1 **TITLE: Acute physical exercise of moderate intensity** 2 **improves memory consolidation in humans via BDNF and** 3 **endocannabinoid signaling**

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Abstract

It is well established that regular physical exercise enhances memory functions, synaptic plasticity in the hippocampus, and BDNF (Brain Derived Neurotrophic Factor) levels. Likewise, acute exercise benefits hippocampal plasticity in rodents, via increased endocannabinoids (especially anandamide, AEA) and BDNF release. Yet, whether acute exercise affects BDNF and AEA levels and influences memory performance in humans, remains to date unknown. Here we combined blood biomarkers, behavioral, and fMRI measurements to assess the impact of acute physical exercise on associative memory and underlying neurophysiological mechanisms. For each participant, memory was tested after three conditions: rest, moderate or high intensity exercise. A long-term memory retest took place 3 months later. At both test and retest, memory performance after moderate intensity exercise was increased compared to rest and high intensity exercise. We also show that memory after moderate intensity exercise benefited from exercise-induced increases in both AEA and BDNF levels: while AEA boosted hippocampal activity during memory recall, BDNF enhanced hippocampal memory representations and long-term performance. These findings confirm previous results on the benefits of acute exercise towards memory consolidation and, by including the contribution of key biomarkers, extend them by explaining neural plasticity mechanisms mediating cognitive enhancement.

Significance statement

Here we show that cycling for half an hour at moderate intensity after encoding new memories improves their retention when tested right after sport, and also 3 months later. This boost in memory performance occurs selectively for moderate

1 intensity exercise, and is not observed after high intensity cycling. We report that
2 exercise-induced memory enhancement is dovetailed by the activation of the
3 hippocampus in the brain, and by an increase in blood concentrations of
4 endocannabinoids (molecules involved in the feeling of euphoria after exercise) and
5 brain-derived neurotrophic factor (BDNF). Consistent with the role of the latter in
6 neural plasticity mechanisms, we show that exercise-induced BDNF increase favors
7 the long-term stabilization of memory representations across brain networks (i.e. 3
8 months after the first memory test).

9 /body

10 **Introduction**

11 Regular physical exercise is a lifestyle factor, which benefits neurocognitive
12 functions and brain plasticity (1) at all ages, and may possibly reduce the risk of
13 cognitive decline associated with Alzheimer's disease (2). Studies in animals support
14 the fact that voluntary regular exercise fosters neurogenesis in the adult
15 hippocampus and improves learning and memory capacities (3). Adult neurogenesis
16 in the human hippocampus has been repeatedly suggested (4, 5), albeit being
17 recently questioned (6). Several lines of evidence converge to suggest that enhanced
18 hippocampal synaptic plasticity is mediated, at least in part, by brain derived
19 neurotrophic factor (BDNF)(7). Specifically, physical exercise increases the levels of
20 BDNF mRNA and protein in the hippocampus and other brain regions (7), and
21 blocking BDNF action in the hippocampus hinders the beneficial effect of exercise on
22 memory (8).

23 Human studies have primarily focused on the long-term effects of exercise on
24 BDNF and cognition. Yet, measuring BDNF levels before and after a period of regular

1 physical training does not account for the kinetics of the upregulation of this growth
2 factor , which is thought to be predominantly fast and transient (9). In particular,
3 BDNF levels are known to rapidly increase in hippocampal subfields in response to
4 exercise (10), together with enhanced long-term potentiation (LTP) and synaptic
5 plasticity (11). These effects may mediate memory enhancement on the timescale of
6 a few hours (12). Associated with LTP induction, exercise also rapidly affects fine cell
7 morphology, especially by increasing the number and size of hippocampal dendritic
8 spines considered to support changes in synaptic strength (13).

9 In addition, physical exercise yields a rapid increase in circulating
10 endocannabinoids, which act on cannabinoid receptors CB1 and CB2 (14-16). Work
11 in animal models have implicated endocannabinoid signaling in exercise-induced
12 adult hippocampal neurogenesis (17) and plasticity mechanisms (18).
13 Endocannabinoids directly mediate different forms of retrograde plasticity (19) and
14 can also modulate non-endocannabinoid-mediated forms of plasticity including LTP
15 (20) and BDNF signaling (21). One recent study directly linked endocannabinoid
16 levels to memory enhancement and hippocampus function in mice by showing that
17 blocking CB1 receptor in the hippocampus disrupted spatial memory performance
18 whereas artificially elevating endocannabinoid concentrations in sedentary animals
19 increased BDNF levels and memory (22).

20 In humans, short periods of exercise, or acute exercise, were reported to have
21 diverse effects on learning, memory, and cognition in humans, ranging from positive
22 to detrimental (1, 23-26). These inconsistent results may primarily be attributable to
23 the use of different exercising intensities and/or overall poor quantification of exercise
24 intensities (27). In a previous behavioral study, we showed that moderate intensity
25 exercise boosted associative memory performance (26). The main aims of the

1 present study were (i) to confirm these effects using an individually-defined
 2 calibration of moderate physical effort (corresponding here to cycling during 30
 3 minutes at 65% of the maximal cardiac frequency measured during VO₂max), and (ii)
 4 to unravel the underlying blood biomarker and neuroimaging correlates. Based on
 5 animal data (reviewed above), we hypothesized that endocannabinoids and BDNF
 6 influence hippocampal functioning after acute physical exercise in humans. As
 7 mentioned above, different exercising intensities have been used in previous work,
 8 but were rarely compared and often poorly characterized. We therefore added an
 9 exploratory high intensity exercising condition to clarify whether the beneficial effects
 10 of exercise on memory performance are specific to moderate intensity or whether
 11 they may also be observed for a high exercising intensity. During the high intensity
 12 condition participants cycled during 15 minutes at 75% of their maximal cardiac
 13 frequency, which corresponds to an effort level above the ventilatory threshold.

14 Here we assessed the influence of different intensities of physical exercise on
 15 memory in 18 participants, by comparing performance during three separate
 16 sessions of moderate intensity exercise, high intensity exercise, and rest period
 17 (according to a cross-over randomized within-subjects design). We used a
 18 hippocampus-dependent associative memory task (26, 28) in which participants
 19 learned 8 series of 6 successive pictures. Participants first saw the 8 series once
 20 during an encoding session (**Fig. 1B**), followed by a 2-alternative forced choice
 21 (2AFC) learning session with feedback (**Fig. 1C – right panel**). After exercise or rest,
 22 associative memory was tested again using a 2AFC on pairs of pictures with different
 23 relational distances (direct, inference of order 1 and order 2; **Fig. 1C**). Sixteen control
 24 trials (used in the decoding analysis) were also included in which the depicted
 25 elements were of a given color (red, blue or green) and participants had to choose

1 among two pictures which one was of the same color as the target picture. Blood
 2 samples were collected before and after each period of exercise or rest to measure
 3 endocannabinoids and BDNF levels. We also tested the effects of acute physical
 4 exercise on long-term memory during a surprise memory retest 3 months after the
 5 last experimental visit (**Fig. 1A**). Functional MRI (fMRI) data was acquired during
 6 memory encoding, learning, test, and retest, and were analyzed using SPM12 (see
 7 Methods), the results from test and retest sessions are reported here. In line with our
 8 previous results (26), we hypothesized that moderate intensity exercise would yield
 9 the largest benefits, especially at immediate test. Further, we expected that such
 10 memory benefits would be associated with exercise-related changes in BDNF and
 11 AEA levels. AEA is known to have transient effects due to its rapid degradation by
 12 metabolic enzymes (29), whereas the reported effects of BDNF are generally long-
 13 lasting (4). We therefore predicted that increases in BDNF levels may underlie long-
 14 term memory effects.

15 **Results**

16 **Test**

17 To test our main prediction about the effect of physical exercise on memory and
 18 provide a replication of our previous behavioral findings (26), percentage of correct
 19 trials (% correct) and efficiency data (see Methods) from the test session were
 20 analyzed using repeated-measures ANOVAs with Exercising Condition (rest,
 21 moderate intensity exercise, high intensity exercise) as repeated measures and
 22 Relational Distance (direct, inference 1, inference 2) as within-subjects factor. We
 23 report a main effect of Exercising Condition for both % correct ($F(2, 102)=4.01$,
 24 $p=0.02$) and efficiency ($F(1, 102)=8.62$, $p<0.01$), but no effect of Relational Distance

1 and no interaction (all $p > 0.05$; **Fig. 2A**). Post-hoc analyses revealed that increased %
 2 correct and higher efficiency after the moderate compared the rest Exercising
 3 Condition ($p_{\text{mod-rest}} = 0.02$ and < 0.01 , respectively), while efficiency was also higher
 4 after moderate intensity exercise compared to high intensity exercise ($p_{\text{mod-high}} < 0.01$).

5 **Blood samples**

6 We measured changes in endocannabinoids and BDNF levels from the blood
 7 samples collected right before and after the rest and exercise sessions (see *S/*
 8 *Appendix* for details). Repeated-measures ANOVAs were performed for each
 9 biomarker with Exercising Condition (rest, moderate, high) as a within-subjects factor.
 10 For AEA, a main effect of Exercising Condition ($F(2, 34) = 39.25$, $p < 0.01$; **Fig. 3A**) was
 11 found. Post-hoc analyses revealed that AEA levels were lower after rest than after
 12 physical exercise ($p_{\text{rest-mod}} < 0.01$, $p_{\text{rest-high}} < 0.01$), with no difference in AEA levels after
 13 moderate and high intensity exercise ($p_{\text{mod-high}} > 0.05$). Please note that AEA during
 14 the rest condition decreased from the first (baseline) to the second (post-rest)
 15 measurement, hence resulting in a negative differential value. This decrease is
 16 consistent with known circadian fluctuations in AEA, whereby AEA levels increase
 17 during sleep and decrease throughout the day (30). For the endocannabinoid 2-
 18 arachidonoylglycerol (2-AG), there was no effect of Exercising Condition ($F(2,$
 19 $34) = 2.90$, $p > 0.05$), consistent with previous descriptions in the literature (15). For
 20 BDNF, we report a main effect of Exercising Condition ($F(2, 34) = 4.78$, $p = 0.01$; **Fig.**
 21 **3D**). Post-hoc analyses revealed that, BDNF levels after moderate and high intensity
 22 exercise differed from after rest ($p_{\text{rest-mod}} = 0.045$, $p_{\text{rest-high}} = 0.01$).

23 **Psychomotor vigilance test (PVT) and Profile of Mood States questionnaire** 24 **(POMS)**

1 We administered the PVT and POMS just before the MRI test session (i.e. about
2 45 min after rest or exercise) to monitor possible condition-dependent differences in
3 vigilance and mood at the time of the test session. For PVT, we replicate our
4 previous results (26) showing no difference in PVT as a function of Exercising
5 Condition (rest, moderate, high), neither in mean or median reaction times, number of
6 lapses, or number of false alarms (one way repeated measures ANOVAs, all
7 $p > 0.05$). For POMS, we report no difference for any of the measured categories
8 (fatigue, tension, confusion, vigor) as a function of Exercising Condition (all $p > 0.05$),
9 suggesting that the physical exercise sessions did not result in significant lasting
10 mood or vigilance changes.

11 **Functional MRI results**

12 **Test**

13 We first conducted a standard general linear model analysis with the data
14 collected during the memory test after rest, moderate intensity exercise, and high
15 intensity exercise modelled as separate sessions. Within each session, we
16 considered correct trials according to Relational Distance (direct, inference 1,
17 inference 2) and control trials as four separate regressors of interest, and included
18 incorrect trials as an additional regressor (**Fig. 1**). When comparing high Relational
19 Distance to low Relational Distance (inference 2 > direct trials) across all sessions,
20 we found increased activity in the right hippocampus [z score=4.35 (18, -38, -8),
21 $p < 0.05$ SVC, see Methods], bilateral parahippocampal gyrus and precuneus (see
22 **Fig. S1** and **Table S1** for exhaustive list of activated regions). No region was
23 activated (at a threshold of 0.001 unc.) when comparing inference 1 to direct trials,
24 and inference 2 to inference 1 trials. Comparisons between Exercising Conditions

1 and interactions between Relation Distance and Exercising Conditions did not yield
2 any significant activation either.

3 As it is known that AEA has a rapid effect on synaptic plasticity in the
4 hippocampus, we tested whether the observed difference in AEA across Exercising
5 Conditions might exert a modulatory influence on brain activity. We thus added
6 individual AEA change as a cofactor in the second-level analyses comparing
7 Exercising Conditions. We found that the increase in AEA after moderate intensity
8 exercise (vs. rest) correlated with the activation in the right hippocampus [z-
9 score=3.72 (38, -14, -20), $p < 0.05$ SVC] (**Fig. 3B**). A similar modulation of
10 hippocampal activity was found for high intensity exercise (vs. Rest) [z-score=4.08
11 (32, -24, -18), $p < 0.05$ SVC], suggesting that AEA increase correlated robustly with
12 hippocampal activation (**Fig. S2**).

13 Next, we used a decoding approach to test whether exercise would affect the
14 coherence of the fine-grained neural representation of correct, incorrect and control
15 trials within the bilateral hippocampus. To test for this, we applied a similar procedure
16 as Van Dongen et al ((23); see *SI Appendix* and Methods section), to classify each
17 single trial, from voxelwise hippocampal activity, into one of three possible outcomes
18 (correct, incorrect or control trial), with a chance level at 33.33%. Focusing on correct
19 trials, we report that decoding accuracy was above chance level after moderate
20 intensity exercise, but at chance level after rest and high intensity exercise (**Fig. 3E**).
21 Post-hoc analyses further showed that decoding after moderate intensity exercise
22 was higher than after both rest and high intensity exercise ($p_{\text{mod-rest}} < 0.001$, $p_{\text{mod-}}$
23 $\text{high} < 0.001$, depicted on **Fig. 3E**). We obtained similar results when we performed the
24 classification on activity from the left or the right hippocampus separately (see **Fig.**
25 **S3**).

1 Because BDNF is known to specifically enhance plasticity mechanisms in the
2 hippocampus, we tested whether BDNF levels may affect the neural representation
3 as measured with decoding results. We report a positive correlation between BDNF
4 enhancement during moderate intensity exercise (calculated as the difference
5 between moderate and rest BDNF values, with baseline values subtracted for each
6 visit) with decoding accuracy after moderate intensity exercise ($R=0.53$, $p=0.02$), but
7 not high intensity exercise ($p>0.05$); **Fig. 3F**.

8 Retest

9 All participants came back for a long-term memory retest session three months
10 later. A repeated-measure ANOVA was performed with Exercising Condition (rest,
11 moderate, high) as within-subjects factor, that revealed a main effect of Exercising
12 Condition ($F(2, 34)=3.32$, $p=0.048$). Post-hoc analyses showed that this main effect
13 was due to participants performing better for the associations learned during the
14 moderate exercise session as compared to those learned during rest session ($p_{\text{mod-}}$
15 $\text{rest}=0.04$); **Fig. 4A**. Moreover, only the trials from the moderate exercise session were
16 remembered above chance level three months later ($t(17)=2.31$, $p=0.03$).

17 We then asked whether physical exercise had some long-term effects on the
18 functional coupling between the hippocampus and other brain regions during the
19 processing of associative memories. We therefore performed a psychophysiological
20 interaction analysis (see Methods) taking as seed region the right hippocampal
21 region that initially showed increased activity for moderate intensity vs. rest as a
22 function of AEA levels (38, -14, -20; see Test results above). We observed increased
23 functional connectivity between the hippocampal seed region and the left superior
24 frontal gyrus [$z\text{-score}=3.10$ (-16, 62, 6), $p<0.001$ unc.] when participants were

1 exposed to associations learned during the moderate intensity exercise session
2 (compared to rest; **Fig. 4B**).

3 Based on the existing evidence that BDNF contributes to neurogenesis and
4 synaptic plasticity (4, 7, 8), we also hypothesized that changes in BDNF levels during
5 the moderate intensity exercise condition (i.e. condition associated with the highest
6 benefit for short-term memory consolidation and hippocampal representations) may
7 potentially promote long-term memory retention. This hypothesis was tested by
8 correlating individual changes in BDNF levels (for moderate intensity vs. rest) to
9 delayed performance increase (i.e., from test to retest) for items initially learned
10 during the moderate vs. rest condition. We report a significant positive correlation
11 ($R=0.55$, $p=0.02$; **Fig. 4C**), while the same correlation for high intensity exercise was
12 not significant ($R=0.31$, $p=0.20$). These results suggest that BDNF increase after
13 moderate intensity exercise may contribute to durable memory enhancement.

14 Discussion

15 We show here that one session of moderate intensity physical exercise compared
16 to a period of rest enhanced associative memory, both at immediate test (2 hours
17 after encoding) and at long-term retest (three months later). These effects may be
18 mediated by the endocannabinoid AEA and the growth-factor BDNF, whose
19 respective concentrations increased after acute exercise. Accordingly, during the
20 short-term test, the increase in plasma AEA concentration correlated with
21 hippocampal activity when associative memories were recalled, and BDNF increase
22 correlated with decoding measures within the hippocampus. Moreover, BDNF
23 increase during moderate intensity physical exercise correlated with better
24 performance at long-term retest. We did not observe such memory benefits for a

1 session of high intensity physical exercise, i.e. when effort levels surpassed the
2 ventilatory threshold. Overall, we show that acute physical exercise at moderate
3 intensity has long-lasting positive effects on the consolidation of associative
4 memories in healthy young human adults. Below, we discuss the neurophysiological
5 mechanisms that could explain these important findings.

6 **Biomarker mechanisms underlying the effects of acute exercise on** 7 **hippocampal plasticity**

8 In a recent study in rodents, Fuss et al. (31) demonstrated that physical exercise
9 induces an acute increase of AEA measured in the plasma, with direct effects on
10 CB1 receptors in the brain. Note that in the same study cerebro-spinal fluid measures
11 did not capture increases in AEA, consistent with AEA being very rapidly metabolized
12 in the brain (29). These results support the fact that plasma measures of AEA, as we
13 performed here, offer a reliable index of AEA activity in the central nervous system.
14 Another rodent study directly linked endocannabinoid signaling to hippocampal
15 memory function, by showing that selectively blocking CB1 receptors in the
16 hippocampus abolished exercise-induced memory effects (22). This study also
17 demonstrated that artificially increasing AEA concentrations (by blocking the Fatty
18 Acid Amine Hydrolase, the enzyme responsible for breaking down AEA) in the
19 hippocampus of sedentary mice mimicked the effects of physical exercise and
20 increased memory performance. Together, these rodent studies elucidate the
21 neurophysiological mechanisms underlying our novel finding that AEA increase in
22 human plasma may reflect direct effects of physical exercise on brain activity,
23 especially in the hippocampus.

1 Traditionally, BDNF has been linked to effects of regular physical exercise,
 2 although it is known that BDNF gene expression is upregulated both after acute and
 3 after chronic physical exercise in rodents (32). It is widely acknowledged that BDNF
 4 enhances synaptic plasticity, especially via LTP (12), which can be induced in a few
 5 minutes and critically contributes to memory consolidation (33). Here we show that
 6 the effects of one single session of exercise may differentially affect both short and
 7 long-term memory retention. On the one hand, BDNF increase after acute moderate
 8 physical exercise correlated positively with decoding accuracy of memory items in
 9 both hippocampi immediately after exercise (test session). On the other hand, BDNF
 10 increase (at test) also correlated with long-term memory differences (at retest) due to
 11 the initial exercising conditions. Specifically, those participants who exhibited larger
 12 increases in BDNF levels at test after the moderate intensity Exercising Condition
 13 remembered the learned associations better at retest three months later.

14 **Acute moderate but not high intensity exercise benefits memory** 15 **consolidation**

16 While characterizing the impact of exercise intensity on cognitive functions is
 17 critical for health recommendations, dementia prevention programs and rehabilitation
 18 strategies, the reported effects remain inconsistent. Some studies suggest that high
 19 intensity training is most efficient (23, 25) while other studies, especially meta-
 20 analyses, indicate that moderate intensity exercise might have more impact (34), and
 21 a recent report show that very mild intensity exercise (at 30% of maximal cardiac
 22 frequency (FcMax)) may already benefit hippocampal memory function (24). Here we
 23 aimed at clarifying this important issue by using a cross-over randomized within-
 24 subjects design according to which each participant was tested at a moderate and at
 25 a high intensity (plus a resting, baseline condition) across distinct sessions where

1 associative memory was also tested. Importantly, here we determined moderate and
 2 high intensity exercise levels with reference to each participant's individual ventilatory
 3 threshold. This threshold was measured using a VO₂max procedure (see Methods),
 4 which is a gold-standard in human physiology research (see 35 for review). Moderate
 5 intensity corresponded to exercise below the ventilatory threshold (65% of individual
 6 VO₂max) and high intensity corresponded to exercise above the ventilatory threshold
 7 (75-80% of individual VO₂max). Here we found strong beneficial effects of moderate
 8 intensity exercise on memory, with a clear difference compared to rest, while the
 9 effects of high intensity exercise appeared to be more complex. Some evidence
 10 suggests that while aerobic exercise training (i.e. below ventilatory threshold) is
 11 beneficial for hippocampal functioning, high intensity training is not (36). One
 12 plausible explanation is that acute high intensity exercise may induce a physiological
 13 stress response (for example a strong increase in cortisol levels) which can impair
 14 memory for previously learned stimuli (37, 38). In this article, rather than
 15 concentrating on the clear-cut positive results between moderate intensity exercise
 16 and rest, we decided to present the findings from both intensities and carefully
 17 discuss below the possible reasons for the differential effects of moderate and high
 18 intensity exercise. We hope that our results and the ideas raised in our discussion will
 19 fuel future debates and investigations in the scientific community.

20 Here we observed that moderate levels of exercise intensity increased both BDNF
 21 and AEA levels and optimized cognitive processes. By contrast, although high
 22 intensity physical exercise further increased the measured concentrations of BDNF
 23 and AEA, performance did not follow this increase. This observation suggests that
 24 large increases in BDNF and AEA concentrations might not be as beneficial for
 25 memory performance. Please note that while, at this point, we cannot exclude that

1 there is no link between BDNF and AEA levels and memory consolidation, this latter
 2 hypothesis is not prevalent in the current literature. In line with our findings,
 3 Mamounas et al.(39) showed that the BDNF dose-response curve follows an inverted
 4 U-shape with intermediate concentrations of BDNF yielding best results for sprouting
 5 of serotonergic neurons in the rodent hippocampus. For AEA, one study using
 6 exogenous AEA administration suggested that related anxiolytic effects also follow an
 7 inverted U-shape dose-response curve with highest concentrations (measured in the
 8 periaqueductal gray) being less effective (40). The findings of the present study also
 9 suggest that submaximal concentrations of both molecules (as obtained after
 10 moderate intensity exercise) yield best effects on neurocognitive functions, here for
 11 hippocampal-dependent memory formation. As mentioned above, we cannot exclude
 12 that other biomarkers may also contribute to the observed effects, such as for
 13 example a large increase in cortisol after high intensity exercise, which may be
 14 detrimental for memory consolidation (37, 38).

15 **Long-term consequences of acute physical exercise on memory** 16 **consolidation**

17 Lasting effects of physical exercise are established for regular physical exercise
 18 protocols, involving several months of training (4, 41). Most of these studies focused
 19 on the possible protective effects of physical exercise in ageing and dementia. Yet,
 20 acute physical exercise has also been reported to have positive short term cognitive
 21 effects (23, 26), albeit not always found in tasks involving hippocampus-dependent
 22 memory (42). Long-term effects of acute physical exercise (at the scale of several
 23 months as we tested here) have to our knowledge not been investigated in humans
 24 so far. Here we found long-lasting effects on memory retention selectively for

1 moderate intensity exercise (i.e. below the ventilatory threshold), which were
2 dovetailed by increased connectivity between hippocampus and prefrontal cortex.

3 **Conclusion**

4 We show that acute moderate intensity physical exercise significantly increased
5 associative memory performance both at short and long term. At short term,
6 hippocampal activation correlated with endocannabinoid AEA while enhanced
7 hippocampal memory representations were associated with a modulation of BDNF.
8 At long term, three months after encoding, memory effects were related to BDNF
9 increase induced by moderate intensity exercise. High intensity exercise did not have
10 such beneficial effects. We conclude that a single session of moderate physical
11 exercise boosts associative memory formation.

12 **Methods**

13 **Participants**

14 We included 20 healthy young male volunteers in this study. All participants gave
15 written informed consent and received financial compensation for their participation,
16 which was approved by the Ethics Committee of the Geneva University Hospitals.
17 Two participants had to be excluded from all the analysis for non-compliance with
18 experimental requirements. The remaining 18 participants were between 18 and 34
19 years old (mean age +/- standard error: 23.03+/-0.92 years). All participants were
20 right-handed, non-smokers, free from psychiatric and neurological history, and had a
21 normal or corrected-to-normal vision. Please note that, for the present experimental
22 design, we estimated the required sample size based on the results from our
23 previous study (26) (see *SI Appendix* for details).

1 Experimental procedure

2 Participants first came to the lab for a VO2max procedure. During this visit,
 3 participants also performed a habituation session of the associative task. Those
 4 participants with a VO2max within the required ranges (see *SI Appendix*) were invited
 5 to come back for three experimental visits separated by one to two weeks, according
 6 to a within-subjects design with the three Exercising Conditions (rest, moderate
 7 intensity exercise, high intensity exercise) randomly counterbalanced across
 8 participants.

9 For each visit, participants arrived at 08:00 AM on an empty stomach, and had a
 10 controlled breakfast with an experimenter (*SI Appendix*). At 09:00 AM, participants
 11 were comfortably installed in the scanner, and started the encoding part of the
 12 associative memory task (see below and **Fig. 1A**) while fMRI data was acquired. At
 13 09:50 AM a qualified medical doctor took a first blood sample. At 10:00 AM
 14 participants were equipped with a Polar RS800CX N device to measure heart rate
 15 and asked to rest or exercise. For the two exercise conditions, participants pedaled
 16 on a cycle ergometer (Ergoline GmbH, Bitz, Germany), the pedaling frequency was
 17 kept between 60 and 80 cycles per minute, which was shown on a small screen in
 18 front of the participant. For moderate intensity exercise, each participant pedaled for
 19 30 minutes, with the load of the ergometer set so that the cardiac frequency of the
 20 participant would be at 60% of his FcMax. For high intensity, participants first warmed
 21 up for 2 minutes at 50% of FcMax then the load was progressively increased over 1
 22 minute to reach 75% of FcMax. Participants pedaled at this intensity for 15 minutes
 23 then they pedaled again at 50% of FcMax for 3 minutes to cool down. For both
 24 exercise conditions, the experimenters checked cardiac frequency every 3-5 minutes
 25 to adjust the resistance of the ergometer if necessary. For the rest condition,

1 participants sat on a chair and were allowed to quietly look at magazines for 30
 2 minutes. To minimize interference with memory, we carefully selected these
 3 magazines so that they were mainly composed of pictures, and that there was little to
 4 be learned from their content. We purposefully did not let participants watch a movie
 5 during rest to minimize motor imagery. At 10:30 AM, the medical doctor took a
 6 second blood sample and fifteen minutes after this, participants performed a
 7 Psychomotor Vigilance Task (PVT) followed by the Profile of Mood States (POMS)
 8 questionnaire. These latter two measures were acquired as control measures to
 9 exclude that difference in fatigue and mood states may explain our memory findings
 10 so they were administered when heart rate and other physiological conditions were
 11 back to baseline, close in time to the second fMRI session when memory was tested.

12 At 11:30 AM, participants underwent a second fMRI session during which memory
 13 for the associative task was tested. A surprise retest fMRI session took place three
 14 months later where participant's memory was tested again; no blood samples were
 15 taken at this time point.

16 **Associative memory task in fMRI:** We adapted an associative memory task (26,
 17 28) consisting of two parts: encoding and test, separated by an exercise (moderate or
 18 high intensity) or rest period (**Fig. 1A**). To avoid interference across experimental
 19 visits for this within-subjects design, we showed different pictures belonging to three
 20 specific themes at each visit: "office", "shoe shop" or "house" (one theme per visit).
 21 The pictures in each theme for the experimental visits were matched in difficulty and
 22 counterbalanced across Exercising Conditions and visits (**Fig. 1B**). Note that for the
 23 habituation session of the task, participants had to memorize 5 series of a "swimming
 24 pool" theme.

1 During the encoding session, participants were first shown 8 series of 6 pictures
2 passively once. Then, participants were presented with the first picture of the series
3 alone (e.g., pen, for the “office” theme; **Fig. 1B**). Then, the same first picture was
4 presented in the upper half of the screen together with two options for the second
5 picture in the series (chair) in the lower half of the screen, one being the correct next
6 picture and the other picture being from a different series (as depicted on the left
7 panel of **Fig. 1C**). Participants had to select the correct next picture by pressing a left
8 or right button. The correct picture was then shown (providing a feedback for each
9 trial), followed by this same picture together with the two next options for the third
10 picture in the series (desk). This continued until the last picture in the series (office
11 building) (*SI Appendix* for details).

12 During the test session, participants were presented with one cue picture and two
13 other pictures, among which they had to select the one belonging to the same series
14 as the cue picture. The two options could represent the immediate next item in the
15 series (direct trials) or could be separated by one or two items from the cue picture
16 (inference of order 1 or order 2 trials; **Fig. 1C**). All types of trials were shown in a
17 randomized order, and were presented in the same format and with the same
18 timeframe as during learning, except that no feedback was provided. In this session,
19 16 trials of the control “color” task were also included.

20 For the delayed retest session, all 18 participants came back for a surprise retest
21 in fMRI three months after the last experimental visit. Participants did not know at test
22 that there would be a retest session. The task was identical to the test sessions,
23 except that pictures of all three themes were now mixed in a random order. For time
24 constraints, we included for each of the eight sequences of pictures of all three

1 themes (24 sequences) two direct trials, two inference order 1 trials and one
2 inference order 2 trial (5 trials) totaling 120 trials overall.

3 **Functional MRI data acquisition and analysis**

4 MRI data were acquired on a 3 Tesla MRI scanner (SIEMENS Trio® System,
5 Siemens, Erlangen, Germany) with a 32-channel head coil. T2*-weighted fMRI 2D
6 images were obtained with a multiband gradient echo-planar sequence acquiring 3
7 slices at a time using axial slice orientation (66 slices; voxel size, 2 x 2 x 2mm;
8 repetition time (TR) = 1880ms; echo time (TE) = 34ms; flip angle (FA) = 60°). A
9 whole-brain structural image was acquired at the end of the first test part with a T1-
10 weighted 3D sequence (192 contiguous sagittal slices; voxel size, 1.0 x 1.0 x 1.0mm;
11 TR = 1900ms; TE = 2.27ms; FA = 9°). Continuous measures of heart rate and
12 breathing rhythm were acquired using a Biopac (Biopac Systems, CA93117, USA).

13 **Conventional fMRI analysis:** Functional images were analyzed using SPM12
14 (Wellcome Department of Imaging Neuroscience, London, UK). This analysis
15 included standard preprocessing procedures (*SI Appendix*). We performed
16 corrections to regress out potential physiological artifacts from heart rate and
17 breathing using Retroicor (43) and RVHcorr (44), respectively. A general linear model
18 (GLM) approach was then used to compare conditions of interest at the individual
19 level, each individual GLM included correct trials separated according to Relational
20 Distance (Direct, Inference 1, Inference 2 trials), control trials and incorrect trails
21 (pooled across Relational Distance), plus 6 movement regressors, 5 heart rate
22 regressors and 1 breathing regressor as regressors of non-interest. Then, contrasts
23 between conditions of interest from each participant were entered a second-level
24 random-effects analysis. All activations are reported at $p < 0.001$ with a cluster size of

1 10 voxels and relevant regions, especially the hippocampus, survived small-volume
 2 correction (SVC) for familywise error ($p < 0.05$) using volumes based on the Anatomy
 3 toolbox of SPM12 (SPM Anatomy toolbox 2.2, Forschungszentrum Jülich GmbH).
 4 Coordinates of brain regions are reported in MNI space.
 5 See *SI Appendix* for PPI and decoding analyses.

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16 **Competing interests**

17 The authors declare no competing interests.

18 **Author contributions**

19 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
 20 K.I performed research; B.M.B., A.B., M.G.L., E.L., A.T., S.S. and K.I. analyzed data;
 21 and all the authors wrote the paper.

1 **Figures**

2
3 **Figure 1 - Experimental design**

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

Figure 2 - Memory performance at test

A) % correct: higher proportion of the percentage of correct trials after moderate intensity exercise than after rest. **B)** Efficiency (% correct / reaction time): higher efficiency after moderate intensity exercise than after rest and high intensity exercise. All bar plots represent mean $\pm$ SEM.

Figure 3 - Increased biomarker levels correlate with hippocampal brain signals after moderate intensity exercise

A) Increased Anandamide level (AEA) after moderate and high physical exercise compared to rest. For all Exercising Conditions Δ AEA corresponds to the difference in AEA between the second blood sample (after exercise or rest) and first blood sample (before exercise or rest). **B)** Increased right hippocampal response [z-score=3.72 (38, -14, -20), $p < 0.05$ SVC] for correct responses after moderate exercise compared to correct responses after rest correlated with the increase in AEA level after moderate exercise. **C)** Correlation between the hippocampal beta values and AEA. **D)** Increased BDNF levels after moderate and high intensity exercise compared to after rest. For all Exercising Conditions Δ BDNF corresponds to the difference in BDNF between the second blood sample and the first blood sample. For display purposes, we represent $\Delta \log \text{BDNF}$. **E)** Higher sensitivity in decoding accuracy of correct trials in the bilateral hippocampus after moderate exercise than rest and high intensity exercise. **F)** Positive correlation between decoding accuracy in the hippocampus and increase in BDNF level after moderate intensity exercise.

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

Figure 4 - Better long-term memory for associations learned after moderate physical exercise, related to prefrontal activation and BDNF signaling

A) Better performance for pictures learned during the moderate intensity visit than for pictures learned 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), $p<0.001$], 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.

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