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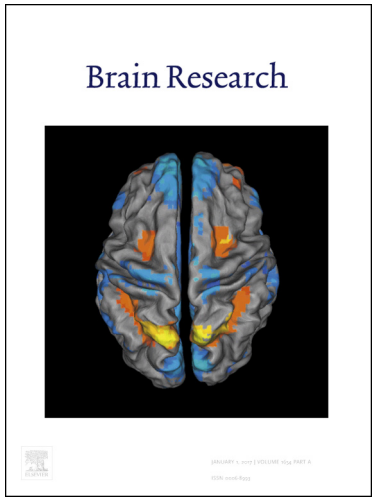

Research report

Environmental enrichment improves cognitive symptoms and pathological features in a focal model of cortical damage of Multiple Sclerosis

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Title: Environmental enrichment improves cognitive symptoms and pathological features in a focal model of cortical damage of Multiple Sclerosis

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1. Introduction

Multiple sclerosis (MS) is a demyelinating disease characterized by neuroinflammation, demyelination and axonal degeneration along with motor, sensitive and cognitive function loss. MS is a very heterogeneous disease that displays different clinical courses, including episodes of relapses and remissions of symptoms (relapsing/remitting MS, RRMS) which can progress to a secondary progressive form (SPMS) that presents disability accumulation and no relapses. Additionally, some patients can experience a persistent progression from the disease onset, known as primary progressive MS (PPMS) (Lublin et al., 2014). Indeed, until now, only one treatment for PPMS patients is available (ocrelizumab) (Frampton, 2017). The lack of more treatments for the progressive forms of MS can be attributed to the lack of animal models recapitulating the key features of these forms. The main challenge in modelling SPMS and PPMS is the impossibility of replicating the chronicity and the cortical injury pattern of these forms, which differ from those of RRMS. Cortical damage in the progressive forms has considerable clinical relevance due to its association with cognitive impairment and disability progression in MS patients (Calabrese et al., 2012; Calabrese et al., 2013; Calabrese and Castellaro, 2017; Nelson et al., 2011). Few animal models, reflecting some characteristics of the cortical pathology have been described in rodents (Gardner et al., 2013; Lagumersindez-Denis et al., 2017; Merkler et al., 2006; Silva and Ferrari, 2018; Silva et al., 2018; Ucal et al., 2017). In particular, we recently developed a new animal model that resembles most of the characteristics of the cortical pathology of progressive MS forms (Silva et al., 2018). This model includes cognitive impairment associated with cortical neuroinflammation, demyelination, neurodegeneration and meningeal inflammation. The sustained peripheral inflammatory stimulus exacerbated the symptoms and allowed the maintenance of the chronicity of cortical lesions (Silva et al., 2018).

So far, no specific pharmacological treatments are available for cognitive deficits (Amato et al., 2013; Sandroff and DeLuca, 2019; Sumowski et al., 2018). Non-pharmacological treatments such as cognitive rehabilitation, exercise and social support, are being evaluated in patients and animal models of neurodegenerative diseases and can act synergistically with pharmacological therapeutics agents (Kotloski and Sutula, 2015). In animal models, the implementation of environmental enrichment (EE) which provides exercise as well as cognitive and social stimulation, has been widely studied. The benefits of EE have been demonstrated in both healthy animals and models of neurodegenerative and psychiatric diseases by exerting positive effects on cognitive domains, such as learning and memory, and improving anxiety-like symptoms (Bruel-Jungerman et al., 2005; De Rosa et al., 2006; Fischer, 2016; Frasca et al., 2013; Jurgens and Johnson, 2012; Kotloski and Sutula, 2015; Larsson et al., 2002; Leggio et al., 2005; Pusic and Kraig, 2014; Sampedro-Piquero and Begega, 2017; Schloesser et al., 2010). It has been demonstrated that EE induces neurotrophins and stimulates adult neural progenitors in healthy animals (Kempermann et al., 1997; Magalon et al., 2007). In pathological conditions, EE promotes neuronal survival and resistance to brain insults (Goncalves et al., 2018; Jacqmain et al., 2014; van Praag et al., 2000; Young et al., 1999). Besides, EE produces changes in neuron morphology, myelination and synaptogenesis during development, adulthood and aging (Bennett et al., 1969; Diamond et al., 1976; Green et al., 1983; Greenough et al., 1986; Juraska and Kopcik, 1988).

In relation to MS models, studies to assess benefits of non-pharmacological interventions were mostly performed in an Experimental Autoimmune Encephalomyelitis (EAE) model. It was described that physical exercise attenuates the progression and pathological hallmarks of EAE, by inhibiting the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-17) in the spinal cord of mice (Souza et al., 2017). Additionally, EE improves remyelination by promoting adult neural progenitor mobilization in the EAE model (Fields, 2008; Magalon et al., 2007). Furthermore, EE enhances CD8+ cytotoxic T lymphocytes activity in secondary lymphoid tissues in EAE (Xiao et al., 2019).

In MS patients, there are no studies addressing the influence of the three EE factors (physical exercise, social and cognitive stimulation) on disease course. Many studies analyse the effect of a single component and very few studies the effect of the combination of two factors (Motl et al., 2016). For many years, motor rehabilitation and exercise has been considered as an approach to improve walking function, and cognitive rehabilitation as an approach to improve cognitive function, separately. However, combined rehabilitation interventions might simultaneously improve walking and cognition, perhaps due to cognitive motor pathways interaction. The combination of physical exercise and cognitive training was demonstrated to improve the cognitive and motor performance in MS patients (Barbarulo et al., 2018; Jimenez-Morales et al., 2017).

We hypothesize that a combined non-pharmacological treatment such as EE can have therapeutic effects on MS. The aim of the present work is to study the effect of EE on a cortical lesion induced by the long expression of IL-1β, further exacerbated by sustained peripheral inflammation. We have analyzed the influence of EE on cognitive impairment, anxiety-like symptoms, neuroinflammation, demyelination and neurodegeneration and found that EE have potent positive effects on all these parameters,

suggesting that EE could be envisaged as a candidate for non-pharmacological intervention in MS with cortical compromise.

2. Results

2.1. Effects of EE on peripheral blood of stimulated animals

To study the effect of EE on peripherally stimulated animals on the behaviour and the pathology of prefrontal cortices of 2 months old rats, adult rats were centrally injected with either the adenoviral vectors expressing human interleukin 1β gene (AdIL-1β) (IL-1β c) or the reporter gene β-galactosidase (Adβ-gal) as a control (β-gal c). Right after the surgery, the animals were randomly distributed in either enrichment environment or standard cage as described in materials and methods. Twenty-one days after the surgery, the animals were peripherally injected with either AdIL-1β or Adβ-gal and relocated in their previous environment (EE or SC). According to the experimental design, four experimental groups were generated: EE IL-1β c/IL-1 iv, EE IL-1β c/β-gal iv, SC IL-1β c/IL-1 iv and SC IL-1β c/β-gal iv. These four groups were analysed at 7 days after the peripheral injection (Fig.1A). As we previously demonstrated, no effect either in behavioural tests or neuroinflammation could be observed in β-gal c/ β-gal iv and β-gal c/IL-1β iv animals (Silva et al., 2018), therefore, we considered that these controls were not advantageous in this work.

All of the animal groups were periodically monitored, and no clinical symptoms, according to the standard (EAE) score, were observed, indicating that the welfare of the animals was consistent with the high standards of the ethical guidelines for animals and were acceptable compared to other MS models, especially EAE. Additionally, the experimental animals did not show signs of ongoing disease. They presented with normal fur, activity, movement, and food consumption

EE moderately attenuated the peripheral inflammatory response to the peripheral IL-1β stimulus, which could be observed as a lesser number of neutrophils in EE IL-1β c/IL-1β iv animals compared with SC IL-1β c/IL-1 iv animals (Fig. 1D). No effects were observed in IL-1β c/βgal iv animals. Concomitantly, the level of the inflammatory marker, mRNA IL-1β dramatically increased in SC IL-1β c/IL-1β iv. This increment significantly decreases in EE IL-1β c/IL-1β iv (Fig. 1 E), demonstrating that EE attenuated the peripheral inflammatory response. No significant increment of mRNA IL-1β was observed in the IL-1β c/β-gal iv rats, regarding housing condition (Fig. 1E).

2.2. EE attenuated the anxiety-like behaviour

To evaluate exploratory activity and anxiety-like symptoms, we performed the open field (OF) and elevated plus maze (EPM) tests. As a general observation, the EE animals were more relaxed and easier to handle. No differences could be found in IL-1β c/β-gal iv animals independently of the environment conditions when OF test was performed. As observed by this test, EE significantly increased the exploratory activity in EE IL-1β c/IL-1β iv compared with SC IL-1β c/IL-1β iv animals (Fig. 2 A-B). IL-1β c/IL-1β iv animals exhibited less anxiety-like symptoms when housed in EE cages when compared with IL-1β c/IL-1β iv rats housed in SC. This anxiety-related behaviour was

shown as a significant increase of the time spent in the center in the OF in EE IL-1β c/IL-1β iv animals compared with SC IL-1β c/IL-1β iv group (Fig 2 A). However, EE did not restore this parameter to the level of EE IL-1β c/βgal iv observed in the OF test. Additionally, the EE IL-1β c/IL-1β iv animals had statistically decreased number of faecal boli and more rearings compared with SC similar animals in EE (Fig. 2 C-D). No significant differences were found in the IL-1β c/β-gal iv animals in both EE and SC for these parameters. These results correlate with our previous work which demonstrated that animals with central IL-1β with no peripheral stimulation have no anxiety-like behavior (Silva et al., 2018).

The anxiety-like symptoms were also tested with the EPM test, which was described as a widely used behavioral assay for innate anxiety behavior of rodents. The EPM is based on rodents' predilection toward dark, closed spaces and an unconditioned fear of heights/open spaces (Walf and Frye, 2007). We used the EPM in a single session for assessing the behavior to this novel situation. Both the IL-1β c/IL-1β iv and β-gal iv group, located in EE exhibited statistically less anxiety-like behavior compared with the animals housed in SC demonstrated as more entries and more time spent in the open arms of the maze in EE animals (Fig 2 E-F). This result is in agreement with studies that affirm that the EPM is a test that has greater sensitivity for the anxiety investigation than the OF test (Walf and Frye, 2007). In particular, EE IL-1β c/IL-1β iv animals have significant more entries to the open arms compared with a similar group in SC. The same scenario was observed in EE IL-1β c/β-gal iv animals, in which the EE improved the entries to the open arms. The same situation could be described with the time in the open arms.

2.3. EE improved cognitive deficits

The EE clearly improves the performance in the NOR test, as evidenced by a significant increase in the time spent in the novel object in animals that were housed in EE compared with the animals housed in the SC. Additionally, DI was also significantly improved in the EE compared with SC (Fig. 3 A-B). Both measured parameters were statistically improved in both groups (IL-1β c/IL-1β iv and IL-1β c/β-gal iv) housed in EE, indicating an improvement in cognitive deficits. These data suggest that even though the EE improves the short term memory, it was not enough to restore it to naive rats' levels, which could indicate a permanent damage induced by cortical lesions.

2.4. Enrichment environment restored anhedonia

The preference to sucrose was significantly greater in the EE IL-1β c/IL-1β iv animals than in the control group located in the SC. However, there was no significant difference in sucrose preference between the IL-1β c/β-gal iv groups located in any environment (Fig. 3 C). The EE restored the sucrose consumption to control levels to IL-1β c/β-gal iv rats (Fig 3 C). This data indicated that peripheral stimulation in SC induced an anhedonic state, reflected by a reduction in sucrose consumption. This state was improved by the EE. All the groups with central injection of IL-1β have low levels of sucrose intake. Therefore, we performed the test in β-gal c/ β-gal iv and β-gal c/IL-1β ivin both conditions, EE or SC (see Suppl Fig.1). These groups have 90% of sucrose

preference demonstrating that the animals injected with IL-1β in the cortex experienced a basal depression state

2.5. Neuroinflammation and glial activation in EE

The long-term expression of IL-1β in the cortex induced inflammation characterized by the considerable recruitment of neutrophils and, to a reduced extent, macrophages as previously described in (Silva et al., 2018). As the lesions evolved, the inflammatory volume decreased and the infiltrate was modified and showed fewer neutrophils and an increasing number of macrophages. However, the peripheral stimulation flared up the neuroinflammation of the central lesion (Silva et al., 2018). As it was previously described, the inflammation was lower in control animals peripherally injected with Adβ-gal (Silva et al., 2018). Therefore, there was no influence of the environment in the development of these small lesions. The EE statistically diminished the inflammatory volume in IL-1β c/IL-1β iv animals compared with animals located in SC (Fig. 4 A-C), which demonstrate that the EE enhanced the evolution of these lesions towards a less inflammatory infiltrate.

Interestingly, EE statistically decreased the microglial activation as evidenced by a lesser number of major histocompatibility complex class II (MHC-II) and Anti CD68 (ED1) positive cells in the central lesions in EE IL-1β c/IL-1β iv animals compared with the animals located in SC (Fig. 4 D-I). ED1 demonstrated the phagocytic activity of microglia/macrophages. Additionally, EE IL-1β c/βgal iv animals presented central lesions with less activated microglia, demonstrating that EE diminishes the microglial activation in central lesions in animals with or without peripheral stimulation compared with the those located in SC (Fig 4 D-I). Furthermore, EE also decreased the astroglia activation, the animals situated in EE have a significantly decreased activation of astroglia in both studied groups (IL-1β c/IL-1β iv and IL-1β c/β-gal iv) (Fig. 4 J-L). Thus, the EE induced less astroglia activation in the cortical lesions of animals with or without peripheral stimulation compared with animals housed in SC.

2.6. EE decreased demyelination in exacerbated central lesions

The demyelination expected in this model was confirmed by myelin associate glycoprotein (MAG) and myelin basic protein (MBP) immunofluorescence (Fig. 5). The animals located in SC exhibited a statistically increment in demyelination, in both groups (SC IL-1β c/IL-1β iv and SC IL-1β c/β-gal iv), when compared with the animals housed in EE. Therefore, the EE either improved the remyelination or decreased demyelination in both EE IL-1β c/IL-1β iv and EE IL-1β c/β-gal iv animals (Fig. 5 A-F).

2.7. EE prevented neuronal loss

To analyse the presence of neurodegenerationin the lesions we quantified FJC-positive cells, the NeuN-positive cells (Fig. 6).

Fluorojade C (FJC) intensity statistically decreased in the animals located in EE, demonstrated that there is less neuronal loss than that observed in animals located in SC in both studied groups (SC IL-1β c/IL-1β iv and SC IL-1β c/β-gal iv) (Fig. 6 A-C). We

also found that the number of NeuN-positive cells statistically decreased in the animals located in SC, demonstrating a lower number of healthy neurons in these animals compared with the animals located in EE (Fig. 6 D-F). Therefore, the EE diminished the neuronal loss in both studied groups.

2.8. Pro- and anti-inflammatory molecules expression in the central lesion

We examined the effect of EE on the expression of pro- and anti-inflammatory molecules in the cortex. As we previously described, we observed an upregulation of the mRNA expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in the ipsilateral cortex (Silva et al., 2018). The EE statistically diminished the expression of IL-1β, TNF-α and IL-6 in both EE IL-1β c/IL-1β iv and EE IL-1β c/β-gal iv control groups. As expected, the IL-1β c/IL-1β iv animals presented a statistically upregulated expression of IL-1 and IL-6 compared with the IL-1β c/β-gal iv group in both EE and SC (Fig. 7 A-C).

It was previously described that, the EE induced the expression of anti-inflammatory molecules, which could in turn collaborate with the enhanced performance in the behavioural tests in the animals exposed to EE (Briones et al., 2013). Therefore, we analyzed the expression of BDNF and TGF-β. Animals exposed to the EE had an increased expression of TGF-β and BDNF in both IL-1β c/IL-1β iv and IL-1β c/β-gal iv groups. The EE IL-1β c/β-gal iv group presented a significantly higher expression of both TGF-β and BDNF compared with IL-1β c/IL-1β iv animals, probably because of the absence of peripheral stimulation (Fig 7 D, F). In concordance with TGF-β expression, we evaluated the mRNA expression of Arginase-1 (Arg-1), as another marker for the M2 phenotype of alternatively activated macrophages. EE statistically upregulated the central expression of Arg-1 in both groups, EE IL-1β c/IL-1β iv and EE IL-1β c/β-gal iv, compared with the same groups located in SC (Fig. 7 E). Taken together, these results suggest that housing rats in an EE modulate neuroinflammation by increasing the expression of anti-inflammatory cytokines and neurotrophic factors and downregulating the mRNA expression of pro-inflammatory ones.

3. Discussion

In this study we found that EE: 1) modulates neuroinflammation by reducing the expression of pro-inflammatory cytokines and enhancing the expression of anti-inflammatory ones, 2) reduces cognitive deficits and anxiety-like symptoms, 3) reduces the peripheral inflammatory response to the stimulus and 4) modulates neurodegeneration, demyelination and glial activation. EE has demonstrated beneficial effects in cognitive behavior, such as memory and learning and improving anxiety-like symptoms, brain structure and function in both healthy and models of neurodegenerative diseases animals (Bruel-Jungerman et al., 2005; De Rosa et al., 2006; Fischer, 2016; Frasca et al., 2013; Jurgens and Johnson, 2012; Kotloski and Sutula, 2015; Larsson et al., 2002; Leggio et al., 2005; Pusic and Kraig, 2014; Sampedro-Piquero and Begega, 2017; Schloesser et al., 2010). In particular, many publications studied the effect of EE in animal models of neurodegenerative diseases, such as Alzheimer, Parkinson, Huntington, amyotrophic lateral sclerosis (Jungling et al., 2017; Kempermann, 2019;

Nithianantharajah and Hannan, 2006; Skillings et al., 2014; Stam et al., 2008; Xu et al., 2016).

Immune peripheral molecules were demonstrated to have an impact in MS models (Murta et al., 2015). We demonstrated that EE reduces the response to systemic stimulation shown as a diminished number of peripheral neutrophils and IL-1β mRNA levels compared with SC IL-1β c/β-gal iv animals. Decreased levels of pro-inflammatory molecules in serum was previously described in a model of Alzheimer disease. This model demonstrated an improved cognitive performance in EE animals (Zhang et al., 2018). EE helped to ameliorate the negative cognitive effects of peripheral infection (Jurgens and Johnson, 2012). In addition, it was demonstrated that EE reduced hippocampal neuroinflammation and improved cognitive performance in a mouse model of peripheral infection with influenza virus when compared to infected mice in standard housing (Jurgens and Johnson, 2012). EE provided beneficial effects on the immune function (Pusic et al., 2016). Additionally, physical exercise attenuated the symptoms of EAE in mice by inhibiting both adaptive and innate immune response, reduction of the oxidative damage, and reestablisment of the brain blood barrier (BBB) (Souza et al., 2017). One study demonstrated that EE improved chemotaxis of phagocytes and lymphocytes and reduces the oxidative stress in immune cells, which strengthened the responses to infection (Arranz et al., 2010). Ultimately, it was demonstrated that EE, in EAE model, modulates thymus and thymocyte development inducing an enhanced activity of CD8+ cytotoxic T lymphocytes in secondary lymphoid tissues (Xiao et al., 2019). This work provides additional evidence that EE could exert immunomodulatory effects.

EE reduced anxiety-like symptoms and enhance cognitive impairment in comparison with animals located in SC. We described that EE reduced the time in the center of the OF along with increasing rearing and decreasing faecal boli. The same situation was previously described revealing that EE reduces the emotional reactivity with a decrease in the levels of freezing and defecation (Larsson et al., 2002). It was described that EE was able to reduce the levels of trait anxiety in adult rodents (Hendershott et al., 2016; Rogers et al., 2017; Sampedro-Piquero and Begega, 2017). We found that EE increases the exploratory behaviour, such as locomotion and rearing, demonstrating that EE animals invested more time in exploring unfamiliar stimuli, which reflects low levels of anxiety (Casarrubea et al., 2013). Also, we described an anti-anhedonic effect in EE animals, which correlated with the induction of BDNF expression in the cortical lesions. In addition, these results are in accordance with previous results showing that EE had an anti-anhedonic effect via induction of BDNF in the prefrontal cortex during the early life (Angelucci et al., 2005; Chu et al., 2018; Yu and Chen, 2011). Furthermore, MS patients that received and integrated cognitive and motor rehabilitation presented positive effects in emotional aspects such as depression in addition to cognitive and motor rehabilitation (Barbarulo et al., 2018; Jimenez-Morales et al., 2017). In addition, in this study, EE enhanced short term memory as demonstrated by improvement of the performance in the NOR test. EE was demonstrated to be a useful tool to improve cognitive function and reduce anxiety-like behaviour in many models of neurodegenerative diseases (Sampedro-Piquero and Begega, 2017). Many authors described that EE animals performed better in the NOR test compared to those animals that were housed in SC (Diniz et al., 2016;

Sampedro-Piquero and Begega, 2017). . Related to MS patients, it was demonstrated that physical exercise and cognitive rehabilitation enhanced cognitive function in patients (Barbarulo et al., 2018; Jimenez-Morales et al., 2017). This work provides evidence that EE could be regarded as an intervention to improve cognitive function and reduce anxiety-like behaviour.

As an advantage, the animal model used in this study and its response to EE can be useful for analysing cognitive deficits and anxiety-like behaviour, without the influence of the motor impairments that characterize EAE, which makes this model not appropriate for testing the symptoms of cortical damage and its behavioural consequences as it was described in Aharoni et al., 2019.

EE reduced cortical neuroinflammation, glial activation and demyelination. In accordance with our results, it was described that chronic exercise reduced the cellular infiltration in the CNS, along with a delay in the onset of disease and a decrease in the demyelination volume in a model of EAE (Pryor et al., 2015). Furthermore, EE induced white matter remyelination both in EAE and lysolecithin (LPC) models (Magalon et al., 2007). EE also reduced neuroinflammation in the cortex of an animal model of mild traumatic brain injury, thus improving cognitive function (Briones et al., 2013). Even though, EE attenuated neuroinflammation in the hippocampus, it had no effect on the cortex in animals peripherally stimulated with lipopolysaccharide (Chabry et al., 2015; Jurgens and Johnson, 2012; Williamson et al., 2012). On the contrary, our results demonstrated that EE impacted on the cortex reducing neuroinflammation and glial activation. The reduction of glial activation by EE was previously demonstrated in several animal models such as, stroke and Alzheimer´s disease and decrease astroglia activation in a model of chronic cerebral hypoperfusion (Hase et al., 2017; Quattromani et al., 2014; Ziegler-Waldkirch et al., 2018). On the other hand, it was described that EE promoted astrogliosis in the peri-infarct neocortex in an ischemic stroke model (Komitova et al., 2006). Therefore, this study provides evidence of a non-pharmacological intervention affecting crucial parameters of MS.

EE attenuated neurodegeneration in cortical lesions. Similar results were described in an EAE model in which chronic exercise, but not EE, reduces motor neuron degeneration and axonal loss (Pryor et al., 2015). Neuroprotection triggered by EE was demonstrated in a model of ischemic stroke by reducing the expression of IL-1β and increasing the expression of GFAP (Goncalves et al., 2018). EE promoted neurogenesis in the hippocampus (Brown et al., 2003; van Praag et al., 2000) and viceversa, social isolation decreased neurogenesis (Leasure and Decker, 2009; Stranahan et al., 2006). As a conclusion, our data support the notion that EE can have neuroprotective effects.

In our model EE improved the remyelination or/and decreased the demyelination rate demonstrated by an increment of myelin density. The mechanisms involved in this phenomena require further experiments. The fact that EE increases production of myelin at all ages, and lessens injury from neurodegenerative disorders including demyelinating disease was described by several authors (Fields, 2008; Pryor et al., 2015; Pusic and Kraig, 2014; Pusic et al., 2016). Additionally, it was demonstrated that EE promoted the mobilization of neural progenitor cells in two different demyelination models (EAE and focal LPC) (Magalon et al., 2007). Several studies have demonstrated that voluntary exercise influenced the development of oligodendrocyte lineage cells in cortical and white matter regions (Mandyam et al., 2007; McKenzie et al., 2014; Tomlinson et al.,

2016). Therefore, our results support the idea that EE is beneficial for remyelination or can decreased demyeliation.

We found that EE modulated the neuroinflammation to the detriment of pro-inflammatory cytokine expression and by increasing the expression of anti-inflammatory molecules. Our data demonstrates that EE dampened IL-1β, TNF-α and IL-6 production in central lesions. At the same time, we evidenced an increment of anti-inflammatory molecules, such as TGF-β, in addition to BDNF and Arg-1. Our data support previous work showing that EE elevated the levels of neurotrophic factors, specially BDNF (Bennett et al., 1969; Greenough and Volkmar, 1973; Ickes et al., 2000; Jha et al., 2011; Kempermann et al., 1997; Pham et al., 2002; van Praag et al., 2000). EE was previously described to reduce the levels of IL-1β and TNF-α in a model of traumatic brain injury, as well as increase the expression of IL-10, concomitantly helping to improve cognitive impairment (Briones et al., 2013). Accordingly, it was demonstrated that EE attenuates influenza virus-induced hippocampal neuroinflammation through decreasing the expression of IL-1β and TNF-α levels with no changes in IL-6 expression, with concomitant increase in the expression of hippocampal BDNF (Jurgens and Johnson, 2012). Also, it was described that Arg-1 positive-microglia were involved in Aβ plaque reduction in a model of Alzheimer´s disease induced by IL-1β expression (Cherry et al., 2015). In addition, the levels of Arg-1 were demonstrated to modulate neuroinflammation in EAE (Ahn et al., 2012). Finally, the expression of Arg-1 and TGF-β was demonstrated to shift microglia/macrophages towards a M2 phenotype (Pusic et al., 2014). Taken all these data together, we believe that our data showed a probable shift to an M2 phenotype by EE.

4. Conclusion

This is the first study that evaluates the effect of EE on a cortical MS animal model, since most of the available studies are focused on white matter. The present study provides evidences that non pharmacological therapies, such as EE presents beneficial effects both at behavioural and cellular levels in a rat model of cortical pathology that represents most of the characteristics of the progressive forms of MS. Effectiveness research in these areas is especially important considering the common and burdensome nature of MS-related cognitive impairment that is poorly-managed by pharmacotherapy (Sandroff and DeLuca, 2019). In this study, we evaluated the effect of EE on cortical pathology for 28 days. Further studies will be necessary to determine the effect of chronic exposure to EE. In addition, these experiments will be useful to estimate the impact of the EE over time. The study of the duration of EE exposure is also a limited in MS patients, because most studies were focused on assessing the short-term benefits of exercise and cognitive rehabilitation and with methodological limitations (Motl et al., 2016; Sandroff and DeLuca, 2019).

Combined and integrative rehabilitation interventions such as EE are currently recommended (Sandroff and DeLuca, 2019; Sumowski et al., 2018) and could be considered as a non-invasive complementary tool to the pharmacological treatment of immune-mediated diseases such as MS. Treatment for impaired cognition in MS patients will require a multifaceted approach including not only behavioural and pharmacological therapies, but also keeping in mind treatment of the whole person which must include

issues such as emotional functions, family relations, personality and a host of real life factors which affect us all (Sandroff and DeLuca, 2019).

Exposure to EE has demonstrated to improve the course of aging as well as neurodegenerative diseases including traumatic brain injury, perinatal asphyxia, Huntington's, Parkinson´s and Alzheimer's diseases (Birch and Kelly, 2019; Fischer, 2016; Frasca et al., 2013; Galeano et al., 2014; Goncalves et al., 2018; Jungling et al., 2017; Mo et al., 2015; Pang and Hannan, 2013; Ziegler-Waldkirch et al., 2018). Recent studies using animal models of Huntington's disease (Mo et al., 2015; Steventon et al., 2015), Alzheimer's disease (Prado Lima et al., 2018; Salmin et al., 2017) and Parkinson's disease (Jungling et al., 2017) provide increasing evidence of neuroprotection in these pathologies with behavioural, cellular and molecular changes induced by EE. Thus, environmental factors could impact on the function of the immune system within the brain and may represent a non-pharmacological agent that could be useful as a complementary tool to the pharmacological treatment used in MS and other neurological diseases.

5. Experimental procedures

5.1. Adenoviral vectors

Adenoviral vectors expressing the human interleukin 1β gene (AdIL-1β) or the reporter gene β-galactosidase (Adβ-gal) as a control were generated as described previously (Ferrari et al., 2004). Stocks were obtained by large scale amplification in HEK293 cells, purified in double caesium chloride gradients and quantified by the plaque assay (final titters: Adβ-gal = 8.41. 10^{11} infective particles/ml; AdIL-1β = 1.1. 10^{11} infective particles/ml). Viral stocks were free of autoreplicative particles as assessed by PCR. Ad-βgal was kindly provided by Dr. J. Mallet (Hospital Pitie Salpetriere, Paris, France).

5.2. Animals and injections

Adult male Wistar rats (2 months old) (Jackson Laboratory, Bar Harbor, Maine, USA), bred for several generations in the Leloir Institute Foundation and in Institute of Translational Medicine and Biomedical Engineerie of the Italian Hospital (IMTIB)'s animal facility, were used in all experiments. Animals were housed under controlled temperature conditions (22 °C ± 2 °C), with food and water provided *ad libitum* and a 12:12 dark:light cycle with lights on at 08.00 h. All surgical procedures and euthanasia were conducted in full compliance with NIH and internal Leloir Institute Foundation and Italian Hospital guidelines and approved by the Institutional Review Board "Cuidado y Uso de Animales de Laboratorio (CICUAL-FIL)" and "Comité de Ética y Protocolos de Investigación Hospital Italiano de Buenos Aires (CEPI- HIBA)", respectively.

For stereotaxic injections, animals were anesthetized with ketamine chlorhydrate (80 mg/kg) and xylazine (8 mg/kg). Adenoviral vectors were delivered with a finely drawn graduated capillary into the left prefrontal cortex (bregma, +1.6 mm; lateral, +2.5 mm; ventral, - 1.6 mm) (Paxinos and Watson, 1986). AdIL-1β and Adβ-gal expressing vectors were diluted in sterile Tris-HCl buffer (Tris-HCl 10 mM, MgCl2 1 mM, pH 7.8)

to a concentration of 1 x10^6 infective particles/µl. Previous work published by our laboratory (Silva and Ferrari, 2018) have shown that this is the minimum dose of both AdIL-1β and Adβ-gal that provides long-term expression of the transgenes with a minimum inflammatory response to the control vector. The volume of the intracortical injection was 1 µl, which was infused over a 5-minute period, with the capillary left in place for another minute to minimize reflux. All surgical procedures were conducted in the morning to avoid possible circadian variation in cytokine expression.

For peripheral injections, animals were briefly anesthetized with isoflurane, and AdIL-1β or Adβ-gal were injected intravenously (iv) at doses of 1.36 x 10^9 infective particles/rat. The final volume of injection was 600 µl: 300 µl for the adenovector solution (in sterile 10 mM Tris–HCl, 1 mM MgCl2, pH = 7.8), followed by 300 µl of saline solution. All iv administrations were performed between 2 pm and 5 pm. The adenoviruses were administered in the periphery at day 21 after injection of adenovirus in the cortex. Five days after the iv injection, a blood smear was performed to confirm the effectiveness of the pro-inflammatory systemic stimulus according to (Murta et al., 2015; Silva et al., 2018). A significant increase in the number of peripheral blood neutrophils in AdIL-1β intravenously injected (IL-1β iv) animals, along with a consequent decrease in the lymphocyte population, was checked as a sign of inflammation. For all experiments in this study, AdIL-1β iv-mediated changes in the blood formula were significantly different from those observed in Adβgal iv-injected animals. Animals were sacrificed 7 days after the systemic stimuli, and their brains were processed for histological and molecular analyses (Fig. 1A).

5.3. Enrichment environmental cages

The EE paradigm provides elements of the three categories of enrichment: 1. Social enrichment, subjects are housed in larger groups of animals compared with standard cages, 2. Cognitive enrichment, which includes exposure to novel stimulation and experiential learning (toys and tunnels). 3. Physical enrichment, consisting of voluntary exercise (access to running wheels). Animals were randomly assigned to the environmental enrichment (EE, n=7–8/cage) or standard cages (SC, n=3-4/cage) conditions after the surgery. Briefly, the enriched environment consisted of a large cage (100 cm x 50 cm x 50 cm) equipped with toys, tunnels, ladders, housing chambers, nesting material and 2 running wheels per cage (Fig. 1B). The toys were changed twice a week. The standard cages consisted of a laboratory polypropylene cage with bedding (55 cm x 33 cm x 20 cm) (Fig. 1C).

5.4. Behavioural Tests

5.4.1. Open field

Spontaneous locomotor activity and anxiety-like behaviour were quantified in rats (n= 10-12/group) using an OF arena, a wooden box of 1 mt x 1 mt with a floor divided into 16 squares. Line crossings, rears and climbing were scored for 5 min. A line crossing was counted when all four paws crossed the square lines. Rearing was scored when the

animals raised both front paws from the floor. Time spent in both central and peripheral squares was also measured. Faecal boli were also counted (Prut and Belzung, 2003).

5.4.2. Novel object recognition test

To evaluate short-term memory, we performed the novel object recognition test (NOR). Each rat (n= 10-12/group) was placed in the arena for 5 minutes with two identical objects. After 2 hours, the rat was re-introduced into the arena, with a new object replacing one of the two previous ones for 3 minutes. The time (measured in seconds) that the animal came in contact with the novel and familiar objects with its nose was recorded and quantified as the novel object exploration time and discrimination index (DI). The DI was calculated as described previously (Antunes and Biala, 2012):

DI= (Time in novel object-Time in familiar object)/(Time in novel object+Time in the familiar object)

Combinations of the objects (novel/familiar) and their location differed among the animals such that right-left preference could be avoided.

5.4.3. Sucrose preference test

The sucrose preference test (SPT) was used for the assessment of anhedonia. The rats were located in groups of five per cage and were freely allowed to consume 2% sucrose for 12 hours. Then, the rats were caged singly with two bottles, one with tap water and the other one with 2% sucrose for 24 hours. The position of bottles was changed every 12 hours. The sucrose preference rate was calculated as sucrose consumption/total liquid consumption x 100%. The test was performed the day before the sacrifice.

5.4.4. Elevated Plus Maze

The EPM was constructed from pine board and painted black. It consisted of two opposing open arms (length:40cm, width:9cm) and two opposing wall-enclosed arms (wall height:15cm) extending off of a centre square (9cm per side) to form a plus shape. The maze was placed on a pedestal (70 cm high) in the center of a rectangular room (3 x 3.7 meters). A video camera was hung from the ceiling directly above the center of the maze. The test was performed the day of the sacrifice (see timeline Fig.1). We used the EPM in a single session for assessing the behaviour to this novel situation. Animals were located in the testing room 1 hour before performing the test. Animals were placed in the center of the EPM facing one of the open arms and then the experimenter left the room. The animals´ behaviour was recorded for 5 minutes. The maze was cleaned with 70% alcohol between trials. Time spend on each arm and the entries were counted.

5.5. Histology

The animals (n= 8–10/group) were deeply anaesthetized as previously described (Murta et al., 2015) and transcardially perfused with heparinized saline, followed by ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB) with a pH of 7.2. Brains were dissected and placed in the same fixative solution overnight at 4 °C and cryoprotected in

30% sucrose in 0.1 M PB solution. Then, the brains were frozen in isopentane and cut using a cryostat into 40um serial coronal sections through the left prefrontal cortex. Sections were mounted on gelatine-coated slides and stained with Cresyl Violet to assess the general nervous tissue integrity and inflammation. For immunohistochemistry, sections were stored in cryoprotective solution at -20°C until needed. To analyse neurodegeneration, we used Fluorojade C (FJC) histochemistry (Millipore, Burlington, MA) according to manufacturer instructions.

5.6. Immunohistochemistry

Free-floating sections were rinsed in 0.1% Triton in 0.1 M PB, blocked in 1% donkey serum for 45 min, and then incubated overnight at 4 °C with primary antibodies diluted in blocking solution. The list of antibodies used is provided in Table 1. After three 10-min washes with 0.1 M PB, the sections were incubated with indocarbocyanine (Cy3) or cyanine Cy2 (Cy2)-conjugated donkey anti-rabbit or anti-mouse antibody, respectively (1:250; Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA) for 2 h at room temperature, rinsed in 0.1 M PB and mounted in Mowiol (Calbiochem, San Diego, CA, USA). Digital images were obtained in a Zeiss LSM 510 laser scanning confocal microscope equipped with a krypton-argon laser.

5.7. Quantitative analysis

The lesion volume was calculated using one every sixth 40μmthick serial section of the entire ipsilateral left prefrontal cortex, according to the anatomical landmarks defined in Paxinos and Watson (Paxinos and Watson, 1986). Photographs of all the sections were obtained using Nikon Eclipse E600 microscope and a CX900 camera (Microbright Field Inc., USA) under 4x magnification objective. The area of one set of serial sections was used for inflammation analysis (Cresyl Violet), The inflammatory was manually delineated and measured using Image J software (Media Cybernetics, Silver Spring, MD). All the measurements were done in a blind manner. The inflammatory average area (IA) was calculated for each animal, and the average volume of inflammation (IV) was estimated by multiplying the IA by the section's width (0.04 mm), and the total number of sections obtained for the whole left prefrontal cortex. For MHC II, NeuN and ED1 positive cells quantification, the 20x objective was used and one picture of the ipsilateral cortex was taken from each section. Laser power, gain and offset conditions were maintained constant for the acquisition of all images. Approximately 12 fields were quantified for each animal using the Zeiss LSM Image Browser. The total number of positive cells was normalized to the total area counted for each animal. In order to quantify GFAP, MAG, MBP and Fluorojade C (FJC) intensity, pictures of the ipsilateral cortex from each section were taken using the 20x objective in the same conditions of laser power, gain and offset, and the intensity was calculated using Image J software as described for MHC II.

5.8. RNA isolation, reverse transcription, and real-time PCR

The animals (n= 5–6/group) were decapitated, their brains quickly removed, and the prefrontal cortex pieces were dissected, snap-frozen in liquid nitrogen, and stored at 80 °C. Brain RNA was prepared by homogenizing the tissue using the GenElute Mammalian Total RNA Miniprep kit (Sigma, St. Louis, MO). Samples were treated with DNAase I to eliminate possible DNA contamination (On- Column DNase I Digestion, Sigma). Blood RNA was prepared by homogenizing 1 ml of blood using the Mouse RiboPure™Blood RNA Isolation Kit (Sigma, St. Louis, MO). Samples were treated with DNAase to eliminate possible DNA contamination (DNA-*free*™ DNA Removal Kit, Sigma). RNA was quantified with a Nanodrop (Nanodrop Technologies, Wilmington, DE, USA), and 0.2 µg of total RNA were reverse-transcribed according to the manufacturer's protocol (Superscript II, Invitrogen, Life Technologies, Carlsbad, CA, USA) for brain tissue and 5 µg for blood samples, using oligo-dT primers. As a control for genomic contamination, a sample without reverse transcriptase was included in each PCR analysis. Relative quantification was performed by real-time PCR using the SYBR-green I fluorescence method and ROX as a passive reference dye. Stratagene MxPro TM QPCR Software and Stratagene Mx3005P equipment were used (Agilent Technologies, Santa Clara, CA, USA). TATA box binding protein (TBP) was used as a housekeeping gene because its expression is not altered by the treatment. All of the samples were assessed in triplicate. Specificity was controlled by melting curves and agarose gels. To calculate the efficiency, LinReg PCR was used (Cikos et al., 2007). The primers for the studied cytokines and neurotrophins are in Table 2.

5.9. Statistical analysis

Results are expressed as mean ± SEM. All experiments were analysed by parametric two-way ANOVA followed by Tukey's multiple comparison test as post-hoc test. Variables were tested for normality (Kolmogorov–Smirnov) and variance homogeneity (Bartlett's test) (Scheirer et al., 1976). The level of statistical significance was set at $p<0.05$. For clarity, statistical analyses of each test are addressed in each figure legend. Statistical tests were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, California, USA).

Author contribution

BAS and CCF conceived and planned the experiments, BAS, CF, MCL performed the experiments. BE helped with the molecular biology techniques. MF did the histological work. PG contributed to the interpretation of the behavioural results. FJP contributed to critical revision of the manuscript. BAS and CF wrote the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

Ahn, M., et al., 2012. Immunohistochemical study of arginase-1 in the spinal cords of Lewis rats with experimental autoimmune encephalomyelitis. Brain Res. 1453**,** 77-86.

Amato, M.P., et al., 2013. Treatment of cognitive impairment in multiple sclerosis: position paper. J Neurol. 260**,** 1452-68.

Angelucci, F., Brene, S., Mathe, A.A., 2005. BDNF in schizophrenia, depression and corresponding animal models. Mol Psychiatry. 10**,** 345-52.

Antunes, M., Biala, G., 2012. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process. 13**,** 93-110.

Arranz, L., et al., 2010. Environmental enrichment improves age-related immune system impairment: long-term exposure since adulthood increases life span in mice. Rejuvenation Res. 13**,** 415-28.

Barbarulo, A.M., et al., 2018. Integrated Cognitive and Neuromotor Rehabilitation in Multiple Sclerosis: A Pragmatic Study. Front Behav Neurosci. 12**,** 196.

Bennett, E.L., Rosenzweig, M.R., Diamond, M.C., 1969. Rat brain: effects of environmental enrichment on wet and dry weights. Science. 163**,** 825-6.

Birch, A.M., Kelly, A.M., 2019. Lifelong environmental enrichment in the absence of exercise protects the brain from age-related cognitive decline. Neuropharmacology. 145**,** 59-74.

Briones, T.L., Woods, J., Rogozinska, M., 2013. Decreased neuroinflammation and increased brain energy homeostasis following environmental enrichment after mild traumatic brain injury is associated with improvement in cognitive function. Acta Neuropathol Commun. 1**,** 57.

Brown, J., et al., 2003. Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. Eur J Neurosci. 17**,** 2042-6.

Bruel-Jungerman, E., Laroche, S., Rampon, C., 2005. New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. Eur J Neurosci. 21**,** 513-21.

Calabrese, M., et al., 2012. Cortical lesion load associates with progression of disability in multiple sclerosis. Brain. 135**,** 2952-61.

Calabrese, M., et al., 2013. Grey matter lesions in MS: from histology to clinical implications. Prion. 7**,** 20-7.

Calabrese, M., Castellaro, M., 2017. Cortical Gray Matter MR Imaging in Multiple Sclerosis. Neuroimaging Clin N Am. 27**,** 301-312.

Casarrubea, M., et al., 2013. Temporal structure of the rat's behavior in elevated plus maze test. Behav Brain Res. 237**,** 290-9.

Cikos, S., Bukovska, A., Koppel, J., 2007. Relative quantification of mRNA: comparison of methods currently used for real-time PCR data analysis. BMC Mol Biol. 8**,** 113.

Chabry, J., et al., 2015. Enriched environment decreases microglia and brain macrophages inflammatory phenotypes through adiponectin-dependent mechanisms: Relevance to depressive-like behavior. Brain Behav Immun. 50**,** 275-287.

Cherry, J.D., Olschowka, J.A., O'Banion, M.K., 2015. Arginase 1+ microglia reduce Abeta plaque deposition during IL-1beta-dependent neuroinflammation. J Neuroinflammation. 12**,** 203.

Chu, F., et al., 2018. The roles of macrophages and microglia in multiple sclerosis and experimental autoimmune encephalomyelitis. J Neuroimmunol. 318**,** 1-7.

De Rosa, V., et al., 2006. Leptin neutralization interferes with pathogenic T cell autoreactivity in autoimmune encephalomyelitis. J Clin Invest. 116**,** 447-55.

Diamond, M.C., et al., 1976. Effects of environment on morphology of rat cerebral cortex and hippocampus. J Neurobiol. 7**,** 75-85.

Diniz, D.G., et al., 2016. Age, environment, object recognition and morphological diversity of GFAP-immunolabeled astrocytes. Behav Brain Funct. 12**,** 28.

Ferrari, C.C., et al., 2004. Reversible Demyelination, Blood-Brain Barrier Breakdown, and Pronounced Neutrophil Recruitment Induced by Chronic IL-1 Expression in the Brain. Am J Pathol. 165**,** 1827-37.

Fields, R.D., 2008. White matter in learning, cognition and psychiatric disorders. Trends Neurosci. 31**,** 361-70.

Fischer, A., 2016. Environmental enrichment as a method to improve cognitive function. What can we learn from animal models? Neuroimage. 131**,** 42-7.

Frampton, J.E., 2017. Ocrelizumab: First Global Approval. Drugs. 77**,** 1035-1041.

Frasca, D., et al., 2013. Traumatic brain injury and post-acute decline: what role does environmental enrichment play? A scoping review. Front Hum Neurosci. 7**,** 31.

Galeano, P., et al., 2014. Life-long environmental enrichment counteracts spatial learning, reference and working memory deficits in middle-aged rats subjected to perinatal asphyxia. Front Behav Neurosci. 8**,** 406.

Gardner, C., et al., 2013. Cortical grey matter demyelination can be induced by elevated pro-inflammatory cytokines in the subarachnoid space of MOG-immunized rats. Brain. 136**,** 3596-608.

Goncalves, L.V., et al., 2018. Environmental enrichment cognitive neuroprotection in an experimental model of cerebral ischemia: biochemical and molecular aspects. Behav Brain Res. 348**,** 171-183.

Green, E.J., Greenough, W.T., Schlumpf, B.E., 1983. Effects of complex or isolated environments on cortical dendrites of middle-aged rats. Brain Res. 264**,** 233-40.

Greenough, W.T., Volkmar, F.R., 1973. Pattern of dendritic branching in occipital cortex of rats reared in complex environments. Exp Neurol. 40**,** 491-504.

Greenough, W.T., et al., 1986. Environmental conditions modulate degeneration and new dendrite growth in cerebellum of senescent rats. Brain Res. 380**,** 136-43.

Hase, Y., et al., 2017. Effects of environmental enrichment on white matter glial responses in a mouse model of chronic cerebral hypoperfusion. J Neuroinflammation. 14**,** 81.

Hendershott, T.R., et al., 2016. Effects of environmental enrichment on anxiety-like behavior, sociability, sensory gating, and spatial learning in male and female C57BL/6J mice. Behav Brain Res. 314**,** 215-25.

Ickes, B.R., et al., 2000. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. Exp Neurol. 164**,** 45-52.

Jacqmain, J., et al., 2014. Pre and post-injury environmental enrichment effects functional recovery following medial frontal cortical contusion injury in rats. Behav Brain Res. 275**,** 201-11.

Jha, S., Dong, B., Sakata, K., 2011. Enriched environment treatment reverses depression-like behavior and restores reduced hippocampal neurogenesis and protein levels of brain-derived neurotrophic factor in mice lacking its expression through promoter IV. Transl Psychiatry. 1**,** e40.

Jimenez-Morales, R.M., et al., 2017. [Cognitive training combined with aerobic exercises in multiple sclerosis patients: a pilot study]. Rev Neurol. 64**,** 489-495.

Jungling, A., et al., 2017. Effects of Postnatal Enriched Environment in a Model of Parkinson's Disease in Adult Rats. Int J Mol Sci. 18.

Juraska, J.M., Kopcik, J.R., 1988. Sex and environmental influences on the size and ultrastructure of the rat corpus callosum. Brain Res. 450**,** 1-8.

Jurgens, H.A., Johnson, R.W., 2012. Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection. Brain Behav Immun. 26**,** 1006-16.

Kempermann, G., Kuhn, H.G., Gage, F.H., 1997. More hippocampal neurons in adult mice living in an enriched environment. Nature. 386**,** 493-5.

Kempermann, G., 2019. Environmental enrichment, new neurons and the neurobiology of individuality. Nat Rev Neurosci. 20**,** 235-245.

Komitova, M., et al., 2006. Enriched environment after focal cortical ischemia enhances the generation of astroglia and NG2 positive polydendrocytes in adult rat neocortex. Exp Neurol. 199**,** 113-21.

Kotloski, R.J., Sutula, T.P., 2015. Environmental enrichment: evidence for an unexpected therapeutic influence. Exp Neurol. 264**,** 121-6.

Lagumersindez-Denis, N., et al., 2017. Differential contribution of immune effector mechanisms to cortical demyelination in multiple sclerosis. Acta Neuropathol. 134**,** 15-34.

Larsson, F., Winblad, B., Mohammed, A.H., 2002. Psychological stress and environmental adaptation in enriched vs. impoverished housed rats. Pharmacol Biochem Behav. 73**,** 193-207.

Leasure, J.L., Decker, L., 2009. Social isolation prevents exercise-induced proliferation of hippocampal progenitor cells in female rats. Hippocampus. 19**,** 907-12.

Leggio, M.G., et al., 2005. Environmental enrichment promotes improved spatial abilities and enhanced dendritic growth in the rat. Behav Brain Res. 163**,** 78-90.

Lublin, F.D., et al., 2014. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology. 83**,** 278-86.

Magalon, K., et al., 2007. Enriched environment promotes adult neural progenitor cell mobilization in mouse demyelination models. Eur J Neurosci. 25**,** 761-71.

Mandyam, C.D., et al., 2007. Methamphetamine self-administration and voluntary exercise have opposing effects on medial prefrontal cortex gliogenesis. J Neurosci. 27**,** 11442-50.

McKenzie, I.A., et al., 2014. Motor skill learning requires active central myelination. Science. 346**,** 318-22.

Merkler, D., et al., 2006. Differential macrophage/microglia activation in neocortical EAE lesions in the marmoset monkey. Brain Pathol. 16**,** 117-23.

Mo, C., Hannan, A.J., Renoir, T., 2015. Environmental factors as modulators of neurodegeneration: insights from gene-environment interactions in Huntington's disease. Neurosci Biobehav Rev. 52**,** 178-92.

Motl, R.W., Sandroff, B.M., DeLuca, J., 2016. Exercise Training and Cognitive Rehabilitation: A Symbiotic Approach for Rehabilitating Walking and Cognitive Functions in Multiple Sclerosis? Neurorehabil Neural Repair. 30**,** 499-511.

Murta, V., et al., 2015. Chronic systemic IL-1beta exacerbates central neuroinflammation independently of the blood-brain barrier integrity. J Neuroimmunol. 278**,** 30-43.

Nelson, F., et al., 2011. Intracortical lesions by 3T magnetic resonance imaging and correlation with cognitive impairment in multiple sclerosis. Mult Scler. 17**,** 1122-9.

Nithianantharajah, J., Hannan, A.J., 2006. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci. 7**,** 697-709.

Pang, T.Y., Hannan, A.J., 2013. Enhancement of cognitive function in models of brain disease through environmental enrichment and physical activity. Neuropharmacology. 64**,** 515-28.

Paxinos, G., Watson, C., 1986. The rat brain in stereotaxic coordinates., Vol., Academic Press, Orlando, FL.

Pham, T.M., et al., 2002. Environmental influences on brain neurotrophins in rats. Pharmacol Biochem Behav. 73**,** 167-75.

Prado Lima, M.G., et al., 2018. Environmental enrichment and exercise are better than social enrichment to reduce memory deficits in amyloid beta neurotoxicity. Proc Natl Acad Sci U S A. 115**,** E2403-E2409.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol. 463**,** 3-33.

Pryor, W.M., et al., 2015. Chronic exercise confers neuroprotection in experimental autoimmune encephalomyelitis. J Neurosci Res. 93**,** 697-706.

Pusic, A.D., Kraig, R.P., 2014. Youth and environmental enrichment generate serum exosomes containing miR-219 that promote CNS myelination. Glia. 62**,** 284-99.

Pusic, K.M., et al., 2014. Spreading depression requires microglia and is decreased by their M2a polarization from environmental enrichment. Glia. 62**,** 1176-94.

Pusic, K.M., Pusic, A.D., Kraig, R.P., 2016. Environmental Enrichment Stimulates Immune Cell Secretion of Exosomes that Promote CNS Myelination and May Regulate Inflammation. Cell Mol Neurobiol. 36**,** 313-325.

Quattromani, M.J., et al., 2014. Enriched housing down-regulates the Toll-like receptor 2 response in the mouse brain after experimental stroke. Neurobiol Dis. 66**,** 66-73.

Rogers, J., et al., 2017. Environmental enrichment reduces innate anxiety with no effect on depression-like behaviour in mice lacking the serotonin transporter. Behav Brain Res. 332**,** 355-361.

Salmin, V.V., et al., 2017. Differential Roles of Environmental Enrichment in Alzheimer's Type of Neurodegeneration and Physiological Aging. Front Aging Neurosci. 9**,** 245.

Sampedro-Piquero, P., Begega, A., 2017. Environmental Enrichment as a Positive Behavioral Intervention Across the Lifespan. Curr Neuropharmacol. 15**,** 459-470.

Sandroff, B.M., DeLuca, J., 2019. Will behavioral treatments for cognitive impairment in multiple sclerosis become standards-of-care? Int J Psychophysiol.

Scheirer, C.J., Ray, W.S., Hare, N., 1976. The analysis of ranked data derived from completely randomized factorial designs. Biometrics. 32**,** 429-34.

Schloesser, R.J., et al., 2010. Environmental enrichment requires adult neurogenesis to facilitate the recovery from psychosocial stress. Mol Psychiatry. 15**,** 1152-63.

Silva, B.A., Ferrari, C.C., 2018. Cortical and meningeal pathology in progressive multiple sclerosis: a new therapeutic target? Rev Neurosci.

Silva, B.A., et al., 2018. A new focal model resembling features of cortical pathology of the progressive forms of multiple sclerosis: Influence of innate immunity. Brain Behav Immun.

Skillings, E.A., Wood, N.I., Morton, A.J., 2014. Beneficial effects of environmental enrichment and food entrainment in the R6/2 mouse model of Huntington's disease. Brain Behav. 4**,** 675-86.

Souza, P.S., et al., 2017. Physical Exercise Attenuates Experimental Autoimmune Encephalomyelitis by Inhibiting Peripheral Immune Response and Blood-Brain Barrier Disruption. Mol Neurobiol. 54**,** 4723-4737.

Stam, N.C., et al., 2008. Sex-specific behavioural effects of environmental enrichment in a transgenic mouse model of amyotrophic lateral sclerosis. Eur J Neurosci. 28**,** 717-23.

Steventon, J.J., et al., 2015. In Vivo MRI Evidence that Neuropathology is Attenuated by Cognitive Enrichment in the Yac128 Huntington's Disease Mouse Model. J Huntingtons Dis. 4**,** 149-60.

Stranahan, A.M., Khalil, D., Gould, E., 2006. Social isolation delays the positive effects of running on adult neurogenesis. Nat Neurosci. 9**,** 526-33.

Sumowski, J.F., et al., 2018. Cognition in multiple sclerosis: State of the field and priorities for the future. Neurology. 90**,** 278-288.

Tomlinson, L., Leiton, C.V., Colognato, H., 2016. Behavioral experiences as drivers of oligodendrocyte lineage dynamics and myelin plasticity. Neuropharmacology. 110**,** 548-562.

Ucal, M., et al., 2017. Widespread cortical demyelination of both hemispheres can be induced by injection of pro-inflammatory cytokines via an implanted catheter in the cortex of MOG-immunized rats. Exp Neurol. 294**,** 32-44.

van Praag, H., Kempermann, G., Gage, F.H., 2000. Neural consequences of environmental enrichment. Nat Rev Neurosci. 1**,** 191-8.

Walf, A.A., Frye, C.A., 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2**,** 322-8.

Williamson, L.L., Chao, A., Bilbo, S.D., 2012. Environmental enrichment alters glial antigen expression and neuroimmune function in the adult rat hippocampus. Brain Behav Immun. 26**,** 500-10.

Xiao, R., et al., 2019. Enriched environment regulates thymocyte development and alleviates experimental autoimmune encephalomyelitis in mice. Brain Behav Immun. 75**,** 137-148.

Xu, H., et al., 2016. Environmental Enrichment Potently Prevents Microglia-Mediated Neuroinflammation by Human Amyloid beta-Protein Oligomers. J Neurosci. 36**,** 9041-56.

Young, D., et al., 1999. Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. Nat Med. 5**,** 448-53.

Yu, H., Chen, Z.Y., 2011. The role of BDNF in depression on the basis of its location in the neural circuitry. Acta Pharmacol Sin. 32**,** 3-11.

Zhang, Y., et al., 2018. The short-term improvements of enriched environment in behaviors and pathological changes of APP/PS1 mice via regulating cytokines. Hum Vaccin Immunother. 14**,** 2003-2011.

Ziegler-Waldkirch, S., et al., 2018. Seed-induced Abeta deposition is modulated by microglia under environmental enrichment in a mouse model of Alzheimer's disease. EMBO J. 37**,** 167-182.

FIGURE LEGENDS

Fig. 1. Experimental design, timeline and assessment of the experiments. **A**. Timeline of the experiments. Animals received an intracortical injection with AdIL-1β c at day 0. After the surgery, the animals were housed either in EE or SC. Twenty-one days after surgery the animals were peripherally injected with AdIL-1β iv or Adβgal iv, as control. Blood smears were performed 5 days after the peripheral injection The studies were performed at 7 days after the peripheral stimulation. Behavioural, histopathological and molecular studies were performed at 30 days after the central injection. **B-C**. Representative picture of a EE (**B**) and SC (**C**). The EE cage includes exposure to novel stimulation and experiential learning (toys and mazes) and access to voluntary exercise (running wheels). **D**. Blood smears were performed 5 days after the peripheral injection. Representative blood smear counts as a control for the effectiveness of the peripheral stimulus. EE decreases the number of peripheral neutrophils in AdIL-1β iv animals This control was repeated in every experiment performed throughout the present work. n= 10-12/group, 2 way-ANOVA, Tukey post hoc test, ***p < 0.0001–0.001. **E**. EE decreased the pro-inflammatory IL-1β mRNA expression in the serum at 5 days after the peripheral stimulation. No changes in IL-1β expression were observed in the IL-1β c/βgal iv group no matter the housing. n= 5-6/group for real time PCR, 2 way-ANOVA, Tukey post hoc test. ns p > 0.05 p*, < 0.01–0.05

Fig. 2. Behavioural tests. Enrichment environmental attenuates the anxiety-like behaviour in peripherally stimulated animals. **A-D**. Open field test. In all the measurements parameters the of IL-1β c/IL-1β iv animals located in EE demonstrated an enhance in the performance of time in the center (**A**) (2 way-ANOVA, Tukey post hoc test), line crossing (**B**), fecal boli (**C**) and rearing (**D**). These results cannot be observed in IL-1β c/βgal iv groups where the environment did not influence the performance of the animals. **E-F**. Elevated plus maze test. The EE enhance the performance of animals of both groups compared with animals located in the SC. These results where demonstrated as an increment in both measurement parameters: entries to the open arms (**E**) (2 way ANOVA, Tukey post hoc test) and time into the open arms (**F**) (2 way-ANOVA,

Tukey post hoc test) compared with control animals. n= 10-12 /group. ns $p > 0.05$, $p^* < 0.01–0.05$, $^{***}p < 0.0001–0.001$, $^{****}p < 0.0001$.

Fig. 3. Behavioural tests. **A-B**. The EE improves cognitive deficits induced by peripheral stimulation in AdIL-1β peripherally injected animals, as evidenced by a significantly increment of time contacts with the novel object in the Novel Object Recognition Test (NOR) in both groups, IL-1β c/IL-1β iv and IL-1β c/βgal iv located in the EE (**A**) (2 way-ANOVA, Tukey post hoc test). **B**. The analysis of the discrimination index (DI) demonstrated that EE statistically improves the performance in the animals located in EE (2 way-ANOVA, Tukey post hoc test). **C**. The sucrose preference was significantly greater in the IL-1β c/IL-1β iv animals located in the EE than in the control group located in the SC 2 way-ANOVA, Tukey post hoc test). No significant difference in sucrose preference between the IL-1β c/βgal iv groups located in any environment. n= 10-12 /group. ns $p > 0.05$, $p^* < 0.01–0.05$, $^{**}p < 0.001–0.01$, $^{****}p < 0.0001$.

Fig. 4. Neuroinflammation and glial activation. **A-C**. The EE statistically reduces the inflammatory volume in IL-1b c/IL-1b iv group (2 way-ANOVA, Tukey post hoc test). The IL-1β c/βgal iv group has very few inflammatory infiltrated in any location. **B–C**. Representative low magnification pictures of the inflammatory lesions stained with Cresyl Violet. The animals located in SC presented an important inflammatory infiltrate compared to IL-1β c/IL-1β iv animals located in EE. **D-F**. Microglia response of the central lesions to EE. **D**. Quantification of MHC-II positive cells confirmed significant decrease in the animals located in EE (2 way-ANOVA, Tukey post hoc test). **E-F**. Representative immunofluorescences against macrophage/microglia marker MHC-II. The animals in SC had more MHC II+ cells in the cortical parenchyma (**E**), than EE group (**F**). **G-I**. ED1 response to EE. **G**. Quantification of ED1 positive cells reveals that animals located in EE have statistically less ED1 positive cells compared with both group located in SC (2 way-ANOVA, Tukey post hoc test). **H-I**. Representative immunofluorescence against macrophage/microglia that have phagocytic activity demonstrate that the animals located in EE (**H**) have less ED1 positive cells compared with SC animals (**I**). **J-L**. Astroglia response to EE. **J.** Quantification of GFAP positive cells demonstrated that glial fibrillary acidic protein (GFAP) intensity is statistically decreased in the animals located in EE in both studied group (2 way-ANOVA, Tukey post hoc test). **K-L**. Representative pictures of GFAP immunofluorescence confirm that EE group (**L**) reduces the astroglia response compared with SC group (**K**). B-C: Scale bar: 200µm. E, F, K, L. Scale bar: 50µm. n= 8-10 /group. ns $p > 0.05$, $p^* < 0.01–0.05$, $^{**}p < 0.001–0.01$, $^{***}p < 0.0001–0.001$, $^{****}p < 0.0001$.

Fig. 5. The enriched environment decreased demyelination. **A-F**. Quantification of the demyelination. **A**. Quantification of MAG positive cells. The enrichment environment induces and increment of MAG intensity in the animals located in EE (2 way-ANOVA, Tukey post hoc test). **B-C**. Representative photograph of the cortex of IL-1β c/IL-1β iv animals located in either SC (**B**) or EE (**C**), where an increase in MAG positive cells can be appreciate in EE animals. **D**. Quantification of MBP intensity. The animals located in the EE exhibited more MBP label compared with animals in SC (2 way-ANOVA, Tukey post hoc test). **E-F**. Representative pictures of the demyelination in the cortex of IL-1β c/IL-1β iv animals located in either SC (**E**) or EE (**F**), visualized by MBP staining, where an increase in MBP intensity can be observed in EE animals. **G.** Quantification of MAG intensity of naïve animals, that demonstrated that the animals located

have more MAG intensity compared to animals located in SC. B-C. Scale bar: 100μm. E-F. Scale bar: 50μm. n= 8-10 /group. p* < 0.01–0.05, **p < 0.001–0.01, ***p < 0.0001–0.001.

Fig 6. Attenuated neurodegeneration can be observed in enriched housed animals. A-C. Neurodegeneration demonstrated by Fluorojade C (FJC) staining. **A**. Quantification of FJC intensity demonstrated significant differences between EE and SC animals (2 way-ANOVA, Tukey post hoc test). A decreased intensity in FJC can be observed in the animals located in EE independently of the treatment. **B-C**. Representative pictures of the FJC staining of SC IL-1β c/IL-1β iv (**B**) and EE IL-1β c/IL-1β iv (**C**) animals. D-F. The EE protects against neurodegeneration. **D**. Quantification of NeuN positive cells. The animals located in EE exhibited a less number of NeuN positive cells compared with SC animals in both treatments (2 way-ANOVA, Tukey post hoc test). **E-F**. Representative picture of Neu N positive cells in SC IL-1β c/IL-1β iv animals (**E**) and EE IL-1β c/IL-1β iv animals (**F**). B-C. Scale bar: 100μm, **E-F**. Scale bar: 50 μm. n= 8-10 /group. p* < 0.01–0.05, **p < 0.001–0.01.

Fig 7. EE decreased the pro-inflammatory cytokine expression as well as it increased the expression of anti-inflammatory molecules in cortical lesions. **A-C**. EE decreases the expression of pro-inflammatory cytokines in both groups, IL-1β c/IL-1β iv and IL-1β c/βgal iv, compared with animals located in SC. IL-1β (**A**), IL-6 (**B**) and TNF-α (**C**) are less expressed in animals located in EE (2 way-ANOVA, Tukey post hoc test). Significant differences can be observed between EE IL-1β c/IL-1β iv and EE IL-1β c/βgal iv animals EE (2 way-ANOVA, Tukey post hoc test). **D-F**. EE induces a higher expression of anti-inflammatory molecules. The expression of TGF-β (**D**), Arg-1 (**E**) and BDNF (**F**) are upregulated in EE located animals in both groups IL-1β c /IL-1βiv and IL-1β c/βgal iv EE (2 way-ANOVA, Tukey post hoc test). Again, a statistically increment can be observed in EE IL-1β/βgal compared with EE IL-1β c/IL-1β iv animals. TBP: TATA box binding protein. n= 5-6 /group. p* < 0.01–0.05, **p < 0.001–0.01, ***p < 0.0001–0.001, ****p < 0.0001.

Supplementary figure legends

Suppl Fig. 1. The sucrose preference performed in β-gal c/ β-gal iv and β-gal c/ IL-1β iv animals located in both EE and SC. The animals that were injected with control adenovirus have a preference to sucrose of around 90%, demonstrating that the animals injected with AdIL-1β in the cortex presented a proinflammatory basal state. No statistically differences could be found in any of the tested groups (2 way-ANOVA, Tukey post hoc test). n= 3-4.

Suppl Fig. 2. Neuroinflammation and glial activation in EE IL-1β c/β-gal iv and SC IL-1β c/β-gal iv groups. **A-B**. Representative low magnification pictures of the inflammatory lesions stained with Cresyl Violet. The animals located in SC (**A**) presented more inflammatory infiltrate compared to IL-1β c/β-gal iv animals located in EE (**B**). **C-D**. Representative immunofluorescences against macrophage/microglia marker MHC-II. The animals in SC had more MHC II+ cells in the cortical parenchyma (**C**), than EE IL-1β c/β-gal group (**D**). **E-F**. Representative immunofluorescence against macrophage/microglia that have phagocytic activity demonstrate by ED1 staining. The SC IL-1β c/β-gal (**E**) have more ED1 positive cells compared with EE IL-1β c/β-gal animals. **G-H**. Representative pictures of GFAP immunofluorescence

confirm that SC IL-1β c/β-gal group (**G**) have an increased astroglia response compared with EE group (**H**). A, B. Scale bar: 200μm. C, D, E, F. Scale bar: 50μm.

Suppl Fig. 3. **A-D**. The enriched environment decreased demyelination in EE IL-1β c/β-gal iv compared with SC IL-1β c/β-gal iv group. **A-B**. Representative photograph of the cortex of IL-1β c/IL-1β iv animals located in either SC (**A**) or EE (**B**), labelled with anti MAG antibody, where an increase in MAG intensity can be appreciate in EE animals. **C-D**. Representative pictures of the demyelination in the cortex of IL-1β c/β-gal iv animals located in either SC (**C**) or EE (**D**), visualized by MBP staining, where an increase in MBP intensity can be observed in EE animals. **E-H**. Neurodegeneration in EE IL-1β c/β-gal iv and SC IL-1β c/β-gal iv animals. **E-F.** Representative pictures of FJC staining in SC IL-1β c/β-gal iv animals (**E**) and EE IL-1β c/β-gal iv animals (**F**). **G-H**. Representative pictures of Neu N positive cells in SC IL-1β c/β-gal iv animals (**G**) and EE IL-1β c/β-gal iv animals (**H**). **A, B, E, F**. Scale bar: 100μm. **C, D, H, G**. Scale bar: 50μm

TABLES

Antibody	**Origin**	**Target**	**Dilution**	**Antigen Retrieval**	**Source**
MHC II	Mouse	MHC Class II restricted macrophages, dendritic cells and B-cells	1:200	no	Serotec
GFAP	Rabbit	Glial fibrillary acidic protein	1:700	no	Dako
NeuN	Mouse	Neuron-specific nuclear protein	1:1000	no	EMD Millipore
ED1	Mouse	Pan-macrophage marker	1:100	no	Serotec
MAG	Rabbit	Mylein associated glycoprotein	1:200	no	Serotec
MBP	Rabbit	Myelin basic protein	1:200	no	Kindly provided by Páez P, School of Medicine and Biomedical Sciences, University at Buffalo

Table 1. Antibody list

GENE	PRIMERS
TATA binding protein (TBP)	Forward : 5'- ACCGTGAATCTTGGCTGTAA Reverse : 5'- CCGTGGCTCTCTTATTCTCA
Interleukin 1 beta (IL1β)	Forward : 5'- TCCATGAGCTTTGTACAAGG Reverse : 5'- GGTGCTGATGTACCAGTTGG
Interleukin 6 (IL6)	Forward: 5'- GCCAGAGTCATTCAGAGCAATA Reverse: 5'- GTTGGATGGTCTTGGTCCTTAG
Tumor necrosis factor alpha (TNFα)	Forward: 5'- GTAGCCCACGTCGTAGCAAA Reverse: 5'- AAATGGCAAATCGGCTGACG
Transforming growth factor beta (TGFβ)	Forward: 5'- ACCAACTACTGCTTCAGCTC Reverse:5´- TGTTGGTTGTAGAGGGCAAG
Brain derived neurotrophic factor (BDNF)	Forward:5´-TCACAGTCCTGGAGAAAGTC Reverse:5´--ATGAACCGCCAGCCAATTCT
Arginase 1 (Arg1)	Forward:5´-TGGACCCTGGGGAACACTAT Reverse:5´- GTAGCCGGGGTGAATACTGG

Table 2. Primers used for cytokine and neurotrophic analyses

Highlights

- EE reduces the peripheral inflammatory response in the serum of systemic stimulated rats

- EE enhances cognitive deficits and anxiety-like symptoms

- EE modulates neurodegeneration, demyelination and glial activation.

- EE modulates neuroinflammation by reducing the levels of pro-inflammatory cytokines and enhancing the levels of anti-inflammatory ones.