Journal of Neurodevelopmental Cognition

Journal of Neurodevelopmental Cognition 4 (2021) 9-15
ISSN: 2645-565X
http://www.jncog.sbu.ac.ir

Cerebellar Plasticity Changes in an Experimental Model of Autism Induced by Maternal Separation

Monireh Mansouri [a*], Faeze Mashayekhi [a], Maryam Ardalan [b]

[a] *Department of Cognitive Psychology, Institute for Cognitive and Brain Sciences, Shahid Beheshti University, Tehran, Iran.*
[b] *Centre for Perinatal Medicine and Health, Institute of Neuroscience and Physiology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden.*

Abstract

Autism is a neurodevelopmental disorder with a complex pathophysiology that affects different areas of the brain. Evidence over the past decade indicated a role for the cerebellum in social skills in connection with neuropathological changes. In this study, we examined cerebellar plasticity in rats that exhibited autistic-like behaviors after maternal separation (MS). We showed that autistic like behaviors were associated with changes in cerebellar volume size. We for the fiest time showed the decreased volume of the cerebellum in the maternal separation animal model of autism, supporting the involvment of the cerebellum in the pathophysioly of the autism.

Keywords: Autism spectrum disorders, Cerebellum, Maternal separation.

1. Introduction

Autism spectrum disorders (ASD) is one of the most common neurodevelopmental disorders (1 in 59 children) with a complex pathophysiology, which is characterized with the main symptoms of social behavioral deficits and stereotyped behaviors [1]. So far, no specific treatment has been developed for improvement of autistic symptoms [2]. Although the role of many genes in autism has been shown [3], recent studies show that important environmental factors such as nutrition, the use of certain drugs such

* Corresponding author
Email addresses: monirehmansouri68@gmail.com
Received: March 2021 *Revised:* June 2021

as sodium valproate and most importantly psychological factors in the first years of life, such as poor communication of parents or caregivers with the child and generally not having an environment rich in sufficient sensory and psychological stimuli can play a role in the development and exacerbation of autism-related behaviors [4]. To understand autism, various animal models have been developed based on genetic manipulation and environmental interventions [5, 6]. Meanwhile, maternal separation has been used as a model to mimic inadequate parental care and to investigate its effect on the development of autistic-like behaviors in animals in several studies and its effects on inducing social behavioral defects and stereotyped behaviors have been shown [7, 8].

We have also shown in previous studies that maternal separation in the first 14 days of life (3 hours daily) in rats leads to autistic-like behaviors in adolescence [4, 9]. Our study [10] also showed that maternal separation causes a change in the plasticity of the brain areas including the hippocampus and the infra-limbic region of the prefrontal cortex. The observed behavioral and structural changes were significantly reduced by oxytocin treatment. However, due to the complex pathophysiology of autism, further studies are needed to explore more mechanistical details underlying the effect of maternal separation on autistic like behaviors.

Due to the causal and phenotypic complexity of the ASD, the study of the other areas of the brain to clearly find changes under existing conditions is of importance. The cerebellum is one of the brain regions highly regarded in autism today [11]. Although it is traditionally involved in motor function, there is ample evidence that is also involved in cognitive functions [12]. Some studies show that the cerebellum is structurally and functionally abnormal in patients with autism [12]. Syndromes that share cognitive symptoms with autism also have genetic mutations associated with abnormal cerebellar growth [13].

Accordingly, the aim of this study was to investigate cerebellar changes in the maternal separation model of autism and the effect of oxytocin treatment on it. In the continuation of the previous study [10] in which autistic like behaviors were induced by maternal separation and the improving effect of oxytocin on the behavioral and structural impairments was reported, the study of the cerebellar structure was performed cereellual samples to investigate the possible effect of maternal separation as well as treatment with oxytocin on this area.

2. Material and methods

As mentioned above this study was performed on the brain samples from the animals which were investigated in previous study. The details of the methods has been described previously [10]. Briefly, the study included four groups: 1. Control-Saline group, 2. Control-Oxytocin group, 3. Maternal Separation-Saline group, and 4. Maternal Separation-Oxytocin group. Pups in the groups 3 and 4 were separated from the mother daily for the first two weeks (PND (post natal day) 1-PND14) of life for 3 hours (9-12 am) and kept in small cages individually, and then were returned to the home cage. After 14 days, the pups were kept with the mother normally until PND21. Control groups were reared normally with the mother until weaning day (PND21). In the oxytocin groups (groups 2 and 4), oxytocin (O3251 Sigma) was injected intraperitoneally at a dose of 1 mg/kg at intervals of 48 hours one day after weaning, ie from PND22 to PND30 (totally 5 times) and in groups 1 and 3 saline was injected with exactly the same program as oxytocin.

In adolescence (PND42-PND50) autism-related behavioral tests including social behavior and repetitive behaviors were done for all groups. On the same day, after the behavioral tests, the animals' brains were fixed with 4% paraformaldehyde using transcardial perfusion and stored in the same fixative solution until the structural studies.

2.1 Tissue processing

To study the structure of the cerebellum, 50-µm-thick sections were cut sagittal from one of the hemispheres which was randomly selected with a systematic sampling fraction of 1/8 using cryostat cutting devise (SCILab, Cool-Cut, SCI85683, England) after storing in a 30% sucrose solution for 72 h.

Brain sections were then Nissl stained with a 0.1% crysel violet solution for quantifying the cerebellum volume and its sub-regions.

2.2 Measuring the Volume of the Cerebellum

The size of cerebellum and its sub-regions including molecular layer (ML) and granular layer (GL) were examined by three-dimensional stereological method using NewCast software and light microscope (Olympus, BX56) applying the Cavalieri estimator method with point counting. Delineation of the area of interest was done on images which were captured using objective lens 4 × and the counting was performed with objective lens 10 ×. Finally, the following formula was used to calculate the volume:

$$V = \sum P.\left(\frac{a}{p}\right).T.\frac{1}{SSF}$$

where the $\sum P$ is the total number of the points counted on the delineated regions per animal, (a/p) is the area per test point, T is the section thickness (50 µm), SSF is the section sampling fraction (1/8).

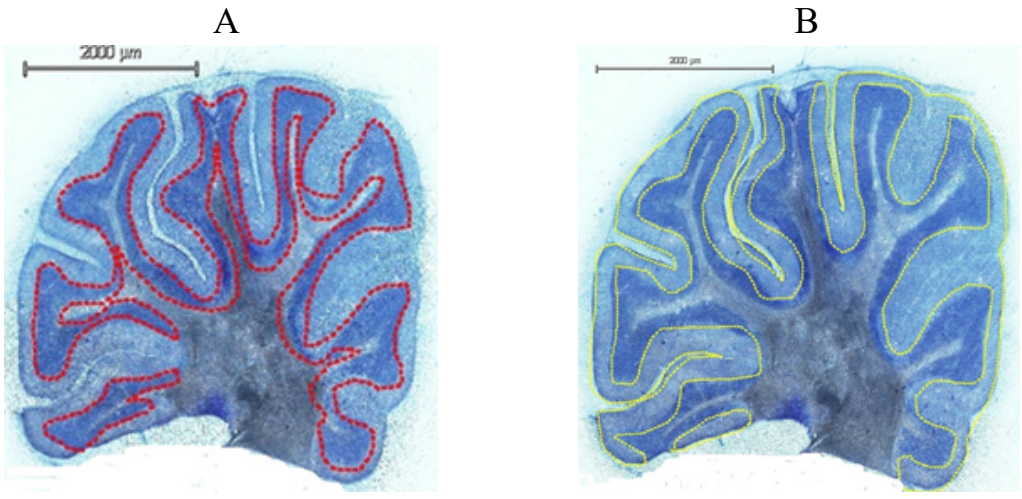

Figure 1. Delineation of the granular (A) and molecular (B) layers of the cerebellum on the images captured with objective lens 4 ×. Scale bar= 2000µm.

2.3 Statistical analysis

All statistical analyzes were performed by SPSS software version 22. For testing the normality of data distribution, Q-Q plot was generated and the homogeneity of the data was checked by Leven's test. Two-way ANOVA analysis and Tukey post hoc test were used to analysis all data. Data are presented as mean ± SEM. The null hypothesis was rejected with $p < 0.05$ as the level of significance.

3. Results

As shown in Fig 2A, there was a significant difference in the volume of the cerebellum between MS and both control groups ($p < 0.001$). There was no significant difference between MS and MS.Oxytocin groups ($p > 0.05$). While we obsereved a significant difference between MS.Oxytocin and both control groups ($p < 0.001$).This means that MS has led to a decrease in the volume of the cerebellum which was not ameliorated following oxytocin treatment.

Analyzing the cerebellum sub-regions demonstrated that neither MS nor oxytocin did not significantly change the size of the molecular layer ($p > 0.05$) (Data not shown). But the volume of the granular layer in the MS group significantly decreased in comparison with control groups ($p < 0.001$). There was no significant difference in the volumes between the MS and MS-oxytocin groups ($p > 0.05$) which showed that oxytocin had no improvement effect on the size of this area (Fig 2B).

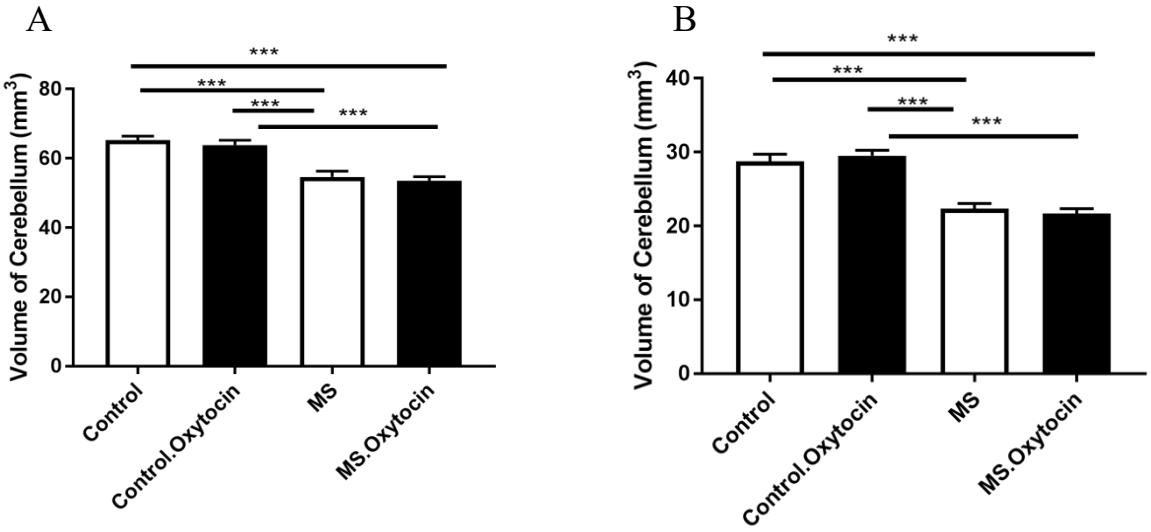

*Figure 2. Effects of MS and oxytocin treatment on the volume of the cerebellum (A) and its granular layer (B). *** p < 0.001. MS: Maternal separation.*

4. Discussion

This study demonstrated that the occurrence of autistic-like behaviors due to maternal separation in rats was associated with changes in cerebellar structure, ie, decreased cerebellar volume in the granular cell layer. Oxytocin injection did not have an improvement effect on the cerebellum structure.

The findings of this study are consistent with the results of previous studies that have shown changes in cerebellar plasticity in social behavior impairments and repetitive behaviors in both human [14, 15] studies and other animal models of autism. For example, in the sodium valproate model of ASD reduced number of the cerebellar Purkinje cells as well as the reduced volume of this area have been shown [16]. A genetic model of ASD also showed a decrease in cerebellar Purkinje cell excitability in mice, which was associated with impaired social and repetitive behaviors [17]. In a human study, MRI imaging showed that the volumes of the hippocampus and parts of the cerebral cortex of the brain in autistic children were larger than in normal children, while the volume of the cerebellum in the same children was smaller than in normal individuals [15]. This is in line with the results of our study, which showed an increase in the volume of the hippocampus and infra-limbic cortex and a decrease in cerebellar volume with the separation of the mother of mice along with the occurrence of autistic-like behaviors. Cerebellum which previously was considered to be involved mainly in movement, today is becoming as an important brain structure that plays a role in the social brain network. The cerebellum has a unique structure, each part of which is dedicated to a specific function due to its special connections with other areas of the brain. For example, lobules VI and VII are involved in cognitive functions [18]. Therefore, any disturbance in the structure and communication of the cerebellum with the cortex will lead to cognitive changes [19]. Interestingly, the cerebellum is also associated with language-related areas and is involved in the proper functioning of language [20]. Therefore, its dysfunction seems likely in disorders such as ASD that are associated with language impairments [21]. In addition to cellular changes, molecular changes have also been confirmed in the cerebellum of autistic patients. For example, decreased levels of the GABA-producing enzyme (glutamic acid decarboxylase 67 (GAD67)) has been shown in the cerebellum of autistic patients, which is an inhibitory neurotransmitter [12]. This type of change is consistent with the disturbance of inhibitory excitatory balance, which is one of the important hypotheses of autism pathology [22].

In the granular cell layer of the cerebellum, excitatory and inhibitory synapses are received from the mossy fibers and Golgi cells respectively. Finally, the synaptic interaction between these neurons forms the cycles of the granular layer [23]. The decrease in the volume of the granular layer may be due to a decrease in the number of neurons or a decrease in the volume of neurons or other cells, which ultimately can lead to disruption of these excitatory and inhibitory neuronal interactions, thereby disrupting the formation of normal cerebellar cycles. Accordingly, we suggest further studies in this regard to find more details which can help in understanding the role of cerebellum in the autism spectrum disorders and the possible therapeutic targets.

5. Conclusion

This study demonstrates the effect of the maternal separation on the structural changes of the cerebellum which is associated with autistic like behaviors in male rats, supporting the role of the cerebellum in the pathophysiology of ASD.

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