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Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males

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Abstract

Physical activity has been reported to improve cognitive function in humans and rodents, possibly via a brain-derived neurotrophic factor (BDNF)-regulated mechanism. In this study of human subjects, we have assessed the effects of acute and chronic exercise on performance of a face-name matching task, which recruits the hippocampus and associated structures of the medial temporal lobe, and the Stroop word-colour task, which does not, and have assessed circulating concentrations of BDNF and IGF-1 in parallel. The results show that a short period of high-intensity cycling results in enhancements in performance of the face-name matching, but not the Stroop, task. These changes in cognitive function were paralleled by increased concentration of BDNF, but not IGF-1, in the serum of exercising subjects. 3 weeks of cycling training had no effect on cardiovascular fitness, as assessed by VO_2 scores, cognitive function, or serum BDNF concentration. Increases in fitness, cognitive function and serum BDNF response to acute exercise were observed following 5 weeks of aerobic training. These data indicate that both acute and chronic exercise improve medial temporal lobe function concomitant with increased concentrations of BDNF in the serum, suggesting a possible functional role for this neurotrophic factor in exercise-induced cognitive enhancement in humans.

Keywords: Exercise; learning; BDNF; hippocampus

Introduction

The benefits that physical activity confers on cardiovascular health are well known, while recent evidence has also demonstrated the ability of exercise to promote brain health. The evidence that physically active older people, particularly those that have been active throughout their lifespan, are at decreased risk of developing Alzheimer's disease and other forms of dementia relative to their sedentary counterparts [1-3] strongly suggests that exercise may be a powerful protective strategy against age-related neurodegenerative decline. In addition to its neuroprotective actions, exercise enhances cognitive function in elderly people and slows the progression of dementia-related cognitive symptoms [4-6]. Thus, exercise may reduce the risk of developing dementia or ameliorate cognitive impairment already present in those suffering from neurodegenerative decline.

Moreover, exercise may also enhance cognitive function in young, healthy, adults. High impact running has been shown to improve vocabulary learning [7], while cycling has been shown to improve performance in a map recognition task [8] and in the Stroop word-colour task [9]. However, Grego *et al*. [8] also showed that prolonged exercise leading to fatigue compromises cognitive function. It has been suggested that intense exercise may facilitate aspects of cognitive function in a manner dependent on an individual's cardiovascular fitness [10]. A recent meta-analysis indicates that cognitive performance may be enhanced or impaired depending on when, relative to an acute exercise bout, performance is measured, the type of cognitive task selected, and the type of exercise performed [11].

Evidence available from animal studies provides some insight into the mechanisms by which exercise may enhance cognition. In rodent models, exercise has consistently been shown to enhance learning and persistently upregulate expression of brain-derived neurotrophic factor (BDNF) in the hippocampus [12-16]. The indisputable importance of the hippocampus in learning and memory and the role of BDNF in mediating hippocampal synaptic plasticity are well established [17-20]; while additional evidence indicates a role for insulin-like growth factor (IGF-1) in mediating the cognitive effects of exercise [21-23]. Interestingly, serum BDNF concentration has repeatedly been reported to increase following exercise in humans [9, 24-26] (for review see [27]), while IGF-1 responses to exercise are more variable [28-30].

Here, we have investigated the effect of acute exercise and aerobic exercise training on cognitive function in young, sedentary men. Given the evidence from animal models that hippocampal function is particularly responsive to exercise intervention, we assessed the impact of acute and aerobic exercise training on performance in a face-name matching task that recruits the hippocampus, and also on circulating concentrations of BDNF and IGF-1, in an attempt to investigate the possible causal links between increased availability of these growth factors and enhancements in cognitive function.

Materials and Methods

Participants

The experimental protocol was approved by the Ethical Committee for Research Involving Human Participants, Faculty of Health Sciences, Trinity College Dublin. Forty-seven healthy male students volunteered to participate (age, height, weight: 22 ± 2 yrs, 180 ± 7 cm, 82 ± 11 kg respectively, mean ± SD). All subjects were sedentary (not involved in any regular physical training) prior to commencement of the study, and each received a routine medical examination before providing written informed consent in accordance with the declaration of Helsinki. Exclusion criteria included any contraindications to intense exercise discovered during the medical examination, intake of prescription medication, history of neurological problems, pre-existing injuries, smoking and intake of recreational drugs. All subjects were required to fast for 2 hours and to refrain from consumption of caffeine for 12 hours prior to testing.

Experimental protocol

Participants were allocated to an exercise group (EX) or a sedentary control group (CON; Fig 1b). During the first testing session all participants performed a set of cognitive tasks, including the face-name matching task and the Stroop task. Following this, the EX group completed a graded exercise test, which served as the acute exercise bout, while CON participants had a 30min rest (Fig 1a). Blood samples were collected from the EX group throughout the testing session, in order to assess the effect of acute exercise on serum BDNF and IGF-1 concentrations. A baseline blood sample was taken at 0min, followed

by the first set of cognitive tests. Another blood sample was taken at 30min, prior to the graded exercise test (30min rest period for CON). A third, post-acute exercise, blood sample was taken at 60min, the second set of cognitive tests was completed and a final blood sample was drawn at 90min (Figure1a).

Two chronic exercise protocols were used to assess the effects of both a 3-week and a 5-week aerobic training intervention on cognition and on serum concentrations of BDNF and IGF-1. These aerobic training programmes were identical in all aspects except for duration. Following the first testing session the EX group was split into the subgroups: C-EX3, C-EX5, A-EX3 & A-EX5 (Fig 1a). The chronic-exercise subgroups, C-EX3 and C-EX5, completed 3 and 5 weeks of aerobic training respectively. The acute-exercise subgroups; A-EX3 and A-EX5, remained sedentary for the corresponding 3 and 5-week intervals and exercised only when performing the graded exercise test. All subgroups repeated the testing session (session 2, as in Fig 1a) following the appropriate 3 or 5-week interval. The CON group remained sedentary both during the testing sessions and during the 3-week or 5-week interval between sessions.

Acute exercise

The acute exercise protocol consisted of a graded exercise test (GXT) to volitional exhaustion, performed on a stationary cycle ergometer (Lode Excalibur, Groningen, Netherlands), to establish maximal oxygen consumption rate (VO_2 max). The initial workload was set at 75W and increased by 50W increments every 3min, until 9min, and subsequently by 25W increments each min until volitional exhaustion was reached. The subject wore a facemask throughout the test in order to collect expired air, which was analysed for

volume and gas composition using an online system (Metalyser, Cortex Biophysik, Leipzig, Germany) and from which VO_2 max was determined as a measure of aerobic fitness. Subjects wore a heart rate monitor (Polar, Oulu, Finland) throughout the test and were verbally encouraged to continue cycling until heart rate (beats.min^{-1}) approached the age predicted maximum (220 – age (yrs)).

Cognitive testing

Subjects were seated before a computer screen and were tested using the Face-Name matching task, followed immediately by the Stroop Word-Colour task.

Face-Name matching task

The face-name task was adapted from a paradigm by Zeineh et al. [31] and included encoding, distractor and recall phases. During the encoding block the subject was presented with images of ten unfamiliar faces paired with names. Each was presented in sequence for 3.5 s via a computer screen display. A 40 s distractor task was performed between each encoding and recall phase to prevent rote rehearsal of the face-name associations. During the distractor task, a fixation cross was presented on the screen at random intervals (0.5 to 3.5 s), and was replaced for 300 ms on screen by a black circle; the subject was required to press a response button on the keypad when presented with a black circle. The recall block consisted of a randomized presentation of the previously-viewed faces but without the paired names. Subjects were requested to recall the correct name and communicate it verbally to the experimenter.

Subjects viewed the same face-name combinations four times per task, providing a maximum possible score of 40. With each subject eventually completing two testing sessions comprising two cognitive trials, four different series of faces and names were used during the study, with no subject repeating the cognitive assessment on the same series of faces. Results are presented as the number of pairs recalled.

Stroop Word-Colour task

The Stroop word-colour task consisted of 2 trials of equal duration with a short (<1 min) inter-trial rest period. Participants were presented with a series of colour words (red, yellow, green, blue) on a computer screen. Words were presented in the same (congruent) or different (incongruent) coloured font. Words appeared on screen for 1.3 s. Subjects were required to inhibit their automatic response to read the word stimulus presented, and instead to report the font colour in which words were presented by pressing colour-coded buttons on a response pad. The protocol consisted of frequent congruent stimuli with randomized infrequent incongruent stimuli, in order to maximize cognitive interference. Results are presented as percentage response accuracy. Tasks were programmed and run using *E-Prime version 1.1* software (Psychology Software Tools, Pittsburgh Tools, Pittsburgh, USA).

Aerobic Training

The chronic-exercise groups, C-EX3 and C-EX5 were required to attend the lab for supervised aerobic cycle training three times per week until session 2. Training was performed on a stationary cycle ergometer for between 30 and 60 min per session. The workload and duration of the exercise were increased

gradually until subjects could maintain a workload estimated to require 60% VO_2max for 60 min. Heart rate was monitored during each training session as a second method of ensuring training intensity was near to the expected workload and as such that the training was sub-maximal and progressive over the entire training period (3 or 5 weeks).

Blood sampling

During each testing session venous blood samples were obtained at t=0, 30, 60 and 90 min via an indwelling catheter located in a forearm vein. Following each sample collection the catheter was flushed with sterile saline (NaCl 0.9% (w/v)) to prevent clot formation within the catheter, while the catheter was cleared of saline prior to each sample collection. Samples were collected into coagulant-free 6 ml vaccutainer specimen tubes, incubated for 20 min at room temperature to allow clotting, then centrifuged at 5000 rpm for 20 min. The resulting supernatant was removed and stored at -80°C for later analysis of the serum concentration of IGF-1 and BDNF.

Analysis of BDNF & IGF-1

Serum concentrations of BDNF ($E_{max}^{®}$ Immunoassay system; Promega Corporation, Madison, WI, USA) and IGF-1 (Human IGF-1 DuoSet ELISA Development kit; R&D Systems Europe, U.K.) were assessed by ELISA. In the case of BDNF, 96-well plates were coated with anti-BDNF monoclonal antibody (1:1000 dilution in carbonate coating buffer; 50 µl) and incubated overnight at 4°C. Plates were washed with Tris-buffered saline-Tween 20 wash buffer (TBS-T; 150 mM NaCl, 50 mM Tris-HCl, and 0.05% v/v Tween 20, pH 7.4) using an

automated plate washer (Columbus Plus, Tecan, Austria), and blocked with block and sample buffer (100 µl) for 1hr at room temperature. Plates were washed with TBS-T and samples and standards were added (50 µl) and incubated for 2 hr at room temperature on an automated plate shaker. After a further five washes with TBS-T, anti-Human BDNF polyclonal antibody was added (1:500 dilution in 1X block buffer; 50 µl) and incubated for 2 hr at room temperature. Plates were rinsed five times with TBS-T, anti-IgY HRP conjugate (1:200 dilution in 1X block buffer; 50 µl) was added and the plates incubated for 1 hr at room temperature on a plate shaker. After a final wash with TBS-T, TMB One solution (50 µl) was added and plates incubated for 30 min on a plate shaker. The reaction was stopped with 1N HCL (50 µl) and the absorbance of samples and standards were read at 450 nm using a plate reader (Sunrise basic, Tecan, Austria).

For the IGF-1 ELISA, 96-well plates were coated with capture antibody (mouse anti-human IGF-1, 1:180 dilution in PBS; 80 µl) and incubated overnight at room temperature. Plates were washed and blocked with block buffer (5% Tween 20, 5% Sucrose in PBS). Samples and standards were added (50 µl) and incubated for 2 hr at room temperature. Plates were washed, incubated with detection antibody (biotinylated goat anti-human IGF-1, 1:180 dilution in reagent diluent; 80 µl) for 2 hr at room temperature, and reacted with Streptavidin-HRP (1:200 dilution in reagent diluent; 80 µl) for 20 min. The reaction was stopped with 1N H_2SO_4 (50 µl). The absorbance of samples and standards were read at 450 nm, standard curves were constructed for each plate and concentrations of BDNF and IGF-1 in the samples were extrapolated from the curves.

Statistical Analysis

Statistical analyses were performed using Graphpad Prism 5 for Mac OSX. Data are expressed as mean ± standard deviation (SD). Group n numbers are indicated in Figure 1b. Where n numbers differ from Figure 1b, it is due to the removal of outliers (values greater than two standard deviations outside the mean) and is clearly indicated in the results. A possible limitation of our study is that *a priori* power calculations were not completed to determine optimal sample size. For analysis of the face-name task and the Stroop task, two-way repeated measures analysis of variance (ANOVA) were used to assess both the effect of trial (the repeated measure) and the effect of group. Where a significant difference occurred, Bonferroni *post hoc* analyses were performed. For the serum analysis, no blood samples were taken from the CON group, hence one-way repeated measures ANOVA with *post hoc* Newman-Keuls were used to analyse serum BDNF changes over time for session 1. Two-way repeated measures ANOVA with *post hoc* Bonferroni were used to analyse BDNF concentrations for session 2, to assess both the effect of time (the repeated measure) and group. A value of $p<0.05$ was considered to be significant.

Results

Acute exercise selectively enhanced cognitive function

Acute exercise induced an enhancement in hippocampal-dependent memory, as assessed by the face-name task (Fig. 2a). There was a significant effect of

trial ($p<0.0001$, $F_{(1,41)}=19.42$; two-way repeated measures ANOVA and *post hoc* Bonferroni; CON n=13; EX n=30), indicating that performance in trial 2 was greater than trial 1. There was also a significant effect of group, ($p=0.0219$, $F_{(1,41)}=5.681$), indicating that exercise altered task performance. *Post hoc* analysis revealed that while CON scores increased across trial, (trial 1: 12.15 ± 3.13 pairs recalled, trial 2: 17.08 ± 6.85 pairs recalled, $p<0.05$), EX improved in the performance of the task to a greater extent, (trial 1: 16.03 ± 5.77 pairs recalled, trial 2: 21.20 ± 7.01 pairs recalled, $p<0.001$). This suggests that, although familiarization with the task may have resulted in an improved score, the acute-exercise intervention resulted in an enhancement in the performance of this task. Results are expressed as mean ± SD of the total number of pairs recalled across the 4 recall blocks, hence 40 is the maximum possible score. Acute exercise did not alter performance of the Stroop word-colour task in either congruent trials (Fig. 2b) or incongruent trials (Fig. 2c). Statistical analysis was by two-way repeated measures ANOVA (CON n=13, EX n=29).

Effect of acute exercise on serum BDNF and IGF-1

Acute exercise in the form of a graded exercise test (GXT) induced an increase in serum BDNF concentration (Fig. 3a). There was a significant difference between timepoints ($p=0.0126$, $F_{(3,93)}=3.813$; one-way repeated measures ANOVA and *post hoc* Newman-Keuls, n=32). *Post hoc* analysis revealed a significant increase in serum BDNF concentration immediately post-GXT (60 min: 1294.0 ± 820.5 $pg.ml^{-1}$), relative to baseline (0 min: 974.7 ± 741.6 $pg.ml^{-1}$, $p<0.05$) and relative to the 30 min timepoint (1004.0 ± 694.1 $pg.ml^{-1}$, $p<0.05$). At 90 min (ie. 30 min post-exercise) serum BDNF was not significantly different

to baseline, 30 min or to 60 min (90 min: 1254.0 ± 871.4 $pg.ml^{-1}$). Results are expressed as mean ± SD. Acute exercise did not alter serum IGF-1 concentration (Fig. 3b) although the degree of inter-individual variation was large. Statistical analysis was by one-way repeated measures ANOVA and *post hoc* Newman-Keuls (0 min: 1087.0 ± 1780.0 $pg.ml^{-1}$, 30 min: 1020.0 ± 1721.0 $pg.ml^{-1}$, 60 min: 1041.0 ± 1718 $pg.ml^{-1}$, 90 min: 1084.0 ± 1744 $pg.ml^{-1}$; n=28). Results are expressed as mean ± SD.

Effect of training on aerobic fitness

Aerobic fitness, as assessed by the VO_2 max test, was altered by the aerobic training paradigms (Fig. 4). There was a significant effect of training ($p=0.0061$, $F_{(1,14)}=10.42$; two-way repeated measures ANOVA and *post hoc* Bonferroni; C-EX3 n=9; C-EX5 n=7). *Post hoc* analysis revealed a significant increase in post-training VO_2 max scores relative to pre-training values in the C-EX5 group (pre-training: 39.70 ± 6.74 $ml.min^{-1}.kg^{-1}$, post-training: 49.26 ± 4.97 $ml.min^{-1}.kg^{-1}$, $p<0.05$) indicating that 5 weeks of training enhanced aerobic fitness. 3 weeks of aerobic training had no effect on VO_2 max scores in the C-EX3 group (pre-training: 44.69 ± 10.36 $ml.min^{-1}.kg^{-1}$, post-training: 47.65 ± 8.01 $ml.min^{-1}.kg^{-1}$). Results are expressed as ml oxygen consumed per min per kg body mass, mean ± SD.

Effect of training on cognitive function

Hippocampal function, as assessed by the face-name task, was not altered by 3 weeks of aerobic training (Fig. 5a). Statistical analysis was by two-way repeated measures ANOVA (CON n=15, A-EX3 n= 5, C-EX3 n=9). However, 5

weeks of aerobic training enhanced performance in the face-name task (Fig. 5b). There was a significant effect of training ($p=0.0172$, $F_{(1,28)}=6.414$; two-way repeated measures ANOVA with *post hoc* Bonferroni; CON n=15, A-EX5 n=8, C-EX5 n=8) indicating that the number of pairs recalled post-training was greater that pre-training. *Post hoc* analysis revealed that there was a significant increase in face-name performance in the C-EX5 group (pre-training: 13.6 ± 4.4 pairs, post-training: 21.1 ± 6.2 pairs, $p<0.05$), while the CON and A-EX5 groups remained unchanged. Results are expressed as mean ± SD.

Effect of training on serum BDNF response to acute exercise

3 weeks of aerobic training had a significant effect on the serum BDNF response to acute exercise (Fig. 6a). There was a significant effect of time ($p=0.0075$, $F_{(3,33)}=4.725$; two-way repeated measures ANOVA with *post hoc* Bonferoni; A-EX3 n=5, C-EX3 n=9) and a significant interaction ($p=0.0109$, $F_{(3,33)}=4.349$). *Post hoc* analysis revealed an increase in the A-EX3 group immediately post-exercise (60 min: 1226.0 ± 957.6 pg.ml^{-1}) relative to 0 min (339.0 ± 389.0 pg.ml^{-1}, $p<0.01$) and relative to 30min (531.1 ± 585.0 pg.ml^{-1}, $p<0.05$). This increase was sustained at 30 min post exercise (90 min: 1059.0 ± 1032.0 pg.ml^{-1}, $p<0.01$). However, serum BDNF concentration did not change from baseline in the C-EX3 group (0 min: 898.3 ± 796.3 pg.ml^{-1}, 30 min: 882.4 ± 821.9 pg.ml^{-1}, 60 min: 878.8 ± 500.6 pg.ml^{-1}, 90 min: 955.7 ± 560.7 pg.ml^{-1}) indicating that 3 weeks of aerobic training altered the profile of the serum BDNF response to acute exercise.

5 weeks of aerobic training also has a significant effect on the serum BDNF response to acute exercise (Fig. 6b). There was a significant effect of time

($p=0.0001$, $F_{(3,42)}=8.973$; two-way repeated measures ANOVA with *post hoc* Bonferroni; A-EX5 n=8, C-EX5 group n=8). *Post hoc* analysis revealed a significant increase in the A-EX5 group immediately post-exercise (60 min: 1485.0 ± 1110.1 $pg.ml^{-1}$) relative to 0 min (608.4 ± 538.9 $pg.ml^{-1}$, $p<0.001$) and relative to 30min (741.8 ± 512.3 $pg.ml^{-1}$, $p<0.01$). This increase was sustained at 90 min (1326.4 ± 1121.0 $pg.ml^{-1}$, $p<0.01$). There was also a significant increase in BDNF concentration in the C-EX5 group, but this did not occur until 90 min (1345.0 ± 650.9 $pg.ml^{-1}$, $p<0.05$ relative to 0 min: 778.4 ± 458.2 $pg.ml^{-1}$, and $p<0.01$ relative to 30 min: 602.7 ± 340.5 $pg.ml^{-1}$) indicating that 5 weeks of chronic exercise has altered the temporal profile of the serum BDNF response to acute exercise. Results are expressed mean ± SD.

Discussion

Here we present evidence that an acute bout of intense aerobic exercise (in the form of a graded exercise test) selectively improves performance in a hippocampal-dependent learning task in parallel with increased BDNF concentration in the serum. We also demonstrate that 5 weeks, but not 3 weeks, of aerobic training improves performance in hippocampal learning and alters the serum BDNF response to acute exercise. We have recently shown that intracerebroventricular injection of exogenous BDNF protein mimics exercise-induced enhancements in hippocampal-dependent learning in the rat [16] and although circulating BDNF originates both from central and peripheral sources [27], evidence suggests that the brain is a major source of circulating BDNF, both at rest and during exercise, in humans [32]. We propose that

exercise-induced enhancements in cognitive function in human subjects may be stimulated via a BDNF-linked mechanism.

Acute exercise

An acute bout of exercise induced an enhancement in cognitive function, as shown by the improvement in face-name task performance. This is in agreement with previous studies which suggest that intense acute exercise enhances learning and memory as assessed by a language-learning model [7], and the Stroop task [9]. Face recognition has been shown to recruit the right medial-temporal lobe (MTL), as evidenced by the inability of patients with right amygdalo-hippocampectomy to recognise previously viewed faces [33]. It has also been repeatedly demonstrated, using high-resolution functional magnetic resonance imaging (fMRI) acquisition and analysis methods, that the face-name association task used in the present study engages the hippocampus [31] and nearby MTL cortical areas, including the amygdala, parahippocampal cortex, perirhinal cortex and entorhinal cortex [34]. Hence, in the present study, the acute exercise-induced cognitive enhancement appears to be selectively MTL-dependent. To our knowledge, this is the first evidence for an acute exercise-induced enhancement in function of these MTL structures in humans.

We observed no change in performance of the Stroop word-colour task, which recruits the anterior cingulate cortex and other frontal regions [35], after an acute exercise bout. In contrast to the present study, Ferris and colleagues [9] have reported post-exercise improvements in the performance of the Stroop word and colour tests. However, given that no control, non-exercising, groups

were included in the study design, it is possible that the improvements they observed were a result of a practice effect.

In agreement with the literature, the serum analysis revealed an acute exercise-induced increase in BDNF concentration in sedentary young men. According to a recent review, 69% of studies in healthy human subjects reported a 'mostly transient' increase in peripheral BDNF concentration following acute exercise [27]. In the present study, acute exercise induced an increase in BDNF that had not quite returned to baseline at 30 min post-exercise, however given that increases in basal BDNF concentrations were not found in the chronic analysis, it may be presumed that the increase in serum BDNF reported here is also transient.

The source of the BDNF increase remains unclear. Evidence indicates that the brain is a major, but not the sole contributor to circulating BDNF [32] and platelets also represent a likely source of serum BDNF, as has consistently been reported [36, 37]. In this context, reports of the ability of BDNF to cross the blood-brain barrier may be of relevance, with movement of BDNF from brain to blood said to occur via bulk flow associated with the reabsorption of the cerebrospinal fluid [38]. It has also been suggested that exercise transiently increases the permeability of the blood brain barrier as demonstrated by an increase in the extravasation of Evans blue albumin into the brain following 30 min of forced swim exercise in rats [39] or post-exercise increases in serum S100β concentration in humans [40].

The acute exercise bout did not alter serum IGF-1 concentrations, although the degree of inter-individual variation was large. This result is in broad agreement with the literature. It has been reported that one hour of

treadmill running had no effect on serum IGF-1 concentrations in the rat, although increased uptake of serum IGF-1 into the brain was reported [21], and this has been causally linked with learning enhancements and the induction of BDNF in the brain post-exercise. Data from human subjects indicates that the IGF-1 response to exercise is highly variable and may depend on exercise intensity, duration and modality. While IGF-1 concentration in the serum has been reported to increase following short bouts of cycling [41, 42] and high-intensity running [43] other studies have reported no increase in serum IGF-1 following running bouts of varying durations and intensities [44-46].

Chronic exercise

A comparison of VO_2 max scores revealed that 5 weeks of aerobic training increased maximal oxygen consumption rates relative to pre-training values, indicating a significant increase in cardiovascular fitness, while 3 weeks of aerobic training did not improve aerobic capacity. As we observed no changes in performance of the Stroop task following acute exercise, we assessed the impact of training on the face-name task alone. 5 weeks of aerobic training enhanced face-name task performance, while 3-weeks of aerobic training had no effect on MTL-dependent learning and memory. This suggests a positive correlation between aerobic fitness and this learning task.

Taken together these results indicate that both acute exercise and 5 weeks of aerobic training enhance MTL-dependent learning. We hypothesized that the mechanism by which physical activity enhances MTL-dependent cognition may be linked with BDNF. An increase in serum BDNF concentration with acute exercise has been demonstrated and was associated with a

concomitant enhancement in the performance of the face-name task. Furthermore, this serum BDNF response to acute exercise was shown to be reproducible in session 2 at both the 3 week and 5 week timepoints. We also monitored IGF-1 following chronic training and saw no change at any timepoint (data not shown). This is consistent with the lack of effect of acute exercise on IGF-1 concentration that we have reported herein.

There is strong evidence from studies using humans or animal models that alterations in BDNF concentration may have functional consequences for cognition. Missense polymorphisms in BDNF in human subjects are linked with decreased synaptic abundance in the hippocampus and poor performance in memory tasks [47]. The binding of BDNF to its TrkB receptor mediates plastic changes involved in recognition memory in sheep [48], while exercise has shown to enhance object recognition learning in association with an increased concentration of BDNF in the dentate gyrus of young rats [15, 16]. Evidence from the literature suggests that BDNF can facilitate neurotransmitter release and enhance synaptic transmission [49, 50], leading to the hypothesis that the acute exercise-induced enhancement in hippocampal function may be mediated by the actions of BDNF on synaptic transmission.

Neither 3 weeks nor 5 weeks of aerobic training had any effect on basal BDNF concentration. Furthermore, following 3 weeks of aerobic training, the ability of acute exercise to increase serum BDNF concentration was abolished. Similarly, 5 weeks of aerobic training altered the profile of the BDNF response to acute exercise, in that the BDNF increase was delayed until 30 min post-exercise. These results indicate that aerobic training is affecting the induction of increased serum BDNF by acute exercise, altering the temporal profile of the

acute-exercise effect. It is unclear whether this is a result of an alteration in the mechanism of BDNF release. It is possible that the effect of chronic exercise on cognition may be mediated by an alternative mechanism involving BDNF. BDNF infusion has been shown to induce neurogenesis in rats [51]. It has been demonstrated that hippocampal neurogenesis also occurs in the dentate gyrus of adult humans [52] and that aerobic exercise training can increase the size of the anterior hippocampus in older adults in parallel with increased concentration of BDNF in the serum [53]. Potential functional consequences of adult neurogenesis must occur as long-term adaptations, rather than acute benefits, due to the time required for newly-generated neurons to mature and become integrated into a network [54]. While this provides an intriguing potential mechanism underlying the effects of exercise training on cognition, experimental limitations inherent in assessing the cellular mechanisms mediating cognition in humans means that investigation of such a hypothesis is currently unfeasible.

The results presented here provide evidence for a link between acute exercise and cognitive function. Acute exercise has been shown to increase serum BDNF and selectively improve MTL-dependent memory. Hence, BDNF is proposed as a mediator of the cognitive enhancements described, possibly through its reported role in short-term mechanisms underlying synaptic plasticity. Furthermore, it has been shown that while the 3-week training programme was insufficient to improve aerobic fitness or augment memory test performance, the 5-week chronic exercise programme resulted in enhanced fitness scores and improvements in MTL-dependent cognition. A role for BDNF

in these improvements in central nervous system function is tentatively suggested.

Figure legends

FIG 1: Outline of the experimental protocol. (a) Sessions 1 and 2 assessed the effects of acute exercise on cognitive function. During session 1, subjects were divided into CON and EX groups and underwent a cognitive testing trial (trial 1), following which EX completed an acute exercise bout while CON rested for a corresponding 30min period. Then all subjects completed a second cognitive testing trial (trial 2). Blood samples were collected from EX at times 0, 30, 60 and 90 min, with acute exercise occurring between times 30 min and 60 min. An overview of the subject groups is shown in (b). Baseline measures of cognitive function and serum BDNF concentration were obtained during session 1. The effect of both 3 and 5 weeks of chronic exercise then was assessed by subdividing the EX group. C-EX3 and C-EX5 completed 3 and 5 weeks of aerobic training respectively, prior to session 2, while A-EX3 and A-EX5 remained sedentary for the corresponding 3-week or 5-week period prior to session 2. The CON group completed session 2 after a 3 or 5-week interval and were sedentary both during the interval and during the testing session.

FIG 2: Effect of an acute exercise bout on cognitive function. (a) Acute exercise enhanced performance of the face-name task. The total number of face-name pairs recalled in trial 2 was greater than trial 1, *$p<0.05$, ***$p<0.001$ (CON n=13, EX n=30). Acute exercise did not alter performance of the Stroop word-colour task in either congruent (b) or incongruent (c) trials (CON n=14, EX n=28). Statistical analysis by two-way repeated measures ANOVA and *post hoc* Bonferroni. Data expressed as mean ± SD.

FIG 3: Effect of acute exercise on serum BDNF and IGF-1 concentrations. (a) Acute exercise (graded exercise test; GXT) induced an increase in serum BDNF concentration, *p<0.05 relative to 0 min, +p<0.05 relative to 30min (n=32). (b) Acute exercise did not alter serum IGF-1 concentrations (n=32). Statistical analysis by one-way repeated measures ANOVA and *post hoc* Newman-Keuls. Data expressed as mean ± SD.

FIG 4: Effect of aerobic training on VO_2 max scores. 3 weeks of aerobic training did not affect fitness, as assessed by VO_2 max scores. 5 weeks of aerobic training significantly increased VO_2 max scores, *p<0.05 relative to pre-training values (C-EX3 n=9, C-EX5 n=7). Statistical analysis by two-way repeated measures ANOVA and *post hoc* Bonferroni. Data expressed as mean ± SD.

FIG 5: Effect of aerobic training on performance of the face-name task. (a) 3 weeks of aerobic training did not affect performance of the face-name task (CON n=15, A-EX3 n=5, C-EX3 n=9). (b) 5 weeks of aerobic training enhanced performance of the face-name task. There was a significant increase in face-name performance in the C-EX5 group, *p<0.05 relative to pre-training value (CON n=15, A-EX5 n=8, C-EX5 n=8). Statistical analysis by two-way repeated measures ANOVA and *post hoc* Bonferroni. Data expressed as mean ± SD.

FIG 6: Effect of aerobic training on serum BDNF response to acute exercise. (a) 3 weeks of aerobic training had a significant effect on the serum

BDNF response to acute exercise. There was a significant increase in serum BDNF in the A-EX3 group immediately post acute-exercise (60 min) which was sustained at 90 min, **p<0.01 relative to 0min, +p<0.05 relative to 30 min. However, serum BDNF concentration did not change from baseline in the C-EX3 group (A-EX3 n=5, C-EX3 n=9). (b) 5 weeks of aerobic training had a significant effect on the serum BDNF response to acute exercise. There was a significant increase in serum BDNF in the A-EX5 group immediately post acute-exercise (60 min) which was sustained at 90 min, ***p<0.001 relative to 0 min, **p<0.01 relative to 0 min, ++p<0.01 relative to 30 min, +p<0.05 relative to 30 min. There was also a significant increase in the C-EX5 group in response to acute exercise, however this did not occur until 30 min post acute-exercise (90 min), *p<0.05 relative to 0 min, ++p<0.01 relative to 30 min (A-EX5 n=6, C-EX5 n=8). Statistical analysis by two-way repeated measures ANOVA with *post hoc* Bonferroni. Data expressed as mean ± SD.

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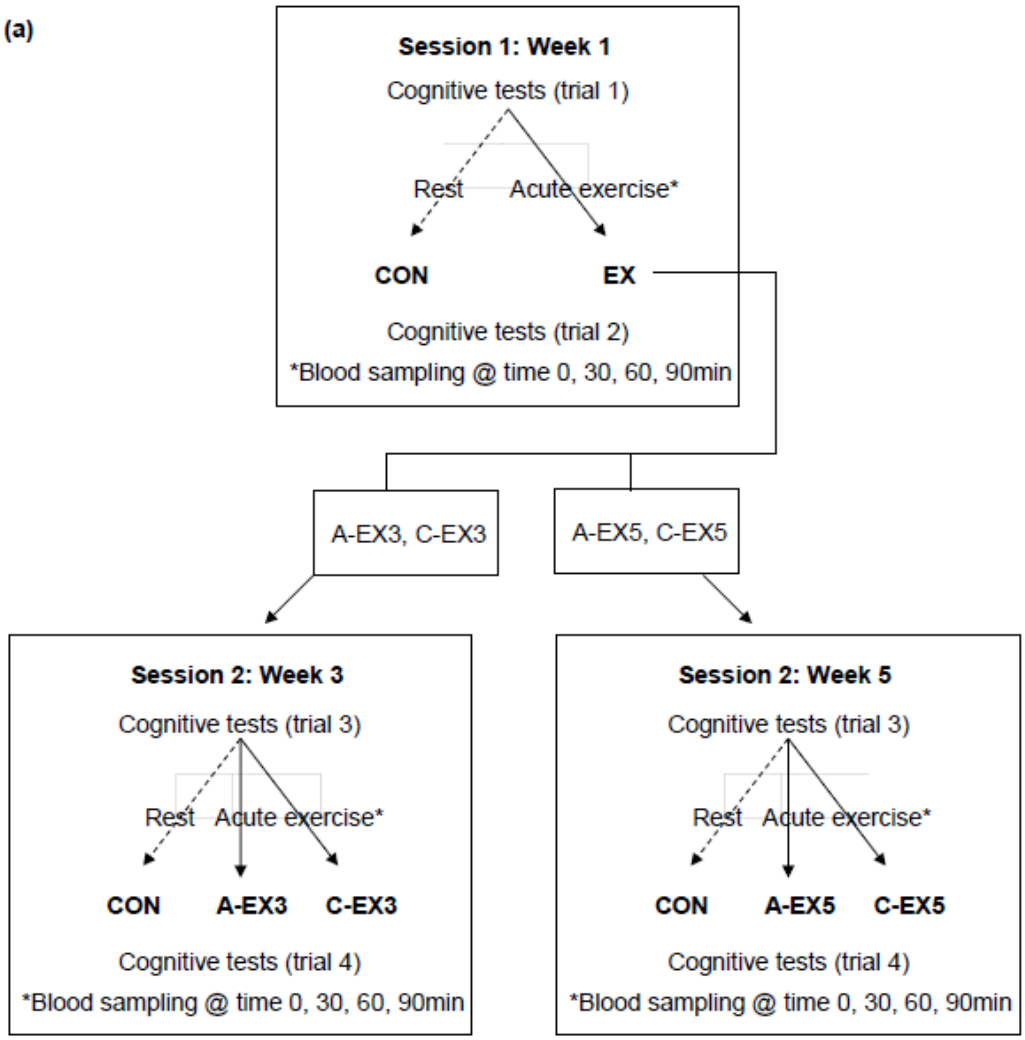


(b)

CON: (n=15) No acute exercise, no aerobic training
EX: (n=32) Acute exercise in week 1, then EX group splits;

A-EX3: (n=5) Acute exercise in weeks 1 & 3; 3 weeks **sedentary**
C-EX3: (n=9) Acute exercise in weeks 1 & 3; 3 weeks **aerobic training**
A-EX5: (n=9) Acute exercise in weeks 1 & 5; 5 weeks **sedentary**
C-EX5: (n=9) Acute exercise in weeks 1 & 5; 5 weeks **aerobic training**

Figure 1

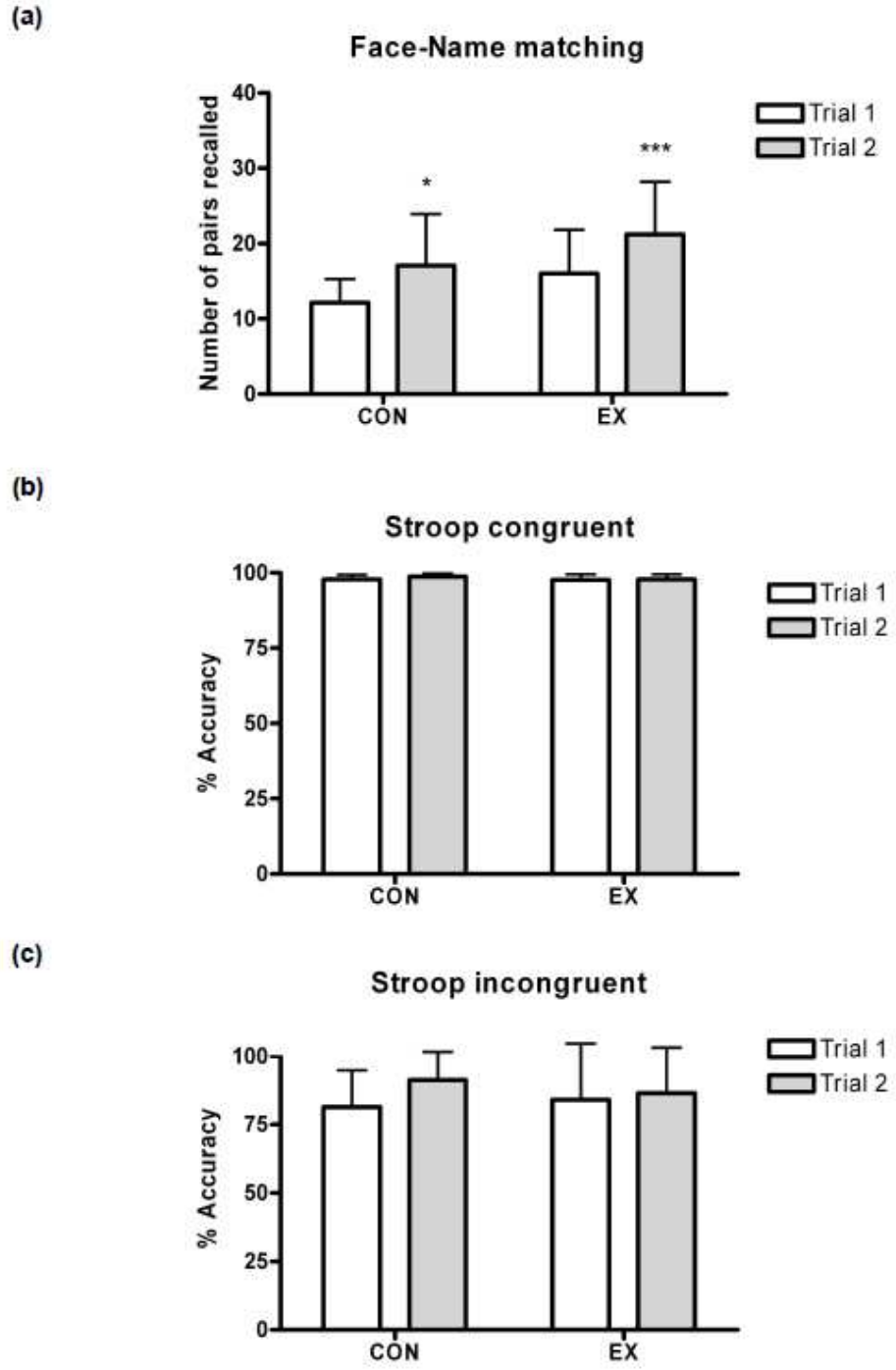
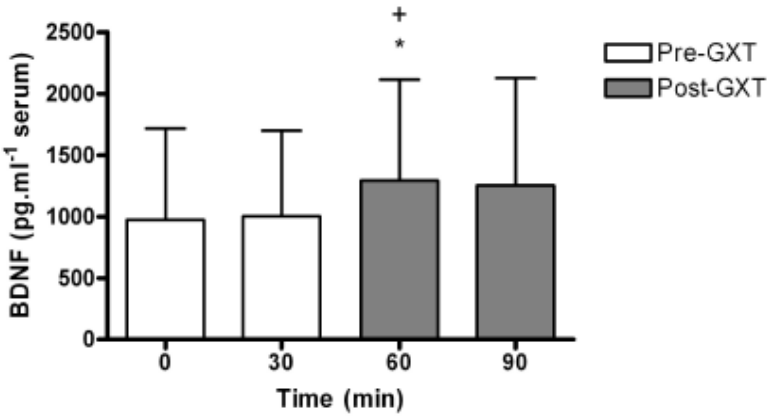


Figure 2

a)



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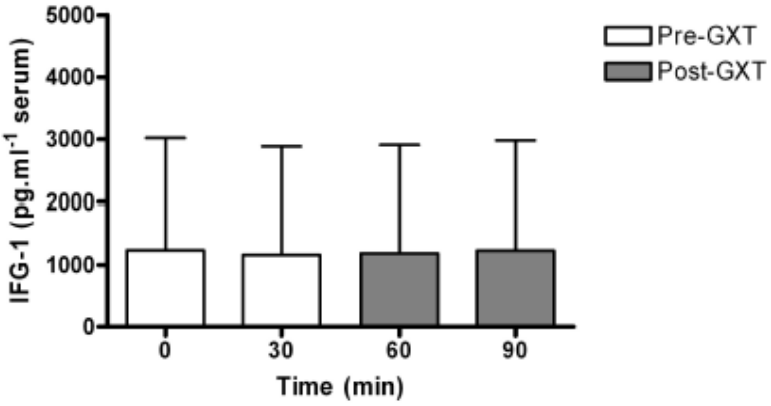


Figure 3

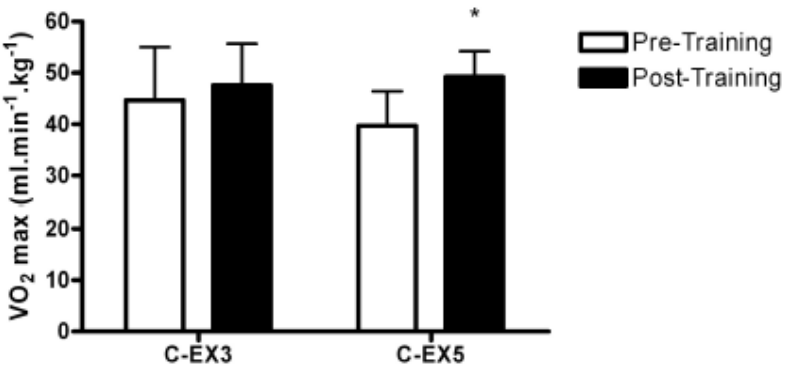
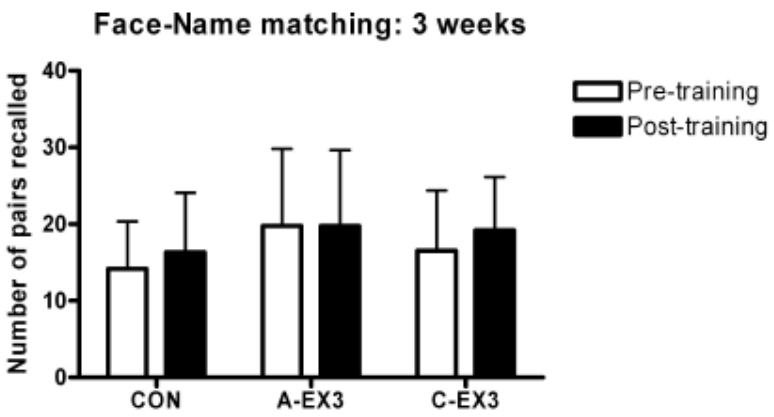


Figure 4

(a)



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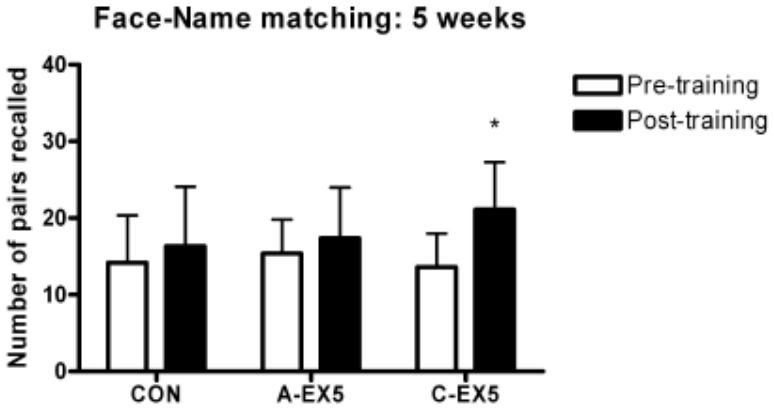

Figure 5

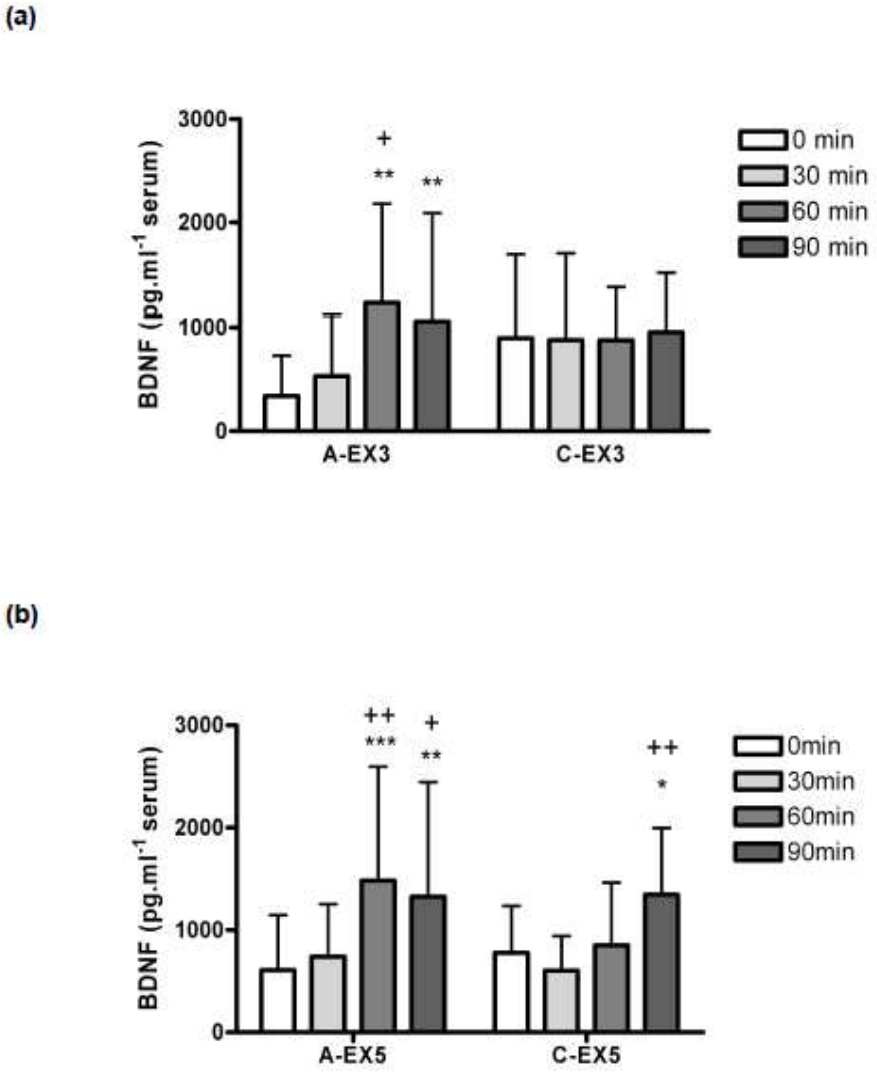

Figure 6

Highlights

An acute bout of aerobic exercise improves performance of the face-name task (an index of hippocampal function), but not the stroop task, in young male subjects.

This cognitive improvement is associated with increased concentration of BDNF in the serum.

Chronic training, resulting in improved VO2max, improves performance of the face-name task and alters the profile of circulating BDNF.

The data suggest a role for BDNF in exercise-induced cognitive enhancement in humans.