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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

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1. INTRODUCTION:

Cognitive impairments, including long term and working memory deficits, are neuropsychiatric features of a majority of TSC patients. Recent studies using *Tsc1* or *Tsc2* heterozygous mutant murine models showed that mTOR disinhibition causes hippocampus dependent cognitive dysfunction. Whereas inhibiting mTOR activities can rescue cognitive impairments, recent clinical studies indicated that the effect of mTOR inhibitors is transient and the efficacy may be limited by their side effects. As such, unraveling the downstream substrates of overactive mTOR will be critical for developing more targeted and effective therapies for the neurocognitive symptoms in TSC. This project is designed to identify molecules or pathways downstream of mTOR that account for synaptic and cognitive deficits in TSC, with the goal of uncovering novel 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. We have found that impaired mTOR-autophagy suppresses 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, and we will thus 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

In **Year 1**, we have examined hippocampus dependent cognitive functions in 1-month old and 3-month old Atg7flox/flox:CamKCre+ (Atg7CKO) and Tsc2+/- mice, using Morris Water Maze, two day radial arm water maze and fear conditioning tests. Both Tsc2+/- and Atg7CKO mice exhibited hippocampus-related cognitive impairment at 3 months of age. These mutant mice however did not develop cognitive deficits at the age of 1 month, when excitatory dendritic spine synapses are exuberant due to the insufficient synapse pruning (Tang et al., 2014). It is likely that the increase in excitatory synapses due to autophagy deficiency contributes to cognitive impairment that occurs in a later life in TSC.

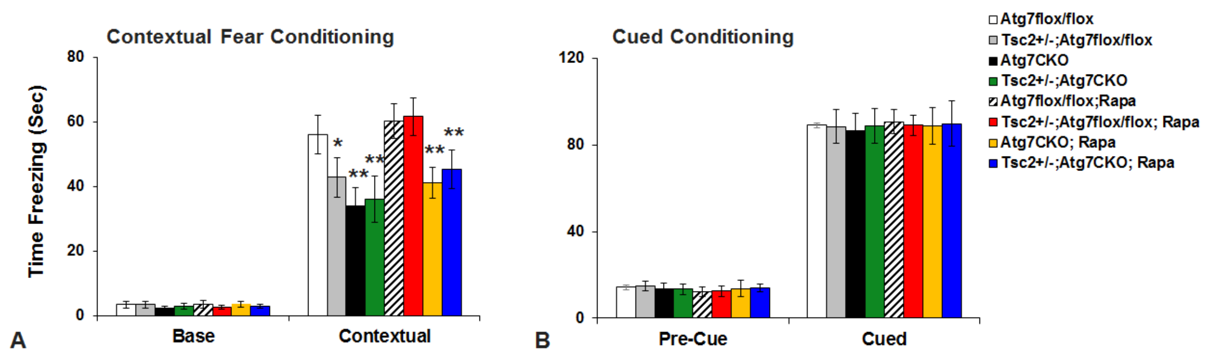

Figure 1. Rapamycin rescues contextual fear Memory deficits in the Tsc2+/- mice but not Atg7CKO and Tsc2+/-;Atg7CKO mice. Vehicle treated mice: Atg7flox/flox, n=12; Tsc2+/-, n=15; Atg7CKO, n=12; Tsc2+/-:Atg7CKO, n=11. Rapamycin treated mice: Atg7flox/flox, n=12; Tsc2+/-, n=20; Atg7CKO, n=13; Tsc2+/-:Atg7CKO, n=11. (**A**) Contextual fear conditioning performed 24 hours after training shows a reduction in freezing in Tsc2+/-, Atg7CKO and Tsc2+/-;Atg7CKO mice. Rapamycin treatment normalized fear memory in the Tsc2+/- mice but not Atg7CKO and Tsc2+/-;Atg7CKO mice. Compared to vehicle treated Atg7flox/flox mice, *, p<0.05; **, p<0.01. 2-way ANOVA; (**B**) No significant differences were observed for cued conditioning between Atg7flox/flox control and Tsc2+/- or Atg7CKO mutant mice. Rapamycin did not affect the cued memory in control and all three mutant mice.

In **Year 2**, we examined whether autophagy deficiency underlies cognitive impairment in Tsc2+/− mice. To disentangle autophagy from other downstream effectors of mTOR, we crossed Tsc2+/− mice to the Atg7CKO mice to produce a Tsc2+/−;Atg7CKO double mutant line. Our previous study showed autophagy defects and spine pruning deficits in all three mutant lines between P20 and P30 (Tang et al., 2014), and that rapamycin normalized autophagy and spine pruning defects in the Tsc2+/− mice to the level of controls but not *Atg7*^{CKO} or Tsc2+/−;Atg7CKO double mutants. We thus hypothesized that neuronal autophagy may be responsible for the development of cognitive function, and that rapamycin treatment would rescue cognitive deficits in the Tsc2+/− mice but would not do so in Atg7CKO and Tsc2+*/-:* Atg7CKO double mutant mice.

We assessed spatial learning and long term memory using Morris Water Maze (MWM), and associative memory using Fear Conditioning to assess associative memory (Puzzo et al., 2014). Mice were treated with rapamycin or DMSO vehicle (5ug/g, ~1ul DMSO/g) through i.p. injection every other day, starting from P21 until 3months of age when cognitive function was examined.

We first investigated the effects of rapamycin on contextual fear memory, a form of associative, hippocampal-dependent memory in which mice have to associate the environment they are exposed to with the occurrence of an aversive stimulus that is delivered during the training phase. Memory was assessed via observation of freezing behavior upon re-exposure to the same context. Consistent with our findings in year1 in naive Tsc2+/- and Atg7CKO mice, we found a reduction in contextual fear memory in vehicle treated Atg7CKO and Tsc2+/- mice (**Figure 1A**). Tsc2+/-;Atg7CKO double mutant mice exhibited significant contextual memory loss at the levels similar to that in Atg7CKO mice, suggesting that there were no additive effects of Tsc2+/- mutation and Atg7 gene depletion mediated autophagy deficiency. Rapamycin rescued contextual memory loss in the Tsc2+/- mice but not in Atg7CKO and Tsc2+/-;Atg7CKO mice (**Figure 1A**). By contrast, cued conditioning, a hippocampus-independent task that was assessed 24 hours after contextual memory by exposing the animals to the tone in a novel environment, which evaluates the function of amygdala, was not affected in all three mutant lines, and rapamycin had not effect on the cued memory in all four mouse lines (**Figure 1B**). Similarly, assessment of the sensory threshold did not reveal any difference between rapamycin- and vehicle-treated mice and between genotypes (not shown), suggesting that treatment with rapamycin does not affect perception of the electric shock. Therefore, autophagy deficiency disrupts hippocampus dependent contextual fear memory in the Tsc2+/- mice.

We next examined hippocampus dependent spatial learning and memory in Tsc2+/-, *Atg7CKO,* and the Tsc2+/-;Atg7CKO double mutants mice, treated with or without rapamycin. During the training phase of MWM, mice were required to learn to find a hidden platform beneath the surface of the water.

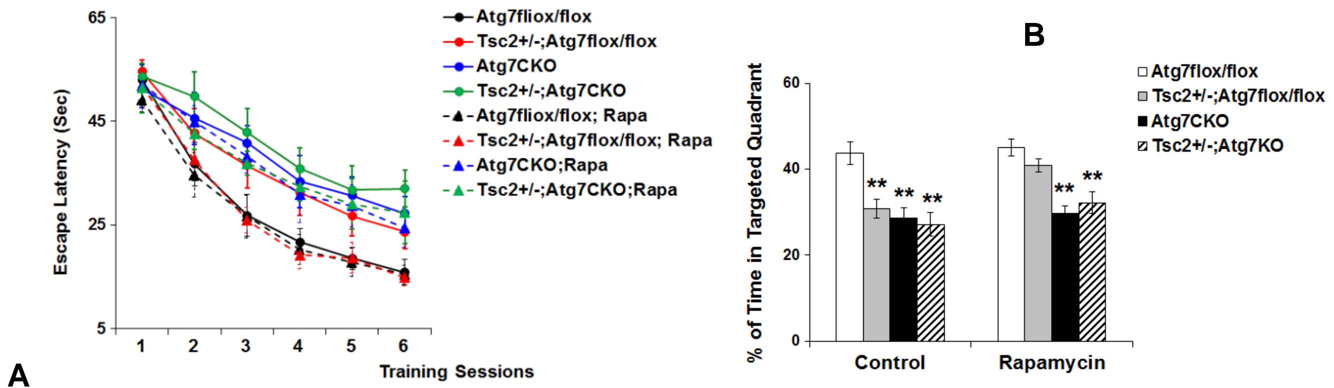

Figure 2. Rapamycin rescues spatial learning and reference memory in the Tsc2+/- mice but not Atg7CKO and Tsc2+/-;Atg7CKO mice. Mice were trained to find a hidden platform in a circular pool of water. Each mouse was given 2 training sessions (4 trials per session, with a 3-hour interval between sessions) per day for 3 consecutive days. The time for the mouse to locate the platform was recorded to analyze escape latency and distance traveled. On day 4, a probe trial was applied by removing the hidden platform from the target quadrant, and the time that testing mice spent in target quadrant was recorded. (**A**) Escape latency to the hidden platform during learning sessions; (**B**) Spatial memory assessed in the probe test. Data are plotted as the mean ± SEM (Vehicle treated mice: Atg7flox/flox, n=12; Tsc2+/-, n=15; Atg7CKO, n=12; Tsc2+/-:Atg7CKO, n=11. Rapamycin treated mice: Atg7flox/flox, n=12; Tsc2+/-, n=20; Atg7CKO, n=13; Tsc2+/-:Atg7CKO, n=11.). Compared to controls, *, p<0.05; **, p<0.01, two way ANOVA.

In vehicle treated mice, compared to *Atg7$^{flox/flox}$* controls, all three mutant mice took longer to find the platform, indicative of impaired spatial learning (**Figure 2A**). When comparing the single sessions, the difference among groups was significant at the 5th ($p = 0.04$) and 6th session ($p < 0.01$). Rapamyin treatment significantly improved learning in the Tsc2+/- mice but not in the Atg7CKO or Tsc2+/-;Atg7CKO mice (**Figure 2A**).

The probe trial, which is used to evaluate reference memory, indicated that all three mutant mouse lines failed to remember where the platform was located in the training sessions, given that all these mutant mice spent a less amount of time in the target quadrant compared to control mice (**Figure 2B**). However, rapamycin treated Tsc2+/- mice spent significant less time in target quadrant, which was comparable to control mice, suggesting a rescue of reference memory. Finally, when performing a trial with a visible platform to exclude motor influences, no differences were found among groups in the latency to reach the platform and speed (not shown).

Taken together, our data strongly support that autophagy deficiency underlies impaired hippocampus dependent cognitive function in the Tsc2+/- mice.

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

In the **year 1** of this project, we characterized CA3-CA1 synapses in P35 *Atg7^{CKO}* mice. We found membrane properties, such as resting membrane potentials, membrane resistance, and membrane capacitance, were similar between control and *Atg7^{CKO}* mice. The NMDA current was significantly enhanced in the *Atg7^{CKO}* mice, resulting in an increase in NMDA:AMPA ratio. We also recorded the miniature EPSCs and found that the amplitude was similar between control and Atg7CKO mice, suggesting that the average synaptic density of AMPA receptors, and thus the synaptic weight of CA1 synapses, remained intact in the Atg7CKO mice. The frequency of mEPSC was however significantly higher in the Atg7CKO mice than in controls, indicating that the number of synaptic contacts or release probability may be substantially increased. Using DiOlistic labeling, we confirmed a remarkable increase in dendritic spine density in the Atg7CKO CA1 neurons which was due to a blockade in postnatal dendritic spine pruning. We further discovered that long-term potentiation (LTP),

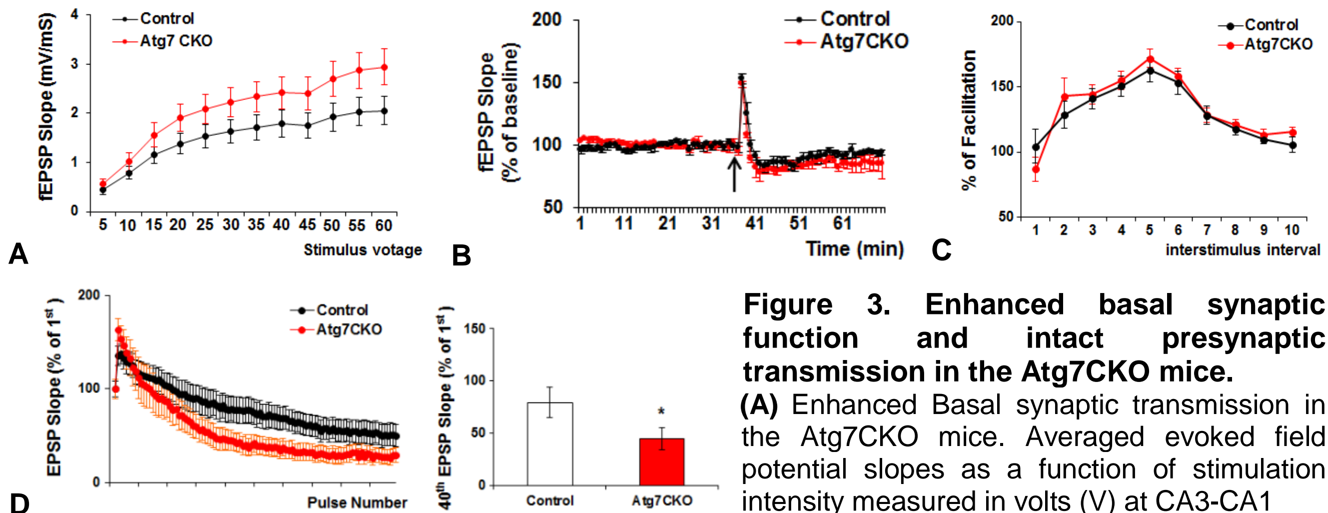

Figure 3. Enhanced basal synaptic function and intact presynaptic transmission in the Atg7CKO mice. **(A)** Enhanced Basal synaptic transmission in the Atg7CKO mice. Averaged evoked field potential slopes as a function of stimulation intensity measured in volts (V) at CA3-CA1 synapses in slices showed significant differences between control and Atg7CKO Mice. (**B**) PTP induced using the tetanic protocol. The magnitude and decay time constant of PTP were similar between control and Atg7CKO mice. The arrow indicates tetanic stimulation. (**C**) Pair pulse facilitation in control and Atg7CKO mice. (D) Synaptic fatigue in control and Atg7CKO mice. *, compared to controls, $p<0.05$. student t test.

an activity dependent synaptic plasticity widely proposed as a cellular model for learning and memory, was impaired in Atg7CKO mice, and that the development of long term depression (LTD) upon activation of group 1 metabotropic glutamate receptors (mGluR-LTD), which is thought to underlie the weakening of synaptic connections, was substantially attenuated in P23-24 Atg7CKO mice immediately following the expression of Cre recombinanse and Atg7 gene depletion. Our findings confirmed an increase in functional excitatory synapses and 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 development of hippocampal synaptic plasticity.

In the **year 2** of this award, we explored the pre- and post- synaptic mechanisms for impaired hippocampal synaptic plasticity, e.g. CA3-CA1 LTP. As an initial step in our analysis, we examined basal synaptic function by plotting the stimulus voltages (V) against the slope of field excitatory postsynaptic potentials (fEPSPs) to generate input-output relations before each experiment. We observed a trend towards enhanced basal synaptic response in the Atg7CKO mice, which was statistically significant at stimulus intensities of 40-60V compared to that of controls (**Figure 3A**).

Post-tetanic potentiation (PTP) is a synaptic parameter indicating presynaptic function. It reflects a period of enhanced transmitter release due to loading of the presynaptic terminal with Ca^{2+} during tetanus stimulus. We induced PTP using theta-burst stimulation (4 pulses at 100Hz, with the bursts repeated at 5Hz and each tetanus including 3 ten-burst trains separated by 15 sec) in 50μM NMDA receptor antagonist D-APV, the same paradigm we used for LTP induction. As shown in **Figure 3B**, no difference in PTP was found between control and Atg7 KO mice, suggesting that presynaptic release in CA1 is not compromised in Atg7 KO mice.

We next examined Pare Pulsed Facilitation (PPF), a presynaptic enhancement of transmission that is observed in the second of two closely spaced stimuli (Katz and Miledi, 1968). PPF reflects a presynaptic build-up of residual Ca++ due to the action potential from the previous depolarization of the terminal, leading to an enhanced transmitter release at the arrival of the

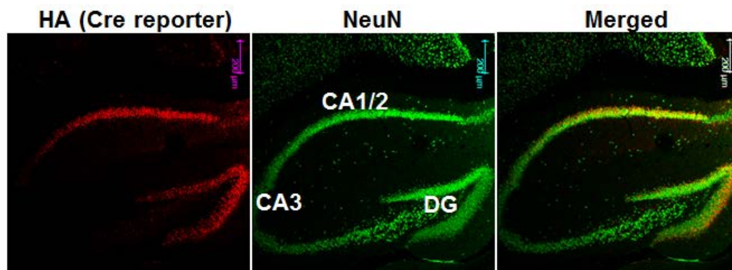

Figure 4. Hippocampal expression of CamkII-Cre recombinanse.

paired stimulus. PPF was induced by paired stimulations at intervals of 10, 20, 30, 40, 50, 100, 300, 500, 1000 ms separately, and was expressed as the ratio between the 2nd and 1st slope of the fEPSPs evoked by the paired stimulation (**Figure 3C**). We failed to detect any differences in PPF between control and Atg7 KO mice, suggesting that presynaptic neurotransmitter release is not compromised in Atg7 knockouts.

As we found no defects in presynaptic transmission in the Atg7CKO mice, we set out to determine whether autophagy is deficient in presynaptic CA3 neurons. This was tested by examining the expression of Cre recombinanse in different hippocampal areas. The CamKII-Cre mice were crossed to the HA-tagged Ribotag mice. The expression of Cre floxed out endogenous Rpl22 allele, resulting in the expression of HA tagged Rpl22 in Cre expressing brain cells. By co-immunostaing for HA and neuronal marker NeuN, we found that CamkII-Cre does not express at CA3 pyramidal neurons (**Figure 4**), suggesting that Atg7 alleles were not floxed out from the presynaptic neurons in the CA3-CA1 circuit.

We also examined synaptic fatigue (SF), a form of short term plasticity that occurs during high frequency stimulation and consists of a decrease in synaptic strength. SF could be caused by a depletion of the release ready pool of presynaptic vesicles or desensitization of postsynaptic glutamate receptors. We induced SF by delivering a 10Hz train for 1 sec during perfusion with D-APV(50μM) and bicuculline (10 μM), and found that SF was more pronounced in Atg7 KO mice (**Figure 3D**). Given that presynaptic transmission remained intact in the Atg7CKO mice, the enhanced SF may suggest a decrease in synaptic localization of AMPA receptors or enhanced desensitization of AMPA receptor at postsynaptic membrane.

Together, our tests of basal synaptic function and short-term forms of synaptic plasticity demonstrate that neuronal autophagy deficiency perturbs the ability of CA1 neurons to respond to controlled presynaptic stimulation. Given the increase in excitatory synaptic density, enhanced basal synaptic transmission and impaired LTP induction, we hypothesize that neuronal autophagy deficiency may occlude further attempts to elicit LTP. This hypothesis will be tested in the next funding period.

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

Our original plan for the Aim3 was to uncover protein substrates that are specifically regulated by mTOR-autophagy that are involved in synapse maturation and the development of cognitive function, with the goal to identify molecular changes due to impaired autophagy that may provide biomarkers for targeted treatment of TSC related cognitive dysfunction bypassing mTOR. In **years 1 and 2** of this project, we had compared genome-wide translation efficiencies between rapamycin treated and untreated control and Tsc2+/- mice and ranked all genes based on their translation efficiency fold-change. Unfortunately, we failed to find altered translation of neuronal enriched genes in the Tsc2+/-; RiboTag mice (n=4). These data confirmed that the Tsc2 heterozygous mutation does not interfere with the translational profile, and that the disrupted protein and synaptic protein homeostasis in the Tsc2+/- mice may be largely due to suppressed protein degradation, instead of exaggerated translation. When both WT and Tsc2+/- mice were treated with rapamyin, we found that only genes containing the TOP-motif are significantly enriched ($p_{adj} < 0.00001$) among the genes that are translationally downregulated by rapamycin treatment (presented in year 1 progress report).

These data were unexpected. As such, we plan to modify our original research strategy on ribosome profiling analysis of rapamycin treated or untreated Tsc2+/-;Atg7CKO mice, and will instead profile the proteome of total hippocampal lysates and excitatory synapses (see plans for the next reporting period), so that we can isolate the contribution of neuronal autophagy specific effects on synapse/neuronal development.

In years 1&2, we had performed the complicate mouse breeding for excitatory synapse specific proteomics. We had crossed Atg7flox/flox mice to PSD95flox/flox mice. The resulting Atg7flox/+;PSD95flox/+ mice were self-crossed to obtain Atg7flox/flox;PSD95flox/flox male breeders. We then crossed the Atg7flox/flox;PSD95flox/flox males to PSD95flox/flox;CamkCre+ females to obtain female Atg7flox/+;PSD95flox/flox;Camkcre+ breeders. By further crossing the male Atg7flox/flox;PSD95flox/flox mice to the female Atg7flox/+;PSD95flox/flox;Camkcre+ mice, we were able to obtain Atg7CKO;PSD95flox/flox and PSD95flox/flox:CamKCre control mice at both ages of postnatal

day 23-24 (P23) and 34-35 (P35) (**n=10 for each group**). We will continue to collect brain tissues from each group at different ages for synapse proteomics or synaptic protein analysis during the next reporting period.

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."

Our studies of basal synaptic function and short-term forms of synaptic plasticity demonstrate that neuronal autophagy deficiency perturbs the ability of CA1 neurons to respond to controlled presynaptic stimulation. Given the increase in excitatory synaptic density, enhanced basal synaptic transmission and impaired LTP induction, we hypothesize that neuronal autophagy deficiency may occlude further attempts to elicit LTP. This hypothesis will be tested in the next funding period. We will study the dendritic spine densities with or without Tetanic high frequency stimuli, so that we can determine whether new synapses can be added during LTP. We will also determine the postsynaptic mechanisms for altered synaptic response by whole-cell patch-clamp recordings of hippocampal CA1 pyramidal neurons.

We will continue to address the molecular mechanisms for altered synaptic and cognitive changes in the Tsc2+/- and Atg7CKO mice. Our original plan was to identify differentially translated genes between rapamycin treated or untreated Tsc2+/- and Tsc2+/-;Atg7^{CKO} mice. As stated above, we failed to detect changes in neuronal translational program in the Tsc2+/- mice treated with or without rapamycin. In the next reporting period, we will proceed to the proteomic analysis of excitatory synapses in both Tsc2+/- and Atg7CKO mouse hippocampus.

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.

Nothing to report.

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.

N/A

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).*

Nothing to report

Other publications, conference papers, and presentations.

Tang G, Li H, Zhang H, Wu X, Arancio O. A role for macroautophagy in postnatal dendritic spine synapse development. Program No. 11.03. 2017 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2017. Online.

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 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: 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: *8* months
Contribution to Project: Dr. Li assisted with mouse breeding and behaviors.
Funding Support: Dr. Li's funding portfolio currently includes 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:

K01 MH096956 (Tang, PI) 05/03/2013-04/31/2018
National Institute of Mental Health (NIMH)
Title: Mitochondrial dysfunction due to aberrant mTOR-autophagy signaling in autism
Role: Principle Investigator

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: N/A

QUAD CHARTS:

Nothing to Report

9. APPENDICES: N/A