GABAergic neurotransmission alterations in autism spectrum disorders

Carla V Sesarini

Instituto de Ciencias Básicas y Medicina Experimental (ICBME), Instituto Universitario del Hospital Italiano de Buenos Aires (HIBA), Potosi 4240 (C1199ACL), CABA, Argentina

Correspondence: Carla V Sesarini
E-mail: carla.sesarini@hospitalitaliano.org.ar
Received: October 04, 2015
Published online: November 09, 2015

Autism spectrum disorders (ASDs) are a group of complex disorders of neurodevelopment characterized by difficulties in social interaction, verbal and nonverbal communication, and repetitive behaviors. In ASD, deficits in social cognition and related cognitive functions would be the resultant of reduced synchronization between brain regions. A possible explanation for ASDs is the disturbance of the delicate balance between excitation and inhibition in the developing brain which may have profound impact in neurobehavioral phenotypes. At least some forms of autism would be caused by a disproportionately high level of excitation (or weaker inhibition) in neural circuits that mediate language and social behavior (local circuits). A more excitable cortex (more weakly inhibited) is functionally more poorly differentiated and could lead to broad ranging abnormalities in perception, memory and cognition, motor control, and seizures. An imbalance between excitation and inhibition could be due to an increase in glutamatergic signaling (excitatory) or reduced inhibitory GABAergic signaling. γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain and some forms of ASDs are associated with alterations of GABAergic signaling. Proper inhibitory GABAergic signaling is essential for normal neural circuit function. If the inhibition is disturbed, excessive excitation results in disturbances in the excitation/inhibition balance and, consequently, dysfunction of cognitive processes. Defects in GABA_A receptor subunits has been associated with neuropsychiatric disorders with variable clinical presentations, supporting the hypothesis that genetic variants in GABA_A receptor subunits may be risk alleles for ASD and epilepsy. Also, expression data and protein levels show a dysregulation in multiple GABA_A and GABA_B receptors in key sites of the brain in subjects with autism which could explain several clinical phenotypes including the presence of seizures, learning disabilities and mental retardation. In this review, we examine the evidence that alterations of GABA_A receptors modified the GABAergic signaling and the excitation/inhibition ratio in ASD.

Keywords: Autism spectrum disorders (ASDs); epilepsy; excitation/inhibition (E/I) balance; GABAergic signaling; GABA receptor subunits

To cite this article: Carla V Sesarini. GABAergic neurotransmission alterations in autism spectrum disorders. Neurotransmitter 2015; 2: e1052. doi: 10.14800/nt.1052.

Copyright: © 2015 The Authors. Licensed under a Creative Commons Attribution 4.0 International License which allows users including authors of articles to copy and redistribute the material in any medium or format, in addition to remix, transform, and build upon the material for any purpose, even commercially, as long as the author and original source are properly cited or credited.
Introduction

Autism spectrum disorders (ASDs) are a group of complex disorders of brain development characterized, in varying degrees, by difficulties in social interaction, verbal and nonverbal communication, and repetitive behaviors. Clinical, neuroanatomical, biochemical, neurophysiological and genetic data suggest that ASDs are neurodevelopmental disorders with a clear neurobiological basis. From a biological point of view, ASDs can be described as a genetic dysfunction that disrupts subtle but global brain circuits affecting the more vulnerable higher order association areas that depend heavily on the precise timing of input from other regions [1].

A possible explanation for ASDs is the disturbance of the delicate balance of inhibition and excitation in the developing brain which may have profound impact in neurobehavioral phenotypes [2]. In this review, we examine the evidence that alterations of GABA<sub>A</sub> receptors modified the GABAergic signaling and the excitation/inhibition ratio in ASD.

Autism spectrum disorders (ASDs)

The prevalence of ASD has been reported as 1:68 (almost 5 times more common among boys) and has increased steadily in the last decade [3]. ASD concordance rate for monozygotic and dizygotic twins is 95.2% and 4.3%, respectively, supporting a genetic etiology [4]. Medical comorbidities include gastroesophageal reflux, food selectivity and neurological disorders (e.g., tics and seizures) [5]. Approximately 30% of children with ASD have epilepsy [6] and between 15-30% of children with epilepsy have autism [7]. Between 50-70% of individuals with ASD have patterns of epileptiform activity even in the absence of a clinical seizure disorder [8]. In families with more than one affected child, the rate of epilepsy is 12.8% in probands and 2.3% in siblings without autism, twice the rate of the general population, suggesting a genetic burden for ASD and epilepsy [9].

Genomic evidence

In recent years, progress has been made in understanding the genetic basis of ASD. Although hundreds of genes and variants have been proposed as predisposing ASD, only 6-15% of patients have an identifiable genetic diagnosis. Current studies suggest multifactorial inheritance, including genetic heterogeneity [10].

The list of genes and chromosomal regions that have been associated with ASD is very extensive and exceeds the aim of the present review. Some relevant data is mentioned below: (1) About 10% of patients also have a monogenic disorder such as Fragile X, Tuberous Sclerosis, Rett syndrome, among others [11]. (2) Almost 2% of individuals with ASD have cytogenetic abnormalities including, but not limited to, chromosomal regions 5p15, 15q11-1q13, 17p11, 22q11.2, of which the 15q11-1q13 locus represents about 1% of cases [12]. (3) Cadherin 10 (CDH10) and cadherin 9 (CDH9) in the 5p14.1 region have been associated with autism, implicating neuronal cell-adhesion molecules in its pathogenesis and indicating that ASDs may result from structural and functional disconnection of brain regions involved in higher-order associations [13]. (4) To date, only two linkage studies have been replicated and involved regions located on chromosome 7q35 and 20p13 [14,15]. (5) De novo copy number variations (CNVs) are present in 10% of cases in simplex families, 3% of probands in multiplex, but only 1% in the general population and could explain a significant proportion of risk factors, especially for sporadic ASDs [16]. De novo CNVs form a larger functional network responsible for synaptogenesis, axonal guidance and related molecular processes [17]. (6) De novo point mutations are mainly of paternal origin (4:1) and positively correlated with paternal age [18], while de novo insertions/deletions (indels) are more common in women diagnosed with ASD and mainly of paternal origin [19]. (7) Private single nucleotide variants are more present in patients than in unaffected siblings with a bias of maternal transmission [20].

Genes associated with ASD can be grouped into three broad categories according if they are involved in structure and synaptic activity, protein synthesis or gene expression regulation [21].

ASD and neural connectivity

The neural and cognitive development involves a delicate balance between the processes of specialization and integration. This developmental process fail in ASD so that the units of information processing in the brain would be disconnected from each other; there would be a deficit in global connectivity and increased connectivity in local networks [22]. Excessive local connectivity may develop in tandem with a deficit of long-distance connectivity, perhaps as a consequence of alterations in synapse formation or pruning [23]. During development, connections are established and retract in response to activity, and connectivity and local architecture are the result of neuronal migration and differentiation [24].

Cortical neuronal networks consist, broadly, of two classes of neurons: excitatory projections, mainly using glutamate as a neurotransmitter, and local circuit inhibitory interneurons,
using mainly GABA as a neurotransmitter. Cortical interneurons play a critical role in modulating neuronal excitability, integrating and generating temporal synchronization, and oscillation between networks of glutamatergic neurons. GABAergic interneurons also contribute to almost all fundamental processes of cortical development; from neuronal proliferation, migration and differentiation to experience-dependent refinement of local cortical circuits [23].

In ASD, deficits in social cognition and related cognitive functions would be the resultant of reduced synchronization between brain regions [26]. In the cognitive neurological level, ASD is a disorder of information processing that impacts the complex or higher order processing with simple information processing intact or increased [27].

**Histological evidence**

In *post mortem* brains of ASD patients anatomical defects have been observed, including thick and poorly laminated cerebral cortex [28], an increase in brain growth during early childhood with reduce rate during late childhood, cerebellar growth and increased density in limbic areas in children under 5 years [29]. This rapid early growth interfere with the normal developmental trajectory of cortical connectivity, coinciding with the processes of synaptogenesis, apoptosis and myelination, and is most marked in the frontal lobe, where patterns of synaptic connectivity of the pyramidal cells usually take years to mature. Consequently, long distance cortico-cortical and cortico-cerebellar connections are affected [30]. It has also been described a larger size of the amygdala [31], where abnormalities in microscopic organization including smaller neurons and more densely populated has been observed [28].

An increase density of basal dendritic spines of pyramidal neurons in layer V in temporal lobe has been described suggesting an increased local excitatory connectivity and would prevent normal development of cortico-cortical and cortico-subcortical communications unbalancing the excitation/inhibition (E/I) ratio [32]. Alterations in spines could be the result of improper synaptic pruning during the postnatal period [33]. It has also been observed lower neuronal density (smaller and densely packed) in the lateral nucleus of the thalamus, cerebellum and corpus callosum [34]. Large cerebellar disturbances and anatomical microscopic pathological changes in this structure has been observed, and may be associated with behavioral manifestations such as hyperactivity and impaired social interactions [35].

Greater amount of minicolumns, smaller and higher in density were observed, suggesting increased short distance connections in frontal and temporal regions [36]. Minicolumns are pyramidal cells arranged vertically and are perhaps best characterized as parallel processors, dynamically grouped with neighboring minicolumns in macrocolumns. Minicolumnar pattern parameters generated during early development of the embryonic brain makes it preeminent to other modes of structure and restricts other higher order structures [37].

Structural studies suggest an abnormal developmental path of brain growth, with evidence of poorly organized white matter (increased cortical thickness and atypical patterns of gyrification), possibly involving abnormalities in neuronal migration, cortical organization and myelination [38].

Evidence from images

Through diffusion tensor imaging (DTI) a reduction of volume and alterations in corpus callosum was observed, suggesting altered interhemispheric connections in children and adults with autism [39, 40]. With functional magnetic resonance imaging (fMRI) alterations in cortical connectivity were observed with a pattern suggesting reduced activity in brain areas involved in executive function [41]. During imitation tasks, altered activity of mirror neurons in the inferior frontal gyrus was observed [42] and deficits in frontal-posterior functional connectivity involved in language comprehension and working memory were also informed [43]. In tasks that require integration of spatial processing and language comprehension, decreased functional connectivity between areas of language and frontal-parietal regions were reported [44].

Face perception is a social function widely studied in ASD since faces represent a primary source of social information and its decoding is a precursor to more complex social inferences. The neural specialization for face perception is evident from three months old while children with ASD show reduced attention to faces from the first year and reduced activity in the fusiform face area during free viewing faces [45]. Furthermore, the concerted action of the fusiform gyrus and amygdala is critical for emotional processing of faces, showing an aberrant amygdala-fusiform system with a regional cortical thickening, indicating decreased structural connectivity between both [46].

It was also described that atypical cortico-cortical connectivity in ASD is not limited to development of white matter connections, but also affects the intrinsic architecture (and connectivity) of gray matter. A significant reduction was observed in the intrinsic cortex wiring, both globally and locally, mainly in the fronto-temporal region, predicting the severity of social and repetitive symptoms, and affecting the
way information is processed, privileging local over global information [47].

**ASD and the excitation: inhibition ratio**

A unifying theory that would explain ASD proposes that the disorder reflects an imbalance between E/I in the brain, particularly in circuits that regulate sensory processing, memory, and social and emotional behavior during critical periods of development [48]. The neural circuits are refined by extraordinary levels of plasticity during sensitive periods of early development, decreasing in adulthood. It is during these critical periods that individual neurons acquire multiple functional properties through experience-dependent maturation [49]. At least some forms of autism would be caused by a disproportionately high level of excitation (or disproportionately weaker inhibition) in neural circuits that mediate language and social behavior (local circuits). A more excitable cortex (more weakly inhibited) is functionally more poorly differentiated and could lead to broad ranging abnormalities in perception, memory and cognition, and motor control. Additionally, a noisy cortex (hyperexcitable) is inherently unstable and vulnerable to epilepsy. An imbalance between E/I could be due to an increase in glutamatergic signaling (excitatory) or reduced inhibitory GABAergic signaling [48]. Several genetic and environmental factors may converge, in different combinations, in different individuals, to produce an altered E/I ratio.

*From systems to animal models*

In ASD patients, minicolumnar organization abnormalities in the prefrontal cortex [34] may reflect defects in GABAergic fibers within and between cortical minicolumns owing to reductions in the neuropil, separating adjacent minicolumns, and could alter local connectivity and lateral inhibition [50]. Processes that increase the numerical or functional balance of inhibitory versus excitatory cells would lead to a state of hyperexcitability [48].

Molecular evidence has reinforced findings that some markers of inhibitory signaling in the brain (GABA-mediated) are reduced in individuals with autism [51] and animal models have also stressed the importance of the GABAergic neurotransmission in ASD. In mouse models of syndromic autism, during brain development, critical periods are disrupted and benzodiazepines improve GABAergic transmission and triggers the opening of a critical period of normal length [52]. In the valproic acid (VPA) animal model, GABAergic pre- and post-synaptic function and modulation are severely compromised raising the possibility that a disorder in the GABAergic system during development contribute to the core symptoms of ASD [53].

**ASD and GABAergic dysfunction**

Despite their different etiologies, ASDs share overlapping symptoms, suggesting deficits in common neurodevelopmental paths. One involves GABA-mediated neurotransmission which plays a crucial role in the establishment of synapses and neural wiring in late prenatal and early postnatal days [54].

Between 20-30% of neurons in the central nervous system are GABAergic [55]. The inhibitory GABAergic interneurons shape the responses from pyramidal cells to inputs, prevent out-of-control excitation, refine cortical receptive fields and are involved in the timing and synchronization of the rhythms expressed as cortical oscillations. Accordingly, disruption of the cortical GABAergic inhibitory interneurons functioning has been linked to various neurodevelopmental disorders, including epilepsy, mental retardation, autism and schizophrenia [56].

The development of GABAergic interneurons is a long process that begins in the embryo, progresses through childhood and is completed in adolescence in all mammalian species. During this period, the interaction between genetic, epigenetic and environmental factors can alter the development of GABAergic circuits [25]. GABAergic interneurons are diverse and can be distinguished based on their cytoarchitecture (basket, chandelier, stellate, etc.) or molecular properties (expressing PV+, calretinin+, calbindin+, and others) and these subtypes have different electrophysiological and functional properties [57].

**GABA**

GABA is the major inhibitory neurotransmitter in the adult mammalian brain. It is synthesized from glutamic acid through a decarboxylation reaction catalyzed by GAD. GABA inhibits neuronal firing by activating two different receptor classes, ionotropic (GABA_A) and metabotropic (GABA_B). GABA_A receptors are located pre-, post- or extra-synaptic as functional heterodimers. The GABA_A receptor function is defined in the development by channels and transporters which control chloride homeostasis [58]. GABA plays key roles at different stages of neural development, affecting migration, maturation and synapse formation of pyramidal cells and interneurons. Furthermore, the precise effect of postsynaptic GABA is dependent on the intracellular concentration of chloride and developmentally regulated through the expression of various co-transporters [56].

Early in development, GABA acts as a trophic factor and modulates neural migration and maturation [59]. In later
stages of development, when synapses are formed, GABA and glutamate release generates a primitive form of oscillatory network events known as giant depolarizing potentials (GDPs) characterized by recurrent membrane depolarization leading bursts of action potentials separated by periods of rest. This early synchronized activity is essential for synaptic wiring and refinement of local neuronal circuits according to the Hebb rule: "neurons that fire together wire together" [60]. GABAergic interneurons exert a great control on excitability of networks and synchronize a large number of main cells that give place to coherent oscillations, supporting different behavioral and higher order cognitive functions [61].

**GABA<sub>A</sub> receptors (GABRA)**

GABA receptors (GABRA) consist of a pentameric assembly of different subunits (α1-α6, β1-β3, γ1-γ3, δ, ε, π, θ and ρ1-ρ3) to form ion chloride channels [62]. Most functional GABRA in cell surface and synapses contain two α subunits, two β subunits and one γ or δ subunit [63]. Approximately 60% of all GABRA have α1β2γ2 subunit combination, between 15-20% have the α2β3γ2 combination, between 10-15% have α3β2γ2, approximately 5% have the α4βnγ or α4βnδ combination and less than 5% have α5β2γ2 or α6β2 / 3γ2 [55].

The binding site for benzodiazepines is formed by one α subunit (α1, α2, α3 or α5) and γ subunit (usually γ2, present in 90% of receptors) [55]. Ligands in the benzodiazepine binding site modify efficacy and/or affinity of agonists, for example GABA, and thus regulate their activity. The α1 subunit would be responsible for the sedative effects of positive allosteric modulators of GABRA system, including diazepam. The α2 and α3 subunits mediate the anxiolytic effects and α5 cognitive and memory deficits [64]. GABRA are the main target for clinically used hypnotics such as zolpidem, barbiturates and many general anesthetics, as well as the actions of anesthetics as propofol and etomidate [55].

Many antiepileptic drugs such as benzodiazepines, phenobarbital and gabapentin act on GABRA through a positive allosteric modulation, thereby improving inhibition mediated by GABRA [65] and have a hypnotic, anxiolytic, anticonvulsant and sedative effect. Other anticonvulsant drugs with anti-anxiolytic effects act indirectly increasing GABAergic transmission by promoting synthesis of GABA or inhibiting its reuptake. Conversely, drugs that antagonize GABRA or inhibit GABA synthesis cause the stimulation of the central nervous system, anxiety and, in high doses, seizures [57].

Because functional GABRA in neuronal surface or synapses assembled from different subunits in region and specific manner during development, mutations or defects in a single subunit may damage or disrupt the assembly and function of the receptors, affecting the delicate E/I balance [58].

A decreased expression of GAD67/GAD65 in parietal and cerebellar cortex of ASD cases has suggested that the inhibitory dysfunction may play a role in a subset of patients [66]. The functional impairment of GABRA is a potential common molecular mechanism and of susceptibility underlying the comorbidity of ASD and epilepsy [58]. Low levels of GABRA may reduce the threshold for the development of epilepsy, associated with autism, as they cause a deficit in inhibitory neurotransmission and, consequently, neuronal hyperexcitability, which together with the hypersynchrony, characterize epileptic neurons [67].

**Post mortem brain studies**

A significant reduction in the density of GABA<sub>A</sub> and GABA<sub>B</sub> receptors and benzodiazepine binding sites in the supra and infra-granular layers in the anterior cingulate cortex (involved in processes of socio-emotional behavior and other associative functions through connections to the prefrontal cortex) in brain of individuals with ASD were described [60, 69]. A significant reduction in the number of GABRA and benzodiazepine binding sites on the surface layers of the fusiform gyrus and posterior cingulate cortex and a decrease in the number of benzodiazepine binding sites in deep layers of the fusiform gyrus, were also observed, suggesting that alterations in inhibitory control in the cortex may contribute to alterations in socio-emotional behavior in ASD [70]. Reduced GABRA and benzodiazepine binding sites in hippocampus were also found [71] and altered packaging of GABAergic interneurons in CA1 and CA3, where defects are associated with the generation of seizures was also described [72].

Changes in the expression of receptor subunits: α1 (GABRA1), α2 (GABRA2), α3 (GABRA3), α4 (GABRA4), α5 (GABRA5), β1 (GABRB1), β3 (GABRB3) and GABA<sub>B</sub>R1 (GABBR1) were observed in superior frontal cortex, parietal and cerebellum [73, 74]. A recent study also reported changes in the expression and reduced protein expression for subunits α6 (GABRA6), β2 (GABRB2), δ (GABRD), ε (GABRE), γ2 (GABRG2), θ (GABRT) and ρ2 (GABRR2) in superior frontal cortex, suggesting that systemic changes in the expression of GABRA subunits would explain cognitive impairment in individuals with ASD [75].

**In vivo studies in ASD patients**
Electro-encephalographic studies (EEG) support GABAergic deficits in the thalamocortical network [76] and with single photon emission computed tomography (SPECT) and a selective benzodiazepine-GABA ligand a reduction in GABRA in upper and medial frontal cortex was observed [77]. Through proton magnetic resonance spectroscopy ($^1$H MRS), low levels of GABA in the left hemisphere has been reported [78], while higher levels of GABA in plasma of patients has also been demonstrated [79]. A reduction in GABA and in GABA/glutamate ratio in frontal lobe was also observed, indicating that ASD would imply not only a reduction in GABAergic activity but also a pathological increased in glutamatergic function [80]. Using PET and a selective ligand of GABRA5, a reduction of this subunit in limbic areas of patients has been shown [81].

Together these findings indicate that alterations of GABAergic signaling in selected microcircuits of brain areas play a key role in the pathogenesis of ASD [60].

**Genetic observations in the GABA$_A$ receptor subunits**

Evidence connecting GABRA in the etiology of ASD were first provided by genetic studies revealing submicroscopic abnormalities in the chromosomes locus 15q11-q13 containing GABRB3, GABRA5 and GABRG3 genes encoding GABRA subunits: β3, α5 and γ3, respectively [12]. Locus 15q11-13 duplications have been observed in ASD patients and association studies in idiopathic autistic patients found significant evidence for a susceptibility allele in the GABRB3 gene [82]. In patients with seizures, 15q11-13 duplications are also common, suggesting a deficit of inhibitory signaling [83].

Knockout mice for Gabra5 and Gabrg3 have normal phenotype, while Gabrb3 knockout have severe neurological abnormalities, hypersensitivity to tactile stimuli, hyperactivity, deficits in learning and memory, and defects in social and exploratory behavior [84]. Also, a mutation in Gabrb3 gene was associated with a 3-6 times greater risk of ASD (with epilepsy), especially when inherited maternally [85], while other GABRA subunits variants, including GABRA4 and GABRB1 (encoding α4 and β1 subunits, respectively), have also been associated with ASD [86], among others.

Genetic abnormalities associated with autism and epilepsy also include duplication or deletion of 1p36 (1p36 deletion syndrome), which includes GABRD subunit. These data suggest that dysfunction of GABRD gene during development promote psychomotor and speech delay, epilepsy and autism [87]. Mutations or variants (polymorphisms) in GABRA subunits (α1, β3, γ2 and δ) have also been associated with epilepsy of various phenotypes and prolonged seizures (status epilepticus) resulted in alterations in the expression and membrane localization of several GABRA (α1, α4, γ2, δ) subunits in dentate granule neurons of the hippocampus [88]. However, higher levels of a single subunit, α1, in the dentate gyrus may inhibit the development of spontaneous seizures after status epilepticus, suggesting that alterations in GABRA subunits are an important component of epileptogenesis [89].

The β3 subunit is abundant during neurodevelopment and its deficiency causes both epilepsy and autism [90]. The severe phenotype produced by GABRB3 gene deletion suggests a critical role in neurodevelopmental disorders. It was suggested that GABRB3 would be involved in the etiology of autism through interaction with GABRD and these results support the hypothesis that GABRA subunit genes are involved in autism, most likely via complex gene-gene interactions [91]. Recently, in an expanded dataset, findings where replicated and also GABRA4 subunit was associated with ASD either independently or in combination with GABRG2 [92]. While multiple GABRA subunits are involved in the pathogenesis of neuropsychiatric disorders, GABRB3 subunit is particularly critical for brain development and pathogenesis of epilepsy and ASD. GABRB3 is an important component of GABRA, especially in the cerebral cortex, hippocampal formation, hypothalamus, cranial nerve ganglia and spinal cord in adults, and even more widespread and abundant in the prenatal and neonatal brain [58].

**Conclusions**

ASDs are neurodevelopmental disorders characterized by E/I imbalance in selective neuronal circuits. Such disequilibrium appears to be mostly related to alterations in the GABAergic signaling. The GABAergic system is a key pathway that is commonly disturbed in many neurodevelopmental disorders, such as ASD and proper inhibitory GABAergic signaling is essential for normal neural circuit function [93]. If the inhibition is disturbed, excessive excitation results in disturbances in the E/I balance and, consequently, dysfunction of cognitive processes.

Defects in GABRA subunits are associated with neuropsychiatric disorders with variable clinical presentations, supporting the hypothesis that genetic variants in GABRA may be risk alleles for ASD and epilepsy. If GABAergic signaling is a specific risk for ASD and epilepsy, GABA would regulate neuronal excitability beyond their normal biology of ionotropic receptor [58]. Also, expression and protein data showed a dysregulation in multiple GABA$_A$ and GABA$_B$ receptors in key sites of the brain in subjects with autism which could explain several clinical phenotypes.
including the presence of seizures, learning disabilities and mental retardation\cite{51}.

Targeting the GABAergic abnormalities may be a unique opportunity for treatment for these disorders.

**Conflicting interests**

The author declares no conflicting interests.

**Acknowledgements**

*In memoriam:* Dr. Pablo F Argibay (Instituto de Ciencias Básicas y Medicina Experimental, Instituto Universitario del Hospital Italiano de Buenos Aires), is gratefully thanked for his suggestions and discussions. Nora Grañana is grateful for the contribution in the project. This work was supported by Instituto de Ciencias Básicas y Medicina Experimental, Instituto Universitario del Hospital Italiano de Buenos Aires.

**References**

2. Rubenstein JLR. Three hypotheses for developmental defects that may underlie some forms of autism spectrum disorder. Curr Opin Neurobiol 2010; 23:118-123.


70. Oblak AL, Gibbs TT, Blatt GJ. Reduced GABAA receptors and benzodiazepine binding sites in the posterior cingulate cortex and fusiform gyrus in Autism. Brain Res 2011; 1380:218-228.


