, C., Ghisi, M., 2007. The Italian versions of the Beck anxiety inventory and the Beck depression inventory-II: Psychometric properties and discriminant power. In:

Lange, M.A. (Ed.), Leading-Edge Psychological Tests and Testing Research. Nova Science Publishers, pp. 27–50.
Souza, P.S., Gonçalves, E.D., Pedroso, G.S., Farias, H.R., Junqueira, S.C., Marcon, R., et al., 2017. Physical exercise attenuates experimental autoimmune encephalomyelitis by inhibiting peripheral immune response and blood-brain barrier disruption. Mol. Neurobiol. 54 (6), 4723–4737.
Stampanoni Bassi, M., Iezzi, E., Mori, F., Simonelli, I., Gilio, L., Buttari, F., et al., 2019. Interleukin-6 disrupts synaptic plasticity and impairs tissue damage compensation in multiple sclerosis. Neurorehabil. Neural Repair 33 (10), 825–835.
Stampanoni Bassi, M., Drulovic, J., Pekmezovic, T., Iezzi, E., Sica, F., Gilio, L., et al., 2020. Cerebrospinal fluid inflammatory biomarkers predicting interferon-beta response in MS patients. Ther. Adv. Neurol. Disord. 13, 1756286420970833. Published 2020 Dec 8.
Tanaka, T., Narazaki, M., Kishimoto, T., 2014. IL-6 in inflammation, immunity, and disease. Cold Spring Harb. Perspect. Biol. 6 (10), a016295. Published 2014 Sep 4.
Tancredi, V., Zona, C., Velotti, F., Eusebi, F., Santoni, A., 1990. Interleukin-2 suppresses established long-term potentiation and inhibits its induction in the rat hippocampus. Brain Res. 525 (1), 149–151.
Thompson, A.J., Banwell, B.L., Barkhof, F., Carroll, W.M., Coetzee, T., Comi, G., et al., 2018. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 17 (2), 162–173.
Wesnes, K., Myhr, K.M., Riise, T., Cortese, M., Pugliatti, M., Boström, I., et al., 2018. Physical activity is associated with a decreased multiple sclerosis risk: the EnvIMS study. Mult. Scler. 24 (2), 150–157.
Xie, Y., Li, Z., Wang, Y., Xue, X., Ma, W., Zhang, Y., et al., 2019. Effects of moderate- versus high- intensity swimming training on inflammatory and CD4+ T cell subset profiles in experimental autoimmune encephalomyelitis mice. J. Neuroimmunol. 328, 60–67.
Ye, J.H., Tao, L., Zalcman, S.S., 2001. Interleukin-2 modulates N-methyl-D-aspartate receptors of native mesolimbic neurons. Brain Res. 894 (2), 241–248.
Yirmiya, R., Goshen, I., 2011. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav. Immun. 25 (2), 181–213.