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# The number of granule cells in rat hippocampus is reduced after chronic mild stress and re-established after chronic escitalopram treatment

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**Abstract**

Stress and depression cause structural changes in the hippocampal formation. Some of these can be reversed by chronic antidepressant treatment. In the present study, we examined the changes in the total number of granule cells and the volume of the granule cell layer after exposing rats to chronic mild stress and chronic escitalopram treatment. Furthermore, we investigated which classes of immature granule cells are affected by stress and targeted by escitalopram. Rats were initially exposed to 2 weeks of CMS and 4 weeks of escitalopram treatment with concurrent exposure to stress. The behavioral changes, indicating a decrease in sensitivity to a reward, were assessed in terms of sucrose consumption. We found a significant 22.4% decrease in the total number of granule cells in the stressed rats. This decrease was reversed in the stressed escitalopram treated rats that responded to the treatment, but not in the rats that did not respond to escitalopram treatment. These changes were not followed by alterations in the volume of the granule cell layer. We also showed a differential regulation of dentate neurons, in different stages of development, by chronic stress and chronic escitalopram treatment. Our study shows that the anhedonia-like state in the CMS rats is associated with a reduced number of granule cells. We conclude that escitalopram acts on specific cellular targets during neuronal differentiation and that recovery from anhedonia-like behavior in rats may be the consequence of an escitalopram mediated increase in specific subtypes of immature dentate neurons.


*Keywords:* Chronic mild stress; Depression; Hippocampus; Escitalopram; Granule cells; Stereology

## 1. Introduction

Stressful life events are related to the onset of depressive episodes (Kendler et al., 1999). Recent studies have shown hippocampal volume reduction in patients with depression (Sheline et al., 2003) and post-traumatic stress disorders. Moreover, hippocampal volume changes were shown to be reversed or prevented by chronic antidepressant treatment (Vermetten et al., 2003). In animal models of depression, stress-induced suppression of hippocampal neurogenesis has been repeatedly shown to be normalized by chronic treatment with antidepressants (van der Hart et al., 2002; Fuchs et al., 2004; Jayatissa et al., 2006). These results suggest a key role of hippocampal neurogenesis in structural brain changes in pathology and treatment of depression.

In our study, we were interested in changes in the developmental progress of hippocampal immature neurons expressing a marker for migrating neuroblasts, doublecortin (DCX). We used the classification of DCX-immunoreactive neurons as D cells previously proposed by Seri et al. (2004). D cells are divided into three morphological categories: mitotic D1 and D2 cells, and postmitotic D3 (Seri et al., 2004; Kempermann, 2006).

Chronic mild stress (CMS) is a valid rodent model of depression (Willner et al., 1992). The CMS procedure involves chronically subjecting rats to a variety of mild stressors. During 1 to 3 weeks of exposure to CMS, rats display a reduction in

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a consumption of a sucrose solution. Decreased sucrose consumption is interpreted as a decrease in sensitivity to reward (Willner et al., 1987). This has been confirmed by decreased rewarding properties of food pellets and amphetamine (Papp et al., 1991), and in increased threshold for intercranial ventral tegmental self-stimulation (Moreau et al., 1992) in CMS rats. In addition, CMS results in the appearance of other symptoms of depression, like decreases in sexual, aggressive (D'Aquila et al., 1994), and investigative behaviors, in locomotor activity (Willner, 1997), and changes in sleep architecture (Cheeta et al., 1997; Gronli et al., 2004). All of the clinically active classes of antidepressants (Willner, 1997) mediate recovery from stress-induced anhedonia. In our hands, the CMS model has an additional feature that enhances its validity. Chronic treatment with the antidepressant escitalopram only reverses the reduction in sucrose consumption in about 50% of the anhedonic rats. The remaining 50% of rats do not respond to the treatment (Jayatissa et al., 2006). The segregation of rats into drug responders and non-responders mirrors clinical drug refraction (Bondolfi et al., 1996; Einarson, 2004). Escitalopram is a selective serotonin reuptake inhibitor. It is the active component of racemic citalopram and is widely used in the clinical setting.

Previously, we reported a positive correlation between sucrose intake and cell proliferation in the granule cell layer of the ventral hippocampus in the CMS rats (Jayatissa et al., 2006). In the present study, using modern design-based stereological tools we demonstrate that there is a decrease in the total number of granule cells in the ventral hippocampus of anhedonic rats, and that the number of granule cells is restored to the level of controls only in rats responding to antidepressant treatment. In addition, we demonstrate an increase in specific subtypes of immature neurons in rats responding to the treatment. These results can serve as bases for designing novel antidepressant drugs with actions directed towards this specific cellular target.

## 2. Material and methods

### 2.1. Animals

Male Wistar rats (Taconic, Denmark), 6–7 weeks old and weighing about 200 g when adaptation for sucrose consumption was initiated and approximately 350 g at the start of stress regime, were used in the study. The animals were singly housed, except when grouping was applied as a stress parameter. Food and water were available ad libitum except when food or/and water deprivation was applied as a stress parameter. The standard 12:12-h light/dark cycle was only changed in the course of stress regime. All the procedures involving animals were approved by Danish National Committee for Ethics in Animal Experimentation (2002/561-575).

### 2.2. Sucrose consumption test

The experimental design is presented in Fig. 1. The animals were first trained to consume a palatable sucrose solution (1.5%). The training lasted 5 weeks. In this period the sucrose test was made twice a week during the first 3 weeks and once a week during the last 2 weeks. The animals were food and water deprived 14 h before the test. The test consisted of a 1-h exposure to a bottle with sucrose solution. During the stress period the sucrose consumption test was performed once a week.

### 2.3. Chronic mild stress protocol

On the basis of sucrose intakes in the three final baseline tests, the animals were divided into two matched groups and placed in separate rooms. One group was exposed to an initial 2 weeks of chronic mild stressors and the other was left undisturbed. The unchallenged group was food and water deprived 14 h before sucrose consumption test; otherwise food and water were freely available.

The stress protocol consisted of 1 period of intermittent illumination, stroboscopic light, grouping, food or water deprivation; 2 periods of soiled cage and no stress; 3 periods of 45° box tilting. All the stressors lasted from 10 to 14 h. After the initial 2 weeks of stress exposure, the unchallenged and stress groups were divided into two matched subgroups and subjected to chronic escitalopram or vehicle administration. Stress was continued during the entire period of treatment. The size of the animal groups were: unchallenged vehicle (U-V) $n = 8$, unchallenged escitalopram (U-Es) $n = 8$, chronic mild stress vehicle (CMS-V) $n = 8$, chronic mild stress escitalopram (CMS-Es) $n = 15$.

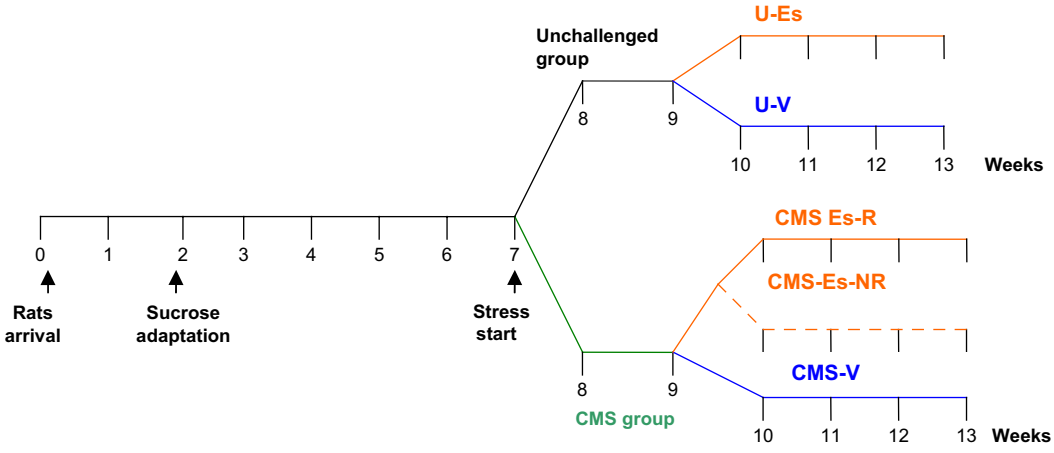


Fig. 1. Experimental design. The time schedule is presented in weeks. The color codes indicate the pharmacological treatment strategies. The blue lines indicate groups treated with vehicle (U-V and CMS-V), the red lines indicate groups treated with escitalopram (U-Es, CMS-Es-R, and CMS-Es-NR). Note that the responder and non-responder subgroups belong to the same group exposed to stress and chronic escitalopram treatment. U, unchallenged ($n = 16$); U-Es, unchallenged escitalopram group ($n = 8$); U-V, unchallenged vehicle group ($n = 8$); CMS, chronic mild stress ($n = 23$); CMS-V, chronic mild stress vehicle group ($n = 8$); CMS-Es-R, chronic mild stress escitalopram responder group ($n = 7$); CMS-Es-NR, chronic mild stress escitalopram non-responder group ($n = 8$).