

## EXPERIMENTAL STUDY

# The impact of serotonergic stimulation on reelin and glutamate decarboxylase gene expression in adult female rats

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**Abstract:** *Background:* Reelin plays an important role in the regulation of synaptic plasticity in adulthood. Administration of 5-methoxytryptamine (5MT), an agonist of serotonin receptors, during natal and neonatal periods results in decreased reelin expression. In adulthood, reelin is expressed by GABAergic neurons.

*Objectives:* The purpose of this study was to reveal the effect of elevated serotonergic stimulation on the expression of reelin and glutamate decarboxylase (GAD1) in adulthood as well as on depressive behavior and spatial cognitive abilities in adult female rats.

*Methods:* Rats were injected with 5MT. A forced swimming test was used for evaluation of the depressive behavior and Morris water maze test was used for evaluation of spatial cognition. Brains were used for measuring the expression of reelin and GAD1.

*Results:* We found a significant decrease in reelin expression in the cerebellum and prefrontal cortex of 5MT-treated rats. GAD1 expression was decreased in the cerebellum of 5MT-treated rats. 5MT-treated rats reached a lower immobility score in the forced swimming test. The Morris water maze test did not reveal any significant differences.

*Conclusion:* We have shown that administration of serotonin receptor agonist resulted in a decreased RELN and GAD1 expression in the cerebellum of adult female rats. We propose that this phenomenon might be relevant in the pathogenesis of autism (Fig. 3, Ref. 38). Full Text in free PDF [www.bmj.sk](http://www.bmj.sk).

**Key words:** serotonin, reelin, glutamate decarboxylase, autism.

Reelin is a neuroprotein with a major role in neuronal migration and prenatal development of neuronal connections (1). It is produced by Cajal-Retzius cells in the hippocampal cortex during neurodevelopment and secreted into the circulation. Reelin exerts its function via two pathways. The proteolysis of extracellular matrix proteins by reelin as a serin protease is crucial for the migrating neurons. Reelin, however, also binds to its receptors on migrating neurons activating the intracellular signaling cascades. Although reelin is considered to act mainly during neurodevelopment, it has been shown that it participates in the regulation of synaptic plasticity in the adult brain (2, 3, 4, 5). Cajal-Retzius neurons degenerate during a three-week period after birth (3). Reelin expression in the adult brain is restricted to GABAergic neurons in the cerebral cortex and hippocampus and in glutamatergic neurons (granule cells) (6) and GABAergic neurons in the cerebellum. Cortical GABAergic interneurons expressing reelin

form heterogenous cell populations with a number of inhibitory synaptic contacts. Reelin-expressing neurons have been identified in hippocampal formation of the adult human brain (7).

Serotonin is a neurotransmitter contributing to the regulation of mood, sleep, and some cognitive functions including memory and learning (8, 9, 10). Rats administered with 5-methoxytryptamine (5MT), an agonist of serotonin receptors, during pregnancy and during ten days after birth showed a decrease in reelin expression in the brain (11). This effect was enabled via synaptic contacts among 5HT neurons and Cajal-Retzius cells expressing the reelin (11). In adulthood, reelin is expressed mainly by GABAergic neurons creating the synaptic contacts with 5HT neurons (12, 13, 14, 15). The primary aim of this study based on a female rat model was to reveal the effect of 5MT administration on reelin expression and on spatial memory. Since reelin is expressed by GABAergic neurons, which express also the glutamate decarboxylase (GAD1, GAD2), we have as well investigated the effect of 5MT administration on GAD1 expression. RELN and GAD genes belong to the group of candidate genes for a neuropsychiatric disorder – autism.

## Methods

### Animals

Female Wistar rats (n = 22; 290–320 g; Dobra Voda, Slovakia) were divided into two groups: 5MT group (5-methoxytryptamine

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**Acknowledgements:** This project was supported by grants AV 4/0038/07, MZSR 07B030702, UK/116/2008, VEGA1/0305/09.

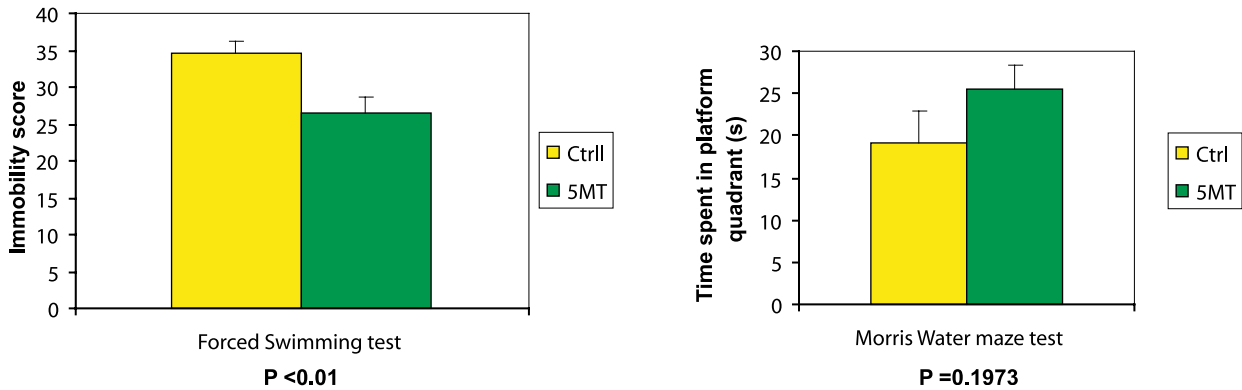


Fig. 1. Behavioral tests. 5MT-treated rats reached a significantly lower immobility score in forced swimming test than control rats ( $p < 0.01$ ). There were no significant differences in the Morris water maze test between 5MT and the control group ( $p = 0.1973$ ).

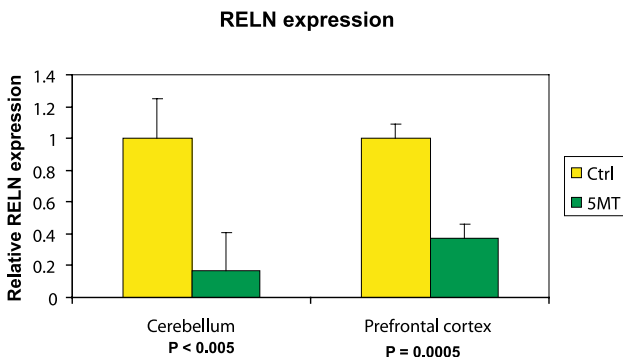


Fig. 2. RELN expression. RELN expression was significantly decreased in the cerebellum ( $p < 0.005$ ) and prefrontal cortex ( $p = 0.0005$ ) of 5MT-treated rats.

treated) of eleven rats and a control group of eleven rats. Both groups had the same climatic conditions, namely temperature of 25 °C and a 12-hr light:dark photoperiod cycle. The experimental protocol was carried out under the guidelines of the Committee on Ethics of the University of Comenius (Bratislava, Slovakia). Animals had free access to food (standard pellet mixture) and water ad libitum.

#### Experimental protocol

For ten days, the experimental rats were daily injected with 5-methoxytryptamine (1 mg/kg of weight s.c.) while the control rats were injected with saline solution at the same dose and with the same timing. A forced swimming test (FST) was performed on day 11. Rats were placed in a deep cylinder of water for three minutes. Every 5 seconds, they were scored for struggling (0 points), swimming (1 point) and immobility posture (2 points). Immobility score consisted of the sum of these scores for each rat. The mean immobility scores of 5MT group were compared to those of control group. (16, 17). A Morris water maze test was performed three days after FST (18). In probe trials, the platform was removed. The time spent in the platform quadrant was measured. After the behavioral testing, the rats were decapitated

under anesthesia (thiopental, i.p., 25–35 mg/kg of weight), and their brains were collected. Total RNA was isolated from samples of cerebellum, prefrontal, frontal and parietal regions of cortex, as well as from hippocampus and medulla.

#### Analyses of gene expression

Total RNA was isolated from tissue samples using TRIreagent (Sigma-Aldrich, St. Louis, MO, USA). Concentration and purity of isolates were assessed spectrophotometrically. Sybr Green one step RT PCR kit (Qiagen, Hilden, Germany) was used for real time PCR on Biorad IQ5 cycler. A melting curve analysis was performed to check the specificity of PCR. The transcripts of target genes were determined relatively to the transcripts of the housekeeping gene (beta actin) using the delta Ct method.

Primers were used with sequences (5'-3') as follows:

RELN-Fw:GCACCAGCCAAAG, RELN-Rev: GTTGCCACCAGCGCAGTAA, GAD1-Fw: GCGGATCCTAATACTACC-AACC, GAD1-Rev: GTCCTTTGCAAGAAACCACAG.

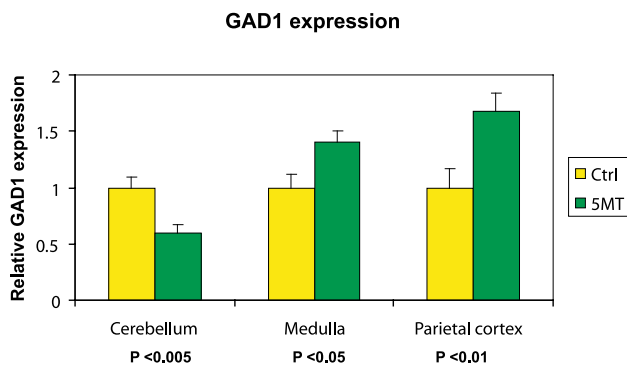
#### Statistical analysis

The unpaired t test (two-tailed) was used to assess the differences of relative expression of RELN and GAD1 between 5MT and the control group. The same test was used to assess the differences between 5MT and control group in immobility scores in FST and in the scoring of time spent in the platform quadrant of the Morris water maze.

#### Results

5MT-treated rats reached a lower immobility score in the Forced Swimming test compared to the control rats ( $t_{20} = 2.98$ ,  $p < 0.01$ ) suggesting lower level of depression in 5MT-treated rats (Fig. 1). The Morris water maze test for assessing the spatial memory did not show any significant differences between controls and 5MT-treated rats, probably due to relatively high interindividual variability (Fig. 1).

RELN expression was significantly decreased in the cerebellum ( $t_{20} = 3.35$ ,  $p < 0.005$ ) and in the prefrontal cortex ( $t_{18} = 4.21$ ,



**Fig. 3. GAD1 expression.** We found a significantly decreased GAD1 expression in the cerebellum of 5MT-treated rats ( $p < 0.005$ ). We found a significant increase in GAD1 expression in the medulla ( $p < 0.05$ ) and parietal cortex ( $p < 0.01$ ) of 5MT-treated rats.

$p = 0.0005$ ) in 5MT-treated rats (Fig. 2). A slight non-significant decrease in RELN expression was shown in the hippocampus, medulla and frontal cortex in 5MT-treated rats. These results suggest a possible inhibitory effect of 5MT treatment on RELN expression in rat brain.

We found a significant decrease of GAD1 in the cerebellum ( $t_{20} = 3.38$ ,  $p < 0.005$ ) in 5MT-treated rats. GAD1 expression was increased in the medulla ( $t_{20} = 2.63$ ,  $p < 0.05$ ) and in the parietal cortex ( $t_{20} = 2.91$ ,  $p < 0.01$ ) in 5MT-treated rats (Fig. 3).

## Discussion

In the present study, we have shown that 5MT administration caused differences in expression patterns of autism candidate genes, RELN and GAD1 in the cerebellum, prefrontal cortex and other brain regions.

The pups whose mothers were treated with 5MT during pregnancy showed brain metabolic and behavioral patterns that resembled the phenotype of autism in humans (19). Reelin expression was decreased in brain lysates of these pups (11). Decreased reelin expression was found also in post mortem autistic frontal and cerebellar regions of cortex, suggesting the importance of reelin protein in autism (20). The influence of elevated serotonin on RELN expression was supposed to be mediated via synaptic contacts between serotonergic neurons and Cajal Retzius cells expressing the reelin during the prenatal life. We aimed to clarify the effect of elevated serotonin on reelin expression in adulthood, when reelin is mainly produced by GABAergic neurons (6). Several studies described the functional as well as physical connections between serotonergic and GABAergic neurons in the midbrain, basal ganglia, auditory cortex and hippocampus (12, 13, 14, 15). We report that daily injections of 5-methoxytryptamine caused a marked decrease in reelin expression in the cerebellum and prefrontal cortex of female rats. We suggest that physical connections between GABAergic and serotonergic neuronal cells mediate this effect. A female rat model was used because the naturally produced estradiol positively modulates the

serotonergic system (21, 22). GABAergic and serotonergic connections were reported to be abnormal in mutant reeler mice lacking the RELN gene (23). These results demonstrate a functional linkage among GABA, serotonergic systems and the reelin pathway, which are all abnormal in autism. In addition, the dysfunctions of reelin, GABAergic and serotonergic systems were observed in post mortem studies of severe psychiatric illnesses (24).

Because of reported involvement of reelin and serotonin systems in the processes of learning and memory (25, 9, 10), we used the Morris water maze to test the spatial memory in 5MT and control rats. 5MT rats performed slightly better, but with high interindividual variability in the measured parameter. This variability might be caused by hormonal differences during the estrous cycle in females. Stronger phenotypic consequences of decreased reelin expression were found during the neurodevelopmental period including abnormal cortical organizations observed in pups of 5MT-treated mothers (11).

Since serotonin plays a role in the pathogenesis of depressive and anxious behaviors, we used the forced swimming test to analyze differences in depressive behavior between 5MT and control rats. 5MT-treated rats scored lower, indicating the generally accepted antidepressant effect of enhanced serotonergic stimulation.

Studies on post mortem autistic brains showed an impairment of the cerebellar structure with marked reduction in Purkinje cell number and glutamate decarboxylase expression (26, 27). A decreased number of Purkinje cells with decreased GABA synthesis might impair the signaling from the cerebellar nuclei towards higher association areas in the cerebral cortex, resulting in cognitive and/or motor abnormalities (26). Decreased GAD1 expression in the brains of autistic, schizophrenic and bipolar patients was observed by various authors (27, 28, 26). We have found decreased GAD1 expression in the cerebellum of 5MT-treated rats in comparison to controls. Increased serotonergic stimulation might cause the decrease of GAD1 expression and GABA levels possibly via contacts among serotonergic and GABAergic fibers. Nevertheless, there are at least three studies showing that serotonin had an opposite effect on GAD1 activity or expression, both *in vivo* (hypothalamus) (29, 30) and *in vitro* (cultured spinal dorsal horn neurons) (31). In the present study, the increased GAD1 expression in the medulla and parietal cortex of 5MT-treated rats has been proved. It seems that abnormal serotonergic stimulation has a region-specific effect on GAD1 expression, while however the phenotypic consequences are unknown. It is also possible that since the forced swimming test represents a strong stressor, the changes in reelin and GAD1 expression have emerged due to serotonergic influence on stress responsiveness.

During the past decade, higher attention has been devoted to the cerebellum in relation to cognition. Patients with cerebellar lesions have strong visuospatial, language, and memory impairments (32). Furthermore, since the cerebellum has a major role in spatial navigation (33), spatial cognitive deficits in autism are supposed to be the consequence of cerebellar abnormalities. In the present study, GABAergic deficits in cerebellum caused by

decreased GAD1 expression did not show any correlation with swimming patterns in both behavioral tests (data not shown).

In conclusion, we have shown that the administration of serotonin receptor agonist resulted in a decreased RELN and GAD1 expression in the cerebellum of adult female rats. This effect was possibly enabled by physical connections between serotonergic and GABAergic neurons. We propose that this phenomenon might be relevant in the pathogenesis of autism. A number of studies reported an increase in blood serotonin levels in autistic patients (34, 35, 36, 37, 38). Based on previously described hyperserotonemic rat model of autism (19) we suggest that peripherally elevated serotonin levels in adulthood could be also relevant. However, the developmental effects of serotonin on CNS remains still the strongest agent in the pathogenesis of some neuropsychiatric disorders. Further studies are needed to explain the effect of elevated serotonin on the gene expression profile in both male and female brains.

We have shown that administration of serotonin receptor agonist caused differences in the expression patterns of autism candidate genes, RELN and GAD1 in the cerebellum, prefrontal cortex and other brain regions in adult female rats. These results underline the relevance of the peripheral serotonergic system in the pathogenesis of neuropsychiatric illnesses.

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Received October 27, 2010.  
Accepted November 27, 2010.