Aplysia Neurons as a Model of Alzheimer’s Disease: Shared Genes and Differential Expression

Nicholas S. Kron1 · Lynne A. Fieber1

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Abstract
Although Alzheimer’s disease (AD) is the most common form of dementia in the United States, development of therapeutics has proven difficult. Invertebrate alternatives to current mammalian AD models have been successfully employed to study the etiology of the molecular hallmarks of AD. The marine snail Aplysia californica offers a unique and underutilized system in which to study the physiological, behavioral, and molecular impacts of AD. Mapping of the Aplysia proteome to humans and cross-referencing with two databases of genes of interest in AD research identified 898 potential orthologs of interest in Aplysia. Included among these orthologs were alpha, beta and gamma secretases, amyloid-beta, and tau. Comparison of age-associated differential expression in Aplysia sensory neurons with that of late-onset AD in the frontal lobe identified 59 ortholog with concordant differential expression across data sets. The 21 concordantly upregulated genes suggested increased cellular stress and protein dyshomeostasis. The 47 concordantly downregulated genes included important components of diverse neuronal processes, including energy metabolism, mitochondrial homeostasis, synaptic signaling, Ca++ regulation, and cellular cargo transport. Compromised functions in these processes are known hallmarks of both human aging and AD, the ramifications of which are suggested to underpin cognitive declines in aging and neurodegenerative disease.

Keywords Beta-amyloid · Tau · Neuroinflammation · Invertebrate model

Introduction
Aging in humans is often accompanied by progressive declines in cognitive capabilities that can result in the inability to perform basic tasks, known clinically as dementia (Weller and Budson 2018). By far the most common of these dementias is Alzheimer’s disease (AD), accounting for up to 80% of dementia cases (Crous-Bou et al. 2017). In addition to neurodegeneration, AD is distinguished from other dementias by the presence of two types of protein aggregates, amyloid-beta (Aβ) plaques and hyperphosphorylated tau protein neurofibrillary tangles, in addition to neurodegeneration (Jack et al. 2017). As of 2014, despite more than 30 years of clinical research, only five drugs had been identified as sufficiently safe and effective for international marketing approval, and these provide mostly modest clinical effects (Schneider et al. 2014). The difficulty in studying this illness in living patients coupled with a complex etiology are major hurdles to the study of AD and development of effective drugs to treat it.

One factor that may contribute to the difficulty in AD research thus far is the inability of many model systems to recapitulate the complex nature of the disease. Medina and Avila (2014) assert that an ideal AD model should be able to integrate the genetic, environmental, and aging factors that contribute to AD disease progression. Unfortunately, many current models often address only one factor in isolation (Medina and Avila 2014). However, invertebrate models offer possible alternatives in modeling the complex states which give rise to AD (Calahorro and Ruiz-Rubio 2011; Fernandez-Funez et al. 2015; Sharma et al. 2017). Not only are these models often faster, cheaper, and in line with ethical efforts to reduce the use of vertebrates in research, but they also offer unique investigative techniques or more amenable environments for study when compared to vertebrate models (Alexander et al. 2014; Gotz and Ittner 2008; Link 2005; Moloney et al. 2010; Prussing et al. 2013; Sharma et al. 2017; Surguchov 2021).
Invertebrate models have provided an alternative approach to traditional mammalian models and have been instrumental in elucidating key components of disease progression in AD and AD-related dementias (ADRD). The tractability of behavioral phenotypes and molecular techniques in *Drosophila melanogaster* and *Caenorhabditis elegans* have made these two popular invertebrate models effective tools in investigating disease mechanisms of AD and ADRD and for drug target discovery in AD and ADRD. For example, the molecular basis for Aβ and tau aggregation and toxicity were elucidated via these model systems (Fernandez-Funez et al. 2015; Hannan et al. 2016).

An underutilized model system in which to study AD and ADRD is the marine gastropod *Aplysia californica* (*Aplysia*). Among the preeminent models for learning, *Aplysia* is a well-described neural model ideal for the integrated study of learning and behavior at the molecular, cellular, neural-circuit, and whole organism levels (Baxter and Byrne 2006; Carew et al. 1983; Castellucci et al. 1970; Cleary et al. 1998; Kindy et al. 1991; Klein et al. 1982; Kupfermann 1974; Moroz 2011; Moroz et al. 2006). Due to an annual life span and a well-mapped nervous system, *Aplysia* has also proven to be an excellent model for investigating the effects of aging on learning, cognitive function, and neuronal physiology (Bailey et al. 1983; Hallahan et al. 1992; KempSELL and Fieber 2014, 2015a, b, 2016; Papka et al. 1981; Peretz et al. 1984; Rattan and Peretz 1981; Srivatsan and Peretz 1996). Molecular studies of the effects of aging on the transcriptomes of sensory neurons (SN) revealed similar aging signatures as those of other animals, including metabolic, proteostatic, and neuro-synaptic impairments similar to those that also occur in AD and ADRD (Greer et al. 2019; Greer et al. 2018; Kron et al. 2020). Furthermore, transcriptomic profiling of individually identified giant neurons in *Aplysia* have allowed for the investigation of the effects of aging on specific neurons (Kadakkuzha et al. 2013; Moroz and Kohn 2010, 2013). As a powerful neural aging model, *Aplysia* offers a unique system in which to study AD and ADRD in the context of the greatest risk factor for AD development.

Previously, cultured *Aplysia* neurons have been demonstrated to recapitulate AD-like tauopathies when transfected with mutant human tau (Shemesh and Spira 2010). These neurons were subsequently used to investigate the efficacy of a potential AD therapeutic (Shemesh and Spira 2011). Similarly, exposure of cultured neurons from closely related *A. kurodai* to mutant human Aβ elucidated the inhibitory effects of Aβ on GABA-induced chloride currents (Sawada and Ichinose 1996). Furthermore, cultured *A. kurodai* sensory-motor neuron co-cultures were used to investigate the formation and deleterious effects of coflin-actin rods, hypothesized to be the precursors to the protein aggregates that typify AD and ADRDs like Parkinson’s disease and amyotrophic lateral sclerosis, via overexpression of the native coflin gene (Jang et al. 2005). Together these studies highlight the applicability of the *Aplysia* model system to allow for the study of AD in the context of behavior, genetics, and aging.

In this study, we further demonstrate that *Aplysia* offers a suitable model for the study of AD and ADRD by combing the *Aplysia* genome for potential orthologs of genes of interest in AD and ADRD. We also compare available molecular aging data of *Aplysia* sensory neurons (SN) to those of late-onset AD (LOAD) to demonstrate the capacity of *Aplysia* neurons to naturally recapitulate the preconditions and risk factors that are believed to contribute to AD development in human aging.

**Methods**

**Aplysia Genome Annotation**

The RefSeq proteome for the latest *Aplysia* genome build (AplCal3.0) was downloaded from the NCBI FTP site (https://ftp.ncbi.nlm.nih.gov/genomes/all/annotation_releases/6500/101/GCF_000002075.1_AplCal3.0/). The human UniProt proteome (UP000005640) was downloaded from the UniProt website (https://www.uniprot.org/proteomes/UP000005640) and used to construct a local blast database using the BLAST+command line tool (version 2.6.0; Camacho et al. 2009). The *Aplysia* proteome was then blasted against the human proteome, selecting only the top hit with an e value of ≤0.001. These *Aplysia*-to-human protein annotations were then imported into the R statistical environment and further annotated to the transcript and gene level for *Aplysia* using the latest gene feature format (gff, gff3, gff4). The human proteome was annotated to the gene level by mapping UniProt protein identifiers to human gene symbols using the org.Hs.eh.db R package (Carlson 2019; R Core Team 2013; Wickham et al. 2019).

**Overlap with Alzheimer’s Genes of Interest**

The putative *Aplysia*-human orthologs generated in the previous section were then intersected with two genome-wide association meta-analysis-derived gene sets of Alzheimer’s-associated genes: Alzgset (Hu et al. 2017) and AlzGene (Bertram et al. 2007).

**Comparison of Aplysia Sensory Neuron Aging and LOAD in the Frontal Lobe**

Gene sets previously identified as differentially expressed in aging in *Aplysia* SN (Greer et al. 2018; Kron et al. 2020) were collected and compared with genes identified as
differentially expressed in LOAD via meta-analysis of six different frontal lobe data sets (Li et al. 2015). In their meta-analysis, Li et al. (2015) considered genes that were identified as significant and had concordant direction of expression change in at least five of the six data sets used. In our comparison with Li et al. (2015), we selected all genes marked as DE and exhibited concordant expression direction in at least two of the three Aplysia data sets (PVC from Greer et al. 2018, and PVC and BSC from Kron et al. 2020), and exhibited concordant expression direction in at least five human data sets from Li et al. (2015).

Results

Aplysia Proteome Annotation

Out of 26,658 unique proteins in the Aplysia RefSeq database, 20,495 proteins mapped to 9116 unique UniProt identifiers, equaling on average 2.3 Aplysia proteins per human protein. Each UniProt protein is mapped to one gene in the UP000005640 reference proteome; thus the ~20,500 Aplysia proteins were mapped to ~9000 human genes.

Among these putative orthologs were several human genes involved in AD and ADRD. An ortholog of amyloid precursor protein (APP) was identified in Aplysia previously, and here we identified two potential APP orthologs (Moroz and Kohn 2010). Similar to Drosophila, but unlike C. elegans, we identified putative Aplysia orthologs of both beta-secretase 1 (BACE1) and all components of the gamma-secretase complex: presenilin (PSEN), nicastrin (NCSTN), presenilin enhancer 2 (PSENEN), and two putative orthologs of anterior pharynx-defective 1 (APH1A). We also identified several potential Aplysia orthologs to the primary alpha secretase A disintegrin and metalloproteinase (ADAM) family members including three orthologs of ADAM10, two orthologs of ADAM12, and seven orthologs of ADAM17. Two potential orthologs of the tau protein gene MAPT were also identified.

Of interest in Parkinson’s disease, six potential orthologs of leucine-rich repeat kinase 2 (LRRK2/PARK8), along with putative orthologs of other Parkinson’s disease-associated genes such as protein deglycase DJ-1 (PARK7/DJ-1), Parkin (PRKN), Parkin coregulated gene protein (PACRG), and synphilin (SNCAIP), were identified. However, a potential ortholog for alpha-synuclein (SNCA/PARK1) was not identified.

Overlap with Alzset and AlzGene

Of the 9000 putative orthologs, 219 were present in Alzset and 364 were present in AlzGene. Alzset and AlzGene share 295 genes, of which 166 were among the ~9000 Aplysia-human orthologs. Considering genes from either data set, a total of 418 AD genes of interest with putative orthologs in the Aplysia genome were identified (Fig. 1). This corresponds to 1207 Aplysia transcripts from 898 Aplysia genes. As noted in the above section, orthologs of PSEN1, APP, and MAPT were present, along with several other Aβ- and tau-associated proteins (Table 1). The full mapping is available in Supplemental Data 1.

Comparison to LOAD Frontal Cortex Study

Comparison of differential expression in three aging Aplysia SN data sets with a meta-analysis of six frontal cortex LOAD (FL LOAD) data sets identified 68 putative gene orthologs concordantly differentially expressed in at least five of the FL LOAD studies and two Aplysia data sets. Of these genes, 21 were concordantly upregulated and 47 concordantly downregulated. Commonly upregulated genes included cellular stress-induced genes such as ANKZF1, BTG1, DDIT4L, and SSR1, as well as elements of the proinflammatory toll/interleukin receptor signaling pathways such as MYD88, NFKBIA, MAP3K8, and BIRC3 (Fig. 2 and Table 2). Commonly downregulated genes were representative of diverse processes including synaptic vesicle dynamics (SYN2, EXOC8, NAPG, SWOP, ARF3), transport of cellular cargo (DCTN6, KIFAP3, RAB6A), energy metabolism...
Table 1  Selection of Aβ- and tau-associated genes present in both the AlzGene and Alzgset databases that have putative *Aplysia* gene orthologs. Human gene symbols are mapped to gene name, putative *Aplysia* ortholog IDs, UniProt accession, Gene Ontology IDs, and Gene Ontology names. Genes represented were annotated for GO BP or MF associated with Aβ or tau, present in the AlzGene and Alzgset gene sets, and annotated to putative *Aplysia* gene orthologs by BLAST + with an e-value of ≤ 0.0001. Genes of high interest in AD are bolded.

| Gene symbol | Gene name | Aplysia gene | UniProt IDs | GO IDs | GO names |
|-------------|-----------|--------------|-------------|--------|----------|
| ADAM10      | ADAM metallopeptidase domain 10 | LOC101859462, LOC101851963, LOC101845573 | O14672 | GO:0.034,205, GO:0.042,987 | Ab formation, amyloid precursor protein catabolic process |
| APH1A       | aph1 homolog A, gamma-secretase subunit | LOC101856754 | Q96B13 | GO:0.034,205, GO:0.042,987, GO:0.042,982 | Ab formation, amyloid precursor protein, amyloid precursor protein metabolic process |
| NCSTN       | Nicastrin | LOC100533532 | Q92542 | GO:0.034,205, GO:0.042,987, GO:0.042,982 | Ab formation, amyloid precursor protein, amyloid precursor protein metabolic process |
| PSEN1       | Presenilin 1 | LOC100533344 | P49768 | GO:0.034,205, GO:0.042,987, GO:0.042,982 | Ab formation, amyloid precursor protein, amyloid precursor protein metabolic process |
| PSENEN      | Presenilin enhancer, gamma-secretase subunit | LOC101854684 | Q9NZ42 | GO:0.034,205, GO:0.042,987, GO:0.042,982 | Ab formation, amyloid precursor protein, amyloid precursor protein metabolic process |
| DYRK1A      | Dual-specificity tyrosine phosphorylation-regulated kinase 1A | LOC106013836 | Q13627 | GO:0.034,205, GO:0.048,156 | Ab formation, tau binding |
| ADRB2       | Adrenocorticotropin receptor beta 2 | LOC101855541, LOC101851894, LOC101852650, LOC118478765 | P07550 | GO:0.001,540 | Amyloid-beta binding |
| APBB2       | Amyloid-beta precursor protein-binding family B member 2 | LOC101847028 | Q92870 | GO:0.001,540 | Amyloid-beta binding |
| BCHE        | Butyrylcholinesterase | LOC101862164, LOC101860246, LOC101862869, LOC101851188, LOC101858624, LOC101862414, LOC101861954, LOC101846738, LOC101862657, LOC10185967, LOC106013051, LOC101851390, LOC101854068, LOC1018479136 | P06276 | GO:0.001,540 | Amyloid-beta binding |
| CST3        | Cystatin C | LOC101857420 | P01034 | GO:0.001,540 | Amyloid-beta binding |
| EPHA4       | EPH receptor A4 | LOC101861456 | P54764 | GO:0.001,540 | Amyloid-beta binding |
| GRIN2B      | Glutamate ionotropic receptor NMDA type subunit 2B | LOC100533244 | Q13224 | GO:0.001,540 | Amyloid-beta binding |
| HSPG2       | Heparan sulfate proteoglycan 2 | LOC101857847, LOC101859116, LOC101861971, LOC101855448, LOC101847382 | P98160 | GO:0.001,540 | Amyloid-beta binding |
| LRPA1       | LDL receptor-related protein associated protein 1 | LOC101847798, LOC101860965 | P30533 | GO:0.001,540 | Amyloid-beta binding |
| NGFR        | Nerve growth factor receptor | LOC106012918 | P08138 | GO:0.001,540 | Amyloid-beta binding |
| SORL1       | Sortilin-related receptor 1 | LOC101857914, LOC118477251, LOC101846105 | Q92673 | GO:0.001,540 | Amyloid-beta binding |
| TLR4        | Toll-like receptor 4 | LOC101847817, LOC101850809, LOC101860761 | Q92673 | GO:0.001,540 | Amyloid-beta binding |
| LDLR        | Low-density lipoprotein receptor | LOC118478465 | P01130 | GO:0.001,540, GO:0.097,242 | Amyloid-beta binding, Amyloid-beta clearance |
| Gene symbol | Gene name | UniProt IDs | GO IDs | GO names |
|-------------|-----------|-------------|--------|----------|
| LRP1        | LDL receptor-related protein 1 | LOC101849041, LOC101849281, LOC101859513, LOC100533545, LOC118478804, LOC18478805, LOC106013813, LOC106013825 | Q07954, GO:0.001.540, GO:0.097.242 | Amyloid-beta binding, Amyloid-beta clearance |
| IDE         | Insulin-degrading enzyme | LOC101845820 | P14735, GO:0.001.540, GO:0.097.242, GO:0.050.435 | Amyloid-beta binding, Amyloid-beta clearance, Amyloid-beta metabolic process |
| BACE1       | Beta-secretase 1 | LOC101859129 | P56817, GO:0.001.540, GO:0.050.435 | Amyloid-beta binding, Amyloid-beta metabolic process |
| CHRNA7      | Cholinergic receptor nicotinic alpha 7 subunit | LOC101851082, LOC101856227, LOC101862541, LOC101856484, LOC101852526, LOC101859946, LOC101852974, LOC106012547, LOC106013357, LOC101853763, LOC101845987, LOC101845835, LOC101857866, LOC101858254, LOC101860243, LOC10185238, LOC101855899, LOC101856895, LOC101856344, LOC1018560583, LOC106012570, LOC10186014, LOC101860352, LOC101853230, LOC101853479, LOC101861149 | P3644, GO:0.001.540, GO:1.904.645 | Amyloid-beta binding, response to amyloid-beta |
| PICALM      | Phosphatidylinositol-binding clathrin assembly protein | LOC101848715 | Q13492, GO:0.001.540, GO:0.048.156 | Amyloid-beta binding, tau binding |
| MME         | Membrane metalloendopeptidase | LOC101861636, LOC101853869, LOC101845751 | P08473, GO:0.097.242, GO:0.050.435 | Amyloid-beta clearance, Amyloid-beta metabolic process |
| ACE         | Angiotensin-converting enzyme I | LOC101850558, LOC101852115, LOC101849400, LOC101863410 | P12821, GO:0.050.435 | Amyloid-beta metabolic process |
| APP         | Amyloid-beta precursor protein | LOC18478801, LOC100533426 | P08067, GO:1.990.000 | Amyloid fibril formation |
| MAPT        | Microtubule-associated protein tau | LOC101864325, LOC100610967 | P10636, GO:1.990.000 | Amyloid fibril formation |
| ABCG1       | ATP-binding cassette subfamily G member 1 | LOC101862516 | P45844, GO:0.042.987 | Amyloid precursor protein catabolic process |
| DHCR24      | 24-Dehydrocholesterol reductase | LOC101864542, LOC101864542, LOC101849310 | Q15392, GO:0.042.987 | Amyloid precursor protein catabolic process |
| BIN1        | Bridging integrator 1 | LOC101856166 | Q00499, GO:0.048.156 | Tau binding |
| CDK5        | Cyclin-dependent kinase 5 | LOC101853437, LOC101864023 | Q00535, GO:0.048.156 | Tau binding |
| GSK3B       | Glycogen synthase kinase 3 beta | LOC100533534 | P49841, GO:0.048.156 | Tau binding |
| PIN1        | Peptidyl-prolyl cis-trans isomerase, NIMA-interacting 1 | LOC101858155 | Q13526, GO:0.048.156 | Tau binding |
(GOT1 and 2, MDH1, CYCS, NDUFA1, PCCB), cyclic-AMP response element-binding protein (CREB)-mediated learning and memory (MAP2K1, PRKACA, CAMK4, ELAV4, Fig. 3) and mitochondrial homeostasis (GDAP1, TUSC2), among others (Table 3). The full gene list is available in Supplementary Data 2.

Discussion

In our screening of the Aplysia genome for orthologs to Alzheimer’s-associated genes we identified 418 putative orthologs. Among these were orthologs of hallmark players in AD progression such as Aβ and tau.

The quintessential hallmark of AD is the formation of Aβ plaques in the nervous system. Aβ is a cleavage product of APP by the single protein beta secretase and the multi-protein gamma secretase enzymes. In contrast to beta and gamma secretases, alpha secretases process APP in a manner that does not produce Aβ. The alpha secretase ADAM10 has been demonstrated to compete with beta and gamma secretases for APP and confers protection from Aβ accumulation and tau hyperphosphorylation (Peron et al. 2018; Yuan et al. 2017). While Aβ plaques associated with AD in humans are not known to occur in invertebrates, endogenous orthologs of APP and associated secretases in Drosophila and C. elegans have been used to investigate the mechanisms by which these enzymes and cleavage byproducts function in normal and pathological conditions. This approach has shed light on the mechanisms of Aβ-related AD pathology, suggesting that Aplysia can be used similarly (Alexander et al. 2014; Calahorro and Ruiz-Rubio 2011; Fernandez-Funez et al. 2015; Link 2005; Prussing et al. 2013).
Neurofibrillary tangles of hyperphosphorylated tau protein are also a hallmark of AD and several ADRDs. Tau neurofibrillary tangles do not naturally occur in invertebrate models; thus previous studies of tau hyperphosphorylation using *Drosophila* and *C. elegans* expressed altered human tau in invertebrate neurons to determine its detrimental effects (Alexander et al. 2014; Calahorro and Ruiz-Rubio 2011; Fernandez-Funez et al. 2015; Hannan et al. 2016; Link 2005; Moloney et al. 2010; Prussing et al. 2013; Sharma et al. 2017). These invertebrate models have been particularly useful in screening for the effects of taupathies in the nervous system (Hannan et al. 2016). Similarly, *Aplysia* SN do not naturally form tau neurofibrillary tangles; however, expression of mutant human tau also has been performed in *Aplysia* SN, which resulted in recapitulation of AD-like taupathies (Shemesh and Spira 2010, 2011). The presence of endogenous MAPT orthologs and the demonstrated capacity to induce taupathies in cultured neurons suggest that *Aplysia* SN may also offer an effective screening tool for the effects of hallmark AD proteinopathies on neurons.

The roughly 400 other orthologs of interest in *Aplysia* offer a broad landscape for functional investigation of the effects of amyloidopathies and taupathies on individual neurons and simple neural circuits. Given the success of translating molecular mechanisms of learning and memory from *Aplysia* to higher vertebrates and humans, the potential for investigation of AD mechanisms in *Aplysia* appears promising (Abrams 2012; Bailey et al. 1983; Ezzeddine and Glanzman 2003; Glanzman 2006; Kupfermann 1974; Lin and Glanzman 1994; Martin et al. 1997; Moroz 2011). This notion is further supported by the shared differential expression of

### Table 2: Gene orthologs upregulated in both *Aplysia* SN aging and FL LOAD. All genes upregulated in two or more aging *Aplysia* SN differential expression data sets and five or more in meta-analysis of human frontal lobe Late Onset AD (FL LOAD) samples by Li et al. (2015). *Aplysia* RefSeq transcript identifiers, their BLAST-assigned putative human orthologs, and the e-value of the match are listed in the first three columns, with alternative names for each human gene in the fourth. The number of data sets in which these orthologs were upregulated is listed in columns 5 (*Aplysia* data sets) and 6 (Li et al. 2015 human FL LOAD data sets). Column 6 groups orthologs into broad categories relevant to aging and AD found in the discussion.

| Aplysia RefSeq Transcript | e-value | Human gene symbol | Other names | Aplysia data sets | FL LOAD data sets | Major category |
|---------------------------|---------|------------------|-------------|------------------|------------------|----------------|
| XM_005091054              | 9.3E-70 | ANKZ1            | ANKZF1, ZNF744 | 3                | 5                | Stress response (ER, ROS) |
| XM_013084296              | 5.3E-09 | BIRC3            | API2, MIHC, cIAP | 3                | 6                | Inflammation |
| XM_013088003              | 7.2E-12 | BIRC3            | API2, MIHC, cIAP | 3                | 6                | Inflammation |
| XM_005111747              | 5.3E-08 | BIRC3            | API2, MIHC, cIAP | 2                | 6                | Inflammation |
| XM_005102233              | 6.5E-22 | BMP1             | mTID, PCP, TLD | 2                | 5                | Inflammation, cholesterol metabolism |
| XM_005112068              | 4.2E-20 | BTG1             | BTG1         | 2                | 6                | Stress response (metabolic, ER, ROS) |
| XM_013080222              | 1.4E-86 | CP3A5            |            | 2                | 5                | Lipid metabolism, cholesterol metabolism |
| XM_005102749              | 1.1E-19 | DDT4L            | DDT4L, REDD2 | 2                | 6                | Stress response (metabolic) |
| XM_013089385              | 5.6E-17 | GA45G            | GADD45G, DDIT-2, CR6 | 3                | 5                | Stress response |
| XM_005111489              | 3.2E-34 | IKBA             | NFKBIA, MAD3, NFKBI | 3                | 5                | Inflammation |
| XM_013089050              | 4.9E-37 | M3K8             | MAP3K8, COT, TPL2 | 2                | 5                | Inflammation |
| XM_005095549              | 0       | MA2B1            | MAN2B1, LAMAN, MANB | 2                | 5                | Proteostasis |
| NM_001204684              | 1.4E-135 | MKNK2         | MKN2, GPRK7 | 2                | 6                | Inflammation |
| XM_005108634              | 2.2E-25 | MLXIP            | MONDOA      | 3                | 5                | Energy metabolism |
| XM_005089580              | 6.6E-05 | MUC1             | CD227, PEM, EMA, EMA, PEMT | 2                | 5                | Stress response (ER), inflammation |
| XM_013081198              | 2.2E-15 | MYD88            | MYD88       | 3                | 5                | Inflammation |
| XM_005097661              | 4.4E-49 | NEO1             | NGN, IGDCC2 | 2                | 5                | Iron accumulation, inflammation |
| XM_005108885              | 4.1E-21 | NFIL3            | E4BP, IL3BP1 | 2                | 5                | Inflammation |
| XM_005096173              | 1.7E-12 | NFKB1            | EBP1       | 2                | 5                | Inflammation |
| XM_005091237              | 1.1E-77 | SSR1             | SSR1, TRAPA | 2                | 5                | Stress response (ER) |
| XM_005110832              | 7.2E-43 | TISB             | ZFP36L1, BRF1, ERF1, TIS11B, BERG36, RNF162B | 3                | 6                | Inflammation, cholesterol metabolism |
genes which are involved in processes known to play key roles in both neuronal aging and AD, including learning and memory, neuronal signaling, transport of cellular cargo, energy metabolism, proteostasis, and neuroinflammation.

Memory impairment associated with AD has been suggested to be the result of synergistic toxicity between Aβ plaques and tau neurofibrillary tangles in cognitive centers like the frontal lobe and hippocampus. Gene transcription as a result of CREB activation is essential for memory formation across Metazoa (Silva et al. 1998). Disruption of CREB signaling in cognitive centers has been observed in AD brains as well as rodent and neuronal models of AD and is suggested to be a major component of AD-associated cognitive impairment (Puzzo et al. 2005; Snyder et al. 2005; Tong et al. 2001; Vitolo et al. 2002; Yamamoto-Sasaki et al. 1999). Similarly, Aplysia SN have been demonstrated to have impaired CREB signaling in aging (Greer et al. 2018; Kempsell and Fieber 2015a). As illustrated in Fig. 3, both aged Aplysia SN and human FL LOAD exhibited downregulation of orthologs of CAMKIV, MAP2K1, and PRKACA. These are critical components of the Ca²⁺/calmodulin (Bito et al. 1996; Hardingham et al. 1998), MEK/ERK (Grewal et al. 2000; Li et al. 2019), and PKA (Turnham and Scott 2016) signaling cascades, respectively, that activate CREB during memory formation. Furthermore, commonly downregulated ELAV4 is a key effector of PKC that plays a critical role in stabilizing the mRNA of CREB target genes, facilitating protein translation and the establishment of CREB-dependent long-term memory in both species (Anderson et al. 2001; Deschenes-Furry et al. 2006; Mirisis et al. 2021; Pascale et al. 2004). Decreased activity and expression of these genes as a result of Aβ and tau has been described previously in AD (Amadio et al. 2009; Gong et al. 2006; Hartmann et al. 2019; Vitolo et al. 2002; Yin et al. 2016b). This suggests that it is the dysregulation of key kinases and their effectors in the CREB signaling cascade that drives the cognitive impairments that typify both Aplysia SN aging and AD.

A mechanism by which AD is believed to impair cognitive function is via the disruption of normal vesicle dynamics and proper trafficking of cellular cargo (Barhet and Mulle 2020; Marsh and Alifragis 2018). Many of the putative orthologs downregulated in aging Aplysia SN and FL LOAD, namely NAPG (Inoue et al. 2015), ARF3 (Kondo et al. 2012), NECP1 (Ritter et al. 2003), and SNX4 (Traer et al. 2007), are involved in endosome formation and trafficking. Others, including NAPG (Stenbeck 1998), SYN2 (Cesca et al. 2010), SVOP (Janz et al. 1998), and EXOC8 (Guo et al. 1999), play key roles in vesicle docking and membrane fusion. Both SYN2 and NAPG have been shown to be disrupted in AD (Nie et al. 2017; Scheff and Price 2003; Sultana et al. 2006). This suggests that normal endo/exocytosis dynamics are affected in aging Aplysia SN as well as FL LOAD, possibly contributing to cognitive impairment. Transport of cellular cargo to and from the

Fig. 3 Orthologs in learning and memory pathway downregulated in common between Aplysia SN aging and FL LOAD. See Fig. 2 caption for diagram description. Commonly downregulated genes included major kinases of CREB1 (PKA, CAMK4, MEK1) and ELAV4, which stabilizes mRNAs of CREB1 target genes. This suggests that CREB1 signaling disruption is a common cause of cognitive impairment in Aplysia SN and LOAD.

As illustrated in Fig. 3, both aged Aplysia SN and human FL LOAD exhibited downregulation of orthologs of CAMKIV, MAP2K1, and PRKACA. These are critical components of the Ca²⁺/calmodulin (Bito et al. 1996; Hardingham et al. 1998), MEK/ERK (Grewal et al. 2000; Li et al. 2019), and PKA (Turnham and Scott 2016) signaling cascades, respectively, that activate CREB during memory formation. Furthermore, commonly downregulated ELAV4 is a key effector of PKC that plays a critical role in stabilizing the mRNA of CREB target genes, facilitating protein translation and the establishment of CREB-dependent long-term memory in both species (Anderson et al. 2001; Deschenes-Furry et al. 2006; Mirisis et al. 2021; Pascale et al. 2004). Decreased activity and expression of these genes as a result of Aβ and tau has been described previously in AD (Amadio et al. 2009; Gong et al. 2006; Hartmann et al. 2019; Vitolo et al. 2002; Yin et al. 2016b). This suggests that it is the dysregulation of key kinases and their effectors in the CREB signaling cascade that drives the cognitive impairments that typify both Aplysia SN aging and AD.

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Table 3 Gene orthologs downregulated in both *Aplysia* SN aging and FL LOAD. All genes downregulated in two or more aging *Aplysia* SN differential expression data sets and five or more in meta-analysis of human frontal lobe FL LOAD samples by Li et al. (2015). See Table 2 for column descriptions. A majority of shared downregulated orthologs are involved in one or more of the following processes: cellular cargo transport, endo/exocytosis, proteostasis, lipid metabolism, energy metabolism, mitochondrial homeostasis, and signaling.

| *Aplysia* RefSeq Transcript | e-value | Human gene symbol | Other names | *Aplysia* FL LOAD data sets | *Aplysia* FL LOAD data sets | Major category |
|-----------------------------|---------|-------------------|-------------|-----------------------------|-----------------------------|----------------|
| XM_005098930                | 0       | AATM              | GOT2        | 3                           | 5                           | Energy metabolism |
| XM_005099066                | 2.5E-46 | ARF3              | ARF3        | 2                           | 6                           | Cellular cargo transport |
| XM_005112446                | 2.6E-25 | CISD1             | ZCD1, mitoNEET | 2 | 5 | Energy metabolism |
| XM_013080281                | 3.6E-21 | CNRP1             | C2orf32     | 2                           | 6                           | Signaling |
| XM_005098434                | 1.3E-59 | CYC               | CYCS        | 2                           | 6                           | Energy metabolism |
| XM_005096347                | 3.9E-65 | DCTN6             | WS3         | 2                           | 6                           | Cellular cargo transport |
| XM_005100966                | 1.1E-107| DECR2             | PDCR, SDR17C1 | 2 | 5 | Lipid metabolism |
| XM_005092530                | 2.1E-146| ELAV4             | ELAVL4, HUD, PNEM | 2 | 6 | Synaptic plasticity, mRNA stabilization |
| XM_005112819                | 4.8E-106| HPRT              | HPRT1, HGPR | 2 | 6 | Nucleotide salvage |
| XM_005102830                | 2.0E-07 | JUP1              | ARM2, HN1   | 2                           | 5                           | Other |
| NM_001204491                | 0       | KAPCA             | PKACA       | 2                           | 5                           | Synaptic plasticity, Ca++ signaling, phosphorylation |
| XM_005106951                | 4.0E-65 | KCC4              | CAMK4, CAMK, CAMK-GR, CAMKIV | 2 | 5 | Synaptic plasticity, Ca++ signaling, phosphorylation |
| XM_005104005                | 0       | KIFAP3            | KIFAP3, KIF3AP, SMAP | 3 | 6 | Cellular cargo transport |
| XM_005102605                | 1.4E-10 | LIAT1             | C17orf97    | 2                           | 5                           | Other |
| XM_005098563                | 4.0E-171| MDHC              | MDH1, MDHA  | 2                           | 6                           | Energy metabolism |
| XM_005089329                | 0       | MP2K1             | MAP2K1, MEK1, PRKMK1, M KK1, MAPKK1 | 2 | 6 | Synaptic plasticity, phosphorylation |
| XM_005098362                | 3.2E-56 | MPN               | MPN         | 3                           | 5                           | Other |
| XM_005089044                | 7.7E-36 | NDUAA             | NDUFa10, CI-42kD | 2 | 5 | Energy metabolism |
| XM_005097418                | 0       | NDUV1             | NDUFV1, UQOR1 | 2 | 5 | Energy metabolism |
| XM_005099251                | 2.6E-103| NCEP1             | NCEP1       | 2                           | 6                           | Endocytosis |
| XM_005097828                | 0       | ODPB              | PDHB, PHE1B | 3                           | 6                           | Energy metabolism |
| XM_013084642                | 3.7E-89 | OTUB1             | OTB1, OTU1  | 3                           | 6                           | DNA damage response |
| XM_013081831                | 0       | PCCB              |             | 2                           | 5                           | Lipid metabolism |
| XM_005089882                | 4.6E-28 | PEX19             | HK33, PXF   | 2                           | 5                           | Lipid metabolism, proteostasis |
| XM_005110189                | 0       | PFKAM             | PFKM, PFKA, PFKX | 2 | 6 | Energy metabolism |
| XM_005109909                | 4.9E-74 | PITH1             | PITHD1, C1orf128 | 2 | 5 | Transcription |
| XM_005097948                | 2.5E-50 | PPAC              | ACP1, LMW-PTP | 2 | 6 | Phosphorylation |
| XM_005097122                | 4.3E-133| RAB6A             | RAB6        | 2                           | 5                           | Cellular cargo transport |
| XM_005093164                | 1.7E-87 | SAMC              | SLC25A26    | 3                           | 5                           | Mitochondrial homeostasis |
| XM_005108342                | 9.4E-28 | SOCC              | SOCCO       | 3                           | 6                           | Autophagy |
| XM_005093202                | 4.4E-78 | SNAG              | NAPG, SNAPG  | 2                           | 6                           | Cellular cargo transport, endocytosis |
synapse in response to synaptic activity is also central to synapse function and health (Guillaud et al. 2020; Hafezparast et al. 2003).

Both aging *Aplysia* SN and FL LOAD exhibit down-regulation of DCTN6, a component of the dynein/dynactin complex that mediates retrograde transport, and RAB6A, the small GTPase that activates dynein-mediated transport (Yamada et al. 2013). This suggests common impairment of retrograde movement of cellular cargo. Similarly, common downregulation of KIFAP3, a key component of the kinesin motor, suggests that anterograde transport is impaired as well (Yamazaki et al. 1996). Furthermore, previously mentioned STAU2 and ELAVL4 both participate in kinesin-mediated transport of mRNAs from the nucleus to neurites (Bronicki and Jasmin 2013; Tang et al. 2001). Anterograde transport of mitochondria and mRNA via kinesins is crucial for synapse health, learning, and memory, and disruptions of this process are associated with several neurodegenerative disorders (Guillaud et al. 2020). Disruption of mitochondrial transport in neurons also impairs mitochondrial homeostasis, which has been suggested to play a central role in many neurodegenerative disorders (Sheng and Cai 2012).

Mitochondrial dysfunction is a classic hallmark of neural aging and AD (Ferguson et al. 2005; Grimm and Eckert 2017; Ojaimi et al. 1999). Due to the energy-intensive activity of neurons, any disruption in metabolic output can adversely affect signaling and synaptogenesis. The downregulation of several genes in common between *Aplysia* SN aging and FL LOAD suggest similar metabolic impairments. Downregulation of PKFM, the enzyme of the first committed step of glycolysis, but upregulation of glucose sensor and PFKM inducer MondoA, suggests common perturbation of glycolysis homeostasis (Sans et al. 2006). Furthermore, two components of the malate-aspartate shuttle (MAS), GOT2 and MDH1, are commonly downregulated. Disruption of MAS results in decoupling of cytosolic and mitochondrial NAD+/NADH ratios, which has been demonstrated to have adverse effects on mitochondrial metabolism and induce senescence (Bradshaw 2019; Broeks et al. 2019; Lautrup et al. 2019; Xu et al. 2020). Another common downregulated gene, PCCB, is critical for proper functioning of the mitochondrial tricarboxylic acid cycle (TCA) and has also been shown to be downregulated in a mouse model of AD (Franco et al. 2019). Dysfunction of PCC results in altered concentrations of TCA intermediates and accumulation of toxic metabolites, which decreases the activity of pyruvate dehydrogenase (PDH), the beta isoform of which is also downregulated (Wongkittichote et al. 2017). In addition to regulators of glycolysis and the TCA cycle, several components of mitochondrial oxidative phosphorylation are also commonly downregulated. These include components of mitochondrial respiratory complex I (NDUFA10, NDUFV1), cytochrome C (CYCS), which links complexes III and IV, and CISD1, which regulates maximal mitochondrial energy output (Kalpage et al. 2019; Paddock et al. 2007; Wang et al. 2017). These transcriptional signatures suggest similar impairment of mitochondrial energy metabolism in both *Aplysia* SN and FL LOAD. In addition to metabolic impairment, mitochondrial dysfunction also contributes to disrupted Ca++ buffering in normal aging and AD (Pandya et al. 2015).

Proper mitochondrial Ca++ regulation is critical not only for proper mitochondrial homeostatic functions but also for synaptic signaling (Gleichmann and Mattson 2011; Marchi et al. 2018; Satrustegui et al. 1996). In neurons, mitochondria act as critical sinks and reservoirs for Ca++
during signaling events. The signaling pathways that target CREB discussed earlier are themselves dependent upon tightly regulated Ca++ signaling (Augustine et al. 2003). Impairment of mitochondrial Ca++ homeostasis has been shown to contribute to AD-associated proteinopathies and has even been suggested to be the proximal cause of AD (Calvo-Rodriguez et al. 2020; Jadiya et al. 2019; Tong et al. 2018).

Three genes downregulated in both aged Aplysia SN and FL LOAD, namely, GDAP1, TUSC2, and GN5B, play an important role in mitochondrial Ca++ regulation, suggesting that aged Aplysia SN suffer similar disruptions of mitochondrial Ca++ dynamics as human FL LOAD (Gonzalez-Sanchez et al. 2019; Kang et al. 2018; Uzhachenko et al. 2014, 2017). Mitochondrial impairment results in energy deprivation, generation of reactive oxygen species (ROS), and elevated Ca++, which contribute to protein aggregation and associated endoplasmic reticulum (ER) stress. Sensors for these stressors converge in a single signaling process known as the integrated stress response (ISR) pathway.

Induction of the ISR results in decreased global translation via phosphorylation of eukaryotic initiation factor 2 (eIF2) and increased transcription of transcription factors in the activating transcription factor family, particularly ATF4 (Costa-Mattioli and Walter 2020; Pakos-Zebrucka et al. 2016). Increased proteostatic stress in AD due to Aβ plaques and tau neurofibrillary tangles has been demonstrated to increase eIF2 phosphorylation, suggesting increased ISR activity in AD (Chang et al. 2002; Ferrer 2002; Hernandez-Ortega et al. 2016; Hoozemans et al. 2005, 2009). Several putative orthologs upregulated in both aged Aplysia SN and FL LOAD are stress-induced genes, including DDIT4L (Cuaz-Perolin et al. 2004; Shoshani et al. 2009; Wang et al. 2003), BTG1 (Cho et al. 2003; Yuniati et al. 2019), SSR1 (Nagasawa et al. 2007), ANKZF1 (Tran et al. 2011; van Haaften-Visser et al. 2017), NFIL3 (Tamai et al. 2014), MUC1 (Olou et al. 2020), GAD45G (Liebermann and Hoffman 2008), and BIRC3 (Hamanaka et al. 2009; Warnakulasuriyarachchi et al. 2004). BTG1 enhances ISR signaling via interaction with ATF4 upon activation (Yuniati et al. 2016). Chronic induction of the ISR and resulting changes in the transcriptional and translational landscape of neurons has been suggested to play a role in disruptions of CREB-mediated learning and memory in AD (Hernandez-Ortega et al. 2016). NFIL3 has been shown to specifically inhibit CREB (MacGillavry et al. 2009). Similarly, upregulation of DDIT4L and NEO1 has been demonstrated to result in decreased neurogenesis with impaired cognitive outcomes (Chen and Shifman 2019; Di Polo 2015; Metzger et al. 2007; Morquette et al. 2015; Shifman et al. 2009). Activation of the ISR also results in the secretion of cytokines that activate receptors in the toll-like and interleukin-like receptor (TIR) family (Abdel-Nour et al. 2019; Deng et al. 2004; Iwasaki et al. 2014). Activation of these TIR initiates signaling cascades that result in the translocation of transcription factors NF-kB and AP-1 to the nucleus and recruitment of pro-survival and proinflammatory genes.

Increased activation of proinflammatory signaling cascades recruited by the ISR has also been demonstrated to be increased in AD (Colangelo et al. 2002). Positive feedback of this proinflammatory loop has been proposed to induce chronic neuroinflammation and contribute to neurodegenerative consequences in AD (Jones and Kounatidis 2017; Ju Hwang et al. 2019; Lindsay et al. 2021; Uddin et al. 2021). For example, induction of miRNAs by NF-kB in AD directly results in the downregulation of previously discovered SYN2 (Lu Kiw 2012). Several genes that participate in and are recruited by the signaling cascades downstream of TIR are upregulated in both Aplysia SN aging and human FL LOAD (Fig. 2), including MYD88, MAP3K8 (Chorzalska et al. 2017), and MKNK2 (Bao et al. 2017; Xu et al. 2018). Furthermore, NEO1 discussed previously exhibits strong proinflammatory effects (Chen and Shifman 2019; Fujita and Yamashita 2017; Shifman et al. 2009).

Most significantly, many core components of the quintessential proinflammatory signaling cascade, NF-kB signaling, are commonly upregulated. NF-kB1, also known as p105, is an NF-kB family protein that, upon phosphorylation as a result of MYD88 activation, is degraded by the proteosome. This liberates MAP3K8, which initiates the AP-1 branch of proinflammatory signaling and produces the p50 NF-kB subunit, which is then recruited into homodimers or heterodimers with p65 to activate downstream NF-kB target genes (Beinke et al. 2004). Several of these target genes are commonly upregulated, including NFκBIA (Hay et al. 1999; Sun et al. 1993), BCL3 (Bours et al. 1993; Caamano et al. 1996; Edwards et al. 2015; Saito et al. 2010), and BIRC3 (Hu et al. 2004; James et al. 2006; Simon et al. 2007). Common upregulation of key genes in this pathway suggest that increased proinflammatory signaling as a result of increased cellular stress is a relevant component of Aplysia SN aging and FL LOAD. However, few of these relationships have been experimentally validated in Aplysia.

While these genes have been observed to play key roles in human neurodegenerative disease, orthologs of these genes have been demonstrated to have conserved function and stress-associated upregulation and function in invertebrate models. Molluscan orthologs of BTG1 (Peng et al. 2014), NFIL3 (Li et al. 2017), MYD88 (Zhang et al. 2015), and BIRC3 (Wang et al. 2016) have been demonstrated to be activated by biotic and abiotic stressors in bivalves. Several other dysregulated orthologs, including NAPG (Clary et al. 1990), SNX4 (Nemec et al. 2017), EXOC8 (Guo et al. 1999), ANKZF1 (Tran et al. 2011), and DDIT4L (Reiling and Hafen 2004) have conserved function between humans and models considered more divergent from humans than Aplysia (Moroz et al. 2006), including ecdysozoans like Drosophila and C. elegans and even yeast. Thus, we believe it plausible that dysregulation of these genes will have similar outcomes in Aplysia SN as observed in human neurons.

Differential expression of genes shared between Aplysia SN aging and FL LOAD represents critical pathways that are
disrupted in aging and neurodegenerative disease, including mitochondrial homeostasis, energy metabolism, vesicle dynamics, cellular cargo transport, Ca++ homeostasis, and synaptic plasticity (Di Paolo and Kim 2011; Haas 2019; Jang et al. 2018; Lopez-Otin et al. 2013; Martinez et al. 2017; Wong et al. 2020; Wu et al. 2019; Yin et al. 2016a). Although the hallmark pathologies of AD are only known in humans, these data suggest that, while the proximal source of neuronal stress may be different, similar transcriptional changes as a result of cellular stress underpin cognitive impairment in both Aplysia SN aging and AD. Indeed, the commonalities between aging Aplysia SN and FL LOAD expression patterns make sense in light of the current understanding that normal brain aging and dementias like AD are parts of a continuum of neurodegenerative outcomes associated with aging (Franceschi et al. 2018). While surface receptors and downstream effectors have diverged and specialized differently over the course of evolution, these data suggest that orthologous signaling cascades and their disruption as a result of age-associated stressors are conserved between the human frontal lobe and Aplysia sensory neurons. We strongly believe that these results, in addition to previous studies, demonstrate the excellent applicability of Aplysia as a multivalent model for the study of AD and ADRD.

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Authors' Contributions Both authors designed this study. Nicholas S. Kron collected and analysed the data. Nicholas S. Kron wrote the manuscript with input from Lynne A. Fieber. Both authors read and approved the final manuscript.

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Availability of Data and Materials Data used in this study is freely available from the cited publications and public databases from which it was sourced as described in the text.

Code Availability Code used for this study is available at the following GitHub repository: [https://github.com/Nicholas-Kron/Kron_Aplysia_Alpheimer-s_Model].

Declarations

Ethics Approval and Consent to Participate Not applicable

Consent for Publication Not applicable

Competing Interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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