

AWARD NUMBER: W81XWH-16-1-0263

TITLE: Impaired mTOR Macroautophagy and Neurocognitive Deficits in Tuberous Sclerosis Complex

PRINCIPAL INVESTIGATOR: Guomei Tang

CONTRACTING ORGANIZATION: TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NY
New York, NY 10032

REPORT DATE: July 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE July 2017		2. REPORT TYPE Annual		3. DATES COVERED 15 Jun 2016 - 14 Jun 2017	
4. TITLE AND SUBTITLE Impaired mTOR-Macroautophagy and Neurocognitive Deficits in Tuberous Sclerosis Complex				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-16-1-0263	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Guomei Tang E-Mail: GT2107@CUMC@COLUMBIA.EDU				5d. PROJECT NUMBER 0010837058	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NY 630 W 168TH ST FL 4NEW YORK NY 10032-3725				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT This study is designed to identify mTOR-downstream molecules or pathways that account for synaptic and cognitive deficits in TSC, with the goal of identifying targets for more specific treatment. We had focused on macroautophagy (autophagy hereafter), a homeostatic catabolic degradation process downstream of mTOR, which is inhibited by hyperactive mTOR in Tsc1/2 deficient mouse brain. During the first project year, we had found significant cognitive impairment in Tsc2+/- mice and Atg7CKO autophagy deficient mice at the age of 3months. These mice however did not show cognitive deficits at 1month of age. Prior to the occurrence of cognitive impairment, Atg7CKO mice exhibited an increase in NMDA:AMPA ratio, increased frequency of miniature EPSCs and increased dendritic spine density, all indicating a blockade in postnatal synapse maturation. Atg7CKO mice moreover showed impaired CA3-CA1 long-term potentiation (LTP) and long term depression (LTD), both of which are well-known electrophysiological surrogates of hippocampus dependent learning and memory. Our findings therefore suggest that Autophagy is essential for synapse maturation and the development of normal synaptic plasticity and cognitive functions. We will continue to examine whether autophagy deficiency may underlie cognitive impairment in Tsc1/2 mutant mice during the next reporting period.					
15. SUBJECT TERMS Tuberous Sclerosis, cognitive impairment, autophagy, neuron, synapse maturation, mTOR					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 14	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	9
5. Changes/Problems.....	10
6. Products.....	11
7. Participants & Other Collaborating Organizations.....	12
8. Special Reporting Requirements.....	13
9. Appendices.....	14

1. INTRODUCTION:

Cognitive impairments, including long term and working memory deficits, are commonly neuropsychiatric features of a majority of TSC patients. Recent studies in *Tsc1* or *Tsc2* heterozygous mutant murine models suggest that disinhibited mTOR is sufficient to cause the cognitive impairments, which promoted the preclinical research to assess therapeutic effects of mTOR inhibitors on TSC related cognitive deficits. The effect of mTOR inhibitors is however transient and the efficacy may be limited by their side effects. Unraveling the downstream substrates of overactive mTOR will be critical for developing more targeted and effective therapies for the neurocognitive symptoms in TSC. This study is designed to identify mTOR-downstream molecules or pathways that account for synaptic and cognitive deficits in TSC, with the goal of identifying targets for more specific treatment while limiting side effects of mTOR inhibitors. We will focus on macroautophagy (autophagy hereafter), a homeostatic catabolic degradation process downstream of mTOR, which is inhibited by hyperactive mTOR in *Tsc1/2* deficient mouse brain. Impaired mTOR-autophagy increases dendritic spine synapse density by suppressing postnatal synaptic pruning, a process necessary for the maturation of functional synaptic connections and neural circuits and required for multiple forms of learning and memory. We propose to study whether impaired autophagy may underlie cognitive impairments in TSC mice by disrupting synapse maturation.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

Tuberous Sclerosis, cognitive impairment, autophagy, neuron, synapse maturation, mTOR

3. ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?

Aim 1: To determine the role of impaired mTOR-autophagy in cognitive dysfunction in *Tsc1* or *Tsc2* (*Tsc1/2*) deficient mice (**Time frame:** months 1-24).

Aim 2: To identify synaptic mechanisms of impaired mTOR-autophagy for cognitive dysfunction in *Tsc1/2* deficient mice (**Time frame:** months 1-24).

Aim3 3: To identify molecular mechanisms of impaired autophagy for synaptic dysfunction in *Tsc1/2* deficient mice (**Time frame:** months 13-36).

What was accomplished under these goals?

Task 1: Determine the role of impaired mTOR-autophagy in cognitive dysfunction in *Tsc1* or *Tsc2* (*Tsc1/2*) deficient mice

We had a delay with the start of this project due to the wait for ACURO animal use approval, and due to an accidental lost the *Atg7*^{flox/+};*Camkcre*⁺ breeding mouse line. We had thus spent 4 months to re-derived the female *Atg7*^{flox/+};*Camkcre*⁺ and then the female *Tsc2*^{+/-};*Atg7*^{flox/+};*Camkcre*⁺ breeders (2016.11-2017.02). We however obtained a cohort of *Camkcre* mediated *Atg7* conditional knockout (*Atg7*CKO) and control mice, as well as a cohort of *Tsc2*^{+/-} and

control mice. These mice were grouped into two different age groups: 1-month old and 3-month old mice, and were tested for cognitive function at different ages. Our major findings are: **1) *Atg7CKO* mice exhibited hippocampus-related cognitive impairment at 3 months of age**, indicated by impaired spatial learning (Fig. 1A) and memory (Fig. 1B) in a Morris water maze (MWM), impaired spatial learning in a 2-day radial arm water maze test (RAWA, Fig 1C) and impaired contextual memory in fear

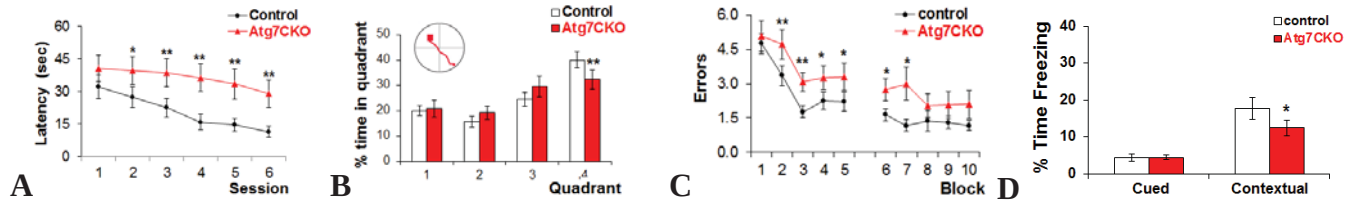


Figure 1. Cognitive deficits in 3month old *Atg7^{CKO}* mice. (A,B) Morris Water Maze test. (A) Escape latency to the hidden platform during learning sessions; (B) Spatial memory assessed in the probe trial for the percentage of time spent in the target quadrant (#4, indicated by the red square) relative to non-target quadrants (#1,#2, #3). (B) 2-day radial arm test. (C) Contextual and Cued fear conditioning test. Data are plotted as the mean $\pm$ SEM (Control, n = 19; *Atg7CKO*, n=14). Compared to controls, *, p<0.05; **, p<0.01, Student t test.

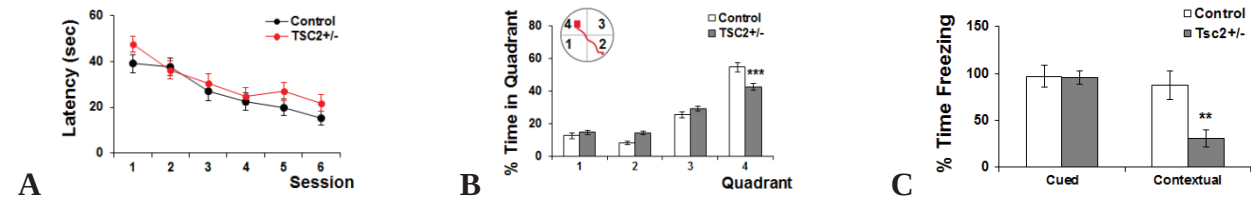


Figure 2. Cognitive deficits in 3month old *Tsc2^{+/-}* mice. (A,B) Morris Water Maze test. (A) Escape latency to the hidden platform during learning sessions; (B) Spatial memory assessed in the probe test. (C) Contextual and Cued fear conditioning test. Data are plotted as the mean $\pm$ SEM (Control, n = 10; *Tsc2^{+/-}*, n=10). Compared to controls, *, p<0.05; **, p<0.01, Student t test.

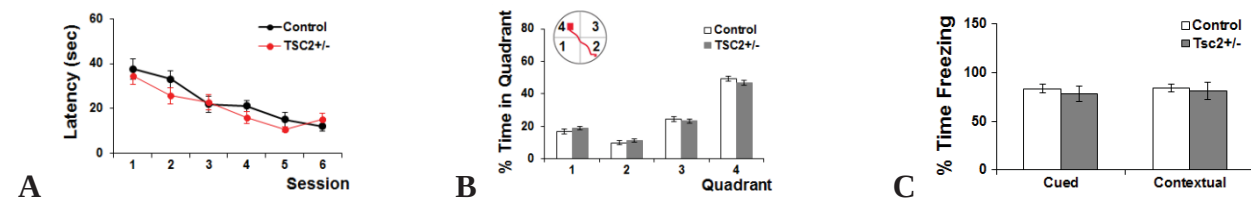


Figure 3. Lack of cognitive impairment in 1month old *Tsc2^{+/-}* mice. (A,B) Morris Water Maze test. (A) Escape latency to the hidden platform during learning sessions; (B) Spatial memory assessed in the probe test. (C) Contextual and Cued fear conditioning test. Data are plotted as the mean $\pm$ SEM (Control, n = 10; *Tsc2^{+/-}*, n=10).

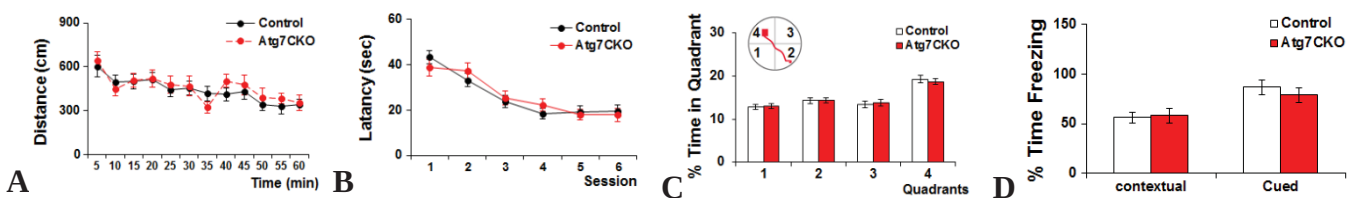


Figure 4. Lack of cognitive impairment in 1month old *Atg7CKO* mice. (A) *Atg7CKO* mice behaved normally in an open field test. (B,C) Morris Water Maze test. (A) Escape latency to the hidden platform during learning sessions; (B) Spatial memory assessed in the probe test. (D) Contextual and Cued fear conditioning test. Data are plotted as the mean $\pm$ SEM (Control, n = 17; *Atg7CKO*, n=14).

conditioning test (**Fig. 1D**); 2) *Tsc2*^{+/-} mice showed hippocampus-related memory at 3 months of age, manifested by a reduction in spatial memory in the MWM test and contextual memory in the fear conditioning test; 3)

using different cohort of mice, we found that *both Atg7CKO and Tsc2*^{+/-} mice did not show cognitive impairment at the age of 1 month (**Fig. 3, 4**). A visible platform trial was administered after the water maze test. All control, Atg7CKO and Tsc2^{+/-} mice reached a visible platform in <1 min ($p > 0.05$), excluding the presence of sensory deficits that may prevent the animals from identifying visual cues. Swimming speed was not reduced in the mutants (not shown), suggesting that the impaired spatial reference memory is a result of defects in cognition but not motility or altered motivation. Both Tsc2^{+/-} and Atg7CKO mice behaved normally in the open field tests (**Fig. 3A**, data for Tsc2^{+/-} mice not shown).

Task 2: Identify synaptic mechanisms of impaired mTOR-autophagy for cognitive dysfunction in *Tsc1/2* deficient mice (Time frame: months 1-24).

We have extended our preliminary study on the characterization of CA3-CA1 synapses in P35 *Atg7*^{CKO} mice. We confirmed the lack of significant differences in resting membrane potentials, membrane resistance, and membrane capacitance between control and *Atg7*^{CKO} mice (**Fig. 5A**). Spontaneous AMPA- and NMDA-mediated EPSCs was recorded at membrane voltages of -70 mV and +40 mV, respectively, and confirmed with either AMPA (CNQX) or NMDA (APV) receptor antagonists. NMDA:AMPA ratio was constructed by dividing the peak NMDA current (at +40 mV) by the peak AMPA current (at -70 mV) for each neuron. We found that in the *Atg7*^{CKO} mice the NMDA current was significantly enhanced (**Fig 5B**), which resulted in an increase in NMDA:AMPA ratio (**Fig 5B**). To determine the average quantal AMPA response of CA1 synapses, we made recordings of miniature EPSCs (mEPSC) in the presence of 50 μ M D-APV and 1 μ M tetrodotoxin (TTX). The mean amplitude of mEPSC events was similar between control and *Atg7*^{CKO} mice (control: 27.6 ± 1.80 pA, $n = 7$; *Atg7*^{CKO}: 29.6 ± 2.05 pA, $n = 7$, $p > 0.05$, Kolmogorov-Smirnov (KS) two-sample test), suggesting that the average synaptic density of AMPA receptors, and thus the synaptic weight of CA1 synapses, remained intact in the *Atg7*^{CKO} mice. The frequency of mEPSC was significantly higher in the *Atg7*^{CKO} mice than in controls (**Figure 5C**, Control: 1.84 ± 0.35 Hz, $n = 7$; *Atg7*^{CKO}: 3.04 ± 0.35 Hz, $p < 0.05$, KS test), indicating that the number of synaptic contacts or release probability may be substantially altered.

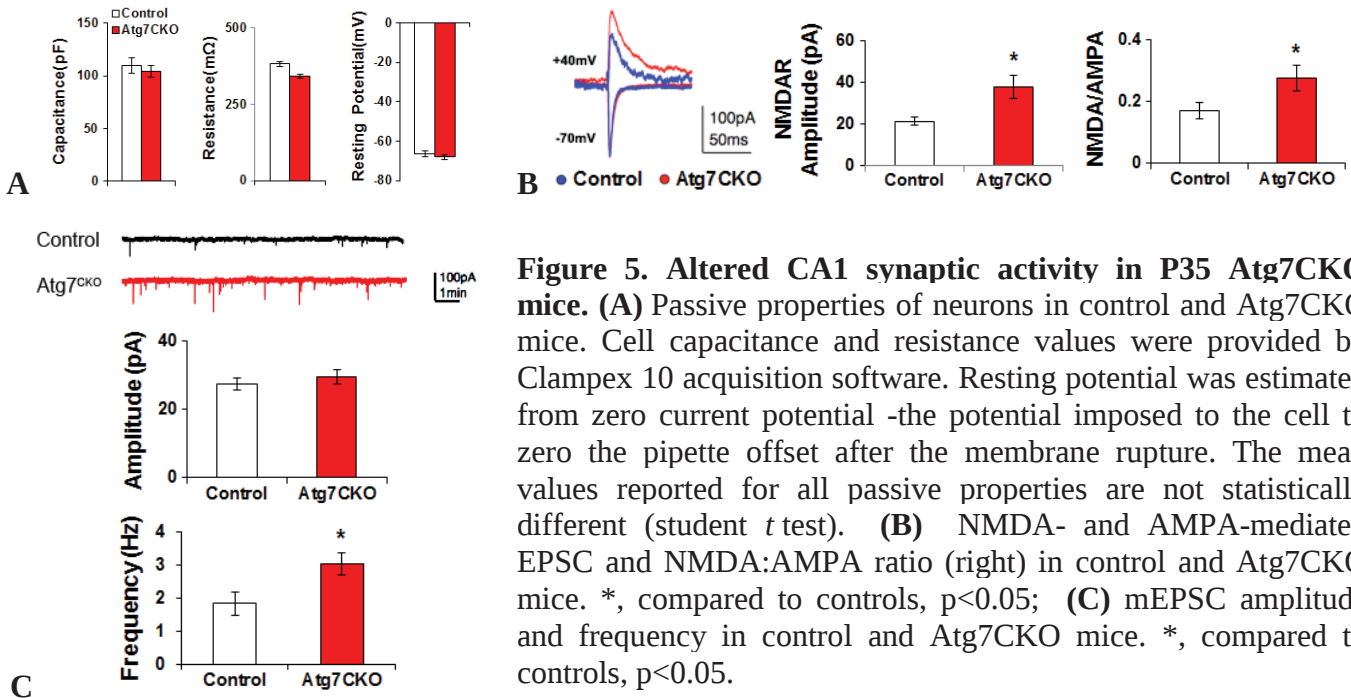


Figure 5. Altered CA1 synaptic activity in P35 *Atg7*^{CKO} mice. (A) Passive properties of neurons in control and *Atg7*^{CKO} mice. Cell capacitance and resistance values were provided by Clampex 10 acquisition software. Resting potential was estimated from zero current potential -the potential imposed to the cell to zero the pipette offset after the membrane rupture. The mean values reported for all passive properties are not statistically different (student *t* test). (B) NMDA- and AMPA-mediated EPSC and NMDA:AMPA ratio (right) in control and *Atg7*^{CKO} mice. *, compared to controls, $p < 0.05$; (C) mEPSC amplitude and frequency in control and *Atg7*^{CKO} mice. *, compared to controls, $p < 0.05$.

We examined dendritic tree complexity and dendritic spine density/morphology using DiOlistic labeling with a Helios Genegun system, as in Tang et al., Neuron 2014. Compared to controls, Atg7CKO CA1 neurons did not show changes in basal dendritic tree complexity (not shown), but a remarkable increase in dendritic spine density (**Fig.6A**). These data were consistent with our above findings of increase mEPSC frequency in the Atg7CKO mice. Altogether, our results support an increase in functional excitatory synapses in P35 Atg7CKO mice. Note that these mice do not develop cognitive impairment at this age when excitatory synapse is overproduced. It is likely that the increase in excitatory synapses due to autophagy deficiency may be the root of cognitive impairment that occurs in a later life. We will seek further evidence during the 2nd year of this award.

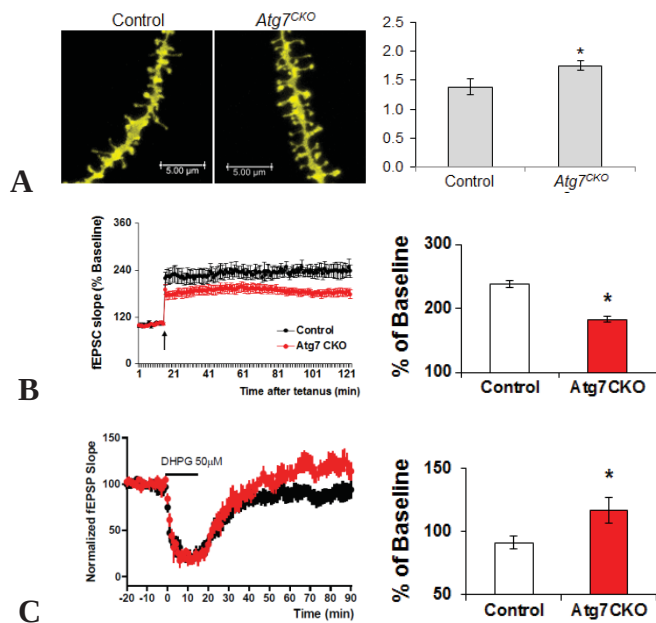


Figure 6 Autophagy and hippocampal synaptic plasticity (A) Increased spine density in CA1 hippocampal neurons in P35 Atg7^{CKO} mice. * compared to control, $p < 0.05$; (C) Averaged theta burst stimulation (TBS) induced LTP was reduced in P35 Atg7^{CKO} mice. * compared to control, $p < 0.05$; (D) Averaged DHPG induced mGluR-LTD was impaired in P24-25 Atg7^{CKO} mice. * compared to control, $p < 0.05$.

cognition. As the selective group 1 mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) effectively induces mGluR, we characterized DHPG-induced mGluR-LTD in control and Atg7CKO mice. Strikingly, autophagy deficiency significantly impaired DHPG-induced mGluR-LTD at hippocampal CA1 synapses: exposure of hippocampal slices from control mice to DHPG at 50 μ M for 10 min induced robust mGluR-LTD at CA1 synapses (**Fig. 6C**). However, in the Atg7CKO mice, the level of DHPG-induced mGluR-LTD was substantially attenuated (**Fig. 6C**).

These results confirmed a blunting of hippocampal synaptic plasticity in Atg7^{CKO} mice that specifically lack autophagy in pyramidal neurons, suggesting that normal autophagy is required for the normal development of LTP and mGluR-LTD in the hippocampus.

Aim 3: Identify molecular mechanisms of impaired autophagy for synaptic dysfunction in *Tsc1/2* deficient mice (Time frame: months 13-36).

It is well-established that specific inhibition of mTORC1 results in sharp translational down-regulation of a set of ribosomal proteins and translation factors through the 4EBP-EIF4E

signaling axis. This interaction is facilitated by 5'-terminal oligopyrimidine (5'-TOP) tracts, which are CT-rich motifs common to the 5'-UTRs of target mRNAs. Our pilot studies computed genome-wide translation efficiencies for the rapamycin treated and untreated control and Tsc2^{+/-} mice and ranked all genes based on their translation efficiency fold-change. Unfortunately, our preliminary results failed to reveal altered translation of neuronal enriched genes in the Tsc2^{+/-}; RiboTag mice (n=2), suggesting that the disrupted protein and synaptic protein homeostasis in the Tsc2^{+/-} mice may be due to suppressed protein degradation, instead of exaggerated translation. When both wt and Tsc2^{+/-} mice were treated with rapamycin, we found that genes containing the TOP-motif are significantly enriched ($p_{adj} < 0.00001$) among the genes that are translationally downregulated by rapamycin treatment (**Fig. 7A**). We next sought to determine whether there are cell type-specific alterations in translation efficiency in response to rapamycin treatment. Our initial hypothesis is that these would be largely restricted to neurons, since the Tsc1 deletion occurs only in Camk2A-expressing cells. **Figs. 2B-C** showed GSEA for translation efficiency fold-change for neuron-specific and oligodendrocyte-specific genes, respectively. Surprisingly, while there are some neuron-specific genes that are translationally downregulated by rapamycin, the effects are much more global and dramatic among genes specific to myelinating oligodendrocytes ($p_{adj} < 0.00001$).

In the second year, we will repeat this measure by using more replicates. If it turns true that the Tsc2 heterozygous mutation does not interfere with the translational profile and that rapamycin exerts some effect on oligodendrocyte phenotype, we will have to modify our original research strategy on ribosome profiling analysis of Tsc1/2 deficient-Autophagy knockout mice, perhaps by profiling the proteome of total hippocampal lysates and excitatory synapses (see our plan for the next reporting period), so that we can isolate the contribution of neuronal autophagy specific effects on synapse/neuronal development.

We had also initiated the complicate mouse breeding for excitatory synapse specific proteomics. We had crossed Atg7^{flox/flox} mice to PSD95^{flox/flox} mice. The resulting Atg7^{flox/+};PSD95^{flox/+} mice were self-crossed to obtain Atg7^{flox/flox};PSD95^{flox/flox} mice (November 2016- February 2017). We next crossed the Atg7^{flox/flox};PSD95^{flox/flox} mice to PSD95^{flox/flox};CamkCre⁺ mice to obtain female Atg7^{flox/+};PSD95^{flox/flox};Camkcre⁺ breeders (March 2017- May 2017).

We are now in the process of crossing Atg7^{flox/flox};PSD95^{flox/flox} mice to the female Atg7^{flox/+};PSD95^{flox/flox};Camkcre⁺ mice (June 2017-present) to obtain Atg7CKO;PSD95^{flox/flox} and PSD95^{flox/flox};CamKCre mice for both synapse proteomics or synaptic protein analysis during the next reporting period.

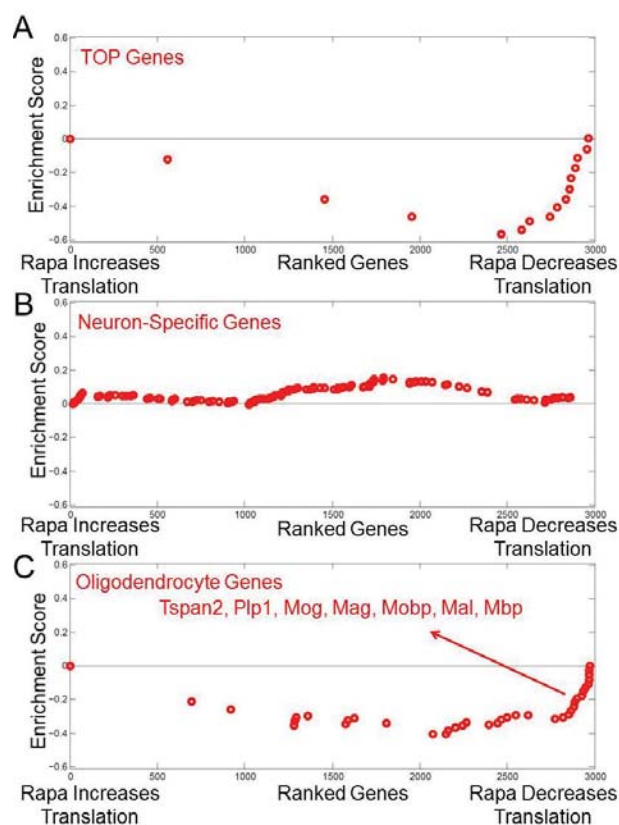


Figure 7 Translational profiling in rapamycin treated mice. (A) Genes translationally downregulated by rapamycin treatment; **(B&C)** Neuronal (B) and oligodendrocyte (C) specific genes that are translationally downregulated by rapamycin.

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

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

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

If this is the final report, state “Nothing to Report.”

In the second year of the award, we will assess whether upregulated mTOR can disrupt cognitive functions through its effects on autophagy (**Aim1**). Both *Tsc1/2* and *Atg7* knockout *Tsc1/2* deficient mice will be treated with or without rapamycin (Rapa) to isolate the effect of autophagy from other downstream events of mTOR. We will measure learning and memory cognitive functions in mice using Morris water maze and fear conditioning tests. Behaviors will be compared among *Tsc2* control, *Tsc2*^{+/-}, *Tsc2*^{+/-}:Rapa, and *Tsc2*^{+/-}:*Atg7*^{CKO}:Rapa mice to isolate the role of mTOR-autophagy in cognitive dysfunction in *Tsc2*^{+/-} mice.

We will examine whether impaired autophagy interferes with CA3-CA1 synapse maturation during the postnatal synapse development in the *TSC1/2* deficient mice (**Aim 2**). Acute brain slices will be cut from the hippocampus of wt mice, rapamycin treated or untreated *Tsc2*^{+/-}, *Tsc2*^{+/-}:*Atg7*^{CKO}, *Tsc1*^{mGFAPCre}CKO, and *Tsc1-Atg7*^{mGFAPCre}DKO mice at postnatal day P19-20 and P35 for the measure of basal synaptic transmission, AMPA:NMDA ratio, pair pulse facilitation. We will assess the morphological changes in dendritic spine synapses using Diolistics in all mouse lines treated with or without rapamycin.

For **Aim 3**, we had proposed to assess the differentially translated genes between rapamycin treated or untreated *Tsc2*^{+/-}, *Tsc2*^{+/-}:*Atg7*^{CKO}, *Tsc1*^{mGFAPCre}CKO and *Tsc1-Atg7*^{mGFAPCre}DKO mice. As stated above, during next reporting period, we will first repeat our preliminary studies to confirm whether *Tsc2*^{+/-} mutation interferes with the translational program in vivo and whether rapamycin can be used to isolate the effect of neuronal autophagy from other downstream cellular and signaling events of mTOR. If this is the case, we will modify our original research strategy on ribosome profiling analysis of *Tsc1/2* deficient-autophagy knockout double mutant mice, perhaps by profiling the proteome of total hippocampal lysates. In tandem with translational or proteome analysis, we will seek to characterize excitatory synapse molecular composition. We will purify flag-tagged synaptosomes specifically from excitatory neurons from *Atg7* deficient mice and in *Tsc2*^{+/-} mice, treated with or without rapamycin.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

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

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Nothing to report

5. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to the accidental loss of mouse breeders, the wait for ACURO approval for animal use, and delays in hiring a postdoc after February 2017, we had been lack of sufficient mice to complete all experiments planned in the first year.

We will complete these incomplete experiments during the 2nd year. To finish all experiments planned in years 1-2 during the next reporting period, we will increase Dr. Hongyu Li’s effort to 100% in the 2nd year Dr. Xiaoping Wu will start his electrophysiological recording in the 2nd year as all mice begin to be available. He will be assisted by a second postdoc who will join the lab in September 2017.

These changes will not have a significant impact on expenditures.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals.

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

Publications, conference papers, and presentations

Report only the major publication(s) resulting from the work under this award.

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Tang G, Kuo SH & Sulzer D (2017). Chapter 11_Autophagy in synaptic structure and function. In Wong E., ed. Autophagy and Signaling. Chapter 11. CRC Press/ Taylor and Francis Books.

Status: *awaiting publication*

Acknowledgement of federal support (*Not Applicable*).

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

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change.”

Name: Guomei Tang

Project Role: Principle investigator

Researcher Identifier (e.g. ORCID ID): 0000-0001-9479-5331

Nearest person month worked: 1.2 months

Contribution to Project: Dr. Tang supervised the project, performed work in mouse breeding, behavioral analysis, molecular biology/RNA analysis and histology.

Funding Support: Dr. Tang's funding portfolio currently includes: NIMH K01 (K01MH096956); The Simons Foundation Autism Research Initiative (SFARI) Pilot award (SFARI 40220); DOD award W81XWH-16-1-0263 and DOD W81XWH-15-1-0112.

Name: Hongyu Li

Project Role: Postdoc

Researcher Identifier (e.g. ORCID ID): N/A

Nearest person month worked: 1 month

Contribution to Project: Dr. Li assisted with mouse breeding and behaviors.

Funding Support: Dr. Li's funding portfolio currently includes The Simons Foundation Autism Research Initiative (SFARI) Pilot award (SFARI 40220) and DOD W81XWH-16-1-0263.

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

If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

The PI has a previously active grant closed in June 2017:

The Simons Foundation Autism Research Initiative (SFARI) Pilot award (#345915)

Sulzer, PI; Tang, PI

Title: "Neuronal translation in Tsc2^{+/-} and Fmr1^{-/-} mutant ASD mouse models"

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.

Nothing to Report

- 9. APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.