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Neurexin Dysfunction in Adult Neurons Results in Autistic-like Behavior in Mice

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SUMMARY

Autism spectrum disorders (ASDs) comprise a group of clinical phenotypes characterized by repetitive behavior and social and communication deficits. Autism is generally viewed as a neurodevelopmental disorder where insults during embryonic or early postnatal periods result in aberrant wiring and function of neuronal circuits. Neurexins are synaptic proteins associated with autism. Here, we generated transgenic βNrx1ΔC mice in which neurexin function is selectively impaired during late postnatal stages. Whole-cell recordings in cortical neurons show an impairment of glutamatergic synaptic transmission in the βNrx1ΔC mice. Importantly, mutant mice exhibit autism-related symptoms, such as increased self-grooming, deficits in social interactions, and altered interaction for nonsocial olfactory cues. The autistic-like phenotype of βNrx1ΔC mice can be reversed after removing the mutant protein in aged animals. The defects resulting from disruption of neurexin function after the completion of embryonic and early postnatal development suggest that functional impairment of mature circuits can trigger autism-related phenotypes.

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental syndrome characterized by restricted and stereotyped behavior patterns, difficulty with social interactions, and deficits in verbal/nonverbal communication. ASD symptoms typically emerge in early childhood and persist through adulthood, suggesting ASD origin could stem from prenatal impairment that develops into enduring postnatal manifestations. Alternatively, dysfunction of postnatal neuronal networks at symptom onset may define the clinical phenotype for this disorder. Thus, it remains unclear whether there are early critical periods where autism develops or if postnatal dysfunction of neuronal circuits is sufficient to produce autism-related phenotypes.

The neurexin family of synaptic plasma membrane proteins forms one class of ASD-associated genes. Neurexins are encoded by three genes (*NRXN1*, *NRXN2*, and *NRXN3*), each of which generates long α- and short β-neurexin proteins from alternative promoters (Tabuchi and Südhof, 2002; Ushkaryov et al., 1992). Deletions and truncating mutations in the *NRXN1* gene affecting α and β isoforms have been linked to autism and other neurodevelopmental disorders (Ching et al., 2010; Gauthier et al., 2011; Schaaf et al., 2012; Szatmari et al., 2007). Moreover, point mutations specific to the *NRXN1β* gene have been identified in ASD patients (Camacho-Garcia et al., 2012, 2013). Neurexins couple presynaptic signaling with binding to postsynaptic partners, such as neuroligins (NLGNs) (Dean et al., 2003; Südhof, 2008). The identification of mutations in *NLGN* and *SHANK* genes in ASD pointed to glutamatergic dysfunction of the *NRNX-NLGN-SHANK* pathway in autism (Durand et al., 2007; Jamain et al., 2003; Laumonnier et al., 2004).

Current genetic data support that hypofunction of neurexin isoforms is a risk factor in autism. However, the brain regions and the developmental stage in which loss of neurexin function leads to autistic-like behaviors are not known. In rodents, neurexin mRNAs are expressed throughout the developing and mature CNS (Ehrmann et al., 2013; Iijima et al., 2011; Püschel and Betz, 1995), which raises the question of whether autism-related symptoms can emerge from neurexin dysfunction after the development has been completed. The manipulation of neurexins is challenging because of their genetic complexity and high number of isoforms (Treutlein et al., 2014). In multifactorial disorders, such as ASD, a combination of DNA variants in a number of genes contributes to the clinical presentation. This poses difficulties when the role of individual mutations is assessed in animal models. Therefore, a dominant-negative approach is well suited to address the effect of impaired neurexin function in vivo. In this study, we generated transgenic mice that express a neurexin-1β mutant in postnatal neurons of brain regions implicated in autism, such as cortex and striatum (Parikshak et al., 2013; Shepherd, 2013; Willsey et al., 2013). Neurexin-1β mutant mice showed impaired glutamatergic transmission in pyramidal cortical neurons and autism-related phenotype. Importantly, the autism phenotype was reversed in young as well as older mice upon inhibiting the expression of mutant neurexin-1β. Our

data indicate that neurexin dysfunction in postnatal forebrain neurons recapitulates the core symptoms of autism, which can be reversed in adult animals when normal neurexin function is resumed.

RESULTS

Characterization of the Neurexin-1β Mutant Protein

Cytoplasmic-tail deletion mutants have been previously shown to inhibit the synaptic function of neurexin variants in cultured neurons (Choi et al., 2011; Dean et al., 2003; Futai et al., 2007). The shared cytoplasmic tail of neurexins interacts with presynaptic scaffolding proteins (Butz et al., 1998), whereas alternative splicing at the extracellular domain modulates the binding to postsynaptic partners, such that maximal binding to neuroligins is exhibited by neurexin-1β variants lacking an insertion at splice site 4 (-S4) (Boucard et al., 2005; Comoletti et al., 2006; Dean et al., 2003). Thus, to uncouple neurexin-1β function, we generated a hemagglutinin (HA)-tagged deletion mutant of neurexin-1β (-S4) that lacks the cytoplasmic tail (Figure 1A). First, we analyzed the distribution of the HA-βnrx1ΔC protein, as it has been suggested that C-terminal sequences are required for synaptic targeting of neurexin-1α (Fairless et al., 2008). The cell-surface localization of the HA-βnrx1ΔC protein was confirmed in nonpermeabilized human embryonic kidney 293T (HEK293T) cells (Figure S1A). Then, we studied the synaptic recruitment of HA-βnrx1ΔC induced by neuroligin-1 (NL1) in nonneuronal cells (Scheiffele et al., 2000). HA-βnrx1ΔC concentrated at synaptic contacts mediated by VSV-NL1 at similar levels as wild-type HA-βnrx1 (Figure 1B). The enrichment of the glutamatergic synaptic vesicle marker vGluT1 to NL1 synapses was not affected by the expression of HA-βnrx1ΔC (Figure 1B), indicating that the cytoplasmic tail of neurexin-1β is dispensable for the recruitment of synaptic vesicles, consistent with a recent report (Gokce and Südhof, 2013).

We reasoned that HA-βnrx1ΔC mutant might compete with endogenous neurexins for the binding to postsynaptic partners but affect other presynaptic parameters such as synapse function due to the absence of the cytoplasmic tail. Consistent with this hypothesis, expression of a neurexin-1β mutant lacking the cytoplasmic domain has previously been shown to decrease release probability in hippocampal neurons, whereas overexpression of wild-type neurexin-1β had no effect (Futai et al., 2007). To directly analyze the effect of HA-βnrx1ΔC mutant on presynaptic release, we studied synaptic vesicle cycle with sypHy (Granseth et al., 2006). We found that expression of HA-βnrx1ΔC mutant in cultured hippocampal neurons decreased action-potential-triggered sypHy fluorescence by ~30%, whereas no effect was observed upon expression of HA-βnrx1 (Figures 1C and S1B). These data indicated that deletion of the intracellular domain of neurexin-1β does not inhibit cell-surface localization or transsynaptic interactions with neuroligins, but it decreases synaptic vesicle release.

Generation of βNrx1ΔC Mice

We used the HA-βnrx1ΔC mutant as a molecular tool to inhibit neurexin function in postnatal neurons. With that purpose, we generated transgenic mice that express HA-βnrx1ΔC in an inducible manner using the Tet-off system (Figure 1D). First, we obtained a mouse line (*TRE-HAβnrx1ΔC*) that expresses the HA-βnrx1ΔC transgene under the control of the tetracycline-responsive promoter element (TRE). TRE-*HAβnrx1ΔC* mice did not express HA-βnrx1ΔC protein in the brain, showing no escape of the transgene (Figure 1E). It has been shown that expression of neurexin-1 (-S4) transcripts is maximal in forebrain regions, including cortex (Ehrmann et al., 2013; Iijima et al., 2011). Therefore, to direct the expression of HA-βnrx1ΔC protein to forebrain neurons, we crossed *TRE-HAβnrx1ΔC* mice with *CaMKIIα-tTA* mice that express the tetracycline transactivator (tTA) in postnatal glutamatergic neurons of the forebrain (Mayford et al., 1996). Double-transgenic *CaMKIIα-tTA*; *TRE-HAβnrx1ΔC* mice (βNrx1ΔC mice) expressed HA-βnrx1ΔC protein in forebrain neurons of the cortex and striatum and showed no detectable expression in the midbrain and cerebellum (Figures 1E and 1F). Compared with endogenous neurexin-1β, exogenous HA-βnrx1ΔC protein is expressed at 100%–200% in cortex and striatum (Figure 1F). Importantly, expression of HA-βnrx1ΔC turns on only in the third postnatal week (Figure 1G). Thus, in our mouse model, neurexin function is unperturbed over the first 2 postnatal weeks when extensive synapse formation takes place. Upon onset, HA-βnrx1ΔC expression is maintained throughout adulthood (Figure 1G) but can be suppressed by doxycycline (Dox) feeding of the mutant mice (Figure 1H).

Synaptic Defects in βNrx1ΔC Mice

Neurexins have been proposed to participate in synapse formation and function by a presynaptic mechanism (Dean et al., 2003; Missler et al., 2003). Therefore, an inhibitory effect in glutamatergic synapses was predicted as a consequence of the expression of the neurexin-1β mutant in cortical glutamatergic neurons. Immunoblot experiments of cortical synaptosomes revealed that HA-βnrx1ΔC is mostly expressed at presynaptic fractions (Figures 2A and 2B), in agreement with a presynaptic role of neurexin proteins. Because the expression of the neurexin-1β mutant begins at a developmental stage when most synapse formation has been completed but of active synaptic plasticity, we analyzed the distribution of synaptic markers and synaptic transmission in βNrx1ΔC mice. The expression of selected synaptic proteins was not significantly altered in βNrx1ΔC mice (Figures S2A and S2B). Then, we performed whole-cell recordings in cortical layer 5/6 (L5/6) pyramidal neurons of the somatosensory cortex, a region that expresses relative high levels of the HA-βnrx1ΔC protein (Figure S2C). The mean amplitude of miniature excitatory postsynaptic currents (mEPSCs) was not altered, suggesting normal quantal content and postsynaptic apparatus. However, the frequency of mEPSCs was reduced by ~50% in βNrx1ΔC mice (Figure 2C). Analysis of the miniature inhibitory postsynaptic currents (mIPSCs) in βNrx1ΔC mice showed a less significant reduction (30%) in the frequency, while the mIPSCs amplitude was unchanged (Figure 2D). Consistent with the activity of tTA in cortical glutamatergic neurons (Mayford et al., 1996), GABAergic interneurons of the somatosensory cortex did not express HA-βnrx1ΔC (Figure S2D), indicating that the reduction in mIPSCs frequency likely reflects a compensatory mechanism