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ORIGINAL ARTICLE

The effect of exercise on memory and BDNF signaling is dependent on intensity

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Abstract

The aims of the present study were to investigate in brain of adult rats (1) whether exercise-induced activation of brain-derived neurotrophic factor (BDNF)/tropomyosin-related kinase B (TrkB) pathway is dependent on exercise intensity modality and (2) whether exercise-induced improvement of memory is proportional to this pathway activation. Wistar rats were subjected to low (12 m/min) or high (18 m/min) exercise intensity on horizontal treadmill (30 min/day, 7 consecutive days) that corresponds to ~40 and 70% of maximal aerobic speed, respectively. Animals treated with scopolamine to induce memory impairment were subjected to novel object recognition test to assess potential improvement in cognitive function. Expressions of BDNF, phosphorylated TrkB receptors, synaptophysin (a marker of synaptogenesis), c-fos (a neuronal activity marker) and phosphorylated endothelial nitric oxide synthase (a cerebral blood flow marker) were measured in prefrontal cortex and hippocampus of different groups of rats. In terms of cognition, our data reported that only the most intense exercise improves memory performance. Our data also revealed that BDNF pathway is dependent on intensity modality of exercise with a gradual effect in hippocampus whereas only the highest intensity leads to this pathway activation in prefrontal cortex. Our study revealed that memory improvement through BDNF pathway activation is dependent on exercise intensity. While reporting that our protocol is sufficient to improve cognition in animals with impaired memory, our data suggest that prefrontal cortex is possibly a more suitable structure than hippocampus when neuroplastic markers are used to mirror potential improvement in memory performance.

Keywords Brain-derived neurotrophic factor · Exercise intensity · Hippocampus · Prefrontal cortex

Introduction

The regular practice of physical exercise (EX) is the most potent non-pharmacological strategy to positively enhance brain health. Indeed, numerous studies have reported positive actions of EX in both animals and humans. From animal studies, EX has been reported to promote memory and learning performance in rodents and to reverse impairment in memory and spatial learning in old or stressed animals (Aguiar et al. 2008; Loprinzi and Frith 2018; Yau et al. 2011). In humans, analysis showed that active individuals may have decreased risk of cognitive impairment and neurodegenerative disorders (Sofi et al. 2011). Moreover, being physically active during the early life has been associated with protection against cognitive decline later in life (Hotting and Roder 2013). Physical EX benefits also to younger adults since it has been shown that exercise improved psychological well-being, cognitive performance (Hogan et al. 2013) and was related to a decreased risk of substance use disorders in adolescent (Nock et al. 2017). The positive effects of EX are linked to the ability of the neural system to modify its organization notably through an increase in synapse number and efficacy (Farmer et al. 2004; van Praag et al. 1999).

Among the molecules responsible for the structural and functional brain changes, brain-derived neurotrophic factor (BDNF) appeared to be an appealing candidate. BDNF acts through its high-affinity for tropomyosin-related kinase B (TrkB) receptor eliciting intracellular signaling cascades that

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mainly impact positively brain function (Reichardt 2006). In response to EX, brain BDNF is produced in neurons but also in cerebral endothelial cells through mechanisms involving increase in neuronal activity and shear stress-dependent expression (Marie et al. 2018; Monnier et al. 2017a, b; Vaynman et al. 2003). In favor of the strong involvement of BDNF in the positive effect of EX on brain health, convincing evidences are given by studies showing that anti-BDNF strategies using antibody against its cognate TrkB receptor negate exercise-associated cognitive benefits (Gomez-Pinilla et al. 2008; Liu et al. 2008). In addition, in human, the val66met polymorphism which is associated with an alteration of BDNF activity-dependent release moderates the cognitive benefits of EX (Erickson et al. 2013; Hopkins et al. 2012).

The typology of the better regimen of EX is not known. In rodents, beneficial effects on brain health have been reported after different type of EX such as treadmill/wheel running or swimming exercise (Liu et al. 2009; Ogonovszky et al. 2005; Vaynman et al. 2004). Of note, brain BDNF elevation in physically trained animals has been described to be dependent of the duration (Sheikhzadeh et al. 2015) while its link with the frequency of training sessions remains controversial (Costa et al. 2012; Dalise et al. 2017). In terms of intensity modality, evidences showed that this parameter is positively associated with a lower risk of cardiovascular diseases (Kemi et al. 2002, 2005), but surprisingly, the connection between EX intensity and brain function improvement is poorly investigated (Pedard et al. 2018). Furthermore, whether cognitive benefits proportionate to changes in the BDNF/TrkB pathway is not known.

Thus, the aims of the present study were to investigate in adult rats (1) whether EX-induced activation of the brain BDNF/TrkB/synaptophysin pathway is dependent on intensity modality and (2) whether EX-induced improvement of memory function is proportional to the levels of activation of this pathway. For this purpose, animals were subjected to horizontal treadmill EX at low (12 m/min, EX12) or high intensity (18 m/min, EX18), 30 min a day for 7 consecutive days. Rats treated with scopolamine to induce memory impairment were assessed for memory performance through the novel object recognition (NOR) test while the expressions of BDNF, phosphorylated TrkB receptors at tyrosine 816 (p-TrkB^{Y816}), synaptophysin (SYN, a marker of synaptogenesis), c-fos (a marker of neuronal activity) and phosphorylated endothelial nitric oxide synthase at serine 1177 (p-eNOS^{S1177}, a marker of cerebral blood flow) were measured in prefrontal cortex (PFC) and hippocampus (HP) of rats either sedentary (SED) or exercised at both intensities.

Materials and methods

Animals

Experiments were carried out on 10-week-old Wistar rats ($n = 69$) according to the French Department of Agriculture guidelines (License 21-CAE-099) and approved by the local ethic committee. They conformed to the European convention for protection of vertebrate animals used for experimental and other scientific purposes. The animals were housed five per cage, kept under a 12-h/12-h light/dark cycle and allowed ad libitum access to food and water. Rats were purchased from Janvier (Le Genest Saint Isle, France).

Exercise training (EX) protocol and groups of rats

After a habituation period to the experimenter and treadmill apparatus (5 days), all animals were first subjected to an incremental to exhaustion exercise test on a treadmill (from 9 m/min to exhaustion by step of 3 m/min for 2 min) to determine their maximal aerobic speed (MAS, m/min). Refractory animals (three rats) to the treadmill exercise during the habituation period were excluded from the experiment. According to their MAS values, animals were allocated to three different groups: SED (sedentary, $n = 34$), low-intensity EX (speed of the treadmill set at 12 m/min, EX12, $n = 16$), high-intensity EX (speed of the treadmill training set at 18 m/min, EX18, $n = 16$). Using these conditions, EX intensity was ~ 40 and 70% of MAS for EX12 and EX18 groups, respectively. In the SED group, animals with different MAS were allocated. Rats assigned to the different EX groups were trained 30 min/day (morning) using a horizontal treadmill for 7 consecutive days while SED rats were kept in their own cage at the proximity of the treadmill apparatus. Of note, no impact of EX was observed in terms of weight between the three groups of rats (data not shown). Animals were separated in two experiments to (1) assess the memory, the anxiety and locomotor activity (Experiment 1, $n = 40$) and (2) measure the biochemical changes of BDNF, p-TrkB^{Y816}, SYN, c-fos, p-eNOS^{S1177} after EX (Experiment 2, $n = 18$). The experimental design is summarized in Fig. 1.

Novel object recognition (NOR) test

Animals from SED ($n = 20$), EX12 ($n = 10$) and EX18 ($n = 10$) groups of Experiment 1 were submitted to the novel object recognition (NOR) test. The test was performed the morning during three consecutive days starting the last day of EX in a dark plastic enclosure (size: 60 × 60 cm with 40 cm side walls) located in a dimly lit room with constant illumination (45 Lux). NOR test (Fig. 1, Experiment

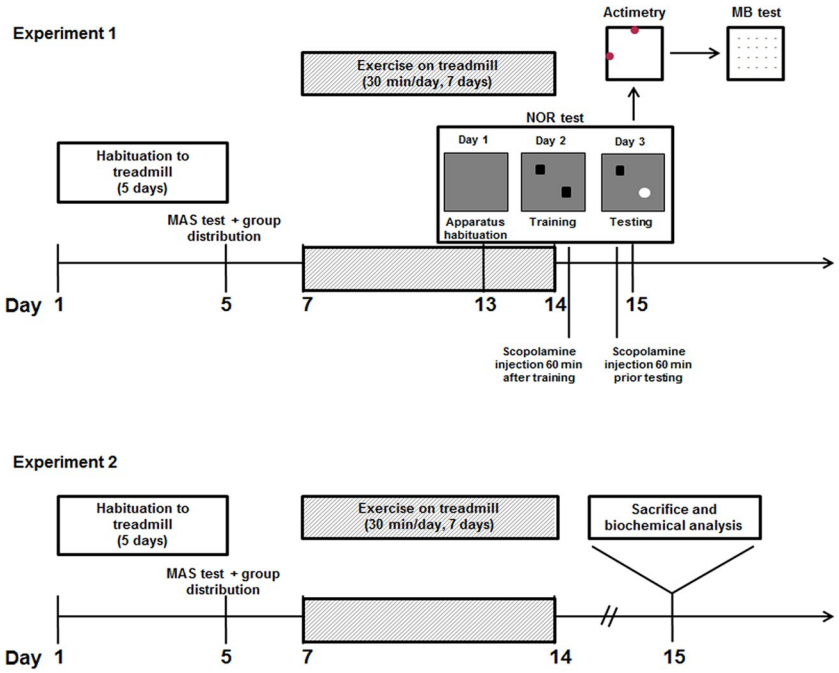

Fig. 1 Schematic experimental design of Experiment 1 and 2. *MAS* maximal aerobic speed, *NOR* novel object recognition test, *MB* marble burying test

1) consisted in a habituation phase (Day 1) during which animals explored for 10 min the empty arena. After 24 h, animals were placed in the center of the previous area with two identical objects to explore during 5 min (Day 2). These objects were placed equidistantly to the opposite corners of the enclosure (27.5 cm between each object, 20 cm between object and corner). Then, animals were immediately replaced in home cages. Twenty-four hours later (Day 3), animals were again positioned in the center of the field in the presence of the same object previously observed, and with a novel object during 2 min.

Position of each object was exchanged between animals to avoid potential confounding spatial clues. The enclosure and objects were cleaned with 70% ethanol before each session and between each animal to minimize odor cues. Time spent exploring each object was recorded. A rat was considered exploring an object when its head was facing the object (< 2 cm) or when it was touching/sniffing the object. The NOR test analyses were performed by two persons blinded from experimental conditions.

Scopolamine, a non-selective post-synaptic muscarinic receptor blocker that impairs cognitive function via diminishing the effectiveness of acetylcholine in the central nervous system in animals and humans (Ebert and Kirch 1998; Klinkenberg and Blokland 2010) was used to induce memory impairment in animals of Experiment 1. Rats were assigned into four groups: SED + saline solution, SED + scopolamine, EX12 + scopolamine, and EX18 + scopolamine. Treatment of scopolamine hydrobromide (1 mg/kg, i.p., 161750010, Acros organics) or saline solution was administered 60 min after training phase (Day 2) and 60 min prior the testing phase (Day 3) of NOR test. The protocol of administration used in our study was designed to disturb both memory consolidation during the training phase as well as the capacity to discriminate the novel object during the testing phase (Ennaceur and Meliani 1992; Martini et al. 2018).

Collection of brain samples

Twenty-four hours after the last treadmill session, rats of Experiment 2 ($n = 26$) were anesthetized with chloral hydrate (400 mg/kg, i.p., Sigma-Aldrich) and transcardially perfused with saline during 5 min to flush out blood from brain vasculature. After decapitation, brains were removed and two brain regions involved in cognition, hippocampus (HP) and prefrontal cortex (PFC) pooled from both hemispheres were quickly dissected on ice glass slide, immediately weighted and frozen at − 80 °C until further use.

Western blotting

Western blotting procedure was performed as previously described (Pedard et al. 2018). HP and PFC were homogenized in 10 volumes of ice cold lysis buffer [100 mmol/L Tris–HCl (pH 7.4), 150 mmol/L NaCl, 1 mmol/L EGTA, 1% triton X-100, 1% protease inhibitor cocktail (P8340, Sigma-Aldrich), 1% phosphatase inhibitor cocktail (78420, Fischer Scientific)]. After homogenization, the protein concentration was measured using the Lowry method. Equal amounts of protein were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and

electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes using Turbo Transblot technology (1704150, Biorad). After blocking non-specific binding sites overnight at 4 °C, with a 5% solution of non-fat dry milk or 7.5% BSA in Tris Buffered Saline (20 mM Tris/HCl, 137 mM NaCl, pH 7.4) containing 0.1% Tween 20 (TBST), membranes were probed with an anti-BDNF (1/3000, rabbit monoclonal antibody, ab108319, Abcam), anti-p-TrkB^{Y816} (1/1000, rabbit monoclonal antibody, ABN 1381, Merck Millipore), anti-c-fos (1/1000, mouse monoclonal antibody, sc-166940, Santa Cruz Biotechnology) anti-SYN (1/3000, rabbit polyclonal antibody, RB-1461-P1, Interchim), anti-p-eNOS^{S1177} (1/1000, mouse monoclonal antibody, 612383, BD Biosciences) and anti-β-actin antibodies (1/10,000, mouse monoclonal antibody, A5441, Sigma-Aldrich). Then, membranes were incubated at room temperature with horseradish peroxidase [111-035-144 (anti-rabbit) and 115-035-166 (anti-mouse), 1:10,000 to 1:50,000 according to the protein, Jackson ImmunoResearch Laboratories]. Protein-antibody complexes were visualized using the enhanced chemiluminescence western blotting detection system (ECL 2, 1151-7371, Fisher Scientific). The band densities were determined by scanning densitometry (GS-800, Biorad Laboratories). Two groups of six rats were analyzed on the same gel, and each gel was run in triplicate. Data were analyzed and a representative immunoblot is shown above each graph.

Importantly, the amount of protein required to detect the protein of interest results in a signal that far exceeds the linear dynamic range when detecting β-actin, thereby eliminating the usefulness of β-actin for the normalization of western blots, β-actin being used here as an internal standard only to verify that proteins were indeed loaded on the gel. The appropriate amounts of total proteins to be analyzed were determined from concentration (increasing amounts of proteins)/response (optical density of the band) curves from two rats both belonging to a particular group (on the same gel). All gels were run in triplicate and identical amount of protein for each sample were loaded in the gel. Data were expressed as arbitrary units and were calculated from a representative gel.

Data and statistical analysis

SigmaPlot 11.0 was used for statistical analysis and GraphPad Prism 6 for all graphs. Data were expressed as means ± standard deviations (SD) for protein expression. Differences between two groups were assessed using parametric *t* test or non-parametric Mann–Whitney test, depending on the normality and equal variance tests.

Pearson's and Spearman's correlations were used to measure the strength of the relationship between paired data, depending on normality distribution. In correlation description, *r* represents Pearson correlation coefficient and r_s, spearman correlation coefficient.

For the NOR test, difference between time spent exploring F and N objects were assessed by a Wilcoxon matched paired signed rank test.

A value of $p < 0.05$ was considered statistically significant.

Results

Exercise intensity effect on memory

Novel object recognition (NOR) test

No statistical difference was obtained concerning the total exploration time at training and testing steps between each group of rats (data not shown). For the testing phase (Day 3), two different objects were presented and the time spent to explore each was recorded. Our data showed that in physiological conditions, the times spent exploring the N and F objects were not statistically different in SED and EX animals indicating that EX did not improve memory in healthy animals (data not shown). However, when treated with scopolamine (Fig. 2), the EX18 group of rats exhibited a significant increase in terms of exploration duration of the N compared to the F object (6.22 ± 3.76 vs. 3.99 ± 1.58, $p < 0.01$).

Of note, no difference was observed in terms of locomotor activity (actimetry) and anxiogenic response (marble burying test) in the different treated groups of animals (data not shown).

Effect of scopolamine treatment on BDNF expression

Since scopolamine has been shown to exert contradictory effect on BDNF expression (Ghosal et al. 2018; Lee et al. 2014), BDNF expression was assessed in the HP and the PFC of SED rats receiving two i.p. injections of scopolamine (1 mg/kg, daily). As shown in Fig. 2b, no variation in BDNF expression could be observed either in the PFC or in the HP.

Exercise intensity effect on BDNF signaling

Hippocampus (HP)

BDNF, p-TrkB^{Y816} and SYN levels were assessed in the HP of SED animals and compared to EX12 or EX18 groups. The results of the effect of EX on BDNF signaling in the HP are presented in Fig. 3.

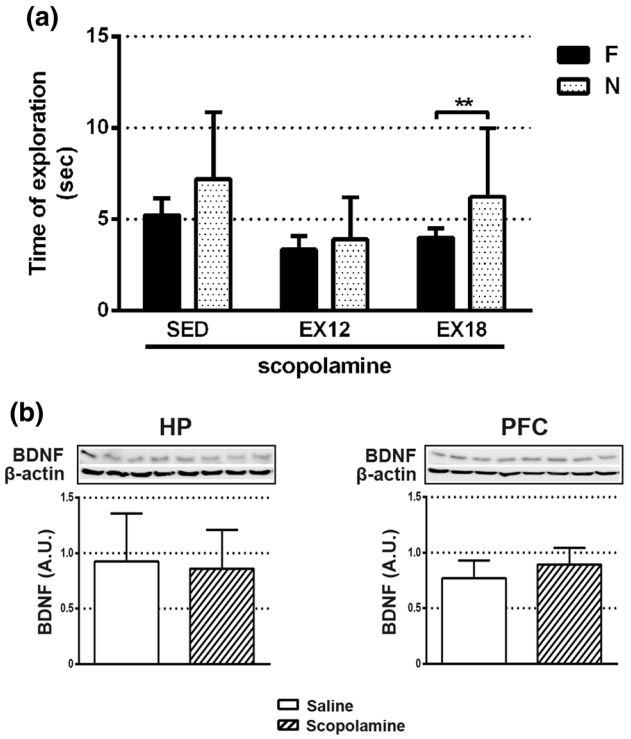

Fig. 2 Effect of exercise on scopolamine-induced memory impairment in the novel object recognition (NOR) test. **a** The times spent to explore the novel (N, dotted) and familiar (F, white) objects during day 3 of novel object recognition test, were compared for each groups: sedentary (SED), exercised at 12 m/min (EX12), exercised at 18 m/min (EX18) treated with scopolamine hydrobromide 1 mg/kg (10 rats/group). **b** BDNF expression in the hippocampus (HP) and prefrontal cortex (PFC) of SED rats treated or not with scopolamine hydrobromide 1 mg/kg. (4 rats/group). *A.U.* arbitrary units. $^{**}p < 0.01$ compared to exploration time of F object

BDNF, p-TrkB^{Y816}, SYN

As shown in Fig. 3, both intensities of EX led to a significant increase in BDNF (Fig. 3a, b), p-TrkB^{Y816} (Fig. 3d, e) and SYN (Fig. 3g, h) expressions. These variations were dependent on EX intensity for BDNF and SYN since for both proteins, EX12 induced an increase in protein levels significantly lower than EX18 (Fig. 3c, i). Concerning p-TrkB^{Y816} expression, no significant intensity effect was observed when p-TrkB^{Y816} expression was compared by plotting EX12 and EX18 groups on the same membrane (Fig. 3f).

c-fos

To verify whether increase in neuronal activity was proportionated to the intensity of EX, c-fos expression was assessed in EX12 and EX18 groups and compared to SED animals. As shown in Fig. 4, both intensities of EX led to a sustained c-fos expression compared to SED values. Indeed, EX12 exhibited an increase of 97% (Fig. 4a) whereas EX18 rats showed an elevation of c-fos expression of 133.5% (Fig. 4b). When compared on the same membrane, no significant difference was obtained between the two modalities of EX (Fig. 4c).

p-eNOS^{S1177}

When p-eNOS^{S1177} was assessed as a marker of shear stress elevation in hippocampal structure (Fig. 4), our results revealed that although both intensities enhanced endothelial NO production, only EX18 reached significance with a 216.3% increase compared to SED group (Fig. 4e). Consistently, when both intensities were analyzed on the same membrane, p-eNOS^{S1177} expression in EX18 group exhibited a strong rise compared to EX12 group (Fig. 4f).

Prefrontal cortex (PFC)

BDNF, p-TrkB^{Y816} and SYN levels were assessed in the PFC of SED group and compared to trained EX12 and EX18 animals (Fig. 5).

BDNF, p-TrkB^{Y816}, SYN

As shown in Fig. 5, among the different groups of trained animals, only EX18 rats exhibited a significant increase in BDNF (Fig. 5a, b), p-TrkB^{Y816} (Fig. 5d, e) and SYN (Fig. 5g, h) expressions as compared to SED animals. When both groups of EX were compared on the same membrane, significant increase in BDNF and p-TrkB^{Y816} expressions were revealed for EX18 compared to EX12 rats (Fig. 5c, f). Concerning SYN expression, EX18 showed no significant augmentation compared to EX12 group (Fig. 5i).

c-fos

As shown in Fig. 6, both groups of trained animals exhibited a significant increase in c-fos expression (+71%, EX12 and +117.1%, EX18) compared to SED values. When EX12 and EX18 were compared on the same membrane, a significant difference of 91% (Fig. 6c) was observed.

p-eNOS^{S1177}

As assessed in Fig. 6, both intensities led to an increase in p-eNOS^{S1177} expression. As compared to SED values, EX12 induced an increase of 79.3% whereas EX18 showed a rise of 227.1% in p-eNOS^{S1177} expression (Fig. 6d, e). When both intensities were compared on the same membrane, p-eNOS^{S1177} expression was significantly higher in EX18 (+126.2%) compared to EX12 animals (Fig. 6f).

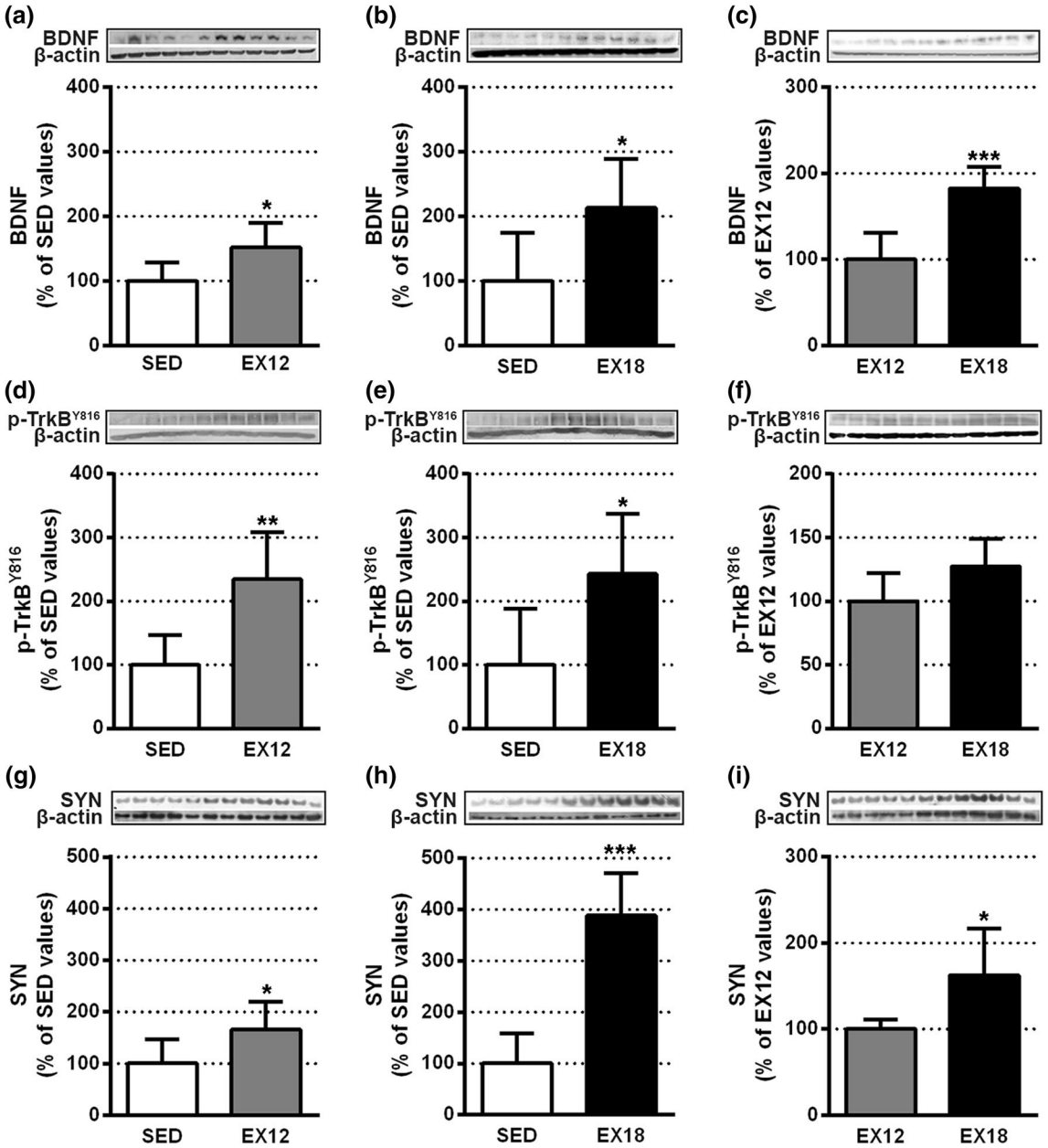

Fig. 3 Effect of exercise on BDNF/p-TrkB^{Y816}/synaptophysin pathway in hippocampus. BDNF (**a**–**c**), p-TrkB^{Y816} (**d**–**f**) and synaptophysin (SYN, **g**–**i**) levels in the hippocampus of trained rats at 12 (EX12) and 18 m/min (EX18) compared to sedentary rats (SED). Corresponding immunoblots of the proteins of interest and β-actin as the internal control are shown above the bar charts. Values are expressed as mean ± SD (6 rats/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to SED or EX12 values

Association between BDNF and SYN expressions in both cerebral structures

To assess whether BDNF expression in the HP and PFC could be linked to neuroplastic changes, association between BDNF and SYN expressions were performed using correlation test (data not shown). Regarding the HP, positive associations were found when values of SED animals were plotted against EX12 ($r = 0.752$, $p = 0.004$) and EX18 ($r_s = 0.792$, $p = 0.001$) groups of animals.

In the PFC, BDNF was positively associated to SYN expression when SED animals were plotted against EX18 ($r = 0.859$, $p = 0.0003$) but not against EX12 ($r = 0.569$, $p = 0.053$) groups of animals.

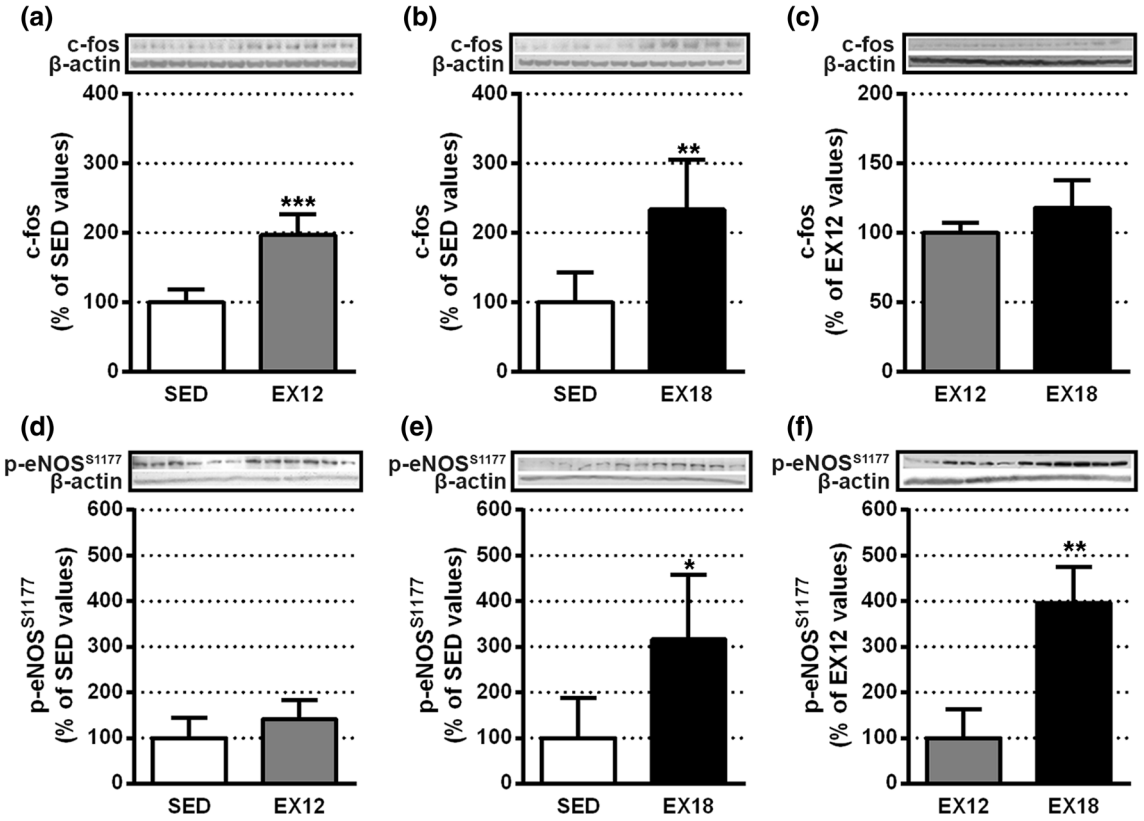

Fig. 4 Impact of exercise intensity on c-fos and p-eNOS^{S1177} expressions in hippocampus. Assessment of c-fos (**a**–**c**), and p-eNOS^{S1177} (**d**–**f**) levels in the hippocampus of sedentary (SED) and trained rats exercised at 12 (EX12) and 18 m/min (EX18). Corresponding immunoblots of proteins of interest and β-actin as the internal control are shown above the bar charts. Values are expressed as mean ± SD (6 rats/group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to SED or EX12 values

Discussion

The present study revealed that only the most intense EX (horizontal treadmill at 18 m/min, 30 min a day, 7 consecutive days) improves memory performance assessed by NOR test and showed in both cerebral structures analyzed, that BDNF/TrkB/SYN pathway is dependent on intensity modality of EX with a gradual effect in the HP whereas in the PFC, only the highest intensity (EX18) led to a significant activation of this pathway.

Our data extend previous results showing the induction of the BDNF/TrkB pathway by EX (Monnier et al. 2017a; Pedard et al. 2018; Prigent-Tessier et al. 2013; Quirie et al. 2012). In our experimental setting, our results provide strong evidences showing that this elevation was dependent on EX intensity since in both cerebral structures, BDNF and p-TrkB^{Y816} expressions were always higher in EX18 compared to EX12 group of animals. However, concerning animals exercised at low intensity, biochemical assessments gave different results according to the cerebral structure analyzed. Indeed, while in the HP, EX at 12 m/min was sufficient to significantly increase the expression of these markers, no statistical effect could be observed in the PFC. This difference of sensitivity to EX between these two brain areas could be explained by structural/histological difference but also by discrepancy in terms of neuronal activation and/or elevation of cerebral blood flow. Indeed, when analyzing neuronal activation through c-fos expression, our data showed in HP that the EX12 regimen already triggered a sustained increase statistically comparable to EX18 whereas in PFC, an intensity-dependent neuronal activation was reported. Concerning cerebral blood flow increase that has been shown to elevate brain BDNF expression through a NO-dependent mechanism (Banoujaafar et al. 2014, 2016), our p-eNOS^{S1177} expression results are coherent with studies on cerebral hemodynamic changes occurring during EX showing that EX induced hyperemia in cortical (Kimura et al. 1994) but also in sub-cortical regions such as the HP (Nakajima et al. 2003; Nishijima and Soya 2006). Consistently, the two cerebral structures analyzed in our study exhibited a similar intensity-dependent activation of eNOS. Consequently, the differences in terms of BDNF/p-TrkB^{Y816} expressions observed at the low intensity might only relate to a disparity in neuronal activation between the two structures.

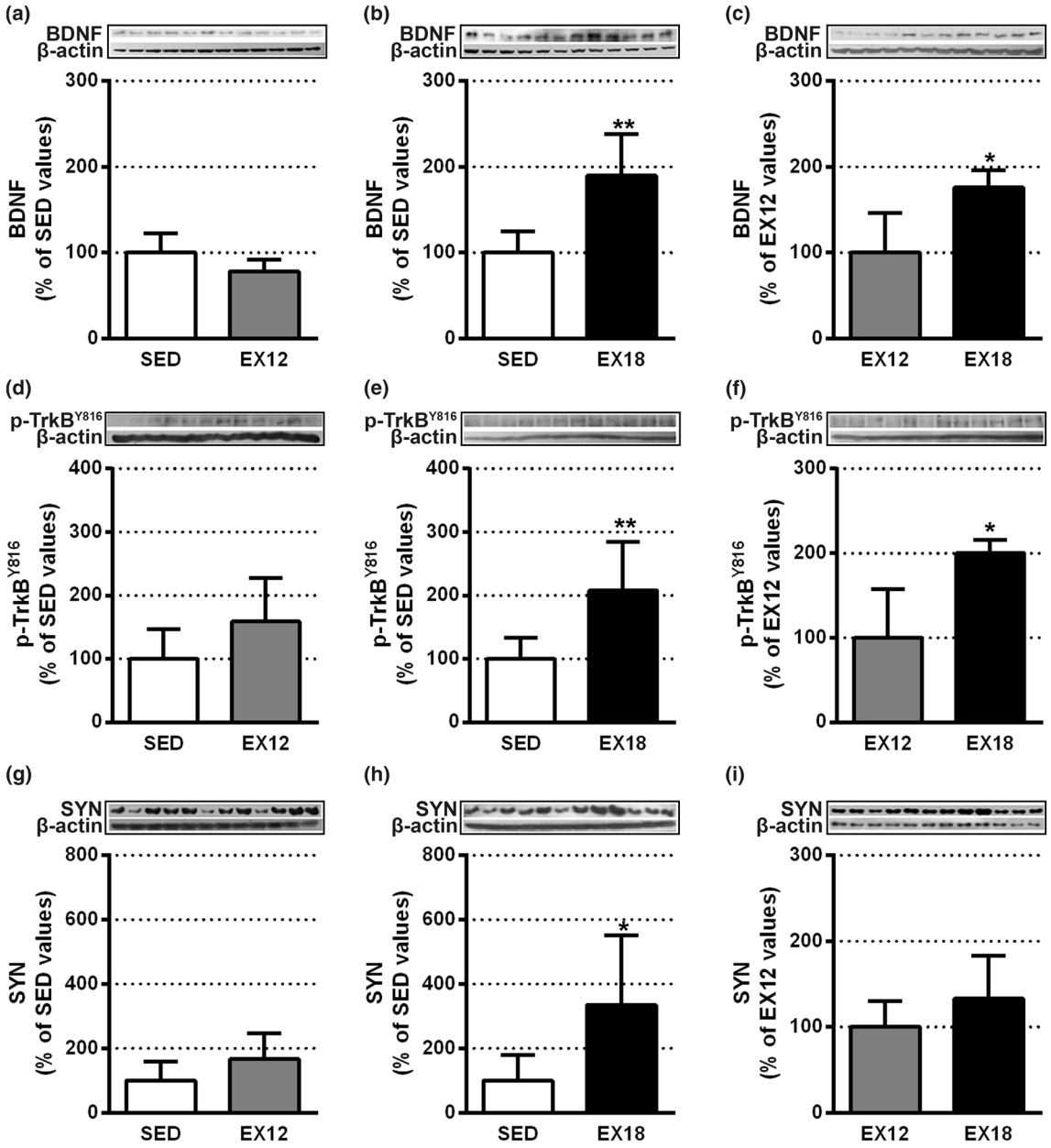

Fig. 5 Effect of exercise intensity on BDNF/p-TrkB^{Y816}/SYN pathway in prefrontal cortex. BDNF (**a**–**c**), p-TrkB^{Y816} (**d**–**f**) and synaptophysin (**g**–**i**) levels in the prefrontal cortex of trained rats at 12 (EX12) and 18 m/min (EX18) compared to sedentary rats (SED). Corresponding immunoblots of the proteins of interest and β-actin as the internal control are shown above the bar charts. Values are expressed as mean ± SD (6 rats/group). *$p < 0.05$, **$p < 0.01$ compared to SED or EX12 values

Although not investigated directly in this work, BDNF/TrkB activation may be coupled to SYN induction as a reflect of synaptogenesis (Banoujaafar et al. 2014) and increase in synaptic activation since it has been reported that blocking BDNF action abrogates exercise-induced SYN expression (Vaynman et al. 2006) and that BDNF knockout mice have a reduced level of SYN in hippocampal synaptosomes (Pozzo-Miller et al. 1999). Consistently, a pattern of SYN expression similar to those of BDNF/TrkB was observed in the two brain areas. The EX regimen performed at the maximal intensity always induced the strongest elevation of SYN expression in both prefrontal and hippocampal regions. A graduated and significant increase was obtained in the HP whereas no statistical effect could be found in the PFC at the low intensity. In addition, when exercised animals were plotted against SED animals, a positive association between BDNF and SYN expressions was found for both intensities in the HP but only at the maximal intensity

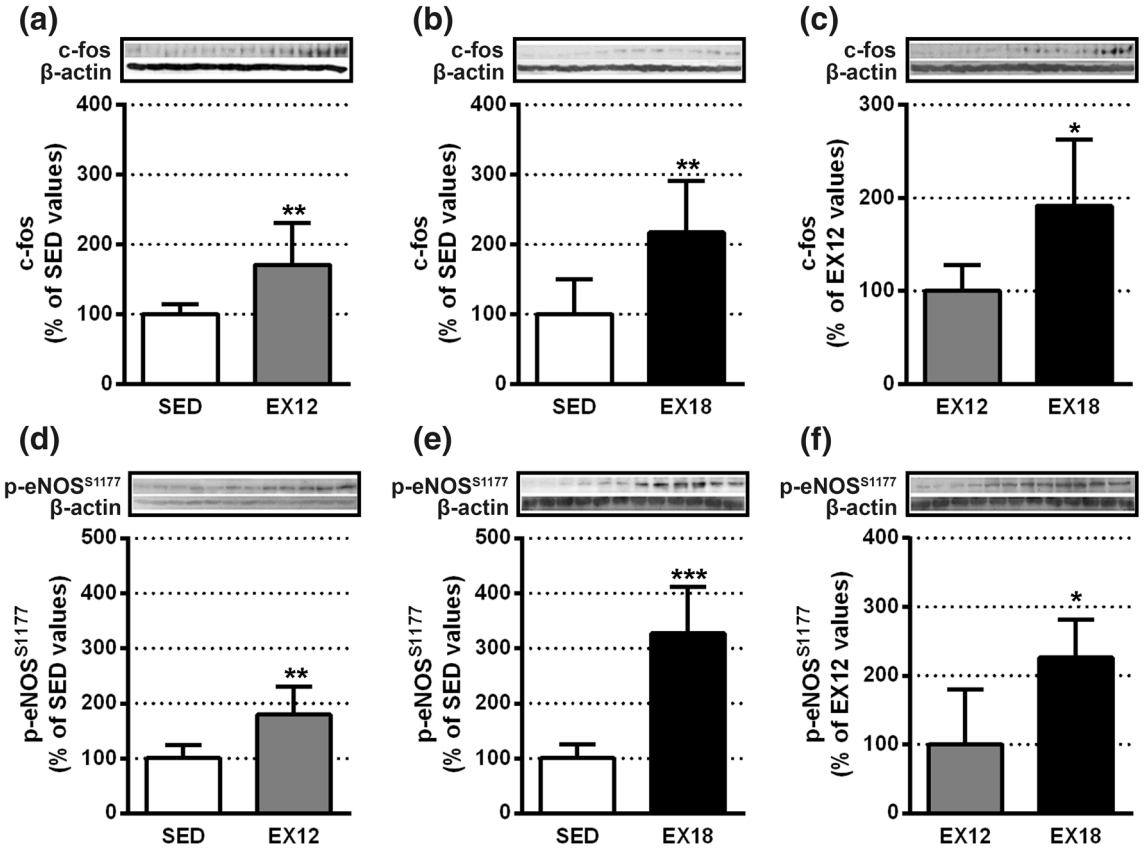

Fig. 6 Impact of exercise intensity on c-fos and p-eNOS^{S1177} expressions in prefrontal cortex. Assessment of c-fos (**a**–**c**), and p-eNOS^{S1177} (**d**–**f**) levels in the prefrontal cortex of sedentary (SED) and trained rats exercised at 12 (EX12) and 18 m/min (EX18). Corresponding immunoblots of proteins of interest and β-actin as the internal control are shown above the bar charts. Values are expressed as mean ± SD (6 rats/group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to SED or EX12 values

in the cortical region. This discrepancy in terms of intensity-dependent-neuroplastic response in these two different brain structures is in line with the previously observed disparity in terms of BDNF/TrkB pathway related possibly to a differential neuronal activation in these two cerebral regions at the low intensity of EX.

Finally, to characterize whether neuroplastic biochemical changes had a direct impact on cognitive function, rats from SED and exercised groups were subjected to NOR test. Of note, since EX by itself was not able to improve cognition in healthy animals (data not shown), animals dedicated to behavioral tests were treated with scopolamine to induce memory impairments. In terms of memory performance, our data showed that only the EX18 group of animals exhibited cognitive improvement as assessed by a significant preference for the exploration of the novel object. Given the prominent role of HP in memory, the absence of effect on cognitive function at the low intensity (EX12) despite an increase in BDNF/TrkB/SYN pathway questions the pertinence of the study of these neuroplastic biochemical markers as a reflect of potential cognitive improvement in this cerebral area. Consistently, this cerebral region has been shown to be not always critical for one-trial object recognition (Dere et al. 2007). In contrast, since the PFC has been reported to be anatomically related to the HP and to operate in parallel in memory consolidation (Preston and Eichenbaum 2013) and according to its important role especially in recent memory tests (Euston et al. 2012), our data showing an induction of the BDNF/TrkB/SYN pathway only at EX18 suggest that neuroplastic biochemical changes in this cerebral region would be a better mirror of potential cognitive improvement that those observed in the HP. In line with this proposal are the data showing that the PFC is needed in recent acquired memories stabilization but could play an even greater role in memory retrieval (Euston et al. 2012). Consistently, when performing object recognition task in rats, chemical disruption of the PFC in its ventromedial part through the infusion of protein synthesis inhibitor or *N*-methyl-D-aspartate (NMDA) receptor antagonist is associated to both consolidation and memory retrieval impairments (Akirav et al. 2006). Besides, it also resonates with a study suggesting in humans that the PFC initiates the processing of target-related

information to facilitate object detection during decision-making procedure (Karimi-Rouzbahani et al. 2019).

As a limitation of the study design, biochemical analyses were not performed on animals treated with scopolamine and submitted to NOR test assessments. Data from the literature show contradictory results concerning scopolamine effect on BDNF expression with an increase 1 h after a single low dose (25 µg/kg) in mice PFC (Ghosal et al. 2018) and a decrease after 14 days of treatment (2 mg/kg, daily) in rats HP (Lee et al. 2014). To verify whether our scopolamine protocol had an impact on BDNF metabolism, BDNF expression was assessed in rats receiving two injections of scopolamine (1 mg/kg, daily). In our experimental conditions, no variation in BDNF expression could be observed either in the PFC or in the HP (Fig. 2b). This absence of effect is in line with data showing oppositely that muscarinic receptor agonist does not change BDNF expression (Di Liberto et al. 2017).

In conclusion, our study revealed that BDNF/TrkB/SYN pathway activation is dependent on intensity modality of EX since the EX18 groups of animals showed the greatest expression of these neuroplastic markers associated with memory improvement as assessed by NOR test. In our experimental paradigm, our data also suggest that the PFC is possibly a more pertinent cerebral structure than the HP when neuroplastic markers are used to mirror improvement in memory performance. As a clinical standpoint, when providing a rationale for the utilization of "assisted" EX to cope for instance in young individuals with memory impairment associated to substance use, intensity modality of EX should be considered as a crucial parameter.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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