TITLE: Acute physical exercise improves memory consolidation in humans via BDNF and endocannabinoid signaling

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Summary

Regular physical exercise enhances memory functions and neurogenesis in the hippocampus, an effect partially mediated by BDNF (Brain Derived Neurotrophic Factor). Acute exercise promotes the release of endocannabinoids (especially anandamide, AEA), which are small lipophilic molecules that enhance BDNF release and have been shown to improve hippocampal plasticity in rodents. How a single session of exercise affects BDNF and AEA levels, and how these biomarkers influence memory performance remains to date unknown. Here we combined blood biomarkers, behavioral and fMRI measurements to assess the impact of moderate and high intensity acute physical exercise on memory and underlying neurophysiological mechanisms in humans. We hypothesized that moderate intensity exercise would optimize memory consolidation and enhance hippocampal activity via BDNF and/or endocannabinoid signaling. We tested associative memory performance in nineteen healthy participants across three visits: each visit consisted of a learning and a test part performed in fMRI separated by a period of exercise (moderate or high intensity) or rest. A long-term memory retest took place 3 months later. We report a selective increase in memory performance after moderate but not after high intensity exercise or rest at test and at long-term retest. Acute exercise boosted both BDNF and AEA levels: AEA increase after moderate intensity exercise correlated with hippocampal activity during retrieval suggesting that AEA may have an acute effect on synaptic plasticity in human. Whereas exercise-induced BDNF correlated with enhanced hippocampal memory representations measured using a decoding approach after moderate intensity exercise and with long-term, memory effects.

Introduction

Physical exercise is a lifestyle factor that boosts neurocognitive functions and brain plasticity (Roig et al., 2013) at all ages, and may possibly reduce the risk of cognitive decline associated with Alzheimer's disease (Lautenschlager et al., 2008). Studies in animals, and more recently in humans, support that voluntary regular exercise fosters neurogenesis in the adult hippocampus, and improves learning and memory capacities (Erickson et al., 2011; Pereira et al., 2007; Uysal et al., 2005; Wu et al., 2007). Several lines of evidence converge to suggest that these effects are mediated at least in part by the brain derived neurotrophic factor (BDNF), which contributes to hippocampal synaptic plasticity (Bekinschtein et al., 2011; Vaynman et al., 2003). Specifically, physical exercise increases the levels of BDNF mRNA and protein in the hippocampus and other brain regions (Alomari et al., 2013; Bekinschtein et al., 2011; Cotman and Berchtold, 2002; Neeper et al., 1995; Soya et al., 2007), and blocking BDNF action in the hippocampus hinders the beneficial effect of exercise on memory (Vaynman et al., 2004).

Most human studies focus on the long-term effects of exercise on BDNF and cognition. Yet, measuring BDNF levels before and after a period of regular physical training cannot account for the dynamic time course of growth factor upregulation, which is thought to be fast and transient (Berg and Bang, 2004; Rojas Vega et al., 2006). In particular, BDNF levels are known to rapidly increase in hippocampal subfields in response to exercise (Cotman and Berchtold, 2002; Neeper et al., 1995), together with enhanced long-term potentiation (LTP) and synaptic plasticity (Farmer et al., 2004; Korte et al., 1996; Liu et al., 2011). These effects may mediate memory enhancement on the timescale of a few hours (van Praag et al., 1999). Associated with LTP induction, exercise also rapidly affects fine cell morphology, especially by

increasing the number and size of hippocampal dendritic spines considered to support changes in synaptic strength (Dietrich et al., 2008; Stranahan et al., 2007; van Praag, 2009).

In addition, physical exercise increases serum concentrations of endocannabinoids, which act on cannabinoid receptors CB1 and CB2 (Dietrich and McDaniel, 2004; Raichlen et al., 2012; Raichlen et al., 2013; Sparling et al., 2003). Binding to cannabinoid receptors may mediate acute anxiolysis and analgesia after running (Fuss et al., 2015). Work in animal models also implicates endocannabinoid signaling in exercise-induced adult hippocampal neurogenesis (Dubreucq et al., 2010; Hill et al., 2010). The presence of the endocannabinoid anandamide (AEA) is essential for neurogenesis and the lack of the enzyme responsible for AEA hydrolysis significantly increases cell proliferation in the dentate gyrus (Aguado et al., 2005; Aguado et al., 2006). One recent study directly linked endocannabinoid system to memory enhancement and hippocampus function in mice by showing that blocking CB1 receptor in the hippocampus disrupts spatial memory performance whereas artificially elevating endocannabinoid concentrations in sedentary animals increases BDNF levels and memory (Ferreira-Vieira et al., 2014).

Short periods of exercise, or acute exercise, were found to yield modest positive effects on learning, memory, and cognition in humans (van Dongen et al., 2016), although existing studies report a wide range of effects, from positive to detrimental (Bosch et al., 2017; Chang et al., 2012; Roig et al., 2013; Tomporowski, 2003; van Dongen et al., 2016). It is important to note that most of these studies used between-subjects comparisons with physical exercise performed at various intensities or durations, which may partly explain disparate outcomes (Basso et al., 2015). Getting a better understanding of the relevant underlying neural mechanisms may help

resolve these apparent inconsistencies while informing design efficient prevention programs. Here we aimed at puzzling out the intricate influence of physical exercise on known blood biomarkers, brain plasticity, and memory in humans. We tested nineteen participants using a cross-over randomized within-subjects design. We used a hippocampus-dependent associative memory task (Bosch et al., 2017; Igloi et al., 2015) in which participants learned series of 6 successive pictures. To assess the influence of different intensities of physical exercise, memory for these series was tested following a moderate intensity exercise session, a high intensity exercise session or a rest period with blood samples taken before and after exercise or rest. We also tested the effects of acute physical exercise on long-term memory during a surprise memory retest 3 months after the last experimental visit (see **Figure 1A**). In line with our previous results (Bosch et al., 2017), we hypothesized that moderate intensity exercise would yield the largest benefits, especially at immediate test. Further, we expected that such memory benefits would be associated with exercise-related changes in BDNF and AEA levels. AEA is known to have transient effects due to its rapid degradation by metabolic enzymes (Labar and Michaux, 2007) whereas the reported effects of BDNF are generally long-lasting (Erickson et al., 2011). We therefore predicted that increases in BDNF levels may underlie long-term memory effects.

Materials and Methods

Participants

Twenty healthy young male volunteers gave written informed consent and received financial compensation for their participation in this study, which was approved by the Ethics Committee of the Geneva University Hospitals. One

participant had to be excluded from all the analysis for non-compliance with experimental requirements. The remaining 19 participants were between 18 and 34 years old (mean age +/- standard error : 23.03+/-0.92 years). All participants were right-handed, non-smokers, free from psychiatric and neurological history, and had a normal or corrected-to-normal vision. They were within the normal ranges on self-assessed questionnaires for depression (BDI (Steer et al., 1997)), anxiety (STAI (Spielberger et al., 1983)), circadian typology (PSQI (Horne and Ostberg, 1976), and reported exercising regularly (at least twice per week). We only included participants whose VO2max was above 40ml/kg/min and below 65ml/kg/min so as to have a homogeneous sample of regularly exercising young adults (see Experimental procedure section below).

Experimental procedure

Participants first came to the lab for a VO2max procedure. During this visit, participants also performed a habituation session of the associative task. Those participants with a VO2max within the required ranges (see above) were invited to come back for three experimental visits separated by one to two weeks, according to a within-subjects design with the three Exercising Conditions (rest, moderate intensity exercise, high intensity exercise) counterbalanced across participants. For each visit, participants arrived at 08:00 AM on an empty stomach, and had breakfast consisting of coffee or tea, orange or apple juice, bread, and jam. Participants were allowed to eat as much as they desired but we controlled that they ate approximately similar amounts for all visits, they were allowed one caffeinated drink only. We did not allow them to eat any lipids to minimize inter-subject variability in endocannabinoid measures which heavily depend on lipid consumption.

At 09:00 AM, participants were comfortably installed in the scanner, and started the encoding part of the associative memory task (see below; **Figure 1A**) while fMRI data was acquired. At 09:50 AM a qualified medical doctor took a first blood sample. At 10:00 AM participants were equipped with a Polar RS800CX N device to measure heart rate and asked to rest or exercise. For the two exercise conditions, participants pedaled on a cycle ergometer (Ergoline GmbH, Bitz, Germany), the pedaling frequency was kept between 60 and 80 cycles per minute, which was shown on a small screen in front of the participant. For moderate intensity exercise, the load of the ergometer was defined so that the cardiac frequency of the participant would be at 60% of his FcMax and the participant pedaled for 30 minutes. For high intensity, participants first warmed up for 2 minutes at 50% of FcMax then the load was progressively increased over 1 minute to reach 75% of FcMax, participants pedaled at this intensity for 15 minutes then they pedaled again at 50% of FcMax for 3 minutes to cool down. For both exercise conditions the experimenters checked cardiac frequency every 3-5 minutes to adjust the resistance of the ergometer if necessary. For the rest condition, participants sat on a chair and were allowed to quietly read magazines for 30 minutes. At 10:30 AM, the medical doctor took a second blood sample and then participants performed a Psychomotor Vigilance Task (PVT) followed by the Profile of Mood States (POMS) questionnaire.

At 11:30 AM, participants underwent a second fMRI session during which memory for the associative task was tested. Participants were asked to keep a regular exercising schedule during at least 5 days before each visit. Compliance was documented by fitness tracker (Fitbit Charge HR, Fitbit, San Francisco, USA). Moreover, they were requested to refrain from intense physical activity for the 48h preceding the experimental visits.

A surprise retest fMRI session took place three months later where participant's memory was tested again; no blood samples were taken at this time point.

Associative memory task in fMRI: We adapted an associative memory task (Bosch et al., 2017; Igloi et al., 2015) consisting of two parts: encoding and test, separated by an exercise (moderate or high intensity) or rest period (**Figure 1A**). To avoid interference across experimental visits for this within subject design, we showed different pictures belonging to three specific themes, one theme per visit: "office", "shoe shop" or "house" (one theme per visit). The pictures in each theme for the experimental visits were matched in difficulty and counterbalanced across Exercising Conditions and visits (**Figure 1B**). Note that for the habituation session of the task, participants had to memorize 5 series of a "swimming pool" theme.

During the encoding session, participants were first shown 8 series of 6 pictures once, one picture at a time (2000 ms per picture), and were asked to encode each series as a whole (**Figure 1B**). Then, during three successive learning blocks, participants were shown the first picture of one series (e.g., pen, for the "office" theme; **Figure 1C**), followed by the same picture with two options for the second picture in the series (chair), one being the correct next picture and the other picture being from a different series. Participants had to select the correct next picture by pressing a left or right button. The correct picture was then shown (providing a feedback for each trial), followed by this same picture together with the two next options for the third picture in the series (desk). This continued until the last picture in the series (office building). Additionally, two control series occurred pseudo-randomly during each block during which participants were shown a picture of a given color (red, green or blue) and had then to choose the picture of the same color (**Figure 1D**). During each learning block, all 8 memory series and 2 control series were

shown once. All stimuli were designed and delivered using a MATLAB Toolbox (Cogent 2000, http://www.vislab.ucl.ac.uk/cogent_2000.php).

During the test session, participants were presented with one cue picture and two other pictures, among which they had to select the one belonging to the same series as the cue picture. The two options could represent the immediate next item in the series (direct trials) or could be separated by one or two items from the cue picture (inference of order 1 or order 2 trials; **Figure 1C**). All types of trials were shown in a randomized order, and were presented in the same format as during learning, except that feedback was not provided. In this session 16 trials of the control “color” task were included.

For the delayed retest session, 18 out of the 19 participants came back for a surprise retest in fMRI three months after the last experimental visit. Participants did not know at test that there would be a retest session. The task was identical to the test sessions, except that pictures of all three themes were now mixed in a random order. For time constraints, only half of the trials of each of the three test sessions were shown at retest, these trials were pseudo-randomly chosen from each series of pictures and included identical numbers of direct, inference 1 and inference 2 trials from each theme.

VO2max measure: A maximal incremental test was performed during a preliminary visit to the laboratory, using an electrically braked cycle ergometer (Ergometrics er800S, Ergoline, Jaeger, Germany). Respiratory gas flows and ventilation were continuously measured at the mouth on a breath-by-breath basis, using a metabolic unit (K4b^2, Cosmed, Italy), consisting of a Zirconium Oxygen analyzer, an infrared CO2 meter and a turbine flowmeter. As recommended by the

manufacturer, the gas analyzers were calibrated with ambient air and with a mixture of known gases (O_2 16 %, CO_2 5 %, N_2 as balance), and the turbine by means of a 3-l syringe. Beat-by-beat heart rate (HR) was continuously monitored by cardiotachography (Polar RS 800 CX, Polar, Finland). Gas exchange variables (V˙O2, V˙CO2, V˙E, and RER) were continuously recorded on a breath-by-breath basis and later averaged over 10-s sliding intervals for further analysis. The initial power output was 50W for 4 min, followed by increases of 25W each 2 min until achievement of 80% of maximal HR predicted by age, then 25W increments each 1 min until volitional exhaustion. The criteria for V˙O2max were RER > 1.1, plateau in V˙O2 (change of <100 mL·min-1 in the last three consecutive 20-s averages), and a HR within 10 beats·min-1 of the maximal level predicted by age. Results of this test were used to select power output for subsequent constant tests, based on the relationship between V'O2 and power output.

Blood samples: Blood was collected into one Becton Dickinson (BD) Vacutainer clot activator tube (CAT; for serum collection) and one BD Vacutainer K_2EDTA 5.4 mg tube (for plasma collection), before and after the rest or exercise period. Seven mL of blood were collected into a BD Vacutainer clot-activator tube (CAT), allowed to clot for 30 minutes at room temperature and centrifuged at 1100 g for 15 minutes at 4°C. Serum was collected from the supernatant in aliquots of 200µL and frozen at -80°C until analysis. Another 5mL of blood were collected into a BD Vacutainer K_2EDTA 5.4 mg tube and centrifuged immediately at 8009g for 10 min. Plasma was collected from the supernatant in aliquots of 200µL frozen at -80°C until analysis. All samples were centrifuged in a Heraeus Biofuge Stratos (ThermoFisher) centrifuge. The Quantikine ELISA Human Free BDNF kits (R&D sytems) were used to quantify

serum BDNF via an enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions.

AEA and 2-AG were extracted from 100 µl of plasma by liquid-liquid extraction, and then separated by liquid chromatography (Ultimate 3000RS, Dionex, CA, USA). Analyses were performed on a 5500 QTrap® triple quadrupole/linear ion trap (QqQLIT) mass spectrometer equipped with a TurboIon-SprayTM interface (AB Sciex, Concord, ON, Canada).

Behavioral analysis

All behavioral analyses were performed using Statistica (Version 12, www.statsoft.com, StatSoft, Inc. TULSA, OK, USA). Repeated-measures ANOVA were performed and Neuman-Keuls post-hoc comparison methods were used. Correlations were performed using the Pearson's R. Non parametric tests were used when normal distribution or equal variance criteria were not met.

Functional MRI data acquisition and analysis

MRI data were acquired on a 3 Tesla MRI scanner (SIEMENS Trio® System, Siemens, Erlangen, Germany) with a 32-channel head coil. T2*-weighted fMRI 2D images were obtained with a multiband gradient echo-planar sequence acquiring 3 slices at a time using axial slice orientation (66 slices; voxel size, 2 x 2 x 2 mm; repetition time (TR) = 1880 ms; echo time (TE) = 34 ms; flip angle (FA) = 60°). A whole-brain structural image was acquired at the end of the first test part with a T1-weighted 3D sequence (192 contiguous sagittal slices; voxel size, 1.0 x 1.0 x 1.0 mm; TR = 1900 ms; TE = 2.27 ms; FA = 9°).

Conventional fMRI analysis: Functional images were analyzed using SPM12 (Wellcome Department of Imaging Neuroscience, London, UK). This analysis

included standard preprocessing procedures: realignment, slice timing to correct for differences in slice acquisition time, normalization (images were normalized to an MNI template), and smoothing (with an isotropic 8-mm FWHM Gaussian kernel) – except for the decoding analysis where we used unsmoothed images (see below). While scanning was not performed right after physical exercise or rest but about 1 h later, we nevertheless performed corrections to regress out potential physiological artifacts from heart rate and breathing using Retroicor (Glover et al., 2000) and RVHcorr (Birn et al., 2006; Birn et al., 2008), respectively. A general linear model (GLM) approach was then used to compare conditions of interest at the individual level, each individual GLM included correct trials separated according to Relational Distance (Direct, Inference 1, Inference 2 trials), control trials and missed trails (pooled across Relational Distance), plus 6 movement regressors, 5 heart rate regressors and 1 breathing regressor as regressors of non-interest. Then, contrasts between conditions of interest from each participant entered a second-level random-effects analysis. All activations are reported at $p<0.001$ with a cluster size of 10 voxels and relevant regions, especially the hippocampus, survived small-volume correction (SVC) for familywise error ($p < 0.05$) using volumes based on the Anatomy toolbox of SPM12 (SPM Anatomy toolbox 2.2, Forschungszentrum Jülich GmbH). Coordinates of brain regions are reported in MNI space.

Psychophysiological Interaction analysis: Psychophysiological interaction (PPI) analysis was computed to test the hypothesis that functional connectivity between a seed region and the rest of the brain differed according to Exercising Condition during the retest session. Therefore, we took as psychological factor the contrast between Moderate intensity exercise and Rest, irrespective of trial type (direct, inference 1 and inference 2 trials). A new linear model was prepared for PPI

analyses at the individual level, using three regressors. The first regressor represented the psychological factor, composed of Moderate intensity exercise vs Rest hits. The second regressor was the activity in the seed region. The third regressor represented the interaction of interest between the first (psychological) and the second (physiological) regressor. To build this regressor, the underlying neuronal activity was first estimated by a parametric empirical Bayes formulation, combined with the psychological factor and subsequently convolved with the hemodynamic response function (Gitelman et al., 2003). The model also included movement parameters. A significant psychophysiological interaction indicated a change in the regression coefficients between any reported brain area and the reference region, related to the correct retrieval after Moderate intensity exercise versus after Rest trials. Next, individual summary statistic images obtained at the first-level (fixed-effects) analysis were spatially smoothed (6 mm FWHM Gaussian kernel) and entered a second-level (random-effects) analysis using ANOVAs to compare the functional connectivity between groups.

Decoding analysis: A decoding procedure was performed on unsmoothed data. For each session of each participant, the timeseries of all voxels within the bilateral hippocampus region of interest, which was defined in the Anatomy toolbox as the union of CA1, CA2, CA3 and DG regions, were extracted. Timeseries were detrended and demeaned, then the movement parameters obtained from realignment and breathing parameters from retroicor and RVHcorr were regressed out. Estimates of the BOLD response for each single trial were then computed to obtain a “voxel by trial matrix”, from which the mean BOLD response for each type of trial (correct, incorrect, and control) was computed per voxel. Decoding accuracy was obtained by first applying a leave one out procedure, computing a mean “voxel by trial type

matrix" for all participants but one. A standard cross-validation procedure was then performed for each trial of the left out participant and it was classified as a trial type where the highest Pearson R correlation was found. Overall, we obtained percentages of trials classified as correct trials, incorrect trials, and control trials for each trial type, which were used for statistical analysis of sensitivity (true positive rate) and specificity (true negative rate). To assess whether there was a laterality effect in the hippocampus, we subsequently ran analyses using the left and right hippocampus as separate regions of interest.

Results

Learning

Hit rates and efficiency (i.e. hit rate divided by reaction time) were analyzed using a repeated measure ANOVA with Learning Blocks (block 1, block 2, block 3) and Visit theme (office, shoe shop, house) as within-subjects factors. Both analyses revealed a main effect of Block (hit rate: $F(2, 36)=26.40$, $p<0.001$; efficiency: $F(2, 36)=19.66$, $p<0.001$), consistent with a progressive learning of the associations, but no effect of Visit theme (hit rate: $F(2, 36)=0.62$, $p=0.54$; efficiency: $F(2, 36)=0.07$, $p=0.93$) and no interaction (hit rate: $F(4, 72)=0.36$, $p=0.84$; efficiency: $F(4, 72)=0.91$, $p=0.46$). Importantly, there was no main effect and no interaction with subsequent physical exercise neither for hit rate nor for efficiency (all $p>0.05$) when this factor was added as repeated measure to the previous ANOVA. Overall, during the third block, participants reached a high level of performance (hit rate ± standard error: 86.97 ± 1.56%), suggesting a good encoding of the series, well above chance level.

Test

Hit rate and efficiency data from the test session were analyzed using two repeated-measures ANOVAs with Exercising Condition (rest, moderate intensity exercise, high intensity exercise) as repeated measures and Relational Distance (direct, inference 1, inference 2) as within-subjects factor. For hit rate, we found a trend for a main effect of Exercising Condition ($F(2, 108)=2.09$, $p=0.12$), no effect of Relational Distance or interaction between both factors (all $p>0.05$; **Figure 2A**). For efficiency, we report a significant effect of Exercising Condition ($F(2, 108)=5.27$, $p=0.006$), no effect of Relational Distance ($F(2, 54)=0.34$, $p=0.71$) and no Exercising Condition x Relational Distance interaction ($F(4, 108)=0.04$, $p=0.99$; **Figure 2B**). Post-hoc analyses revealed that participants performed better in the moderate than in the rest and in the high Exercising Condition ($p_{mod\text{-}rest}=0.03$, $p_{mod\text{-}high}=0.006$). Taken together, these results indicate that moderate exercise could offer a favorable condition for memory consolidation processes.

Psychomotor vigilance test (PVT) and Profile of Mood States questionnaire (POMS)

We report no difference in PVT as a function of Exercising Condition (rest, moderate, high), neither in mean or median reaction times, number of lapses, or number of false alarms (one way repeated measures ANOVAs, all $p>0.05$) suggesting that participants did not significantly differ in vigilance state after rest or physical exercise. For the POMS questionnaire, there was no difference for any of the parameters that we measured (Fatigue, Tension, Confusion, Vigor; repeated measures ANOVAs with POMS parameters and Exercising Condition as repeated measures, all $p>0.05$) showing that the physical exercise sessions did not result in excessive fatigue or tension.

Blood samples

Blood samples were taken right before and after the rest and exercise sessions. The first blood sample served as a baseline measure (especially for endocannabinoids known to substantially fluctuate according to diet and other environmental factors). The values reported here were obtained for each visit by subtracting the first blood sample (baseline measure) from the second blood sample. Repeated-measures ANOVA were performed for each biomarker that was measured with Exercising Condition (rest, moderate, high) as within-subjects factor.

For the endocannabinoid Anandamide (AEA), a main effect Exercising Condition ($F(2, 36)=8.30$, $p=0.001$; **Figure 3A**) was found. Post-hoc analyses revealed that all Exercising Conditions differed significantly from each other (all $p<0.05$). For the endocannabinoid 2-Arachidonoylglycerol (2-AG), there was no effect of Exercising Condition ($F(2, 36)=3,2289$, $p>0.05$), consistent with previous descriptions in the literature (Sparling et al., 2003). For the Brain Derived Neurotrophic Factor (BDNF), we report a main effect of Exercising Condition ($F(2, 36)=4.6$, $p=0.02$; **Figure 4A**). Post-hoc analyses revealed that, as for AEA, all Exercising Conditions differed significantly from each other (all $p<0.05$).

Heart rate and breathing analysis

During the rest period participants' heart rate was at 34.4+/-3.9% of their maximal heart rate as assessed by the VO2max procedure (see Materials and Methods). During moderate and high intensity physical exercise, participants pedaled at 68.7+/-1.1 % and 77.7+/-1.8% of their maximal heart rate, respectively. One way repeated-measures ANOVA revealed a significant difference in heart rate between the 3 Exercising Conditions (rest, moderate, high intensity exercise; $F(2, 36)=1865.7$,

p<0.001). Post-hoc analyses confirmed that the three Exercising Conditions differed from each other (all p<0.001). We also recorded heart rate during the test part and found no difference in heart rate as a function of the Exercising Condition (all p>0.05), suggesting that all participants' heart rate was back to baseline at test (i.e., at least 60 min after the completion of the exercise session).

Functional MRI results

We first performed standard general linear model analysis with data collected after rest, moderate intensity exercise, and high intensity exercise modelled as separate sessions. Within each session, we considered correct trials according to Relational Distance (direct, inference 1, inference 2) and control trials as four separate regressors of interest, and included incorrect trials as an additional regressor. When comparing high Relational Distance to low Relational Distance (inference 2 > direct trials) across all sessions, we found increased activity in the right hippocampus [z score=3.64 (18, -38, -8), p<0.05 SVC], bilateral parahippocampal gyrus and precuneus (see supplemental **Figure 1**, and **Table 1** for exhaustive list of activations). No region was activated (at a threshold of 0.001 unc.) when comparing inference 1 to direct trials, and inference 2 to inference 1 trials. Comparisons between Exercising Conditions and interactions between Relation Distance and Exercising Conditions did not yield any significant activation either.

As it is known that AEA has a rapid effect on synaptic plasticity in the hippocampus, we tested whether the observed difference in AEA across Exercising Conditions might exert a modulating influence on brain activity. We thus added individual AEA change as a cofactor in the second-level analyses comparing Exercising Conditions. We found that the increase in AEA after moderate intensity

exercise (vs. rest) correlated with the activation in the right hippocampus [z-score=3.89 (38, -14, -20), $p<0.05$ SVC], **Figure 3B**. A similar modulation of hippocampal activity was found for high intensity exercise (vs. Rest) [z-score=3.41 (32, -22, -20), $p<0.05$ SVC], suggesting that AEA increase correlates robustly with hippocampal activation, **supplemental Figure 2**.

A decoding approach was used to test whether exercise would affect the classification of single trials (as correct, incorrect, or control trials; see Materials and Methods section) from activity within the bilateral hippocampus region. For a classification with 3 possible outcomes, chance level was at 33.33%. Decoding of correct trials was at chance level after rest and high intensity exercise but significantly above chance level after moderate intensity exercise (**Figure 4B**). Two repeated-measures ANOVA were performed on sensitivity and specificity of our decoding results within the bilateral hippocampus with Exercising Condition (rest, moderate, high) as within-subjects factor. We report a main effect of sensitivity ($F(2, 36)=11.24$, $p<0.001$), with post-hoc analysis showing that decoding after moderate intensity exercise is higher than after both rest and high intensity exercise ($p_{mod-rest}<0.001$, $p_{mod-high}<0.001$, depicted on **Figure 4B**). Specificity did not yield any main effect of Exercising Condition ($F(2, 36)=0.88$, $p=0.423$). Similar results were obtained when performing decoding from activity in the left and in the right hippocampus separately, with better decoding sensitivity after moderate exercise than rest or high intensity exercise (see **Supplemental Figure 3**).

Because decoding was done in the hippocampus and BDNF is known to specifically enhance plasticity mechanisms in the hippocampus, we tested for a relationship between these two variables. We report a positive correlation between BDNF enhancement during moderate intensity exercise (calculated as the difference

between moderate and rest BDNF values, with baseline values subtracted for each visit) with decoding accuracy after moderate intensity exercise (R=0.57, p=0.01), **Figure 4C**.

Retest

Long-term memory was assessed in a retest session three months later. Eighteen subjects came back for the retest session. Retest was similar to the test sessions but it comprised a subset of trials from all three experimental visits (see Materials and Methods section). For the analysis of the retest session, trials were not separated according to Relational Distance as no behavioral effect related to this was previously found. A repeated-measure ANOVA was performed with Exercising Condition (rest, moderate, high) as within-subjects factor, that revealed a main effect of Exercising Condition ($F(2, 34)=3.32$, $p=0.048$). Post-hoc analyses showed that participants performed better after moderate Exercising Condition than after rest ($p_{mod\text{-}rest}=0.04$; no other comparison was significant); **Figure 5A**. It was also assessed whether participants performed above chance level during each of the three Exercising Conditions and only those trials learnt during the moderate exercise condition were remembered above chance level three months later ($t(17)=2.31$, $p=0.03$).

We asked whether physical exercise had some long-term effects on the functional coupling between the hippocampus and other brain regions during the processing of associative memories. A psychophysiological interaction analysis was performed (see Materials and Methods) taking as seed region the right hippocampal activation for moderate intensity vs. rest correlated with AEA increase reported above (38, -14, -20) and we report that the left superior frontal gyrus [z-score=3.16 (-16, 62, 6), $p<0.001$ unc.] shows such pattern of increased functional connectivity for

associations learnt during the moderate intensity exercise condition (compared to the Rest) session (**Figure 5B**).

We also hypothesized that changes in BDNF may have long-lasting effects on neurogenesis and synaptic plasticity, potentially increasing long-term memory retention. This hypothesis was tested by correlating changes in BDNF levels after moderate intensity (vs. after rest) to delayed performance increase (i.e., from Test to Retest) for moderate vs. rest Exercising Condition. We report a significant positive correlation (R=0.47, p=0.042) see **Figure 5C**, while the same correlation for high intensity exercise was not significant (R=0.005, p=0.98). These results suggest that BDNF increase after moderate intensity exercise may contribute to memory enhancement.

Discussion

We show here that one session of moderate intensity physical exercise but not high intensity physical exercise enhances associative memory, both at immediate test (2 hours after encoding) and at long-term retest (three months later). These effects may be mediated by the endocannabinoid AEA and the growth-factor BDNF, whose respective concentrations increased after acute exercise. Accordingly, during the short-term test, the increase in serum AEA concentration correlated with hippocampal activity when associative memories were recalled, and BDNF increase correlated with decoding measures within the hippocampus. Moreover, BDNF increase at test correlated with better performance at long-term retest. Overall, we show that acute physical exercise at moderate intensity has long-lasting positive effects on the consolidation of associative memories in healthy young human adults.

Below, we discuss the neurophysiological mechanisms that could explain these important findings.

Biomarker mechanisms underlying the effects of acute exercise on hippocampal plasticity

In a recent study in rodents, Fuss et al. (2015) demonstrated that physical exercise induces an acute increase of AEA measured in the plasma, with direct effects on CB1 receptors in the brain. Note that in the same study cerebro-spinal fluid measures did not capture increases in AEA, consistent with AEA being very rapidly metabolized in the brain (Labar and Michaux, 2007). These results support the fact that plasma measures of AEA, as we performed here, may reflect the effect of AEA on the brain. Another rodent study directly linked endocannabinoid signaling to hippocampal memory function, by showing that selectively blocking CB1 receptors in the rodent hippocampus abolished exercise-induced memory effects (Ferreira-Vieira et al., 2014). The same study also demonstrated that artificially increasing AEA concentrations (by blocking the Fatty Acid Amine Hydrolase (FAAH), the enzyme responsible for breaking down AEA) in the hippocampus of sedentary mice mimicked the effects of physical exercise and increased memory performance. Together, these rodent studies illuminate the neurophysiological mechanisms underlying our novel finding that AEA increase in human plasma may have direct effects on brain activity, especially in the hippocampus.

Traditionally, BDNF has been linked to effects of regular physical exercise, although it known that BDNF gene expression is upregulated both after acute and after chronic physical exercise in rodents (Molteni et al., 2002). Here we show that the effects of one single session of exercise may differentially affect both short and

long-term. On the one hand, BDNF increase after acute physical exercise correlated positively with decoding accuracy of memory items in both hippocampi immediately after exercise (test session). On the other hand, BDNF increase also correlated with long-term memory increase between Exercising Conditions (retest session), suggesting that those participants who exhibited larger increases in BDNF levels at test remembered the learnt association better at retest three months later.

How can we explain that an acute modulation of BDNF levels affects memory? It is widely acknowledged that BDNF enhances synaptic plasticity, especially via LTP (van Praag et al., 1999), which can be induced in a few minutes and critically contributes to memory consolidation (Bliss and Lomo, 1973). BDNF facilitates LTP by activating signaling pathways (including MAPK and Akt) (Mei et al., 2011), promoting cytoskeleton changes (Rex et al., 2007), and enhancing protein synthesis required for vesicle trafficking and the release of neurotransmitters (Bekinschtein et al., 2007). Several studies have now confirmed that physical exercise per se and via BDNF signaling boosts synaptic plasticity and especially LTP (Liu et al., 2011; van Praag et al., 1999). LTP heavily depends on glutamatergic NMDA receptors. On the one hand, physical exercise increases the expression of both NR2A and NR2B subtypes of the NMDA receptor in the hippocampus (Farmer et al., 2004; Molteni et al., 2002). On the other hand, NMDA receptors have been shown to be modulated by BDNF at hippocampal synapses (Levine et al., 1998). A study on mice lacking the NMDA receptor NR2A subtype showed that these knockout mice did not present an increase in neurogenesis or in BDNF levels after exercise, as opposed to wild type mice (Kitamura et al., 2003). Note that increases in AEA can also elevate BDNF levels in the hippocampus (Ferreira-Vieira et al., 2014; Tantimonaco et al., 2014)

Effect of exercise intensity

While characterizing the impact of exercise intensity on cognitive functions is critical for health recommendations, dementia prevention programs and rehabilitation strategies, the reported effects remain inconsistent. Some studies suggest that high intensity training is most efficient (Tomporowski, 2003; van Dongen et al., 2016) while other studies, especially meta-analyses, indicate that moderate exercise might have more impact (Chang et al., 2012). Here we aimed at clarifying this important issue by using a cross-over randomized within-subjects design according to which each participant was tested at a moderate and at a high intensity (plus a resting, baseline condition) across distinct sessions where associative memory was also tested.

Another concern relates to the definition of intensity, which is far from being consistent across studies, especially considering different forms of exercise performed (rowing compared with cycling or running), as highlighted in a recent meta-analysis (Szuhany et al., 2015). In the few studies where different exercise intensities were compared, some evidence suggests that moderate activity may be associated with lower risks for cerebrovascular events (hemorrhagic and ischemic) than strenuous physical exercise (Armstrong et al., 2015). Further, intense exercise may have detrimental effects particularly in the presence of cerebrovascular and metabolic risk factors (Lucas et al., 2015) or increased risk for Alzheimer's disease (Norton et al., 2014; Schmidt et al., 2013). Here, we considered moderate intensity as exercise below the ventilatory threshold (about 60-65% of individual VO2max) and high intensity as exercise above the ventilatory threshold (about 75% of individual VO2max). These criteria have the advantage of carefully defining individual exercise threshold through VO2max measure and comparing exercise below (moderate intensity) and above (high intensity) the ventilatory threshold, a threshold standardly used in physiological measurements (see Svedahl and MacIntosh, 2003 for review).

Here we observed that moderate levels of exercise intensity increased both BDNF and AEA levels and optimized cognitive processes. High intensity physical exercise also increased the measured concentrations of BDNF and AEA, but performance did not follow this increase. One possible interpretation for the maximal beneficial effect of moderate intensity exercise is that the action of these molecules follows an inverted U shape. In line with this hypothesis, Mamounas et al. (2000) showed that the BDNF dose-response curve follows an inverted U shape with intermediate concentrations of BDNF yielding best results for sprouting of serotoninergic neurons in the rodent hippocampus. For AEA, one study suggests that its anxiolytic effect also follows an inverted U-shape dose-response curve with highest concentrations (measured in the periaqueductal gray matter) being less effective (Moreira et al., 2007). The main findings of the present study provide further support for intermediate concentrations of both molecules having a maximal effect on neural functions, here for hippocampal-dependent memory formation. Of course, we cannot exclude that other biomarkers may also contribute to the observed effects, such as for example a large increase in cortisol after high intensity exercise, which may be detrimental for memory consolidation (Het et al., 2005).

Possible confounding factors due to fatigue or carry-over effects of exercise

Fatigue and reduced vigilance are known to affect cognitive performance. We sought to minimize any potential effect of exercise-related fatigue (i) by scheduling the Test part of the protocol 1 hour after the end of the physical exercise session; (ii) by including only participants who were exercising regularly and whose VO2max levels were above 45L/kg/min, so that exercise intensity and duration would not be exhausting for them. We also specifically measured fatigue and vigilance level in our participants and found that neither POMS scores for fatigue nor PVT did differ after

moderate or high intensity exercise. We also checked that heart rate and breathing rhythm of all our participants were back to baseline levels when the test session started. Nevertheless, to exclude any contaminations of heart rate or breathing on our fMRI data, we carefully regressed out these effects using Retroicor (Glover et al., 2000) and RVHcorr (Birn et al., 2006; Birn et al., 2008).

Conclusion

We show that acute moderate but not high intensity physical exercise significantly increases associative memory performance both at short and long term. At short term, hippocampal activation correlated with endocannabinoid AEA while enhanced hippocampal memory representations were associated with a modulation of BDNF. At long term, three months after encoding, memory effects were related to exercise-induced BDNF increase. We conclude that a single session of moderate physical exercise boosts associative memory formation.

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Disclosures

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Competing interests

The authors declare no competing interests.

Author contributions

B.M.B, A.B, G.F., S.S. and K.I. designed research; B.M.B, A.B., M.G.L., N.I. and K.I performed research; B.M.B., A.B., M.G.L., A.T., S.S. and K.I. analyzed data; and B.M.B, A.B, A.T., S.S. and K.I., wrote the paper.

Figures

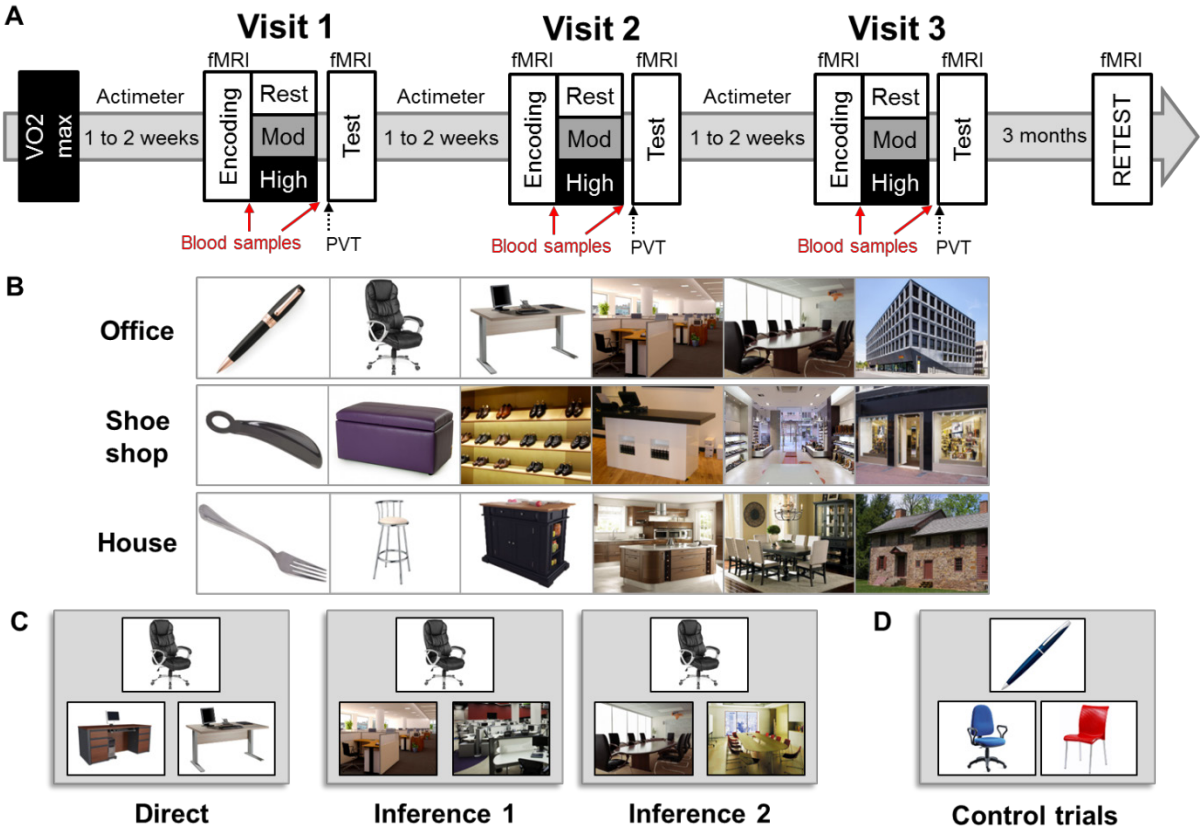


Figure 1 - Experimental design

A) Overview of the experimental protocol composed of five visits: three experimental visits preceded by a VO2max visit and a retest visit performed three months after the last experimental visit. All experimental visits started at 9AM and were composed of two MRI sessions (encoding and test) separated by a physical exercise or rest session. Physical exercise was either of moderate intensity (30 minutes cycling at 60% of FcMax) or of high intensity (15 minutes cycling at 75% of FcMax). Blood samples were taken twice at each experimental visit, before and after exercise or rest. PVT and POMS questionnaire were administered after exercise or rest. **B)** Examples of series of pictures for each theme (upper line: office, middle line: shoe shop, lower line: house). **C)** Examples of direct trials (left), inference of order 1 (middle) and 2 trials (right). Direct trials were used during the learning, test, and retest sessions, inferences 1 and 2 trials were used during test and retest sessions. **D)** Example of control trials.

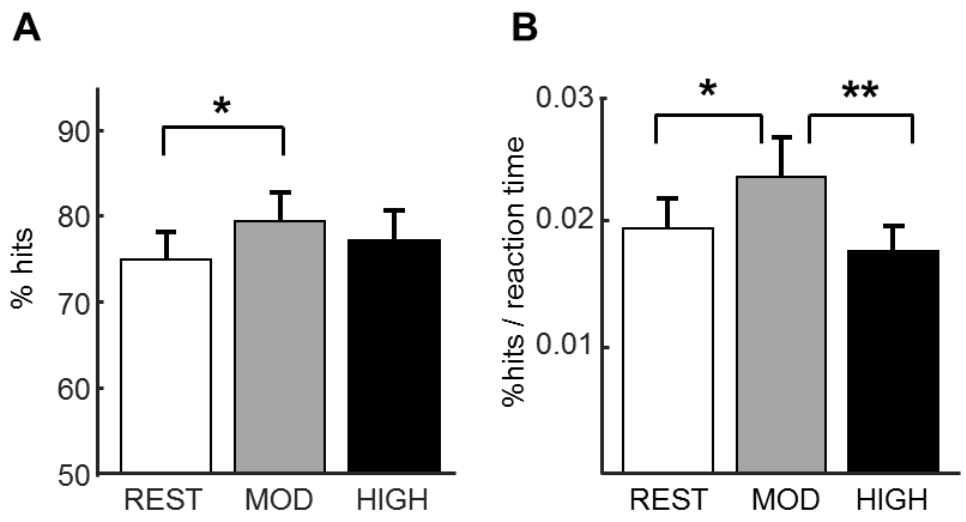


Figure 2 - Better performance after moderate intensity exercise at test

A) Performance: Better performance after moderate intensity exercise than after rest.
B) Efficiency: Better efficiency (%hits / reaction time) after moderate intensity exercise than after rest and high intensity exercise.

*: $p<0.05$, **:$p<0.01$, ***:$p<0.001$

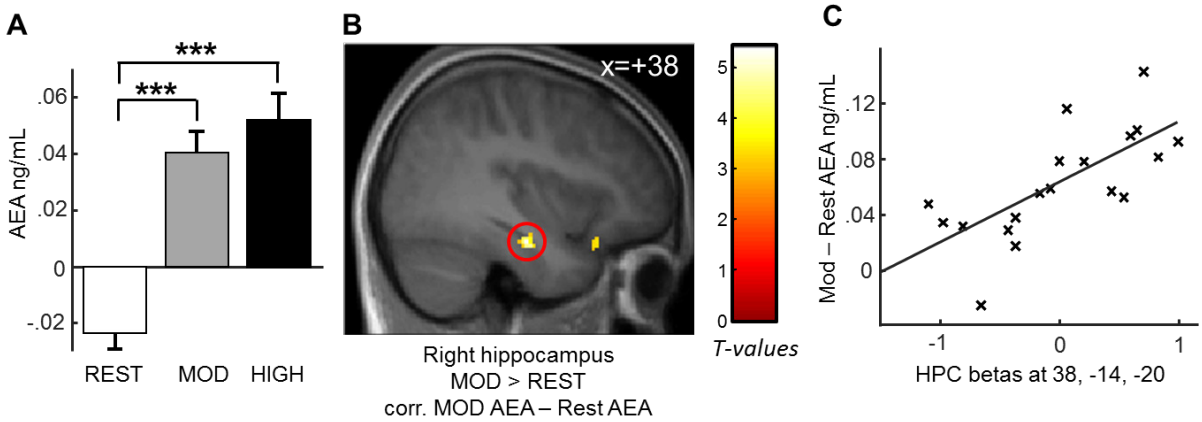


Figure 3 - Increased anandamide levels correlate with hippocampal activation after moderate intensity exercise

A) Increased Anandamide level (AEA) after moderate and high physical exercise compared to rest. **B)** Increased right hippocampal response [z-score=3.89 (38, -14, -20), p<0.05 SVC] for hits after moderate exercise compared to hits after rest correlated with the increase in anandamide level after moderate exercise. **C)** Correlation of the hippocampal beta values with increase in anandamide. Activation map displayed on the mean T1 anatomical scan of the whole population. For display purposes, hippocampal activations are thresholded at P<0.005.

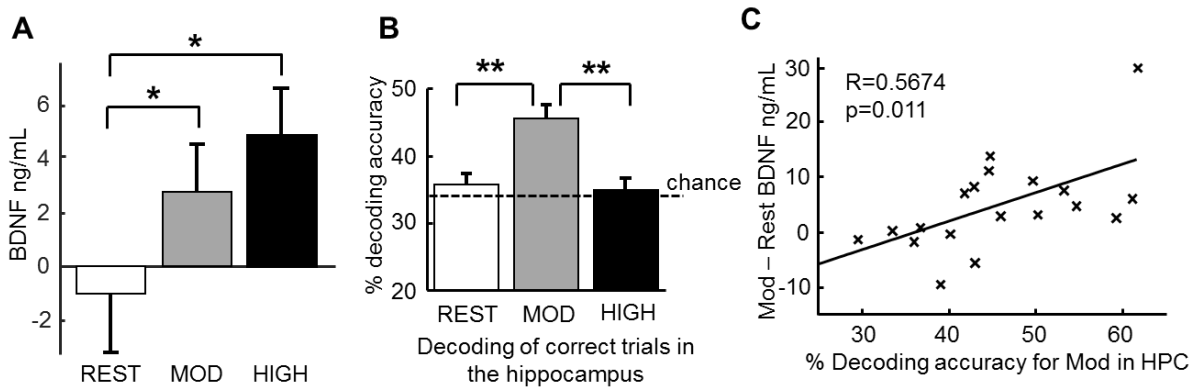


Figure 4 - Increased BDNF levels correlate with decoding accuracy in the hippocampus after moderate physical exercise

A) Increased BDNF levels after moderate and high intensity exercise compared to after rest. **B)** Better sensitivity of decoding accuracy of correct trials in the bilateral hippocampus after moderate exercise than rest and high intensity exercise. **C)** Positive correlation between decoding accuracy in the hippocampus and increase in BDNF level after moderate intensity exercise.

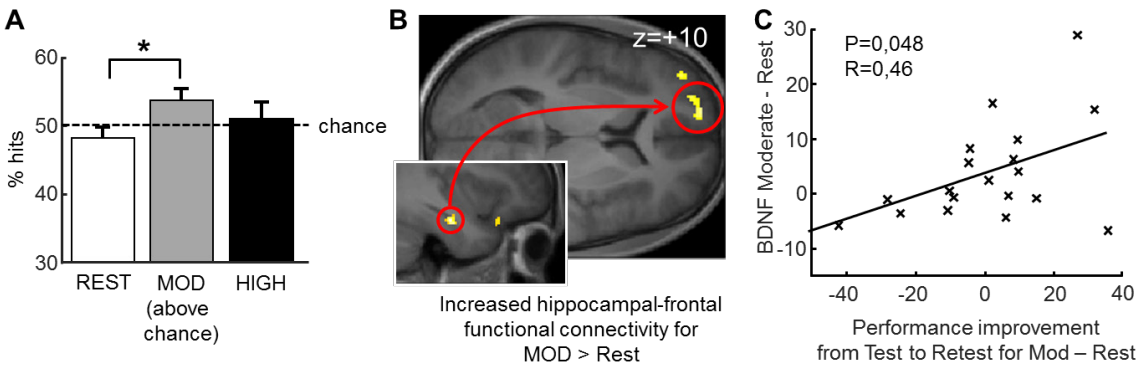
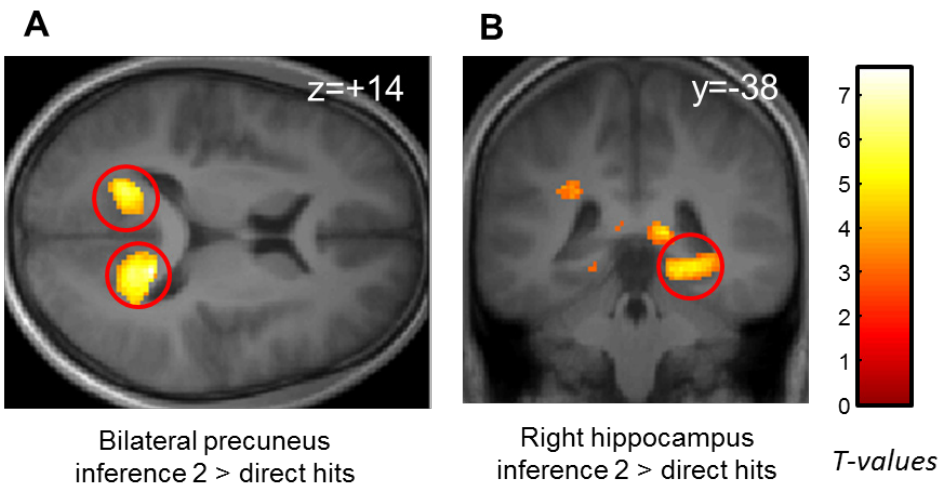


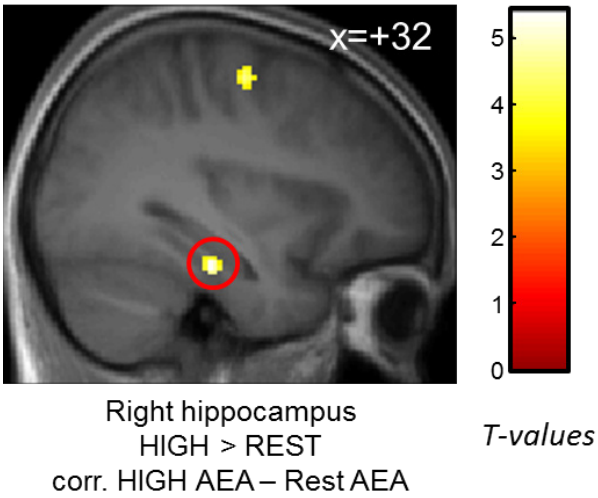
Figure 5 - Better long-term memory for associations learnt after moderate physical exercise, related to prefrontal activation and BDNF signaling

A) Better performance for pictures learnt during the moderate intensity visit than for pictures learnt during the resting visit. Performance after moderate exercise is significantly above chance level. **B)** PPI for the retest session, using the seed in the left hippocampus from Figure 3B. Increased functional coupling with the left superior frontal gyrus [z-score=3.16 (-16, 62, 6), $p<0.05$ SVC], selectively after moderate exercise compared to after rest . **C)** Performance improvement from Test to Retest for moderate exercise compared to Rest correlates with BDNF enhancement from Moderate exercise to Rest.



Supplemental Figure 1 - Brain correlates of increasing Relational Distance

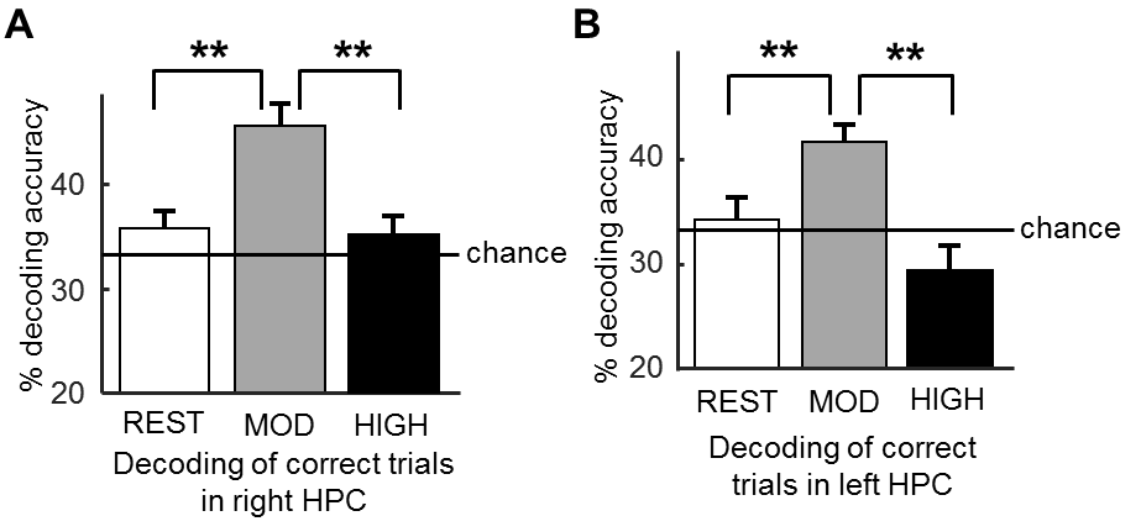
A) Bilateral precuneus activation for increasing Relational Distance (inference 2 hits > direct hits). **B)** Right hippocampal activation for increasing Relational Distance (inference 2 hits > direct hits) [z score=3.64 (18, -38, -8), $p<0.05$ SVC].



Supplemental Figure 2 - Hippocampal response correlated with endocannabinoid increase after high intensity exercise

Increased right parahippocampal (extending into hippocampus) response [z-score=3.41 (32, -22, -20), p<0.05 SVC] for hits after high intensity exercise compared to hits after rest correlated with the increase in anandamide level after high intensity exercise.

Activation map displayed on the mean T1 anatomical scan of the whole population. For display purposes, hippocampal activations are thresholded at P<0.005.



Supplemental Figure 3 – Better decoding accuracy in both left and right hippocampi after moderate intensity exercise

A) Better sensitivity of decoding of correct trials in the right hippocampus after moderate exercise than rest and high intensity exercise. ANOVA F(2, 36)=8,4039, P=0,001, post-hoc $p_{Mod\text{-}Rest}$=0,048 $p_{Mod\text{-}High}$<0,001. B) Better sensitivity of decoding of correct trials in the left hippocampus after moderate exercise than rest and high intensity exercise. ANOVA F(2, 36)=6,7875, p=,003, post-hoc $p_{Mod\text{-}Rest}$=0,002 $p_{Mod\text{-}High}$=0,004.

Brain Region	Lat.	cluster size	unc. p-value	SVC p-value	peak T	peak Z	X	Y	Z
Increasing relational distance (inference 2 hits > direct hits)									
Precuneus	Right	614	1,8E-07		7,59	5,09	16	-46	14
Precuneus	Left	480	5,9E-05		4,82	3,85	-20	-48	-4
Hippocampus	**Right**	**203**	**6,3E-06**	**0,017**	**4,37**	**4,37**	**18**	**-38**	**-8**
Subiculum	Right	21	1,4E-04	0,001	3,64	3,64	26	-28	-20
Lingual gyrus	Right	49	8,8E-05		3,75	3,75	16	-82	-6
Occipital gyrus	Right	39	1,6E-04		3,60	3,60	-12	-92	0
moderate intensity exercise > rest corr. with changes in AEA									
Hippocampus	**Right**	**13**	**5E-05**	**0,008**	**5,36**	**3,89**	**38**	**-14**	**-20**
high intensity exercise > rest corr. with changes in AEA									
Parahippocampus	Right	13	1,7E-04		4,70	3,58	34	-24	-22
Hippocampus (extending from parahippocampus)	**Right**			**0,039**			**32**	**-22**	**-20**
Middle Occipital Gyrus	Right	11	3,4E-04		4,34	3,40	42	-76	34
Retest PPI moderate > rest									
Inferior Frontal Gyrus	Right	52	7,7E-04		3,72	3,16	-16	62	6

Table 1 - Activation tables

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