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Exercise prevents raphe nucleus mitochondrial overactivity in a rat depression model

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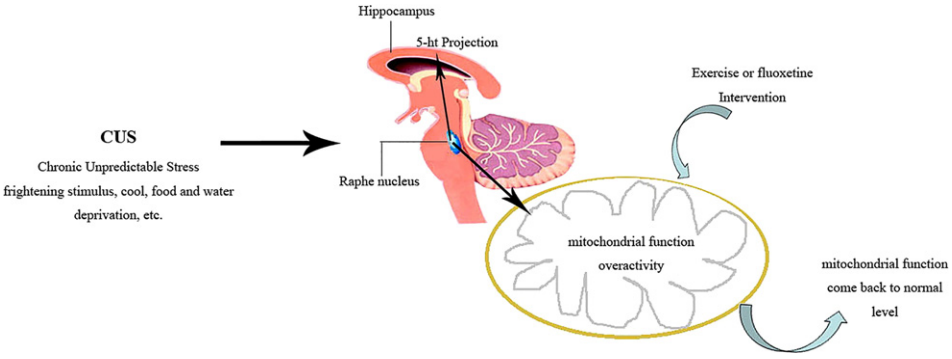
HIGHLIGHTS

- Mitochondrial dysfunction may underlie depression. Raphe nuclei may be involved.
- Chronic unpredictable stress (CUS) increased raphe nucleus mitochondrial function.
- The control, exercise and fluoxetine groups did not differ significantly.
- CUS in rats may cause overactivation of the mitochondria in the raphe nuclei.
- Exercise training may suppress these changes.

GRAPHICAL ABSTRACT



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ABSTRACT

Monoamine deficit and mitochondrial dysfunction may underlie depression. Serotoninergic neurons from raphe nuclei project widely and may be involved in depression. This study used chronic unpredictable stress (CUS) in rats as a model of depression to assess the effects of CUS, exercise and fluoxetine on mitochondrial function and serotonin levels in the raphe nuclei. Rats were divided into 4 groups (6 per group): control (C); depression (D), CUS for 28 days; depression + exercise (DE), treadmill exercises from days 11–28 of CUS; depression + fluoxetine (DF), fluoxetine (5 mg/kg/d i.g.) from days 11 to 28 of CUS. Behavioral changes were assessed using body weight, sucrose consumption tests (anhedonia) and open field tests (locomotor/exploratory behavior). Raphe nucleus mitochondrial function was determined using the respiratory control ratio, ATP synthesis rate, and activities of superoxide dismutase and glutathione peroxidase. Serotonin levels were measured in the raphe nuclei and hippocampus. On day 28 of CUS, body weight was higher in group C than in groups D, DE and DF ($P < 0.001$), and higher in group DE than in group D or DF ($P < 0.05$). Sucrose consumption was higher in group C than in groups D, DE and DF ($P < 0.001$), higher in group DE than in groups D ($P < 0.001$) or DF ($P < 0.05$), and higher in group DF than in group D ($P < 0.05$). All measures of mitochondrial function were increased in group D compared with the other groups ($P < 0.01$). Hippocampal serotonin was lower in group D than in the other groups ($P < 0.01$); levels in the raphe nuclei were elevated in group DE compared with the

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remaining groups ($P < 0.001$). CUS in rats may cause overactivation of the mitochondria in the raphe nuclei, and exercise training may suppress these changes.

1 . Introduction

Depression is a common affective disorder that presents with depressed mood, anhedonia, decreased energy, disturbed sleep or appetite, feelings of low self-worth and poor concentration. It has been estimated that 350 million people worldwide are affected by depression, and that nearly a million deaths annually are attributable to suicide [1]. For many years, pharmacologic therapy has been based on the widely accepted theory that a deficit of monoamines (serotonin, noradrenaline and dopamine) in the brain contributes to the pathogenesis of depression [2]. Selective serotonin re-uptake inhibitors (SSRIs), such as fluoxetine, are considered first-line agents for the treatment of depression, since they have superior efficacy to placebo, and fewer or less serious adverse effects than monoamine oxidase inhibitors (MAOIs) and tricyclic anti-depressants [3–8].

Although the monoamine hypothesis has been a cornerstone theory to explain the pathogenesis of depression, its limitations have become increasingly recognized. In recent years, interest has grown in the possibility that mitochondrial alterations may contribute to the development and/or maintenance of neuropsychiatric disorders, including depression [9], and there is now a school of thought that an interaction between monoamines, mitochondrial dysfunction and inflammation may underlie depression [10]. Depression has been suggested to result from a complex interplay of numerous factors, including inflammatory cytokines, oxidative and nitrosative stress (which cause lipid peroxidation and damage to DNA and proteins), reduced levels of anti-oxidants such as glutathione and glutathione peroxidase, and mitochondrial damage [11]. Although the exact mechanisms linking alterations in mitochondrial function to the pathogenesis of depression are not known, it has been suggested that changes in mitochondrial activity are associated with decreased energy production, reduced anti-oxidant capacity and increased oxidative stress, resulting in neuronal damage that underlies the development of the mood disorder [12]. Thus, the role of mitochondrial dysfunction and oxidative damage are emerging areas of research in this field.

There is now substantial evidence that exercise may improve symptoms in individuals diagnosed with depression, with effects comparable to those of anti-depressant medication [13–17]. Exercise has also been reported to have beneficial actions in animal models of depression. For example, in a rat model of depression, exercise was demonstrated to improve or reverse depressive symptoms and impaired spatial performance [18]. Interestingly, a recent study in a rat model of depression has shown that in addition to having beneficial effects on depression-related behavioral changes, exercise improved mitochondrial function and increased anti-oxidant enzymes in the brain, raising the possibility that the anti-depressive actions of exercise may, in part, be due to a reversal of mitochondrial dysfunction [19].

Various brain regions have been implicated in the pathogenesis of mood disorders [20], with the raphe nuclei of the upper brainstem thought to play a key role. Serotoninergic neurons of the raphe nuclei project widely, including to the forebrain, and are thought to regulate a variety of functions, including mood, memory and learning [21]. However, little is known about the changes in mitochondrial function and secretory activity of these nuclei during depression, and whether exercise or SSRIs might suppress or reverse any such changes. Therefore, we have used a validated rat model of depression, which involves the use of chronic unpredictable stress (CUS), to determine whether alterations in serotonin levels and mitochondrial function in the raphe nuclei parallel the behavioral changes evident in rats following exposure to CUS. Furthermore, we have investigated whether exercise was able to influence the effects of CUS on serotonin levels and mitochondrial activity, and compared any actions of exercise with those of fluoxetine.

2 . Materials and methods

2.1 . Animals

The protocols in this study were approved by Tianjin Medical University (Tianjin, China). Male 4-month-old Sprague–Dawley rats, weighing 300–400 g, were obtained from the Animal Center at Tianjin Medical University (Tianjin, China) and were housed singly in standard rodent cages with food and water provided *ad libitum*. The animals were maintained in a temperature- (23 ± 3 °C) and humidity- ($55 \pm 10\%$) controlled environment, with a 12-hour light–dark cycle (light commencing at 7 a.m.). Rats were allowed 1 week to acclimate before use in experiments. All procedures were in accordance with local, international and institutional guidelines. Care was taken to minimize the number of animals used and their suffering.

2.2 . Experimental protocol

The protocol used for these experiments is illustrated in Fig. 1. The animals were first trained, over a 10-day period, to consume a 1% sucrose solution. Rats were then randomly assigned to 1 of 4 groups (6 rats per group): control (C), depression (D), depression/exercises (DE), and depression/fluoxetine (DF). The animals in the 3 depression groups (D, DE, DF) were exposed to CUS (see below) for 4 weeks. Rats in group C were left undisturbed during the 4-week period, except when regular cage cleaning, weighing or behavioral testing were carried out. From day 11 to day 28 of the period of CUS, rats in the DE group engaged in treadmill exercises, and those in the DF group received intragastric injection of the anti-depressant, fluoxetine (see below). For rats in all groups, weighing and sucrose tests were performed at the following times: on the day before commencement of CUS; day 10 of the period of CUS; and at the end of the period of CUS. An open field test (OFT) was also performed at the end of the period of CUS. 48 h after the end of the period of CUS, the animals were sacrificed to allow isolation of mitochondria from the raphe nucleus, and measurement of 5-HT in the hippocampus and raphe nucleus.

2.3 . Chronic unpredictable stress (CUS)

The animals in the 3 depression groups (D, DE and DF) were exposed to the CUS protocol [22,23] shown in Table 1.

2.4 . Fluoxetine

Fluoxetine was obtained from the Hospital Center at Tianjin Medical University (Tianjin, China). From day 11 to day 28 of the period of CUS (a total of 18 days), rats in the DF group were injected intragastrically with fluoxetine at a dosage of 5 mg/kg/d (time of administration, 8:30–9:00 a.m.). The conditions used for CUS were the same as in the other two depression groups (D and DE).

2.5 . Exercise training

From day 11 to day 28 of the period of CUS (a total of 18 days), rats in the DE group were run on a six-lane treadmill (Tianjin Institute of Sport Medicine, Tianjin, China), for 45 min per day [24–27]. The speed