Biological Psychiatry: Celebrating 50 Years

Commentary

The Up and Down of the *N*-Methyl-D-Aspartate Receptor That Causes Autism

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Anomalous synaptic formation, signaling, and plasticity characterize several neurodevelopmental diseases like autism spectrum disorder (ASD), and it is now clear that accurate control of synaptic development is critical for the formation of a neuronal network that exhibits regular activity and healthy brain function. ASD is a composite neurodevelopmental disorder that is clinically defined by impairments in social relationships, including emotional deficits and stereotyped behaviors. Mutations in many genes codifying for synaptic proteins have been identified in patients with ASD (1,2). Among these genes, an emerging role has been taken by three members of the *Shank/ProSAP* family that codify for master scaffold proteins, located at the postsynaptic density of glutamatergic synapses. Mutations in *SHANK* genes are clearly associated with ASD and intellectual disability with differing degrees of severity, *SHANK3* mutations being the most severe and *SHANK1* being the least severe (3). Most human behavioral and neurological symptoms have been replicated in mice with deletions and mutations in *Shank* genes. All mouse models present with impairments in social communication, memory flexibility, and motor behavior generally associated with synaptic functional alterations that are considered causative of the behavioral deficiencies.

SHANK proteins are scaffold proteins of the glutamatergic synapses, and therefore it is not surprising that the deletion of these genes causes alterations to the activity of different glutamate receptors (4). This has been clearly demonstrated in three *Shank2* knockout (KO) mice recently generated in which, even though opposite results were displayed, the mice showed an increased or decreased level of *N*-methyl-D-aspartate receptor (NMDAR) efficacy in various brain areas (5–7). Interestingly, in one of these mouse models it was possible to rescue the behavioral deficits by pharmacologically increasing the NMDAR activity in adult mice (7). However, most of the studies have characterized adult KO mice despite the clear demonstration of SHANK proteins regulating synapse formation. In this issue of *Biological Psychiatry*, Chung *et al.* (8) studied the synapses during development in the absence of *Shank2* with an expression peak around postnatal day 14 (P14). Given that ASD is a neurodevelopmental disorder, it is necessary to understand how the altered expression of synaptic proteins interferes with the development of synapses and circuits during development. Chung *et al.*'s (8) findings are surprising and unexpected, revealing a new possible molecular mechanism that elucidates the pathogenesis of ASD.

In the first set of experiments, Chung *et al.* (8) compared the brain transcriptomic between wild-type and KO mice at two different ages: at P14, when the expression of *Shank2* gains the maximum level and synaptogenesis is still ongoing, and at P21, around weaning, when the synaptogenesis is essentially completed. This analysis revealed that only at P14 is there a differential expression of a set of genes associated with excitatory postsynaptic compartments, postsynaptic membrane, and postsynaptic density genes, as well as some genes associated with NMDAR activation and NMDAR-dependent synaptic plasticity.

The presence of SHANK2 during synaptogenesis is therefore vital to the regulation of several synaptic genes. The most exciting finding, however, was discovered when Chung *et al.* (8) explored the excitatory synaptic transmission mediated by NMDARs and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPARs) in the hippocampus and medial prefrontal cortex, two brain regions implicated in several cognitive functions. At P14, in specific synapses of both areas, they found that the ratio of NMDAR- to AMPAR-mediated synaptic transmission was higher in the *Shank2* KO mice than in the wild-type mice because the NMDAR component had increased—results displaying exactly the opposite of what they found at P24 in juvenile mice, where the NMDARs were hypofunctioning.

Therefore, in a condition in which most of the synaptic parameters are unchanged (but with a slight reduction of total glutamatergic synapses in medial prefrontal cortex neurons), Chung *et al.* (8) found a rapid temporal switch of NMDARs from hyper- to hypofunction close to the period of synapse maturation and brain circuit consolidation (between P14 and P24) in the hippocampus and medial prefrontal cortex of *Shank2* KO mice. Chung *et al.* (8) were also able to demonstrate that this up and down of the NMDAR function correlates to increased (at P14) and decreased (at P24) phosphorylation of GluN2B at Ser1303 and GluN1 at Ser896. These two posttranslational modifications were previously demonstrated to be involved in regulating the delivery of NMDARs to synapses (9). Also at P14, the GluN2B-containing receptors are mostly increased at the synapses—but the reason *Shank2* deletion in these mice causes this alteration on NMDAR subunit phosphorylation remains to be determined.

That brings us to the most important section of the article. NMDARs are druggable targets, and the most efficient experiment to conduct is to block the NMDARs with a specific antagonist, such as memantine, at an early stage of the development in order to try to demonstrate the correlation between early hyperactivity of the NMDAR with the hypoactivity in the juvenile mice. When Chung *et al.* (8) treated the *Shank2* KO mice for 14 days (from P7 to P21), the NMDAR currents were normal at P25 up to P31. Even more importantly, the 2-week treatment with memantine was able to rescue the

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social behavioral impairments observed in adult mice. Thus, in *Shank2* KO mice, early NMDAR hyperfunction at P14 is a causal mechanism for the NMDAR hypofunction and social deficits at juvenile (P28) and adult (P56) stages.

These important findings tell us that drugs for the treatment of severe neurodevelopmental disease could differ depending on the age of the patient, and that some drugs can be beneficial at one age but detrimental at another age. A second crucial point is to highlight the importance of studying the neuronal functional alteration during brain development in mouse models of neurodevelopmental diseases. Thus, the accurate analysis of the molecular alteration during synaptogenesis is critical for the development of pharmacological treatments, especially if the mutated genes codify for synaptic proteins or for proteins that indirectly control synapse formation.

However, Chung *et al.* (8) pointed to other issues that need to be resolved. Two other different *Shank2* KO mice were available, one mutant lacking exon 7 and the other lacking exon 24. Both mice showed normal NMDAR function at P14 but enhanced NMDAR function at P21–P24 for the exon 7 mutant (5,7), while the exon 24 mutant had reduced NMDAR function at P21, similar to the exons 6 and 7 mutant mouse used by Pappas *et al.* (6). Why does this difference occur? A possible explanation could be that the *Shank2* gene can generate at least two splice variants and that the expression of one of these is increased in the exon 7 mutant mice. However, there is no possible explanation for the exon 24 mutant mice. This is certainly a general problem for the mice carrying *Shank* gene mutations—especially for the *Shank3* KO mice, considering that the *Shank3* gene has several splice variants and considering that several differences in behavioral and molecular mechanisms have been described for these mice. There is also evidence that in humans, different mutations on either *SHANK2* or *SHANK3* genes are associated with different pathological behavior. This reinforces the hypothesis that patients with different *SHANK* mutations might need different pharmacological treatments.

Despite the completeness of the study, some questions without answers remain. To fully demonstrate that the hyperfunction of the NMDAR is directly subsequent to hypofunction and related behavioral alterations, we need to know what would happen if *Shank2* is deleted after P24, when synapses are mature. If one were to guess, one would assume that these mice would not show social impairments. However, they might still show hyperactivity not rescued by memantine treatment, and they can be used as model to find a specific treatment for this selected and isolated behavioral alteration.

Furthermore, the molecular mechanism that links the up state to the down state of NMDAR activity at the synapses was not fully investigated. Chung *et al.* (8) showed that the GluN1 and GluN2B are differentially phosphorylated in *Shank2* KO mice, but we do not know which kinase and/or phosphatase is involved and why these enzymes are probably wrongly activated in *Shank2* KO mice; this might help to identify other pharmacological targets.

Finally, memantine was not able to rescue the hyperactivity of the *Shank2* mice, a behavioral alteration that is present in all *Shank2* KO mice. This implies that the rescue of the NMDAR dysfunction is not sufficient to fully cure the *Shank2* KO mice. Thus, ASD and all neurodevelopmental diseases in general will probably require multiple drug treatments, making the development of pharmaceutical therapies for these diseases a challenge. However, this remarkable study is helping find a possible path to a solution.

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Article Information

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