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Full Length Research Paper

Differential behavioral outcome of anxiety tests in runner rats treated with corticosterone

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Both clinical and pre-clinical studies have suggested that physical exercise is beneficial for lowering anxiety. However, some animal studies fail to demonstrate the anxiolytic effect of physical exercise. The inconsistencies among studies may be due to differences in animal models and behavioral tests. Previously, injection of corticosterone (CORT) for 14 days induced depression-like behavior to varying based on was shown. Animal model was used to investigate whether CORT treatment induced anxiety-like behavior and to examine the anxiolytic effect of voluntary running using two behavioral measurements: open field test and elevated plus maze. Results indicated that running reduced anxiety-like behavior in vehicle-treated animals in both tests. Treatment with CORT showed a significant anxiogenic effect in the open field test regardless of the doses, but not in the elevated plus maze. Running reduced anxiety in theopen field test, but showed no effect in elevated plus maze in the CORT-treated rats. The data suggest the discrepancy of these two anxiety-related measures in the animal model of stress.

Key words: Corticosterone (CORT), stress, voluntary running, open field test, elevated plus maze, anxiety-like behavior.

INTRODUCTION

Hippocampus is not only involved in cognitive functions, but also plays an important role in emotional regulation (Mac, 1949) and anxiety state (Bannerman et al., 2004). In our daily life, we always face challenges that are anxiogenic, for example sitting for an examination, job interviews and public presentations. Anxiety is a normal response to a stressor that helps an individual to promptly cope with the demanding situation. Anxiety disorder presents as an excessive pathological form of fear consisting of a fear state with excessive reaction for the actual threat. Anxiety disorder frequently presents in clinical psychiatry with a 30% occurrence rate. In addition, patients with co-occurring symptoms of anxiety always show worsened severity of depression (Mineka et al., 1998).

In animal studies, it has been shown that exposure to acute uncontrollable stressors cause a variety of behaviors called learned helplessness such as exaggerated shock elicited freezing; deficits in shuttle box escape learning. These kinds of stress responses have been suggested to represent the animal analogs of human anxiety (Maier and Watkins, 1998). Physical exercise is known to reduce the signs and symptoms of anxiety disorder and depression with support from a number of human studies (Dun et al., 2001; Merali et al., 2003;

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Blumenthal et al., 2007). Recently, it has been reported that exercise could exert similar anxiolytic effect as antidepressant treatment in animals (Duman et al., 2008). Preventative and curative effects of physical exercise in depression- and anxiety-like behaviors in animal model have also been reported (Binder et al., 2004; Dulawa et al., 2004). In vehicle-treated rats, four or eight weeks of wheel running is able to alter anxiety behavior in the open field test, but not in the elevated plus maze (Burghardt et al., 2004). Chronic running (6-week) reduces anxiety-like behaviors in terms of exaggerated shock-elicited freezing and shuttle box escape deficits in rats exposed to uncontrollable stress (McKinney and Bunney, 1969). However, inconsistent effects of exercise in the animal models of anxiety have also been found in different studies in which animals with voluntary wheel running show anxiolytic effect (Duman et al., 2008; Salam et al., 2009), no effect (Chiang et al., 2010) or anxiogenic effect (Burghardt et al., 2004). The differences in animal models (normal or stressed animals), animal species, and behavioral tests may contribute to the inconsistencies among those studies.

Psychiatric patients suffering from depressive disorders are often associated with hyperactivation of hypothalamus-pituitary-adrenal axis (HPA axis) that leads to elevated plasma corticosteroids. Animal models with repeated stress have been widely used to study the neurobiological changes following stress exposure (Nestler et al., 2002). However, this model may increase the experimental variability since individual differences in the activation of HPA axis in response to stress exposure may result in different levels of CORT elevation among different individuals exposed to the same stressor. The alternative way is to use repeated exogenous CORT administration as a mean to mimic the effect of stress that associated with elevated CORT levels. This model could provide an effective control over the increases in blood CORT levels in the animals and increase depression-like behavior in rats in a dose-dependent manner (Yau et al., 2011b).

However, exogenous administration of CORT has been reported to both increase (Calvo et al., 1998) or decrease (Andreatini and Leite, 1994) the anxiety level in the elevated plus-maze. It is unclear whether the discrepancy is due to dose effect of CORT treatment. It has previously been reported that treatment with different doses of CORT for 14 days produced a graded increase in depression-like behavior and plasma CORT level (Yau et al., 2011b). Furthermore, wheel running or antidepressant treatment could counteract CORT-induced depression-like behavior and depression-impaired memory in the rats. Since there is no experimental data on measuring anxiety-like behavior in animals treated with different doses of CORT, in the present paper, we used different doses of CORT administrations to examine whether there was an increase in anxiety-like behavior in CORT-treated rats; and to characterize the effects of voluntary running on anxiety-related behaviors in the open field test and the elevated plus maze (the two behavioral paradigms that are commonly used in anxiety tests).

In vehicle-treated rats, the results showed that voluntary running reduced anxiety-like behaviors in the open field test and elevated plus maze. In the CORT-treated rats, the anxiety level was significantly increased by CORT treatment and decreased by running in the open field test. However, in the elevated plus maze, neither CORT treatment nor voluntary running affected the anxiety level in the CORT-treated rats. The data may indicate a discrepancy in measuring the anxiety-like behavior between the open field tests and elevated plus maze in the CORT-injected rat model of stress.

MATERIALS AND METHODS

Animal group and treatment

Adult Sprague Dawley (250 ± 20 g, 6 to 7 weeks old) male rats were obtained from the Laboratory Animal Unit, University of Hong Kong. All experimental procedures were approved and followed the guidelines of the Committee on the Use of Live Animals in Teaching and Research, University of Hong Kong. Rats were kept on a 12 h light-dark cycle with *ad libitum* access to food and water. The animals were divided into runner and non-runner groups and were treated with different doses of CORT (30, 40 and 50 mg/kg) or sesame oil for 14 days (n = 8 to 10/group). The day after the CORT treatment (day 15), the rats were subjected to the open field test and elevated plus maze to examine anxiety-like behavior.

Corticosterone (CORT) treatment

CORT (Sigma-Aldrich, USA) at different doses (30, 40 and 50 mg/kg) and sesame oil were prepared and injected subcutaneously according to the method of Hellsten et al. (2002). The dose of 40 mg/kg has been reported to elevete blood levels of CORT over a 24 h period (Sapolsky et al., 1985). A stock of CORT emulsion was prepared by suspending CORT in sesame oil, followed by vortex and sonication. Injection (0.8 ml/ rat) was made subcutaneously in the neck region daily at 4:00 p.m. The CORT treatment was started at the same time when the rats were allowed to run. The control rats received daily injections of sesame oil.

Voluntary wheel running

Runner rats were housed singly in polyethylene cages equipped with running wheels (diameter, 31.8 cm; width, 10 cm; Nalgene Nunc International, NY). Animals were allowed to familiarize with living condition with locked wheels for 3 days prior to the treatment. The locked wheels were then unlocked for 14 days in the runner groups or kept locked for the non-runner groups. Wheel revolutions were recorded by computer using VitalViewer software (Mini Mitter Company, Inc, OR). Non-runners were housed with locked running wheels in cages identical to that of runners.

Open field test

The behavioral test was conducted in a quiet and dimmed room with constant illumination; the test was performed from 2:00 to 4:00 p.m. After 2 h of habituation in the test room, each rat was individually placed into the center of the open field arena (90 × 90

cm with 40 cm-high side walls). The locomotor activity was recorded for 5 min by a video camera. The arena was then cleaned with 70% ethanol and dried with towel for each trial. Results were analyzed using the Smart Junior software (Panlab, Spain). The open field was broken into two zones for analysis: the peripheral and central zones. Anxiety-like behaviors were indicated by the time spent in the peripheral and central areas, while locomotor activity was indicated by average traveling speed and distance over 5 min in the arena.

Elevated plus maze

Each rat was tested in the elevated plus maze 2 h after the open field test in the same room under the same lighting condition. The elevated plus-maze consisted of four arms made of Plexiglas, elevated 50 cm above the floor. Two arms were open (0.5 cm high edges), while the other two arms were enclosed with 36 cm high wall. Each animal was placed onto the center of the apparatus facing an open arm and was then evaluated for 5 min test. The maze was wiped with 70% ethanol and dried between each trial. The behavior was recorded with a video camera and then analyzed using Smart Junior system (Panlab, Spain). Anxiety-like behavior was evaluated through percent time spent in open and closed arms.

Statistical analysis

Two-way analysis of variance (ANOVA) with CORT treatment and running as between-subject factors was performed with Fisher's LSD post-hoc test. A repeated measure ANOVA was applied for analyzing body weight change and running activity over the 14-day treatment period. Student's t-test was only applied for comparison between vehicle-treated non-runners and runners. The statistical difference was determined with P-value < 0.05. Data were presented as mean± standard error of mean (SEM).

RESULTS

Change of body weight and adrenal gland weight

Weight loss of the adrenal gland which occurs after CORT treatment indicates the efficacy of subcutaneous injections of CORT. Repeated measures of ANOVA revealed a significant change in body weight gain during the 14-day treatment period (Figure 1a and b), effect of day: $F_{3,57}$ = 19.2, P = 0.00090). Significant interaction between day and CORT treatment on body weight change was also observed (day X CORT: $F_{3,57}$ = 44.9; P = 0.00053). CORT treatment and running decreased body weight gain during the treatment (effect of CORT: $F_{3,57}$ = 57.2, P = 0.0077; effect of running: $F_{1,57}$ = 7.7, P = 0.00050). The adrenal gland weight was expressed as the ratio of adrenal gland weight to body weight. Two-way ANOVA indicated that the ratio of adrenal gland to body weight was significantly decreased by CORT treatment (effect of CORT: $F_{3,57}$=142.7, P=0.00033) (Figure 1c), but was not affected by running (effect of running, $F_{1,57}$ = 1.1, P = 0.300).

Running activity of CORT-treated runners

Repeated measures ANOVA indicated that all rats increased their running activity during the treatment period (Figure 2: $F_{3,38}$ = 14.8, P = 0.00042). The runners showed increased running activity during the treatment period (effect of day: $F_{3,38}$ = 5.8, P = 0.0060). Runners with CORT treatment showed a relative higher running activity when compared to runners with vehicle treatment; however, there was no significant effect of CORT on increasing running activity (effect of CORT: $F_{3,38}$ = 1.4, P = 0.29).

Running and CORT treatment did not affect locomotor activity in the rats

Results showed that neither CORT treatment nor running for 14 days affected traveling distance (Figure 3a: effect of running: $F_{1,50}$ = 1.0, P = 0.75; effect of CORT: $F_{3,50}$ = 1.8, P = 0.16) and travelling speed (Figure 3b: effect of running: $F_{1,50}$ = 1.4, P = 0.24; effect of CORT: $F_{3,50}$ = 1., P = 0.15) in the open field test. This data suggests that the difference in anxiety-like behavior was not an artifact of altered locomotor activity following CORT treatments.

Anxiety behavior of the CORT-treated runners and non-runners in the open field test

Anxiety-like behavior of the rats was tested in the open field test and elevated plus maze following the 14-day treatment. In the open field test, CORT, nor running showed no significant effect on the time spent in the central area (Figure 3c: effect of CORT: $F_{3,56}$ = 0.8, P = 0.52; effect of running: $F_{1,56}$ = 1.4, P = 0.25). However, there was a relative increase in time spent in the central area in the vehicle-treated runners when compared with the vehicle-treated non-runners (student's t-test, P < 0.05), indicating running reduced anxiety level in the vehicle-treated rats.

Time spent in the peripheral area was increased by CORT (Figure 3d: effect of CORT: $F_{3,47}$ = 3.0, P = 0.042). Post hoc analysis showed that the rats treated with 30 and 40 mg/kg CORT demonstrated a significant increase in time spent in the peripheral area when compared with the vehicle-treated rats (P < 0.05). The main effect of running was also observed (effect of running: $F_{1,47}$ = 16.7, P = 0.00021), indicating running decreased anxiety level in CORT-treated rats in the open field test. There was a significant decrease in time spent in the peripheral area in the vehicle-treated runners when compared with the vehicle-treated non-runners (student's t-test, P < 0.05).

Effect of CORT and running on anxiety-like behavior in elevated plus maze

There was no significant effect of CORT and running on the locomotor behavior in the elevated plus maze (data not shown). Running and CORT treatment was ineffective

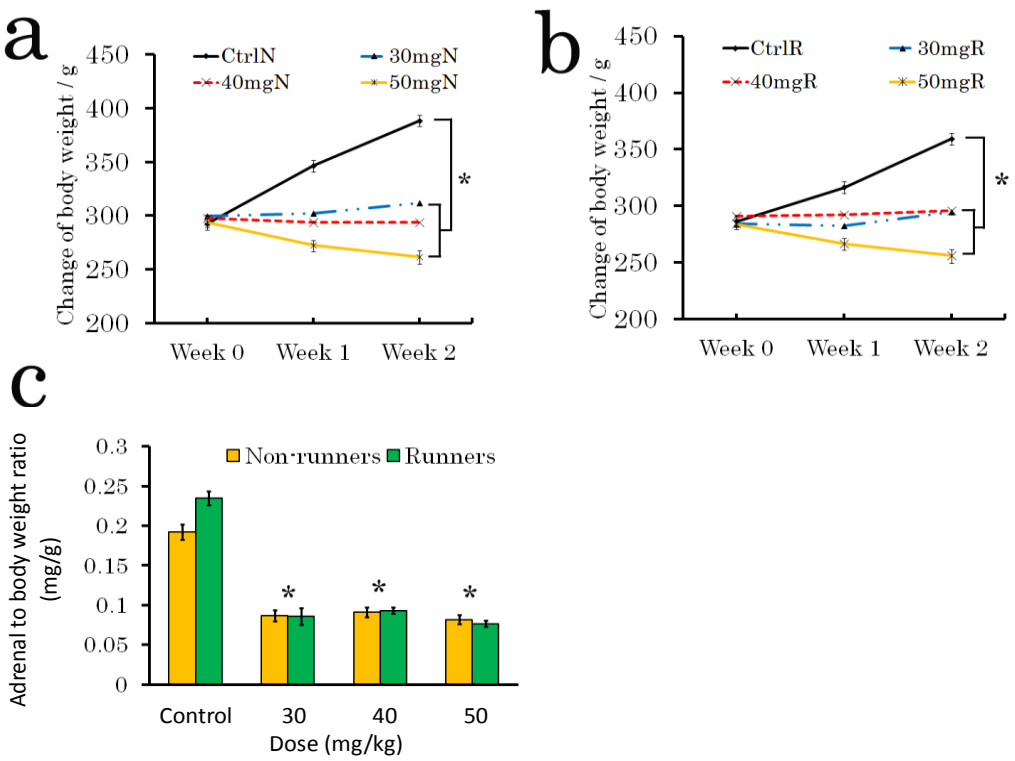

Figure 1. Change of body weight and adrenal weight of the CORT-treated runners and non-runners. (a and b) Body weight gain was significantly decreased by CORT treatments in the non-runners and runners. Running reduced body weight gain. (c) Adrenal to body weight ratio was an indicator for the efficacy of CORT administration. A significantly decreased adrenal weight was found in the CORT-treated rats when compared with the vehicle-treated rats. Ctrl: Vehicle treatment; 30, 40 and 50 mg: doses of CORT per body weight (kg). N: non-runners; R: runners. Value are respresented as mean±SEM. *P < 0.005 compared to the vehicle-treated group.

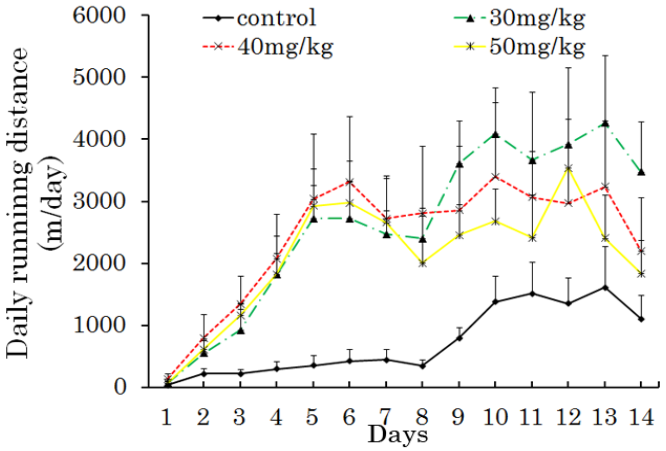

Figure 2. Voluntary running activity of the CORT- and vehicle-treated rats. The running activity was increased in the first week, and was then maintained at the level of 2 to 4 km/day. The rats treated with CORT showed a relatively higher running activity as compared to the vehicle-treated control. Data are expressed as daily mean running distance (m) per day mean ± SEM.

ineffective in altering the time spent in the open arms (Figure 4a: effect of running: $F_{1,51}$ = 0.1, P = 0.71; effect of CORT: $F_{3,51}$ = 0.6, P = 0.63) and in the closed arms (Figure 4b: effect of running: $F_{1,54}$ = 1.5, P = 0.23; effect of CORT: $F_{3,51}$ = 0.4, P = 0.34). The CORT-treated runners and non-runners did not show an increase or decrease in time spent in the open arms and closed arms, indicating that CORT treatment alone or running in CORT-treated rats did not exert effects on anxiety-like behavior in the elevated plus maze. Furthermore, CORT treatment showed no effect on time spent in the center (Figure 4c: effect of CORT: $F_{1,51}$ = 1.11, P = 0.35). Conversely, running showed a main effect on time spent in the center (effect of running: $F_{1,51}$ = 6.06, P = 0.018). Running showed an anxiolytic effect in the vehicle-treated runners as evidenced by relatively decreased percentage of time spent in the closed arms, and increased time spent in the open arms when compared with the vehicle-treated non-runners (student's t-test: P < 0.05).

DISCUSSION

In the present study, we used the open field test and elevated plus maze to examine: (1) whether different doses of repeated CORT treatment could induce anxiety-

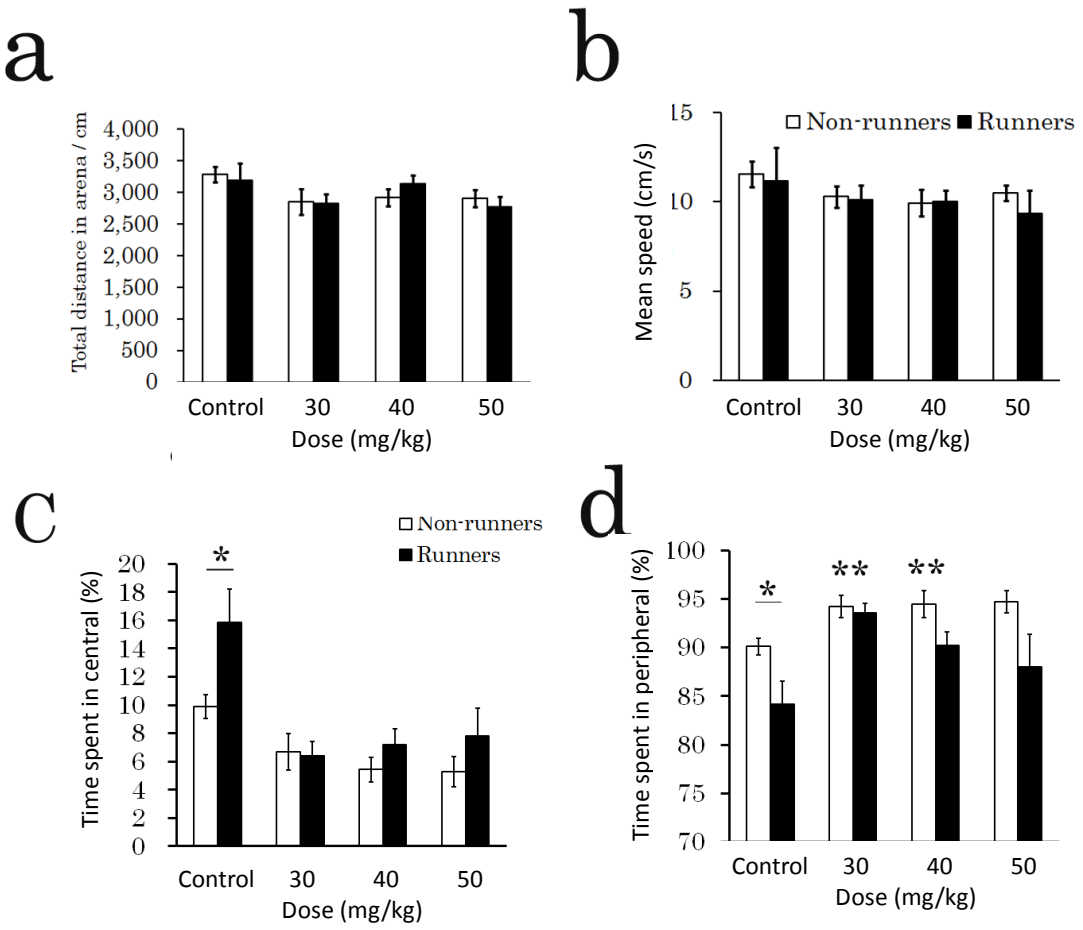

Figure 3. Locomotor activity and anxiety-like behavior of runners and non-runners in the open field test. (a) CORT treatment did not affect locomotor activity in the runners and non-runners. There was no significant difference in the mean speed of the CORT-treated runners and non-runners. (b) Total distance in the arena of the CORT-treated rats remained in the similar level as the vehicle-treated rats. (c) The CORT-treated rats showed no difference in time spent in the central area of the arena when compared with the vehicle-treated rats. However, running significantly increased the time spent in the central area in the vehicle-treated rats, but not the CORT-treated rats. (d) Rats treated with CORT showed increased time spent in the peripheral area. Runners showed a significant decrease in time spent in the peripheral area of the arena when compared to the non-runners. The vehicle-treated runners showed significantly decreased time spent in peripheral areas when compared with the vehicle-treated non-runners. Student's t-test: *P < 0.05 compared to the vehicle-treated non-runners. One-way ANOVA: **P < 0.05 compared to the vehicle-treated rats.

like behaviors; and (2) the counteractive effect of voluntary wheel running on CORT-induced anxiety-like behavior. The vehicle-treated runners showed an increase in time spent in the center of the open field test, in the open arm and the center of the elevated plus maze, respectively.

The findings confirmed that voluntary running for two weeks was associated with a reduced anxiety-like behavior as previously shown in the vehicle-treated rats by other group (Salam et al., 2009). However, CORT treatment increased anxiety level in the open field test, but not in the elevated plus maze. The results raise the question on the discrepancy between these behavioral tests for anxiety-like behavior in animal model of stress.

Although exercise has been shown to reduce anxiety in humans (Dunn et al., 2001; Manger and Motta, 2005) and in exercised animals (Binder et al., 2004), some studies fail to show the anxiolytic effect of exercise (Burghardt et al., 2004; Chiang et al., 2010). Contradictory results on the effects of exercise on anxiety behavior have been reported in some animal studies (Andreatini and Leite, 1994; Calvo et al., 1998). Contradictory results are also found in the open field test and elevated plus maze, for example, same mouse strain was defined as anxious in the elevated plus maze, but non-anxious in the open field test (Trullas and Skolnick, 1993, Rogers et al., 1999). Although these two tests are commonly applied to examine anxiety-like behavior in the animals, the differences in anxiogenic property of these two apparatuses have been reported. Carola et al. (2002) suggested that

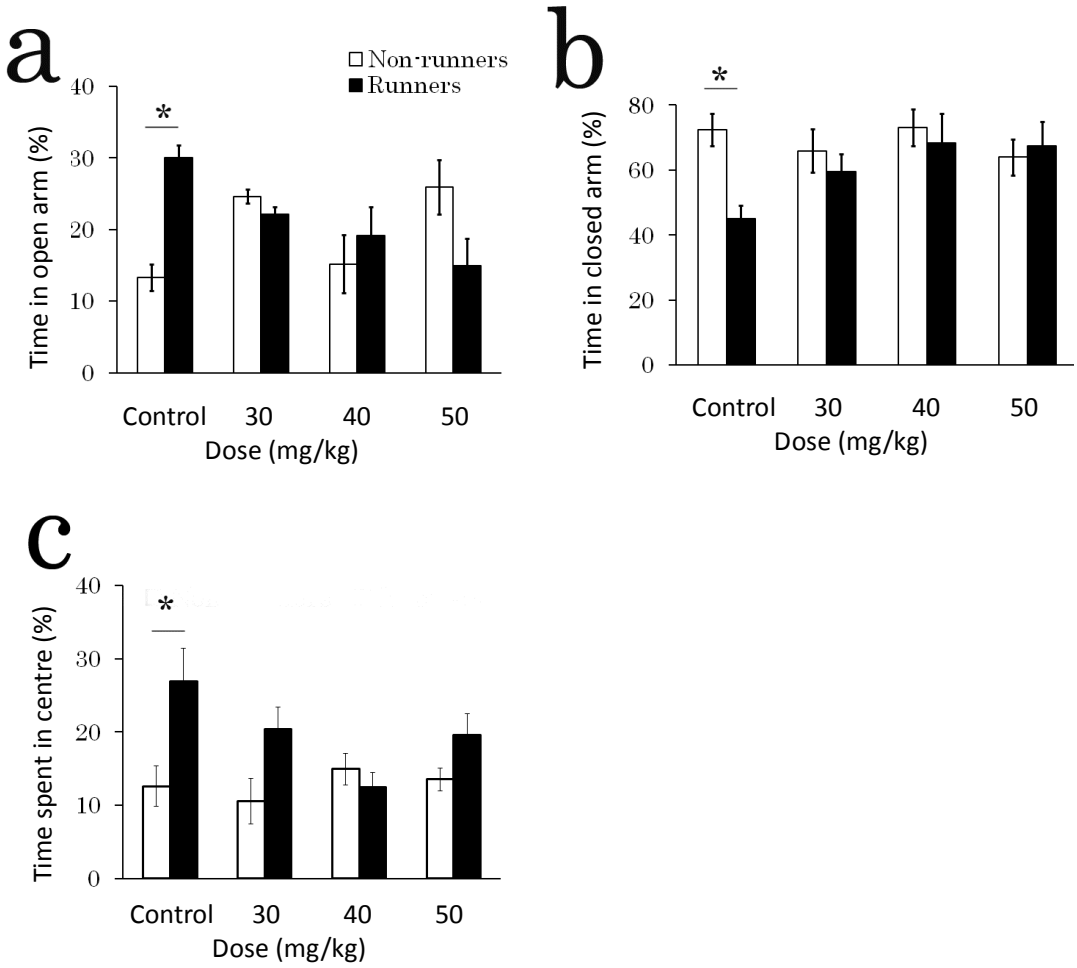

Figure 4. Running decreased anxiety-like behaviour in the vehicle-treated rats, but not CORT-treated rats in the elevated plus maze. Running and CORT treatment did not affect the percentage time spent in the open arms (a) and closed arms (b), respectively. Student's t-test revealed significant increases in time spent in open arms and center (c), and a decrease in time spent in closed arm in the vehicle-treated runners when compared to vehicle-treated non-runners. *P < 0.05 compared to runner counterpart by student's t-test.

discrepancies between these two tests may be due to differences in analyzing criteria among different studies or it is possible that the elevated plus maze may be more anxiogenic to experimental animals than the open field test.

Aversive stress in the open field test is mainly due to the novelty in a new environment, whereas aversive stress in the elevated plus maze is due to novelty and the height of the maze that comprise the behavior of risk assessment during the test, suggesting a stress coping to the animals during the test (Rodgers, 1997). In the elevated plus maze, the animals are placed in the centre of the plus maze as the starting point where animals initially engage in high level of risk assessment, therefore, the design of the elevated plus maze offers more choices and causes a higher level of stress to the animals as compared to the open field test. Nosek et al. (2008) conclude that the differences in the stressfulness between the open field test and elevated plus maze may contribute to the differential outcome. Nosek et al. (2008) suggested that the open field test would be a better measure of passive coping, while the elevated plus maze would be a more sensitive measure of active coping in response to stress.

A consistent result was observed in the vehicle-treated rats that running reduced anxiety level in both the elevated plus maze and open field test. The differential behavioral outcome between these two tests in the CORT-treated rats may suggest a differential stress response between these two tests in our CORT-injected rats. Pre-exposure to the open field test may contribute as a confounding effect since pre-exposure to different novel environment (e.g. the open field) immediately before testing in the elevated plus maze may alter the motor activity in the elevated plus maze and lead to a greater preference of entering the open arms of the maze

(Pellow et al., 1985). However, Walf and Frye (2007) reported that pre-exposure to other testing environment does not alter subsequent behavior of rats and mice in the elevated plus maze. To avoid any possibility of different experience or stressor exposure altering the behavior of rats in the elevated plus maze, the rats were left in the transport cages for 2 h before testing to ensure that the experimental rats have similar level of stress exposure before the behavioral test. Therefore, it is unlikely that the order effect will be a confounding variable to our study. Prut and Belzung (2003) suggested that the open field test is not a valid test for anxiety as it is not sensitive to some effective compounds (e.g. alprozolam and selective serotonin reuptake inhibitor (SSRI)) in treating anxiety disorders, they also concluded that the open field test may be a test for rodent model of normal anxiety.

Increased open field locomotion and decreased anxiety measures following wheel running have been found in the animals after 24 h of wheel running (Dishman et al., 1996; Burghardt et al., 2004). In this study, there was no difference in locomotor activity between the CORT-treated runners and non-runners as indicated by similar activity levels in the open field test and elevated plus maze, though there was a higher running activity in the CORT-treated rats as compared to the vehicle-treated rats.

The presented data shows that CORT treatment significantly increased anxiety-like behavior (increased time spent in peripheral) in the open field test regardless of the doses of CORT treatment, but not in the elevated plus maze. The counteractive effect of running on reducing anxiety-like behavior in CORT-treated rats was only observed in the open field test, but not in elevated plus maze. Gregus et al. (2005) reported that CORT (40 mg/kg) treatment for 21 days in non-running rats significantly increased immobility time in the forced swim test, but showed minimal effect on anxiety-like behavior in the open field test. The hippocampus has been known to play a crucial role in anxiety and depression. The dorsal and ventral hippocampi exhibit discrete functions in regulating learning and emotion, respectively. It has been reported that lesions of dorsal hippocampus led to relatively a weak anxiolytic-like effect, while lesions of ventral hippocampus produced a robust anxiolytic-like effect (Bertoglio et al., 2006; Pentkowski et al., 2006). Recently, it has been suggested that adult neurogenesis in the dorsal and ventral hippocampus might function in a dissociated way (Duncko et al., 2007) in which the dorsal hippocampus is likely specialized for memory functions, whereas the ventral hippocampus is dedicated to anxiety functions (Engin and Treit, 2007). Our previous study showed that physical exercise in terms of running wheel could protect the animals from detrimental effects of CORT via restoring adult neurogenesis and dendritic atrophy in the dorsal part of the hippocampus (Yau et al., 2011a). Several studies reported that a decrease in hippocampal neurogenesis is associated with an increase in anxiety-related behaviors (Ageta et al., 2008; Bergami et al., 2008; Revest et al., 2009). In particular, Revest et al. (2009) demonstrated an increased anxiety-like behavior in transgenic mice with an inducible reduction in neurogenesis throughout the rostro-caudal axis of the dentate gyrus. It is possible that depressive behavior and memory impairment is more dependent on the dorsal part of the hippocampus, while anxiety behavior is more dependent on the ventral part of the hippocampus.

We have previously reported that the dorsal hippocampal neurogenesis was increased in rats with two-week voluntary wheel running (Yau et al., 2011b). This study demonstrated that running decreased anxiety-like behavior in vehicle-treated rats; the promoting effect of running on adult neurogenesis and structural changes in the ventral hippocampus could possibly contribute to the observed effects of wheel running in our vehicle-treated rats. However, this speculation warrants further investigation with examination on the effects of specific blockade of ventral or dorsal hippocampal neurogenesis on emotional behavior.

In summary, these results have demonstrated that voluntary running reduced anxiety-like behaviors in the vehicle-treated rats in two behavioral paradigms: the open field test and the elevated plus maze. The CORT treatment increased anxiety level in the open field test, but showed an effect in the elevated plus maze. Running primarily reduced anxiety-like behavior in the open field test, but not in the elevated plus maze in the CORT-treated rats. The data indicated a differential anxiety-like behavior measured by these two tests in the CORT-treated rats. Extra attention should be given to the discrepancy of these two anxiety-related measures in animal model of stress since the physiological basis of anxiolytic effect of exercise will only be revealed with consistency of effects of exercise across the anxiety-related measures.

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