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A synaptic trek to autism

Thomas Bourgeron[1,2]

Autism spectrum disorders (ASD) are diagnosed on the basis of three behavioral features namely deficits in social communication, absence or delay in language, and stereotypy. The susceptibility genes to ASD remain largely unknown, but two major pathways are emerging. Mutations in *TSC1/TSC2*, *NF1*, or *PTEN* activate the mTOR/PI3K pathway and lead to syndromic ASD with tuberous sclerosis, neurofibromatosis, or macrocephaly. Mutations in *NLGN3/4*, *SHANK3*, or *NRXN1* alter synaptic function and lead to mental retardation, typical autism, or Asperger syndrome. The mTOR/PI3K pathway is associated with abnormal cellular/synaptic growth rate, whereas the NRXN–NLGN–SHANK pathway is associated with synaptogenesis and imbalance between excitatory and inhibitory currents. Taken together, these data strongly suggest that abnormal synaptic homeostasis represent a risk factor to ASD.

Addresses
[1] Human Genetics and Cognitive Functions, Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France
[2] University Denis Diderot, Paris 7, Paris, France

Corresponding author: Bourgeron, Thomas (thomasb@pasteur.fr)

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Introduction

Autism affects about 0.7% of children and is characterized by deficits in social communication, absence or delay in language, and stereotyped and repetitive behaviors. Beyond this unifying definition, lies a spectrum of disorders/conditions, ranging from severe impairments to mild personality traits. Autism spectrum disorders (ASD) are diagnosed before three years of age, a period characterized by intense synaptogenesis in the human brain [1]. This review reports recent genetic and neurobiological findings that highlight two routes leading to ASD: abnormal cellular/synaptic growth and imbalance between inhibitory and excitatory synaptic currents.

Abnormal cellular/synaptic growth in ASD

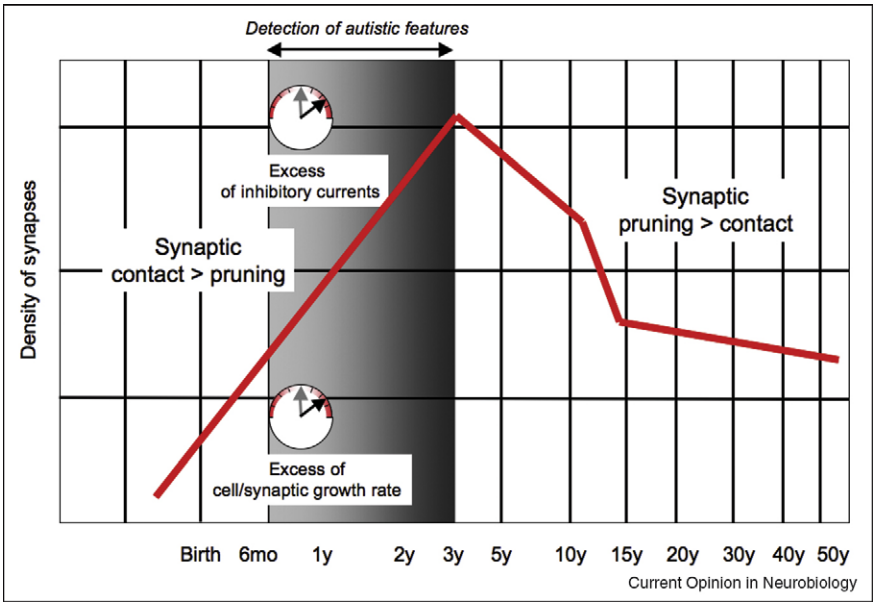
The hypothesis that abnormal cellular/synaptic growth may increase the risk of having ASD, was first suggested by the recurrent observation of macrocephaly in 10–30% of the patients with ASD [2–4]. The head circumference may be normal at birth, but during the first four years of life, an overgrowth of the brain is observed [5,6]. The nature of the macrocephaly — too many neurons, glial cells, synapses, or larger cells — remains difficult to establish. However, studies on neurofibromatosis, tuberous sclerosis, and Cowden/Lhermitte–Duclos syndromes have provided interesting information on the link between abnormal growth rate and ASD [7]. These genetic syndromes associate both susceptibility to ASD and macrocephaly and are caused by mutations in the tumor suppressor genes *NF1*, *TSC1/TSC2*, and *PTEN* [7]. In tuberous sclerosis, mutations of *TSC1/TSC2* induce cortical developmental malformations called tubers. These tubers were originally thought to be the cause of ASD when their locations in the brain were overlapping areas important for social communication and language. However, studies in mice showing that loss of *Tsc1/Tsc2* or *Pten* results in neuronal hypertrophy have led to the hypothesis that susceptibility to ASD was not because of the tubers, but to an abnormal shape and size of the neurons [8•,9].

Interestingly, NF1, TSC1/TSC2, and PTEN act in a common pathway as negative effectors of the rapamycin-sensitive mTOR–raptor complex (mTORC1), a major regulator of cellular growth in mitotic cells [10]. Mutations are predicted to enhance the mTORC1 complex, a signal activated by a sequential kinase cascade downstream of phosphoinositide-3 kinase (PI3K) pathway. This pathway may also be modulated by serotonin since macrocephaly and abnormal behaviors are exacerbated in mice with both *Pten* and serotonin transporter mutations [11]. A stimulating hypothesis proposed by Kelleher and Bear, suggests that the increase of the mTOR pathway could lead to abnormal synaptic function owing to an excess of protein synthesis at the synapse [10].

Abnormal balance between inhibitory and excitatory currents in ASD

The possibility that alteration of synaptic functions could lead to ASD was first indicated by the phenotypic overlap between autism, fragile X syndrome, and Rett syndrome [12,13]. In addition, the key role of the excitatory/inhibitory currents in ASD was further supported by the observation that 10–30% of patients with ASD have epilepsy [14]. The synaptic hypothesis was confirmed by the identification of mutations affecting the postsynaptic cell adhesion molecules Neuroligins (NLGN) in individuals with ASD [15••,16]. At the functional level, the mutations

Figure 1



Schematic representation of the different phases of synaptogenesis in the human brain. During the first three years of life, an excess of cell/synaptic growth rate and inhibitory currents could increase the risk of ASD. Mutations within the mTOR/PI3K pathway lead to an excess of synaptic/cell growth. Mutations within the NRXN–NLGN–SHANK pathway lead to abnormal synaptogenesis and excess of inhibitory currents. The arrows entering the red zone illustrate the excess of synaptic/cell growth and inhibitory currents during early brain development.

were found to alter the property of the NLGN to trigger synapse formation in cultured neuronal cells [17]. NLGN mutations probably concern a limited number of cases (<1% of the individuals), but following these initial results, mutations in other synaptic proteins such as *SHANK3*, *NRXN1*, *CNTNAP2*, *CNTN3/4*, and *PCDH9/10* were identified in patients with ASD [18–25]. Interestingly, *NRXN1* codes for the presynaptic binding partner of NLGNs, CNTNAP2 (Caspr2) possess strong homology to NRXN and SHANK3 is a scaffolding protein of the postsynaptic density that binds to NLGN and regulates the size and shape of dendritic spines [26].

Only limited data are available for understanding the role of these proteins in the human brain, but studies using neuronal cell culture and animal models have provided crucial information. Firstly, NLGNs and NRXNs enhance synapse formation *in vitro* [27••], but are not required for the generation of synapses *in vivo* [28••]. Therefore, NLGNs may not establish synapses, but may contribute to the activity-dependent formation of neural circuits [29•]. Secondly, NLGNs and NRXNs are emerging as central organizing molecules for excitatory glutamatergic and inhibitory GABAergic synapses in the mammalian brain [30,31]. The mutant mice carrying a R451C *Nlgn3* mutation identified in two brothers with ASD displays an increased number of GABAergic synapses and inhibitory currents [32]. An imbalance of inhibition and excitation was also observed in MeCP2 knockout [33] and in several mice proposed as model of autism such as the *Caps2* knockout [34] or mice subject to prenatal valproate treatment [35]. Interestingly, the link between GABA function and spine pruning has been identified during a critical period of brain development when individual experience is essential for the normal development of the neuronal network [36]. Therefore, impaired inhibitory–excitatory balance can be manifest as a shifted critical period for brain development [37] or an alteration of sensory processing, such as reduced gamma oscillations in FMRP knockout mice [38] as seen also in ASD [39]. Taken together, these results strongly suggest that synapse homeostasis and specificity play an important role in the susceptibility to ASD.

Atypical neuronal networks in ASD

In the human cerebral cortex, the first synapses are evident at the 40th day after conception. Thereafter, the rate of synapse formation and pruning exhibit distinct phases, the most dramatic change takes place during the perinatal period (Figure 1). During the first three years of life, synaptic contacts are formed, but only some will be stabilized. This selection process represents a key step in the cognitive development of the child. The NLGN–NRXN–SHANK pathway is probably required during this stabilization phase of the synapse in response to neuronal activity. Strikingly, the role of the NLGN–NRXN–SHANK pathway in the development of social interaction seems to be conserved in other species.

Indeed, knockout mice for *Nlgn4* display reduced social interactions and ultrasonic vocalizations (USV) at the adult stage [40••]. Mice carrying the R451C mutation in *Nlgn3* display normal [41] to reduced social interaction [32] at the adult stage and a reduction of isolation calls in pups [41]. However, knockout *Nlgn4* and mutant knockin *Nlgn3* display normal to enhanced learning when compared with wild-type mice [32,40••]. The same is true for the mice carrying a null mutation of *Shank1*, which exhibits increased anxiety-related behavior, but show enhanced spatial learning [42].

One of the main challenges for basic scientists and clinicians is to understand how far abnormal cell/synaptic growth and synaptic function could be reversed. Remarkably, in mice with *Tsc1/Tsc2* or *Pten* mutations, the use of rapamycin, a specific inhibitor of mTORC1, can prevent and reverse neuronal hypertrophy, resulting in the amelioration of the behavior [43•,44•]. Similarly, abnormal synaptic functions could be reversed in adult mice model for fragile X or Rett syndrome [45•,46,47]. The possibility to reverse the social and USV alterations of the Nlgn3/4 mutant mice has not been tested yet, but the recent results obtained on mice model for fragile X or Rett syndrome provide new hopes for the treatment of ASD.

New routes to ASD?

Two main pathways were identified in the susceptibility to ASD, but most probably many other tracks can lead to this complex syndrome. Furthermore, even when a pathway is identified, the diversity of genotype–phenotype relationships observed in patients with ASD indicates that other modulators such as serotonin and/or melatonin may play crucial roles in the onset and severity of ASD [48,49•]. The recent results have shed light on the origin of ASD and we are confident that new pathways will be identified soon to better understand the many facets of ASD.

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