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Syngap1 Haploinsufficiency Damages a Postnatal Critical Period of Pyramidal Cell Structural Maturation Linked to Cortical Circuit Assembly

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ABSTRACT

BACKGROUND: Genetic haploinsufficiency of *SYNGAP1*/*Syngap1* commonly occurs in developmental brain disorders, such as intellectual disability, epilepsy, schizophrenia, and autism spectrum disorder. Thus, studying mouse models of *Syngap1* haploinsufficiency may uncover pathologic developmental processes common among distinct brain disorders.

METHODS: A *Syngap1* haploinsufficiency model was used to explore the relationship between critical period dendritic spine abnormalities, cortical circuit assembly, and the window for genetic rescue to understand how damaging mutations disrupt key substrates of mouse brain development.

RESULTS: *Syngap1* mutations broadly disrupted a developmentally sensitive period that corresponded to the period of heightened postnatal cortical synaptogenesis. Pathogenic *Syngap1* mutations caused a coordinated acceleration of dendrite elongation and spine morphogenesis and pruning of these structures in neonatal cortical pyramidal neurons. These mutations also prevented a form of developmental structural plasticity associated with experience-dependent reorganization of brain circuits. Consistent with these findings, *Syngap1* mutant mice displayed an altered pattern of long-distance synaptic inputs into a cortical area important for cognition. Interestingly, the ability to genetically improve the behavioral endophenotype of *Syngap1* mice decreased slowly over postnatal development and mapped onto the developmental period of coordinated dendritic insults.

CONCLUSIONS: Pathogenic *Syngap1* mutations have a profound impact on the dynamics and structural integrity of pyramidal cell postsynaptic structures known to guide the de novo wiring of nascent cortical circuits. These findings support the idea that disrupted critical periods of dendritic growth and spine plasticity may be a common pathologic process in developmental brain disorders.

Keywords: Autism spectrum disorder, Development, Epilepsy, Intellectual disability, Mouse model, Synapse, *Syngap1*

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Hundreds of genetic loci are believed to contribute to commonly diagnosed neurodevelopmental disorders (NDDs), such as intellectual disability (ID), autism spectrum disorder (ASD), epilepsy, and schizophrenia (SCZ). Recent genomic sequencing studies in enriched patient populations have shown that damaging mutations commonly occur in patients with these developmental brain disorders (1–11). Collectively, these studies have demonstrated that certain commonly mutated genes, while rare in overall occurrence, have an unusually large effect on brain function during development. Based on these findings, mouse models of these mutations may provide unique insight into the pathobiology of NDDs. Indeed, because similar mutations in the same gene can lead to distinct psychiatric disorders (12,13), identification of pathobiological processes in these mouse models may also provide insight into the shared neurobiology of related, but distinct, developmental brain disorders.

Damaging mutations in SYNGAP1/*Syngap1* are causally linked to a spectrum of developmental brain disorders, including ID, ASD, epileptic encephalopathy, and SCZ (1,3,4,11,14–20). Indeed, *Syngap1* has been highlighted as a high-impact neurodevelopmental disease gene based on the convergence of several developmental brain disorders seen in patients with these damaging mutations (12,13). De novo nonsense mutations in *Syngap1* that lead to genetic haploinsufficiency are one of the most common causes of sporadic and nonsyndromic ID. Current estimates indicate that pathogenic *Syngap1* mutations account for 2% to 8% of sporadic ID cases (2,3,14,17,19) and up to 1% of epileptic encephalopathy cases (1), suggesting that there are tens of thousands of undocumented individuals carrying these mutations. By reducing expression of SynGAP protein, haploinsufficiency causes a severe comorbid syndrome with commonly overlapping

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diagnoses of ID, ASD, and epilepsy (19). However, less severe *Syngap1* mutations, such as splicing mutations, are linked to milder forms of cognitive impairment, such as those seen in SCZ (10). Furthermore, missense mutations that moderately impact SynGAP protein function lead to relatively less severe forms of ID (19). Together, the genomic data compiled from enriched patient populations point to a relationship between the extent of *Syngap1* genetic damage and severity of mental dysfunction.

There is a convergence of findings indicating that abnormal function of dendritic spine synapses contribute importantly to intellectual disability and related brain developmental disorders (21). Many high-risk loci for NDDs encode proteins that directly regulate dendritic spine synapse function (22). *Syngap1* encodes a neuron-specific RasGAP (SynGAP) that is predominantly expressed in glutamatergic neurons of the mammalian forebrain (23,24). Within these neurons, the protein is trafficked exclusively to dendritic spine synapses (24) where it negatively regulates excitatory synaptic function and dendritic spine size through suppression of the Ras/extracellular signal-regulated kinase pathway (25–27). While it is known that two intact copies of *Syngap1* are essential for proper mouse brain development and cognitive maturation (25,26), it remains unclear how haploinsufficiency of this gene impacts key neurodevelopmental substrates known to influence cognitive maturation, such as the structural development of dendrites and spines and their relationship to cortical circuit assembly.

METHODS AND MATERIALS

All procedures were performed in accordance with the Scripps Research Institutional Animal Care and Use Committee. Complete methods, experimental procedures, and statistical analyses are reported in Supplement 1.

RESULTS

To investigate the potential impact of *Syngap1* mutations on structural alterations of developing neurons, we first tracked apical dendritic arborization in young and adult somatosensory cortical pyramidal cells in wild-type (*wt*) and *Syngap1* heterozygous knockout (*het*) (28,29) mice (Figure 1A–E). Arbors traced from postnatal day (PND)21 *het* neurons exhibited increased total neurite length (Figure 1B,C) and higher complexity (Figure 1D) and occupied a larger volume (Figure 1E) compared with *wt* neurons from mice of the same age. Strikingly, *het* arbors in these young mice already had several features of older neurons, such as higher order branching and adult-like dendritic length (Figure 1B–E), indicating that the neuronal phenotype of PND21 *het* mice resulted from early maturation of arbors rather than abnormal overgrowth of dendrites. We observed that soma size predicted dendritic length in *wt* neurons at both ages but not in *hets* (Figure 1F), consistent with disruptions to growth-related processes in *Syngap1* mutants. To probe for possible signaling abnormalities underlying accelerated neuronal growth in *hets*, we examined levels of phosphorylated S6, a marker of mammalian target of rapamycin complex 1-mediated cellular growth and protein synthesis (30,31). We found that PND4 *het* mice had elevated phosphorylated S6 immunoreactivity in L5 neurons compared with *wt*s (Figure 1G). Our results in L5 neurons are consistent with a recent report demonstrating that SynGAP suppresses protein synthesis in cultured forebrain neurons (32). Together, these findings indicate that *Syngap1* mutations cause accelerated growth and/or early differentiation of developing deep cortical pyramidal cells in vivo.

We next investigated the relationship between altered dendrite maturation and spine morphogenesis in *het* L5 pyramidal neurons. We observed a striking developmental shift in both spine formation and spine pruning in *het* neurons relative to *wt*s (Figure 2A,B), indicative of an overall acceleration in the normal development of neuronal structure. Peak supernumerary spine density and the final density after pruning were indistinguishable between genotypes (Figure 2B). However, the timing of each of these key developmental milestones was clearly shifted in *hets*, with each happening at least 1 week earlier than in the cortex of *wt* mice, and appeared to map onto the time course of accelerated dendritic elongation (Figure 1B–F). Filopodia and spine steady-state density exhibit an inverse relationship in developing neurons because filopodia can be precursors of spines (33). Thus, their levels fall as dendritic spine density increases. Interestingly, we also observed a premature age-dependent reduction in filopodia density in *het* mice that was inversely related to dendritic spine density (Figure 2C), consistent with a dramatic early structural maturation of deep cortical pyramidal neurons. Finally, we also observed significantly longer dendrites in very young *het* neurons without spines (Figure S1 in Supplement 1), indicating that coordinated increases in spine density and dendritic elongation are influenced by a common upstream factor, such as the rate of postmitotic differentiation.

We next investigated if early structural maturation of neurons in *het* mice was associated with lower levels of dendritic plasticity. To test this idea, we first determined if whisker-related activity contributed to the disparities between *wt* and *het* spine density at PND21. Whisker deprivation during the period of peak spinogenesis in *het*s (PND7–21) did not normalize spine density, though this experimental manipulation did reduce peak spine density equally in each genotype (Figure 3A–C). These data indicated that intrinsic processes in the *het* brain, rather than environmental influences, drive developmental increases in steady-state spine density in superficial dendrites of deep cortical pyramidal cells. As expected, whisker deprivation caused a 2.5-fold increase in *wt* filopodia density at PND21 (Figure 3D), a cellular response associated with the functional remapping of sensory networks in response to altered experience (34). Remarkably, this response was entirely absent in *het* mice (Figure 3D), consistent with a reduction in the capacity to reorganize cortical circuits.

It is unclear how transient alterations to steady-state spine density and altered dendritic plasticity in developing *Syngap1* mutants could impact cognitive ability. One possibility is that these steady state measures reflect altered spine and filopodia dynamics, which could impact organization and target specificity of developing neural circuits. Indeed, the dynamic formation and elimination of dendritic spines reflects functional reorganization of neural circuits (33,34). Therefore, we next performed transcranial in vivo serial dendritic spine imaging to determine how *Syngap1* haploinsufficiency impacts dendritic