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14. ABSTRACT
We are testing the hypothesis that the balance between excitation and inhibition is skewed in TSC brains and that this synaptic dysregulation is caused by altered protein synthesis. We have found that the imbalance between excitation and inhibition using biochemical assays and electrophysiology. Furthermore, calcium imaging show hyperexcitable neuronal activities in TSC-1 deleted neurons. These results further support our hypothesis. Correction of this synaptic dysregulation will hopefully provide a new therapeutic target to neurological symptoms in TSC such as epilepsy, mental retardation and autistic behaviors.

15. SUBJECT TERMS- Tuberous Sclerosis, Synaptic Plasticity, Protein Synthesis

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INTRODUCTION: The goal of this study is to identify therapeutic targets for neurological symptoms of tuberous sclerosis complex (TSC) such as epilepsy, mental retardation and autistic $_{behaviors}$. TSC is caused by mutations in two genes, *tsc1* (Hamartin) and *tsc2* (Tuberin), both of which play important roles in protein synthesis and cell growth. Since TSC proteins are negative regulators of mTOR, an enhancer for protein synthesis, mutations causing hypofunction of *TSC1* or *TSC2* can theoretically induce abnormally increased production of proteins. Recent work suggests that TSC mutants show abnormalities in dendritic spine formation, glutamate receptor kinetics, and synaptic plasticity. Enhanced mTOR signaling, which has also been observed in TSC mutants, may be responsible for these defects. Although enhancement of mTOR likely leads to increased protein synthesis, it is unknown, which neuronal proteins mTOR regulates and how malfunction in the TSC/mTOR pathway could result in the neuronal defects and neurological symptoms observed in TSC. Seizures related to TSC are rooted in an imbalance between excitation and inhibition and infantile spasm in TSC is treatable with Vigabatrin, a GABA transaminase inhibitor. This indicates that abnormal protein synthesis may skew the balance between excitation and inhibition in the TSC brain. Thus, we are testing the hypothesis that the balance between excitation and inhibition is skewed in TSC and that this synaptic dysregulation is caused by altered protein synthesis.

BODY:

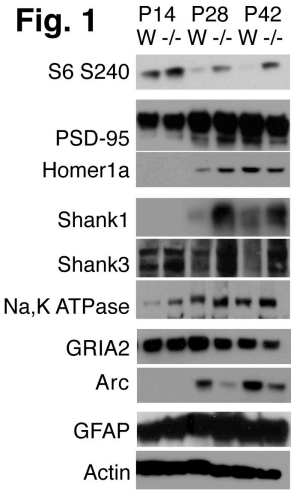
Specific Aim I: To test if balance between synaptic proteins associated with excitation or inhibition are altered in TS

Study Design: There are proteins that undergo activity-dependent protein synthesis. I will use immunoblotting to compare levels of these proteins between *tsc1*$^{null\text{-}neuron}$ and WT. I will also examine both excitatory and inhibitory synapse associated proteins in brains of mutant mice as compared to WT.

Method: synaptoneurosome preparation, immunoblotting.

Milestones and Time line: Predicted outcome is that mutants will show increases in proteins associated with activity-dependent synthesis. I will also look for evidence of up-regulation of excitatory synaptic proteins and/or down-regulation of inhibitory synaptic proteins in the mutant. Immunoblottings of selected synaptic proteins will be performed in the first 6 months of the first year.

Accomplishment: During previous funding period, we collected protein samples from the brains of neuronspecific *Tsc1* null mutant mice throughout the development including postnatal day (P) 14, 28 and 42. We have been using immunoblotting to identify alterations in synthesis of synaptic proteins. As an indicator of successful gene recombination of neuron-specific Tsc1 null mutant, we observed an increased phosphorylation of a ribosomal protein S6 as expected (Fig.1). However, contrary to our predictions, many synaptic proteins showed variable expression patterns. We have found that PSD-95 and Homer-1a are up-regulated in neuron-specific *Tsc1* null mutant mice as compared with WT at P28 while both proteins were down regulated at P42 (Fig.1). Arc showed decreases at both P28 and P42 (Fig. 1). Arc protein is critical for endocytosis of AMPA receptors (AMPAR), thus a reduction in Arc suggest that AMPA receptors abnormally accumulates on postsynaptic membrane. As will be discussed in the next section of Specific Aim I, our biotinylation assay has shown that Tsc1 deleted neurons have a higher amoung of surface GluA2 (GluR2) subunit of AMPARs than WT. It is likely that an insufficient amount of Arc results in accumulation of GluA2 at synapses and causes hyperexcitability (See Fig. 2 and 3).



Developmental profile of synaptic proteins.

Shank 1 and 3 are up-regulated as compared to WT (Fig.1) at both P28 and P42. Shank proteins are scaffolding molecules and reside deep inside the postsynaptic density and bind other postsynaptic density proteins such as SAPAPs and Homers (*1*). SAPAPs bind PSD-95, which is the major scaffolding protein of NMDA, AMPA and kainate receptors (*2*). Homer intereacts with metabotropic glutamate receptors as well as IP3 receptors (*2*). By interacting with both SAPAP/PSD-95 and Homer, Shank integrates all types of glutamate

receptors into a single large protein complex (*1*). Thus, Shank is known as "master scaffold".

Interestingly, Shank proteins have been implicated in autism. For example, both Shank 2 and 3 are mutated in families with autism (*3, 4*). Shank 3 is also associated with Phelen-McDermid syndrome, which presents with autistic behaviors and is caused by copy number variation of chromosome 22q13 (*1*). Mice deleted with Shank3 have recently been shown to self-injurious repetitive grooming and deficits in social interaction (*5*). These symptoms are reminiscent of obsessive-compulsive disorder as well as stereotypy in autism. Shank3 deleted mice also show poor social interaction. Furthermore, two patients with a microscopic duplication of 22q13 including the Shank3 gene manifested with infantile hypotonia, developmental delay and growth deficiency (*6*).

Shank1 knockout mice have smaller dendritic spine (*7*). Overexpression of Shank1 causes enlargement of dendritic spines (*8*). Neurons with decreased expression levels of Tsc-1 or –2 using RNAi showed abnormally enlarged spines (*9*). Thus, we will focus on correlating abnormal expression of Shank proteins, and abnormal dendritic morphology as well as functional alterations in *Tsc1*-deleted neurons.

Shank proteins binds IP3 receptor, which is a Ca2+ channel at smooth ER and regulates intracellular Ca^{2+} signaling. We are setting up to perform calcium imaging as proposed in Specific Aim III. Shank proteins also binds cdc42, which regulates actin polymerization. It will be interesting to determine in the future whether accumulation of actin is altered in Tsc1-deleted neurons using live imaging of actin tagged with fluorescent protein(s) such as fluorescent recovery after photobleaching (FRAP) (*10*).

We have also found that the Na^+, K^+-ATPase is up-regulated in *Tsc1*-deleted neurons (Fig. 1). The Na^+, K^+-ATPase maintains the Na^+ and K^+ gradients that are critical for maintenance of neuronal excitability and conduction of the action potential and uptake of neurotransmitters, regulation of cell volume, pH, and Ca^{2+} concentrations (*11*). Na^+, K^+-ATPase is highly dependent on intracellular ATP. An impaired activity of Na^+, K^+-ATPase results in the inability to maintain transmembrane Na^+ and K^+ gradients and an increased water influx leading to intracellular swelling. One of the well-documented morphological changes in Tsc1-deleted neurons is an enlargement of neuronal cell body. In the future, it will be interesting to test if up-regulation of Na^+, K^+-ATPase results in imbalance between supply and consumption of intracellular ATP.

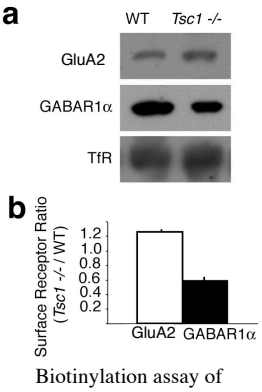
Study Design: I will look at the surface expression of both glutamate and GABA receptors using biotinylation assay in cultured neurons, comparing basal surface expression of these receptor subunits between WT and TSC neurons. Next, AMPAR internalization will be examined after bath application of NMDA (NMDAR dependent internalization) or application of DHPG (mGluR dependent internalization). GABA receptor internalization will be examined after high KCl exposure. If surface expression changes differ between WT and TSC mice, I will pretreat the cultures with rapamycin before an induction protocol to see if deviant surface receptor expression can be corrected.

Method: dissociated cortical neuronal culture, biotinylation assay

Milestones and Time line: I predict that mutants will show up-regulation of excitatory synaptic proteins and/or down-regulation of inhibitory synaptic proteins. I will look for the differences in turnover of excitatory and inhibitory receptors in response to the above stimulation protocol. The biotinylation assay will be performed in the second 6 months of first year.

Accomplishment: In the current period, we have continued the biotinylation assay to examine the amount of surface receptors. surface expression of an AMPAR subunit GluA2 (GluR2) was increased in Tsc1-deleted neurons as compared to WT while GABA receptor α1 subunit was decreased (Fig. 2). On the same membrane, transferrin receptor levels were constant and serve as a loading control. These results are consistent with our hypothesis that the balance between excitation and inhibition is deviated to excitation.

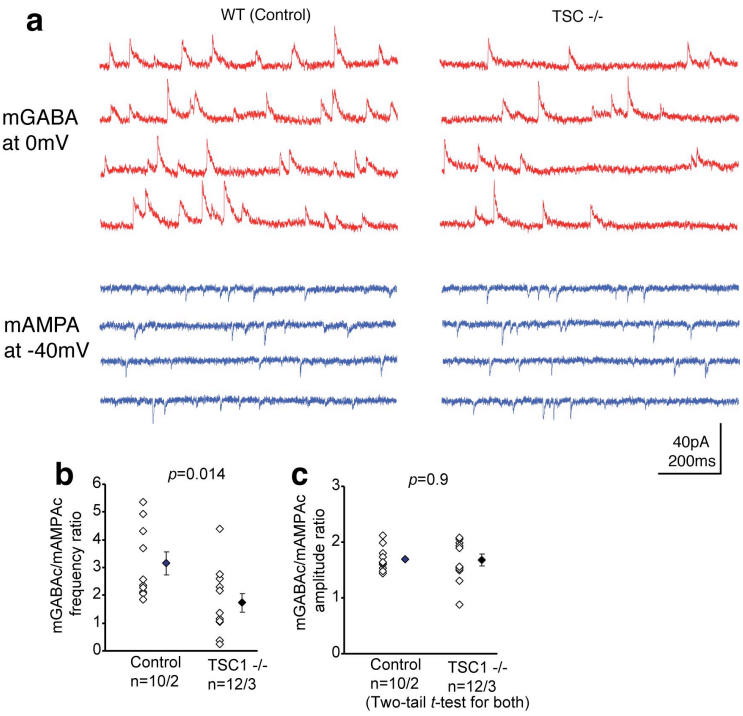
Fig. 2



Biotinylation assay of surface receptors.

To further confirm the above findings, I have collaborated with Dr. Jiang-Ping Zhao who is an electrophysiologist at the Constantine-Paton lab. We used acute visual cortical slices at postnatal day 28 and recorded miniature current activities of AMPARs (mAMPAc) and GABARs (mGABAc) in layer 2/3 pyramidal neurons (Fig. 3a). Frequency ratio between mGABAc and mAMPAc was significantly reduced (Fig. 3b: two tailed t-test, p = 0.014) in TSC1 -/- neurons as compared to WT while amplitude ratio was not different (Fig. 3c: two tailed t-test, p = 0.9). These results is fully consistent with the biotinylation assay (Fig. 2) and indicate that excitation-inhibition balance is deviated toward excitation. However, this deviation toward hyperexcitability was not corrected at least 30 minute after rapamycin treatment (100 μM). This may be because the effect of rapamycin requires longer time and / or concentration. Alternatively, it is possible that hyperexcitability in TSC is not simply treatable with rapamycin but may require a direct intervention to either reduce excitation or enhance inhibition.

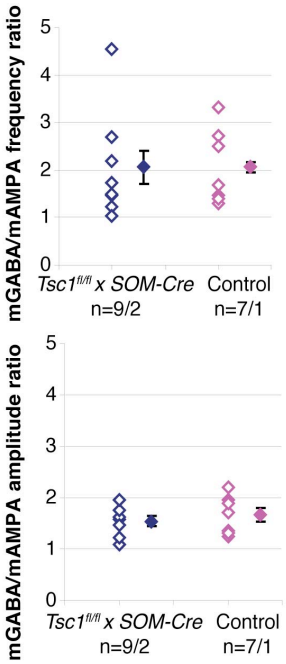
Fig. 3



Miniature GABA and AMPA currents in P28-30 VC L2/3 pyramidal neurons in *Tsc1*$^{fl/fl}$ x *Syn-Cre* mice. mGABAc/mAMPAc frequency ratio (b) is significanlty reduced.

The above results suggest that inhibitory neurons are especially susceptible to *Tsc1* deletion in the brain. However, there is a wide variety of interneurons and they are classified based on morphologies, molecular markers or electrophysiological properties. Inhibitory neuronal subclassses have distinct and complementay roles in cortical computation, and are likely to play different roles in pathphysiology of neurological conditions. For example, large basket cells terminate their axons to the soma of pyramidal neurons and are fast-spiking. These cells are positive for paralbumin (PV). Somatostain (SOM) positive interneurons target distal segments of apical dendrites. PV neurons suppresses pyramidal neurons by dividing responses while SOM cells simply reduces response level by subtraction (*8*). Recent advance in mouse genetics offers an analysis of subpopulations of inhibitory cells based on molecular markers (*12*). We have generated SOM cell specific *Tsc1* knockout mouse by crossing Tsc1 fl/fl mouse with SOM-Cre strain. We did not see significant diffferences in ratio of minature current frequency and amplitude between GABA receptors and AMPA receptors (Fig. 4). This result indicate that local suppression of an apical dendrite may not account for altered excitation-inhibition balance in TSC. We are in the process of generating PV cell specific *Tsc1* knockout mouse.

Fig. 4



Miniature GABA and AMPA currents in P28-30 VC L2/3 pyramidal neurons in *Tsc1*$^{fl/fl}$ x *SOM-Cre* mice. Neither frequency nor Amplitude ratio of mGABAc/mAMPAc is different.

Specific Aim II: To identify any synaptic proteins with enhanced protein synthesis in TS neurons

Study Design: Using S^{35} methionine, I will label newly synthesized proteins in cultured cortical neurons, ultimately performing 2-D gel electrophoresis on synaptic protein extracts to compare labeled protein levels between WT and TS samples. I will focus on protein spots that show higher signal(s) in TSC compared to WT and identify these differentially expressed proteins using mass spectrometry. If these

comparisons do not reveal significant differences, I will use protocols that stimulate activity-dependent protein synthesis.

Method: synaptoneurosome preparation, dissociated cortical neuronal culture, ^{35}S-Methionine pulse-chase labeling, 2-D gel and assessment of protein synthesis, SILAC (Stable isotope labelling with amino acids in cell culture).

Milestones and Time line: Protein spots with significantly higher ^{35}S-Methionine signal(s) in TSC compared to WT will be selected and identified using mass spectrometry. I predict that some of them will be synaptic proteins involved in excitatory and inhibitory neurotransmission. While I discussed SILAC as an alternative approach, this technique allows comparison of proteins quantitatively and exclusively. However, this approach may have to be delayed till an independent phase because of the expense involved. Thus, I will start with ^{35}S-Methionine labeling method in the second half of the first year and continue on the second year. Then, I will spearhead SILAC from the last half of second year to third year.

Accomplishment: I have collected brain tissues to perform proteomic analysis. I proposed to study this specific aim mainly after I start my own laboratory. I have been very actively searching for a tenure-track faculty position. In the meantime, I have collaborated with Rory Kirchener, a graduate student in the Constantine-Paton laboratory, who studies activity dependent synapse formation using deep sequencing analyses of mRNAs (RNAseq). We isolated mRNA from brains of *TSC1*$^{fl/fl}$ x *Syn-Cre* mice and WT at P28 and conducted RNAseq using the MIT Biomicrocenter Illumina II. We found more than 30 transcripts highly dysregulated in the cortex of the Tsc1^{null-n} mice. These include: immediate early genes such as fosb, egr2 and 3; neuronal proteins such as stxbp2 and cortistatin; and growth factor signaling molecules such as igfbp3 and erbb3. Among these transcripts, the serotonin (5-hydroxytryptamine:5-HT) receptor subtype 2C (5-HT$_{2C}$R) has become my major interest. It is approximately 3 times up-regulated in the RNAseq data and I also confirmed upregulation of the protein level in immunoblotting.

5-HT$_{2C}$R is a G-protein coupled receptor driving Ca^{2+} release from dendritic stores via PLC and IP3 receptors. The up-regulated 5-HT$_{2C}$R is particularly pertinent to developmental diseases because: (1) Serotonergic neuronal input is present in the embryonic brain and early neonatal brain producing bursting release of 5-HT as the cortical cytoarchitecture develops. In fact cortical barrels in somatosensory cortex do not form if this 5-HT input is removed. (2) 5-HT has been known for decades as a major factor in pathophysiology of autism where it is elevated in CSF (Veenstra-VanderWeele and Blakely). (3) 5-HT$_{2C}$R has a PDZ-binding domain and has been shown to interact with PSD-95, the major scaffolding protein for the ion passing glutamate receptors NMDA and AMPA. These receptors are critical mediators of learning, memory, sensory processing, and cognition. (4) Both serotonin and 5-HT$_{2C}$R have been implicated in developmental plasticity in visual and other neocortical regions (*13, 14*). (5) 5-HT$_{2C}$R is an intriguing molecule from the standpoint of RNA biology as its transcript undergoes RNA editing and splicing (*15*). How these mechanisms are associated with TSC-mTOR in the developing synapse are currently unknown. In situ hybridization of 5-HT$_{2C}$R suggests that in the developing mouse brain the receptor is highly expressed in neocortical layers 2/3 (Allen Brain Atlas). Using immunohistochemstry, I have confirmed that the 5-HT$_{2C}$R protein shows punctate distribution which is similar to PSD-95. Since 5-HT$_{2C}$R is a major regulator of intracellular Ca^{2+} release, I will focus on this receptor in Aim III using Ca^{2+} imaging.

Specific Aim III: To characterize neuronal functions of dysregulated proteins in TS neurons

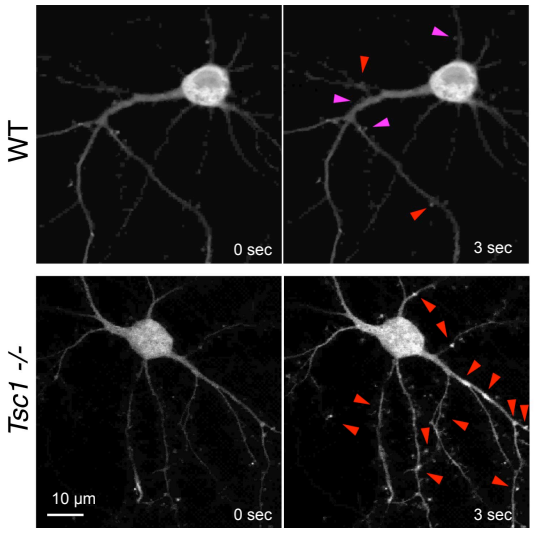
Study Design: Proteins found to be overexpressed in the mutant will be knocked down with siRNA in TSC knockout neurons in order to rescue synaptic defects. Biotinylation assays will be used to access surface expression between neurons with or without siRNA. Finally, I will determine whether these genetic manipulations of dysregulated proteins up or down regulates transmission in cultured neurons using Ca^{++} imaging with and without protein knock-down.

Method: dissociated cortical neuronal culture, biotinylation assay, Calcium imaging.

Milestones and Time line: I predict gene knockdown of overproduced proteins in mutant neurons will correct deviation of surface receptors in biotinylation assays and abnormal Ca^{++} influx in Ca^{++} imaging. I will perform the experiments in Specific Aim III in third and fourth years.

Accomplishment: As reported in the accomplishment of Specific Aim I, we have found that Shank1 and 3 are up-regulated in *Tsc1*-deleted brains. As Shank proteins interact with IP3 receptors, which regulate an intracellular Ca^{2+} release from ER. Thus, it is possible that intracellular Ca^{2+} level is dysregulated in *Tsc1*-deleted neurons. I have started Ca^{2+} imaging using an genetcally endoded Ca^{2+} indicator GCaMP protein. I transfected the DNA construct into genotyped culture neurons and serially imaged neurons at the interval of 3 seconds (Fig. 5). WT neurons show scattered and transient signal increases in multiple dendritic spines while intensities of the cell body appear relatively stable (Fig. 5 top right). On the other hand, *Tsc1* -/- neurons show occasional synchronized intensity increases through out dendrites as well as the soma (Fig. 5 top right). Interval of the synchronized intensity change is 13.5 $\pm$ 3.4 seconds (N=5 cells) in *Tsc1* -/- neurons while it is 35 $\pm$ 10 seconds (N=4 cells)(p=0.047). This preliminary finding indicates that *Tsc1* -/- neurons are more hyperexcitable than WT and I will collect more data to confirm it.

Fig. 5

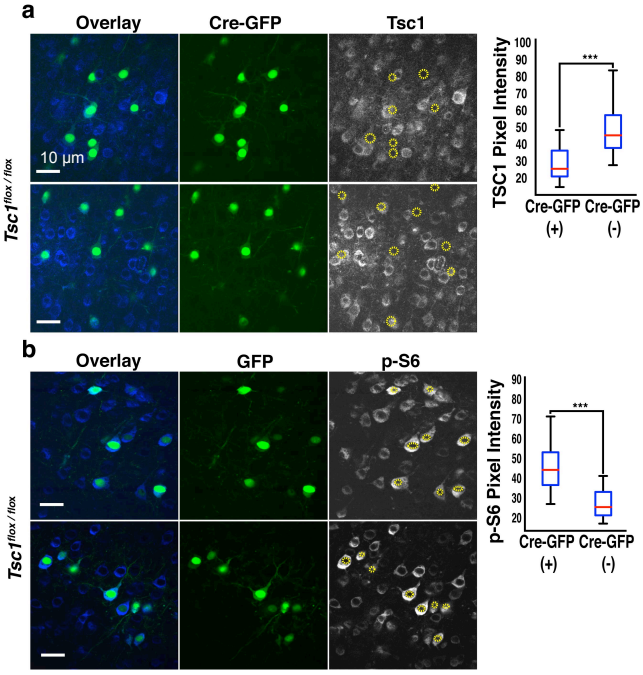


GCaMP3 shows changes in intracellular Ca2+. Note Tsc1 -/- neuron show a synchronous increase throughout dendrites (red arrows) while WT shows increases (red arrows) or decreases (purple arrows) in a few spines.

We will next combine this method with pharmacologic and genetic intervensions such as RNA interference to modify glutamate receptor signaling proteins and serotonin receptor 5-HT_{2C}R. As discussed in Aim II, 5-HT_{2C}R regulates intracellular Ca2+ dymamics through IP3 receptor on endoplasmic reticulum. To study the neuromodulatory effect of serotonin through 5-HT_{2C}R in these neurons, I will first treat dissociated neurons with several different 5-HT_{2C} R antagonists with known dosages in rodents including agomelatine,CEPC, RS-102,221, SB-242,084 and Lu AA24530 (tedatioxetine). Some of these have already been used in humans. I will also test risperidone because this dopamine antagonist also possess anti-serotonergic properties and is the first FDA approved medication for behavioral improvement in patients with autism. I will determine whether and how these antagonists change the Ca^{2+} responses and whether they have similar effects on WT neurons. RNA interference against HT_{2C}R will also assess the suppression of 5-HT_{2C}R in WT and *Tsc1* deleted neurons. These experiments will assess the potential of 5-HT_{2C} R blocker as a therapeutic for neurological symptoms of TSC.

In addition to dissociated cultured neurons, I also plan to perform Ca2+ imaging in cortical slices because they preserve architecture of neuronal circuitry better. To achieve this, I am combining a Cre-lox recombination system and *in utero* electroporation. Specifically, I electroporated a DNA construct encoding Cre-recombinase tagged with green fluorescent protein (Cre-GFP) into WT or $Tsc1^{fl/fl}$ mutant fetuses at embryonic day 15.5 (E 15.5). I confirmed in mutant brains that Cre-GFP expressing neurons showed suppression of the TSC1 protein and increased phosphorylation of ribosomal protein S6, indicating an enhanced mTOR signaling as a result of suppressed TSC1 function (Fig. 6).

Fig. 6

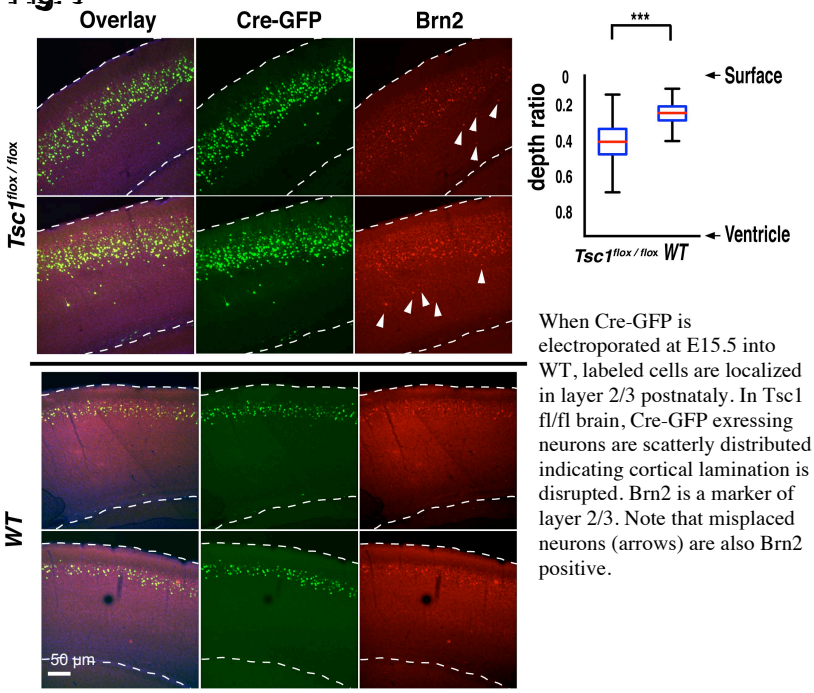


Cre-GFP positive Tsc1 fl/fl neurons show reduces in TSC1 proteins and increase in S6 phosphorylation.

Postnatally, WT Cre-GFP positive neurons were located in cortical layer 2/3. However, mutant brains showed a scattered distribution of Cre-GFP positive neurons (Fig. 7). Furthermore, these *TSC-1* deleted cells also expressed a marker protein for layer 2/3 despite their malpositions.

We also examined slices prepared from embryonic brains after electroporation at E15.5. In WT slices, Cre-GFP positive neurons had already started migrating at E18 and migrated to the cortical plate after 24 hours in vitro. However, in the brains of the *Tsc1*^{tm1Djk}/J mouse electroporated with Cre-GFP, the majority of *TSC1*$^{-/-}$ neurons are still located in the ventricular or intermediate zones at E18.5 plus 24 hours in vitro. The malpositioning of neurons at E18 plus 24 hours *in vitro* can be corrected by rapamycin treatment. To our surprise, live imaging of slices prepared from E18 embryonic cortex revealed that migration velocities were comparable between WT and mutant.

Fig. 7



When Cre-GFP is electroporated at E15.5 into WT, labeled cells are localized in layer 2/3 postnataly. In Tsc1 fl/fl brain, Cre-GFP exressing neurons are scatterly distributed indicating cortical lamination is disrupted. Brn2 is a marker of layer 2/3. Note that misplaced neurons (arrows) are also Brn2 positive.

We argue that abnormal cortical lamination is a previously unrecognized feature of TSC and may contribute to neurological symptoms. Indeed, a recent pathological study on human postmortem brains also reports widespread alterations in cortical cytoarchitecture outside of tubers (Marcotte et al. 2012). These incidental finding were presented in Society for Neuroscience Meeting 2012 (New Olreans).

KEY RESEARCH ACCOMPLISHMENTS:

- Shank1 and 3 are up-regulated in *Tsc-1*-deleted brains.
- Na^+, K^+-ATPase is up-regulated in *Tsc-1*-deleted brains.
- Arc is down-regulated in *Tsc-1*-deleted brains.
- Serotonin receptor 5-HT_{2C}R is upregulated in *Tsc-1*-deleted brains.
- We have found that surface expression of GluR2 is up-regulated while that of GABAR 1α subunit is down-regulated. Furthermore, we have found that frequency ratio of GABAR currents to AMPARs is reduced in *Tsc-1*-deleted neurons. The results indicate that the excitation-inhibition balance is shifted toward excitation in Tsc-1 deleted neurons. However, conditional deletion of *Tsc1* in somatostain positive interneuron does not show the deviation of the excitation-inhibition balance. We are currently assessing paralbumin positive interneurons specific *Tsc1* knockout mice. Identification of susceptible cell type and synapse population will facilitate finding a better anti-epileptic drug for patients with TSC.
- Using Ca^{2+} imaging with a genetically encoded Ca^{2+} indicator GCaMP3, we have found that Tsc-1 deleted neurons in primary cultures show frequent surges of intracellular Ca^{2+} in soma and dendrites. This result also confirms hyperexcitability in TSC neurons. In the future, we will study the contribution of 5-HT_{2C}R to abnormal dendritic Ca^{2+} dynamics using antagonists. The results will have an implication for autistic symptoms and other cognitive impairments in TSC.

REPORTABLE OUTCOMES:

Manuscripts:

Yoshii, A. and Constantine-Paton, M. (2010). Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Dev Neurobiol 70*, 304-322.

Zhao, J.P., Constantine-Paton, M., and **Yoshii, A.** Abnormal balance between excitation and inhibition in tuberous sclerosis. *In preparation*.

Invited Seminars:

3/21/11 Activity dependent synapse formation and its implications for neurodevelopmental disorders. An invited seminar, Mount Sinai School of Medicine, New York.

9/5/11 Eye-Opening Induced Synapse Formation in the Central Viual System. An invited seminar, RIKEN Brain Science Institute, Wako, Japan.

1/30/12 Activity-dependent synapse formation and its implications for neurodevelopmental disorders. An invited seminar, George Washington University, Washington D.C.

1/26/13 Activity-dependent synapse formation and its implications for neurodevelopmental disorders. Keio Neuroscience Symposium, Keio University Hospital, Tokyo, Japan.

2/13/13 Molecular mechanisms underlying activity-dependent synapse formation in the developing brain. An invited seminar, University of Iliois College of Medicine, Chicago, IL.

Abstract: Cox, R.L., Calderon de Anda, F., Constantine-Paton, M., and **Yoshii, A.** TSC-1 deletion results in abnormal positioning of cortical neurons. Program No. 444.23. 2012 Neuroscience Meeting Planner. New Orleans, LA: Society for Neuroscience, 2012. Online.

CONCLUSION:

We have found dysregulated syntheses of synaptic proteins in the neuron-specific *Tsc1* mutant mice (Aim I). Remarkably, Shank 1 and 3 , postsynaptic proteins associated with autism, are up-regulated in Tsc1-deleted neurons. However, contrary to the initial hypothesis, some porteins are down-regulated *Tsc1* mutant brains.

We have made a major progress in Specific Aim I and confirmed that surface expression of an AMPAR subunit GluA2 is up-regulated while GABAR α1 subunit is down-regulated. A reduction in the Arc protein may be responsible for the increase in GluR2. Our electrophysiological study have also revealed that the frequency ratio between excitation and inhibition is deviated to hyperexcitability (Aim II). In the future, we will aim to identify subpopulation of interneurons that accont for suppressed inhibition. So far, we have ruled out somatostatin positive neurons.

Intracellular Ca^{2+} increases more frequently and tends to be synchroously distributed throughout dendrites in *Tsc1* mutant neurons (Aim III). RNAseq and protein anlyses revealed abnormally high synthesis of 5-HT_{2C}R, which regulates Ca^{2+} release from ER through IP3 receptors. Both serotonin pathway and TSC are associated with autism and we will continue to study abnormal Ca^{2+} dynamics in TSC using 5-HT_{2C}R antagonists.

Collectively, these results support our hypothesis that synthesis of synaptic proteins is dysregulated and results in imbalance between excitation and inhibition. Correction of this synaptic dysregulation will hopefully provide a new therapeutic target to neurological symptoms in TSC such as epilepsy, mental retardation and autistic behaviors.

Personnel supported by the award:

Akira Yoshii

Updates on Career Transition:

After the mentored phase was completed in March 2011, I could not secure a faculty postion timely because the job market has been unprecedently competitive situation, therefore I have asked to hold the independent phase. I am currently negotiating a job offer with University of Illinois Medical School.

REFERENCES:

1. T. M. Boeckers, J. Bockmann, M. R. Kreutz, E. D. Gundelfinger, ProSAP/Shank proteins - a family of higher order organizing molecules of the postsynaptic density with an emerging role in human neurological disease. *J Neurochem* **81**, 903 (Jun, 2002).
2. E. Kim, M. Sheng, PDZ domain proteins of synapses. *Nat Rev Neurosci* **5**, 771 (Oct, 2004).
3. C. M. Durand *et al*., Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. *Nat Genet* **39**, 25 (Jan, 2007).
4. S. Berkel *et al*., Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. *Nat Genet* **42**, 489 (Jun, 2010).
5. J. Peca *et al*., Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*, (Mar 20, 2011).
6. N. Okamoto *et al*., 22q13 Microduplication in two patients with common clinical manifestations: a recognizable syndrome? *Am J Med Genet A* **143A**, 2804 (Dec 1, 2007).
7. A. Y. Hung *et al*., Smaller dendritic spines, weaker synaptic transmission, but enhanced spatial learning in mice lacking Shank1. *J Neurosci* **28**, 1697 (Feb 13, 2008).
8. C. Sala *et al*., Regulation of dendritic spine morphology and synaptic function by Shank and Homer. *Neuron* **31**, 115 (Jul 19, 2001).
9. S. F. Tavazoie, V. A. Alvarez, D. A. Ridenour, D. J. Kwiatkowski, B. L. Sabatini, Regulation of neuronal morphology and function by the tumor suppressors Tsc1 and Tsc2. *Nat Neurosci* **8**, 1727 (Dec, 2005).
10. A. Yoshii, M. Constantine-Paton, BDNF induces transport of PSD-95 to dendrites through PI3K-AKT signaling after NMDA receptor activation. *Nat Neurosci* **10**, 702 (Jun, 2007).
11. E. E. Benarroch, Na+, K+-ATPase: functions in the nervous system and involvement in neurologic disease. *Neurology* **76**, 287 (Jan 18, 2011).
12. H. Taniguchi *et al*., A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. *Neuron* **71**, 995 (Sep 22, 2011).
13. L. Kojic *et al*., Columnar distribution of serotonin-dependent plasticity within kitten striate cortex. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 1841 (Feb 15, 2000).
14. J. F. Maya Vetencourt *et al*., The antidepressant fluoxetine restores plasticity in the adult visual cortex. *Science* **320**, 385 (Apr 18, 2008).
15. J. J. Rosenthal, P. H. Seeburg, A-to-I RNA editing: effects on proteins key to neural excitability. *Neuron* **74**, 432 (May 10, 2012).