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Anhedonia was associated with the dysregulation of hippocampal HTR4 and microRNA *Let-7a* in rats

Mei Bai [a], Xiong-Zhao Zhu [a,c,*], Yi Zhang [a,d], Sheng Zhang [a,d], Li Zhang [a,d], Liang Xue [a,d], Mingtian Zhong [a,d], Xiuwu Zhang [b]

[a] Medical Psychological Institute, Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China
[b] Departments of Radiation Oncology, University of Maryland, School of Medicine, Baltimore 20221, USA
[c] National Technology Institute of Psychiatry, Central South University, Changsha, 410011, P.R. China
[d] Key Laboratory of Psychiatry and Mental Health of Hunan Province, Central South University, Changsha, 410011, P.R. China

HIGHLIGHTS

- Anhedonia was related to dysregulation of hippocampal *Let-7a* and HTR4 in rats.
- The hippocampal HTR4 level might be regulated by microRNA *Let-7a* in rats.
- Different stressors (MD and CUPS) induce distinct depressive phenotypes in rats.
- MD but not CUPS affect hippocampal *Let-7a* and HTR4 in rats.

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ABSTRACT

Objective: Depression is a serious mental illness. However, the molecular mechanisms responsible for the development of depression remain unknown.
Methods: In this study, animal models of depression were established using maternal deprivation (MD) and chronic unpredictable stress (CUPS). Behavioral performance of rats was monitored by open field test, forced swim test, and sucrose consumption test. The expression of serotonin receptor-4 (*Htr4*) mRNA and *Let-7a* microRNA was detected by real-time PCR, while *Htr4* protein level was measured by Western blot.
Results: In the open field test, rats subjected to MD and CUPS exhibited significant decreases in vertical activity. CUPS rats spent less time in the central area and excreted more fecal pellets than MD and control rats. In the forced swim and sucrose consumption tests, CUPS and MD rats exhibited significantly longer floating time and consumed less sucrose than control rats. MD rats exhibited significantly shorter floating time and consumed less sucrose than CUPS rats. MD rats showed significantly lower *Htr4* mRNA and protein expression and significantly higher *Let-7a* level in the hippocampus than control rats. *Htr4* mRNA and protein expression negatively correlated with *Let-7a* expression. *Htr4* mRNA expression positively correlated with sucrose preference rate, while *Let-7a* expression negatively correlated with the sucrose preference rate.
Conclusion: Anhedonia, not despair or a decline in exploratory interest, may be associated with upregulation of *Let-7a* and downregulation of *Htr4* expression in the hippocampus. The hippocampal *Htr4* level may be regulated by *Let-7a* in rats.

1. Introduction

Depression is a serious mental illness with a lifetime prevalence rate of about 17% [1]. Serotonin deficiency in the synaptic clefts of the central nervous system is believed to be an important risk factor for the onset of depression [2]. This reasoning gives rise to the use of selective serotonin reuptake inhibitors (SSRIs) as first-line therapy for depression in the clinic. SSRIs work primarily by inhibiting the reuptake of serotonin into the presynaptic cell and increasing the level of serotonin available in the synaptic cleft to bind to the postsynaptic receptor. However, it is still unclear why up to 40%–50% of patients with depression show little or no response to SSRIs [3]. A recent study discovered lower *Htr4* gene expression at the neuronal surface in p11 knockout mice that

* Corresponding author. Tel.: +86 731 85292126; fax: +86 731 85361328.
E-mail address: xzxzhu@yahoo.com (X.-Z. Zhu).

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displayed depression-like behaviors [4]. Moreover, administration of HTR4 protein agonist RS 67333 was able to normalize depressive rats' performance in forced swim test and sucrose consumption test, and the HTR4 agonist was found to work faster than SSRIs [5]. HTR4 protein is distributed specifically in limbic regions of the brain and is abundantly found in the hippocampus [6], a key brain region associated with the development of depression. We therefore hypothesized that hippocampal HTR4 protein may be a key player in the development of depression.

A growing number of studies have verified that environmental factors serve a crucial role in the development of depression [7]. Environmental factors, including chronic stress during early life and adulthood, may affect the expression of various genes through epigenetic mechanisms such as microRNAs (miRNAs). Mature miRNAs (about 22 nucleotides in length) are endogenous, non-coding, single-stranded RNA molecules, which have emerged recently as a major class of gene expression regulators closely associated with the development of depression [8]. Given the fact that miRNAs always induce translational repression or mRNA degradation by binding to the seed sequence at the 3′-UTR or the coding sequences of their target mRNAs [9], it is possible to predict potential regulatory miRNAs for *Htr4* by using bioinformatic analysis for seed complementarity [10]. Results from the bioinformatic analysis demonstrated that miRNA *Let-7a* may regulate *Htr4* (Fig. 1A). Recent studies demonstrated that *Let-7a* was upregulated in the frontal cortex of mice after exposure to acute stress [11], and *Let-7a* was the only miRNA affected by both acute and chronic stressors in the central amygdala [12]. However, it is unclear whether hippocampal *Let-7a* and *Htr4* expression was regulated by various depressogenic stressors and whether the expression of *Let-7a* and *Htr4* correlates with the phenotype of depression.

In this study, we adopt two well-known stress paradigms, maternal deprivation and chronic unpredictable stress, to investigate hippocampal *Let-7a* and *Htr4* expression and their correlation with depressive behaviors.

2. Materials and methods

2.1. Animals and grouping

Ten Sprague–Dawley rats at the age of 3 months were provided by the Animal Center of Central South University and housed in polycarbonate cages under standard conditions in accordance with the Guide for Care and Use of Laboratory Animals (Chinese Council). After fertilization, rats were checked at 9:00 every day for delivery. Rat offspring born before 9:00 were designated as postnatal day 1 (PND 1). All experiments were conducted in accordance with an approved protocol from Central South University. Every effort was made to minimize the number of animals used. Newborn male offspring from 10 pregnant rats were mixed and randomly divided into three groups: maternal deprivation group (MD, N = 6), chronic unpredictable stress group (CUPS, N = 6), and control group (C, N = 6). MD rats received maternal deprivation for 2 weeks after birth. CUPS rats received chronic unpredictable stress for 3 weeks after the rats reached 10-weeks old. Control rats received only standard husbandry care.

2.2. Maternal deprivation (MD)

The MD paradigm was carried out as previously described [13]. Briefly, rat offspring were separated from their mothers for 6 h daily from PND 1 to PND 13 (the separation occurred at 9:00–15:00). To block communication between mothers and offspring, each offspring was placed in a new cell (32 cm × 32 cm × 14 cm, divided into four cells of the same size) covered with sawdust in a new room and later returned to their home cage when the experiment concluded at 15:00. All experiments were conducted in a temperature-controlled room (25 ºC). After PND21, rats were grouped and housed socially with the same gender until adulthood (10 weeks).

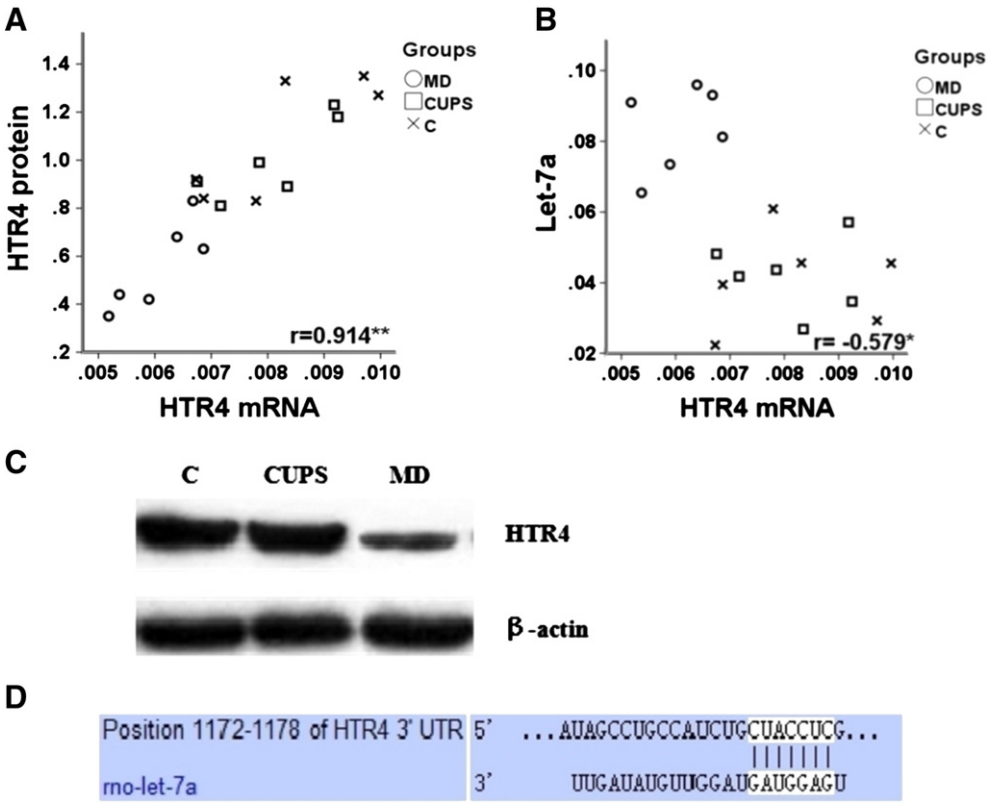

Fig. 1. Correlations between genes' expressions. (A) Correlation between HTR4 mRNA expression and HTR4 protein expression. (B) Correlation between HTR4 mRNA expression and *Let-7a* expression. (C) Representative Western blots of HTR4 and β-actin protein expression. (D) Bioinformatic analyses of *Let-7a* binding site at the 3′ UTR of *HTR4* gene.