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Research report

# Blood oxygen level-dependent signals via fMRI in the mood-regulating circuit using two animal models of depression are reversed by chronic escitalopram treatment

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## HIGHLIGHTS

- Both CUMS and MS rat groups demonstrated depression-like behaviours and cognitive impairment, which could be modified by chronic escitalopram treatment.
- Increased BOLD activation was observed in some brain regions of MS and CUMS animals, such as the bilateral hypothalamus, limbic system, hippocampus and frontal lobe, which are parts of mood-regulating circuit.
- The MS- and CUMS-induced increases in BOLD activation were partially attenuated by chronic escitalopram treatment.

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## ABSTRACT

*Background:* People exposed to stressful experience are at increased risk of the development of depression. A number of functional imaging studies have found disturbances in the mood-regulating circuit of the stress-exposed depressed patients, although few animal imaging studies have been undertaken addressing the brain functional changes of depression.
*Methods:* Two rat models of depression: maternal separation (MS) and chronic unpredictable mild stress (CUMS), imitating early life stress and adult stress respectively, were administered with escitalopram. The differences in functional brain changes were determined by blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI).
*Results:* Increased BOLD activation was observed in some brain regions of MS and CUMS animals, such as the bilateral hypothalamus, limbic system, hippocampus and frontal lobe, which were parts of mood-regulating circuit. Furthermore, the MS- and CUMS-induced increases in BOLD activation were partially attenuated by chronic escitalopram treatment.
*Conclusions:* These results suggested hyperactivation of mood-regulating circuit at baseline in the depressed animals exposed to stressful experience, and escitalopram can at least partially reverse these effects.



## 1. Introduction

Depression is the most common psychiatric illness that involves disturbance of mood [1]. Brain regions involved in mood-regulating circuit such as hippocampus, temporal lobe, amygdala, caudate, anterior cingulate cortex, and frontal cortex have demonstrated their role in the onset and/or maintenance of depression [2].

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Considering that the complex brain processes do not merely arise from "switching on" or "switching off" of individual brain structures, the consequence of depression may be attributed not only to focal changes within certain brain regions, but also to disturbances of function within them.

Some functional imaging studies have found disturbances in the mood-regulating circuit of the depressed patients, and antidepressants could restore the integrity of the mood-regulating circuit [3–5]. However, the retrospective clinical studies were limited in that they did not provide direct evidence of a cause-effect relationship. In addition, these studies put patients associated with different kinds of stress together which could not distinguish the effects of them. Animal studies are indispensable to improve our understanding of the consequences of depression, although few animal imaging studies have been undertaken to address the brain functional changes of depression. The only study [6] investigating brain alterations in flinders sensitive line (FSL) rats, a genetic animal model of depression, found FSL rats exhibited greater BOLD activation in the cortical amygdala and hyperactivation in the insular cortex when innate fear was induced, suggesting enhanced neuronal responses in depression.

Epidemiologic studies indicate that people exposed to stressful experience are at increased risk of the development of depression [7]. In our previous studies [8,9], we used two rat models of depression: maternal separation (MS) and chronic unpredictable mild stress (CUMS), imitating early life stress and adult stress respectively. The findings of both studies showed that the two models demonstrated reduced concentration of neuronal marker *N*-acetylaspartate (NAA) in the hippocampus without changes in hippocampal volume. Blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has been extensively applied to study the brain and its functional organization in healthy and disease states, and has distinct advantages over other imaging techniques [10]. In the present study, we investigated the functional brain changes associated with the two above-mentioned animal models.

Selective serotonin reuptake inhibitors (SSRIs) are found to be safe and effective in the treatment of depression. Anand et al. [3,5] found that SSRIs sertraline had effects on the alterations of mood-regulating circuit in depression patients. Moreover, a functional imaging study with positron emission tomography (PET) by Mayberg et al. [11] found decrease metabolism in hippocampus after treatment with the SSRIs fluoxetine. Escitalopram is the oxalate of (S)-citalopram, works faster and more efficacious than citalopram, and has been become a first-line treatment of depression. Among all the SSRIs, escitalopram has highest selectivity on the recovery inhibition of 5-HT, and to our knowledge, it has never been tested on functional brain changes of depression. Our previous studies [8,9] found that escitalopram could reverse the effects of MS and CUMS on the neurochemistry of hippocampus. Therefore, a further aim of the present study was to determine whether functional brain changes in the two animal models determined by BOLD could be ameliorated by escitalopram treatment.

## 2. Materials and methods

### 2.1. Animals

In the early life stress experiments, timed-pregnant Sprague-Dawley rats (Animal House Center, Southeast University, China) were provided on gestation days 16–18 that were individually housed. In the experiments of chronic unpredictable mild stress, adult male Sprague-Dawley rats (Animal House Center, Southeast University, China) weighing 200 g were housed 4 to a cage. All animals were in a temperature ($21 \pm 2\,^{\circ}C$) and humidity ($55\% \pm 5\%$) controlled room on a 12-h light/dark cycle (lights on at 07:00 am) with food and water provided *ad libitum*. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Jiang Su Animal Care and Use Committee.

### 2.2. Experimental procedures

#### 2.2.1. Early life stress experimental procedure

In early life stress procedure, the day of delivery was designated as postnatal day (PND) 0. As previously described [12,13], on PND 1, litter composition was standardized to 10 pups (5–6 males and 4–5 females). All pups were randomly assigned to MS or control group from PND 2 to 14 inclusive. For MS pups, dams were first removed and placed in an adjacent cage. Pups were then transferred to a plastic container lined with bedding from the home cage and placed in an incubator maintained at roughly 34 °C, a temperature consistent with nest measurements. After the separation period, MS pups were returned to their home cages, where they were reunited with their dams. MS was carried out for a period of 180 min between 08:00 am and 11:00 am Control pups remained with their dams over this period.

From PND 43 to 60, MS and control animals received daily administrations of saline or escitalopram. There were 4 experimental groups (n = 12–14, 6–7 animals of each sex): control + saline, control + escitalopram, MS + saline, MS + escitalopram. Behavioural tests (sucrose preference test, forced swimming test, Morris water maze test) were undertaken on adult animals (PND 61–75), with an interval of 3–5 days between tests to minimize any stressful effects from the previous test. Then animals were exposed to MRI scan in random order.

#### 2.2.2. CUMS experimental procedure

The CUMS procedure contained 9 different stressors randomly arranged day and night across 42 consecutive days: 20 h food and water deprivation, 18 h water deprivation, 17 h of 45° cage tilt, overnight illumination, 21 h wet cage, 5 min swimming in water at 4 °C, 30 min on a 160 Hz rocking bed, 1 min tail pinch and 2 h restrict movement. The behavioural tests were performed and scored by trained and experienced observers who were blind to the condition of the animals.

The rats were randomly divided into 4 groups: control + saline, control + escitalopram, CUMS + saline and CUMS + escitalopram (n = 6 per group). Two weeks after the beginning of the CUMS regimen, rats received escitalopram or saline treatment once a day for 4 weeks. A sucrose preference test and a forced swimming test were administered at 3 time points: before stress (baseline), before drug administration (week 2) and at the end of the experiment (week 6). The Morris water maze test and MRI scan were carried out at the end of the experiment.

### 2.3. Pharmacological administration

Escitalopram oxalate tablets (H. Lundbeck A/S, Copenhagen, Denmark) were dissolved in saline (0.9%) and administered by gavage at a dose of 10 mg/kg body weight [14].

### 2.4. Behavioural tests

#### 2.4.1. Sucrose preference test

In the sucrose preference test, the animals were allowed to consume water and a 1% sucrose solution for 1 h after 20 h food and water deprivation, following 48 h of exposure to both water and sucrose solution. The positions of the two bottles (right/left) were varied randomly across animals and were reversed after 30 min. The sucrose preference was calculated according to the following