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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

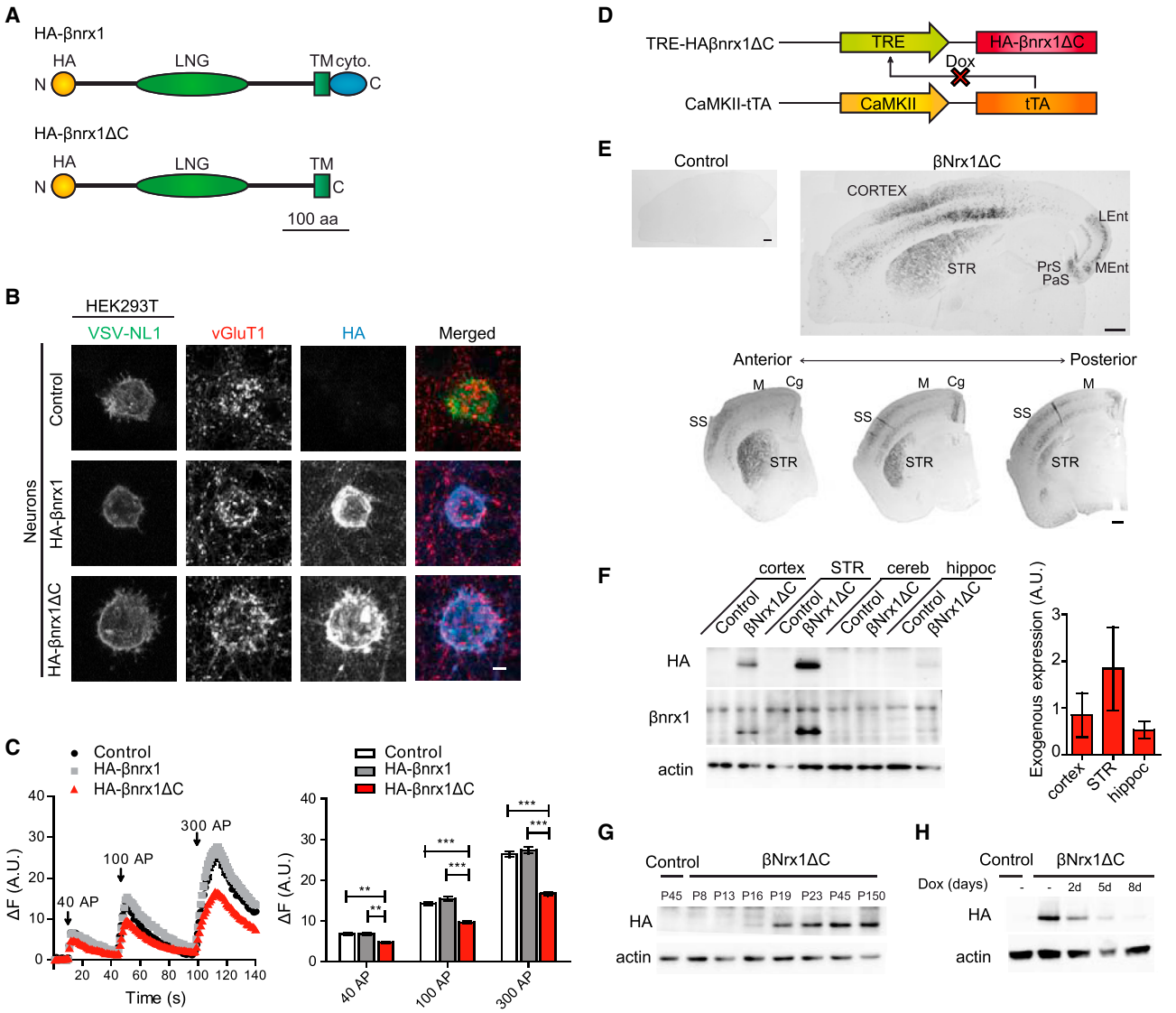

Figure 1. βNrx1ΔC Mouse

(A) HA-tagged βnrx1 and βnrx1ΔC proteins. LNG, laminin/neurexin/sex hormone binding globulin domain; TM, transmembrane domain; cyto, cytoplasmic tail.

(B) Recruitment of HA-βnrx1 and HA-βnrx1ΔC to HEK293T cells expressing VSV-NL1. Hippocampal neurons infected with lentivirus expressing HA-βnrx1 or HA-βnrx1ΔC and cocultured with HEK293T cells transfected with VSV-NL1 were immunostained with VSV (green), vGluT1 (red), and HA (blue) antibodies.

(C) Time kinetics (left graph) and peak amplitudes (right graph) of sypHy fluorescence elicited by 40, 100, and 300 action potentials in hippocampal neurons cotransfected with sypHy and empty vector (control), HA-βnrx1, or HA-βnrx1ΔC. Average of >400 synapses obtained from 12 experiments from four independent cultures.

(D) Experimental design of βNrx1ΔC mouse.

(E) Horizontal (top) and coronal (bottom) brain sections from control (*TRE-HAβnrx1ΔC*) and βNrx1ΔC mice (*CaMKIIα-tTA*; *TRE-HAβnrx1ΔC*) stained with HA antibody. Cg, cingulate cortex; LEnt, lateral entorhinal; M, motor cortex; MEnt, medial entorhinal; PaS, parasubiculum; PrS, presubiculum; SS, somatosensory cortex; STR, striatum.

(F) Immunoblots with HA and βNrx1 antibodies showing expression of HA-βnrx1ΔC and endogenous neurexin-1β proteins in lysates from cortex, striatum, cerebellum, and hippocampal formation. The lower band recognized by the βnrx1 antibody represents exogenous HA-βnrx1ΔC. Graph shows quantitation of HA-βnrx1ΔC expression normalized to endogenous βNrx1 (n = 3).

(G) Cortical lysates from control and βNrx1ΔC mice analyzed by immunoblotting with the HA antibody at the indicated postnatal time.

(H) Inhibition of HA-βnrx1ΔC expression in βNrx1ΔC mice fed with Dox.

Scale bars represent 5 μm in (B) and 500 μm in (E). *p < 0.05, **p < 0.01, ***p < 0.001. All error bars are SEM. See also Figure S1.

to counterbalance decreased excitation (Lau and Murthy, 2012). Moreover, the electrophysiological experiments indicate that the reduced strength of glutamatergic transmission in βNrx1ΔC mice occurs by a presynaptic mechanism mediated by the expression of the neurexin-1β mutant, providing a functional validation of the genetic manipulation.

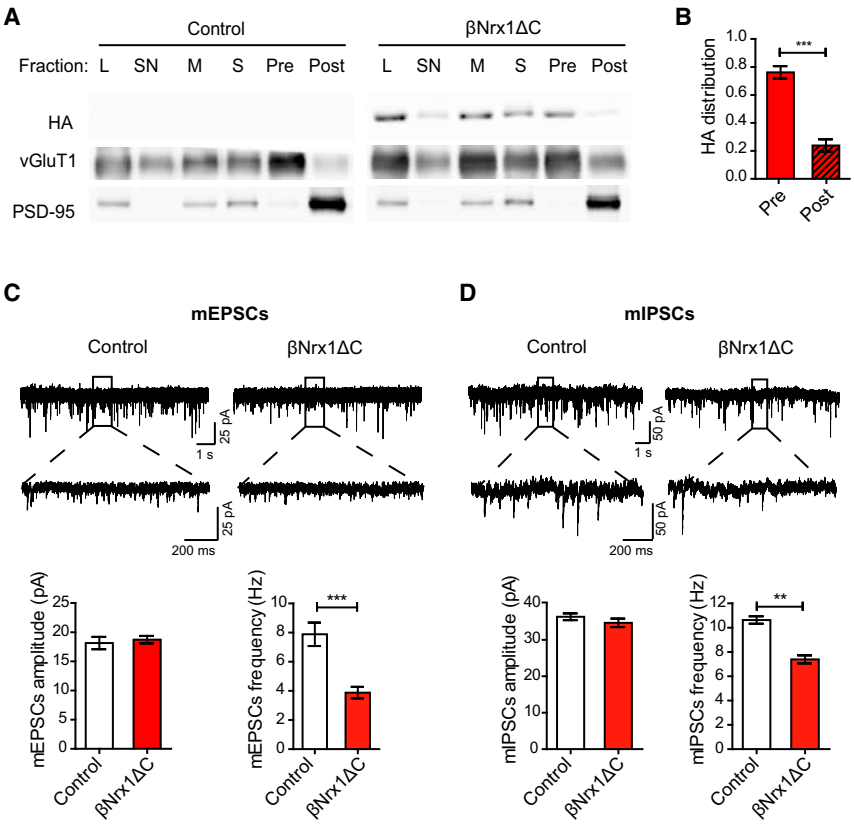

Figure 2. Synaptic Defects in βNrx1ΔC Mice

(A) Cortical synaptosomes from control and βNrx1ΔC mice immunoblotted with HA, vGluT1, and PSD-95 antibodies, as indicated. L, lysate; SN, supernatant; M, membrane; S, synaptosome; Pre, presynaptic fraction; Post, postsynaptic fraction.

(B) Normalized HA-βnrx1ΔC expression in presynaptic and postsynaptic fractions (n = 4).

(C and D) Representative traces and quantitations of frequency and amplitude of mEPSCs (C) and mIPSCs (D) recorded from L5/6 pyramidal neurons of the somatosensory cortex. For mEPSCs: n = 9 cells from three control mice and n = 8 cells from three βNrx1ΔC mice. For mIPSCs: n = 16 cells from four control mice and n = 20 from four βNrx1ΔC mice.

**p < 0.01, ***p < 0.001. All error bars are SEM. See also Figure S2.

Autistic-like Behavior of βNrx1ΔC Mice

We next analyzed the behavior of βNrx1ΔC mice. Body weight and gross brain morphology were not affected in the βNrx1ΔC mouse (Figure S3A and data not shown). In the rotarod test, βNrx1ΔC mice displayed normal motor learning and did not exhibit an anxiety-like phenotype in the open field (Figures 3A and 3B). In the novel-object recognition test, the percentage of time interacting with a novel object was similar in βNrx1ΔC and control mice, although the total interaction time with any object was significantly increased in βNrx1ΔC mice (Figure 3C).

To uncover autism-related symptoms, βNrx1ΔC mice were compared with littermate controls using behavioral tests with relevance in autism (Silverman et al., 2010). As deficits associated with autism appear early in life but persist through adulthood, we analyzed two cohorts of mice at different ages: young (2–4 months old) and aged (7–9 months old) mice. Furthermore, the analysis of two independent cohorts of mice offered an additional control for the behavioral studies.

Increased repetitive behavior and social impairment are symptoms of patients with autism. Interestingly, in the self-grooming test young βNrx1ΔC mice showed increased repetitive phenotype compared to control mice (Figure 3D). Similarly, self-grooming was also increased in aged βNrx1ΔC mice (Figure 4A). Social behavior in mice can be analyzed in the three-chamber test (Yang et al., 2011). In the sociability assay, the tested mouse can freely move and interact with a caged object or mouse placed in side chambers and the time in close interaction with each stimulus is quantified. As expected, control mice interacted longer time with the mouse than with the object (Figure 3E). Unlike control animals, young βNrx1ΔC mice spent similar time interacting with both stimuli, showing increased interaction with the object and decreased interaction with the mouse (Figure 3E). This lack of preference for the animated stimulus was also replicated in aged βNrx1ΔC mice (Figure 4B). In the social preference test, the object is replaced by a nonfamiliar mouse. While control mice showed preferential interaction with the novel mouse, young and aged βNrx1ΔC mice showed reduced interaction with the novel mouse (Figures 3F and 4C). However, the number of entries to each chamber and the traveled distance were similar in control and βNrx1ΔC mice of both ages (Figures S3B and S4A).

Deficits in communication are common in autistic patients. In rodents, communication depends on olfactory cues. When exposed to a novel odor, mice initiate sniffing and then habituate to its novelty during repeated presentations. In the olfactory habituation/dishabituation test, mice are exposed to sequential presentations of nonsocial and social odors (Yang and Crawley, 2009). Control mice sniffed for a longer time a cotton swab containing a social odor (urine A) than nonsocial odors (water, vanilla, or orange blossom extracts) (Figures 3G and 4D). Interestingly, in βNrx1ΔC mice, the time spent sniffing nonsocial odors was increased to the level of the social odor (Figures 3G and 4D). To rule out a general olfactory impairment, we analyzed the time to find a buried food pellet and found no differences between control and βNrx1ΔC mice (Figures S3C and S4B). These data indicate that impaired neurexin function in late postnatal forebrain neurons is sufficient to cause an autistic-like phenotype that persists into adulthood.

Reversion of the Autistic-like Phenotype

The enduring behavioral deficits observed in βNrx1ΔC mice could originate from a temporarily restricted effect during postnatal development or rather from a continuous dysfunction of

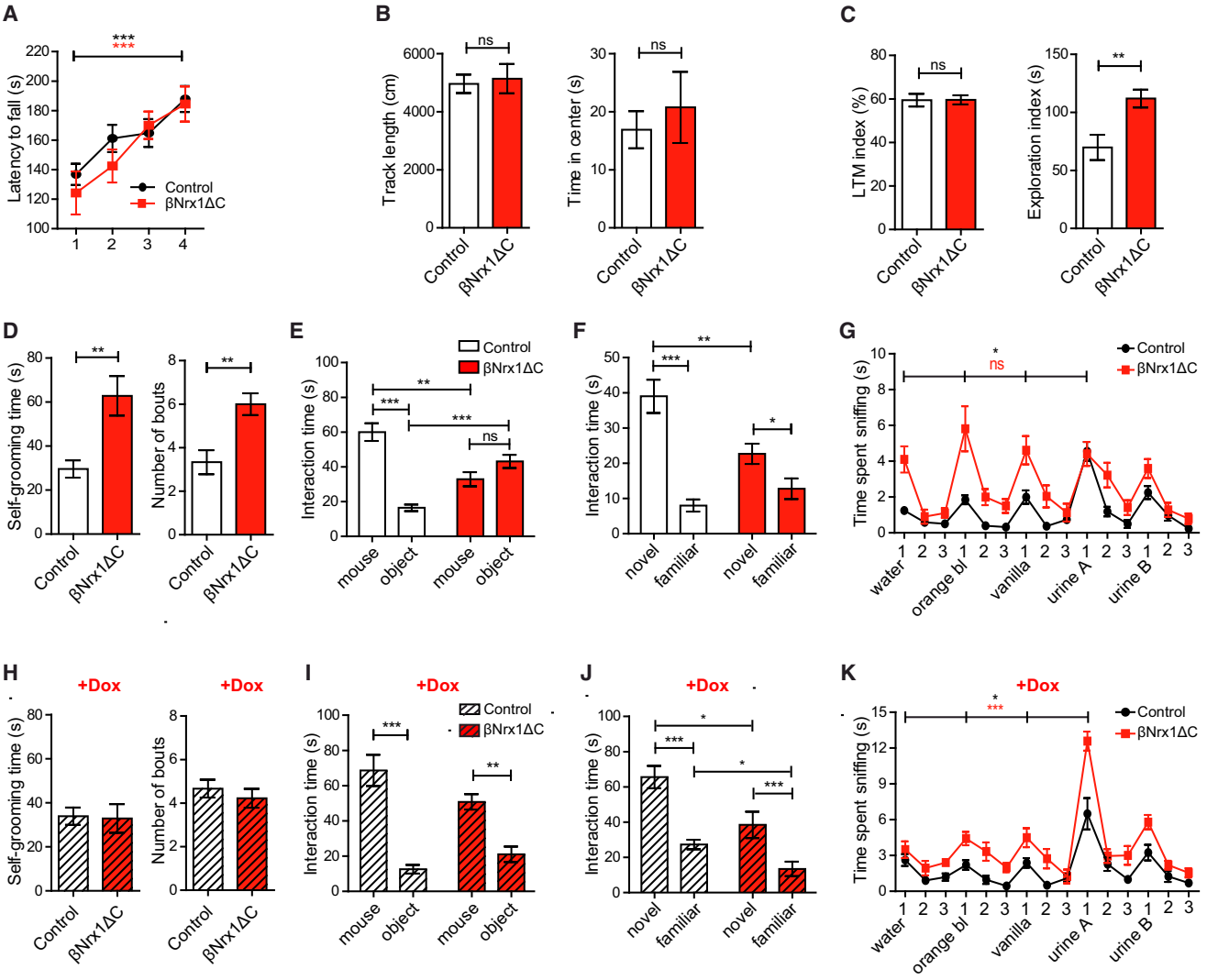

Figure 3. Increased Self-Grooming and Impaired Social Interaction and Preference for Social Odors in the βNrx1ΔC Mice

(A) Accelerating rotarod test. Data obtained from two trials per day during 4 consecutive days.

(B) Open field test. Locomotor activity (track length) and time in center were similar in control and βNrx1ΔC mice.

(C) Novel-object recognition test showed no differences in control and βNrx1ΔC mice in long-term memory index (LTM). Exploration index analysis resulted in increased interaction time of βNrx1ΔC mouse.

(D) Increased time spent in self-grooming and number of bouts in βNrx1ΔC mice.

(E) Social interaction in the three-chamber test. Graph shows time in close interaction with a caged object or a mouse. βNrx1ΔC mice displayed similar time interacting with a nonsocial (object) than with a social (mouse) stimulus.

(F) Analysis of social preference in the three-chamber test. βNrx1ΔC mice showed decreased interaction with a novel mouse.

(G) Olfaction habituation/dishabituation test. Quantitation of time spent sniffing a sequential presentation of nonsocial odors (water, orange blossom, and vanilla extracts) and social odors (urine A and urine B). Whereas control mice displayed significantly more time sniffing a social odor (urine A), βNrx1ΔC mice displayed no difference among the first exposure to the tested odors.

(H–K) Control and βNrx1ΔC mice were fed with Dox for 14 days before re-evaluation of the behavioral phenotype. (H) Treatment with Dox reduced self-grooming and frequency of bouts of βNrx1ΔC mice to control levels. (I) βNrx1ΔC mice treated with Dox showed preference for the animal versus the object in the three-chamber sociability test. (J) Treatment with Dox increased the interaction of βNrx1ΔC mice with the novel mouse compared with the familiar mouse. (K) In the olfaction habituation/dishabituation test, Dox-treated βNrx1ΔC mice showed increased time sniffing a social odor (urine A) compared to nonsocial odors. n = 10–13 (A–C) and n = 9 (D–K) mice per genotype at 2–4 months of age.

*p < 0.05, **p < 0.01, ***p < 0.001. All error bars are SEM. See also Figure S3.

adult neurons in the mature brain. In the former, inhibition of transgene expression in mature βNrx1ΔC mice would fail to rescue the behavioral deficits, which would be reversed, even in old mice, in the case that continuous dysfunction caused the autistic-like phenotype of mutant mice. To distinguish between these possibilities, we re-evaluated the behavioral

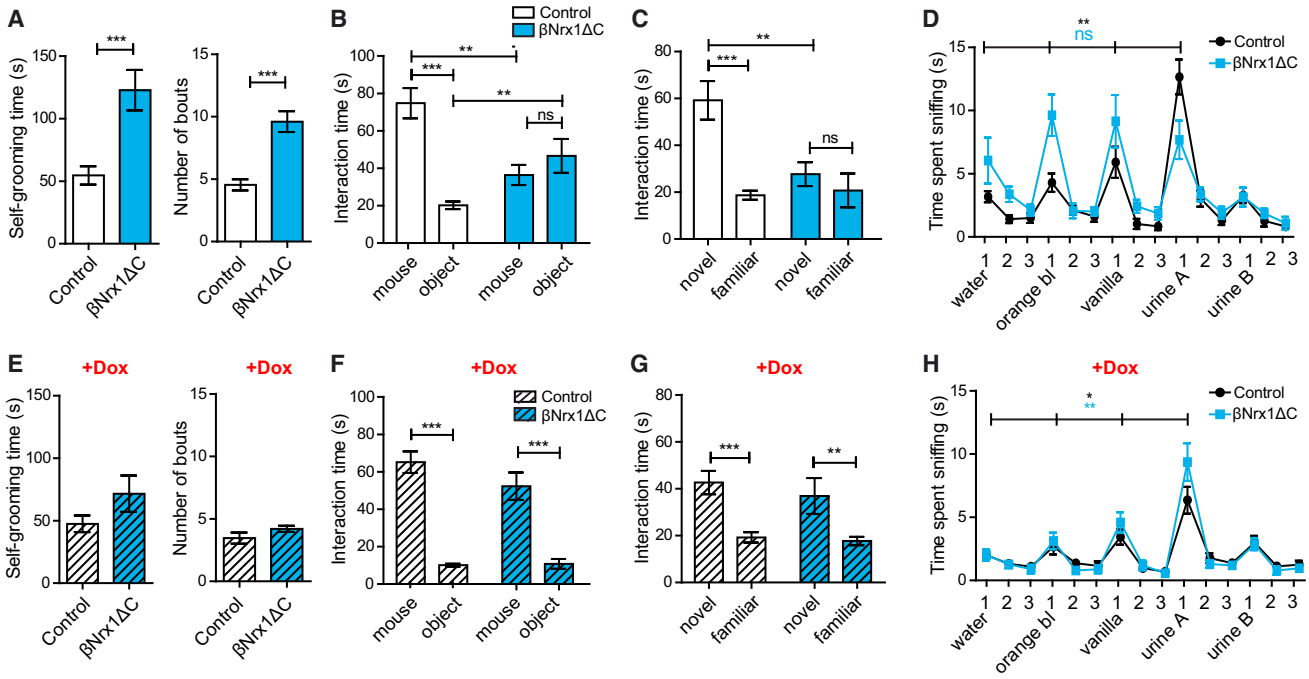

Figure 4. Reversion of the Autistic-like Phenotype in Aged βNrx1ΔC Mice

(A) Time spent in self-grooming and frequency of bouts in aged control and βNrx1ΔC mice.

(B and C) In the three-chamber test, aged βNrx1ΔC mice showed similar time interacting with the mouse and the object (B) and with the novel and familiar mouse (C).

(D) In the olfaction habituation/dishabituation test, βNrx1ΔC mice exhibited no preference for a social odor (urine A) compared with nonsocial odors.

(E–H) Reversion of the autistic-like phenotype in aged βNrx1ΔC mice. Analysis of self-grooming (E), sociability (F), social preference (G), and the olfaction habituation/dishabituation test (H) in control and βNrx1ΔC mice after treatment with Dox. n = 10–13 mice per genotype. Mice were analyzed at 7–9 months and re-evaluated at 9–11 months following treatment with Dox for 14 days.

*p < 0.05, **p < 0.01, ***p < 0.001. All error bars are SEM. See also Figure S4.

phenotype after switching off transgene expression. A 2-week treatment with Dox resulted in the effective inhibition of HA-βnrx1ΔC expression in synaptosome fractions (Figure S4C). Interestingly, the autism phenotype was reversed in young and aged βNrx1ΔC mice after Dox treatment (Figures 3H–3K and 4E–4H). Self-grooming in βNrx1ΔC mice was reduced to control levels after Dox treatment (Figures 3H and 4E). Notably, social interaction deficits were recovered in βNrx1ΔC mice. In the three-chamber test, Dox-treated βNrx1ΔC mice showed significant increased interaction with the mouse versus the object at both tested ages (Figures 3I and 4F; Movies S1, S2, and S3). Similarly, Dox treatment of young and aged βNrx1ΔC mice increased the interaction with the novel mouse compared with the familiar mouse (Figures 3J and 4G). In the olfactory habituation/dishabituation test, Dox-treated βNrx1ΔC mice decreased the time spent sniffing nonsocial odors compared with the time spent sniffing the social odor (Figures 3K and 4H), similar to the pattern observed in control mice. However, Dox treatment had no effect in control mice in any of the behavioral tasks (Figures 3 and 4). These data further confirmed that the behavioral deficits observed in βNrx1ΔC mice are due to the expression of the mutant HA-βnrx1ΔC protein rather than a positional effect of the transgene. The reversion of the behavioral deficits indicated that continuous absence of neurexin function in adult forebrain neurons leads to the autistic-like phenotype observed in βNrx1ΔC mice.

DISCUSSION

Mutations in the *NRXN1* gene have been increasingly found in ASD patients. Although neurexin-1α knockout mice that show autistic-related behaviors have been generated (Etherton et al., 2009; Grayton et al., 2013), animal models for neurexin-1β had not been reported. The data shown here further extends a role for neurexin-1 proteins in autism. Behavioral analysis of the neurexin-1β mutant mice uncovered increased repetitive behavior and deficits in social interaction. In the three-chamber arena, βNrx1ΔC mice presented defects in sociability and social preference tests, shown as similar time interacting with an object and a mouse and by a decreased interaction with a novel mouse, respectively. Despite normal habituation/dishabituation and detection of an olfactory cue (food pellet), βNrx1ΔC mice showed similar response to social and nonsocial odors, reminiscent of the exaggerated response to sensory stimuli found in ASD (Marco et al., 2011). The behavioral phenotype occurs in the absence of deficits in locomotion and motor learning, anxiety, and recognition memory, indicating that βNrx1ΔC mice were not globally impaired. Although mutant neurexin-1β could

be interacting with ligands shared by different neurexin isoforms, these data support a role for synaptic dysfunction of neurexin-1β in autism.

The rescue of ASD-associated symptoms in a number of mouse models has challenged the irreversibility concept of autism (Blundell et al., 2010; Gkogkas et al., 2013; Guy et al., 2007; Peñagarikano et al., 2011; Won et al., 2012). However, the developmental stage where perturbations of ASD-associated genes lead to the onset of the autistic phenotype remains largely unknown. The results shown here provide insight into the identification of the developmental period and the brain regions in which dysfunction of neurexin produces a behavioral phenotype associated with autism. Because of the restricted spatiotemporal pattern of the genetic manipulation, our data indicate that normal neurexin function in adult forebrain neurons is critical to prevent the onset of autistic-like behaviors in mice. Although most ASD cases are sporadic, the identification of Mendelian syndromes that display symptoms of autism has contributed to the elucidation of the neurobiological basis of ASD. Interestingly, postnatal mouse models of Mendelian ASD genes have been established that recapitulate the core disease symptoms (Chen et al., 2001; Kwon et al., 2006; McGraw et al., 2011; Tsai et al., 2012). The neurexin-1β C-terminal deletion described here represents a model of nonsyndromic autism, even though C-terminal deletions reported so far in ASD patients also affect the transmembrane domain (Schaaf et al., 2012; Wiśniowiecka-Kowalnik et al., 2010). Together with the results described for Mendelian ASD models, these findings would indicate the relevance in ASD pathology of preserving normal function of ASD genes in mature brain. The high prevalence of autism and the absence of an effective treatment pose challenges for the care of patients affected by this lifelong condition. Mutations in ASD-associated genes are mostly found in heterozygosis, offering the stimulation of the normal allele's function as a potential therapeutic approach. Our data showing reversion of the autistic-like phenotype in adult βNrx1ΔC mice indicate that autistic symptoms arising from dysfunction of neurexins may benefit from therapeutic interventions aimed to restore normal neurexin function, not only during infancy but also in adult patients.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice

The HA-βnrx1ΔC construct contains an HA tag at the N terminus of the mature protein and lacks the last 55 residues of the cytoplasmic tail. A DNA fragment containing the TRE promoter, the coding sequence of HA-βNrx1ΔC and a WPRE fragment (woodchuck hepatitis virus posttranscriptional regulatory element) was injected into the pronucleus of FVB/N zygote for transgenic mouse production. *TRE-HAβnrx1ΔC* mice were backcrossed with C57BL/6J mice for four generations followed by mating with *CaMKIIα-tTA* mice in a C57BL/6J background (Mayford et al., 1996) that led to the generation of βNrx1ΔC mice (*CaMKIIα-tTA/TRE-HAβnrx1ΔC*). Animals were kept at 22°C on a 12 hr dark/light cycle and food and water were provided ad libitum. In some experiments, Dox (SAFE) was provided in the diet (0.625 g/kg). Mice were used according to animal care standards and all protocols were approved by the Committee of Animal Use for Research at the University of Seville (Spain).

Behavioral Analysis

Behavioral studies were performed in two independent cohorts of male βNrx1ΔC and littermate control mice of different ages: 2–4 months (young mice) and 7–11 months (aged mice). To avoid any effect due to the insertion of the transgene, littermate *TRE-HAβnrx1ΔC* mice were included in the control population along with nontransgenic littermates. In rescue experiments, control and βNrx1ΔC mice were fed with Dox for 2 weeks. To eliminate memory effects on the behavioral tasks, the initiation of treatment with Dox in aged mice was delayed for 2 months (age at the re-evaluation time, 9–11 months). All behavioral tasks and quantitation analysis were performed by researchers blind to the genotype of the mice.

Electrophysiology

Whole-cell patch-clamp recordings were performed in coronal slices of postnatal day 27 (P27) to P32 control and βNrx1ΔC mice. mIPSCs and mEPSCs were recorded from L5/6 pyramidal neurons of the somatosensorial cortex. The recordings and analysis of the electrophysiological experiments were performed by a researcher blind to the genotype.

Statistical Analysis

Student's t test was used for comparisons between two groups. One-way ANOVA was used in the analysis of live imaging in cultured hippocampal neurons. Two-way repeated-measures ANOVA with Bonferroni's post hoc comparison was performed in the rotarod, three-chamber, and olfaction habituation/dishabituation tests. Data were analyzed using SPSS 13.0 (IBM) and Prism5 software (GraphPad Software). All data are shown as mean ± SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.06.022.

AUTHOR CONTRIBUTIONS

L.G.R., E.R.-L., J.L.N.-G., and F.G.S. designed experiments. L.G.R. and E.R.-L. characterized mutant neurexin protein, analyzed transgenic mice, and performed behavioral experiments. J.L.N.-G. performed electrophysiological recordings. F.G.S. supervised the project and wrote the manuscript with input from all the authors.

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