



# How physical and motor training affect cognitive performance: lessons from an inflammatory molecule

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Our phenotype includes not only physical features, but also behavioral outputs such as motor schemes and learned skills, and is the result of a complex interaction between genetic background and environment. In fact, a good fraction of last decades' biomedical efforts were dedicated to understanding how different elements (e.g., genetic polymorphisms, lifestyle components) participate in this interplay, ultimately contributing to affect a given phenotype up to the point of steering it towards pathology. Genetic and epigenetic elements are relatively easy to analyze individually using a reductionist approach, for instance via loss- or gain-of-function studies in cellular and animal models; the effects of nutrients, drugs, pollutants can be similarly tested as single specific stimuli. Not as easy is to establish in rigorous terms and to understand the molecular mechanisms through which different levels of education, social interaction, exposure to a playful environment, psychological stress, to voluntary physical activity, may impact our brain phenotype, whose combined complexity is hard to reproduce in the experimental setting.

However, all the elements listed above can be simulated in animal laboratory models, typically rodents, using environmental enrichment (EE). EE is defined by comparison with the standard rearing condition of rats and mice, which are normally provided only some bedding and nesting material, in addition to their number being limited to small groups (3–5 individuals on average). On the other hand, EE is based on the use of wide cages, equipped with (i) one or more running wheels for voluntary physical exercise, (ii) maze-like tunnels, (iii) objects that are regularly changed to stimulate explorative behavior and curiosity. These cages host large groups of animals (at least 10), which are free to experience the different sensory and motor stimuli available to them (Sale et al., 2014).

It is not surprising that EE has dramatic effects on the brain, both during development and adulthood, which, in general, empower plasticity, the process by which neural circuits adapt their response to variation in external inputs. The main results of EE on the brain are (i) accelerated development of sensory systems, (ii) improved learning and memory abilities, (iii) in genetic models of neurological diseases, attenuation of the pathological phenotype (Sale et al., 2014).

With specific regard to physiological brain aging, others and we demonstrated that EE improves the function of neural circuits, their plasticity, and reduces the deposition of amyloid- $\beta$  oligomers (Sale et al., 2014).

Going back from laboratory models to human subjects to design an EE-like protocol has obvious difficulties, since standard rearing and EE are extreme conditions, while no average individual has been reared or has lived for extended periods in an almost empty room. However, appropriate sensorimotor stimulation protocols, involving physical as well

as psychological training, have been defined also for human subjects, and are nowadays implemented as a highly effective intervention to delay the progression of Alzheimer's disease. A successful example is represented by the "Train the Brain" project, carried out in Pisa (Italy) since 2009, and consisting in a Randomized Control Study aimed at looking for possible benefits of cognitive training and physical exercise in a social setting, on subjects with mild cognitive impairment. This non-invasive, multi-domain approach was indeed effective in reducing cognitive decline, gray matter loss, and in increasing cerebral blood flow in the hippocampus and parahippocampal areas, as well as in ameliorating task-related brain activity and reducing neuropsychiatric symptoms (Train the Brain Consortium, 2017; Cintoli et al., 2021).

Although less surprising, given the widely demonstrated relationship between physical activity and metabolic/cardiovascular health, it is worth emphasizing that elderly mild cognitive impairment subjects enrolled in Train the Brain also showed an improvement of cardiovascular parameters such as endothelial function, and carotid artery elasticity (Bruno et al., 2018). This well illustrates the global action of EE, with the result on brain function arising from the combination of direct cognitive stimulation and indirect effects on peripheral organs.

These encouraging results provide an effective protocol, which can be easily introduced with a minimum of equipment also in small communities with no quick access to large healthcare facilities. However, they also prompt researchers to understand how EE on laboratory rodents and its counterparts on humans can achieve their positive effects on brain function. How brain-oriented stimuli (i.e., sensory stimulation, cognitive training, mental sports) can achieve a positive effect on neural circuits has been elucidated with a good level of detail, and points to a key role of (i) brain-derived neurotrophic factor (BDNF), and (ii) modulation of the synaptic excitation/inhibition ratio (Sale et al., 2014). Therefore, the key point to be addressed is understanding how peripheral organs benefiting from EE can indirectly improve the status of the brain, beyond the trivial aspects of healthier cardiovascular and respiratory systems in exercised elderly.

In this regard, the levels of circulating hormones, metabolites, and molecules involved in inflammation finely tune the function of all organs, including brain, and are crucial determinants of health. Indeed, blood as the main determinant of disease but also as a therapeutic target is a line of medical thought, known as humoral pathology, historically dating back to Hippocrates. The attempt to rejuvenate old bodies by young blood administration is the application of this concept in the aging context.

Parabiosis, i.e., the physical connection of the blood circles of 2 animals is a formal way to experimentally investigate whether blood-

borne factors can affect brain aging. In a landmark experiment, Villeda and colleagues used parabiosis to demonstrate that age-related changes in the systemic milieu are responsible for the age-related decline in adult neurogenesis, impairment in hippocampal synaptic plasticity, and cognitive function (Villeda et al., 2011). By using a proteomic approach, they could give a name to those blood-borne factors whose changes in concentration correlated with changes in brain performance. Among them, the C-C motif ligand (CCL) chemokines CCL11, CCL12, CCL19, and CCL11/eotaxin-1, were all found to be elevated in aged mice. Indeed, when CCL11 was administered to young mice, neurogenesis was inhibited and learning and memory capacities were impaired (Villeda et al., 2011). An increase in cytokine and chemokine circulating levels is typical of an inflammatory response, the physiological reaction of the organism to an insult, that displays its highest adaptive value in acute conditions, when tissues need to be repaired following an injury or when we need to get rid of infections by pathogens. However, when sustained for a long time, as it often occurs in chronically diseased or elderly individuals, high inflammation causes damage to organs through multiple routes that involve increased signaling of cytokines and chemokines. Elevated levels of the pro-inflammatory cytokines interleukin-1, interleukin-6, tumor necrosis factor- $\alpha$ , and chemokines have been implied in the onset of type I diabetes, rheumatoid arthritis, lupus nephritis, psoriasis, and systemic sclerosis. Even adipokines, such as leptin, have a quasi-neurotrophic effect on neurons, and loss of leptin sensitivity leads to impaired synaptic plasticity (Maffei and Mainardi, 2019).

Strikingly, inflammation also modulates the severity of neuropathological states and is a determinant of Alzheimer's disease pathogenesis (Vainchtein and Molofsky, 2020). CCL11 participates in such a process by suppressing endogenous tissue repair mechanisms critical for normal central neural system function (Huber et al., 2018). Several different routes can be used by a systemic inflammatory response to communicate with the central nervous system. Notably, the blood-brain barrier shows a relatively high permeability to CCL11 (Huber et al., 2018). Once into the brain, these factors may interact with microglia by switching it from a potentially protective phenotype to an aggressive inflammatory phenotype, with tissue damage and cell loss, ultimately promoting synapse loss and neurodegeneration. Of note, changes in the permeability of the blood-brain barrier can facilitate the access of proinflammatory molecules to the brain parenchyma, which may exacerbate their effect on glia and neurons.

The role of EE on the modulation of inflammation of the immunomodulatory response has received less attention than its effects on brain health and behavior. All the studies converge on the modulation of the immune system, but effects are largely dependent on the age of animals, length, and variations introduced in the EE protocol, which may or not include olfactory and taste stimuli (Singhal et al., 2014). More definitive conclusions are reached when the immunomodulatory response is investigated in models exposed to a single component of EE, i.e., physical exercise, which induces an enhancement of the humoral anti-inflammatory response (Singhal et al., 2014).

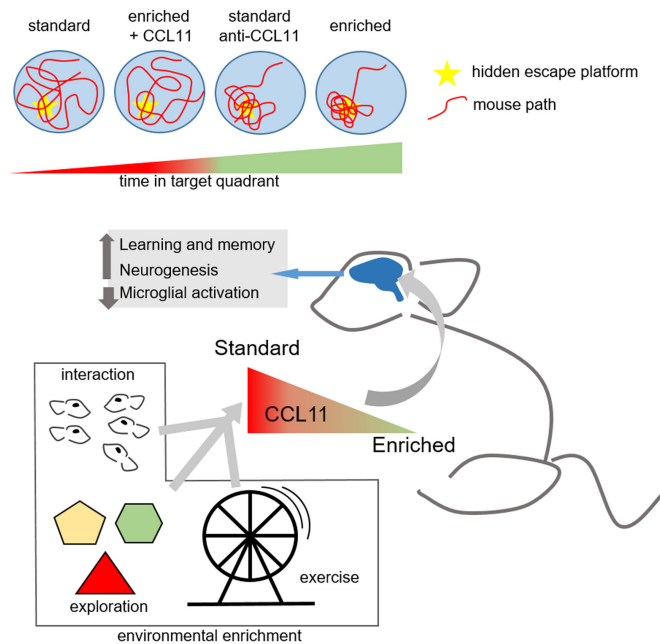
This evidence can be summarized in the following key messages: (i) EE can empower the brain and prevent cognitive decline; (ii) EE affects brain health also by modulating peripheral signals whose levels can be sensed by the brain; (iii) systemic inflammation varies with aging, has been implied in neurodegeneration, and is a target of EE.

A recent study from our group establishes a causal role for a single inflammatory mediator in converting enhanced stimulation coming from EE into enhanced cognitive function in elderly mice. The observation that subjects enrolled in the Train the Brain study showed a small, but significant reduction in the circulating levels of CCL11 after 7 months of training, prompted us to further analyze this finding in aged mice exposed to EE. After confirming that EE-reared, aged mice showed a reduction in plasma CCL11, we challenged them in the Morris water maze to test spatial learning and memory. As expected, EE mice performed better than standard-reared controls. Strikingly, keeping CCL11 levels high during EE using injections of anti-CCL11 antibodies erased the improvement in long-term maintenance of spatial memory. Conversely, treating standard-reared mice with periodic injections of anti-CCL11 antibodies simulated exposure to EE (Scabia et al., 2021). Our experiments demonstrate that the reduction of circulating CCL11 is necessary for EE to exert its benefits on memory and sufficient to reproduce them on standard-reared animals (Figure 1).

We also looked for possible cellular targets of CCL11. In addition to confirming its impact on hippocampal neurogenesis, consistently with Villeda et al. (2011), we also observed in mice exposed to EE a lower density of Iba-1-immunoreactive microglial cells, indicating reduced microglial activation (Scabia et al., 2021; Figure 1). We hypothesize that this finding may indicate a link between CCL11 and modulation of synaptic stripping by microglia, a process that is involved in cognitive deficits associated with neurological and neurodegenerative disorders. Microglia express chemokine (C-C motif) receptor 3 (CCR3), the main receptor for CCL11, in addition to expressing CCL11 itself (Zhu et al., 2017). Moreover, knocking out CCR3 in a transgenic mouse model of Alzheimer's disease rescued impaired spatial memory (Zhu et al., 2017). Finally, also astrocytes express a variety of chemokine receptors, including CCR3 (Dorf et al., 2000).

We think that CCL11 can be considered as a prototypical example of a messenger bridging systemic inflammation with brain function via multiple actions on neurons and glial cells. Interfering with its – as far as we currently know – detrimental action leads to dramatic improvements in the learning and memory performance of healthy and diseased mice. This evidence calls for further studies, firstly aimed at elucidating the molecular details converting CCL11 binding to CCR3 into decreased neurogenesis and, possibly, increased microglial phagocytosis of synapses. In this regard, CCL11 can directly act on microglia through CCR3 or indirectly modulate the microglial phenotype via inflammatory mediators released by its primary target, i.e., eosinophils.

In addition, the main source(s) of CCL11 at the level of peripheral organs and brain have to be identified, a point that is particularly relevant, given its capability to cross the blood-brain barrier (Huber et al., 2016), and the direct



**Figure 1 | Key findings on CCL11 as a modulator of the effects of environmental enrichment on the aged brain.** Enhanced social interaction, sensory exploration and physical exercise are experienced by aged mice exposed to environmental enrichment, which also results in lower levels of plasma CCL11. The reduction in CCL11 is responsible for higher hippocampal neurogenesis and reduced microglial activation. At the behavioral level, mice exposed to environmental enrichment show a better performance in a spatial memory task (Morris water maze), and lower CCL11 is a necessary and sufficient factor for this beneficial effect to occur, as demonstrated by treating enriched mice with exogenous CCL11, and standard-reared mice with anti-CCL11 monoclonal antibodies, respectively. CCL11: Chemokine (C-C motif) ligand 11.

correlation between increased CCL11 in plasma and brain (Scabia et al., 2021). We envisage that clarifying these points will indicate new avenues to improve brain function in aging and age-related pathologies.

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**Date of submission:** November 4, 2021

**Date of decision:** December 24, 2021

**Date of acceptance:** January 14, 2022

**Date of web publication:** April 29, 2022

<https://doi.org/10.4103/1673-5374.339486>

**How to cite this article:** Maffei M, Mainardi M (2022) How physical and motor training affect cognitive performance: lessons from an inflammatory molecule. *Neural Regen Res* 17(12):2689-2690.

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C-Editors: Zhao M, Liu WJ, Wang Lu; T-Editor: Jia Y