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Effects of exercise in experimental autoimmune encephalomyelitis (an animal model of multiple sclerosis)

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Abstract

Exercise training has improved many outcomes in “clinical” research involving persons with multiple sclerosis (MS), but there is limited understanding of the underlying “basic” pathophysiological mechanisms. The animal model of MS, experimental autoimmune encephalomyelitis (EAE), seems ideal for examining the effects of exercise training on MS-disease pathophysiology. EAE is an autoimmune T-helper cell-mediated disease characterized by T-cell and monocyte infiltration and inflammation in the CNS. To that end, this paper briefly describes common models of EAE, reviews existing research on exercise and EAE, and then identifies future research directions for understanding the consequences of exercise training using EAE.

Keywords

Experimental autoimmune encephalomyelitis; Multiple sclerosis; Exercise

1. Introduction

Multiple sclerosis (MS) is an immune-mediated neurological disease with an estimated prevalence of 1 per 1000 adults in the U.S. (Page et al., 1993; Mayr et al., 2003). This neurological disease typically begins with periods of inflammation within the central nervous system (CNS) that results in axonal demyelination and transection. Such an expression is consistent with the relapsing–remitting course of MS (Mäurer and Rieckmann, 2000). There are further neurodegenerative processes presumably resulting from insufficient neurotrophic support that results in axonal and neuronal loss (Frohman et al., 2006). The damage and its location within the CNS can result in walking and cognitive dysfunction, symptoms such as fatigue, pain, and depression, and reduced quality of life (QOL) and participation in activities of daily living (Lublin, 2005).¹

There is an abundance of research from clinical trials demonstrating the beneficial effects of physical activity and exercise training on outcomes in persons with MS (Motl and Pilutti, 2012). For example, our laboratory has demonstrated that exercise training has improved walking mobility (Snook and Motl, 2009), depression (Ensari et al., 2013), fatigue (Pilutti et al., 2014), and QOL (Motl and Gosney, 2008) based on meta-analyses of clinical trials including persons with MS. This research provides evidence for “clinical” benefits of exercise training, but does not widen our understanding of exercise training effects on the “basic” pathophysiology of MS. For example, exercise training seemingly exerts beneficial effects in MS through immunomodulation and regulation of neurotrophic factors that reduce axo-neuronal degeneration and promote neuroprotection, respectively, in MS (Castellano and White, 2008). Indeed, exercise training and physical activity have increased expression of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), in the hippocampus of mice and rats (Cotman and Berchtold, 2002; Gibbons et al., in press), and insulin-like growth factor-1 (IGF-1) in the brain of rats (Carro et al., 2000). Hippocampal neurogenesis, in particular, is a commonly documented effect of exercise in the rodent brain (Cotman et al., 2007; Gibbons et al., in press) and exercise has further been associated with neuroprotective effects by increasing gene expression that promotes synaptic plasticity (Cotman et al., 2007). Exercise training, both acute and chronic, and physical activity may further influence the number and function of many cells of the innate immune system including neutrophils, monocytes, and natural killer (NK) cells (Walsh et al., 2011). There is additional evidence that lymphocytosis occurs during and after exercise, with effects on numbers of both T and B cells and alterations in pro and anti-inflammatory cytokine balance (Walsh et al., 2011). However, effects of exercise training and physical activity on the peripheral immune system for protection against infections and disease seem to be highly dependent on parameters such as volume and intensity (Pedersen and Hoffman-Goetz, 2000; Terra et al., 2012). Such hypotheses and observations could be examined using an animal model of MS and thereby provide a basic science or pre-clinical understanding of exercise training effects on MS disease pathophysiology.

The most common animal model for examining the mechanisms of action for treatment effects on MS pathophysiology is experimental autoimmune encephalomyelitis (EAE). EAE is a T-helper (Th) cell-mediated autoimmune disease characterized by T-cell and monocyte infiltration in the CNS associated with local inflammation (Robinson et al., 2014). The autoimmune molecular targets identified have been proteins expressed by myelin-producing oligodendrocytes in the CNS, resulting in primary demyelination of axonal tracks, impaired axonal conduction in the CNS, and progressive hind-limb paralysis (Robinson et al., 2014). The EAE model has been extremely useful for studying disease pathogenesis and potential therapeutic interventions (Robinson et al., 2014). Indeed, variations of the EAE model that produce different patterns of clinical MS presentation are often used in preclinical research for identifying the benefits and dissecting the mechanisms of new therapeutic interventions

¹Multiple sclerosis (MS); central nervous system (CNS); experimental autoimmune encephalomyelitis (EAE); quality of life (QOL); T-helper (Th); Theiler's Murine Encephalomyelitis (TMEV); antigen presenting cells (APCs); proteolipid protein (PLP); myelin basic protein (MBP); myelin oligodendrocyte protein (MOG); complete Freund's adjuvant (CFA); chronic relapsing EAE (CR-EAE); guinea pig spinal cord tissue (GPSC); days post-induction (dpi); spontaneous inhibitory postsynaptic currents (sIPSCs); brain-derived neurotrophic factor (BDNF); tumor necrosis factor- α (TNF- α); nerve growth factor (NGF); glial cell line-derived neurotrophic factor (GDNF).

for subsequent translation into human clinical trials of MS (Steinman and Zamvil, 2006). This model would further be beneficial for dissecting the possible mechanisms of exercise training effects in MS.

Researchers have speculated that exercise training exerts its consequences through pathophysiological mechanisms within the CNS (Heesen et al., 2006; White and Castellano, 2008; Motl and Pilutti, 2012) and this can be most readily studied through research involving EAE. To that end, the purpose of this paper is to generate a case for EAE as a model for understanding the possible pathophysiological mechanisms of exercise training and physical activity effects in humans with MS. We briefly describe common models of EAE that might be considered for such research, review existing research on exercise and EAE, and lastly identify future research directions for understanding the benefits of exercise training using EAE.

2. Common models of EAE

Over the past 60 years, researchers have developed different animal models for understanding mechanisms and targets of therapeutic interventions in MS (Denic et al., 2011; Simmons et al., 2013). These include the autoimmune EAE model, virally induced models such as Theiler's Murine Encephalomyelitis (TMEV) infection, and toxin-induced models of demyelination (Denic et al., 2011). To date, EAE is the most extensively studied murine model of MS as many of the pathologies observed in the CNS of mice resemble those observed in the CNS of persons with MS (McCarthy et al., 2012). The classic EAE model focuses on the role of CD4⁺ T cells as a major effector cell and explains the strong correlation of MS susceptibility to MHC class II alleles (Simmons et al., 2013). Mice are most commonly used in EAE due to the availability of transgenic and knockout mice for mechanistic studies (Robinson et al., 2014). There are many pathophysiologic forms of EAE in mice with different patterns of clinical presentation depending on the animal species and strain, priming protein or peptide, and route of immunization (Gold et al., 2006). These different models have been used to study disease development and specific histopathological characteristics with relevance to MS, and to dissect mechanisms of potential therapeutic interventions (Steinman and Zamvil, 2006; McCarthy et al., 2012).

Two routes of immunization are widely used for inducing EAE in mice: active induction by immunization with myelin antigens and passive induction by adoptive transfer of pre-activated myelin-specific T cells into naïve mice (McCarthy et al., 2012). Peripheral immunization of mice with myelin antigen in active EAE results in breakdown of peripheral immune tolerance and further activation of myelin antigen-specific T cells in secondary lymphoid organs. After initial activation, these T cells differentiate into effector T cells and leave the secondary lymphoid organs. Effector T cells express integrins (Yednock et al., 1992) that allow the cells to cross the blood–brain barrier and are reactivated by antigen presenting cells (APCs) presenting myelin antigens in the CNS (Kawakami et al., 2004). This activation enables the effector T cells to express pro-inflammatory cytokines such as IFN- γ that locally damage the surrounding nervous tissue (Lees et al., 2008). Chemokines are produced by T cells, leading to recruitment of nonspecific effector cells (i.e., monocytes, macrophages, and neutrophils) into the CNS (Kroenke et al., 2008). Together, these

inflammatory cells are responsible for destruction of the myelin-sheathed surrounding axons and lesion formation. The adoptive transfer of activated, myelin-specific Th cells from immunized donors into naïve syngeneic receptors can directly induce the 'effector phase' of EAE (Raine et al., 1984; Zamvil et al., 1985; Cross and Raine, 1990). The clinical features of passive EAE are identical to those of active EAE; yet passive EAE induction is a more direct way of establishing T cell effector function in the CNS.

There is variation in the specific strains of mice and encephalitogenic peptides capable of eliciting EAE. EAE is often actively and passively induced in the SJL mouse through immunization with CNS homogenate, proteolipid protein (PLP), myelin basic protein (MBP), encephalitogenic epitopes of PLP (PLP_{139–151}, PLP_{178–191}), myelin oligodendrocyte protein (MOG_{92–106}), or MBP_{84–104}, in a suspension with complete Freund's adjuvant (CFA) (Robinson et al., 2014). The disease follows a predictable course, with initial symptoms appearing 10–15 days post-induction, followed by ascending hind limb and tail paralysis that progresses to the fore limbs with accompanying weight loss. The disease in SJL mice is characterized by a relapsing–remitting course of paralysis. In C57BL/6 mice, MOG_{35–55} is an encephalitogen that presents in the clinical form of a chronic progressive disease course in both active and passive EAE induction. This is characterized by sustained priming antigen-specific T cell responses. The description of clinical disability, or of disease course as relapsing–remitting or chronic progressive, is based on a standard, 6-point clinical grading scale; Grade 0 = asymptomatic; Grade 1 = decreased tail tone or weak tail only; Grade 2 = hind limb weakness (paraparesis); Grade 3 = hind limb paralysis (paraplegia) and/or urinary incontinence; Grade 4 = weakness of front limbs with paresis or paraplegia (quadriparesis) and/or atonic bladder; and Grade 5 = paralysis of all (4) limbs (paraplegia) and/or quadriplegic with reduced mental alertness and/or increased respiratory effort and/or moribund (Miller and Karpus, 2007; McCarthy et al., 2012). The onset and progression of clinical disability in models of relapsing–remitting and chronic progressive EAE are the result of inflammation and demyelination mainly manifesting in the spinal cord and brain (Simmons et al., 2013). Importantly, inflammation and demyelination, and overall clinical pattern of EAE immunopathology, vary among the models (i.e., type of animal species, route of immunization, and target antigen) (Gold et al., 2006). No single EAE model can fully replicate the spectrum of inflammatory and neurodegenerative mechanisms seen in MS, but rather a combination of different methods and models is necessary to identify particular features of the complex pathogenesis of EAE and recognize targets for therapeutic interventions in MS (Gold et al., 2006; Simmons et al., 2013).

3. Exercise and EAE

To our knowledge, there are five published studies of exercise and EAE (Le Page et al., 1994, 1996; Rossi et al., 2009; Bernardes et al., 2013; Patel and White, 2013). These studies are quite varied as two focused on exercise as a physiological stressor that might worsen disease development (Le Page et al., 1994, 1996), whereas the other three focused on exercise as a therapeutic intervention for attenuating disease activity and its expression (Rossi et al., 2009; Bernardes et al., 2013; Patel and White, 2013). The first study of exercise and EAE examined whether severe forced endurance treadmill running, as a physiological stressor, worsened the incidence and development of chronic relapsing EAE

(CR-EAE) (Le Page et al., 1994). Eight-week-old Lewis rats ($n = 109$) were randomly assigned into one of four groups: control females ($n = 27$), control males ($n = 27$), exercised females ($n = 27$), and exercised males ($n = 28$). EAE was induced in all rats with guinea pig spinal cord tissue (GPSC) and Freund's adjuvant on day 0. On days 1 to 10 post-induction, rats in the exercise group ran on a treadmill ($60\text{--}120\text{ min}\cdot\text{day}^{-1}$, $15\text{--}30\text{ m}\cdot\text{min}^{-1}$), whereas rats in the control group were sedentary, but exposed to the same environment (i.e., noise, handling, light, temperature, access to food and water) as exercised rats. Clinical signs and body weight were examined every two days until 16 days post-induction (dpi) in both exercised and control rats. There was a statistically significant delay of the onset and first day of maximum severity of CR-EAE in exercised female and male rats based on the clinical assessment of EAE ($p = 0.001$). The duration of CR-EAE in exercised rats further demonstrated a statistically significant decrease compared to controls (3.2 days vs. 4.2 days; $p = 0.001$). However, the peak severity of clinical disability was not significantly different between exercised and control rats ($p > 0.05$) and this was further confirmed by the fact that the maximal body mass loss was not significantly different between exercised and control rats. Taken together, this study demonstrated that exercise performed as a stressor during onset of disease did not exacerbate EAE symptoms, but rather weakly delayed the progression and diminished the duration of CR-EAE.

The follow-up study examined the effects of different doses of stress (i.e., severe or moderate exercise) performed before or after EAE induction (Le Page et al., 1996). The researchers essentially observed if the effect of physical exercise on EAE expression varied with the time of stress application (i.e., after or before induction of EAE by cell transfer). EAE was passively induced in eight-week-old female Lewis rats by transfer of lymph node cells from donor animals primed with guinea pig MBP to naïve recipients to elicit a monophasic form of CR-EAE in a series of four experiments: Experiment 1: effect of two days of severe exercise ($250\text{--}300\text{ min}\cdot\text{day}^{-1}$, $15\text{--}20\text{ m}\cdot\text{min}^{-1}$) on the day of cell transfer and next day following cell transfer ($n = 19$); Experiment 2: effect of two days of severe exercise before cell transfer ($n = 18$); Experiment 3: effect of five days of running at constant speed ($15\text{--}25\text{ m}\cdot\text{min}^{-1}$, $2\text{ h}\cdot\text{day}^{-1}$) or running at variable speed ($20\text{ m}\cdot\text{min}^{-1}$ for 3 min followed by 2 min at $35\text{ m}\cdot\text{min}^{-1}$ for $1\text{ h}\cdot\text{day}^{-1}$) between cell transfer and onset of clinical signs (day 0 to 4) ($n = 28$); Experiment 4: effect of five days of variable speed running exercise performed between cell transfer and onset of clinical signs ($n = 20$). The four experiments included the same control condition of sedentary rats exposed to an identical environment as exercised rats. When two consecutive days of severe exercise were performed *after* cell transfer (Experiment 1), time of onset of disease and day of maximal severity of clinical disability were both significantly delayed ($p = 0.008$ and $p = 0.016$, respectively) when compared to control rats. There were no statistically significant effects between exercise and control in Experiments 2, 3, and 4. This suggests that the physical stress of forced exercise might have an effect on the immediate effector phase of EAE when it is applied during the development of EAE.

The first study on the possible therapeutic nature of exercise examined the effect of *voluntary* exercise on clinical disability as well as the preservation of cannabinoid CB1 receptor sensitivity and dendritic integrity in the striatum (Rossi et al., 2009). To do this,

eight-week-old C57BL/6 female mice ($n = 20$) were induced with chronic EAE by subcutaneous immunization of MOG_{35–55} in incomplete Freund's adjuvant containing tuberculosis. Pertussis toxin was injected on the day of immunization and two days later. Pertussis toxin is required in the C57BL/6 mice with MOG_{35–55} model of EAE to contribute to the initiation of the disease (Hofstetter et al., 2002). Mice were randomized in the EAE group ($n = 10$) or in the EAE plus exercise group ($n = 10$). Mice in the EAE plus exercise group were housed in cages equipped with a running wheel to allow for voluntary running. The control group involved sedentary mice reared in a standard cage (i.e., no running wheel). Clinical disability and electrophysiological effects of exercise were compared with those of EAE alone at 20 and 50 dpi. Mice in the exercise group demonstrated lower clinical disability scores in both acute and chronic stages of the disease compared with controls ($p < 0.05$). The exercise group had attenuated synaptic defects when comparing the action of the cannabinoid CB1 receptor agonist HU210 on striatal GABA-mediated spontaneous inhibitory postsynaptic currents (sIPSCs). Exercise completely reversed the inhibition of HU210 responses induced by EAE, at both 20 and 50 dpi ($p < 0.01$), possibly due to the effect of exercise-driven dopamine release. Exercising EAE mice further presented increased spine density compared to control EAE mice ($p < 0.01$). To our knowledge, this is the only study to demonstrate that the clinical, dendritic, and synaptic defects of EAE may be attenuated by *voluntary* wheel running.

The fourth study investigated the effect of forced treadmill running on clinical disability scores and differences in protein expression (i.e., brain-derived neurotrophic factor [BDNF], tumor necrosis factor- α [TNF- α], nerve growth factor [NGF]) in CR-EAE (Patel and White, 2013). Eight-week-old female Lewis rats ($n = 40$) were randomly assigned to one of four groups: EAE-exercise, EAE-sedentary, control-exercise, and control-sedentary. The Lewis rats in the EAE groups ($n = 20$) were induced with CR-EAE by MOG_{33–55} and CFA the day prior to the first session of exercise training. Exercising rats then engaged in progressive treadmill running for ten consecutive dpi. Treadmill running began with 60 min of running at 30 m min^{-1} on days 1 and 2 and 90 min (30 m min^{-1}) on days 3 to 10. Clinical disability (scale 0–5; 0 = normal, 5 = death) and body mass were scored daily. There was no statistically significant difference in clinical disability score between EAE-exercise and EAE-control groups (disability score = grade 1.0; decreased tail tone or weak tail only). There further were no significant differences in BDNF and TNF- α concentration between EAE-exercise and EAE-control groups, but total brain NGF concentration was significantly greater in the EAE-exercise group ($p < 0.05$). The absence of significant difference in clinical disability scores, however, makes it problematic to relate the effects of exercise to protein modulation in relation to clinical disability.

The most recent study of exercise examined the effect of six weeks of forced swimming performed both before and after chronic EAE induction on inflammatory leukocyte recruitment, lesion size and demyelination processes, and concentration of cytokines and BDNF (Bernardes et al., 2013). Female C57BL/6 mice (six- to eight-weeks-old) were randomly allocated into four groups: EAE + Exercise, EAE + No Exercise, Control + Exercise, and Control + No Exercise. Both exercise groups of mice were subject to a swimming training protocol of 30 min day^{-1} , 5 days week^{-1} , for 6 weeks. The average

workload of the swimming protocol was set up with an average of 7% of the animal's body weight. The control groups involved mice being placed on a flat surface inside a swimming pool for the same length of time as the exercised group. The mice in the EAE groups were injected with MOG_{35–55} complete with CFA in the fifth week of the swimming training protocol and pertussis toxin was injected intraperitoneally on the day of immunization and after 48 h. Saline injections were given to the control groups at similar times. The exercise training continued until 10 dpi. Intravital microscopy and histopathology were performed on the EAE (exercise and sedentary) and control (exercise and sedentary) groups at 10 or 14 dpi. Results demonstrated an attenuated disease severity and weight loss ($p < 0.05$) in the EAE + Exercise group. There was no significant difference in leukocyte rolling or adhesion or lesion development between exercised and unexercised groups in both the brain and spinal cord on 10 or 14 dpi. While comparison of the EAE groups (Exercise and No Exercise) demonstrated a few statistically significant effects of exercise in modulating the production of cytokines TNF, IL-1 β , IL-6 and IL-10, there was no clear, consistent pattern in cytokine levels in either the brain or spinal cord. There was a statistically significant increase in BDNF production and decrease in demyelination in both the brain and spinal cord between the exercised and unexercised EAE mice at both 10 and 14 dpi. These results are contradictory in that the researchers suggest enhanced anti-inflammatory function via cytokine modulation in the CNS associated with exercise, however, cytokine levels in the spinal cord, which best correlate with clinical disease opposed to the brain (Recks et al., 2013), demonstrated increases in both pro- (TNF and IL-6) and anti-inflammatory (IL-10) cytokines at 14 dpi in the EAE +Exercise group.

4. Limitations and future research

The existing research on exercise training and physical activity in EAE is promising, but has notable limitations that obfuscate conclusions from the literature (See Table 1). One limitation of the research involves the different models of EAE induction and types of exercise training (i.e., design heterogeneity). The first two studies (Le Page et al., 1994, 1996) adopted an older model of disease induction in rats and only examined if severe endurance treadmill running as a physiological stressor, performed post-EAE induction, worsened development of CR-EAE. Two of the three studies that focused on exercise as a therapeutic intervention in EAE included mice and only examined the effect of exercise post-EAE induction (Rossi et al., 2009; Patel and White, 2013). This limits human translation and likely underestimates the beneficial effect of exercise, as exercise training occurred only for a short period after disease induction. Only one study has examined the effect of exercise training administered before and after EAE induction (Bernardes et al., 2013); however, forced swimming as a mode of exercise training would seem to increase stress in the animal and could therefore independently cause alterations in disease expression. Only one study (Rossi et al., 2009) examined the beneficial effect of *voluntary* wheel running. Collectively, the heterogeneity of questions (exercise as a stressor vs. therapeutic intervention), applied models of EAE (active vs. passive induction), susceptible animals (rats vs. mice), exercise stimuli (forced vs. voluntary), and period of exercise relative to EAE induction (post-EAE vs. pre-post EAE combined) can make conclusions on outcomes (i.e., disability, immune regulation, neurotrophic factors) difficult. However,

existing research in EAE and human trials supports a systematic, long-term investment in this line of research.

An important consideration for future researchers involves examining the effects of exercise and physical activity on the release of glucocorticoids and glucocorticoid sensitivity that can influence immune function, and how this might differentially influence disease outcomes between rodents and humans. Indeed, dependent on the intensity and duration, physical activity and exercise training can represent different degrees of physiological stress with different degrees of hypothalamic–pituitary–adrenocortical (HPA) axis activation (Cook et al., 2013). HPA axis activation plays an important role in the control of exaggerated inflammation and might impact symptom burden and disease outcomes in the EAE model. HPA axis activation may further differ between different strains of rats and mice (Sternberg et al., 1989; Droste et al., 2003), and therefore comparative studies of exercise training and physical activity may provide conflicting results between strains and species. This might explain why exercise and its variation in parameters (i.e., chronic or acute, force or voluntary, intense or mild) may cause differential alterations in the immune system and yield differential effects in EAE (Pérez-Nievas et al., 2010; Terra et al., 2012).

Future research should identify and differentiate the effects of voluntary wheel running independently compared with it as part of an enriched environment (e.g., presence of a running wheel along with toys, tunnels, hiding places, and odorants) on EAE and its expression. Indeed, one study reported that an enriched environment that included activity wheels reduced functional impairment and increased neural progenitor cell mobilization in mice using the EAE model (Magalon et al., 2007), and both activity wheels and an enriched environment may have independent effects on neurogenesis and brain plasticity (Olson et al., 2006). Such examination will be important for delineating if voluntary exercise itself has disease-modifying effects in EAE, or if such effects simply reflect environmental enrichment.

There are certainly many areas of future research inquiry regarding exercise training and EAE, and we propose a guide for initial research. Firstly, we recommend using the mouse model of EAE as mice have become the dominant organism used due to the availability of knockout and transgenic mice as tools to investigate immune regulation and the pathophysiological effect of inflammation on axonal integrity (Gold et al., 2006). Secondly, active induction of EAE would be most valuable to begin with as this will allow researchers to study the initial phase of the immune response of EAE in relation to exercise training. Passive induction will be valuable in the future for more targeted studies of immunopathologic mechanisms and will allow for concentration on the role of specific T-cell lineages (Katsnelson, 2012). We further specifically recommend applying active induction of EAE in SJL mice, as this elicits a relapsing–remitting disease course similar to the most common type of MS in humans (“Relapsing–remitting MS (RRMS),” n.d.). Research on *voluntary* exercise training (i.e., voluntary wheel running) is warranted as this would best mimic real, volitional physical activity in humans (Cook et al., 2013). Research examining the effects of voluntary exercise training delivered either before or after EAE induction as well as both before and after EAE induction using a cross-over design is important as previous research has mainly focused on the disease modifying effect of

exercise in EAE. There is no definitive information on the *preventative vs. disease modifying, restorative* effect of exercise on the development and progression of EAE, and there might be independent and additive effects. There may be value in the application of in-vivo magnetic resonance imaging (MRI) in exercised EAE mice as, to our knowledge, this has not been done. Previous research has used MRI to identify levels of gray matter, ventricle width, and inflammatory infiltrates using techniques such as cryogenically-cooled quadrature-resonator and T2-weighted analysis in EAE (MacKenzie-Graham et al., 2012; Waiczies et al., 2012; Lepore et al., 2013). Such research could widen our understanding of anatomical brain changes following exercise training in mice with EAE, as MRI is currently accepted as a valid outcome measure for MS and has been applied in exercise and MS (Prakash et al., 2007, 2010; Dalgas and Stenager, 2012). Identification of structural change and neurogenesis (Huehnchen et al., 2011) in the hippocampus of mice with EAE post-exercise may provide insight as clinical signs of cognitive dysfunction have been associated with hippocampal changes in MS (Prakash et al., 2011).

The continued investigation of exercise effects in the different models of EAE is necessary as the hallmark features of the disease (i.e., inflammation, demyelination, and axonal injury) vary between models (Gold et al., 2006). For example, passive MOG-induced EAE induction in rats elicits a demyelinating autoantibody response, whereas in C57BL/6 mice, there is no demyelinating response (Gold et al., 2006). It therefore can be seen as advantageous for future researchers to examine the effects of exercise on specific pathological features using the most suitable EAE model. This might permit an understanding of how exercise influences disease pathophysiology by comparing effects across different EAE models.

More information on the effects of exercise through brain neurotrophic factors, such as NGF, glial cell line-derived neurotrophic factor (GDNF), and BDNF, are necessary as these factors may play a role in lesion pathogenesis and neuronal growth and survival (Makar et al., 2014). Indeed, studies on humans with MS have primarily measured BDNF in the serum, but this may not represent levels in the brain, spinal cord, or cerebrospinal fluid (Lühder et al., 2013). The examination of immune markers, cytokine modulation, and dendritic pathology will provide further evidence regarding the immunological effects of exercise in EAE on both the CNS and peripheral immune systems. Careful, state-of-the art immune flow cytometric assays examining the effect of exercise on Th1 vs. Th17 immune response (Hunter et al., 2014) and potential differences in inflammatory cell infiltration and accumulation in the periphery and CNS (Getts et al., 2014), as well as number of cells of the oligodendrocyte lineage (Robinson et al., under review) are warranted to help separate effects of exercise on immune response vs. CNS.

5. Conclusions

We have provided a summary of common models of EAE that have previously been applied in examinations of MS pathophysiology and treatment, and therefore can be considered for future research in exercise and physical activity. We further analyzed existing research on the effects of exercise and physical activity in EAE. Some of the existing data support a beneficial effect of exercise on the clinical symptoms associated with EAE, but no

consistent evidence exists to support the beneficial effects of exercise on the immune system and specific histopathologic changes in the CNS that contribute to the attenuated clinical disease severity. The continued investigation of exercise and EAE will provide further evidence and conclusions regarding the basic science and pathophysiological effects of exercise of significance for persons living with MS.

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Table 1

Description of existing research on exercise in EAE.

Study	Animal species	Mode/strain of EAE induction	Type of EAE	Type of exercise/ time of delivery/ duration	Analysis	Results
Le Page, C., Ferry, A., Rieu, M., 1994. Effect of muscular exercise on chronic relapsing experimental autoimmune encephalomyelitis. <i>J. Appl. Physiol.</i> 77, 2341–2347.	Male and female eight-week-old Lewis rats	Active/guinea pig spinal cord tissue (GPSC)	Chronic–relapsing (CR)	Forced treadmill running/post disease induction/10 days	Clinical disability	Delay of onset of clinical disability and diminished duration of disease in exercised mice
Le Page, C., Bourdoulous, S., Beraud, E., Couraud, P.O., Rieu, M., Ferry, A., 1996. Effect of physical exercise on adoptive experimental auto-immune encephalomyelitis in rats. <i>Eur. J. Appl. Physiol.</i> 73, 130–135.	Female eight-week-old Lewis rats	Passive/MBP primed Lewis rat lymph nodes	Monophasic	Severe and moderate forced treadmill running/pre and post disease induction/two and five days	Clinical disability	Two days of severe treadmill running performed after disease induction delayed time of onset of disease and day of maximal severity of clinical disability in exercised mice
Rossi, S., Furlan, R., De Chiara, V., Musella, A., Lo Giudice, T., Mataluni, G., Cavasinni, F., Cantarella, C., Bernardi, G., Muzio, L., Martorana, A., Martino, G., Centonze, D., 2009. Exercise attenuates the clinical, synaptic and dendritic abnormalities of experimental autoimmune encephalomyelitis. <i>Neurobiol. Dis.</i> 36, 51–59.	Female eight-week-old C57BL/6 mice	Active/MOG ₃₅₋₅₅	Chronic	Voluntary wheel running/post disease induction/50 days	Clinical disability, sensitivity of cannabinoid CB1 receptors, dendritic pathology, and inflammatory infiltrates	Lower clinical disability and attenuated dendritic and synaptic defects in exercised mice
Patel, D.I., White, L.J., 2013. Effect of 10-day forced treadmill training on neurotrophic factors in experimental autoimmune encephalomyelitis. <i>Appl. Physiol. Nutr.</i> 38, 194–199.	Female eight-week-old Lewis rats	Active/MOG333-55	Chronic–relapsing (CR)	Forced treadmill running/post disease induction/10 days	Clinical disability, BDNF, NGF, TNF- α concentration	No significant difference in clinical disability or BDNF and TNF- α concentration; greater total brain NGF concentration in exercised mice
Bernardes, D., Oliveira-Lima, O.C., Silva, T.V. da, Faraco, C.C.F., Leite, H.R., Juliano, M.A., Santos, D.M. dos, Bethea, J.R., Brambilla, R., Orian, J.M., Arantes, R.M.E., Carvalho-Tavares, J., 2013. Differential brain and spinal cord cytokine and BDNF levels in experimental autoimmune encephalomyelitis are modulated by prior and regular exercise. <i>J. Neuroimmunol.</i> 264, 24–34.	Female six- to eight-week-old C57BL/6 mice	Active/MOG35-55	Chronic	Forced swimming/pre disease induction/6 weeks	Clinical disability, leukocyte adhesion or rolling, lesion development, cytokine and BDNF concentration	Attenuated disease severity and weight loss; increase in BDNF production and decrease in demyelination in both brain and spinal cord in exercised mice