CEREBELLUM AS A CENTRAL CULPRIT IN AUTISM SPECTRUM DISORDERS

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ABSTRACT
Autism spectrum disorder (ASD) represents an early-onset neurodevelopmental disorder characterized by decreased social and communication skills, impaired motor function and repetitive and stereotyped movements and behavior. Cerebellum has been long recognized for its fundamental role in the coordination of movements and motor learning and recent research supports also its involvement in emotion and cognition. Studies regarding ASD have reported a cerebellar implication as well, since the main symptoms of this pathology are often associated with a cerebellar structural or functional disruption. ASD individuals can present alterations of the higher-order cognitive, affective and motor skills, domains in which cerebellum strongly participates. In this work, we briefly discuss the cerebellar anatomical, physiological and network anomalies associated with ASD behavioral manifestations, alterations that were discovered using genetic, neuroimaging, electrophysiological and post-mortem histological studies, as well as animal models of the disease. Our research tries to identify possible mechanisms involved in the development of this pathology and offers a better understanding of the cerebellar contribution to ASD.

Keywords: cerebellum, Purkinje cells, autism, neurodevelopment.

INTRODUCTION

The cerebellum represents a crucial part of the rhombencephalon, being highly involved in the coordination of movements, posture, equilibrium and muscle tone. However, in the last centuries, research studies have offered important evidence of its participation not only in motor functions, but also in cognitive and emotional ones, such as memory, language, sensorial perception, time perception, action planning, attentional control and emotion [1, 2].

The high order function of the cerebellum has been proven through anatomical, electrophysiological and functional neuroimaging studies that showed several neural circuits connecting multiple brain regions, including the brainstem, midbrain, thalamus and premotor areas [1, 2], but, most interestingly, the limbic system with the cerebellum. The projections of the deep cerebellar nuclei ascend the superior cerebellar peduncle and reach the medial mammillary nucleus, the hypothalamus, the accumbens, the hippocampus, the dentate gyri and the septal nuclei, suggesting an important modulatory role of the cerebellum in affective disorders. Moreover, cerebellar damage has been associated with various behavior changes, such as executive functioning impairments (deficient process of making plans, abstract reasoning, working memory and reduced verbal fluency), spatial cognition deficiencies, important personality changes, such as blunting of affect or disinhibition and improper behavior, and dysprosodia and agrammatisms.

Researchers considered these clinical aspects as forming the "cerebellar cognitive

affective syndrome" [3]. Moreover, there has been previously described a pediatric case, where the agenesis of the cerebellum caused no motor abnormalities, but concerned only cognitive functions such as memory and language [3]. Also, decades ago it was discovered that stimulation of the vermis could ameliorate aggressive behavior. Further research showed that modulation of the vermis and the fastigial nuclei, the oldest cerebellar nuclei, situated the closest to the midline, in the antero-superior cerebellum, altered the activity of the limbic circuit [4]. Functional magnetic resonance imaging and positron emission tomography revealed that the activation of the vermis was associated with anxiety and unpleasant emotions in humans, while the damage of the vermis in rat pups impaired the social and emotional behavior in their adulthood [4].

The autism spectrum disorders (ASD) are characterized by both inadequate social behaviors and motor deficits, including repetitive movements, therefore a new hypothesis has emerged: cerebellum might be playing a central role in the occurrence of this sort of diseases. The cerebellum presents a high vulnerability to toxic in utero environments, neonatal brain injury and several other genetic and epigenetic factors, given its prolonged period of development: it is one of the first neural structures to begin the cellular differentiation and among the last ones to mature [4, 5]. This particular growth indicates that even small changes in the early development of the nervous system can form aberrant neural morphologies and abnormal circuits that are able to cause complex developmental brain disorders with negative effects on behavior [4]. However, further research must be done in order to discover the manner in which certain areas in the cerebellum contribute to behavior and to bring to light the existence of certain areas that play a role in both motor and non-motor behaviors [4, 5]. The development of ASD symptoms has been associated most frequently with a decrease in the Purkinje cell population, but the amount of cell loss that is able to trigger this pathology is not currently known [4, 5]. The Purkinje cells are neurons located in the cerebellar cortex, which receive excitatory input from the climbing fibers (directly) and granule cells (indirectly). They connect also with the Golgi, stellate and basket cells, which control the flow of information through the cerebellar cortex. The role of the Purkinje cells is highly important, as they realize the inhibition of the deep cerebellar nuclei by releasing GABA at their level and reducing the transmission of the nerve impulses, therefore reducing the cerebellar output. Purkinje neurons are thus able to regulate and coordinate the motor movements. Moreover, the large glutamatergic deep cerebellar nuclei axons project contralaterally towards the midbrain and the limbic structures and represent the only way in which behavior can be influenced by the cerebellum [4], therefore any reduction in the Purkinje neurons activity can allow a robust activation of the cerebellar output, able to exert a big excitatory effect on all the limbic system related structures and consecutively on the prefrontal cortex, which regulates impulses and behavior [5]. The feedback loop that the cerebellar cortex creates with the deep cerebellar nuclei, the thalamus and the cerebral cortex is strategically organized: the ventral dentate passes the information from the posterior cerebellum to neocortical association areas, such as prefrontal cortex, the posterior part of the parietal cortex, while the interpositus nuclei (globose and emboliform) transmit the motor information from the anterior cerebellum to the sensorimotor cortices. Furthermore, the fastigial nucleus, together with the vermis, seems to be highly associated with vital structures (brainstem and thalamus) that coordinate the vestibular and oculomotor control, posture and equilibrium and with paralimbic and autonomic

cerebral areas, proving once again the appurtenance of these structures to the limbic system [4].

Interestingly, the substantial growth of the cerebellum happens in the third semester of pregnancy and continues in the early postnatal period (up to 12-18 months postnatal), stages associated with increased occurrence of aversive environmental factors and brain injuries known to favor ASD development. More, it was demonstrated that the cerebellar injury at birth can augment the risk of developing ASD up to 36 times, when compared to the general population [4]. Post-mortem studies indicated that the Purkinje cell loss occurs before receiving afferents from the climbing fibers (originating in the inferior olive), in the early perinatal period. Other researchers support also this affirmation: it was previously demonstrated that the number of stellate and basket cells is preserved in autistic brains, suggesting that the Purkinje cells might have well migrated and properly settled, after their synthesis [5]. Moreover, the inferior olivary neurons remain unaffected, therefore no degeneration took place consecutive to a possible death of Purkinje cells connected to them [5]. However, not all ASD patients present a decrease in the total number of Purkinje cells, suggesting that in some cases the disorder might be due to the deficient maturation of the functional connections and subsequent abnormalities in the wiring of the cerebellum, occurred therefore after the differentiation (which starts at 24 weeks of gestation), proliferation and migration of Purkinje cells [5]. Other studies revealed that the Purkinje cell density in lobules IV, V, VI (sensorimotor processing areas) and crus I and II (which constitute lobule VIIa of the posterior cerebellar lobe and modulate social and behavioral planning) was decreased in post-mortem autistic human brains, when compared to control, especially in crus I and II regions. In addition, the density was decreased also in lobule X (in the flocculonodular lobe, known to be associated with vestibular regulation and eye movement) in autistic males and this reduction was correlated with the ADI-R score of the social eye contact of the patient, suggesting its contribution to particular clinical features of ASD. In addition, males presented a lower overall Purkinje cell density, when compared to females [5]. A study regarding lobule VIIa reported that this structure plays an important role in other human non-motor functions also: crus I region lesion determined attention impairments and crus II region determined visuospatial and verbal memory impairments in 100% of the studied cases [5]. Lobule VII has been demonstrated to cooperate with the prefrontal cortex through bidirectional connection. Moreover, researchers propose that even the volume and growth of the lobule VII throughout human evolution is proportional with the frontal cortical expansion, evidence that supports the existence of a frontoparietal attention network [5, 6].

Many ASD patients can present a small volume of the vermis or hypoplasia of the vermal lobules VI and VII and low Purkinje cell counts in the vermis [4,5,6]. Also, decreased gray matter density was found in the cerebellar hemispheres and the posterior vermis of ASD patients [5]. Recent studies indicated also that the posterior part of the cerebellum had a robust connection with the prelimbic, orbitofrontal and anterior cingulate cortex and the perturbation of the cerebello-prefrontal circuit increases the risk of autism [5]. More specifically, in mice, lobule VI and crus I region were found to form ascending disynaptic pathways in several areas. Injection of anterograde transsynaptic viruses in the lobule VI induced the expression of fluorescent tracers in the anterior cingulate, prelimbic and infralimbic cortices and orbitofrontal cortex, confirming the similar distribution found in humans [6]. However, lobule VI projects preferentially towards the prelimbic

and orbitofrontal, areas known to bring a big contribution to the reward expectation and value-based decision making, being therefore able to influence the reversal learning. On the other side, the injection of fluorescent viruses in the crus I region induced their expression also in the anterior cingulate, infralimbic and prelimbic cortices, but the distribution of the fibers was found to be robust in the anterior cingulate cortex, a region highly contributing to flexible and affective cognition [7]. Moreover, the crus I seems to send powerful projections also to the somatosensory cortex and were demonstrated to respond to orofacial stimuli, therefore the received sensory information might have provided or triggered the development of social preference in the early life. The existence of these circuits suggest that cerebellum could influence the neural activity in distant neocortical regions, therefore influence behavior. An investigation of the social behavior in mice, done using a three-chamber apparatus in which the experimental subject was placed in the middle, between chambers with either a mouse or an object inside, revealed that the disruption of crus I and II regions during the juvenile life can determine profound indifference towards the other mouse. The control groups presented a mouse-over-object preference and the perturbation of any of the cerebellar lobules in adult mice did not affect the social preferences, suggesting that social behavior in adult mice do not depend on the local activity in lobule VI, VII, crus I and crus II and the only capable of changing the social preference is the developmental disruption. Moreover, the results suggested that cerebellum could exert a processing role that can in time lead the long-term maturation of novelty-seeking and contribute to a malleable cognition [8].

Autistic spectrum disorder patients may present repetitive and stereotyped movements and behaviors, as well as motor impairments. Researchers propose that these abnormalities have occurred consecutive to a distorted learning of motor control during development. Experiments involving elementary motor learning tasks in which the movements were intentionally perturbed in order to cause deviant manifestations were performed while the information was processed simultaneously by proprioceptive and visual inputs, in normal and ASD children. Results showed that all children were able to learn from the error and changed their motor commands on the next try to fulfill the task, but ASD individuals performed better than the normal children at learning from perturbed proprioceptive information and worse at visual error processing. Moreover, given the fact that the volume of the sensorimotor cerebellum was found to be smaller than normal in ASD children, the results of the performed trial (which is highly sustained by the cerebellar function) indicated that the impaired acquisition of the motor coordination in ASD can happen due to an abnormal sensorimotor signaling. A research article studying the associative sensory learning in mice using genetic autism models showed that defects in associative temporal binding of sensory events are common in all the used models [9]. Several autism susceptibility genes that co-express in the cerebellum during post-natal period seem to be responsible for the associative learning, therefore mutations affecting the cerebellum were chosen for experiments, as well as mutations associated with Rett syndrome or cortical dysplasia-focal epilepsy syndrome, all of which displayed reminiscent phenotypes of human autism, such as abnormal social and repetitive behaviors. Since autistic patients present difficulties at matching distinct senses and integrating information from both hearing and vision on subsequent time scales, tests that rely on making fast associations between a light and a puff of air in the eye were performed in mice. The results showed that mice exhibited

an impaired eye blink reaction (e.g. latency to the onset) after the light administration, suggesting a disrupted multisensory learning in autism caused by cerebellar genetic mutations that affect the local plasticity [9].

The pathological development of the neural system of ASD individuals may happen due to an abnormal maturation of GABAergic cerebellar Purkinje cell transmission. During the prenatal period and in very early stages of development, the γ-aminobutyric acid generates the depolarization of the membrane when activating the GABA A receptors, therefore both glutamate and GABA are able to induce excitation in immature neurons, facilitating the proliferation, migration, maturation and differentiation of neurons and stimulating the synthesis of new connections between neurons. After birth, the adequate timing of GABA transition from depolarizing to hyperpolarizing seems to be vital for finding the balance between excitation and inhibition and offering a proper development [10]. The functional switch of GABA takes place in two phases, one during the delivery and the second one soon after the birth. The immature neurons present a high level of NKCC1 (Cl^- importer) which determines the leakage of Cl^- when activated by GABA, resulting in the depolarization of the cell. The mature neurons present high levels of KCC2 (Cl^- exporter), which decrease the intracellular concentration of Cl^-, triggering therefore the Cl^- influx and hyperpolarizing the cell [10]. However, the complete mechanism is not yet fully understood [8]. Studies sustain that the GABA shift is abolished by maternal immune activation and that any delay in the shift from depolarizing-excitatory to hyperpolarizing-inhibitory may lead to the development of autism spectrum disorders [11]. The normal developmental shifts have been proven to take place in the subcortical and cortical structures, including the cerebellum and cerebellar Purkinje cells, which showed post-mortem abnormalities in ASD individuals and in rodent valproate model of autism [12], in which mice cannot regulate their chloride gradient, resulting in distorted hippocampal excitability. The Purkinje cells are the most affected in the cerebellum, as they continue to develop after birth and influence the regression of the climbing fiber input and the dendritic expansion. The elimination of the climbing fiber-Purkinje cell synapses had a similar progress in males and females, indicating that the GABAergic signaling is not sex-dependent at this level and cannot interrupt this fundamental developmental process. The normal wild-type and the valproate-model of mice in both male and female demonstrated that, in Purkinje cells, the GABA A receptor channel shifts from high-conductance (depolarizing, in the newborn) to low-conductance (hyperpolarizing, in juvenile and adult mice), but the shift is delayed after prenatal exposure to valproate in both sexes and also delayed in females, when compared to males. The results suggested a sex-dependent developmental timing, which might be even more affected in ASD and other neurodevelopmental disorders. Moreover, these results supported the cerebellar dysfunction as an important factor in the ASD occurrence. There are multiple theories regarding the changes in conductance that occur during neonatal period and the factors that influence differently the brain of males and females, but still further research must be done to elucidate the full mechanisms. First, the estradiol is known to promote the dendritic growth, spinogenesis and synaptogenesis during neonatal life [13]. Moreover, the expression of the insulin receptors and insulin-like growth factor-1 (IGF-1) receptors in the cerebellum increases between day 0 and day 7 and decreases (is down-regulated) in day 14 in male mice, while the same receptors in females suffer no difference during day 0 to day 7 and increase in day 14 [14]. This finding might represent

one of the reasons why a difference in chloride homeostasis is noticeable between males and females, since IGF-1 is known to accelerate the developmental switch between NKCC1 and KCC2 transporters of chloride [13]. In conclusion, the chloride equilibrium, the gradient modifications and the GABA switch in the Purkinje neurons can influence the development of the brain by altering the properties of the Purkinje cell, changes associated with the ASD.

Neuroimaging studies indicated numerous abnormalities in the cerebello-cortical operating circuits of ASD individuals. Functional MRI demonstrated decreased activity in the cerebrocerebellar motor networks during demanding tasks, such as finger sequence tapping [12], reduced connectivity in crus I and lobule VI at rest [13] and low activation of crus I region during facial and vocal stimuli processing [14] and executive functioning [15], when comparing to normal. Moreover, a significantly reduced cerebrocerebellar connectivity was found during verbalization in ASD patients [16]. However, cerebrocerebellar overconnectivity was observed during resting-state fMRI [17] and a high activation of the cerebello-thalamic circuitry was noticed during a visual task involving a saccadic eye movement [18].

Together, all these findings indicate that the early disruption of the cerebellum can trigger important changes in the cerebello-cortical circuitry, causing definitive sensorimotor and cognitive alterations and substantial developmental impairments. Cerebellar dysfunction has been regularly associated with the ASD symptomatology, therefore cerebellar neuromodulation can be regarded as a potential therapeutic target in the ASD.

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