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14. ABSTRACT

Mutations in the human TSC2 gene cause tuberous sclerosis complex (TSC), a developmental disorder characterized by tumor susceptibility and neurological manifestations. To better understand the disease, we generated an animal model in which the mouse Tsc2 gene is disrupted exclusively in excitatory neurons of the forebrain. We investigated how heterozygous and homozygous Tsc2 mutations affect the development of mutant excitatory neurons as well as other surrounding brain cells, in vivo and in vitro. We found that heterozygous mutations of Tsc2 in the excitatory neurons of the forebrain have modest effects on their growth, whereas homozygous loss disrupts the maturation not only of excitatory neurons and their synapses, but also disrupts the development of inhibitory neurons and their synapses. These combined effects likely contribute to altered neuronal activity and increased seizure susceptibility in TSC.

15. SUBJECT TERMS
Tuberous Sclerosis Complex, animal model, neuronal development, synapse, TSC2

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1. INTRODUCTION:

Mutations in the *TSC2* gene cause tuberous sclerosis complex (TSC), a developmental disorder characterized by tumor susceptibility in multiple organs and frequent neurological manifestations, such as seizures, intellectual disability, and autism. The brain of TSC patient is characterized by lesions (cortical tubers or tumors) containing abnormal homozygous mutant cells, whereas the majority of the brain cells carry heterozygous mutations and appear morphologically normal. We hypothesized that heterozygous mutations in the *TSC2* gene disrupt the normal development and function of excitatory neurons without affecting their size. Homozygous loss-of-function mutations, on the other hand, not only profoundly alter their size and intrinsic development, but also disrupt cell-cell communication with other cell types. These non cell-autonomous mechanisms exacerbate defects in synaptic function and cognition, and possibly contribute to the formation of cortical tubers and tumors in the TSC brain.

2. KEYWORDS:

Tuberous Sclerosis Complex, animal model, neuronal development, synapse, TSC2

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The overall goal of this study was to define the cellular abnormalities of excitatory neurons that are deficient in TSC2 activity, and to understand how they impact the development of other neuronal and glial subtypes in the cerebral cortex.

For our study we utilized NEX-*Tsc2,* a conditional *Tsc2* knock out mouse line in which gene deletion occurs specifically in embryonic forebrain excitatory neurons, the most abundant cell type in the developing cerebral cortex. We proposed to characterize the neurodevelopmental defects of heterozygous and homozygous NEX-*Tsc2* mice, including cell autonomous abnormalities in the development of excitatory neurons, as well as non-cell autonomous effects on inhibitory neurons and non-neuronal cell types. In collaboration with Dr. Anne Anderson, we also planned to use video-EEG recordings to determine whether heterozygous and homozygous NEX-*Tsc2* mice exhibit seizures or increased susceptibility to chemoconvulsants. Our work includes in vivo studies (Specific Aim 1= Major Task 1), and in vitro culture studies (Specific Aim 2 = Major Task 2).

The goal of Major Task 1 was to generate and fully characterize the brain phenotype of heterozygous and homozygous NEX-*Tsc2* mutant mice. The goal of Major Task 2 was to analyze neuronal and neuro-glia cultures obtained from these mutant mice.

What was accomplished under these goals?

Major Task 1: *In vivo* characterization of heterozygous and homozygous NEX-*Tsc2* mice

We generated experimental NEX-Tsc2 mice by breeding $Cre^{+/+}$;$Tsc2^{flox/wt}$ mice with heterozygous mice $Cre^{-/-}$;$Tsc2^{flox/wt}$. This breeding strategy generated 100% $Cre^{+/-}$ mice that were either wild type (WT = $Tsc2^{wt/wt}$), heterozygous (HT = $Tsc2^{fl/wt}$) or homozygous (KO = $Tsc2^{fl/fl}$) for *Tsc2*. Pups were born at the expected Mendelian ratio (25% WT, 50% HT, and 25% KO). As expected HT mice appeared indistinguishable from WT controls, whereas KO mutant mice appeared runt and some died prematurely at approximately 12-15 days after birth (P12-15). Despite some mortality, we were able to obtain multiple sets of mice of each genotype (WT, HT and KO) for our study. To maintain the colony and regenerate breeder mice of the appropriate genotype we also interbred separately $Cre^{+/+}$;$Tsc2^{flox/wt}$ mice as well as $Cre^{-/-}$;$Tsc2^{flox/wt}$ and selected $Tsc2^{flox/wt}$ from each progeny. These breeders were regenerated twice per year to ensure fertility.

We analyzed body weight, brain weight and the ratio of brain/body weight in a cohort of NEX-Tsc2 mice at postnatal day (P) 16, and determined that heterozygous mice are completely normal with regard to these parameters, whereas homozygous mutants are smaller but their brain/body weight is much larger than in control mice. This indicates that homozygous Tsc2 mutations cause an enlargement of the brain, but heterozygous mutations do not affect brain size or overall animal growth and health.

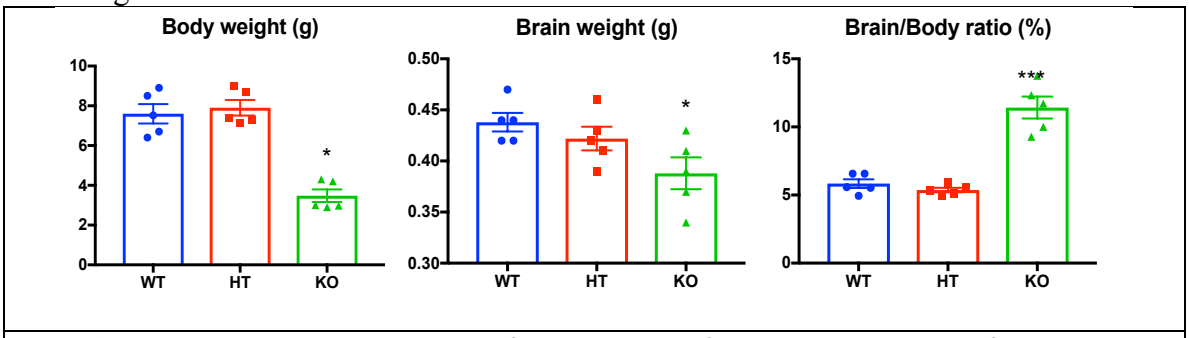

Figure 1. Body and brain weights in a cohort of NEX-Tsc2 mice. Statistical analysis was performed using the GraphPad Prism 7 software (n=5 mice per genotype group). Body weight values were analyzed using Kruskal-Wallis with Dunn's multiple comparisons test, whereas brain weight and brain/body ratios were analyzed using ordinary one-way ANOVA with Tukey's multiple comparisons test. Adjusted p values of WT vs. HT were not significantly different for any parameter measured; KO values were significantly different than WT or HT. *=p<0.05; ****= p<0.0001.

To analyze neuronal development and synaptogenesis in NEX-Tsc2 mice, we isolated the brain of P16 mice of the 3 genotypes, extracted RNA and proteins, conducted RT-PCR analysis of neuronal markers, and examined cell and synaptic markers in both whole lysates as well as crude synaptosome preparations by Western blot analysis. RT-PCR analysis was conducted after extracting total RNA samples from the cerebral cortex or the hippocampus of NEX-Tsc2 mice using gene specific primers. Specific proteins were detected using the quantitative LI-COR Odyssey Fc imaging system. Blots will be stained with REVERT™ Total Protein Stain (Li-COR) to control for protein loading, followed by primary antibodies and secondary antibodies conjugated to near-infrared fluorophores (Li-COR). Statistical analysis was performed by ordinary one-way ANOVA with Dunnett's post-hoc multiple comparisons using the GraphPad Prism7 software.

RT-PCR analysis of neuronal markers in the forebrain of NEX-Tsc2 mice.

The data plotted below show that there is a significant decrease in *Tsc2* mRNA levels in heterozygous and homozygous mutant both, in the cerebral cortex (Fig. 2) and in the

hippocampus (Fig. 3). However, there was no significant difference in the expression levels of the neuronal markers βIII Tubulin (*TuJ1)* (p>0.05).

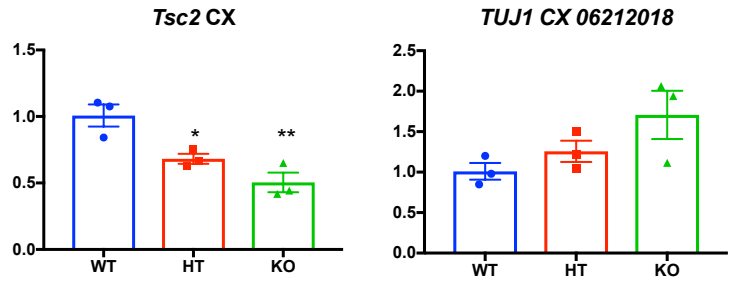

Figure 2. RT-PCR analysis of Tsc2 and TuJ1 expression in the cerebral cortex. One-way ANOVA with Dunnett's multiple comparisons, n=3 samples per genotype, *p<0.05, **p< 0.01.

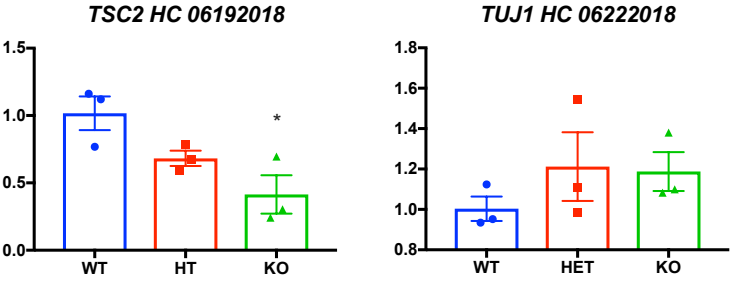

Figure 3. RT-PCR analysis of Tsc2 and TuJ1 expression in the hippocampus. One-way ANOVA with Dunnett's multiple comparisons, n=3 samples per genotype, *p<0.05.

Western blot analysis of neuronal markers in the whole forebrain of NEX-Tsc2 mice.

Consistent with the data above, Western blot data show that there is no significant difference in the protein expression of neuronal markers such as TuJ1 in NEX-Tsc2 mutant mice, despite the deficit in Tsc2 expression. Furthermore, there was no defect in the expression of the dendrite marker Map2 or the axonal markers Tau or NFH in either HT or KO mice (Fig. 4).

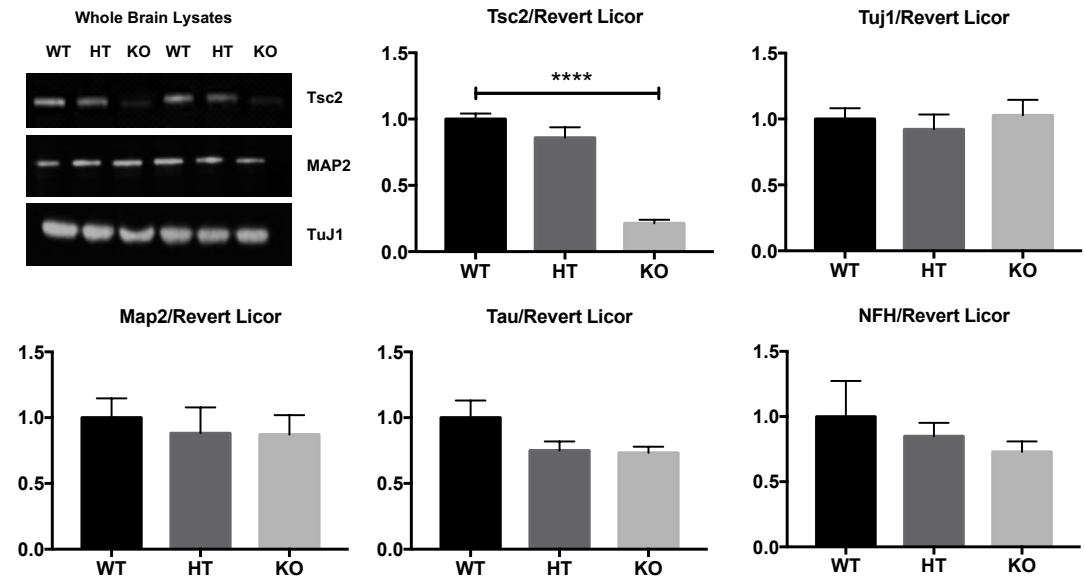

Figure 4. Western blot analysis of neuronal markers in whole forebrain homogenates of NEX-Tsc2 mice. One-way ANOVA with Dunnett's multiple comparisons, n=5 samples per genotype, ****p<0.0001.

Western blot analysis of synaptic markers in whole forebrain lysate of NEX-Tsc2 mice.

Next, we analyzed synaptic markers by Western blotting. The data below show that there is no significant difference between genotypes regarding the expression levels of general presynaptic marker Synaptophysin (Syn), inhibitory-specific presynaptic marker VGAT and Synaptotagmin2 (Syt2), excitatory-specific postsynaptic marker PSD95 and inhibitory-specific postsynaptic marker Gephyrin. Nevertheless, the expression levels of most of these markers appeared consistently decreased in homozygous mice. However, there was a significant decrease in the levels of the excitatory-specific presynaptic marker VGlut2. These results suggest that Tsc2 deficiency in excitatory neurons results in a strong deficit in the formation of excitatory synapses, but it may also affect other synapses.

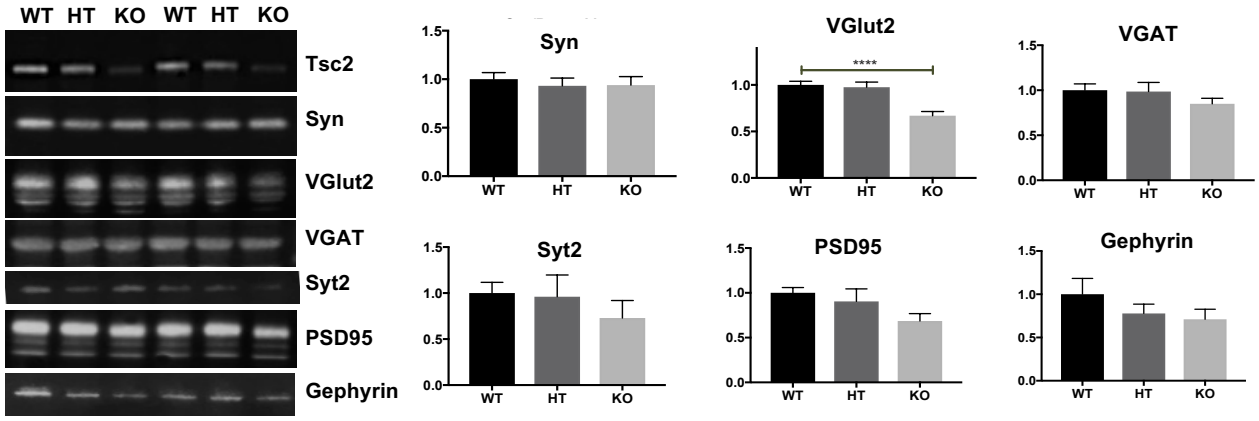

Figure 5. Western blot analysis of synaptic markers in whole forebrain homogenates. One-way ANOVA with Dunnett's multiple comparisons, *n*=5 samples per genotype, ****p<0.0001.

Western blot analysis of synaptic markers in synaptosomes of NEX-Tsc2 mice.

To examine synaptic markers in more detail we prepared crude synaptosome fractions of the forebrain of P16 NEX-Tsc2 mice and conducted Western blot analysis of the same markers. The data confirm that there is no significant difference between genotypes in the expression levels of general presynaptic marker Synaptophysin (Syn) or inhibitory-specific presynaptic marker VGAT, but there is a significant decrease in the excitatory-specific presynaptic marker VGlut2 and the inhibitory-specific marker Synaptotagmin2 (Syt2) in homozygous mice. Also, the data confirm that excitatory-specific postsynaptic marker PSD95 is not altered, but indicate that inhibitory-specific postsynaptic marker Gephyrin is decreased, although the difference was significant only when homozygous values were compared to heterozygous values. Overall the results suggest that Tsc2 deficiency in excitatory neurons results in a strong deficit in the formation of excitatory synapses, and possibly also affect inhibitory synapses.

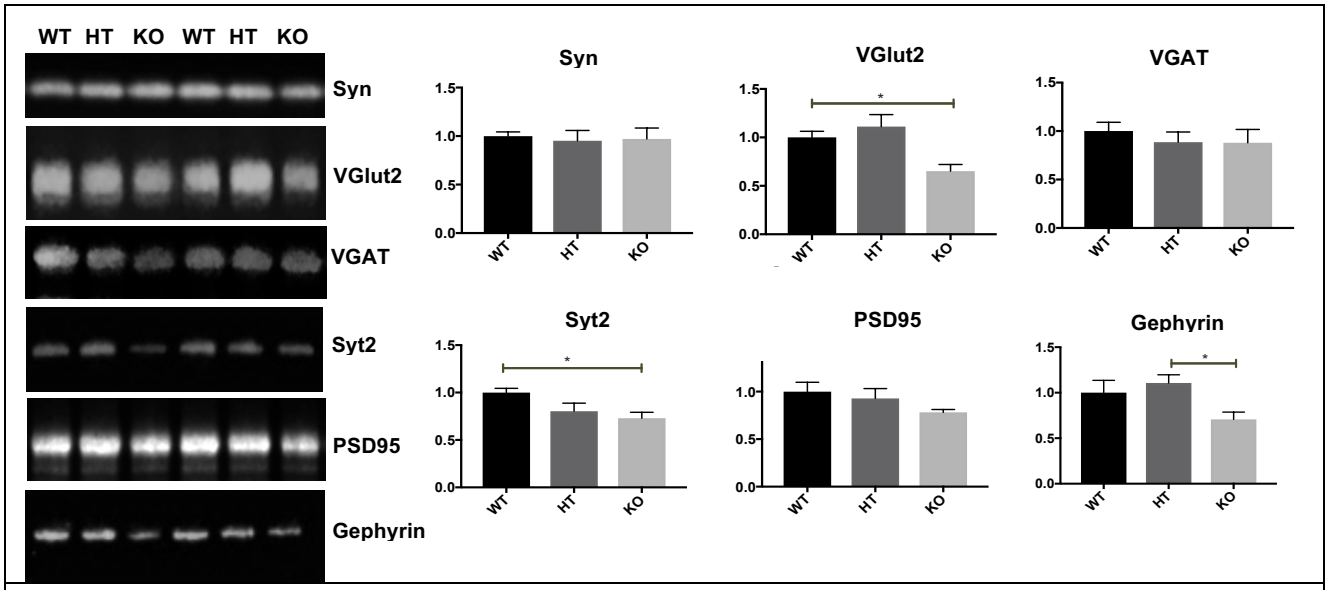

Figure 6. Western blot analysis of synaptic markers in synaptosome fractions. One-way ANOVA with Dunnett's multiple comparisons, *n*=5 samples per genotype, except for Syn (*n*=3-4 samples per genotype), *p<0.05.

The data above indicate that the Tsc2 mutation in homozygosity not only disrupts the development of excitatory neurons (cell-autonomous) but it may also interfere with the development of interneurons forming inhibitory synapses on mutant cells (non cell-autonomous). To explore this possibility, we conducted RT-PCR and Western blot analysis of GAD67 (an inhibitory neuron marker) mRNA and protein expression in the forebrain of P16 mice.

RT-PCR analysis of *GAD1* (GAD67) in the forebrain of NEX-Tsc2 mice.

The data below show that there is no significant decrease in *GAD1 (GAD67)* mRNA levels in heterozygous and homozygous mutant both, in the cerebral cortex or hippocampus (Fig. 7). However, there was a modest decrease in the homozygous hippocampus.

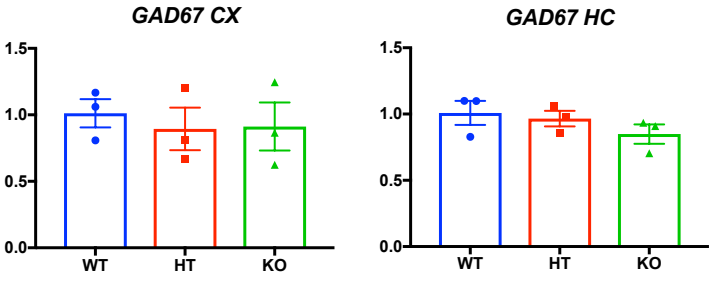

Figure 7. RT-PCR analysis of the *GAD1* gene (encoding GAD67) expression in the cerebral cortex (CX) and hippocampus (HC). One-way ANOVA with Dunnett's multiple comparisons, *n*=3 samples per genotype.

Western blot analysis of GAD67 expression in whole forebrain lysate of NEX-Tsc2 mice.

To examine the levels of GAD67 protein we conducted Western blot analysis of the same whole forebrain homogenate samples used for the above analysis of synaptic markers and some previous samples. The data show that there is a modest decrease in GAD67 expression in homozygous samples. However, the significance of these results depends on the statistical tool

utilized. One-way ANOVA comparing all 3 genotypes indicates a marginal but not significant difference (p= 0.0574), whereas an unpaired t test comparing only WT to KO samples indicates a significant difference (p=0.0197).

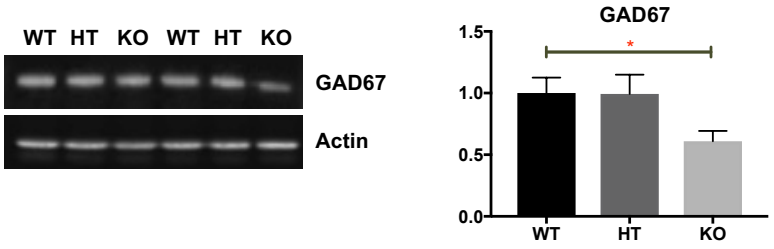

Figure 8. Western blot analysis of GAD67 expression in whole forebrain homogenates. One-way ANOVA with Dunnett's multiple comparisons (p>0.05), unpaired t test WT versus KO *p<0.05, *n*=8-9 samples per genotype.

Immunofluorescence analysis of GAD67 expression in the forebrain of NEX-Tsc2 mice.

To further investigate the expression of GAD67 in the forebrain we perfused 3 sets of P16 NEX-Tsc2 mice, prepared brain sections with a cryostat, and conducted immunofluorescence. Epifluorescence images reveal the presence of sparse GAD67+ cells in the cortex and hippocampus of WT and KO mice. Confocal images further revealed the cell bodies of these GAD67+ inhibitory neurons as well as their presynaptic terminals decorating the cell bodies of GAD67- excitatory neurons in both brain regions.

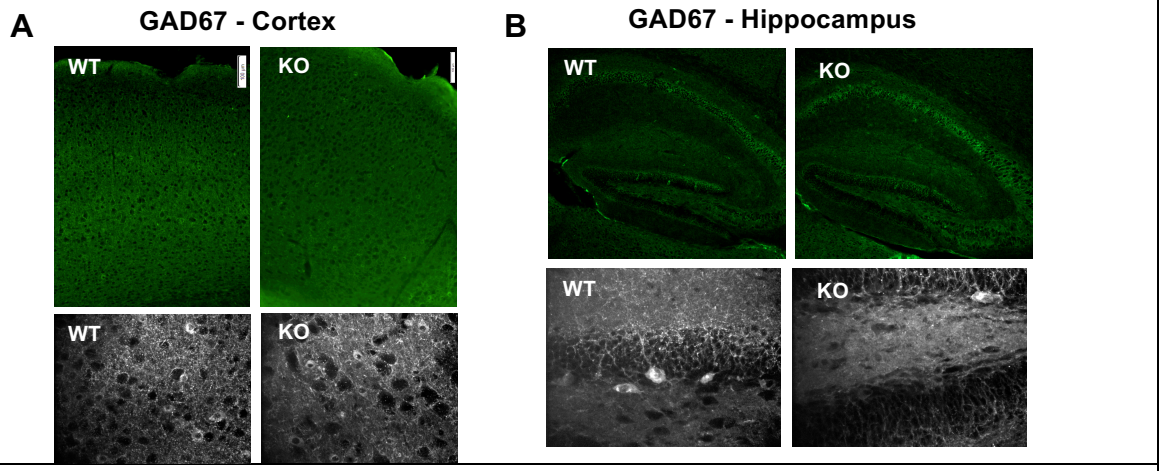

Figure 9. Immunofluorescence analysis of GAD67 expression in the cerebral cortex (A) or hippocampus (B) of NEX-Tsc2 mice. Top panels (green) are epifluorescence images at 10x magnification; bottom panels are confocal images at the 20X magnification.

At first glance GAD67 expression appeared mostly normal in KO mice, however, there seemed to be a decrease specifically in the dentate gyrus of the hippocampal formation. To determine if this was the case we analyzed the number of GAD67+ cells in the whole hippocampus proper and in the dentate gyrus alone. The data show that there was a significant deficit in GAD67+ inhibitory neurons specifically in the KO dentate gyrus (Fig. 10).

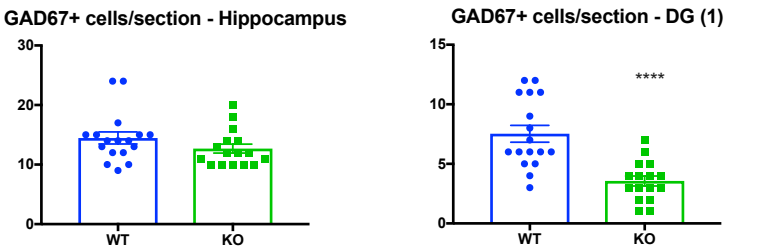

Figure 10. Quantification of GAD67+ cells by immunofluorescence in the whole hippocampus or dentate gyrus (DG) of NEX-Tsc2 mice. Data were analyzed by unpaired t tests; n= 16-17 sections from 3 mice/genotype. ****p<0.0001.

Together, the data indicate that the loss of Tsc2 expression in KO mice results in a reduced number of inhibitory neurons, specifically in the dentate gyrus of the hippocampus.

Seizures and EEG abnormalities.

Recurrent spontaneous seizures were evident in KO mice beginning at P11 and observed until death (Figure 11A). No epileptiform activity was detected in WT or HT mice. Epileptiform activity in KO mice appeared initially as isolated interictal spike activity that evolved into frequent polyspike discharges. Whereas exploratory time in KO mice was normal on the first day of recording, subsequent days consisted of longer periods of immobility interrupted by brief periods of exploration. Immobility coincided with epochs of epileptiform activity. Quantitative EEG analysis revealed that total EEG power (0.5-50 Hz) was decreased compared to WT in KO from P11 to P14 and in HT mice at P14 (Figure 11 A- C). KO mice showed significantly reduced power in delta frequencies at P11 compared to WT and HT mice (1-3 Hz; Figure 11C), while HT are comparable to WT at this age. At P14, spectral power was significantly reduced in delta and theta frequencies in both KO and HT relative to WT (KO: 1-6 Hz; HT: 1-4 Hz; Figure 11D).

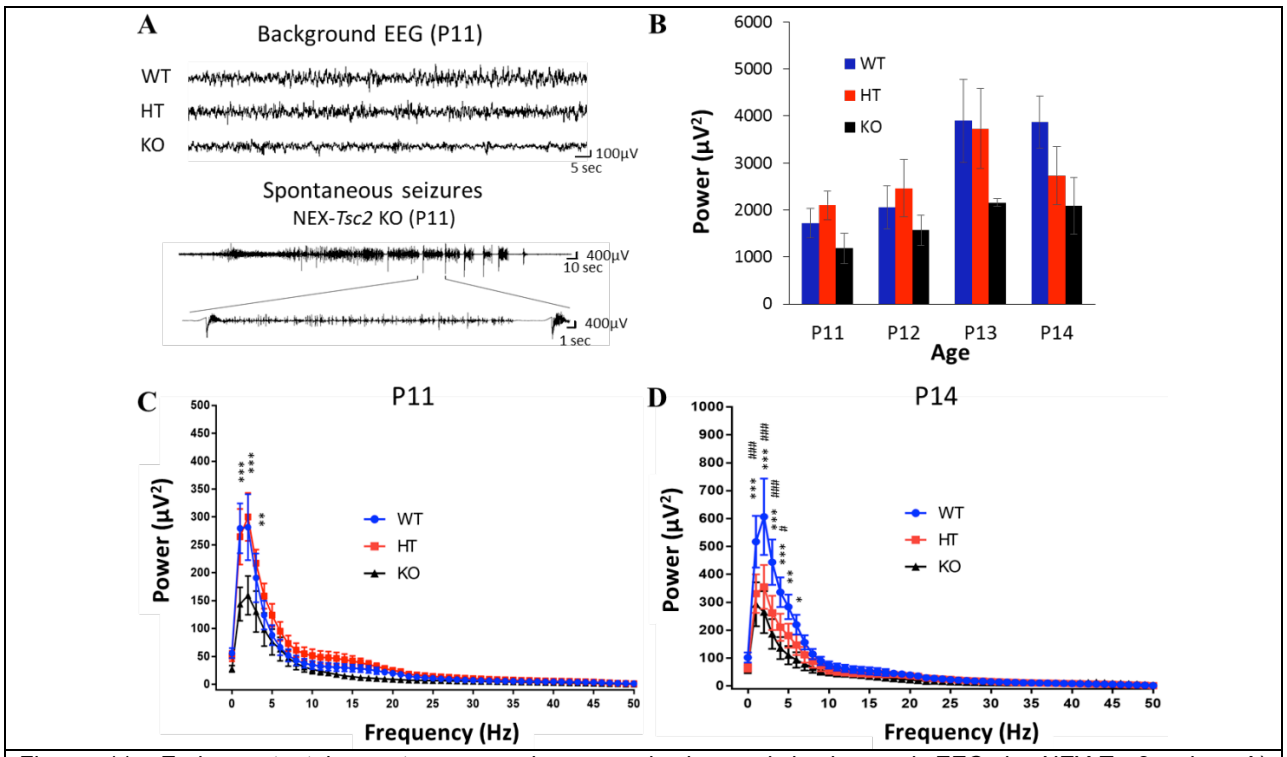

Figure 11. *Early postnatal spontaneous seizures and abnormal background EEG in NEX-Tsc2 mice.* A) Representative cortical EEG from WT, HT, and KO mice at P11 as well as EEG activity from a KO during a spontaneous tonic-clonic seizure. B) Total cortical power (0.5-50 Hz) from WT, HT, and KO mice at P11-P14. Spectral power analysis at C) P11 and D) P14 showed a decrease in delta frequencies in KO mice at P11. At P14, reduced power compared to WT is found in an increased range of frequencies in KO mice, while HT mice showed a reduction in delta frequencies compared to WT mice. Two-way ANOVA; WT vs KO: *p<0.05, **p<0.01; ***p<0.001; WT vs HT: #p<0.05, ###p<0.001; P11: n=6-10/group; P14: n=4-10/group; Data represent mean ± SEM.

Seizure susceptibility in NEX-Tsc2 mutant mice.

To assess seizure threshold, separate cohorts underwent seizure induction with the chemoconvulsant pentylenetetrazol (PTZ; 50mg/kg intraperitoneal) at P12 or P15 (Figure 12). KO mice are more vulnerable than WT and HT mice. Almost all KO mice at both age groups enter status epilepticus (Figure 12, C and D), fail to recover and continue to exhibit electrographic seizure bursts and severe tonic clonic seizures without regaining posture even at 1 hour post PTZ (Figure 12, A and B). Latency to the first seizure varied widely between 1 and 10 minutes with no difference among genotypes.

Data from our pilot experiments at P49 showed HT mice were more susceptible to PTZ than WT mice. Although all mice from both groups developed tonic-clonic seizures after PTZ, 60% of HT mice died whereas all WT mice recovered within 1 hr (n =5 WT, 7 HT). EEG analysis at this time revealed that surviving HT mice displayed an increased frequency of rhythmic spiking compared to WT mice.

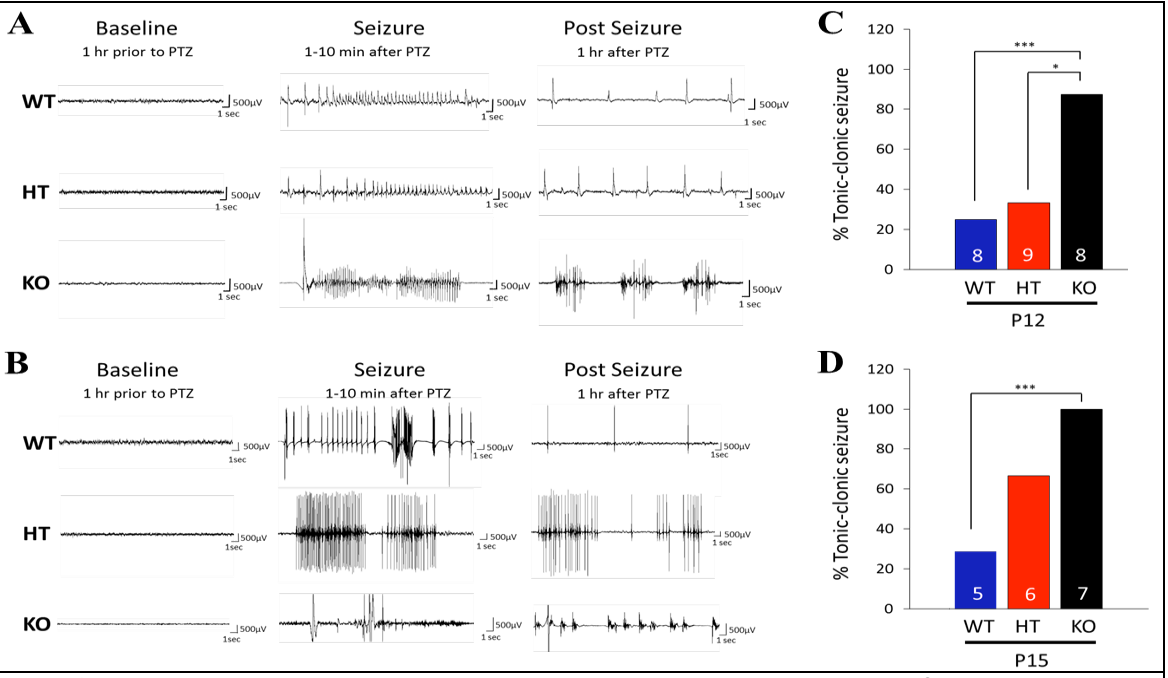

Figure 12. *Increased PTZ seizure susceptibility in juvenile NEX-Tsc2 mutant mice.* After a single injection with PTZ, mice were observed for 1 hour with seizure activity scored using EEG activity (A,B) and behavior (C, D) in mice age P12 (A, C) or P15 (B, D). EEG activity was recorded before, during, and after PTZ-induction. Chi Square test; *p<0.05, ***p<0.001.

Major Task 2: *In vitro* analysis of neuronal abnormalities and neuro-glia interactions in NEX-*Tsc2* cultures

Immunofluorescence analysis of excitatory neuron maturation.

We prepared hippocampal cultures from newborn NEX-Tsc2 mice of all genotypes, and stained them with the neuronal cell body/dendrite marker Map2, and the axonal marker Tau. Excitatory neurons could be easily identified based on their cell body morphology. We first measured soma size and dendrite complexity (Sholl analysis) based on the Map2+ signal. The data from multiple independent experiments reveals that HT neurons are very slightly larger than WT, and have a modest increase in the number of proximal dendrites. Mutant KO excitatory neurons, on the other hand, are very enlarged, and present with dramatically increased dendrite complexity at several distances from the some (Fig. 13).

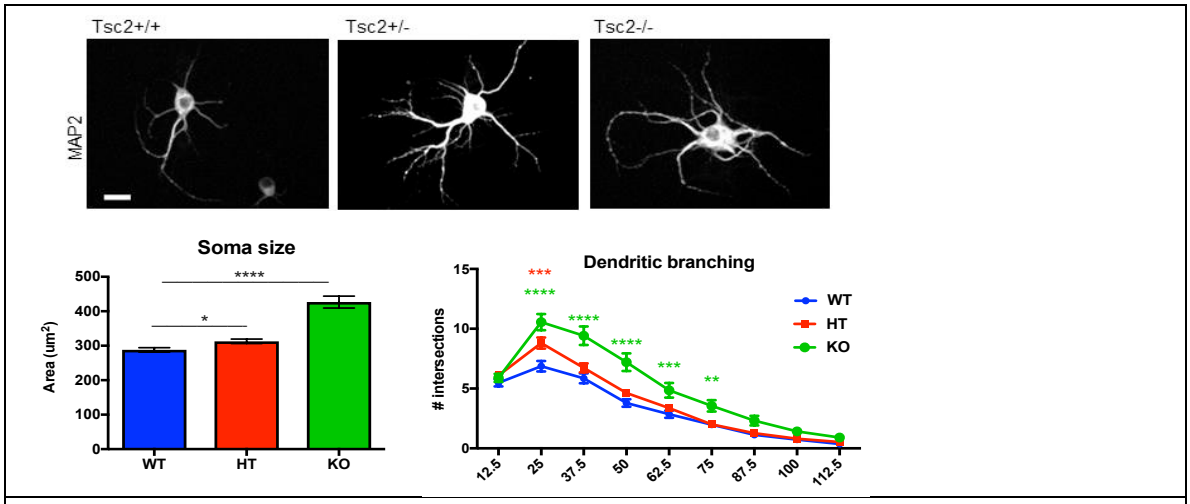

Figure 13. Immunofluorescence analysis of Map2-stained hippocampal excitatory neurons. Analysis of the soma size was conducted using Kruskal-Wallis tests. Dendrite branching was analyzed by two-way ANOVA followed by Dunnett's multiple comparison tests. Values on the X axis represent distances from the soma (μm). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

We then measured axonal length based on the Tau signal. The data from multiple independent experiments reveals that HT neurons are normal with regard to axonal extension. Mutant KO excitatory neurons, on the other hand, present with dramatically increased axonal elongation (Fig. 14).

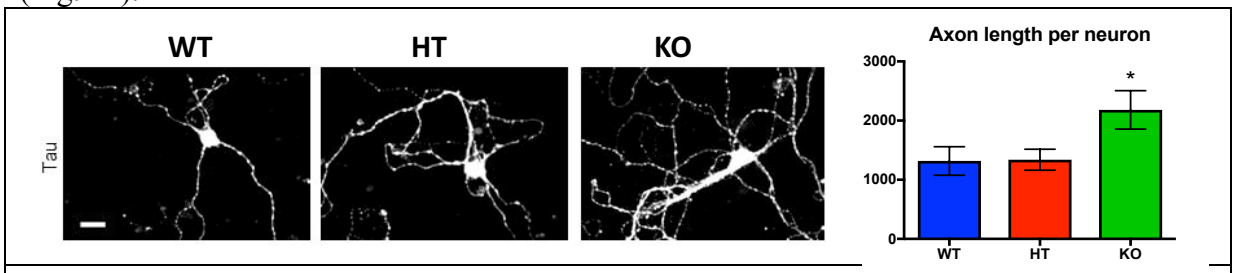

Figure 14. Immunofluorescence analysis of Tau-stained hippocampal excitatory neurons. Total axonal length was analyzed using one-way ANOVA. *p<0.05.

Western blot analysis of signal transduction.

We prepared cortical cultures from newborn NEX-Tsc2 mice of all genotypes, extracted proteins and analyzed them by Western blotting probing for antibodies that reveal the activity of the Akt/mTOR signaling pathway. The data from multiple experiments revealed that signaling in HT cultures appears normal, although a non-statistically significant increase in phosphoS6K, a readout of mTORC1, was noted. Signaling in KO cultures was very abnormal and included a non-statistically significant decrease in phosphoAkt, a non-statistically significant increase in phosphoS6K, and a strong statistically significant increase in phosphoS6, a downstream target of mTORC1 (Figure 15). These data indicate that HT excitatory neurons, the main component of these cultures, are mostly normal, whereas KO neurons are abnormal at the signal transduction level, as expected. Specifically, mTORC1 activity in these neurons is highly increased.

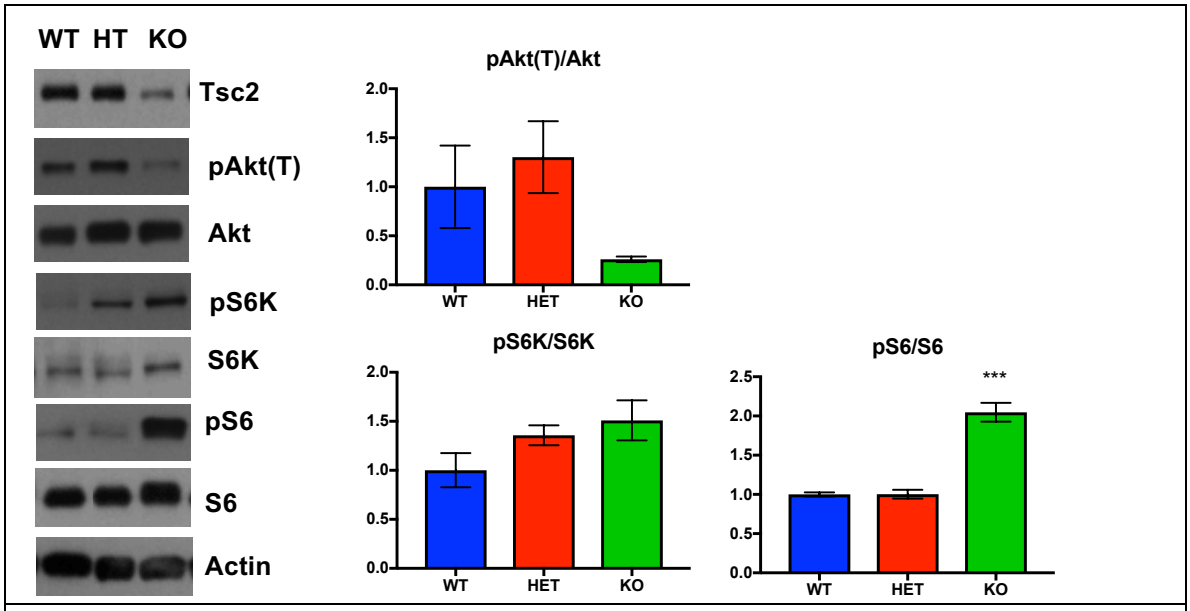

Figure 15. Western blot analysis of cortical cultures. Levels of phosphorylated Akt, S6K or S6 were normalized to the total levels of the corresponding proteins. Data were analyzed by one-way ANOVA. ***p<0.001.

High content imaging analysis of signal transduction.

We used high-content analysis of microscopy images to further investigate mTORC1 signaling in NEX-Tsc2 cultures. These cultures were stained by double immunofluorescence with NeuN antibodies to label all neuronal nuclei, and with phosphoS6 antibodies conjugated to AlexaFluor 488 to probe mTORC1 activity. Random images containing several hundred neurons per genotype were collected using the INCell Analyzer 6000 (GE). The phosphoS6 signal intensity in all neurons was then measured and plotted. The data confirmed that phosphoS6 levels (and thus mTORC1 activity) are strongly elevated in KO but not in HT neurons (Fig. 16).

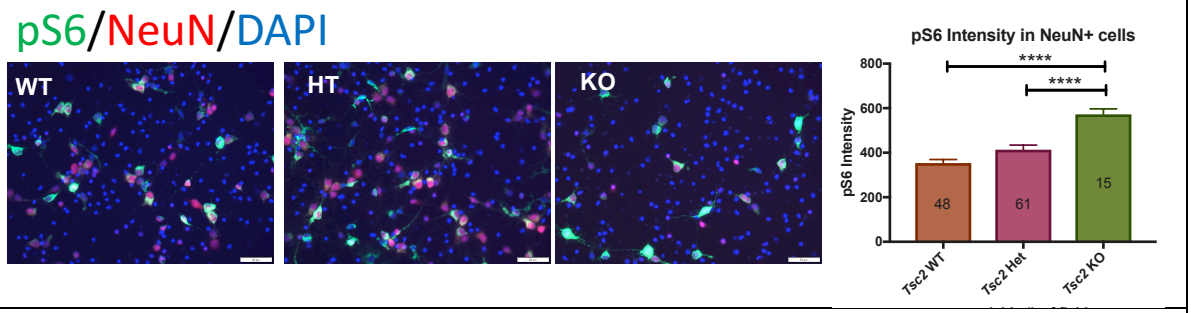

Figure 16. PhosphoS6 (pS6) signal intensity by high-content analysis of microscopy images. Levels of pS6 were measured in NeuN+ neurons from NEX-Tsc2 cortical cultures. Data were analyzed by the Kruskal-Wallis test. n= 15-61 random fields.****p<0.0001.

High content imaging analysis of signal transduction in inhibitory neurons.

We used high-content analysis of microscopy images to investigate mTORC1 signaling in NEX-Tsc2 specifically in the inhibitory neurons present in cortical cultures. These neurons were identified by immunofluorescence with Gad67 antibodies and were double labelled with phosphoS6 antibodies conjugated to AlexaFluor 488 to probe mTORC1 activity. Random

images containing several hundred neurons per genotype were collected using the INCell Analyzer 6000 (GE). The phosphoS6 signal intensity in all Gad67+ neurons was then measured and plotted. The data revealed that phosphoS6 levels (and thus mTORC1 activity) are strongly elevated in the inhibitory neurons present in KO but not in HT cultures (Fig. 17). These findings suggest that Tsc2-null excitatory neurons surrounding the genetically normal inhibitory neurons in KO cultures disrupt mTORC1 signaling by non cell-autonomous mechanisms.

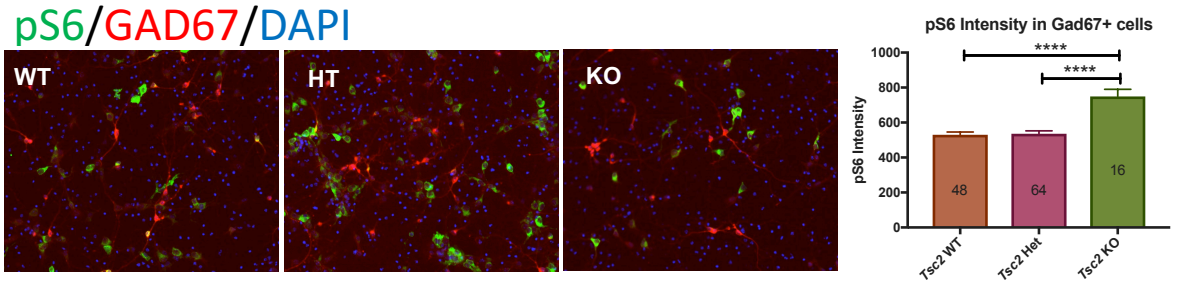

Figure 17. PhosphoS6 (pS6) signal intensity in inhibitory neurons measured by high-content analysis of microscopy images. Levels of pS6 were measured in Gad67+ neurons from NEX-Tsc2 cortical cultures. Data were analyzed by the Kruskal-Wallis test. n= 16-64 random fields.****p<0.0001.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

Nothing to report

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our findings revealed several neurodevelopmental abnormalities resulting from the cell autonomous loss or deficiency in Tsc2, as well as non cell-autonomous defects in inhibitory neurons surrounding KO excitatory neurons. 1) Complete loss of Tsc2 in excitatory neurons disrupts their development, resulting in their hypertrophy, coupled with a disruption in VGlut2+ excitatory synapses, and increased mTORC1 activity. 2) KO excitatory neurons also disrupt the development of inhibitory neurons (which are genetically normal in the NEX-Tsc2 line), resulting in deficits in Gephyrin+ inhibitory synapses, loss of Gad67+ neurons in the dentate gyrus of the hippocampus, and altered mTORC1 activity. Together these defects may explain the elevated seizure activity reported in KO mice. Furthermore, we noted subtle alterations in the development of HT neurons, such as increased soma size, dendrite branching and phosphoS6K levels, indicating that these neurons are not entirely normal. These abnormalities may explain the

reported increased susceptibility to seizures noted in PTZ experiments with HT mice. Our findings elucidate the mechanisms that underlie the altered neuronal activity and seizures that are frequently associated with TSC.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  Nothing to report

- **Journal publications**

  Nothing to report

- **Books or other non-periodical, one-time publications**

  Nothing to report

- **Other publications, conference papers and presentations**

  Nothing to report

- **Website(s) or other Internet site(s)**

  Nothing to report

- **Technologies or techniques**

  Nothing to report

- **Inventions, patent applications, and/or licenses**

  Nothing to report

- **Other Products**

  Nothing to report

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Gabriella D'Arcangelo
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	darcangelo
Nearest person month worked:	12
Contribution to Project:	Dr. D'Arcangelo planned and supervised this study
Funding Support:	NSF

Name:	Beth Crowell
Project Role:	Senior lab technician
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	30
Contribution to Project:	Ms. Crowell managed the NEX-Tsc2 mouse colonies and performed the in vivo experiments in the D'Arcangelo lab
Funding Support:	

Name:	Avery Zucco
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	Mr. Zucco performed the in vitro experiments in the D'Arcangelo lab
Funding Support:	

Name:	Amber Levine
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	20
Contribution to Project:	Ms. Levine performed some of the seizure experiments in the Anderson lab
Funding Support:	NIH and foundation

Name:	Julianah Ajose
Project Role:	Research Technitian

Researcher Identifier (e.g. ORCID ID):
Nearest person month worked: 6
Contribution to Project: Ms. Ajose assisted with the seizure experiments in the Anderson lab
Funding Support: NIH and foundation

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

The PI received the following new grant:

- NSF Proposal #IOS-1755184 (D'Arcangelo G)
  Sponsor: National Science Foundation
  Title: Collaborative Research: Structure and function of Reelin in brain development
  Project period: 8/1/18-7/31/21
  Total costs: $ 272,000
  % Effort: 10%

What other organizations were involved as partners?

This project was conducted in collaboration with the lab of Dr. Anne Anderson at the following academic institution:
Organization Name: Baylor College of Medicine
Location of Organization: Houston, TX
Partner's contribution to the project: Collaboration

7. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Since this is a collaborative award, we have included in this report the results of the work conducted by the Collaborating/Partnering PI.

QUAD CHARTS: N/A

8. APPENDICES: None