IDENTIFICATION OF LEARNING-INDUCED CHANGES IN PROTEIN NETWORKS IN THE HIPPOCAMPUS OF A MOUSE MODEL OF ALZHEIMER’S DISEASE

E Ferreira1, DM Shaw1,2 and S Oddo1,2

INTRODUCTION

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder and the number one cause of dementia in the United States. To this end, it is estimated that > 13 million people will have this disease in the United States by 2050.1 Histopathologically, AD is characterized in part by the presence of extracellular neuritic plaques formed by β40 and β42 peptides. These peptides are generated as the result of sequential cleavage of the amyloid precursor protein (APP) through the amyloidogenic processing pathway.2 The other hallmark lesion of AD is the accumulation of intracellular neurofibrillary tangles consisting of hyperphosphorylated tau.3 These lesions often develop early in AD pathogenesis in the medial temporal lobe structures of the entorhinal cortex and hippocampus.4 One of the earliest clinical manifestations of AD is represented by impairments in memory formation, and as the disease progresses other cognitive domains become impaired, ultimately leading to a bedridden AD patient.5 To date, the precise mechanisms underlying the decline of learning and memory associated with AD are not known.

Immediate early genes (IEGs) play a key role in memory formation and consolidation.6 Most IEGs encode transcription factors that regulate the expression of other genes involved in the establishment of long-term memories.7 Growing evidence suggests that the alterations in these mechanisms may be an early event associated with AD pathogenesis. For example, cAMP-response element-binding protein-regulated transcription is impaired in multiple mouse models of AD, and restoring its function is sufficient to rescue cognitive deficits in 3×Tg-AD mice, a widely used animal model of AD.8-10 In this work, we sought to identify learning-induced changes in protein levels that may be responsible for underlying cognitive deficits in AD.

The use of high-throughput quantitative proteomics has offered significant insights into furthering the understanding of AD pathogenesis at a global molecular level.11,12 These studies have yielded significant results when comparing the proteome of the AD brain versus control cases. For example, the proteomic analysis of human samples compared to control cases has led to the identification of potential biomarkers that can aid in differentiating mild cognitive impairment from AD, as well as biomarkers with both diagnostic value and prognostic value regarding mild cognitive impairment to AD progression.13,14 Proteomic studies in mice have led to important insights of AD pathogenesis as well, such as characterization of the proteomic changes in the hippocampus throughout AD pathogenesis when comparing non-transgenic (NonTg) to transgenic mice with AD-like phenotypes.15 The quantitative proteomic strategy of using isobaric tag for relative and absolute quantitation (iTRAQ) has been a particularly powerful proteomics tool. As an example, iTRAQ method has been applied to specific regions of the mouse brain (that is, hippocampus, parietal cortex, cerebellum) in normal versus AD-like mice to yield the identification of numerous proteins found to be differentially regulated in AD-like mice in a site-specific manner, with most of these proteins found to be involved in molecular transport, nervous system development, synaptic plasticity and apoptosis.16

While these studies have generated significant knowledge regarding proteomic changes due to AD pathogenesis, there is a clear lack of quantitative proteomic studies designed specifically to identify the learning-induced proteomic changes affected by AD. This is a critical distinction, because events in AD pathogenesis are linked to memory formation and consolidation.17 To better understand how AD pathogenesis impedes memory, we employed iTRAQ to measure proteomic differences induced by
MATERIALS AND METHODS

Mice

The generation of the 3 × Tg-AD mice has been described previously. All mice were housed four to five to a cage at 23 °C, kept on a 12 h light/dark cycle and were given ad libitum access to food and water. In our colony of 3 × Tg-AD mice, males show a large neuropathological variability, even between littermates. In contrast, female 3 × Tg-AD mice do not show such large variability and their phenotype changes as a function of age in a predictable manner. Therefore, only female mice were used for the experiments described here. All animal procedures were approved by The Institutional Animal Care and Use Committee of Arizona State University.

Protein extraction from mouse hippocampus for iTRAQ labeling

Mice were killed by CO2 asphyxiation 2 h after final MWM training. Their brains were removed and sagittally bisected. The left hippocampus was removed and used for iTRAQ labeling; the right hippocampus was removed and stored at −80 °C until use for western blot analysis.

Hippocampi were homogenized and lysed in a lysis buffer containing 8 M urea, 50 mM HEPES, pH 8.5. After centrifugation at 20 000g and 4 °C for 60 min, the protein concentration of the supernatant was determined using BCA assay (Thermo Scientific, San Jose, CA, USA) with a resolution of 100 mM TEAB, pH 8.5 − 2 M urea followed by digestion overnight at 37 °C with trypsin in a 1:50 enzyme-to-substrate ratio (Promega, Madison, WI, USA, V5113). After digestion, the peptide mixtures were acidified with trifluoroacetic acid (TFA) to 1%, and subjected to C18 solid-phase extraction (Sep-Pak, Waters, Milford, MA, USA). Finally, the desalted peptide samples were dried in a vacuum concentrator and stored at −20 °C for peptide iTRAQ labeling.

iTRAQ protocol

The desalted peptides were dissolved in 100 μl of 100 mM TEAB, pH 8.5. The peptide concentration was measured using a MicroBCA assay (Thermo Scientific), and 100 μg of digested peptides for the samples were incubated with 40 μl TMT reagents (Thermo Scientific) for 1 h at room temperature. Reactions were quenched by adding 8 μl of 5% hydroxyamine and incubating for 15 min. TMT-labeled samples (S1, S5, S9, S13, S17, S21) were combined at a 1:1:1:1:1:1 ratio, desalted and dissolved in an isoelectric focusing (IEF) buffer consisting of 5% glycerol and 2% IPG buffer (pH 3–10, GE Healthcare, Arlington Heights, IL, USA). The peptide mixtures were loaded into 24 wells over a 24-cm Immobiline Dry Strip, pH 3–10 (GE Healthcare), and separated on a 3100 OFFGEL Fractionator (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer’s instructions. A total of 24 peptide fractions were obtained, acidified using 1% TFA and desalted prior to liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis. The dried peptides were dissolved in 20 μl of 0.2% formic acid and subjected to nLC-MS/MS analysis. The peptides were separated with the homemade C18 reverse-phase column packed with 15 cm of ReproSil-Pur C18-AQ resin (3 μm, 120 Å, Dr Maisch, Ammerbuch, Germany). Peptides were eluted with a 2 h gradient of 6–30% acetonitrile in 0.1% formic acid at a flow rate of 300 nl min−1 using an Easy nLC 1000 system (Thermo Scientific). The eluted peptides were analyzed directly with a Q Exactive (Thermo Fisher Scientific, Anthem, AZ, USA). The spray voltage was set to 2.0 kV. Full-scan MS survey spectra (m/z 300–1600) in positive mode were acquired at a resolution of 30 000 after accumulation of 1 000 000 ions, followed by 4 CID fragmentation (collision-induced dissociation; normalized collision energy, 35%; activation time 30 ms, isolation width 1.0 m/z) and 4 HCD fragmentation (higher-energy collisional dissociation, normalized collision energy 70%; maximum inject time 300 ms; mass resolution 7500; activation time 30 ms) for the 4 most intense peptide ions selected from the survey scan in the Orbitrap.

Data processing, statistical analysis and bioinformatics

Raw MS data files were processed with Proteome Discoverer (Version 1.4, Thermo Fisher Scientific) and searched against Uniprot mouse protein sequence database. The parameters were set as follows: fixed modifications, carbamidomethylation (C), TMT/+/229-D-K and TMT/+/229-D-N terminal; oxidation (M) (variable); the enzyme specificity was set to trypsin; the maximum missed cleavages were set to 2; the precursor ion mass tolerance was set to 10 p.p.m. while MS/MS tolerance was 0.5 Da. The false positive rate was set as 0.01. A TMT 6-plex quantitation method in Proteome Discoverer was applied for HCD-based peptide quantitation. This allowed for quantitative measurement of each detected protein as relative abundance ratios. Ratios were used to determine 1.2-fold change levels and significance of these fold-changes (P < 0.05) using an online distribution-free permutation-based quantitative proteomics P-value calculator (http://qppc.di.uq.edu.au). To account for false discovery, Benjamini–Yekutieli method was used.

ConsensusPathDB-mouse (http://cpdb.molgen.mpg.de/MCPDB) is a bioinformatics web-tool that integrates interaction networks in Mus musculus, including binary and complex protein–protein, genetic, metabolic, signaling, gene regulatory and drug–target interactions, as well as biochemical pathways. Data originate from 16 public resources for interactions and interactions curated from the literature. Overrepresentation analysis was used to identify pathway-based sets in which the 164 proteins of interest were enriched.

STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) is a database of known and predicted protein interactions. The interactions include direct (physical) and indirect (functional) associations; they are derived from four sources: genomics, high-throughput experiments, coexpression and previous knowledge. Proteins of interest were mapped to the STRING10.0 M. musculus database to identify high-confidence protein–protein interaction networks using confidence analysis.

The PANTHER (Protein Analysis Through Evolutionary Relationships) Classification System was used to carry out gene ontology (GO) analysis. PANTHER is designed to classify proteins (and their genes) to facilitate high-throughput analysis. Proteins of interest were classified according to family and subfamily, molecular function and biological process. Detailed methods of how proteins are classified have been previously described.

Protein extraction and western blots

Frozen hippocampi were homogenized using a dounce homogenizer in T-PER buffer (Thermo Scientific) supplemented with a protease inhibitor cocktail tablet (Roche, Indianapolis, IN, USA) and phosphatase inhibitors. Samples were then centrifuged at 25 000g for 30 min at 4 °C. The supernatant was stored as the soluble fraction and used for western blot experiments for validation of iTRAQ data.

Proteins from the soluble fraction were loaded on precast SDS–polyacrylamide gel electrophoresis gels and run under reducing conditions, after which they were transferred to a nitrocellulose membrane. Membranes were incubated in a 5% milk solution in T-TBS (0.1% Tween 20, 100 mM Tris, pH 7.5; 150 mM NaCl) 1 h at 25 °C, washed and incubated in primary antibody overnight at 4 °C. Membranes were washed in TBS-T for 30 min and incubated in goat anti-mouse IRDye 680LT (LI-COR Biotechnology, Lincoln, NE, USA) or goat anti-rabbit IRDye 800CW (LI-COR Biotechnology). Protein densitometry was calculated by dividing the integrated intensity of the protein of interest by integrated intensity of beta-actin loading control.

Antibodies

PLC-y1, eIF4H, a-Parvin and 6-actin were obtained from Cell Signaling Technology (Danvers, MA, USA).

RESULTS

Identification of learning-induced proteome changes

Learning-induced changes in protein expression are known to be at the basis of memory formation and consolidation.19–21
We sought to identify the learning-induced changes in protein expression between 3×Tg-AD mice and NonTg mice. To this end, we used 12–15-month-old mice (n = 4 per genotype). At this age, the 3×Tg-AD mice have well-documented cognitive decline, associated with the build-up of amyloid-β deposits and neurofibrillary tangles (Supplementary Figure 1 and refs 10,18,22). Mice were trained in the spatial version of the MWM for 5 consecutive days, four training trials per day. Thirty minutes after the last training trials, mice were killed and their hippocampi quickly removed and frozen in dry ice. These mice that underwent learning will hereafter be referred to as 3×Tg-AD-L and NonTg-L mice. Per each genotype, control mice were killed directly from their home cage and are hereafter referred to as Naive mice, or 3×Tg-AD-N and NonTg-N. The left hippocampi were then processed for iTRAQ as described in Materials and methods section and Supplementary Figure 2.

We first compared NonTg-N mice with NonTg-L mice to identify changes in protein levels that are related to learning and memory. A total of 3183 proteins were identified and, of these, 321 satisfied the arbitrary cut-off criterion of a 1.2-fold change in levels between NonTg-L and NonTg-N. The 1.2-fold cut-off is a value routinely used in data analyses of iTRAQ data.23–25 Of the 321 proteins that met this first criterion, 192 had a P-value < 0.05 (Figure 1). To determine whether the same proteins were also differently regulated by learning in 3×Tg-AD mice, we repeated the same experiments comparing the steady-state levels of the hippocampal proteome between 3×Tg-AD-N mice and 3×Tg-AD-L mice. We detected 3184 different proteins in the hippocampus of 3×Tg-AD mice. Of these, 133 satisfied the 1.2-fold change in protein levels. All of these had adjusted P-values < 0.05 between 3×Tg-AD-N and 3×Tg-AD-L mice (Figure 1). We postulated that some of the 192 proteins whose levels changed after learning in NonTg mice might be involved in memory formation and consolidation. Therefore, we asked which of these 192 proteins were not differentially expressed when compared 3×Tg-AD-N and 3×Tg-AD-L mice. We found that of these proteins, 28 were also found to have altered levels between 3×Tg-AD-N and 3×Tg-AD-L mice (Supplementary Table 1). This yielded a total of 164 unique proteins whose levels were altered following learning in the hippocampus of NonTg mice, and not in 3×Tg-AD mice (Figure 1). The top 20 of these proteins are shown in Table 1 and the complete list is in the Online Supplementary Material. We also identified 105 unique proteins whose levels were altered following learning in the hippocampus of 3×Tg-AD mice but not in NonTg mice. The complete list of these proteins is in the Online Supplementary Material. We hypothesize that some of the 164 proteins that are differentially expressed following learning in NonTg mice but not in 3×Tg-AD mice may contribute to the memory deficits in 3×Tg-AD mice.

Functional classification of proteins regulated in hippocampus during learning and memory

To gather insight into the biological functions of the 164 proteins found to have altered expression during learning in the hippocampi of NonTg mice but not in those of 3×Tg-AD mice, we conducted GO analysis using the PANTHER classification system database. The PANTHER classification system classifies proteins and their respective genes, by molecular function, biological process, cellular component and protein class to facilitate high-throughput analysis of a large set of proteins/genes.26,27 GO analysis of the 164 proteins of interest showed that these proteins represent numerous and diverse classes of proteins (Figure 2). The largest proportion of the protein class identified represents nucleic acid-binding proteins, suggesting that regulation of gene expression may be disproportionately affected during learning and memory in 3×Tg-AD mice. Consistent with this observation, we also found that some of the 164 differentially regulated proteins are part of the transporter and enzyme modulator proteins, suggesting that the proteins with altered levels during spatial learning may have an important role in protein/RNA trafficking, as well as changes in metabolism. This is further supported when viewing our proteins of interest by their biological process classification, as the highest percentage of proteins are involved in metabolic processes. The next highest percentage of biological process classifications is cellular process, which includes regulation of cell cycle, cell growth, cell communication and cell proliferation, further indicating that these proteins regulated during learning and memory are disproportionately linked to processes involving growth. The classification of these proteins by cellular component indicates that the majority reside in organelles. Last, the classification of the 164 proteins of interest by their molecular function further corroborates the idea that the majority of these proteins are involved in metabolism, transport, regulation of growth and regulation of gene expression as the majority of them have catalytic activity function or binding activity (Figure 2). Taken together, these results indicate that the proteins significantly affected during learning by AD-like pathology are involved in gene expression, metabolism, cell growth and...
protein transport. The altered regulation of these protein classes would significantly impact the synthesis and regulation of proteins required for memory consolidation during a spatial learning training exercise such as MWM.28

Pathway analysis of proteins regulated in hippocampus during learning and memory

To determine the specific cellular pathways in which our proteins of interest play significant roles, we used the online proteomics tool Consensus Path Database (mouse) enrichment analysis function. This database integrates protein–protein interaction networks using data originating from 16 public resources.29 Enrichment analysis using the 164 proteins with learning-induced changes in protein levels in NonTg hippocampi resulted in the finding that these proteins are significantly enriched in RNA-processing pathways such as processing of intron-containing pre-mRNA (P < 0.001), mRNA splicing (P < 0.001) and mRNA processing (P < 0.01), as well as gene expression and metabolism pathways (Table 2). To further identify the protein networks most affected during learning by AD-like pathology, we used the STRING v10. This web-based database and tool maps protein interaction networks based on known and predicted protein–protein interactions.30 Figure 3 shows the protein network resulting from STRING analysis, to identify the key cellular processes and protein interactions view of the 164 proteins, as well as their roles in modifying gene expression and cellular pathways in which our proteins are involved. The STRING analysis includes proteins involved in synthesis and regulation of phosphatidylinositol phosphates (that is, Pip4k2b, Plc1). The inclusion of three associated proteins (Arfgap3, Mrpl38 and Mrpl40) involved in mitochondrial protein synthesis suggests that learning-induced increases in metabolic needs may be affected by AD pathogenesis.

To validate the proteomics data obtained by iTRAQ, we measured learning-induced changes in three randomly chosen proteins, α-parvin, eIF4H and PLC-γ1, by western blot in the hippocampi of NonTg-N and NonTg-L mice. We found that the steady-state levels of α-parvin and PLC-γ1 were significantly increased by learning in the hippocampi of NonTg mice (P < 0.05), as detected by western blot. In contrast, the levels of eIF4H were not statistically significant between NonTg-N and NonTg-L (Supplementary Figure 3). These western blot data were consistent with changes detected with iTRAQ (gray bars in Supplementary Figure 3).

DISCUSSION

In an effort to identify key cellular networks most affected by AD pathogenesis during learning and memory, we employed a quantitative proteomic approach, iTRAQ, to find learning-induced changes in hippocampal protein level that are unique between NonTg and 3 × Tg-AD mice. The overexpression of the two transgenes in 3 × Tg-AD mice may be sufficient to alter the expression of proteins within the networks we identified in our analyses. However, the expression of proteins that are differentially regulated solely because of the tau and APP transgenes would not change between 3 × Tg-AD-N and 3 × Tg-AD-L. In other words, our analytical approach greatly reduces the identification of false positive hits driven solely by the presence of the transgenes in 3 × Tg-AD mice.

We identified 162 proteins that were differentially expressed after learning in NonTg mice but not in 3 × Tg-AD mice. We used them as input for GO analysis, as well as pathway overrepresentation analysis, to identify the key cellular processes and protein networks where these proteins of interest are enriched. Top hits for GO analysis revealed that learning-induced alterations to the hippocampal proteome are overrepresented by regulators of gene expression, metabolism, cell growth and protein transport. Consistent with GO analysis results, pathway analysis using protein interaction databases and bioinformatic web-tools showed that these proteins of interest were significantly enriched

Table 1. Top 20 proteins showing greatest learning-induced change in expression levels unique to NonTg mice

| UniProt ID | Gene symbol | Gene name | Normalized | FC in NonTg-L relative to NonTg-N | Learning-induced regulation |
|-----------|-------------|-----------|------------|----------------------------------|-----------------------------|
| Q88B89    | Clic6       | Chloride intracellular channel protein 6 | 0.000999901 | 3.405049197 | Up |
| A2AJ0     | Map7d1      | MAP7 domain-containing protein 1 | 0.002997003 | 2.614151998 | Up |
| P02802    | Mt1         | Metallothionein-1 | 0.002997003 | 2.505048383 | Up |
| Q9ER35    | Fn3k        | Fructosamine-3-kinase | 0.006993007 | 2.301152791 | Up |
| Q99J77    | Nans        | N-acetylneuraminic acid synthase | 0.001998002 | 2.179955834 | Up |
| Q9CQ10    | Chmp3       | Charged multivesicular body protein 3 | 0.007992008 | 2.175759902 | Up |
| Q31UGX3   | Nat8l       | N-acetylaspartate synthetase | 0.001998002 | 2.148748441 | Up |
| Q8BQ30    | Ppp1r18     | Phoshostin | 0.005994006 | 2.028196723 | Up |
| Q60673    | Ptpn        | Receptor-type tyrosine-protein phosphatase-like N | 0.002997003 | 2.02741379 | Up |
| Q5UK5     | Rab6        | Rab-like protein 6 | 0.002997003 | 2.025783874 | Up |
| Q7TN79    | Akap7       | A-kinase anchor protein 7 isoform gamma | 0.002997003 | 2.025783874 | Up |
| Q8K2M0    | Mrpl38      | 39 S ribosomal protein L38, mitochondrial | 0.011098901 | 1.941987474 | Up |
| Q3UJB9    | Educ4       | Enhancer of mRNA-decappping protein 4 | 0.011988012 | 1.905276998 | Up |
| Q9ESW5    | Agk         | Acylglycerol kinase, mitochondrial | 0.028971029 | 1.621647401 | Down |
| Q5SK18    | Srrm1       | Serine/arginine repetitive matrix protein 1 | 0.018981019 | 1.559280245 | Down |
| Q91W2I    | Eif3h       | Eukaryotic translation initiation factor 3 subunit H | 0.026973027 | 1.479532814 | Down |
| Q8E060    | Gpcpd1      | Glycophosphocholine phosphodiesterase GPCPD1 | 0.038961039 | 1.431324152 | Down |
| Q9WVM2    | Plek2       | Pleckstrin-2 | 0.044955045 | 1.37844914 | Down |
| Q9JVL1    | Bag3        | BAG family molecular chaperone regulator 3 | 0.046953047 | 1.31003492 | Down |

Abbreviation: NonTg, non-transgenic.
in regulation of gene expression networks such as mRNA translation initiate on, pre-mRNA transport and splicing, as well as pathways within metabolic networks such as mitochondrial protein synthesis and regulation of phosphatidylinositol. Taken together, these results indicate that gene expression pathways, specifically regulation of pre-mRNA transport and splicing, as well as initiation of translation, play a prominent role in the learning and memory pathways affected by AD pathogenesis.

Of the GO and pathway analysis results reported above, the most prominent and overrepresented cellular networks affected by AD pathology involve a host of RNA-processing events, including formation of spliceosome with intron-containing pre-mRNA, transport of mRNA out of the nucleus and initiation of mRNA translation for protein synthesis. Consistent with our finding, strong published evidence indicates that alterations in protein translation may contribute to the pathogenesis of AD.31 We, and others, have previously shown that the mammalian target of rapamycin, a master regulator of protein translation,32 is upregulated in human AD brains.18,33–35 These alterations in protein translation may lead to the changes of proteins involved

Table 2. Pathways enriched by proteins identified with learning-induced hippocampal level changes

| Pathway name                                | Pathway set size | Candidates contained | P-value         | Pathway source |
|----------------------------------------------|------------------|----------------------|-----------------|----------------|
| Processing of capped intron-containing pre-mRNA | 144              | 7 (4.9%)             | 3.38 x 10^-4   | Reactome       |
| mRNA splicing                               | 112              | 6 (5.4%)             | 5.46 x 10^-4   | Reactome       |
| α-myoinositol-5 phosphate metabolism         | 19               | 3 (15.8%)            | 6.44 x 10^-4   | MouseCyc       |
| mRNA processing                             | 163              | 7 (4.3%)             | 7.12 x 10^-4   | Reactome       |
| PIP metabolism                              | 54               | 4 (7.4%)             | 1.46 x 10^-3   | MouseCyc       |
| Gene expression                              | 720              | 14 (2.0%)            | 4.52 x 10^-3   | Reactome       |

Figure 2. Pie chart depicting the functional classification of differentially regulated hippocampal proteins during learning and memory between NonTg and 3×Tg-AD mice. The iTRAQ-identified changes in the hippocampal proteome were characterized using gene ontology (GO) analysis. Subcellular and functional categories were based on the annotations of GO using the PANTHER Classification System for the categories of Biological process, Molecular function, Protein class and Cellular component.
in AD pathogenesis and learning and memory. To this end, previous studies have shown that the mRNA transcripts of IEGs involved in learning and memory consolidation, such as Arc, are regulated by localized transport at dendritic compartments of active synapses. This targeted sequestration of IEG mRNA transcripts at dendritic compartments allows for rapid downstream IEG expression in the nucleus of a neuron activated by behavioral events, such as MWM training. This makes the transport of mRNAs, as well as mRNA translation machinery, to dendritic compartments crucial for synaptic plasticity required for learning and memory consolidation. Together, our data suggest that the development of neuropathology in the hippocampi of 3 × Tg-AD mice may affect the learning-induced differential regulation of these RNA-processing pathways and thus impede IEG mRNA expression and downstream signaling cascades that ultimately cause deficits in learning.

In addition to events that affect mRNA transport and translation of initiation, RNA splicing is also a major pathway overrepresented within protein–protein networks of our proteins of interest. Indeed, a report of a proteomics study analyzing the insoluble proteome of human AD brains revealed a significant enrichment of RNA spliceosome proteins, in particular the U1 small nuclear ribonucleoprotein. Comparisons of RNA splice variants between human AD brains to control cases showed dysregulated RNA processing, including unspliced RNA species, within AD brains. This could potentially lead to accumulation of unspliced transcripts that are involved in cellular pathways key to memory consolidation.

The findings reported here point to several major protein networks significantly altered in the hippocampus of 3 × Tg-AD mice affecting mRNA transport and translation initiation, RNA splicing, and protein–protein interactions. These networks involving synaptic membrane ion channels, mitochondrial protein synthesis and most prominently RNA processing, transport and translation are crucial for learning and memory consolidation. The disruption of these networks in AD pathogenesis may contribute to the development of memory deficits in these mice.
translation present avenues for future studies to further elucidate the precise molecular mechanisms responsible for learning deficits in AD pathogenesis. One opportunity for further investigation presented by these data are the investigation of the RNA profiles of 3 × Tg-AD mice during learning and memory events in comparison to NonTg mice. Specifically, examining splicing alterations within mRNA transcripts of genes involved in APP processing, tau phosphorylation, autophagy and proteasomal degradation pathways between NonTg and 3 × Tg-AD mice would be an important comparison to examine. Further study of synaptic membrane dynamics is also warranted, specifically looking at transport of IEG mRNA to dendritic compartments.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

ACKNOWLEDGMENTS
This work was supported by grants to SO by the National Institutes of Health (RO1 AG037637).