EFFECTS OF VOLUNTARY RUNNING ON PLASMA LEVELS OF NEUROTROPHINS, HIPPOCAMPAL CELL PROLIFERATION AND LEARNING AND MEMORY IN STRESSED RATS

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Abstract—Previous studies have shown that a 2-week treatment with 40 mg/kg corticosterone (CORT) in rats suppresses hippocampal neurogenesis and decreases hippocampal brain-derived neurotrophic factor (BDNF) levels and impairs spatial learning, all of which could be counteracted by voluntary wheel running. BDNF and insulin-like growth factor (IGF-1) have been suggested to mediate physical exercise-enhanced hippocampal neurogenesis and cognition. Here we examined whether such running-elicited benefits were accompanied by corresponding changes of peripheral BDNF and IGF-1 levels in a rat model of stress. We examined the effects of acute (5 days) and chronic (4 weeks) treatment with CORT and/or wheel running on (1) hippocampal cell proliferation, (2) spatial learning and memory and (3) plasma levels of BDNF and IGF-1. Acute CORT treatment improved spatial learning without altered cell proliferation compared to vehicle treatment. Acute CORT-treated non-runners showed an increased trend in plasma BDNF levels together with a significant increase in hippocampal BDNF levels. Acute running showed no effect on cognition, cell proliferation and peripheral BDNF and IGF-1 levels. Conversely, chronic CORT treatment in non-runners significantly impaired spatial learning and suppressed cell proliferation in association with a decreased trend in plasma BDNF level and a significant increase in hippocampal BDNF levels. Running counteracted cognitive deficit and restored hippocampal cell proliferation following chronic CORT treatment; but without corresponding changes in plasma BDNF and IGF-1 levels. The results suggest that the beneficial effects of acute stress on cognitive improvement may be mediated by BDNF-enhanced synaptic plasticity that is hippocampal cell proliferation-independent, whereas chronic stress may impair cognition by decreasing hippocampal cell proliferation and BDNF levels. Furthermore, the results indicate a trend in changes of plasma BDNF levels associated with a significant alteration in hippocampal levels, suggesting that treatment with running/CORT for 4 weeks may induce a change in central levels of hippocampal BDNF level, which may not lead to a significant change in peripheral levels. © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: corticosterone, BDNF, IGF-1, hippocampal cell proliferation, voluntary running, stress.

INTRODUCTION

Physical exercise enhances learning and memory and exerts antidepressive effects (Ernst et al., 2006; Yau et al., 2011a). Animal studies have shown that free wheel running improves spatial learning in concurrence with changes in hippocampal plasticity, including increased neurogenesis, enhanced long-term potentiation (LTP) (van Praag et al., 1999a,b), and elevated expression of brain-derived neurotrophic factors (BDNF) (Cotman and Berchtold, 2002; Farmer et al., 2004; Cotman et al., 2007).

Enhanced hippocampal neurogenesis from physical exercise (van Praag et al., 1999b) is putatively regarded as a basis for improving cognitive function (van Praag et al., 1999a, 2005) and countering depression (Ernst et al., 2006). In contrast, reduced neurogenesis by chronic stress or by high doses of glucocorticoid treatment (Gould et al., 1992, 1998) has been associated with cognitive deficits and linked to the psychopathology...
of clinical depression (Duman et al., 1997; Ernst et al., 2006). Ablation of neurogenesis in animal studies further indicates that hippocampal neurogenesis potentially mediates the beneficial effects of exercise on cognitive performance and depressive disorders (Clark et al., 2008; Lafenetre et al., 2010; Yau et al., 2011b).

Several neurotrophic factors, such as BDNF and insulin-like growth factor (IGF-1) have been suggested to be potential mediators for the exercise-derived hippocampal plasticity (Colman and Berchtold, 2002; Colman et al., 2007). Increased neurogenesis and improved cognitive function by exercise are in parallel with BDNF up-regulation in the brain (Colman and Berchtold, 2002; Vaynman et al., 2004; Zheng et al., 2006), which possibly caused by the exercise-triggered uptake of IGF-1 into the brain (Carro et al., 2000). In a study conducted by Trejo et al. mutant mice with low levels of serum IGF-I showed reduced hippocampal neurogenesis together with impaired spatial learning which could not be reversed by running (Trejo et al., 2008). This suggests that exercise-induced neurogenesis may be mediated by the entry of peripheral IGF-1 into the brain (Trejo et al., 2001). Thus, peripheral BDNF and IGF-1 levels are increasingly seen as potential biomarkers for evaluating the exercise-elicited cognitive improvement in studies involving human subjects.

Clinical observation has demonstrated a decrease in blood BDNF level in depressive patients and an increase in serum BDNF level after antidepressant treatment (Martinowich et al., 2007; Molenkamp et al., 2011). Since it is not feasible to directly measure the alteration of hippocampal neurogenesis in live human brain tissues, clinical studies have most exclusively relied on examining the serum or plasma levels of neurotrophins following interventions. It has recently been shown that the alteration in blood BDNF level can reflect its release from the brain (Klein et al., 2011) where it contributes to the increase in plasma BDNF in response to exercise (Rasmussen et al., 2009). Given the positive association between hippocampal neurogenesis, levels of neurotrophins (e.g. BDNF and IGF-1) and cognitive improvement after exercise intervention suggested by other studies, it would be interesting to investigate whether alterations of peripheral neurotrophins are accompanied by the corresponding changes of hippocampal cell proliferation. A positive correlation would justify the rationale to apply such peripheral biomarkers in evaluating cognitive state following exercise for translational studies on human subjects.

In a rat model of stress using repeated corticosterone (CORT) injections, we have previously shown that voluntary wheel running for 14 days could restore the decrease in hippocampal cell proliferation and attenuate learning impairment together with enhanced expression of BDNF protein levels (Yau et al., 2011b). However, it is unclear whether peripheral changes of BDNF and IGF-1 correspond to alteration in hippocampal cell proliferation. Hence, we investigated the effects of acute and chronic CORT treatments, with or without running on hippocampal-dependent learning and memory, cell proliferation in the hippocampus, and peripheral levels of BDNF and IGF-1.

EXPERIMENTAL PROCEDURES

Animals and housing conditions

All experimental procedures were approved and followed the guidelines of the Committee on the Use of Live Animals in Teaching and Research, The University of Hong Kong. Adult male Sprague–Dawley rats (250 ± 20 g) were housed individually with unlocked running wheels for runners or locked wheels for non-runners. Animals were divided into several groups including: the vehicle-treated non-runners (CtrlN) and runners (CtrlR), the 40 mg/kg CORT-treated non-runners (40 mgN) and runners (40 mgR). These were then further subdivided into either acute (5 days) or chronic (28 days) treatment groups (n = 4–6 per group). All rats were kept on a 12-h light–dark cycle with ad libitum access to food and water.

Animal treatments

After adaptation in the cages with locked wheels for 2 days, the rats received daily treatments with sesame oil or 40 mg/kg CORT and subjected to unlocked or locked wheels for 5 days (acute treatment) or 28 days (chronic treatment). The treatment was started on Day 3 and ended on Day 7 for acute treatment or on Day 30 for chronic treatment. CORT (Sigma–Aldrich, St. Louis, MO, USA) was prepared and injected subcutaneously according to the method of Hellsten et al. (2002). Bromodeoxyuridine (BrdU, 50 mg/kg body weight) was dissolved in normal saline and administered intraperitoneally in 12-h intervals during the last 3 days of the treatments for labeling proliferating cells. This was followed by the Morris water maze task. Changes in body weight were monitored weekly, and the adrenal weight was measured after sacrificing the animals (see Fig. 1).

Exercise training

Runners were housed singly in cages equipped with running wheels (diameter 31.8 cm; width 10 cm; Nalgene Nunc International, Rochester, NY, USA). Rats were allowed to run freely for 5 or 28 days and wheels were then locked during the water maze task. Wheel revolutions were recorded using the VitalViewer software (Mini Mitter Company, Inc., Bend, OR, USA). Non-runners were housed individually in identical cages with locked wheels.

Morris water maze task

Spatial learning and memory was examined using the water maze task with a hidden platform 0.5 cm below the water surface. The water was made opaque by adding non-toxic ink. The test was conducted for five consecutive days with four trials per day with the platform located in the same position. Animals were brought into the testing room 30 min prior to the test for adaptation. The starting points were randomly assigned to each animal every day. The latency for the animals to reach the platform was recorded by a trained observer. If the animals did not find the platform after a maximum of 60 s, it was brought to the platform for 20 s and assigned a score of 60 s. The animals were then returned to the cage and given a 4-min inter-trial rest.

On the sixth day, a probe trial was performed with the platform removed from the tank. Time spent in the target area in the probe trial was analyzed using SmartJunior software (Panlab, Spain). The target area was defined as a circular area with the diameter of 20 cm measured from the center of the platform as previously applied (Yau et al., 2011b).
 Sucrose preference test

The test was performed after 3 or 4 weeks of CORT treatments. The rats were firstly supplied with 1% sucrose in place of water for 24 h. They were then offered simultaneously one bottle containing 1% sucrose and one bottle with tap water for 24 h. The position of the two bottles was swapped after 12 h. The consumption of water and sucrose was recorded by weighing the bottles before and after the test. The sucrose preference was presented as the percentage of total amount of liquid consumption.

Tissue preparation

Twenty-four hours after the end of the water maze task, animals were anesthetized with a mixture (2:1, v/v) of ketamine 200 mg/ml and xylazine and transcardially perfused with 0.9% saline solution, followed by 4% paraformaldehyde for 20 min. Brains were removed from the skull and postfixed overnight at 4 °C. After that, the brains were placed in 30% sucrose solution until they sank. Twelve series of 40-μm coronal sections were cut with a freezing sliding microtome (Model 860; American Optical, Buffalo, NY, USA) and the brain slices were stored in cryoprotectant until use. The cryoprotectant consists of 200-ml 0.1 M phosphate-buffered saline (PBS), 150-g sucrose and 150-ml ethylene glycol, with the final volume adjusted to 500 ml with 0.1 M PBS.

Immunohistochemistry and immunofluorescence

BrdU labeling and double staining were performed as previously described (Lau et al., 2007; Yau et al., 2011b). Briefly, the sections were boiled in 0.01 M citric acid (pH 6.0) at 95 °C for 30 min, followed by three rinses of 0.01 M PBS. The sections were then incubated with 2 N HCl at 37 °C for 30 min in an oven and subsequently neutralized with boric acid buffer (pH 8.5) for 15 min at room temperature. After three rinses with PBS, the sections were incubated with mouse anti-BrdU antibody (1:1000, Roche Molecular Biochemicals, Essex, UK) overnight at room temperature. The sections were then washed and incubated with biotinylated goat anti-mouse antibody (1:200, Vector Laboratories, Inc., Burlingame, CA, USA). This was followed by incubation with an avidin–biotin complex (Vector Laboratories), and finally the sections were visualized with diaminobenzidine. For co-labeling, the sections were incubated with primary antibodies: rat anti-BrdU antibody (1:1000, Abcam, Cambridge, MA, USA) and rabbit anti-doublecortin (DCX, 1:200, Cell Signaling, Danvers, MA, USA) overnight in blocking diluent. Sections were then rinsed and incubated with Alexa Fluor 488-conjugated goat anti-rabbit and Alexa Fluor 568-conjugated goat anti-rat IgG (1:200, Molecular Probes, Eugene, OR, USA) overnight at room temperature. After rinsing, sections were coverslipped with mounting medium.

Quantification of BrdU-labeled cells and neuronal differentiation

Brain sections were counted in one-in-twelve series of coronal sections as reported previously (Yau et al., 2011b). All BrdU-positive cells were counted under 40× objective using the StereoInvestigator (MicroBrightField, Williston, VT, USA), and only those within the granular cell layer (GCL) and two cell diameters below the GCL were counted. The resulting numbers were then multiplied by 12 to obtain the total number of BrdU-positive cells in the dentate gyrus.

For phenotypic analysis, thirty BrdU-positive cells per animal were randomly selected from the dorsoventral axis of the dentate gyrus and analyzed for the co-expression of BrdU and DCX using fluorescence microscopy (Axioplan, Zeiss, Oberkochen, Germany) with 40× magnification. A cell with a BrdU-positive nucleus stained together with a DCX-positive surrounding soma was determined as a double-labeled cell. The proportion of DCX-positive cells over the 30 BrdU-positive cells was obtained as the indicator for neuronal differentiation. The counting was performed in a blinded manner.

Plasma BDNF and IGF-1 measurements

Blood samples were collected 1 h after injection of CORT on the last day of the treatment as previously performed (Yau et al., 2011b). In brief, animals were anesthetized using the mixture of ketamine and xylazine (2:1 ratio) and blood was collected from the tail vein within 3 min using a heparinized needle. Blood
samples were then centrifuged (Eppendorf centrifuge 5417R, USA) at 1000×g for 20 min at 4 °C. Plasma aliquots were then stored at −80 °C until use. The BDNF and IGF-1 levels were determined using commercially available ELISA kits (BDNF: Chemikine BDNF Sandwich ELISA Kit, Millipore, Ballerica, CA, USA; Mouse/Rat IGF-1 Quantikine ELISA Kit, R&D System, Minneapolis, MN, USA). Sample neurotrophin levels were calculated from the standard curve transforming the absorbance into neurotrophin concentrations, which presented in the unit of pg/ml.

**BDNF enzyme-linked immunosorbent assay.** Briefly, the BDNF standards, plasma samples (200-fold dilution) or 50-μg hippocampal protein sample brought to 100 μl using sample diluent were added to 96-well plate and incubated overnight with shaking at 4 °C. After washing the plate with wash buffer, the diluted biotinylated mouse anti-BDNF monoclonal antibody was added to the plate incubated at room temperature for 3 h. After washing, the diluted streptavidin–horseradish peroxidase (HRP) conjugate solution was added and incubated for 1 h at room temperature. TMB/E substrate was incubated for 15 min, followed by stop solution. The absorbance was immediately measured at 450 nm.

**IGF-1 enzyme-linked immunosorbent assay.** The IGF-1 standard and samples (1000-fold dilution) were added to 96-well plate and incubated with 150-μl assay diluent for 2 h at 4 °C. After washing, the plate was incubated with cold IGF-1 conjugate for 1 h at 4 °C, followed by washings and 30-min incubation with substrate solution at room temperature. The reaction was stopped by adding stop solution and absorbance was measured at 450 nm and corrected by subtracting reading at 570 nm.

**Statistical analysis**

Two-way ANOVA with running and CORT treatment as the between-subject factors was performed with post hoc Fisher’s LSD test. The data of body weight change and spatial learning were analyzed using repeated measures ANOVA with duration as the within-factor, and exercise and CORT treatment as the between-subject factors. Student’s t-test was applied to compare two sets of data. Values were presented as mean ± SEM.

**RESULTS**

**Changes in body weight and adrenal weight by CORT treatment and running**

The adrenal to body weight ratio was measured to assess the efficacy of CORT injection. Acute CORT treatment for 5 days significantly decreased adrenal weight to body weight ratio when compared with the vehicle treatment (effect of CORT: F(1, 22) = 9.956, P = 0.0037; Fig. 2a). Running did not affect the adrenal weight to body weight ratio (effect of running: F(1, 22) = 0.169, P = 0.686) and there was no interaction between CORT and running (CORT × running: F(1, 22) = 0.0004, P = 0.983).

Chronic CORT treatment also significantly decreased the adrenal weight to body weight ratio (effect of CORT: F(3, 21) = 16.8, P = 0.00687; Fig. 2b). In contrast, chronic running significantly increased the adrenal weight to body weight ratio (effect of running: F(3, 21) = 66.253, P = 0.000288; interaction CORT × running: F(3, 21) = 64.732, P = 0.000338). Post hoc test indicated that the CtrlR group showed a significant increase in the ratio compared with the CtrlN group (P < 0.005), indicating that running increased the adrenal weight in the normal rats. CORT treatment significantly decreased the ratio in both non-runners and runners when compared to the vehicle-treated non-runners and runners, respectively (P < 0.005). All rats showed an increase in the body weight gain during the 28-day treatment (repeated measures ANOVA; effect of day: F(4, 92) = 236.315; P < 0.005). Running did not affect the body weight gain, but CORT significantly decreased body weight gain (effect of running: F(1, 23) = 0.007, P = 0.936; effect of CORT: F(1, 23) = 72.283, P < 0.005; running × CORT: F(1, 23) = 6.626, P = 0.019). Post hoc test showed that CORT treatment significantly decreased body weight gain compared with the vehicle treatment (Fig. 2c, Day 1, F(3, 23) = 0.434, P = 0.731; Day 2, F(3, 23) = 10.275, P < 0.005; Day 3, F(3, 23) = 21.276, P < 0.005; Day 4, F(3, 23) = 35.516, P < 0.005; Day 5, F(3, 23) = 37.887, P < 0.005).

**Depression-like phenotype of the CORT-treated rats**

We have previously validated the stress level and depression-like behavior of the CORT-treated rats, where the result indicates a significant elevation in CORT level and an increase in immobility time in the forced swim test following 14-day CORT injection (Yau et al., 2011b). In the present study, we assessed the behavior of anhedonia, a hallmark symptom of depression, which is characterized by a decrease in sucrose preference compared to water in the sucrose preference test. The rats with 3-week and 4-week CORT treatment showed a decrease in sucrose preference compared with the vehicle-treated rats (Fig. 2d, Student’s t-test, P < 0.05), indicating that chronic CORT treatment induced depression-like behavior in the animals.

**Running activity in the vehicle-treated and CORT-treated rats**

Both the vehicle-treated and CORT-treated rats gradually increased their running distance during the first week in acute and chronic treatment. Rats with acute CORT treatment showed a higher running activity relative to the vehicle-treated rats, with significant differences observed on Day 4 and Day 5 (Fig. 3a, P < 0.05). The total running distance was significantly higher in the CORT-treated rats relative to the vehicle-treated rats (Fig. 3b, Student’s t-test, P < 0.05). Rats with chronic CORT treatment displayed a higher initial running activity during the first 2 weeks compared to the vehicle-treated rats (Fig. 3c, P < 0.05). However, there was no significant difference in the total running distance in the vehicle treatment group compared to the CORT treatment group (Fig. 3d, P > 0.05).

**Effect of acute and chronic treatment with CORT and running on hippocampal cell proliferation**

Neither running nor CORT treatment for 5 days affected hippocampal cell proliferation in the rats (Fig. 4a, effect of running: F(3, 20) = 1.372, P = 0.257; effect of
Fig. 2. Change of body weight and adrenal weight after treatment. (a) Acute treatment with CORT significantly decreased adrenal to body weight ratio compared with the vehicle treatment. (b) Chronic treatment with CORT significantly decreased the ratio compared with the vehicle treatment. Running increased adrenal weight in the normal rats. *P < 0.005 compared to the CtrlN group and CtrlR group, respectively; †P < 0.005 compared to the CtrlN group; ††P < 0.005 main effect of CORT. (c) The CORT-treated rats gained less body weight than the vehicle-treated rats during the treatment period. *P < 0.005 when compared to the 40-mgN group and 40-mgR group, respectively. (d) CORT treatment for 3 or 4 weeks significantly decreased sucrose consumption compared with the vehicle treatment. *P < 0.05 compared to the vehicle-treated control group. CtrlN or CtrlR: the vehicle-treated non-runners or runners; 40-mgN or 40-mgR: 40 mg/kg CORT-treated non-runners or runners.

Fig. 3. Running activity of the rats with vehicle or CORT treatment. (a, b) Acute treatment with CORT increased the running distance when compared with the vehicle-treated rats with a significant difference observed on Day 4 and Day 5. Acute CORT treatment increased total running activity compared to the vehicle treatment. (c, b) The CORT-treated rats showed higher running activity relative to vehicle-treated rats during the first 2-weeks of running. However, there was no difference in total running distance between the vehicle and chronic CORT treatment. *P < 0.05 compared to the vehicle-treated rats.
CORT: $F(3, 20) = 1.504, P = 0.237$). The data indicated that acute treatment with CORT and running showed no effect on modulating the proliferating rate of hippocampal progenitor cells.

Chronic running significantly increased the number of BrdU-positive cells in the dentate gyrus compared with the non-runners (Fig. 4c, effect of running: $F(3, 19) = 10.649, P = 0.00517$). In contrast, chronic treatment with CORT significantly suppressed hippocampal cell proliferation (effect of CORT, $F(3, 19) = 9.318, P = 0.00806$). Post hoc analysis showed a significant increase in the number of BrdU-positive cells in the CtrlR group compared with the CtrlN group ($P = 0.023$), indicating that running for 28 days significantly increased hippocampal cell proliferation in normal rats. Conversely, the 40-mgN group showed a significant decrease in the number of BrdU-positive cells in the dentate gyrus compared to the CtrlN group ($P = 0.046$), indicating that CORT suppressed hippocampal cell proliferation. The 40-mgR group exhibited a significant increase in BrdU-positive cells compared to the 40-mgN group ($P = 0.05$), indicating running counteracted the suppressive effect of CORT treatment on hippocampal cell proliferation.

**Effect of acute and chronic treatment on hippocampal neuronal differentiation**

We examined the neuronal differentiation by quantifying the ratio of BrdU-positive cells expressing the immature neuronal marker: DCX. Acute treatment with CORT or running showed no effect on the ratio of DCX to BrdU-positive cells, suggesting that neuronal differentiation was affected by neither acute CORT treatment nor running (Table 1 and Fig. 5a, effect of running: $F(3, 18) = 0.465, P = 0.506$; effect of CORT: $F(3, 19) = 9.318, P = 0.00806$).

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**Fig. 4.** Effect of acute and chronic treatments on hippocampal cell proliferation. (a) Representative images showing immunohistochemical staining of BrdU-positive cells in the dentate gyrus of the hippocampus. (b) Acute treatment with CORT or running did not affect the number of BrdU-positive cells in the dentate gyrus. (c) Representative images of BrdU-positive cells after chronic treatment. (d) Chronic running (CtrlR) significantly increased the number of BrdU-positive cells in the dentate gyrus when compared with the normal non-runners (CtrlN). CORT treatment without running (40 mgN) robustly decreased the number of BrdU-positive cells, whereas running restored the number of BrdU-positive cells in rats treated with 40 mg/kg CORT (40-mgR). *$P < 0.05$, **$P < 0.005$ compared to the CtrlN group. #P < 0.05 to the 40-mgN group. Scale bar = 10 $\times$: 100 $\mu$m; 40 $\times$: 30 $\mu$m. CtrlN or CtrlR: the vehicle-treated non-runners or runners; 40 mgN or 40 mgR: 40 mg/kg CORT-treated non-runners or runners.
In contrast, chronic running significantly increased neuronal differentiation as indicated by a significant increase in the ratio of DCX to BrdU-positive cells in the runners compared with the non-runners (Table 1 and Fig. 5b, effect of running: \( F(1, 20) = 5.740, P = 0.031 \)) whereas chronic treatment with CORT suppressed neuronal differentiation (effect of CORT: \( F(1, 20) = 34.77, P = 0.000389 \)). Post hoc analysis showed that running significantly increased neuronal differentiation in the vehicle-treated rats compared with the non-runner counterparts \((P = 0.039)\). The 40-mgN and 40-mgR groups both showed a significant decrease in the ratio compared with the CtrlN group \((P < 0.05)\).

**Table 1. Neuronal differentiation of proliferating cells in the dentate gyrus**

<table>
<thead>
<tr>
<th></th>
<th>Acute</th>
<th>Chronic</th>
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<tbody>
<tr>
<td>Number of BrdU+ and DCX+ cells</td>
<td>Co-labeled ratio</td>
<td>Number of BrdU+ and DCX+ cells</td>
</tr>
<tr>
<td>CtrlN</td>
<td>20 ± 1.56</td>
<td>0.66 ± 0.050</td>
</tr>
<tr>
<td>CtrlR</td>
<td>20 ± 1.69</td>
<td>0.71 ± 0.030</td>
</tr>
<tr>
<td>40 mgN</td>
<td>19 ± 3.41</td>
<td>0.62 ± 0.041</td>
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<tr>
<td>40 mgR</td>
<td>19 ± 1.89</td>
<td>0.64 ± 0.052</td>
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Acute CORT treatment improved spatial learning, but not memory consolidation

Two-way ANOVA with repeated measures showed that all rats learned to locate the hidden platform during the learning phase (Fig. 6a, effect of day: \( F(4, 24) = 85.575, P = 0.000230 \)). A significant interaction between day and CORT treatment was observed \((\text{day} \times \text{CORT}): F(4, 24) = 4.836, P = 0.00158\), but without a main effect of running \((\text{effect of running}: F(3, 24) = 0.586, P = 0.454)\). However, acute treatment with CORT showed a main effect on spatial learning \((\text{Fig. 6a, effect of CORT: } F(3, 24) = 5.136, P = 0.0353)\). Post hoc analysis confirmed that the 40-mgN and 40-mgR groups both showed a significant increase in the ratio of DCX to BrdU-positive cells in the runners compared with the non-runners \((P = 0.039)\). The 40-mgN and 40-mgR groups both showed a significant decrease in the ratio compared with the CtrlN group \((P < 0.05)\).
showed a significant decrease in the escape latency compared with the CtrlN group ($P = 0.013$ and $P = 0.026$, respectively). However, in the probe trial test, acute treatment with CORT or running did not affect time spent in the target area, indicating that memory consolidation was not affected by acute running or CORT treatment (Fig. 6b).

**Running reversed chronic CORT-impaired spatial learning**

Animals with chronic treatment showed that all rats learnt to locate the position of the hidden platform during the 5-day training period (Fig. 6c, effect of day: $F(4, 24) = 29.541$, $P = 0.00087$). Two-way ANOVA indicated a significant difference between the non-runners and runners (effect of running: $F(3, 24) = 6.076$, $P = 0.033$). The 40-mgN group showed a significant increase in the escape latency compared with the CtrlN or CtrlR groups, respectively (Day 1: $P = 0.024$ and $P = 0.035$ and Day 2: $P = 0.030$ and $P = 0.015$, respectively), indicating impaired spatial learning after chronic treatment with CORT. In contrast, running decreased escape latency as evidenced by the decreased escape latency in the CtrlR group compared with the 40-mgN group (Day 4, $P = 0.014$). Running also reversed CORT-impaired spatial learning in the 40-mgN group as evidenced by a significant decrease in escape latency in the 40-mgR group compared with the 40-mgN group (Day 2, $P = 0.009$). The data indicated that running significantly counteracted the impairment in spatial learning induced by CORT treatment.

In the probe trial, two-way ANOVA indicated insignificant effects of running or CORT treatment on the time spent in the target area (Fig. 6d, effect of running: $F(3, 24) = 1.923$, $P = 0.182$; effect of CORT: $F(3, 24) = 0.002$; $P = 0.961$), suggesting that chronic running and CORT treatment did not affect memory consolidation.

**Peripheral and central levels of BDNF after acute and chronic treatment**

We next investigated the linkage between cognitive enhancement and increase in peripheral BDNF and IGF-1 levels in the rats with running. We measured the plasma BDNF level since it has been reported that the cortex and hippocampus are the major sources for the increased plasma BDNF level in response to physical exercise (Rasmussen et al., 2009). Two-way ANOVA indicated a significant main effect of CORT on plasma BDNF level in acute treatment (Fig. 7a, effect of CORT: $F(3, 20) = 4.726$, $P = 0.042$). There was a significant increase in plasma BDNF level in the 40-mgN group relative to the CtrlR group ($P = 0.011$) following acute treatment. There was an increased trend in plasma BDNF level in the 40-mgN group relative to the CtrlR group ($P = 0.011$) following acute treatment. There was an increased trend in plasma BDNF level in CORT-treated non-runners compared to
the vehicle-treated non-runners, and this trend was accompanied with a significant elevation in hippocampal BDNF levels (Fig. 7c, Student’s t-test, $P < 0.05$ compared to the vehicle-treated non-runners).

In contrast, there was no significant change in plasma BDNF level following chronic treatment with CORT or running (effect of running: $F(3, 18) = 0.602$, $P = 0.580$; effect of CORT: $F(3, 18) = 0.024$, $P = 0.903$). However, there was a decreased trend in plasma BDNF level in the CORT-treated non-runners, and this decrease was associated with a significant decrease in hippocampal BDNF level (Fig. 7d, Student’s t-test, $P < 0.05$ compared to the vehicle-treated non-runners).

**Plasma level of IGF-1 after acute and chronic treatment**

There was no significant changes in plasma levels of IGF-1 following acute treatment with running and/or CORT (Fig. 7b, acute: effect of running: $F(3, 21) = 0.307$, $P = 0.587$; effect of CORT: $F(3, 21) = 3.009$, $P = 0.101$), or following the chronic treatment with running and/or CORT (Fig. 7b, effect of running: $F(3, 18) = 2.564$, $P = 0.132$; effect of CORT: $F(3, 18) = 2.648$, $P = 0.126$).

**DISCUSSION**

The present study investigated the effects of acute and chronic running on cognitive improvement and the corresponding changes of peripheral BDNF and IGF-1 levels in the CORT-injected animal model of stress. To the best of our knowledge, this is the first study examining the linkage between peripheral BDNF and IGF-1 levels and changes in hippocampal cell proliferation in rats after running. Consistent with our previous findings (Yau et al., 2011b), the result showed that chronic running reversed the suppression of hippocampal cell proliferation and impaired spatial learning by CORT treatment. However, no significant alterations of peripheral BDNF and IGF-1 levels after running for 28 days were observed. Interestingly, our data showed that acute CORT treatment increased spatial learning without altering hippocampal cell proliferation, which was accompanied by a significant increase in hippocampal BDNF levels, whereas chronic treatment with CORT impaired spatial learning and memory, and decreased hippocampal cell proliferation in association with a significant decrease in hippocampal BDNF level, but with no significant changes of peripheral BDNF and IGF-1 levels.

**The animal model of stress mimicked by subcutaneous CORT injection**

The CORT-injected animal model of stress has been repeatedly reported to significantly increase plasma CORT level and cause depression-like behavior in a dose-dependent manner (Johnson et al., 2006; Yau et al., 2011b). The depressionogenic effects of the CORT injection paradigm are known to be time- and dose-dependent. Treatment with 40 mg/kg CORT for 14
or 21 days considerably increases depression-like behavior, whereas neither 20 mg/kg CORT nor 10 mg/kg CORT treatment produces significant increase in depression-like behavior (Gregus et al., 2005; Johnson et al., 2006). Our previous report has shown that treatment with 20 mg/kg CORT for 14 days exerts a beneficial effect on hippocampal plasticity (Yau et al., 2011c). It has also been reported that acute CORT treatment could produce antidepressant-like effect (Dunn and Swiergiel, 2008; Stone and Lin, 2008) whereas chronic CORT treatment for 3 weeks or longer significantly increased depression-like behavior (Zhao et al., 2008, 2009). The present study showed that the rats treated with CORT for 3 or 4 weeks demonstrated a behavior of anhedonia as evidenced by decreased sucrose preference. Our data also showed that acute (5 days) treatment with CORT significantly improved hippocampal-dependent learning whereas chronic (28 days) treatment with CORT led to learning impairment, in agreement with results reported by others that short-term and long-term CORT treatments differentially lead to positive and negative effects on animal behavior (Zhao et al., 2009). With reproducible findings from the same animal model, the protocol of repeated treatment with CORT has been validated as a reliable method for inducing stress in rodents in a controllable manner (Sterner and Kalynchuk, 2010).

Effects of acute treatment on hippocampal cell proliferation, neurotrophins and spatial learning and memory

Our results demonstrated the differential effects of acute and chronic treatment with 40 mg/kg CORT on hippocampal plasticity. The acute treatment with CORT improved spatial learning that associated with higher plasma BDNF levels and a significant increase in hippocampal BDNF levels, but did not affect hippocampal cell proliferation, suggesting that the positive influence of acute stress on spatial learning may not necessarily involve an enhancement in hippocampal cell proliferation under acute stress condition. It is known that acute stress is able to promote memory formation (Shors, 2001) and enhances acquisition up to 2 days after stressor cessation (Servatius and Shors, 1994). Acute exposure to stress has also been clinically shown to facilitate spatial navigation performance (Duncko et al., 2007). These improvements in spatial learning triggered by acute stress might be due to the transient activation of NMDA receptor (Shors and Mathew, 1998) or increased BDNF level. In fact, the association between the increase in hippocampal BDNF levels and improvement in spatial learning following acute CORT treatment in the present study suggests that the acute effect of stress on improving cognition may possibly be mediated by BDNF-enhanced synaptic plasticity. Our result echoed other reports that mRNA level of BDNF in the hippocampus can be rapidly increased by a short-time exposure to immobilization stress, suggesting this rapid change in BDNF expression may be a form of neuronal plasticity for coping with new stimuli (Marmigere et al., 2003) as BDNF is well-known for its critical involvement in the induction of LTP and synaptic plasticity (Kovalchuk et al., 2002).

The present data also showed that a higher level of peripheral BDNF corresponded to significant increase in hippocampal BDNF level and improvement in spatial learning following acute CORT treatment. However, there was no significant change in plasma BDNF level following 5-day running. A human study has shown that plasma BDNF level was increased after rowing exercise for 4 h and then returned to resting levels after recovery for 1 h (Rasmussen et al., 2009), indicating the transient increase in plasma BDNF in response to acute exercise. Acute treatment (5 days) in the present study may not be able to trigger substantial changes in peripheral levels of neurotrophins.

There was no change in plasma IGF-1 levels after the acute treatment with running and/or CORT. The responses of peripheral IGF-1 to interventions (e.g. exercise) are inconsistent among studies with some reporting no alteration in serum IGF-1 levels (Banfi et al., 1994; Griffin et al., 2011), while others reported an increase in IGF-1 levels after intensive exercise training in human subjects (Hartmann and Singrun, 1968; Schwarz et al., 1996). It has been reported that rats with one-hour treadmill running displayed increased uptake of IGF-1 into the brain without affecting basal level of serum IGF-1 (Carro et al., 2000). It is possible that regulations of IGF-1 level in response to intervention may depend on the duration and intensity (Griffin et al., 2011), and a significant change in peripheral IGF-1 may only be observed after intensive interventions.

Effect of chronic treatment on hippocampal cell proliferation, neurotrophins and spatial learning and memory

Our previous study has reported that running counteracted CORT-impaired spatial learning and neurogenesis following 2 weeks of treatment (Yau et al., 2011b). The beneficial effect of running on promoting hippocampal neurogenesis has also been shown in a genetically depressed rat model (Bjornebekk et al., 2005). We here confirmed our previous observation that treatment with 40 mg/kg CORT over 4-weeks significantly decreased hippocampal cell proliferation and caused behavioral deficit in the water maze task that was reversed by running. From phenotypic analysis of newly proliferating cells, we showed that treatment with CORT over 4 weeks suppressed the ratio of DCX/BrdU co-labeled cells, indicating reduced neuronal differentiation by chronic treatment with CORT. The results echoed the finding by Wong and Herbert that high levels of glucocorticoid decrease neuronal differentiation of the surviving cells (Wong and Herbert, 2006). The suppressive effect of CORT on cell proliferation may result from the decreased hippocampal BDNF levels, since BDNF is an important neurotrophic factor for promoting neuronal survival and growth (Barde, 1994), the effects of which could be inhibited by high levels of CORT (Rasmusson et al., 2002; Jacobsen...
and Mork, 2006). In fact, we have observed a significant decrease in hippocampal BDNF level in rats with chronic CORT treatment. Furthermore, our previous study has shown that running restored hippocampal cell proliferation in association with restored hippocampal BDNF level following a 2-week CORT treatment (Yau et al., 2011b). As a result, the number of newborn neurons that can functionally integrate into neuronal circuitry of the hippocampus was decreased, leading to impairment in hippocampal function, and consequent behavioral deficits under stress condition (Yau et al., 2011b).

Prolonged elevation of corticosteroid reduces the availability of hippocampal BDNF level (Rasmusson et al., 2002; Jacobsen and Mork, 2006) and suppresses the synthesis of IGF-1 in the liver (the main source of peripheral IGF-1) (Yakar et al., 1999). In the present study, we demonstrated that chronic CORT treatment significantly impaired spatial learning, suppressed cell proliferation and decreased hippocampal BDNF level, together with relative lower peripheral BDNF and IGF-1 levels compared to the vehicle-treated non-runners. Furthermore, running promoted hippocampal cell proliferation in the absence of changes in peripheral BDNF and IGF-1 levels in the vehicle-treated and CORT-treated rats. Our results indicated a trend in changes of peripheral BDNF levels together with a significant alteration in hippocampal cell proliferation following chronic treatment with CORT. However, there are studies showing that animals with 4-week exposure to stressful event displayed decrease in serum and hippocampal BDNF concentrations in association with morphological changes that presented in depressive disorder (Sheline et al., 1999; Duman and Monteggia, 2006), such as reduced hippocampal volume and neurogenesis, CA3 dendritic retractions and decreased spine density (Blugeot et al., 2011). It has been reported that changes in blood BDNF levels reflect the changes in the hippocampus (Blugeot et al., 2011). We observed a decrease in hippocampal cell proliferation, and impairment in spatial learning together with a trend toward lower levels of plasma BDNF and IGF-1 in the non-runners with chronic CORT treatment. It is possible that the change in hippocampal BDNF levels was too small to be reflected in plasma levels in our CORT-treated rats. The changes of central BDNF might not be detectable in peripheral samples, though hippocampal BDNF levels have been significantly decreased following the 4-week CORT treatment. Furthermore, we have found that rats treated with 50 mg/kg CORT for 2 weeks showed a considerable decrease of BDNF levels in both hippocampus (Yau et al., 2011b) and plasma (Yau et al., unpublished data). It is possible that the changes in brain BDNF levels in our 40 mg/kg CORT-treated rats may be too small to be reflected in the peripheral levels.

The results of studies reporting changes in peripheral levels of BDNF and IGF-1 after interventions are controversial. It has been reported that serum BDNF levels were up-regulated after exercise intervention in humans (Tang et al., 2008; Knaepen et al., 2010), whereas those of IGF-1 varied after exercise (Kraemer et al., 1995; Abellan et al., 2006). However, there are also reports showing no change in the basal BDNF level in healthy subjects with a 5-week aerobic exercise training (Griffin et al., 2011). Furthermore, there is no change in serum BDNF level in depressive patients reflecting the therapeutic effects of physical exercise after 12-week training (Toups et al., 2011). Oppositely, it has been reported that aerobic exercise increased the size of hippocampus in association with increased serum BDNF level in older subjects (Erickson et al., 2011). It is possible that interventions triggering substantial changes in brain levels of neurotrophins may be critical to induce significant changes to the peripheral levels.

CONCLUSION

Our results suggest that the beneficial effects of acute stress on cognitive improvement may be mediated by BDNF-enhanced synaptic plasticity in a new cell-independent manner, whereas the detrimental effects of chronic stress on cognitive impairment may be due to suppressed hippocampal cell proliferation and BDNF levels. Enhancement in spatial learning and elevation in hippocampal BDNF levels were accompanied by a corresponding increased trend in the plasma BDNF level following acute CORT treatment. Conversely, impairment in spatial learning, and suppression in hippocampal cell proliferation and decrease in hippocampal BDNF levels were not associated with significant peripheral changes of BDNF levels following chronic CORT treatment. Furthermore, the beneficial effects of running on hippocampal cell proliferation in normal and CORT-treated rats were in absence of significant alteration in peripheral levels of neurotrophins. As discussed above, it is possible that mild change in central levels of neurotrophins after 4-week treatment with running or CORT may not be reflected in peripheral levels. Since responses of peripheral BDNF and IGF-1 to exercise training are variable and may depend on duration and intensities as suggested by Griffin et al. (2011), conditions triggering substantial changes in the brain which can be reflected in peripheral neurotrophin levels warrant further investigation in an attempt to show BDNF or IGF-1 as biomarkers for the effects of exercise on cognitive improvement.

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