A single bout of aerobic exercise promotes motor cortical neuroplasticity

Michelle N. McDonnell, Jonathan D. Buckley, George M. Opie, Michael C. Ridding and John G. Semmler

J Appl Physiol 114:1174-1182, 2013. First published 14 March 2013;
doi: 10.1152/japplphysiol.01378.2012

Journal of Applied Physiology publishes original papers that deal with diverse area of research in applied physiology, especially those papers emphasizing adaptive and integrative mechanisms. It is published 24 times a year (twice monthly) by the American Physiological Society, 9650 Rockville Pike, Bethesda MD 20814-3991.
 ESSN: 1522-1601. Visit our website at http://www.the-aps.org/.

J Appl Physiol 114: 1174–1182, 2013;
doi:10.1152/japplphysiol.01378.2012.

A single bout of aerobic exercise promotes motor cortical neuroplasticity

Michelle N. McDonnell,[1] Jonathan D. Buckley,[2] George M. Opie,[3] Michael C. Ridding,[4] and John G. Semmler[3]

[1]*International Centre for Allied Health Evidence, University of South Australia, Adelaide, South Australia, Australia;* [2]*Nutritional Physiology Research Centre, University of South Australia, Adelaide, South Australia, Australia;* [3]*Discipline of Physiology, School of Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia;* [4]*Neuromotor Plasticity and Development, The Robinson Institute, School of Paediatrics and Reproductive Health, The University of Adelaide, Adelaide, South Australia, Australia*

Submitted 14 November 2012; accepted in final form 27 February 2013

McDonnell MN, Buckley JD, Opie GM, Ridding MC, Semmler JG. A single bout of aerobic exercise promotes motor cortical neuroplasticity. *J Appl Physiol* 114: 1174–1182, 2013; doi:10.1152/japplphysiol.01378.2012.—Regular physical activity is associated with enhanced plasticity in the motor cortex, but the effect of a single session of aerobic exercise on neuroplasticity is unknown. The aim of this study was to compare corticospinal excitability and plasticity in the upper limb cortical representation following a single session of lower limb cycling at either low or moderate intensity, or a control condition. We recruited 25 healthy adults to take part in three experimental sessions. Cortical excitability was examined using transcranial magnetic stimulation to elicit motor-evoked potentials in the right first dorsal interosseus muscle. Levels of serum brain-derived neurotrophic factor and cortisol were assessed throughout the experiments. Following baseline testing, participants cycled on a stationary bike at a workload equivalent to 57% (low intensity, 30 min) or 77% age-predicted maximal heart rate (moderate intensity, 15 min), or a seated control condition. Neuroplasticity within the primary motor cortex was then examined using a continuous theta burst stimulation (cTBS) paradigm. We found that exercise did not alter cortical excitability. Following cTBS, there was a transient inhibition of first dorsal interosseus motor-evoked potentials during control and low-intensity conditions, but this was only significantly different following the low-intensity state. Moderate-intensity exercise alone increased serum cortisol levels, but brain-derived neurotrophic factor levels did not increase across any condition. In summary, low-intensity cycling promoted the neuroplastic response to cTBS within the motor cortex of healthy adults. These findings suggest that light exercise has the potential to enhance the effectiveness of motor learning or recovery following brain damage.

neuroplasticity; exercise; transcranial magnetic stimulation

THERE IS INCREASING EVIDENCE that exercise exerts a wide range of positive effects on plasticity and neuroprotection within the nervous system (60). Experiments in humans have demonstrated that regular exercise leads to improved learning (56), enhanced cognitive flexibility (37), enhanced motor cortex plasticity (10), as well as preserving cognitive function and reducing the risk of dementia (20, 46). Furthermore, exercise is particularly effective at improving several cognitive domains in older adults (1, 11). Animal studies confirm that aerobic exercise has a neuroprotective effect and activates growth hormones that increase the survival of neural stem cells (4) and increases the blood flow to the dentate gyrus, supporting neurogenesis in the hippocampus (44). The potential for exercise to promote recovery following brain damage is as yet unexplored, but preliminary work concerning Parkinson's disease is promising (27).

The mechanisms whereby aerobic exercise can act on multiple systems of the brain are not completely understood and are likely to involve several mechanisms. One neurotrophic factor that has an important role in the health, survival, and function of neurons is brain-derived neurotrophic factor (BDNF) (3). Interestingly, exercise has been shown to influence BDNF levels. For example, several days of running-wheel exercise in rats led to increased levels of BDNF in the hippocampus, spinal cord, cerebellum, and cortex (12), and increased levels of BDNF may partially explain the neurogenesis seen in the hippocampus of exercising mice (36). Individuals who regularly take part in large amounts of vigorous physical activity have a greater neuroplastic response to noninvasive brain stimulation techniques than sedentary individuals, suggesting that, over the long term, aerobic exercise may promote increased neural density and survival in the human cortex (10).

A pivotal question is whether these changes, which may result in enhanced neuroplasticity, will occur after a single session of exercise. It has been demonstrated that a single session of cycling has positive behavioral effects on learning (56), as well as on concentration and cognitive function (51). Similarly, 30 min of aerobic exercise improved executive function in depressed patients (34) and improved response time and accuracy in a working memory task (47). It is likely that BDNF has a critical role in mediating exercise-induced changes in learning via its effects on long-term potentiation (22). Activity-dependent secretion of BDNF can, however, be reduced by the presence of the common single nucleotide polymorphism, Val^{66}Met (19), which also reduces some forms of brain plasticity (9, 19, 32). It is worth noting that moderately vigorous exercise also results in increased cortisol production (48), and both acute (50) and chronically elevated cortisol levels are associated with impairments in neuronal plasticity, largely evident through memory deficits (7, 31).

We hypothesized that a single session of aerobic exercise would promote neuroplasticity in the motor cortex of healthy, moderately active adults. Exercise has the potential to act globally within the brain, not just in the specific circuits controlling exercising muscles, due to the ability to trigger critical molecular and cellular processes that support brain plasticity (33). We, therefore, tested neuroplasticity mechanisms in a small hand muscle after a single bout of lower limb cycling. We assessed excitability and plasticity within the primary motor cortex of healthy adults using two forms of transcranial magnetic stimulation (TMS), a noninvasive brain

Address for reprint requests and other correspondence: M. N. McDonnell, *i*CAHE, Univ. of South Australia, GPO Box 2471, Adelaide SA 5001, Australia (e-mail: michelle.mcdonnell@unisa.edu.au).

stimulation technique that can probe changes in effectiveness of synaptic connections in the brain, revealing short-term plasticity (17). The patterned stimulation involved repetitive TMS, specifically continuous theta-burst stimulation (cTBS), which results in a suppression of cortical excitability through mechanisms believed to be similar to long-term depression (LTD) (29). The TMS was performed before and after exercise, compared with a sedentary condition, to determine whether exercise can promote neuroplasticity. Two exercise intensities were investigated: moderate-intensity exercise, which may be more likely to increase both BDNF (33) and cortisol, or low-intensity exercise, which may not increase BDNF levels, but is unlikely to raise cortisol levels (48). We also measured circulating levels of BDNF and cortisol before and after exercise, as they may influence the plasticity response (50).

METHODS

Participants

Healthy adults, aged 18–60 yr, were invited to participate in the study using recruitment flyers. The upper limit of 60 yr was chosen due to recent evidence that the human motor cortex shows age-related reduction in neuroplastic capacity over this age, compared with young and middle-aged subjects (21). Participants were screened for their suitability to undertake exercise using the Sports Medicine Australia preexercise screening system (52), and the International Physical Activity Questionnaire (14) short-form was used to calculate metabolic equivalents (METs) over the past 7 days. Participants who accumulated more than 3,000 MET-minutes per week were excluded from the study, as they met the criteria for "high" levels of physical activity. Other exclusion criteria included history of neurological disease or epilepsy, metallic implants in the head, and conditions such as hypertension or other cardiovascular disease that would preclude participating in exercise. Using a crossover design, all participants were randomly allocated to participate in each of the three conditions, with sessions separated by at least 1 wk (see Fig. 1 for time line of experiments). All experiments were conducted in accordance with the standards established by the Declaration of Helsinki and were approved by the relevant local Human Research Ethics Committees.

Baseline Assessment

Participants attended a familiarization session and screening for eligibility, where they provided informed consent. After completing the screening questionnaires, participants were asked to provide a sample of buccal cells using a cheek swab (Isohelix, Cell Project Harrietsham, Kent, UK) to enable analysis of the common Val^{66}Met *BDNF* polymorphism. Genotyping was carried out with known positive controls from the buccal cell samples. The rs6265 single nucleotide polymorphism was genotyped using a commercially available Taqman genotyping kit (41). The assay was performed according to manufacturer's instructions.

Participants underwent a submaximal cycle exercise test, which required them to pedal a cycle ergometer (Ergomedic 828E, Monark Exercise AB, Stockholm, Sweden) at a constant rate (50 rpm) at three different workloads designed to increase heart rate to ~55, 65, and 75% of age-predicted maximal heart rate (220 − age) (23). The cadence of 50 rpm was chosen, as this is commonly used for submaximal exercise testing (2). Heart rates were recorded at the end of each minute, and the steady-state heart rate for each workload was used to extrapolate the workload required to take participants up to their age-predicted maximal heart rate. The regression equation was then used to accurately estimate the workloads required to achieve 75% of age-predicted maximal heart rate for the intervention. This workload was used for the "moderate"-intensity condition, and the workload was halved, and the exercise duration was doubled for the "low"-intensity condition to ensure that both exercise conditions were matched in terms of the work completed.

Intervention

The experimental sessions involved exercising at one of three intensities: low intensity for 30 min, moderate intensity for 15 min, and a rest condition where participants remained seated for 30 min. Participants cycled at the same cadence as the submaximal exercise testing, 50 rpm, which may be more suitable for the elderly or those with neurological conditions. During each exercise session, heart rate and Borg's 6–20 ratings of perceived exertion scale were recorded every 5 min.

TMS

Single-pulse TMS was used to measure changes in corticospinal excitability in each of the three experimental sessions. Fifteen TMS pulses were applied over the optimal scalp site for evoking responses [motor-evoked potentials (MEPs)] in the right first dorsal interosseus muscle. A figure-of-eight coil was used with the handle oriented posteriorly and at 45° to the midsagittal line, ensuring optimal activation of cortico-motoneuronal cells. Resting motor threshold was defined as the minimum stimulus intensity required to produce a MEP of at least 50 μV in the relaxed first dorsal interosseus (FDI) in at least 5 of 10 trials (49). Active motor threshold was defined as the minimum stimulus intensity required to produce a MEP of at least 200 μV during a low-level isometric contraction of the FDI (20% of the maximal voluntary contraction) in at least 5 of 10 trials. Postexercise, cTBS was delivered according to established protocols (17, 18, 29). In short, low-intensity stimuli (80% active motor threshold) were delivered in a basic pattern of short bursts (3 stimuli) of moderate-frequency (50 Hz) stimuli applied every 200 ms. Continuous application of this pattern for 40 s induces a lasting decrease in motor cortical excitability, thought to be due to a LTD-like effect on cortical synapses (29). This paradigm was chosen due to its ability to produce a robust, significant change in cortical excitability (17) by decreasing the excitability of cortical circuits, which generate I waves, in particular I1 (18). Theta burst stimulation was delivered as soon as practicable after the exercise or rest condition on each occasion. Using supramaximal ulnar nerve stimulation, maximal M waves (n = 5) were recorded in the FDI muscle immediately after MEPs were recorded (Fig. 1). The optimal site to stimulate the ulnar nerve was first located using a bar electrode and then adhesive electrodes, which were secured with tape at this site. The stimulating current was increased until the M wave was maximal, and the intensity was then increased by 20% and maintained at this level for the remainder of the experiment.

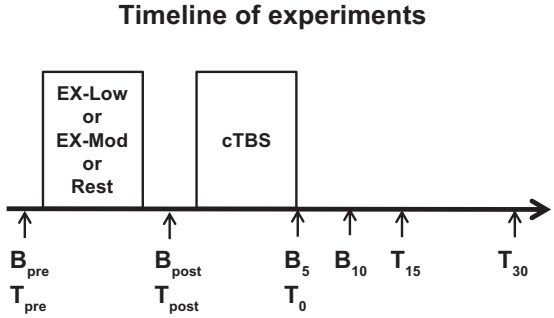

Fig. 1. Time line of experiments, indicating when bloods (B) were taken [pre- (B_{pre}) and postexercise (B_{post}), and at 5 (B_5) and 10 min (B_{10}) following cessation of exercise] and measures of cortical excitability (T) [pre- (T_{pre}) and postexercise (T_{post}) and at 0 (T_0), 15 (T_{15}), and 30 min (T_{30}) following continuous theta burst stimulation (cTBS)]. EX-Low, low-intensity exercise; EX-Mod, moderate-intensity exercise.

Table 1. *Baseline characteristics by genotype*

	Genotype	
	Val/Val	Met allele
n	10	15
Age, yr	27.8 ± 7.9	26.1 ± 8.4
Sex (F/M)	6/4	10/5
Physical activity (IPAQ), MET-min/wk	1,867 ± 783	1,393 ± 1,030
Handedness (EHI score, 0–100)	90 ± 11.7	89.0 ± 19.1
RMT, %MSO	42.6 ± 6.7	41.9 ± 9.7
1-mV TMS intensity, %MSO	51.3 ± 8.0	50.5 ± 11.4
AMT, %MSO	52.0 ± 7.7	54.3 ± 12.9

Values are means ± SD; *n*, no. of subjects. F, female; M, male; IPAQ, International Physical Activity Questionnaire; MET, metabolic equivalents; EHI, Edinburgh Handedness Inventory; RMT, resting motor threshold; MSO, maximum stimulator output; TMS, transcranial magnetic stimulation; AMT, active motor threshold.

Blood Sampling and Analysis

On each occasion, venous blood samples were taken from the antecubital vein via an indwelling catheter. Blood samples were taken before and immediately after exercise and at 5 and 10 min following cessation of exercise. Following collection of each sample, the catheter was flushed with sterile saline to prevent clot formation. This saline was later drawn off and discarded before obtaining the next sample. Samples were collected in a coagulant-free 10-ml serum tube (Vacuette, Greiner bio-one,) and stored on ice until the end of the experiment. Serum was then separated by centrifuging the blood at 1,800 *g* (Universal 32R, Hettich Zentrifugen) for 10 min at 4°C and stored at −80°C until analysis.

Serum BDNF and cortisol concentrations were analyzed using commercially available enzyme-linked immunosorbent assay kits, Chemicon (Temecula, CA) and Alpco Diagnostics (Salem, NH), respectively, according to the manufacturer's instructions. To minimize the assay variance, serum levels were analyzed in duplicate in a single session by a technician blinded to the nature of the exercise conditions. BDNF content was quantified against a standard curve calibrated with known amounts of BDNF, and samples were diluted 1:50–1:200 before assaying. The detection limit for BDNF was 0.4 μg/dl, and intra- and interassay coefficients of variation were 3.7 and 8.5%, respectively. The detection limit for cortisol was 7.9 pg/ml, and intra- and interassay coefficients of variation were 2–10% and 3–9%, respectively.

Data Analysis

The peak-to-peak amplitude of MEPs and M waves was measured in each trial. Trials in which electromyographic activity preceded MEPs were excluded from analysis. The average MEP and M-wave amplitude from each time point were calculated for each participant. Data analysis was conducted using SPSS Statistics (version 18.0; SPSS, Chicago, IL). Data that were skewed were transformed appropriately, if required before analysis. Linear mixed-model ANOVA with repeated measures were used to investigate the effects of exercise condition on MEP and M-wave amplitudes across time points. This was performed separately for the effect of exercise, with factors condition (low-, moderate-intensity, or control conditions) and time (before or after exercise). A second ANOVA was used to investigate the effect of cTBS on MEP and M-wave amplitude with factors condition (low-, moderate-intensity, or control) and time (before, after and 15 and 30 min post-cTBS). Condition and time were treated as fixed effects, and each subject was considered as a random effect. Additional ANOVA were repeated for mean values of BDNF and cortisol with four time periods: before and after exercise, and 5 and 10 min after exercise. Post hoc analyses were conducted using Fisher's least significant difference test to analyze significant main effects and interactions where necessary. The significance level was set at $P < 0.05$ for all comparisons, and data are shown as means ± SE.

RESULTS

Baseline characteristics of participants are shown in Table 1. Groups were well matched at baseline for all demographics, and there were no significant differences between all baseline measures (independent samples *t*-test, all $P > 0.05$). TMS characteristics at baseline are the average for all three experimental conditions.

Effect of Exercise

Cycling at a moderate-intensity resulted in a mean ± SD heart rate of 149 ± 13 beats/min, which equates to 76 ± 16% of each individual's age-predicted maximal heart rate. Participants reported this exercise intensity as 15 ± 1 on the Borg ratings of perceived exertion 6–20 scale, indicating a "hard" level of exertion. In contrast, the low-intensity exercise condition resulted in a mean heart rate of 110 ± 10 beats/min or 58 ± 5% of age-predicted maximal heart rate. Participants reported this level of intensity to be 11 ± 2, or "light" exertion.

M waves. Analysis of M-wave amplitude before and after each intervention revealed a significant effect of time ($F_{1,114} = 10.9$; $P = 0.001$) and condition ($F_{2,114} = 7.4$; $P = 0.001$), but no time × condition interaction ($F_{2,114} = 2.3$; $P = 0.109$). M waves were significantly smaller after exercise (average M-wave amplitude for all conditions across time: pre = 20.0 ± 0.9 mV vs. post = 18.3 ± 0.9 mV), and in the two exercise conditions (average M-wave amplitude across time points for each condition: moderate = 18.1 ± 0.9 mV; low = 18.8 ± 0.9 mV) compared with the control condition (20.4 ± 0.9 mV). Pairwise comparisons revealed that M-wave amplitude did not differ following the control condition ($P = 0.747$), whereas it decreased significantly in both exercise conditions ($P < 0.05$), as seen in Fig. 2.

MEPs. Due to the significant change in M-wave amplitude before and after exercise, MEP amplitude was normalized to M-wave amplitude for the remainder of the analyses. Data were skewed right, so analyses were performed on the square-root-transformed data. There was no main effect for time

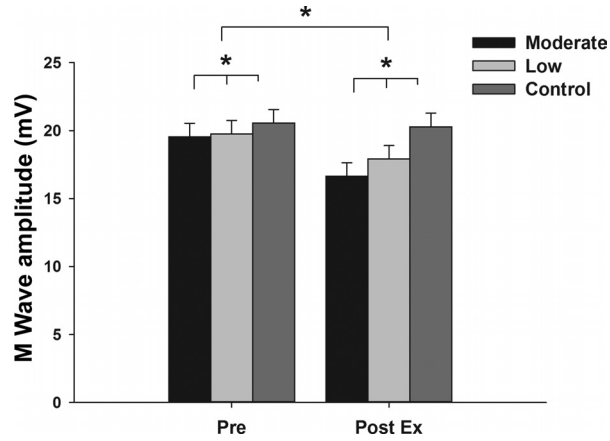

Fig. 2. Mean ± SE M-wave amplitude before (Pre) and after exercise (Post Ex) for the moderate-intensity, low-intensity, and control conditions. There were significant differences between the groups at baseline, and all M waves decreased over time (*$P < 0.05$).

($F_{1,100} = 0.1$; $P = 0.75$), condition ($F_{2,99} = 21.5$; $P = 0.22$), or a time × condition interaction ($F_{2,99} = 0.01$;= 0.99) when comparing MEPs recorded before and immediately following exercise or the control period (data not shown).

Effect of cTBS and Exercise

M waves. Amplitudes of M waves for the three conditions were compared at four time points: pre- and post-cTBS, and 15 and 30 min post-cTBS. It is important to note that the pre-cTBS measure is the same as the postexercise time point described above, as shown in Fig. 1, thus accounting for the smaller amplitude of M waves in the exercise conditions compared with the control conditions. The data were normally distributed, and analysis revealed main effects for condition ($F_{2,228} = 15.6$; $P < 0.001$) and time ($F_{3,229} = 3.4$; $P = 0.02$), but no condition × time interaction ($F_{6,228} = 1.3$; $P = 0.252$). M waves gradually increased in size over time, with post hoc analysis showing that the M waves at 30 min were significantly larger than those at preexercise ($P = 0.005$). Furthermore, M waves were significantly smaller in the moderate-intensity condition compared with the control and low-exercise conditions ($P < 0.001$ for both comparisons) (Fig. 3).

MEPs. Data were again normalized to M-wave amplitude, and square-root transformation was performed. Some observations were missing due to technical difficulties with M-wave recordings, and these were assumed in the mixed model to be missing at random. Figure 4 shows raw MEP amplitude data from a representative participant. This figure demonstrates the differences between conditions, with exercise increasing MEP amplitude following exercise, but not control, conditions, and cTBS inhibiting MEP amplitude in the low-intensity and control conditions only.

For all participants, normalized MEP amplitudes were different between conditions ($F_{2,159} = 34.5$; $P = 0.01$). Both the moderate-intensity (mean response ± SE: 2.14 ± 0.11, $P = 0.01$) and low-intensity (2.14 ± 0.10, $P = 0.006$) MEP responses across all time points were significantly larger than the control condition (1.86 ± 0.11), as shown in Fig. 5.

MEP amplitudes did not show a significant change over the four time points across the three groups ($F_{3,172} = 1.8$; $P = 0.14$). Post hoc pairwise comparisons were performed to investigate our a priori hypothesis that MEP amplitudes would decrease in all groups immediately post-cTBS and to determine whether there were any differences between the groups. The decrease in MEP amplitude observed immediately after cTBS was only statistically significant for the low-intensity exercise condition ($P = 0.02$), which can be seen in Fig. 5.

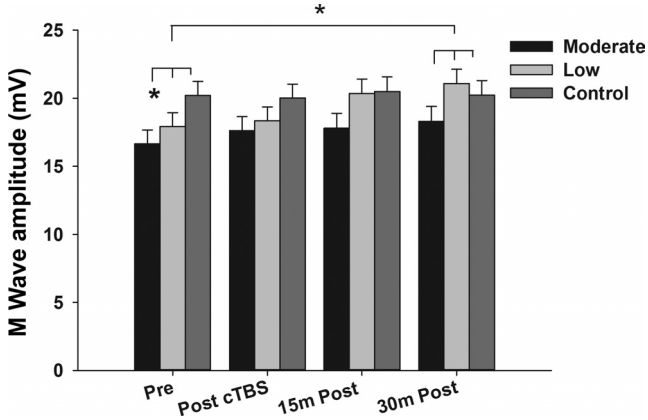

Fig. 3. Mean ± SE M-wave amplitude before (Pre), after (Post), and at 15 (15m Post) and 30 min following cTBS (30m Post). There were significant differences between the groups and over time (*$P < 0.05$) as M waves gradually increased in both moderate-intensity and low-intensity exercise conditions.

Genotyping for the *BDNF* polymorphism was performed in all participants, who were then classified as Val/Val ($n = 10$) or having the Met allele (i.e., Val/Met or Met/Met, $n = 15$). We repeated the mixed-model analysis on normalized MEP following cTBS, including the factors *BDNF* genotype, time, and condition, which did not reveal a main effect of genotype ($P = 0.17$), nor any genotype × time, genotype × condition, or genotype × condition × time interactions (all $P > 0.70$).

Blood results. Cortisol levels were normally distributed, and mixed-model analysis of variance revealed main effects for time ($F_{3,239} = 4.70$; $P = 0.003$) and condition ($F_{2,239} = 24.71$; $P < 0.001$). Cortisol levels decreased over the course of the experiment for low-intensity ($F_{3,239} = 3.84$; $P = 0.01$) and control conditions ($F_{3,239} = 3.94$; $P = 0.009$), as shown in Fig. 6*A*, but pairwise comparisons revealed that cortisol levels in the moderate-intensity condition did not decrease over time ($F_{3,239} = 0.646$; $P = 0.59$). This time × condition interaction was, however, not significant ($F_{6,239} = 1.86$; $P = 0.09$).

Serum levels of BDNF required square-root transformation to achieve a normal distribution. Subsequent analysis revealed a decline in BDNF levels for all conditions over time, evident as a main effect for time ($F_{3,243} = 43.80$; $P < 0.001$). There were no differences between conditions ($F_{2,243} = 2.06$; $P = 0.13$) and no time × condition interaction ($F_{6,243} = 0.81$; $P = 0.57$) (see Fig. 6). We repeated the analysis of serum BDNF levels with the factor group (Val or Met). This revealed a main effect for group for each of the three experimental conditions ($P < 0.001$) due to significantly lower baseline values of BDNF in serum for the Met group (e.g., control condition baseline serum BDNF was 45,615 ± 11,142 pg/ml for Val group and 16,454 ± 3,525 pg/ml for the Met group). Serum BDNF levels decreased over time in all conditions, regardless of genotype (Fig. 6*B*).

DISCUSSION

The results of the present study support the hypothesis that low-intensity aerobic exercise can promote neuroplasticity within the motor cortex of healthy adults. The stimulation paradigm chosen for this study, cTBS, is believed to be analogous to LTD of synaptic connections in animal studies (5) as it satisfies the requirement for activation of *N*-methyl-D-aspartate receptors (28), input specificity, and duration (>60 min) (29). This suggests that exercising at a low intensity, for a duration of 30 min, is able to modulate motor cortical plasticity.

The magnitude of MEP reduction immediately after cTBS was 8% of the baseline MEP normalized to M wave for the control condition, 18% in the low-intensity condition, while the moderate-intensity condition increased by 1%. We interpret this reduction in MEP amplitude as a decrease in cortical excitability, rather than spinal excitability, because the cTBS paradigm delivers subthreshold stimuli, which do not activate spinal motoneurons (29). This decrease in excitability is less

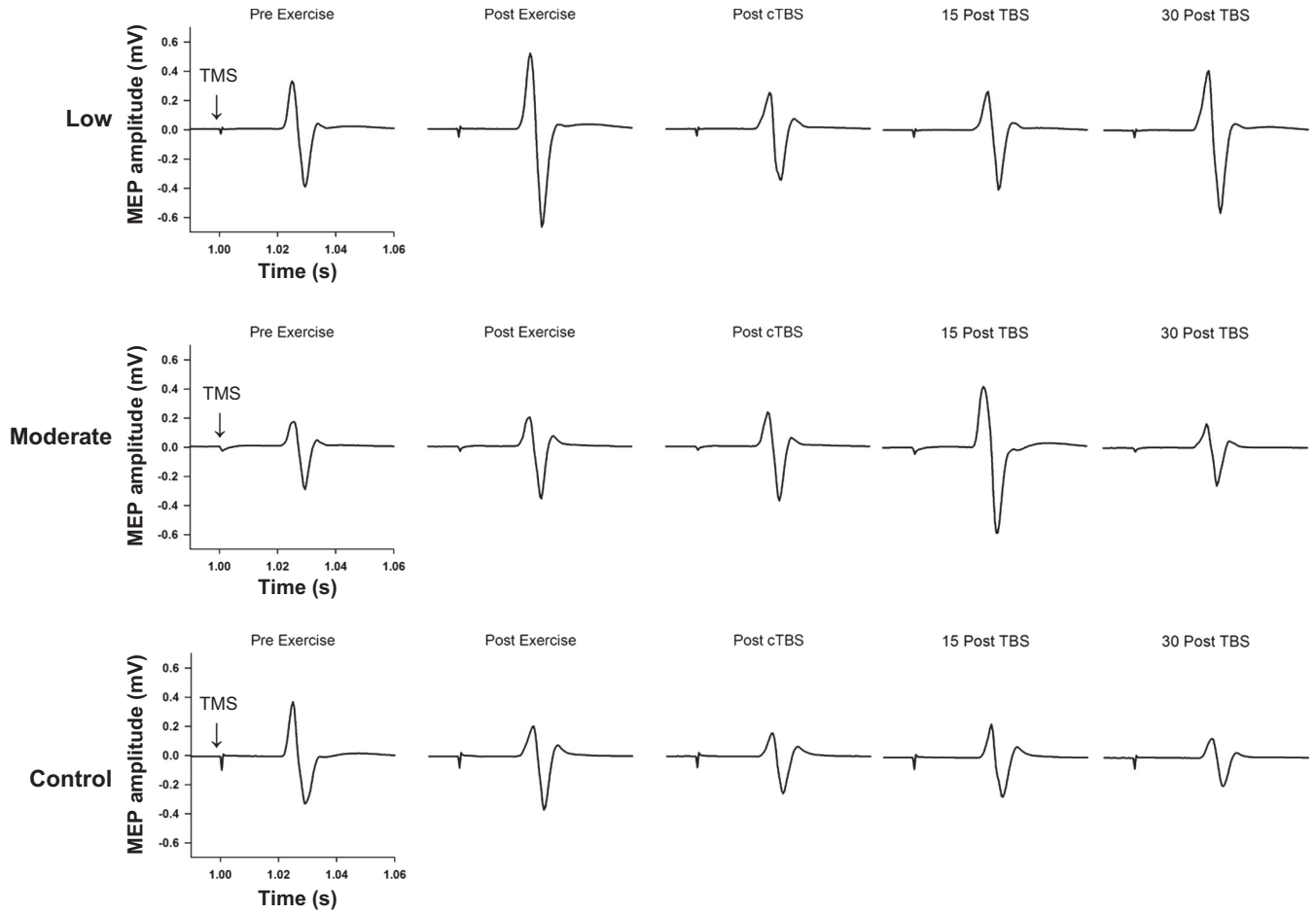

Fig. 4. Raw motor-evoked potential (MEP) data from a representative participant (genotype Val/Val) obtained before and after low- and moderate-intensity exercise, and in a no-exercise control condition. Exercise resulted in a 49% increase in MEP amplitude in the low-intensity condition, 35% increase following the moderate-intensity, and 16% decrease in the control condition. However, M waves decreased in amplitude in the high-intensity condition in particular, so the normalized results were 51% increase in normalized MEP amplitude for the low-intensity condition, 26% increase in moderate intensity, and 16% decrease in the control condition. Following cTBS, the change in raw MEP amplitude for each condition (low intensity 47% inhibition, moderate intensity 3% facilitation, control 28% inhibition) was similar to the normalized values (low 47% inhibition, moderate intensity 1% inhibition, control 31% inhibition). Blood results for this participant followed the general trend with decreased cortisol and brain-derived neurotrophic factor (BDNF) levels over time for all experimental conditions, except for a transient increase in BDNF levels immediately postexercise in the moderate-intensity condition (57,800 to 73,900 pg/ml), which returned to baseline within 5 min of completing the exercise. TMS, transcranial magnetic stimulation; TBS, theta burst stimulation.

than originally reported using a similar cTBS paradigm (29), and the effect was relatively short-lasting, with the maximal inhibition noted immediately after cTBS, and responses returning to baseline at 15 min post-cTBS. Evidence is emerging that responses to both cTBS and intermittent TBS are highly variable between individuals and may be related to intraindividual differences in the recruitment of interneuronal networks (17, 24, 58).

We hypothesized that, to facilitate plasticity induction, the exercise needed to be sufficiently intense to increase BDNF (55, 56) and yet not so high as to increase cortisol levels, which are likely to interfere with both BDNF expression (39) and plasticity induction (50). We observed that, when preceded by a period of exercise at ~55% of the maximal heart rate (at a "light" to "somewhat hard" exercise intensity), cTBS resulted in an immediate, but transient, suppression of MEP amplitudes that was not observed in the other two experimental conditions. We had hypothesized that increased circulating levels of BDNF may be associated with increasing exercise intensity and play a role in facilitating neuroplastic processes. However, in contrast to our original hypothesis, serum levels of BDNF decreased significantly across time for all three conditions. Most studies investigating the effect of acute exercise on circulating concentrations of BDNF report a dose-response relationship, with higher intensity exercise resulting in greater increases in BDNF concentrations (33). However, only 44% of studies of low- to moderate-intensity exercise demonstrate increased BDNF concentrations (33). We had expected that the moderate-intensity condition, at 75% of age-predicted maximal heart rate, would have been sufficient to increase BDNF levels, but other studies reporting increases in BDNF tended to perform a graded exercise test until exhaustion (33), which may be required to induce a significant increase in BDNF concentrations.

The decrease in BDNF concentrations over time, while unexpected, may in fact be related to changes in cortisol. The baseline blood sample was collected shortly after insertion of a cannula, which remained in the antecubital vein for the remainder of the experiment. This allowed for painless and quick sampling of blood immediately after the exercise, but the

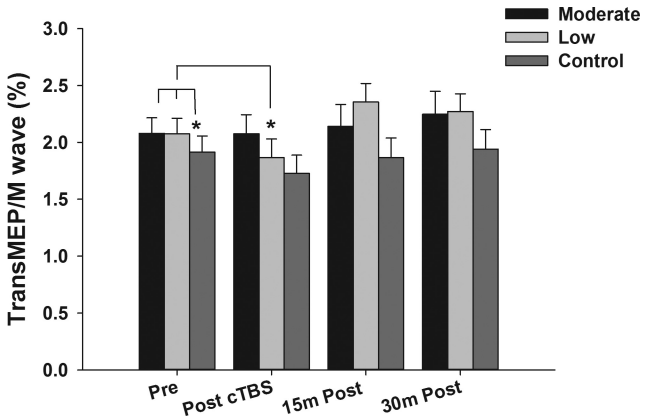

Fig. 5. Change in normalized MEP amplitudes over time, following square root transformation. MEP amplitudes decreased following cTBS for both the control and low-intensity conditions, but only the low-intensity condition changed significantly. The control condition MEP amplitudes were smaller than the exercise conditions at baseline (*$P < 0.05$). Values are means ± SE.

baseline sample was collected almost immediately after the cannula had been inserted and secured in place. Baseline cortisol levels are likely to have been elevated at the commencement of the experiment due to the venepuncture (38), and subsequent cortisol levels decreased significantly over time in the rest and low-intensity conditions. It is unlikely that the stress response associated with venepuncture would have a direct impact on baseline circulating levels of BDNF, but it has been demonstrated in animal studies that corticosterone administration interferes with BDNF mRNA expression (25). Therefore, the anticipated exercise-induced increases in BDNF may have been suppressed by the high cortisol levels at baseline.

While there was no difference in the serum BDNF response between treatments, there was a clear differential in the cortisol response between exercise conditions. In the control condition, there was a significant reduction in serum cortisol levels immediately postexercise, most likely reflecting a return to baseline levels after the stress associated with the venepuncture. This trend for a reduction in cortisol was also observed in the low-intensity condition, and for both of these conditions there was a continued decrease over the course of the experiment. In contrast, the moderate-intensity condition demonstrated a 10% increase in cortisol levels immediately postexercise, with a further increase 5 min later, and a subsequent decrease 10 min postexercise. We suggest that this elevation in circulating cortisol may have inhibited the neuroplastic response to cTBS observed in the moderate-intensity condition, as previously reported with a similar plasticity-inducing noninvasive brain stimulation technique (50).

Our finding that the presence of the Val^{66}Met polymorphism on the *BDNF* gene did not influence the neuroplastic response to cTBS is consistent with some recent work in this area. Kleim and colleagues (32) were the first to note that those with the Val^{66}Met polymorphism had reduced training-dependent plasticity, which was further supported by Cheeran and colleagues (8), who confirmed reduced long-term potentiation-like plasticity in those with the Met allele. However, and in contrast to these results, it has recently been shown that carriers of the Met allele responded no differently when tested with a quadripulse TMS plasticity-inducing paradigm (40). In addition, a recent study examined the influence of *BDNF* polymorphisms on the response to three different experimental protocols, involving repetitive TMS and motor practice. This work demonstrated that carriers of the Met allele did not respond differently to Val^{66}Val carriers (35). This could be due to specific interactions of the *BDNF* genotype with other single nucleotide polymorphisms that influence cortical plasticity (e.g., Ref. 57). We did, however, find that those with the Met allele had significantly lower serum BDNF levels at baseline, which has been previously reported (43).

We note that the control condition had significantly smaller MEPs at baseline across all participants. Given that the session orders were randomized and experimental procedures were identical for each session, we are unable to account for this observation other than by intersession variations in electrode placement. However, it is possible that the knowledge given to participants at the start of the experiment that they would not have to undertake exercise on this occasion may have contributed to this effect.

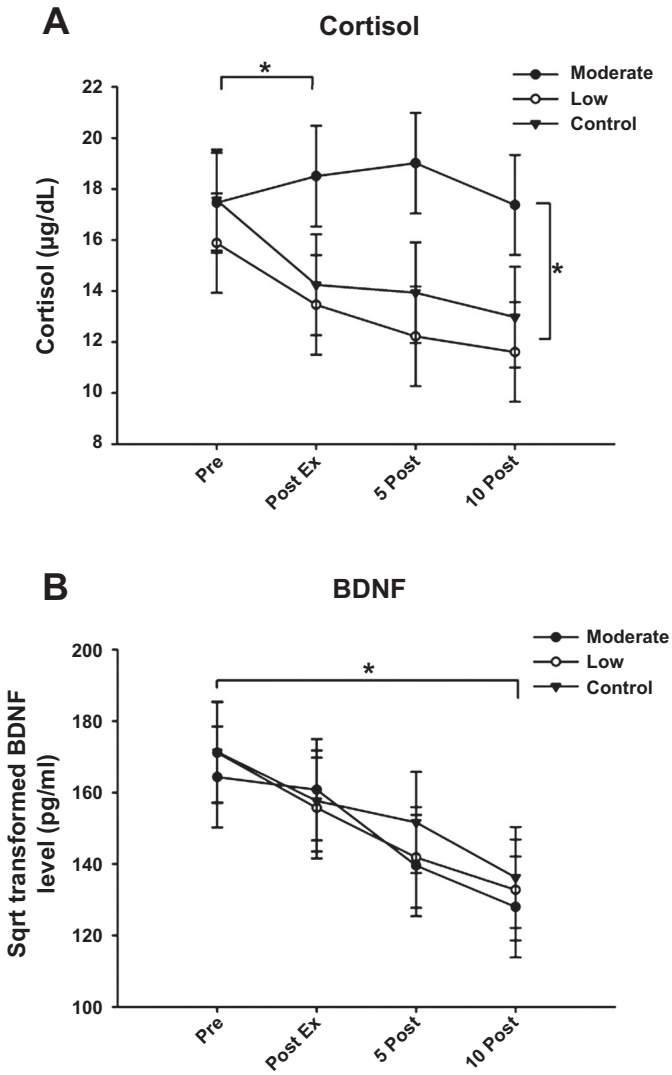

Fig. 6. Cortisol (*A*) and BDNF (*B*) levels over the time course of the experiments with moderate-intensity condition shown with solid circles, low intensity with open circles, and the control condition as solid triangles. There were significant differences for cortisol over time and between conditions, and for BDNF over time (*$P < 0.05$). Values are means ± SE.

Responses to single-pulse TMS were recorded throughout the experiment to examine corticospinal excitability. Previous studies have reported that repetitive, nonskilled exercise results in significant depression of excitability in the corticospinal projection to muscles involved in the task, without any changes in M-wave amplitude (59). In addition, lower limb fatiguing exercise can influence cortical excitability and cortical inhibition for the nonexercised upper limb, with the exercise initially facilitating and then depressing cortical excitability for both hand and arm muscles for up to 20 min (54). It is unlikely that there was significant fatigue in either of the exercise conditions, and, consistent with this, we found no change in FDI MEPs following exercise.

In contrast, exercise reduced the amplitude of M waves in FDI, most dramatically in the moderate-intensity condition, with a mean decrease of 14% compared with 8% after low-intensity exercise, and the control condition remaining essentially unchanged. Repeated measurement of M waves alone can be associated with reduced amplitude over the course of an experiment (15). However, this is unlikely to be a factor here, as the M waves did not change in the control condition. The reason for the reduction in the exercise conditions is not clear, but changes in body temperature may play a role. Direct warming of the lower limbs has been shown to decrease maximal M-wave amplitude by 12% (16), a finding confirmed with warming of the upper limbs (6). Exercising in a hot environment to increase body temperature can also lead to reduced ability to generate force in the upper limb, as shown by reduced maximal voluntary contractions when performing a handgrip task after exercising on a cycle ergometer (42). Experiments in the present study were carried out in an air-conditioned laboratory, and, although skin temperature was not measured, participants were noted to sweat profusely during the moderate-intensity condition in particular. The gradual return of M-wave amplitudes toward baseline over the remaining 30 min of the study would be consistent with a decrease of body temperature back to normal levels following exercise.

A limitation of the present study is that we did not have additional experimental conditions where participants underwent exercise but not cTBS. Therefore, we cannot rule out delayed changes in cortical excitability that were not evident immediately after exercise. Our initial hypothesis was that, for exercise to have a role in priming the brain, changes in the cortical environment that might facilitate plasticity would be required (e.g., neurotrophic factors). The primary outcome of interest, LTD-like neuroplastic change was not, however, associated with an increase in serum BDNF levels. This has been reported previously (33, 56), but we acknowledge that measuring peripheral BDNF is perhaps an inadequate marker of cortical BDNF. It is likely that other mechanisms are involved in the low-intensity exercise facilitation of short-term plasticity reported in this study. For example, upregulation of other neurochemicals following exercise, such as insulin-like growth factor I and vascular endothelial growth factor, known to modulate neuroplasticity and learning (13) and angiogenesis (26), respectively, may be important. Exercise also has a crucial role in reducing proinflammatory conditions (45), which impair growth factor signaling (13). Increased flow velocity through the cerebral blood vessels also occurs during exercise (30), delivering required nutrients to the active brain and possibly overcoming any deficiency that might be present before exercise.

In conclusion, low-intensity cycling, but not rest or moderate-intensity exercise, promoted cTBS-induced neuroplasticity within the motor cortex of healthy adults. Aerobic exercise has the potential to prime the motor cortex to enhance the effectiveness of motor learning or recovery following brain damage. This may have clinical implications for neurological rehabilitation, for example following stroke, where exercise to prime the motor cortex enhances motor function of the upper limbs (53). Increasing physical activity is an important yet often overlooked aspect of secondary prevention following stroke, and these data suggest that low-intensity exercise, which does not increase cortisol levels, may have a role in improving brain health as well as cardiovascular health.

ACKNOWLEDGMENTS

We thank Dr. Ashleigh Smith for assistance with early experiments, and the late Dr. John Petkov for assistance with statistical analysis.

GRANTS

M. N. McDonnell and M. C. Ridding are supported by National Health and Medical Research Council of Australia fellowships and this research was funded by a grant from the Brain Foundation of Australia.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.N.M., J.D.B., M.C.R., and J.G.S. conception and design of research; M.N.M., G.M.O., and M.C.R. performed experiments; M.N.M., G.M.O., and J.G.S. analyzed data; M.N.M., J.D.B., G.M.O., M.C.R., and J.G.S. interpreted results of experiments; M.N.M. prepared figures; M.N.M. drafted manuscript; M.N.M., J.D.B., G.M.O., M.C.R., and J.G.S. edited and revised manuscript; M.N.M., J.D.B., G.M.O., M.C.R., and J.G.S. approved final version of manuscript.

REFERENCES

1. **Angevaren M, Aufdemkampe G, Verhaar HJ, Aleman A, Vanhees L.** Physical activity and enhanced fitness to improve cognitive function in older people without known cognitive impairment. *Cochrane Database Syst Rev* 16: CD005381, 2008.
2. **Astrand PO, Ryhming I.** A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during sub-maximal work. *J Appl Physiol* 7: 218–221, 1954.
3. **Binder DK, Scharfman HE.** Brain-derived neurotrophic factor. *Growth Factors* 22: 123–131, 2004.
4. **Blackmore DG, Golmohammadi MG, Large B, Waters MJ, Rietze RL.** Exercise increases neural stem cell number in a growth hormone-dependent manner, augmenting the regenerative response in aged mice. *Stem Cells* 27: 2044–2052, 2009.
5. **Bliss TV, Collingridge GL.** A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31–39, 1993.
6. **Bolton CF, Sawa GM, Carter K.** The effects of temperature on human compound action potentials. *J Neurol Neurosurg Psychiatry* 44: 407–413, 1981.
7. **Brunner R, Schaefer D, Hess K, Parzer P, Resch F, Schwab S.** Effect of corticosteroids on short-term and long-term memory. *Neurology* 64: 335–337, 2005.
8. **Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R, Rothwell JC.** A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS. *J Physiol* 586: 5717–5725, 2008.
9. **Cirillo J, Hughes J, Ridding M, Thomas PQ, Semmler JG.** Differential modulation of motor cortex excitability in BDNF Met allele carriers following experimentally induced and use-dependent plasticity. *Eur J Neurosci* 36: 2640–2649, 2012.

10. **Cirillo J, Lavender AP, Ridding MC, Semmler JG.** Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. *J Physiol* 587: 5831–5842, 2009.
11. **Colcombe S, Kramer AF.** Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci* 14: 125–130, 2003.
12. **Cotman CW, Berchtold NC.** Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci* 25: 295–301, 2002.
13. **Cotman CW, Berchtold NC, Christie LA.** Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci* 30: 464–472, 2007.
14. **Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P.** International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 35: 1381–1395, 2003.
15. **Crone C, Johnsen LL, Hultborn H, Orsnes GB.** Amplitude of the maximum motor response (Mmax) in human muscles typically decreases during the course of an experiment. *Exp Brain Res* 124: 265–270, 1999.
16. **Dewhurst S, Riches PE, Nimmo MA, De Vito G.** Temperature dependence of soleus H-reflex and M wave in young and older women. *Eur J Appl Physiol* 94: 491–499, 2005.
17. **Di Lazzaro V, Dileone M, Pilato F, Capone F, Musumeci G, Ranieri F, Ricci V, Bria P, Di Iorio R, de Waure C, Pasqualetti P, Profice P.** Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. *J Neurophysiol* 105: 2150–2156, 2011.
18. **Di Lazzaro V, Pilato F, Saturno E, Oliviero A, Dileone M, Mazzone P, Insola A, Tonali PA, Ranieri F, Huang YZ, Rothwell JC.** Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. *J Physiol* 565: 945–950, 2005.
19. **Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR.** The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112: 257–269, 2003.
20. **Etgen T, Sander D, Huntgeburth U, Poppert H, Forstl H, Bickel H.** Physical activity and incident cognitive impairment in elderly persons: the INVADE study. *Arch Intern Med* 170: 186–193, 2010.
21. **Freitas C, Perez J, Knobel M, Tormos JM, Oberman L, Eldaief M, Bashir S, Vernet M, Pena-Gomez C, Pascual-Leone A.** Changes in cortical plasticity across the lifespan. *Front Aging Neurosci* 3: 5, 2011.
22. **Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B.** Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66: 198–204, 2010.
23. **Gore CJ, Booth ML, Bauman A, Owen N.** Utility of pwc75% as an estimate of aerobic power in epidemiological and population-based studies. *Med Sci Sports Exerc* 31: 348–351, 1999.
24. **Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC.** The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex.* In press.
25. **Hansson AC, Sommer WH, Metsis M, Stromberg I, Agnati LF, Fuxe K.** Corticosterone actions on the hippocampal brain-derived neurotrophic factor expression are mediated by exon IV promoter. *J Neuroendocrinol* 18: 104–114, 2006.
26. **Hillman CH, Erickson KI, Kramer AF.** Be smart, exercise your heart: exercise effects on brain and cognition. *Nat Rev Neurosci* 9: 58–65, 2008.
27. **Hirsch MA, Farley BG.** Exercise and neuroplasticity in persons living with Parkinson's disease. *Eur J Phys Rehabil Med* 45: 215–229, 2009.
28. **Huang YZ, Chen RS, Rothwell JC, Wen HY.** The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol* 118: 1028–1032, 2007.
29. **Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC.** Theta burst stimulation of the human motor cortex. *Neuron* 45: 201–206, 2005.
30. **Ide K, Secher NH.** Cerebral blood flow and metabolism during exercise. *Prog Neurobiol* 61: 397–414, 2000.
31. **Keenan PA, Jacobson MW, Soleymani RM, Mayes MD, Stress ME, Yaldoo DT.** The effect on memory of chronic prednisone treatment in patients with systemic disease. *Neurology* 47: 1396–1402, 1996.
32. **Kleim JA, Chan S, Pringle E, Schallert K, Procaccio V, Jimenez R, Cramer SC.** BDNF val66met polymorphism is associated with modified experience-dependent plasticity in human motor cortex. *Nat Neurosci* 9: 735–737, 2006.
33. **Knaepen K, Goekint M, Heyman EM, Meeusen R.** Neuroplasticity–exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects. *Sports Med* 40: 765–801, 2010.
34. **Kubesch S, Bretschneider V, Freudenmann R, Weidenhammer N, Lehmann M, Spitzer M, Gron G.** Aerobic endurance exercise improves executive functions in depressed patients. *J Clin Psychiatry* 64: 1005–1012, 2003.
35. **Li Voti P, Conte A, Suppa A, Iezzi E, Bologna M, Aniello MS, Defazio G, Rothwell JC, Berardelli A.** Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. *Exp Brain Res* 212: 91–99, 2011.
36. **Marlatt MW, Lucassen PJ, van Praag H.** Comparison of neurogenic effects of fluoxetine, duloxetine and running in mice. *Brain Res* 1341: 93–99, 2010.
37. **Masley S, Roetzheim R, Gualtieri T.** Aerobic exercise enhances cognitive flexibility. *J Clin Psychol Med Settings* 16: 186–193, 2009.
38. **Meeran K, Hattersley A, Mould G, Bloom SR.** Venepuncture causes rapid rise in plasma ACTH. *Br J Clin Pract* 47: 246–247, 1993.
39. **Murakami S, Imbe H, Morikawa Y, Kubo C, Senba E.** Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci Res* 53: 129–139, 2005.
40. **Nakamura K, Enomoto H, Hanajima R, Hamada M, Shimizu E, Kawamura Y, Sasaki T, Matsuzawa D, Sutoh C, Shirota Y, Terao Y, Ugawa Y.** Quadri-pulse stimulation (QPS) induced LTP/LTD was not affected by Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) gene. *Neurosci Lett* 487: 264–267, 2011.
41. **Neves FS, Malloy-Diniz L, Romano-Silva MA, Campos SB, Miranda DM, De Marco L, Figueira PG, Krebs MO, Correa H.** The role of BDNF genetic polymorphisms in bipolar disorder with psychiatric comorbidities. *J Affect Disord* 131: 307–311, 2011.
42. **Nybo L, Nielsen B.** Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol* 91: 1055–1060, 2001.
43. **Ozan E, Okur H, Eker C, Eker OD, Gonul AS, Akarsu N.** The effect of depression, BDNF gene val66met polymorphism and gender on serum BDNF levels. *Brain Res Bull* 81: 61–65, 2010.
44. **Pereira AC, Huddleston DE, Brickman AM, Sosunov AA, Hen R, McKhann GM, Sloan R, Gage FH, Brown TR, Small SA.** An in vivo correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proc Natl Acad Sci U S A* 104: 5638–5643, 2007.
45. **Petersen AM, Pedersen BK.** The anti-inflammatory effect of exercise. *J Appl Physiol* 98: 1154–1162, 2005.
46. **Podewils LJ, Guallar E, Kuller LH, Fried LP, Lopez OL, Carlson M, Lyketsos CG.** Physical activity, APOE genotype, and dementia risk: findings from the Cardiovascular Health Cognition Study. *Am J Epidemiol* 161: 639–651, 2005.
47. **Pontifex MB, Hillman CH, Fernhall B, Thompson KM, Valentini TA.** The effect of acute aerobic and resistance exercise on working memory. *Med Sci Sports Exerc* 41: 927–934, 2009.
48. **Rojas Vega S, Struder HK, Vera Wahrmann B, Schmidt A, Bloch W, Hollmann W.** Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Res* 1121: 59–65, 2006.
49. **Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH, et al.** Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol* 91: 79–92, 1994.
50. **Sale MV, Ridding MC, Nordstrom MA.** Cortisol inhibits neuroplasticity induction in human motor cortex. *J Neurosci* 28: 8285–8293, 2008.
51. **Schneider S, Vogt T, Frysch J, Guardiera P, Struder HK.** School sport–a neurophysiological approach. *Neurosci Lett* 467: 131–134, 2009.
52. **Sports Medicine Australia.** *Sports Medicine Australia Pre-Exercise Screening System*, edited by Department of Health and Ageing. Canberra, ACT, Australia: Government of Australia, 2005.
53. **Stinear CM, Barber PA, Coxon JP, Fleming MK, Byblow WD.** Priming the motor system enhances the effects of upper limb therapy in chronic stroke. *Brain* 131: 1381–1390, 2008.
54. **Takahashi K, Maruyama A, Hirakoba K, Maeda M, Etoh S, Kawahira K, Rothwell JC.** Fatiguing intermittent lower limb exercise influences corticospinal and corticocortical excitability in the nonexercised upper limb. *Brain Stimul* 4: 90–96, 2011.
55. **Tang SW, Chu E, Hui T, Helmeste D, Law C.** Influence of exercise on serum brain-derived neurotrophic factor concentrations in healthy human subjects. *Neurosci Lett* 431: 62–65, 2008.

56. **Winter B, Breitenstein C, Mooren FC, Voelker K, Fobker M, Lechtermann A, Krueger K, Fromme A, Korsukewitz C, Floel A, Knecht S.** High impact running improves learning. *Neurobiol Learn Mem* 87: 597–609, 2007.
57. **Witte AV, Kurten J, Jansen S, Schirmacher A, Brand E, Sommer J, Floel A.** Interaction of BDNF and COMT polymorphisms on paired-associative stimulation-induced cortical plasticity. *J Neurosci* 32: 4553–4561, 2012.
58. **Zafar N, Paulus W, Sommer M.** Comparative assessment of best conventional with best theta burst repetitive transcranial magnetic stimulation protocols on human motor cortex excitability. *Clin Neurophysiol* 119: 1393–1399, 2008.
59. **Zanette G, Bonato C, Polo A, Tinazzi M, Manganotti P, Fiaschi A.** Long-lasting depression of motor-evoked potentials to transcranial magnetic stimulation following exercise. *Exp Brain Res* 107: 80–86, 1995.
60. **Zigmond M, Smeyne RJ.** Foreword: exercise and the brain. *Brain Res* 1341: 1–2, 2010.