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A circuitry and biochemical basis for Tuberous Sclerosis symptoms: From Epilepsy to Neurocognitive deficits

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Abstract

Tuberous sclerosis complex (TSC) is an autosomal dominant monogenetic disorder that is characterized by the formation of benign tumors in several organs as well as brain malformations and neuronal defects. TSC is caused by inactivating mutations in one of two genes, *TSC1* and *TSC2*, resulting in increased activity of the mammalian Target of Rapamycin (mTOR). Here, we explore the cytoarchitectural and functional CNS aberrations that may account for the neurological presentations of TSC, notably seizures, hydrocephalus, and cognitive and psychological impairments. In particular, recent mouse models of brain lesions are presented with an emphasis on using electroporation to allow the generation of discrete lesions resulting from loss of heterozygosity during perinatal development. Cortical lesions are thought to contribute to epileptogenesis and worsening of cognitive defects. However, it has recently been suggested that being born with a mutant allele without loss of heterozygosity and associated cortical lesions is sufficient to generate cognitive and neuropsychiatric problems. We will thus discuss the function of mTOR hyperactivity on neuronal circuit formation and the potential consequences of being born heterozygote on neuronal function and the biochemistry of synaptic plasticity, the cellular substrate of learning and memory. Ultimately, a major goal of TSC research is to identify the cellular and molecular mechanisms downstream of mTOR underlying the neurological manifestations observed in TSC patients and identify novel therapeutic targets to prevent the formation of brain lesions and restore neuronal function.

Keywords

Tuberous Sclerosis Complex; tuber; mental retardation; neurogenesis; mTOR; epilepsy; autism; SEGA; seizures; FMRP; spine; dendrite; migration; differentiation; stem cell; progenitor cell

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Introduction

Tuberous Sclerosis Complex (TSC) is an inherited developmental disease characterized by discrete lesions in diverse tissues, including the skin, heart, kidney, lung, and brain (Crino et al., 2006). The incidence of TSC is estimated to be between 1:6,000 and 1:10,000 individuals (O'Callaghan et al., 1998). TSC is caused by inactivating mutations in one of two genes, *TSC1* and *TSC2*, which encode for the proteins hamartin and tuberin, respectively (European Chromosome 16 Tuberous Sclerosis Consortium, 1993; van Slegtenhorst et al., 1997). Inactivating mutations in *TSC1* or *TSC2* subsequently lead to hyperactivity of the mTOR pathway (Kwiatkowski, 2003a, b). Most patients are born with at least one detectable mutation and are thus heterozygous for either *TSC1* or *TSC2*. Very often, there are subsequent inactivating mutations of the other functional allele (Green et al., 1994; Sepp et al., 1996). This process, known as loss of heterozygosity (LOH), occurs somatically in a subset of cells, and is often detectable within peripheral and brain lesions (Kwiatkowski and Manning, 2005; Tsai and Crino, 2012), but another mechanism leading to *TSC1* or *TSC2* haploinsuffiency or alteration in another component of the TSC pathway, such as inflammation or epigenetic alterations, may also occur, but these mechanisms need further investigations (Crino, 2013).

Although TSC affects many organ systems, the neurological symptoms (i.e., seizures, mental retardation, autism, and hydrocephalus) account for the most significant mortality and morbidity (de Vries, 2010; Orlova and Crino, 2010). Seizures are observed in the vast majority of patients. They often begin during the first year of life as infantile spasms and are often unresponsive to conventional pharmacological interventions (Curatolo et al., 2012; Curatolo and Moavero, 2010). In addition, more than 50% of affected children exhibit mental retardation and cognitive delay, with many (~40%) exhibiting autistic traits (Curatolo et al., 2010; Greenstein and Cassidy, 1986; Weber et al., 2000). Presently, there are no known cures for TSC. However, thanks to an increasing understanding of the disease etiology, treatments are now on the horizon (Khwaja and Sahin, 2011).

This review explores the cytoarchitectural and functional CNS aberrations that may account for the neurological presentations of TSC, notably seizures, hydrocephalus, and cognitive and psychological impairments. In addition, this review expounds upon alterations that may be independent of gross anatomical disturbances, including changes in neuronal connectivity and plasticity that may account for cognitive and psychiatric impairments in TSC.

Following background on mTOR signaling and the genetics of the disease, approaches recapitulating the LOH-associated brain lesions will be presented. In particular, a combination of technical approaches is being used to recapitulate cortical and subcortical lesions. Most notably, *in utero* and neonatal electroporation are discussed in relation to identifying defects in neuronal positioning, morphogenesis, and functional connectivity. The next sections deal with the identification of cellular and molecular correlates of cognitive and psychiatric deficits that may arise independent of neurological lesions. In particular, the effect of TSC1/2 dysfunction on integration, connectivity, and plasticity, and the interaction of the TSC-mTOR signaling cascade with another key autism-related pathway may provide mechanistic insights into novel therapies.

TSC-mTOR signaling

TSC1, TSC2, and TBC1D7 form a heteromeric complex that can bind to and stimulate the GTPase Ras homolog enriched in brain Rheb (Dibble et al., 2012; Inoki et al., 2003; Nakashima et al., 2007; Zhang et al., 2003) (for reviews see (Kwiatkowski and Manning, 2005; Tee et al., 2002) (Figure 1). The heteromeric complex functions as a GTPase Activating Protein (GAP), which drives Rheb from an active GTP-bound state to an inactive

GDP-bound state. Active Rheb directly activates the mTOR kinase by altering substrate affinities (Sato et al., 2009). Thus, the GAP complex acts as a negative regulator of Rheb and thus mTOR. mTOR is a shared component of two complexes, mTORC1 and mTORC2 (Laplante and Sabatini, 2012a). These two biochemically distinct complexes vary in their downstream substrates; however, the actions of the TSC GAP are predominantly linked to mTORC1 signaling (Laplante and Sabatini, 2012b). mTORC1 is thought to regulate protein translation through the direct phosphorylation of eIF4E-binding protein 1 (4E-BP1) and p70 S6 Kinase 1 (S6K1), which phosphorylates the ribosomal protein S6. Activation of both 4E-BP and S6K1 are required for appropriate growth factor-dependent translation of mRNA transcripts and cell growth (Hentges et al., 2001).

In TSC, canonical inactivating mutations in *TSC1* or *TSC2* result in hyperactivation of mTORC1, constitutive phosphorylation of 4E-BP1, and activation of ribosomal protein S6 through S6K1 phosphorylation. The result is sustained translation of growth-promoting transcripts. Despite the fact that several non-canonical pathways are activated as well, inhibition of mTORC1 through rapamycin, as detailed below, is sufficient to reverse nearly all phenotypes in animal models. As a result, the contribution of mTORC2 has not been extensively explored in regards to TSC. However, like any drug, rapamycin is imperfect and could potentially, depending on dose and length of treatment, result in mTORC2 inhibition. Regardless, mTORC1 would appear to be the primary target of the TSC GAP. Finally, it remains unaddressed whether mTORC2 inhibition may also reverse cellular phenotypes seen in TSC models and which mTORC1 (noted mTOR throughout the review) substrates are required for each respective cellular process.

TSC Neurogenetics: a mosaic brain

A seminal discovery is that inactivating mutations in *TSC1* and *TSC2* are the primary cause of TSC (European Chromosome 16 Tuberous Sclerosis Consortium, 1993). TSC is inherited in an autosomal dominant fashion, which obeys a typical Mendelian distribution but has a variable penetrance. Mutations in other elements of the TSC-mTOR pathway have been hypothesized (e.g., Rheb and TBC1D7), but have not been found (Dibble et al., 2012; Qin et al., 2011; Qin et al., 2010). However, the broad neurological spectrum and unique combination of renal, cutaneous, and neural lesions suggest that a complex genetic mechanism is responsible for TSC.

Genetically, 85% of the patients meeting diagnostic criteria fall into several categories: 1) ~1/3 of the patients have inherited mutations, which can be detected in parents (Au et al., 2004), 2) a small proportion (2-3%) of patients have *de novo* mutations occurring as a result of germline mosaicism (Rose et al., 1999), and 3) the remaining patients are thought to have mutations that sporadically arose in very early somatic cells and may not equally affect all organ systems (Sampson and Harris, 1994; Verhoef et al., 1999). As a generalized rule, all patients are considered to be born with a mutant allele despite some organs exhibiting with somatic mosaicism.

Both the nature of the mutations acquired from parents or as a result of germline mosaicism and the timing of the somatic mutations are thought to contribute to the phenotypic diversity observed in TSC patients. For example, patients with mutations in the *TSC2* gene tend to have a worse prognosis than those with a *TSC1* mutation (Jansen et al., 2008; Jozwiak et al., 2006; Qin et al., 2011; Sancak et al., 2005). On the other hand, some mutations may alter regions of the encoded proteins that are required for functional output, subcellular localization, protein interactions, or for integrating upstream activating and inhibiting signals. In contrast, subtle mutations could contribute to the stability, levels, or even the enzymatic kinetics of the GAP. Thus, some mutations would render the GAP complex

inactive while others would result only in minor consequences (Goedbloed et al., 2001; Mayer et al., 2004; Sancak et al., 2005; Verhoef et al., 1998; Vrtel et al., 1996).

To render the genetics more complex is the fact that early studies pointed toward Knudson's two-hit hypothesis resulting in LOH during development. This suggests that lesions or tumors form when patients with an inherited mutation suffer a second mutation (Knudson, 2001). Evidence suggests that two hits occurring in TSC alleles may account for brain lesions, including cortical tubers and subependymal giant cell astrocytoma (SEGA) (Henske et al., 1997; Jozwiak et al., 2004; Roberts et al., 2004). Importantly, it should be noted that inactivating mutations resulting in a dominant negative function could result in *TSC1* or *TSC2* haploinsufficiency and brain lesions without LOH. The timing and location of a second hit could lead to differences in lesion size and location that could also explain differences in neurological outcomes. In addition, considerable debate still exists about other potential environmental and genetic modifiers that may influence patient outcomes.

In conclusion, TSC patients are most commonly born with at least one single functional allele of *TSC1* or *TSC2*. As part of a second hit, the functional allele often becomes mutated in a founder somatic cell resulting in LOH or haploinsufficiency and lesion formation. The cortical lesions discussed below are associated with seizures and presumably contribute to the severity of the disease.

Seizures and cortical malformations in TSC

Post-mortem analysis of epileptic brains has revealed that a wide spectrum of structural abnormalities is associated with seizures (Andrade, 2009; Andrade and Minassian, 2007; Bentivoglio et al., 2003). For example, gray matter sub-band heterotopia, lissencephaly, and focal cortical dysplasias (FCDs) are associated with epilepsy (Palmini et al., 2004). TSC is a subtype of FCDs, which were historically classified as disorders of glioneuronal proliferation and differentiation (Wong, 2008).

Epileptic seizures occur in 75% of TSC patients and ~30-40% of the cases are unresponsive to conventional drug therapies (Holmes and Stafstrom, 2007; Jansen et al., 2006; Jansen et al., 2007). The majority of TSC patients present with infantile spasms. Although children often outgrow these spasms, they typically acquire other seizure types. Besides surgery, few options exist for the treatment of these patients because they are pharmacoresistant to classic therapies. Thus, a major goal for TSC research has been aimed at understanding the etiology of cortical lesions and identifying novel treatments.

Cortical tubers are focal cerebral cortical malformations that are thought to form *in utero* during corticogenesis (Kwiatkowski and Short, 1994; Wortmann et al., 2008). They are currently targeted for resection in pharmacologically intractable epileptic TSC patients, and their removal resolves seizures in a subpopulation of patients (Bollo et al., 2008). Indeed, cortical tubers co-localize by magnetic resonance imaging (MRI) with focal inter-ictal discharges detected by electroencephalography EEG (Jansen et al., 2003; Jansen et al., 2006; Jansen et al., 2005). In agreement, reduced metabolism and elevated neurotransmitter precursor uptake in seizure areas overlap with PET scans (Chugani et al., 1998; Kato et al., 1997). Candidacy for surgical resection includes the presence of focal seizures and hence EEG alterations and the identification of a single epileptiform tuber. However, as detection methods become more sensitive, there is a realization that most surgeries present with a much more complex scenario (Bollo et al., 2008). Regardless, the range for successfully reducing seizures following surgery is 22-67% according to a recent review (Evans et al., 2012). Furthermore, cognition and social responsiveness (i.e. autistic behaviors) are inextricably correlated with early seizure onset and the number of seizures in TSC. As a result, early surgical intervention could potentially improve other neurological outcomes

(van Eeghen et al., 2012). However, whether epilepsy causes autism or cognitive defects is still a point of contention. Indeed, the correlation could be due to similar network or biochemical abnormalities.

How cortical tubers contribute to seizure activity or epileptogenesis remains unclear. It has been suggested that cortical tubers do not contribute themselves to seizure initiation as tubers seem to be electrically silent (Major et al., 2009). However, it remains unknown whether young tubers may be epileptogenic and progressively lead to short- and long-distance generations of seizure foci accompanied by progressive silencing of the tuber activity over time and progressive inflammation (Boer et al., 2008). Indeed, cortical tubers were showed to display decreased levels of $GABA_A$ receptors despite increase GABA levels perhaps as a compensatory mechanism (Mori et al., 2012). In addition, cortical giant cells or cytomegalic cells display an immature complement of glutamate receptors and may thus display altered maturation (Talos et al., 2008). Alternatively, during cortical tuber formation, the surrounding cortex may experience seizure-prone re-organization and synaptic activity changes. For example, normal appearing neurons in or around human tubers display increased excitatory synaptic drive (Cepeda et al., 2010). In addition, it was reported that synaptic excitation was altered in a direction that favors seizure generation in TSC brain tissue regardless of cortical tubers (Wang et al., 2007). Finally, the contribution of reactive astrocytes as a determinant of seizure initiation/worsening or a consequence of seizure activity remains unclear (Feliciano et al., 2011; Wong and Crino, 2012).

Histologically, tubers are characterized by gliosis, loss of lamination, a unique type of cell known as a giant cell, and cytomegalic neurons that are ectopically located and dysmorphic (Figure 2 and 3) (Mizuguchi, 2007; Mizuguchi and Takashima, 2001; Yamanouchi et al., 1997a; Yamanouchi et al., 1997b). Additional anatomical alterations are illustrated in Figure 2. Near the base of the cortical tuber, clusters of cytomegalic neurons commonly form white matter nodules (or heterotopias) that are accompanied by hypo-myelination. Occasionally tubers become cystic and even calcified (Rott et al., 2002). Since the TSC1/TSC2 complex negatively regulates mTOR activity (Baybis et al., 2004; Crino, 2004; Tee et al., 2002), the enlarged neurons of cortical tubers have high mTOR activity as measured by increased ribosomal protein S6 phosphorylation.

Mouse models of TSC brain lesions and discrete lesion formation via *in utero* electroporation

To recapitulate the neurological manifestations observed in TSC patients and to gain a better understanding of the pathological etiology, several transgenic mouse models have been generated (Table 1). Transgenic mice with TSC genes removed from specific cell populations have been intensively studied. For example, in one of the first murine TSC models both *Tsc1* alleles were removed in cells expressing the mouse glial fibrillary acidic protein (GFAP)-promoter (Uhlmann et al., 2002). The mouse *gfap* promoter is not expressed during embryonic life and thus essentially targets astrocytes and adult neural progenitor cells. These mice displayed severe seizures and reduced survival. A subsequent study by Meikle et al. (2007) used mice carrying a mutant and a conditional (floxed, fl) *Tsc1* allele crossed with mice carrying a *synapsin I* promoter-driven Cre recombinase (SynI-Cre) allele (Meikle et al., 2007). These mice lost *Tsc1* in neurons starting at ~embryonic day (E) 12.5 and displayed severe alterations that mimicked many of the TSC brain alterations in humans (e.g., seizures and enlarged and dysplastic cortical neurons). Another model was generated by crossing mice that have a mutant and a conditional *Tsc2* allele with mice expressing Cre under the human *gfap* promoter, resulting in the loss of the conditional allele in radial glia (embryonic neural progenitor cells) at ~E12 and their progeny, including neurons and astrocytes (Way et al., 2009). These mice were severely runted, developed macrocephaly, and died between 3 and 4 weeks of age, presumably from seizures. At the cellular level,

there were cortical and hippocampal lamination defects, hippocampal heterotopias, enlarged dysplastic neurons and glia, abnormal myelination, and astrocytosis. A more recent model used mice expressing the *nestin* promoter driving Cre crossed with either mice containing a mutant and a conditional *Tsc1* allele, or two conditional *Tsc1* alleles (Anderl et al., 2011; Goto et al., 2011). While the former mice die by postnatal day (P) 1, the latter survive longer and develop almost all TSC pathological hallmarks including giant cells (over time). Additional models include mice that express Cre driven by the *Emx1* or the *Dlx5/6* promoter, in which recombination essentially occurs in neural progenitor cells generating glutamatergic neurons and astrocytes, and in GABAergic neuronal precursors, respectively (Carson et al., 2012; Fu et al., 2012; Magri et al., 2011; Way et al., 2009).

These models are in agreement with several concepts. First, LOH using a mutant background results in ectopic neuronal positioning or mislamination. Second, neurons are enlarged and dysplastic, and show enhanced mTOR pathway activity. Third, either seizures or susceptibility to seizures is a key phenotype. In addition, almost all of these effects are dependent on mTOR hyperactivity as prenatal or postnatal rapamycin reverses the above defects.

Discrete lesion formation through *in utero* electroporation

These studies provide an important step towards understanding TSC pathology. However, one major limitation of the above mouse models is the inability to recapitulate the discrete nature of the lesions seen in patients. To generate discrete TSC-like lesions, we used *in utero* electroporation (IUE) (Feliciano et al., 2011; Walantus et al., 2007). IUE consists of introducing DNA constructs to the lateral ventricles of embryos while *in utero*. Using pulses of current across the head of the embryo through the uterine wall, the DNA plasmids are introduced into the neural progenitor cells lining the ventricles (i.e., electroporation). IUE is a versatile approach because any plasmids can be electroporated into a desired region and to distinct cell types depending on the embryonic stage at the time of electroporation and the cell-type-specific promoter used (Tabata and Nakajima, 2001). In addition, to recapitulate LOH a double-hit strategy was used by targeting transgenic mice carrying a conditional and a mutant (mut) *Tsc1* allele. Thus, by introducing a Cre-encoding plasmid through IUE, the conditional *Tsc1* allele was knocked out in embryonic neural progenitor cells. Electroporations can be reliably performed as early as E12.5 (Figure 3) and as late as E16. In agreement with published data, neurons were ectopically localized throughout the cortex and generated large clusters in and above the corpus callosum (i.e., white matter), which we refer to as white matter nodules (Feliciano et al., 2011) (Figure 3). Furthermore, the tuber-like lesions displayed mosaicism characterized by the presence of $Tsc1^{null}$ neurons with elevated phosphorylated S6, enlarged somas, and hypertrophic dendritic trees in a heterozygote brain. This technique represents a significant advancement in the field, allowing the creation of discrete lesions and the control of the timing of LOH and the affected region (Wong, 2012). For example, while we have focused on the somatosensory cortex, the anterior cingulate cortex can be targeted, allowing for behavioral studies and cognitive deficit assessment. However, it should be added that some aspects of cortical tubers, including gliosis, demyelination, and the presence of giant cells, were absent in this model. It remains to be seen whether the differences are a limitation or an advantage of this model distinguishing mTOR-dependent pathology from the side effects of seizures. Nevertheless, this approach can be applied in a similar manner to a spectrum of ages, including neonates (see section on SEGA), which allows for rapid modeling and experimentation supplementing existing transgenic mouse models.

Discrepancies between models

The *mgfap, hgfap* and inducible *nestin* promoter-driven models report enhanced phosphorylated S6 levels in astrocytes (Goto et al., 2011; Uhlmann et al., 2002; Way et al., 2009). In contrast, the IUE model (henceforth referred to as "tuber-like") and two Emx1 models report no activation of the mTOR pathway in astrocytes despite removing *Tsc1* from labeled astrocytes (Carson et al., 2012; Feliciano et al., 2011; Magri et al., 2011). These results suggest that perhaps astrocyte activation is secondary to seizures. However, additional explanations include the possibility that mTOR may have different substrates in neurons and astrocytes or that TSC may activate mTOR-independent pathways in each cell type. However, the ability of rapamycin to revert many TSC defects does not support the latter hypothesis. Coincidentally, several recent studies reported that convulsants such as pentylenetetrazol and pilocarpine can also activate mTOR in astrocytes (Zeng et al., 2009; Zhang and Wong, 2012). An additional missing piece to the tuber puzzle is that *bona fide* giant cells were absent in all but the *nestin* model (Goto et al., 2011). In this model, the authors elegantly showed that mixed marker-expressing giant cells, which were highly vacuolated, appeared in the cortex only after months of seizures (4 months). The clear demonstration that it takes months for these cells to appear suggests that their role in the pathogenesis of TSC is minimal. Further studies on their importance are, however, clearly warranted.

Collectively, the generation of multiple inducible transgenic mouse models has facilitated the study of brain defects in TSC. These models permit selective ablation of TSC genes in specific cell types, allowing examination of the role of TSC1 and TSC2 during development and the study of TSC pathogenesis. Neurological defects exhibited by these mice include ectopic neural positioning, mislamination, enlarged neurons with enhanced mTOR activity, and seizures. The IUE model employed by our laboratory addresses a limitation of previous models, as the induction of LOH by introducing a plasmid that deletes *Tsc1* from embryonic progenitor cells on a mutant background recapitulates the discrete lesions seen in patients. The efficacy of rapamycin in rescuing many of the defects observed in these models supports the conclusion that many of the neurological manifestations associated with TSC are reversible and mTOR-dependent.

Subependymal nodules (sen) and sega

SENs are among the most common brain lesions associated with TSC (95% of the patients) and are contiguous with the lateral ventricles (LVs) (Bender and Yunis, 1980). SENs contain enlarged neurons and glia as well as giant or multinucleated cells similar to those observed in tubers. These multinucleated cells may present markers for both neural and glial lineage (Taraszewska et al., 1997). SENs may calcify and harden over time or enlarge. Lesions that are less than 10 mm in diameter are considered nodules. Lesions that are greater than 10 mm with more than 5 mm of growth are defined as SEGAs. SEGAs are classified as benign, slow-growing, grade I astrocytomas (Ess et al., 2005), express both neuronal and astrocytic markers, and have a low proliferative index (Jozwiak et al., 2008).

Continued growth of SEGAs may block the flow of the cerebral spinal fluid (CSF) resulting in hydrocephalus and surgical intervention to remove the SEGA (Campen and Porter, 2011). Treatment with rapamycin can ablate growth and even reduce the size of SEGAs and SENs, but these therapeutic gains are reversed once treatment is halted (Franz et al., 2006). In addition, Everolimus, a 40-O-(2-hydroxyethyl) derivative of rapamycin, has been recently approved for use in treating SEGAs. Everolimus selectively blocks mTORC1 activity, whereas rapamycin is less selective and carries side effects.

Origin and modeling SENs and SEGAs

SENs were recently modeled by using *Emx1*-Cre transgenic mice crossed with *Tsc1*$^{fl/mut}$ mice (Magri et al., 2011). Gliogenesis and neurogenesis are protracted through embryonic development into the first year of life in the subependymal zone (SEZ, also more routinely called the subventricular zone or SVZ) in humans and though adulthood in rodents (Bonfanti and Peretto, 2011; Gould, 2007). During the first two to three postnatal weeks in mice (a ~10 day-old mouse is a newborn infant), NPCs contribute to gliogenesis as well as olfactory bulb and subcortical (e.g., piriform cortex, nucleus accumbens) neurogenesis and to some extent cortical (De Marchis et al., 2004; Feliciano and Bordey, 2012; Pathania et al., 2010; Seki and Arai, 1991). Considering that SEGAs in TSC patients demonstrate biallelic mutation of *TSC1* or *TSC2* genes resulting in increased mTOR activity (Chan et al., 2004), two recent studies hypothesize and tested whether neonatal neurogenesis contributes to SEN and SEGA generation (Feliciano et al., 2012; Zhou et al., 2011).

Both reports used *Tsc1*$^{fl/fl}$ mice crossed with mice expressing an inducible Cre (CreERT2) under the *nestin* promoter. The studies found that removal of the conditional *Tsc1* alleles resulted in mTOR up-regulation and the formation of nodules along the SEZ wall and SEGA-like lesion at the base of the lateral ventricle. Zhou et al. (2011) further found that the SEZ and nodules contained ectopic neurons and that proliferation was not altered, leading them to conclude that the formation of SENs result from migration deficits. Migratory alterations of newborn neurons were recently confirmed as detailed in the next section (Feliciano et al., 2012; Lafourcade et al., 2013).

In conclusion, SEN and SEGA can arise from neural progenitor cells in the embryonic and neonatal following TSC1 loss in mice. Considering that P7-P10 in mice corresponds to a newborn infant, these SEZ derived malformations may arise perinatally in humans.

OLFACTORY HAMARTOMAS AND ECTOPIC NEURON DIFFERENTIATION

Individuals with TSC display lesions (referred to as nodules or hamartomas) in the forebrain, such as the olfactory and basal ganglia structures (Braffman et al., 1992; Cusmai et al., 1990; de León et al., 1988; Gallagher et al., 2009; Inoue et al., 1998; Raznahan et al., 2007; Ridler et al., 2004). Importantly, using neonatal electroporation to delete *Tsc1* or express a constitutively active Rheb selectively in NPCs of the SVZ, we also reported the presence of heterotopia along the migratory path to the olfactory and olfactory structures, micronodules in the olfactory bulb, and ectopic neurons in the nucleus accumbens and the cortex (not examined in Rheb condition) (Feliciano et al., 2012; Lafourcade et al., 2013). Neonatal electroporation was recently described and visually documented (Feliciano et al.; Lacar et al., 2010). *Tsc1* removal was performed in *Tsc1*$^{fl/mut}$ mice while Rheb was expressed in wild-type mice. This is important as it suggests that the reported defects can result from mTOR upregulation in individuals born without a mutated allele.

Ectopic neurons were absent or rare in the control brains using our approach. In the nucleus accumbens, neurons were located at the base of the lateral ventricle and around the anterior commissure. In the olfactory structures and along the migratory path, fluorescent cells were identified as neurons based on NeuN immunostaining and patch clamp recording. NeuN expression was observed earlier than in the control conditions, suggesting premature differentiation of newborn neurons despite their ectopic location. Ectopic neurons displayed an enlarged morphology and biophysical properties of projection neurons, suggesting that they may be glutamatergic, but this remains to be examined. Despite being ectopic, these neurons survived, received synaptic currents, and integrated in the surrounding circuit. These studies also reported that neurons in the olfactory bulb exhibited hypertrophic dendritic trees. Finally, most of the defects were prevented by rapamycin treatment,

suggesting their mTOR-dependence. Interestingly, a recent study also suggested that *PTEN* deletion in NPCs of the SVZ using inducible *nestin*-Cre mice led to premature newborn neuron differentiation associated with migration arrest (Zhu et al., 2012). The defect was also mTOR-dependent. Nevertheless, differences exist between these two studies, such as the dramatic expansion of the SVZ in the PTEN study, which may result from differences in pathway activation. For example, *PTEN* and *Tsc1* deletion lead to Akt activation and repression, respectively (Endersby and Baker, 2008).

The mechanisms downstream of mTOR by which TSC1/2 and Rheb alter SEZ neurogenesis remain unknown and should clearly be examined. By concentrating on developing therapies that target specific downstream elements of the mTOR pathway, fewer side effects and greater efficacy may be achieved.

In conclusion, neonatal loss of *Tsc1* in SEZ neural progenitor cells leads to severe malformations in olfactory structures that resemble those seen in TSC patients and altered neuronal dendritogenesis. Due to the relative simplicity of generating these defects, this model system can allow investigators to identify conserved downstream molecules responsible for cell ectopic placement, premature differentiation, and hypertrophic dendrites in hope to rescue circuit alternations in TSC.

Altered connectivity in TSC: contribution to neurological deficits?

Cognitive decline/deficits and neuropsychiatric problems could result from tuber burden and related seizures. However, it has become evident that these cognitive dysfunctions are not fully explained by tuber burden and may result from the heterozygous state. This hypothesis suggests that loss of a single copy of *TSC1/2* can result in defects in connectivity and/or biochemical function (see next section) at synapses. The structural foundations of communication and connectivity in the CNS are the dendrites, synapses, and axons. While dendrites behave as antennae and receive signaling inputs, the axons are the wiring relays of the connective network. One way to assess changes in connectivity, therefore, is to analyze changes to the development, maintenance, and morphology of these structures. We discuss here the function of the TSC-mTOR pathway on these structures in wild-type neurons and then the defects reported for these structures in the context of TSC, including both LOH and heterozygous models.

Dendrites

There is strong *in vitro* evidence for the importance of mTOR signaling in dendritic arborization (Jaworski et al., 2005; Kumar et al., 2005; Urbanska et al., 2012),. However, it was mentioned that deletion of *Tsc1* in hippocampal pyramidal neurons postnatally (P14-16) in $Tsc1^{fl/fl}$ mice did not alter dendritic branching (Bateup et al., 2011).

In the context of TSC, *Tsc1* loss driven in *SynI-Cre* $Tsc1^{fl/mut}$ mice causes sporadic cortical pyramidal neurons with increased apical dendrite thickness and abnormal polarity (Meikle et al., 2008; 2007). Furthermore, more complex basal dendrites are observed in pyramidal neurons and olfactory bulb granule neurons following inducible deletion of *Tsc1* in perinatal neural progenitor cells in $Tsc1^{fl/mut}$ mice (Feliciano et al., 2012; 2011; Goto et al., 2011). Thus, when comparing these studies, it remains to be examined whether the mTOR-induced increase in dendritic arborization is limited to a developmental time-window.

Spines

Spines are submicron membranous protrusions located primarily on dendrites of many neuronal types where >90% of excitatory synapses terminate (Harris and Kater, 1994). These primary sites of communication between two neurons are highly plastic, existing in a

variety of shapes and sizes and adapting constantly to the experience and wiring needs of the organism. Disruptions in dendritic spines have been linked to mental retardation and autistic phenotypes (Kelleher and Bear, 2008).

Removal of *Tsc1* in postmitotic pyramidal neurons from *Tsc1*$^{fl/fl}$ mice resulted in fewer spines and increased spine length and head width in rodent hippocampal slice cultures (Tavazoie et al., 2005). There was also a similar but less pronounced phenotype following removal of only one *Tsc1* allele, suggesting that the *Tsc1* loss-induced spine defects are gene dosage-dependent. Furthermore, rapamycin partially blocked the effects of *Tsc1* loss on spine defects, implicating mTOR in the regulation of dendritic spines. However, the same group reported that *Tsc1* ablation in post-differentiated CA1 pyramidal neurons following *in vivo* injection of a Cre-encoding virus in the same *Tsc1*$^{fl/fl}$ mice led to no significant changes in spines density or morphology (Bateup et al., 2011). Thus, the effects of *Tsc1* on spine density and morphology are currently unclear and may be region- and time-dependent.

In TSC patients, cortical biopsy and postmortem cortex have shown fewer spines on abnormally shortened dendrites of principal projection neurons found in tubers (Huttenlocher and Heydemann, 1984; Machado-Salas, 1984). Consistent with this finding, conditional ablation of *Tsc1* in *Tsc1*$^{fl/mut}$ mice early in cortical development also reduces spine densities in ectopic and dysplastic pyramidal neurons in the cortex without affecting spine length (Meikle et al., 2008). The effect is rapamycin-sensitive, implicating the involvement of mTOR signaling. In Purkinje cells, both homozygous and heterozygous deletion of *Tsc1* during development significantly increased spine density at 4 weeks of age (Tsai et al., 2012a).

Axons

White matter abnormalities are observed in TSC patients using diffusion tensor imaging, suggesting problems in axonal architecture and poor myelination (Krishnan et al., 2010; Widjaja et al., 2010). Axonal targeting is dependent on both intrinsic cues and on contextual signaling cues from the surrounding environment. Nie et al. (2010) reported that molecules involved in the TSC-mTOR pathway tend to aggregate in the axonal processes of some neuronal cell types. One of the first studies on the role of the Tsc1/2-mTOR pathway in axon specification reported that inactive (phosphorylated) *Tsc2* compartmentalizes in the neurite destined to become the cell's axon along with activated Akt and activated S6K1 (Choi et al., 2008). However, it is unknown whether phosphorylation at a single site serves as a predictor of activity levels for *Tsc2*. Regardless, the same study reported that when *Tsc1* or *Tsc2* are overexpressed in cultured neurons, mTOR activity is suppressed and axon formation is reduced. In contrast, when *Tsc1* or *Tsc2* expression are knocked down, neurons express multiple axons. When *Tsc1* is removed specifically from neurons *in vivo*, axons spread abnormally throughout the cortex instead of concentrating in the intermediate zone (Choi et al., 2008). In further support of the TSC complex playing a role in cell polarization and axon specification, *Tsc1/2* knockdown leads to an mTOR-dependent increase in SAD kinase, which is known to play a crucial role in axon development (Kishi et al., 2005). Additionally, mTOR regulates the translation of Tau and collapsing response mediator protein 2 (CRMP2), the expression of which determines cell polarity and axon specification (Morita and Sobue, 2009). In *Drosophila melanogaster*, when *Tsc1* is removed in photoreceptor neurons that project to a wild type brain, defects in photoreceptor axon guidance are seen at several different developmental stages (Knox et al., 2007).

In the context of TSC, *Tsc1* loss driven in *SynI-Cre Tsc1*$^{fl/mut}$ mice led to persistent growth of axons presumably responsible for demyelination, but axonal projections appeared normal (Meikle et al., 2007). In *Emx1*-Cre mice crossed with *Tsc1*$^{fl/mut}$ mice, pyramidal neurons showed abnormal projections and disarranged neurites and axons, but analysis of the defects

remains to be explored (Magri et al., 2011). In mice heterozygous for *Tsc2*, retinal ganglion cells (RGCs) have elevated mTOR activity. RGC projections to the geniculate nucleus, which develop postnally and normally target the geniculate nucleus, are shifted in these mice. These aberrant connections are found to be caused by misregulated EphA-ephrin-A signaling, which can normally activate TSC via ERK (Nie et al., 2010).

In conclusion, it has been demonstrated that *Tsc1* loss during development has a detrimental effect on connectivity. However, whether *Tsc1* plays a role in circuit plasticity in postmitotic neurons remains unclear. In the context of TSC, it is clear that tuber cells display abnormal connectivity, but defects in connectivity have not been clearly shown in *Tsc1* heterozygous mice. In addition, the full cohort of downstream molecules disrupted in TSC that result in altered connectivity has yet to be identified. There are some clues as to which molecules involved in axon formation are disrupted in TSC, but their correlates in dendrite formation remain unknown. It is also currently unknown whether loss of *Tsc1* has different effects on a mutant or wild-type background, and whether being born heterozygous has a different impact on connectivity than acquiring an inactivating mutation in an allele post-development does.

Collectively, these studies demonstrate that changes in TSC-mTOR signaling likely result in aberrant network connectivity in TSC, but very little has been explored in heterozygote animals, which do not display cortical malformation (tubers).

Evidence for a biochemical basis of neurocognitive dysfunction in TSC

While the relationship between severe “second-hit” pathologies and cognitive performance is clear (O’Callaghan et al., 2004), they only partially account for the reduced cognitive performance observed in affected individuals (Joinson et al., 2003). Moreover, TSC patients with normal IQ exhibit subtler problems such as dypraxia, speech delay, memory impairment and dyscalcula (Jambaque et al., 1991). It is therefore possible that *TSC1* or *2* heterozygosity, independent of major pathology, contributes to impaired cognitive function independent of more severe lesions.

Neurocognitive dysfunction in TSC rodent models

Pharmacological and genetic mTOR manipulations and TSC mouse models have deficits in cognitive and autistic behaviors as well as impaired synaptic plasticity (summarized in Table 2) (reviewed by (Hoeffer and Klann, 2010b)). Plasticity is the cellular correlate of learning and memory; it is often measured through induction of long-term potentiation (LTP) or long-term depression (LTD), which describes a strengthening or weakening of synaptic strength, respectively. Some of the discrepancies among models likely arise from differences in animal models and also protocols used to induce synaptic plasticity. Importantly, $Tsc2^{wt/mut}$ mice and rats, and $Tsc1^{wt/mut}$ mice (wt for wild-type) display social interactions and learning and memory defects in the absence of anatomical defects or seizures (Goorden et al., 2007)(see Table 2 for references). While plasticity has not been examined in $Tsc1^{wt/mut}$ mice, the $Tsc2^{wt/mut}$ rats and mice display impaired synaptic plasticity. This deficit was rescued by rapamycin treatment of $Tsc2^{wt/mut}$ mice (Ehninger et al., 2008). *hgfap* × $Tsc2^{fl/mut}$ mice, which display several cortical abnormalities, exhibited cognitive deficits that were rescued with rapamycin treatment. Intriguingly, in this model, the rescue depended on the timing of drug treatment (Way et al., 2012). It is also intriguing that the degree of decrease in TSC2 levels (using hypomorphic conditional mice), which also display cortical abnormalities, correlated with behavioral abnormalities in anxiety, social interaction and learning assays (Yuan et al., 2012). Mice with cortical abnormalities also display social and cognitive impairments that were rapamycin-sensitive and different from those in heterozygote mice, which do not show gross anatomical alterations.

Collectively, these data suggest that alterations in connectivity independent of cortical lesions as discussed above or biochemistry of the synapse (see below) may account for some of the cognitive and social deficits. In addition, cognitive and social deficits can be rescued with rapamycin treatment.

A biochemical hypothesis for the cognitive deficits in TSC

Within cortical lesions, TSC-complex inactivation leads to unchecked mTOR activity and runaway protein synthesis resulting in the cytomegaly and architectural distortions that contribute to epilepsy and impaired cognition. Apart from these lesions, emerging data suggest that altered mTOR activity and protein translation in the heterozygous state may also contribute to impaired cognition. Indeed, the fact that rapamycin corrected plasticity and learning defects in lesionless *Tsc2*$^{+/-}$ mice (Ehninger et al., 2008) suggests that mTOR activity is altered in the *TSC1* or *2* heterozygous state and contributes to impaired learning and behavioral memory. It is notable that altered mTOR signaling, and subsequently affected protein synthesis, is a shared feature of a number of other neurodevelopmental disorders with high rates of autism and mental retardation: mTOR signaling is increased in TSC, Phosphatase and Tensin Homologue Hamartoma Syndromes, Neurofibromatosis and Fragile X Syndrome (Gipson and Johnston, 2012) and decreased in models of Rett syndrome (Ricciardi et al., 2011). Moreover, altered gene dosage of many of the components of the mTOR pathway leads to abnormal plasticity and or learning in mouse models (*Tsc1, Tsc2, Fkbp1a, S6k1/2, Eif4e-bp2*)(Hoeffer and Klann, 2010a).

Despite extensive characterization of the TSC-mTOR-protein translation axis and documentation of mTOR hyperactivity in the *TSC1/2* null state, the activity of the mTOR in the heterozygous state has received little attention. A simple model would predict that mTOR signaling is intermediate between the *Tsc* wildtype and null state, however, this has not been rigorously demonstrated. Elevated phosphoS6 $S^{235/236}$ has been observed in *Tsc2*$^{+/-}$ mice (Ehninger et al., 2008), however, these residues can also be phosphorylated by RSK (Roux et al., 2007) and PKA (Moore et al., 2009), and therefore may not be as faithful a readout of mTOR activity as phosphoS6 $S^{240/244}$. While the rapamycin rescue of learning is indicative of elevated mTOR signaling in *Tsc2*$^{+/-}$ mice, a separate study directly examined protein synthesis in acute hippocampal slices from *Tsc2*$^{+/-}$ and found that basal protein translation is reduced in these animals. Paradoxically, this reduction in protein translation was reversed by inhibition of mTOR (Auerbach et al., 2011).

Taken together these data suggest that TSC complex heterozygosity may be sufficient to alter mTOR signaling, protein translation, plasticity, learning and memory. The precise molecular events underlying these disturbances, however, remain to be elucidated (Auerbach et al., 2011; Gipson and Johnston, 2012; Hoeffer and Klann, 2010b; Moore et al., 2009; Ricciardi et al., 2011; Roux et al., 2007).

Conclusions

Despite the seemingly complex neurological presentation of TSC, three unifying themes have propelled the field toward a greater understanding of the etiology. First, inactivating mutations in *Tsc1* or *Tsc2* are the genetic cause of TSC. Most of the patients are born with a mutant *Tsc1* or *Tsc2* allele leading to different degrees of loss of function. A second hit occurring during development leads to LOH or severe haploinsuffiency in neural progenitor cells and their progeny, resulting in the formation of cortical and ofactory bulb lesions as well as SENs and SEGAs during perinatal life. Clearly the timing and location of the second hit will determine the extent of the lesions and their location, which will affect the occurrence and severity of seizures and likely contribute to progressive cognitive impairments. The cortical lesions contribute to epileptogenesis, perhaps through circuit

reorganization, but the mechanism remains to be identified. Independent of the second hit, being born with a mutant allele may be sufficient to create circuit (dendrites, spines, axons) and biochemical alterations associated with neuropsychiatric and neurocognitive deficits. Second, inactivating mutations lead to increased mTOR kinase signaling and likely changes in parallel signaling pathways (e.g., Notch (Karbowniczek et al., 2010; Ma et al., 2010) and ERK (Chevere-Torres et al., 2012a)). These pathways need to be further examined. Finally, perhaps one of the most exciting findings is that mTOR inhibition may be sufficient to prevent lesion and seizure formation and reverse cognitive deficits in rodents. Thus, early detection of *Tsc1/Tsc2* mutations and early intervention using mTOR pathway inhibitors should provide patients and their families the greatest tools in the fight against TSC. Nevertheless, one of the important avenues of investigation is to identify mTOR-downstream molecules responsible for specific defects such as in migration, spines, or synaptic biochemistry. This would allow more specific treatment while limiting drug-induced side effects.

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Abbreviations

CNS	Central Nervous system
CreERT2	Inducible Cre
CSF	Cerebral spinal fluid
EEG	Electroencephalography
4E-BP1	eIF4E-binding protein 1
E	Embryonic day
fl	Floxed
FCDs	Focal cortical dysplasias
FMR1	FMRP gene
FMRP	Fragile X mental retardation protein
FXS	Fragile X syndrome
GFAP	Glial fibrillary acidic protein
GAP	GTPase Activating Protein
hgfap	*Human gfap*
IUE	*In utero* electroporation
LV	Lateral ventricle
LOH	Loss of heterozygosity
LTD	Long-term depression
LTP	Long-term potentiation
MRI	Magnetic resonance imaging
mTOR	mammalian Target of Rapamycin
mgfap	*Mouse gfap*
mGluR-LTD	Metabotropic glutamate receptor class I long term depression

mTORC1 or mTORC2	mTOR complex 1 or 2
P	Postnatal day
PP2A	Protein phosphatase 2A
RGCs	Retinal ganglion cells
Rheb	Ras homolog enriched in brain
S6K1	p70 S6 Kinase 1
SEGA	Subependymal giant cell astrocytoma
SEN	Subependymal nodules
SEZ	Subependymal zone
SynI-Cre	*Synapsin I* promoter-driven Cre
***Tsc1*$^{fl/fl}$**	Floxed *Tsc1* alleles (transgenic mice)
***Tsc1*$^{fl/mut}$**	Floxed and mutant *Tsc1* alleles
***Tsc1*$^{wt/mut}$**	Wildtype and mutant *Tsc1* alleles
TSC	Tuberous sclerosis complex
TSC1 or TSC2 TSC	*gene 1 or gene 2*

Highlights

1. Generation of cortical tuber-like lesions using in utero electroporation in mutant *Tsc1* mice
2. Increased mTOR activity altered neurogenesis and circuit formation
3. Circuit dysfunction and biochemical dysregulation at synapses may account for cognitive and psychiatric impairments in tuberous sclerosis complex

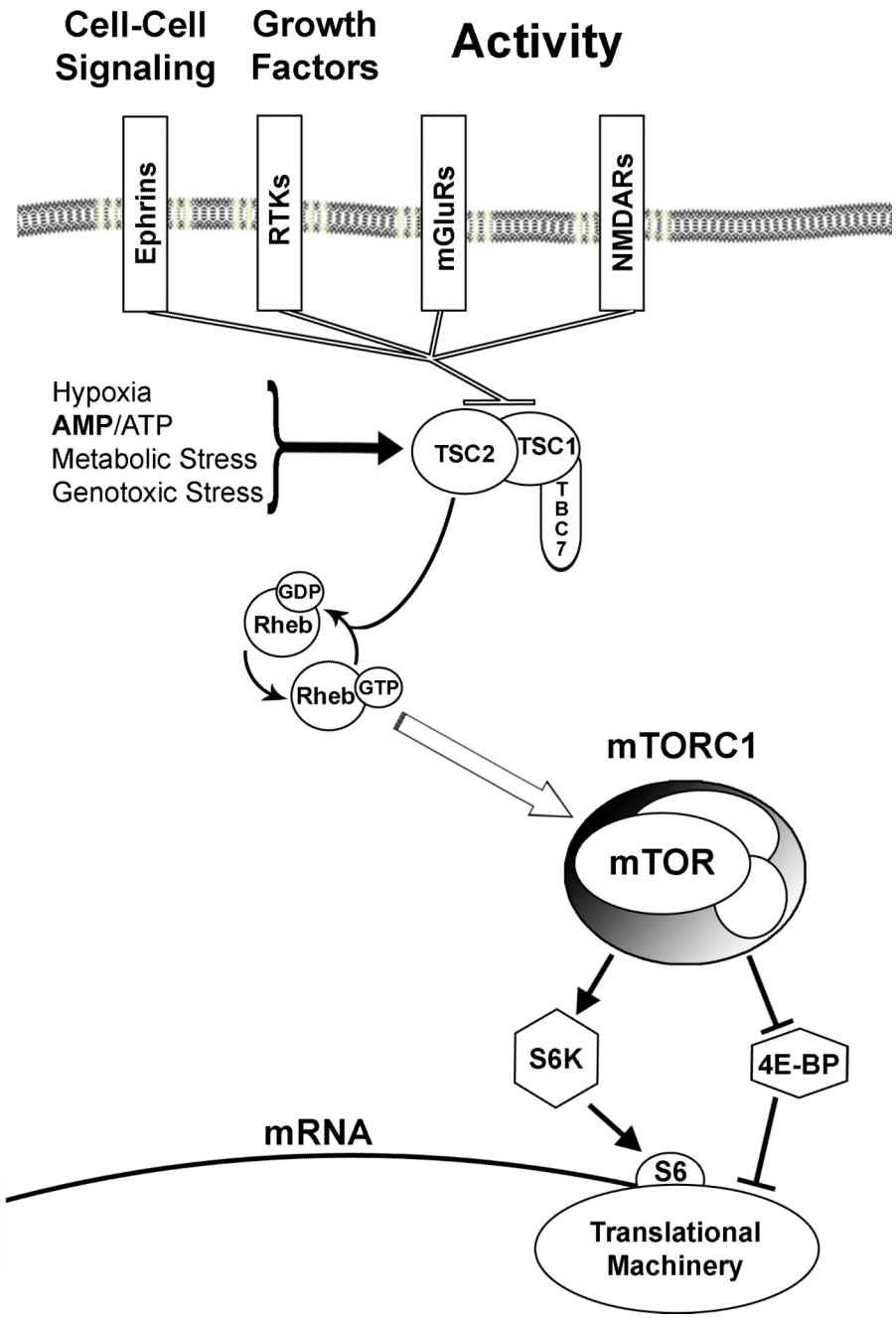

Figure 1. Simplified mTOR signaling pathway

Activation of several receptors on the cell membrane including (but not limited to) ephrin, growth factor receptor, mGluR, and NMDA receptor (NMDAR) leads to inhibition of the hamartin/tuberin/TBC1D7 (TSC1/TSC2) complex. By contrast, hypoxia, AMP/ATP ratio, metabolic stress, and genotoxic stress lead to increased TSC1/TSC2 complex activity. Inhibition of the complex activity relieves a block on Rheb activity by allowing it to become GTP-bound (active)_resulting in mTORC1 activation. Upon activation, mTORC1 phosphorylates 4E-BP1 and S6K1 leading to CAP-dependent translation.

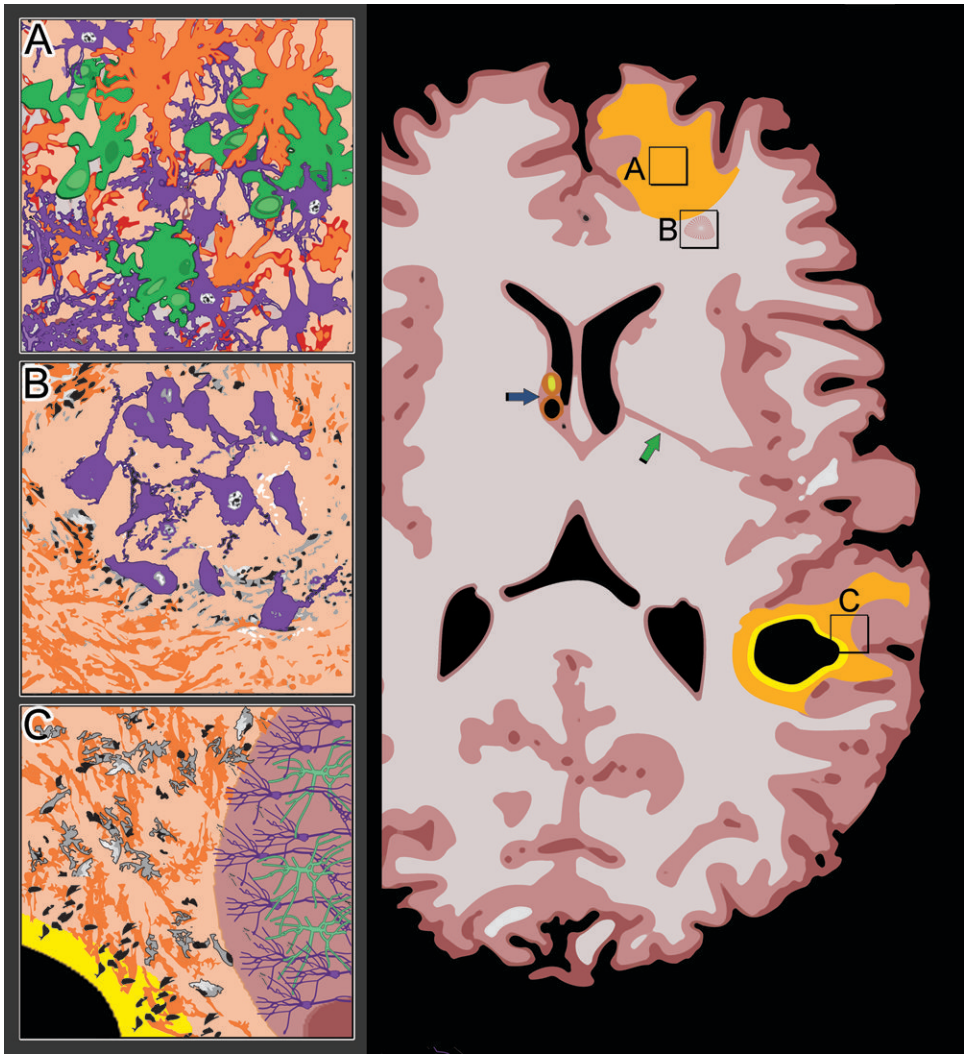

Figure 2. Tuber-like lesion model

Schematic representation of some of the neuropathologies associated with TSC. **(A)** Cortical tubers are characterized by cytomegalic neurons (purple) multinucleated giant cells (green) and gliosis (orange with red border). **(B)** White matter heterotopias often lie at the base or near the base of cortical tubers and are characterized by abnormally oriented cytomegalic neurons (purple) and hypomylenation (note the lack of glia, orange, in the center of the heterotopias). **(C)** The center of tubers may become necrotic and form cerebrospinal fluid-filled cysts. The perimeters of these cysts may calcify (bright yellow) and are often abutted by necrotic, tuberous tissue. In the adjacent, nontuberous, tissue, excitatory neurons (purple) and interneurons (green) are normally organized. Other pathologies can include subependymal nodules, which can become calcified (yellow) and/or cystic (blue arrow), and radial migration lines which are characterized by abnormal grey matter extensions from periventricular regions to the overlying cortex. Radial migration lines are thought to be comprised of arrested neurons and abnormal glia organized along formal developmental routes of migration.

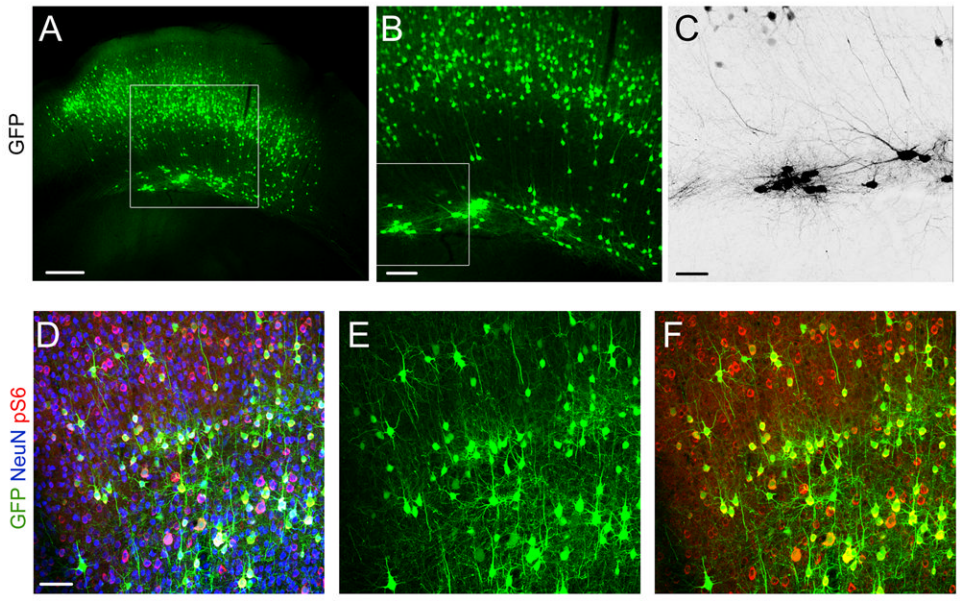

Figure 3.

(A-C) 4x image (A), 10x image from the white square shown in A (B), and 20x image in part from the white square shown in B (C) images of a P28 brain following IUE of Cre and GFP at embryonic day 12.5. Note the mislamination of cells throughout the cortex, including cell clusters above the corpus callosum. **(D-E)** Most GFP-positive cells (D-F) stained positive for the neuronal marker NeuN (D) and have elevated levels of phospho-S6 (D, F). Scale bars: 140 μm (A), 70 μm (B), 35 (C), 30 μm (D-F)

Table 1

Neurodevelopmental Rodent models of Tuberous Sclerosis Complex

Promoter	Initiation	Cell Type	Gene	Features	References
Emx1-Cre	E10.5	Cortical NSCs	*Tsc1*	ML, M, C, H, RG, S, P	(Carson et al., 2012; Magri et al., 2011)
Nestin-Cre	E10.5	Cortical NSCs	*Tsc1*	ML, M, C, H, RG, S, P	(Anderl et al., 2011)
Synapsin I-Cre	E12.5	Neurons	*Tsc1*	ML, M, C, H, S	(Meikle et al., 2007)
hgfap-Cre	E13.5	Cortical NSCs	*Tsc2*	ML, M, C, H, RG, S	(Way et al., 2009)
Nestin-rTet-Cre	E13.5	Cortical NSCs	*Tsc1*	M, C	(Goto et al., 2011)
Dlx5/6-Cre	E13.5	Cortical NSCs (GABAergic)	*Tsc1*	M, C	(Fu et al., 2012)
mgfap-Cre	E14.5	Astrocytes	*Tsc1/Tsc2*	M, RG, S	(Uhlmann et al., 2002)
In utero electroporation	E15/16	Cortical NSCs	*Tsc1*	ML, M, C	(Feliciano et al., 2011; Tsai et al., 2012b)
Neonatal electroporation	P0-1	SVZ NSCs	*Tsc1*	ML, C, P	(Feliciano et al., 2012)
αCamKII-Cre	P5	Forebrain Neurons	*Tsc1*	M, C, RG	(Ehninger et al., 2008; McMahon et al., 2012)
L7-Cre	P6	Cerebellar Purkinje Cells	*Tsc1*	C	(Tsai et al., 2012a)
Nestin-Cre/ERT2	P7/30	NSCs	*Tsc1*	M, C, P	(Feliciano et al., 2012; Zhou et al., 2011)
Mash1	P7	TACs	*Tsc1*	M, C, P	(Zhou et al., 2011)

Abbreviations: Mislamination: ML; Macrocephaly: M; Cytomegaly: C; Hypomyelination: H; Reactive Gliosis: RG; Seizures: S; Periventricular abnormalities: P.

Table 2

Plasticity and behavioral effects due to pharmacological or genetic mTOR manipulations or in the context of TSC mice.

Conditions	Plasticity	Behavior	References
mTOR manipulations			
Rapamycin in rats	↓ L-LTP	↓ Spatial learning	(Cammalleri et al., 2003; Qi et al., 2010; Tang et al., 2002)
Rapamycin C57BL/6 mice	↓ mGluR-LTD	↑ Learning and memory	(Halloran et al., 2012; Hou and Klann, 2004)
Tsc1$^{fl/fl}$ with Cre virus in cultured hippocampal slices	↓ L-LTP	↓ Hippocampus-dependent learning	(Zeng et al., 2007)
Tsc1$^{fl/fl}$ with Cre virus injected into pyramidal neurons in vivo	↓ mGluR-LTD		(Bateup et al., 2011)
Tsc1$^{fl/fl}$, *Tsc1*$^{fl/wt}$ × Cre line in Purkinje neurons		↑ Autistic-like behavior	(Tsai et al., 2012a)
*Syn*Cre *x Tsc2*$^{fl/mut}$		↓ anxiety, ↑ hyperactivity in the open field, abnormal social interactions (↑ time spent with novel animal), ↓ reversal learning	(Yuan et al., 2012)
FKBP12 cKO mice (mTOR up)	↑ L-LTP	↑ Contextual fear memory, ↑ Autistic/obsessive-compulsive behavior	(Hoeffer et al., 2008)
4E-BP1-KO mice	↑ mGluR- LTD, ↓ L- LTP, ↑ E-LTP	↓ Hippocampus-dependent memory	(Banko et al., 2006; Banko et al., 2005)
TSC heterozygote rodents			
Tsc2$^{wt/mut}$ rats	↓ E-LTP	↑ Episodic-like memory, ↑ responses to chemically-induced kindling	(von der Brelie et al., 2006), (Waltereit et al., 2006)
Tsc2$^{wt/mut}$ mice	↑ L-LTP	↓ Hippocampus-dependent learning	(Ehninger et al., 2008)
Tsc1$^{wt/mut}$ mice	N.D.	↓ Hippocampus-dependent learning, ↓ Social behavior	(Goorden et al., 2007)
Tsc1$^{wt/mut}$ mice and *Tsc2*$^{wt/mut}$ mice	N.D.	↓ Social interactions recovered by rapamycin treatment, intact motor and sensory function	(Sato et al., 2012)
Tsc2 ΔRG mice	↓ mGluR-LTD	↓ Social behavior, motor learning skills, and spatial learning	(Chevere-Torres et al., 2012a; Chevere-Torres et al., 2012b)
Inducible *Tsc1*$^{fl/mut}$ or *Tsc2*$^{fl/mut}$ mice			
*Syn*Cre × *Tsc2*$^{fl/mut}$	N.D.	Greater decrease in TSC2 levels than *Tsc2*$^{fl/fl}$ × SynCre correlating with worse behavioral abnormalities than *Tsc2*$^{fl/fl}$ × SynCre above	(Yuan et al., 2012)
hgfap × *Tsc2*$^{fl/mut}$ (*Tsc2* removal in embryonic neural progenitor cells)	N.D.	↓ Spatial memory and context discrimination rescued by rapamycin treatment	(Way et al., 2012)

Abbreviations: cKO: conditional knockout; E-LTP: early phase LTP; L-LTP: late phase LTP; mGluR-LTD: metabotropic glutamate receptor dependent LTD; ND: not determined; *Tsc2* ΔRG: dominant/negative TSC2 that binds to TSC1, but has a deletion and substitution mutation in its GAP-domain, resulting in inactivation of the complex.