UTJMS 2025 December 15, **14**(S4):e1-e16 doi:10.46570/utjms-2025-1901

Tracking Neural Deterioration in proNGF-Overexpressing Mice: Hippocampal Serotonergic Dysfunction and Synaptic Loss Drive Neurodegeneration

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Received 12/10/2025
Accepted for publication 12/10/2025
Published 12/15/2025

Abstract

ProNGF, the precursor protein of mature nerve growth factor (NGF), plays a complex role in neural signaling and has been implicated in neurodegeneration through its apoptotic signaling with the p75NTR receptor. For example, in Alzheimer's disease increased levels of proNGF are associated with cholinergic neuron loss and excitatory/inhibitory imbalance. This study deploys a bioinformatic analyses of microarray data from TgproNGF#3 mice models that express furin-resistant proNGF. The TgproNGF#3 mouse mimics the pathological accumulation of the precursor NGF protein observed in these diseased brains. Our analysis revealed that proNGF accumulation in the hippocampus and entorhinal regions leads to early downregulation of genes critical for neuronal communication, such as pion and KCNAB2, and later to the upregulation of stress-related and signaling genes, including Neurod1, and Gnai1. These shifts in gene regulation suggest that while the brain attempts to counteract the excess in proNGF, its delay and inefficacy may contribute to early-stage Alzheimer's disease processes (1-4).

Keywords: Alzheimer’s Disease, Animal Model, Pathways, Transcriptomic Profiles, DEGs, Nerve Growth Factor, Hippocampus, Gnai1, KCNAB2, Neurodegeneration

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1. Introduction

Nerve Growth Factor (NGF) is a chemical regulator in a multitude of neuronal functions and processes. NGF is essential for regulating neuronal development and plays a crucial role in synaptic flexibility by influencing the effectiveness of synaptic transmission, which is a foundational factor in processes such as learning, memory consolidation, and neuronal circuit remodeling. NGF is derived from the enzymatic cleavage of the precursor protein proNGF by matrix metalloproteinases (MMPs). Then, NGF binds to the tropomyosin kinase receptors (TrkR) to promote neuronal survival. In the dentate gyrus of Alzheimer's disease (AD) mouse models, accumulation of proNGF promotes activation of the p75 neurotrophin receptor (p75NTR) which induces activation of apoptotic pathways that contributes to neuronal cell death, to protect against DNA damage or infection. The balance between proNGF and mature NGF is critical as it determines neuronal health and influences signaling pathways. In the TgProNGF#3 AD mouse model, a genetic deletion promotes the accumulation of proNGF and triggers excessive neuronal loss through apoptotic pathways (1, 2, 5-9).

In AD, neurons are damaged and lost leading to impairments in memory, cognitive processing, and behavior. AD impacts over 7 million individuals per year and is characterized by the presence of neurofibrillary tangles and beta-amyloid plaques both of which contribute to neural degeneration and cognitive decline, and are thus considered hallmarks of AD (10). Beta-amyloid plaques form via the amyloid precursor proteins or peptides that aren't properly cleaved by specific enzymes. They become adherent to neuronal debris in the brain to form structures, known as plaques. The amyloid-Beta plaques, start forming in the neocortex and eventually traverse their way to the hippocampus, but they are not the sole cause of neurodegeneration (11). Pathological forms of β-amyloid, known as oligomers, accelerate the hyperphosphorylation of Tau protein, which is toxic to the surrounding neural environment. Ultimately, this combination of β-amyloid and hyperphosphorylated tau drives progressive neurodegeneration and neuronal death (12). The presence of these toxic protein aggregates likely impairs cells from regulating the stress response, resulting in cell death due to 'burnout' (13).

The hippocampus is a central point of interest for elucidating AD progression. In this study, we reanalyzed microarray dataset from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) repository (accession GSE70757), that was originally obtained and derived from a study on TgproNGF#3. These transgenic are genetically manipulated via alterations in the *Thy1* gene to cause the mice to lose the ability to cleave proNGF to NGF, inducing a furin-resistant proNGF acculmulation. The authors of the original study dissected entorhinal-hippocampus samples from the proNGF and WT mice and ran microarrays to assess RNA expression (3). The authors probed effects of proNGF accumulation the neural circuitry between the inhibitory parvalbumin-expressing interneurons (PVIN) within the dentate gyrus and the cholinergic neurons that project from the basal forebrain as a major contributor to neurodegenerative phenotypes (3).

The basal forebrain cholinergic neurons (BCFN) are excitatory neurons that release the neurotransmitter acetylcholine and project to the parvalbumin interneurons of the dentate gyrus; a subsection of the hippocampus that plays a critical role in the ability to form and recollect memories. Previous studies have elucidated a relationship between perturbations within the dentate gyrus and AD progression. For example, using this model we have found the cholinergic neurons originating from the basal forebrain regulates activity of parvalbumin interneurons, which in turn modulates the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). The control of GABA release then affects the firing of excitatory granule cells in the dentate gyrus, which in turn affects the circuitry essential for both memory formation and maintenance in the hippocampus (3, 14-16).

The excessive production of proNGF protein in PVINs are sent in a retrograde fashion through the PVIN-cholinergic synapses, leading to the degradation of cholinergic axonal projections. The cholinergic neurons of the basal forebrain rely on neurotrophic support from mature NGF via binding to the TrkA receptor. The binding of NGF on TrkA induces the activation of PI3K/Akt, MEK/ERK, and PLCγ pathways for maintenance. The accumulation of proNGF promotes causes hyperactivation of the low affinity p75NTR, via proNGF binding, leading to an hypoactivity of the TrkA receptor due to less NGF available for binding. Both the p75NTR and TrkA pathways may work to counterbalance one another, yet in the case of proNGF accumulation,

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this causes activation of p75NTR mediated apoptosis via c-Jun N-terminal kinases (JNK) signaling, leading to subsequent p53, Jax-like protein and caspase activation. The increase in activation of apoptotic pathways overrides neurotrophic support needed for basal forebrain cholinergic neurons to survive. The diminished cholinergic activity to the parvalbumin interneurons (PVINs) induced a hyperexcitation of the excitatory granule cells in the dentate gyrus, which project to the CA3 and CA4 regions of the hippocampus through their axons called “mossy fibers,” ultimately diminishing granule cell communication causing a diminishment in memory formation and recollection (3, 20-25).

In addition, the TgproNGF#3 mice were used to mimic specific molecular phenotypes of AD, providing valuable insight into the mechanism by which proNGF-mediated apoptosis disturbs the excitatory/inhibitory (E/I) balance between the cholinergic neurons and PVINs. A reduction in parvalbumin interneuron activity within the dentate gyrus leads to granule cell dysregulation, memory impairments, diminished spatial awareness and attention, recall errors, and notably an increased risk of seizures. Therefore, exploring the connection between proNGF and the p75NTR pathway is critical in understanding the progression of AD, and the neurodegenerative phenotype that follows (15, 17-19).

Despite the advances in understanding the role of proNGF in AD, some key gaps remain, including how proNGF processing enzymes like plasmin and matrix metalloproteases are regulated and localized. Although these enzymes are well characterized, their regulatory nature and spatial dynamics remain unclear. This lack of clarity restricts our understanding of the induction of proNGF accumulation and disease progression. Thus, TgproNGF#3 transgenic mouse model mimics the progressive accumulation of proNGF and is essential for studying how proNGF influences gene expression timing related to synaptic maintenance between basal forebrain cholinergic neurons and dentate gyrus parvalbumin interneurons, ultimately affecting granule cell activity that may contribute to age-related cognitive decline.

We applied cutting-edge bioinformatic analyses to assess differentially expressed genes, gene pathways, and network changes related to synaptic maintenance specifically altered by the progression of proNGF accumulation. Using this dataset, we analyzed gene expression profiles across developmental stages (1 vs 3 months and 3 vs 12 months) for TgproNGF#3 and wild-type mice. Data was compiled and visualized in RStudio’s 3PodR tool to enable comparisons between proNGF accumulation and wild type mice, helping identify gene expression changes on synaptic maintenance genes associated with the early onset and progression of Alzheimer’s-like neurodegeneration.

2. Methods

2.1 Data Access and Brain Samples

Transcriptomic data from the GEO repository (accession GSE70757), were originally obtained and derived from a study on transgenic mice overexpressing furin-resistant proNGF, referred to as TgproNGF#3, compared to wild-type controls (3). This model has progressive loss of PVINs in the dentate gyrus, disruption of excitation/inhibition balance, and detectable epileptiform activity starting at 1 month of age (3). Hippocampal microarray datasets collected at 1, 3, and 12 months from TgproNGF#3 mice (n = 4 at 1 and 3 months; n = 3 at 12 months) and matched wild-type (WT) controls (n = 4, 4, and 3, respectively) were reanalyzed.

2.2. Differential Gene Expression

Differential gene expression analysis was performed by using the *limma* pipeline (version 3.64.1) in R (version 4.5.1). Briefly, the expression data was retrieved from the experimental dataset using GEOQuery, followed by quantile normalization. *Limma* was used to obtain differentially expressed genes (26-28).

2.3. Pathway Analysis

Pathway analysis was performed using the Gene-set Enrichment Analysis (GSEA) and Enrichr. GSEA takes a ranked list of genes and returns an enrichment score based on the running enrichment score over a given gene set. Enrichr takes in a list of genes only. We analyzed the top and bottom 10% differentially expressed genes by logFC values to Enrichr (29-31).

2.4. Data Availability

All data and preprocessed scripts are available at https://github.com/Blah/Paper.

3. Results

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To investigate and understand the basis of proNGF-induced neurodegeneration, we performed a time-based gene expression analysis using entorhinal-hippocampal data from TgproNGF#3 mice compared to wild-type (WT) mice matched in ages at 1 month, 3 months, and 12 months. The primary goal was to identify the genes related to synaptic maintenance that were most prominent in the early stage (1m vs 3m) and late stage (3m v 12 m) to track genes that play a role in the progression of disease.

3.1. Differential Gene Expression of Early and Late Genes in wild-type and TgproNGF#3 mice

In wild type (WT) mice, we identified 1,596 genes that were differentially expressed "early" genes, and 2,138 genes that were differentially expressed "late" genes. In the TgproNGF#3 mice, we identified 1,277 genes that were differentially expressed between 1m vs 3m (early) and 3,599 genes differentially expressed between 3m vs 12m (late) (Table 1).

Next, we compared age progression in WT and TgproNGF#3 at early (1 m vs 3 m) and late (3m vs 12m) stages (Figure 2).

3.2. Prominent early-stage genes in TgproNGF#3

In 1m vs 3m (Figure 3), we identified four genes of interest differentially expressed between proNGF and WT mice, including KCNAB2 (LFC = -1.076, raw p-value = 0.035). This gene expresses the voltage-gated potassium channel subunit beta-2 (Kvβ2), which regulates neurotransmitter release and affects neuronal signaling. Another downregulated gene of interest was pion, pigeon homologue protein (LFC = -0.456, raw p-value = 0.019). The pion and KCNAB2 genes were not differentially expressed in the WT mice in early (1m vs 3m) stages (LFC = +0.035, raw p-value = 0.850 and LFC = -0.007, raw p-value = 0.988, respectively) (33).

3.3. Prominent late-stage genes in TgproNGF#3

Notable late-stage genes of interest (Figure 3) include NeuroD1, which is a transcription factor involved in neuronal differentiation and development (LFC = +0.597, raw p-value = 0.006). Another upregulated gene of interest was Guanine Nucleotide-Binding Protein G(i) Subunit Alpha (Gnai1) (LFC = +0.411, raw p-value 0.012), which encodes for the inhibitory alpha subunit of the heterotrimeric G-protein (35, 36).

We also found that KCNAB2 transcripts went from being downregulated in the early stage to upregulated in the late stage (LFC = +1.316, raw p-value = 0.012), suggesting a time-dependent regulation of gene (32).

3.4. Significant genes associated with abrogation in serotonergic pathways

The serotonergic system has been implicated in neurogenerative diseases such as AD (37, 38). We found a significant switch in differential gene expression for 5-hydroxytryptamine (serotonin) receptor 2A (5HTR2A). The 5HTR2A gene was significantly downregulated in the early stage (LFC = -1.457, raw p-value < 0.001), but upregulated in late stage (LFC = +2.401, raw p-value < 0.001). 5HTR2A was a leading-edge gene associated with 9 up-regulated pathways in the late stage. Significant pathways included post synaptic membrane (NES=1.63) and presynaptic membrane (NES=1.63). This finding suggests that reported abrogation in synaptic integrity may stem from dysregulation of the serotonergic system, which may play a critical role in the progression of AD-like pathology (Figure 4).

4. Discussion

By analyzing gene expression over time in TgproNGF#3 mice, we uncovered various changes before the typical onset of AD-like cognitive deficits (3). Our findings suggest that proNGF accumulation affects genes critical in maintaining synaptic function, balance of excitatory and inhibitory synapses, and associated stress responses. The genes most effected throughout proNGF pathology progression were Pion, Gnai1, KCNAB2, and Neurod1, resulting in synaptic and cellular vulnerabilities to appear before neuronal death, which might offer insight into how these genes play a role in early neurodegenerative disease progression on a molecular level.

In the publication from which the original dataset that we reanalyzed, some examples of genes of interest in AD-like TgProNGF#3 included Slc6a13 and Lama1, suggesting a phenotype of significant GABAergic neuronal loss and structural laminin protein degradation. Additionally, pathways associated with synaptic transmission were significantly downregulated, such as genes encoding glutamatergic transmission, long term potentiation (LTP) and aggrecan protein encoding diminishments. These changes in gene expression coincide with structural evidence demonstrating the neuronal networks of proNGF mice underwent degeneration within the dentate gyrus. The proNGF induced degradation of cholinergic projections derived from the entorhinal cortex,

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which in turn diminished cholinergic activation of the parvalbumin interneurons (PVINs) within the dentate gyrus, causing significant excitatory/inhibitory (E/I) imbalance and diminishment of synaptic connections (3).

In this study, we set out to determine if there were additional pathway themes that could be detected by reanalyzing the differential gene expression between the time points, such as 1m vs 3m and between 3m vs 12m. Our goal was to observe if we could identify a distinct genetic signature for early and late stages of disease progression.

4.1. Molecular signatures of neurodegenerative processes identified in proNGF mice.

Overall, the differential gene expression data shows that proNGF mice undergo significant changes in neuronal function over time. Initially, there are clear signs that neurons may not be signaling or communicating efficiently, followed by an increased sign of inflammation, a key feature of early-onset AD. We identified several important genes (Pion, KCNAB2, Gnai1, and Neurod1) that may provide insight into how the pathology is initiated and progresses (Figure 5). In our efforts to elucidate genetic signatures that relate to the progression of proNGF mediated AD-like pathology observed in the proNGF mice, we identified three genes (KCNAB2, Neurod1 and Gnai) that were downregulated at the early stages, followed by upregulation in the later stage.

An idea has emerged that increases in KCNAB2 expression may be indicative of a neuroprotective mechanism to decrease AD risk in humans (32). The KCNAB2 gene responsible for the expression of the voltage-gated potassium channel beta-2 subunit (Kvβ2). KCNAB2 is highly expressed within the hippocampal formation, particularly dense within the axonal projections of the dentate gyrus and found to remain consistently expressed throughout the lifetime of the mouse (39). Thus, a decrease in KCNAB2 expression in proNGF mice at early stage (1m vs 3m) suggests proNGF-mediated pathology may be initiated. Overproduction of proNGF allows for this protein to be transported in a retrograde fashion from the axonal junction to the soma, causing the degradation of the cholinergic projections to the dentate gyrus. ProNGF-mediated destruction of the projections to the PVINs of the dentate gyrus may induce excitotoxic processes within the neural circuits associated with the dentate gyrus such as the hippocampal CA3 region to cause neurodegeneration (3). In our study, the KNCAB2 gene changed from being downregulated in the early stage, to upregulated in the late stage of proNGF-mediated neurodegeneration. Such upregulation could demonstrate the potential compensatory actions that may occur due to the excitotoxic processes that occurred in early stage, and since potassium channels are crucial for hyperpolarization, the neurons may have a mechanism to try to induce hyperpolarization to compensate for the loss of inhibitory parvalbumin interneurons.

Another gene of interest was the pigeon homologue protein (Pion). This gene is responsible for the expression of GSAP (gamma-secretase activating protein) which selectively increases the formation of neurotoxic βamyloid (Aβ), playing a significant role in regulating Aβ-plaque formation (33, 40). In the proNGF model, Pion was significantly downregulated in early and late intervals. This finding suggests that the pro-NGF induced neurodegenerative mechanism may not be through the accumulation of Aβ-plaque.

Some genes were only differentially expressed in the late stage, including Gnai1 and NeuroD1. Gnai1 encodes the Guanine Nucleotide-Binding Protein G(i) Subunit Alpha protein (Gαi1), the inhibitory alpha subunit of the heterotrimeric G-protein. This G-protein inhibits adenylate cyclase activity for muscarinic acetylcholine receptor (mAChR) signaling. Gnai1 was identified as part of a coordinated protein to protein interaction network to play essential role in the pathological aspect of Parkinson's Disease (41), schizophrenia-induced neurodegeneration (42, 43), and AD (44, 45). Gnai1 found to be significantly upregulated in the late interval (3m vs 12m) of our study suggests the proNGF-mediated neurodegeneration may be related to deficiencies in the coupling between cholinergic synapses within the dentate gyrus in the proNGF mice.

Our late interval gene of interest in the proNGF was NeuroD1, which encodes for a transcription factor involved in the regulation of neuronal differentiation and development. NeuroD1 was significantly regulated in the late interval. In the 5xFAD AD mouse model, targeting the NeuroD1 gene can reprogram cortical reactive astrocytes into functional neurons (34, 46). For example, enhancements in NeuroD1 expression, mitigated neuron loss in 5xFAD mice (47). Our findings of NeuroD1 upregulation in the late interval in our study suggest that astrocyte activation may have occurred due to neuronal damage within this region to try to mitigate damage from neurodegeneration.

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4.2. Overall Themes and Patterns Found Within the Dataset

Several key patterns emerge from our analysis of gene expression in proNGF-expressing mice, providing insight into the molecular changes that may result in early-stage neurodegeneration. We observed that the accumulation of proNGF induced a downregulation of genes involved in neuronal communication and neurotransmitter release, as well as genes that support cell stability (Figure 4). In addition, we identified a 5HTR2A as a leading-edge gene driving a downregulation of pathways related to the function and maintenance of the serotonergic system. The serotonergic system plays a critical role in diminishments in learning and memory consolidation for AD. In previous AD studies, prefrontal regions of the brain have a reduction in the number of serotonergic receptors, suggesting perturbations in critical serotonergic projections that may contribute to cognitive decline (48-50).
Notably, the serotonergic system can influence neuronal activity in a multitude of ways (Figure 6). Activation of the Gq coupled to the serotonergic 5-HT2a receptor induces phospholipase C, activity which in turn enhances DAG and IP3 mediated enhancement in neuronal excitability. 5HTR2A is a significant factor in the genetic signature of the early stage (1m vs 3m) in proNGF mice (Figure 3).

4.3. Limitations

Mouse models remain a cornerstone of AD research; however, important limitations must be acknowledged. For example, transgenic mouse models used to study AD generally overexpress genes based on known AD pathology. This makes it difficult to track the critical moments in which AD progression turns from early to late-stage. Additionally, many studies using AD mouse models have used behavioral assays to assess learning and memory, yet these tests do not capture the full spectrum of cognitive symptoms, such as language loss, executive dysfunction, or personality changes. Finally, many advancements made using AD mice models have been difficult to translate into human therapies. One example of this was the failure of BACE1 (β-secretase) inhibitors in clinical trials, despite strong preclinical success in mouse models (51-54). In human clinical trials, the BACE1 inhibitors such as verubecestat, elenbecestat, and atabecestat failed to show cognitive benefit and were discontinued due to adverse effects, including cognitive worsening and toxicity (55). This highlights a major disconnect between preclinical efficacy and clinical outcomes.

4.4. Conclusions

In summary, we explored the changes in genes and pathways triggered by proNGF overexpression (Figure 7). Under physiological conditions, proNGF is processed and cleaved to produce mature NGF, which binds to TrkA receptors to support neuronal survival, synaptic plasticity, and memory function. In contrast, in pathological conditions a disruption in this balance via binding to the p75NTR receptor triggers a cascade of detrimental effects, including interneuron apoptosis, structural degeneration of cholinergic projections to parvalbumin interneurons, and inflammation. These molecular and cellular changes may contribute to early impairments in excitatory-inhibitory balance, leading to cognitive deficits reminiscent of early-stage AD disease. By comparing the differentially expressed genes between the early-stage and late-stage, we identified potential pathways in which hippocampal neurons try to adapt to this proNGF accumulation, and the effects of accumulation of proNGF on synaptic structure and maintenance of synapses. This pathway highlights how alterations in neurotrophin processing can serve as a mechanistic link between molecular dysfunction and behavioral symptoms, emphasizing the importance of early intervention in neurodegenerative disorders. We identified genes that were specific to early and late stages of proNGF mediated neurodegeneration.

4.5. Future Directions

Future directions include identifying differentially expressed genes at a level of neuronal subtypes. This would help to identify more specifically how the degradation of cholinergic projections to the parvalbumin interneurons affects gene expression for TgproNGF#3 mice. Another direction could include determining the full scale of how the serotonergic system is affected, including effects on 5HT release and receptor activation. In addition, it would be useful to identify which population of cells are most affected by the upregulation of 5HTR2A expression. This would allow us to further understand magnitude of how the hippocampus is affected when the inhibitory circuitry is unchecked due to the loss of cholinergic projections to the parvalbumin interneurons of the dentate gyrus, which is a critical signaling node CA3 hippocampal regions which may play a large part in AD pathology and progression of the disease state.

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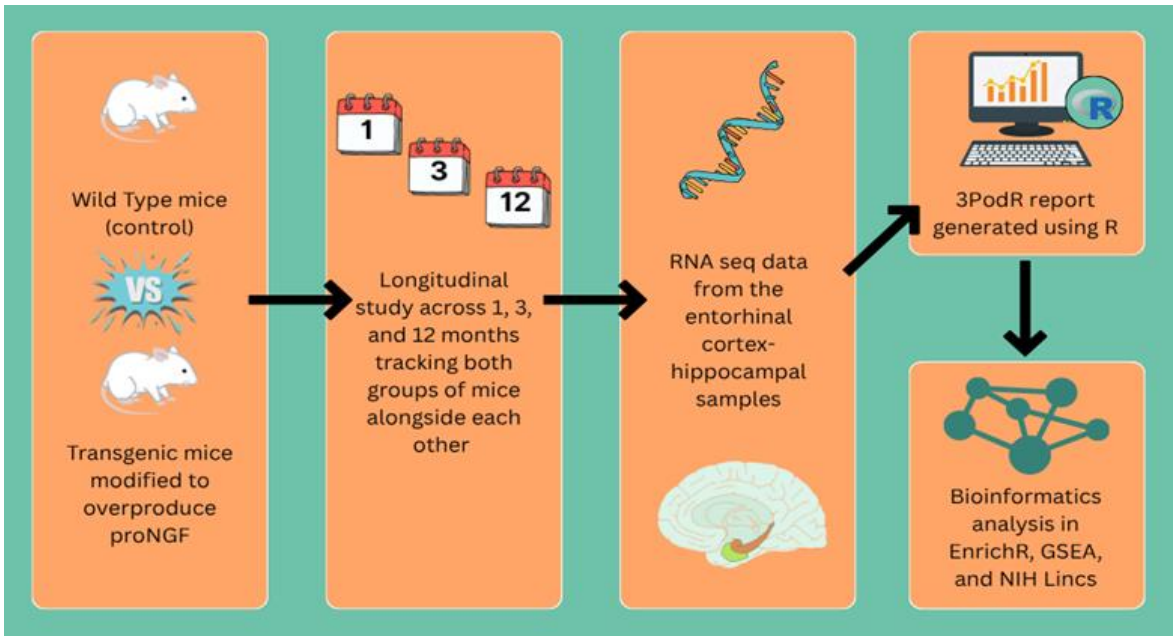

Figure 1: Workflow Schematic for the Study. The dataset (GSE70757) was obtained from the NIH GEO Database. We performed differential gene expression analysis using limma and then processed the data further using the 3PodR pipeline developed by the Cognitive Disorders Research Lab.

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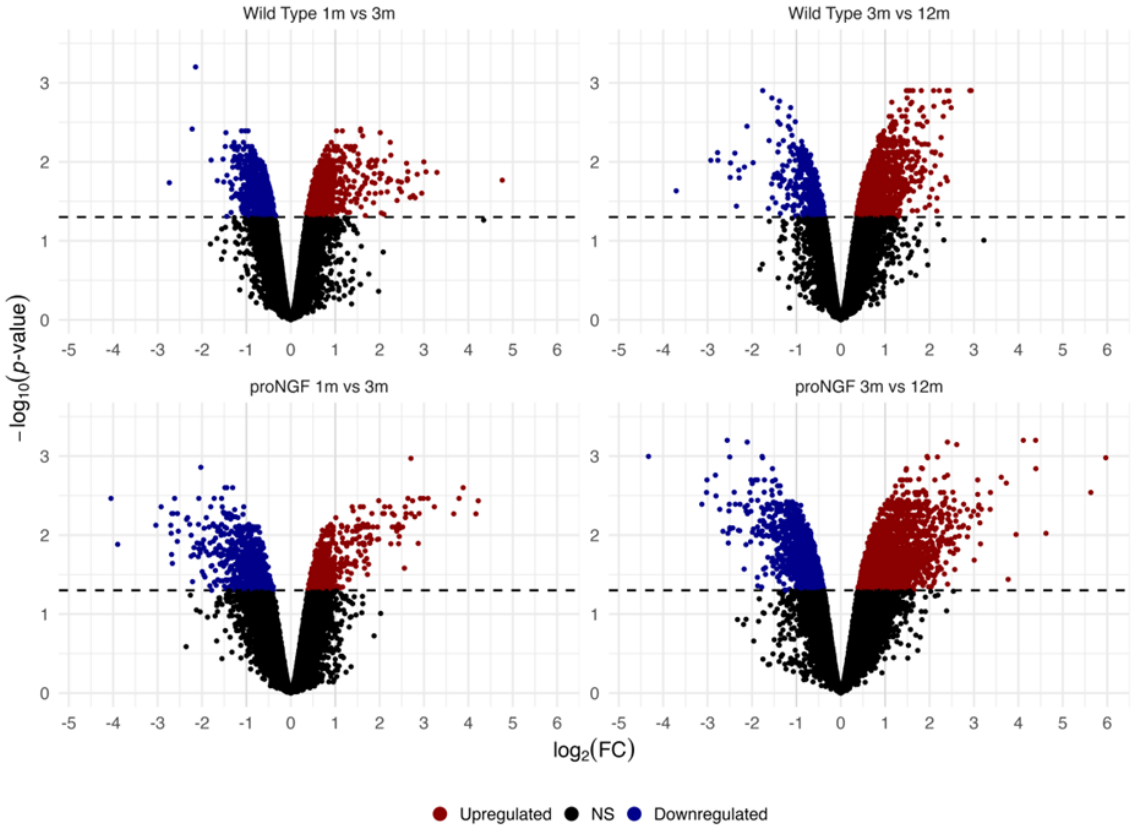

Figure 2: Volcano plots illustrate differential gene expression between transgenic and wild-type mice at two time points. The x-axis shows the $\log_2$ fold change of gene expression, and the y-axis displays the $-\log_{10}$ of the adjusted p-value. Upregulated genes are marked in red, and downregulated genes in blue.

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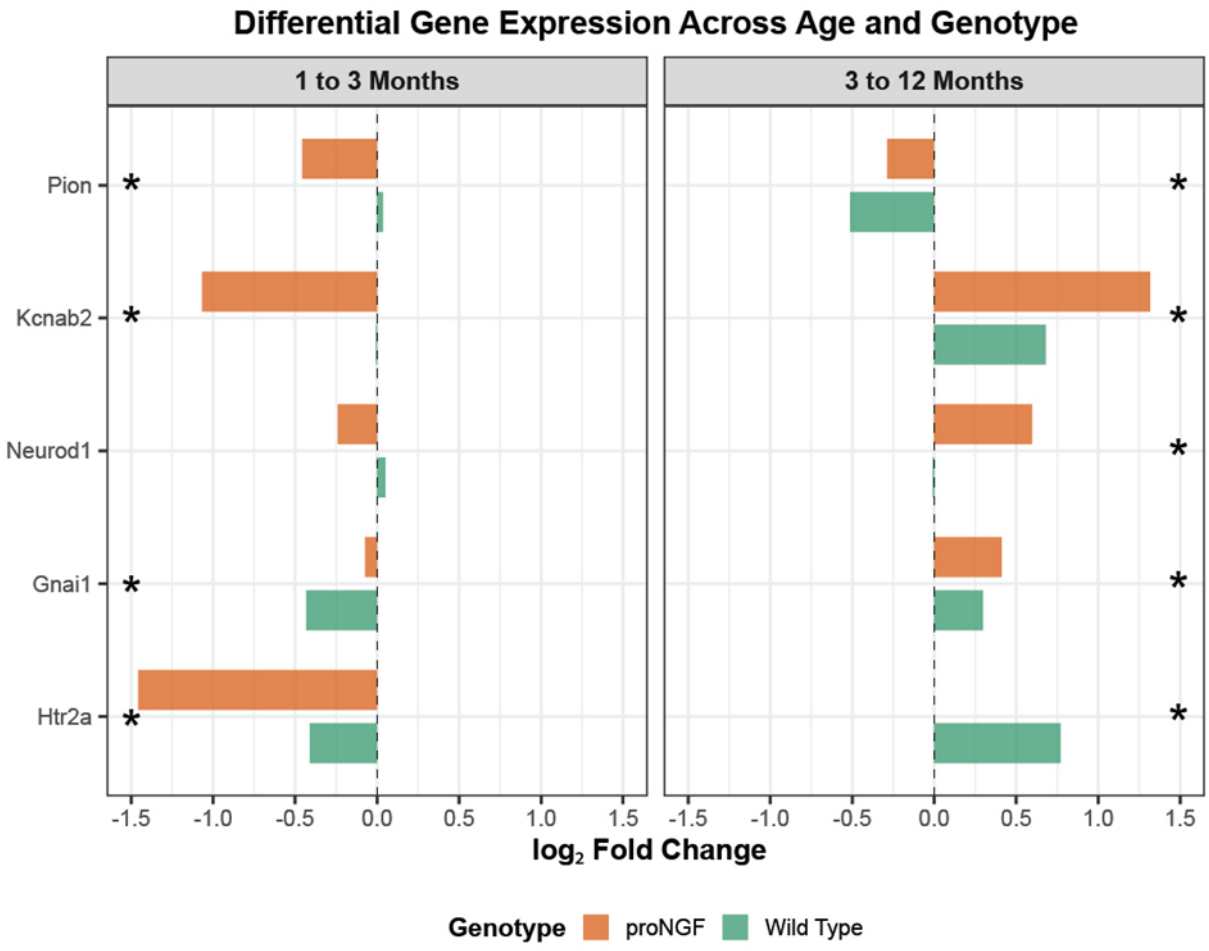

Figure 3: Notable selected gene expression in TgproNGF#3 mice across developmental stages. Faceted horizontal bar plot displays significantly regulated genes in TgproNGF#3 mice at 1 vs 3 months and 3 vs 12 months. Log_2 fold changes of selected genes (Pion, Kcnab2, Neurod1, Gnai1) are shown for male WT (green) and TgproNGF#3 (orange) mice at early (1–3 months) and late (3–12 months) time points. Positive values indicate upregulation relative to baseline, and negative values indicate downregulation. Asterisks denote genes that reached significance based on corrected p-values.

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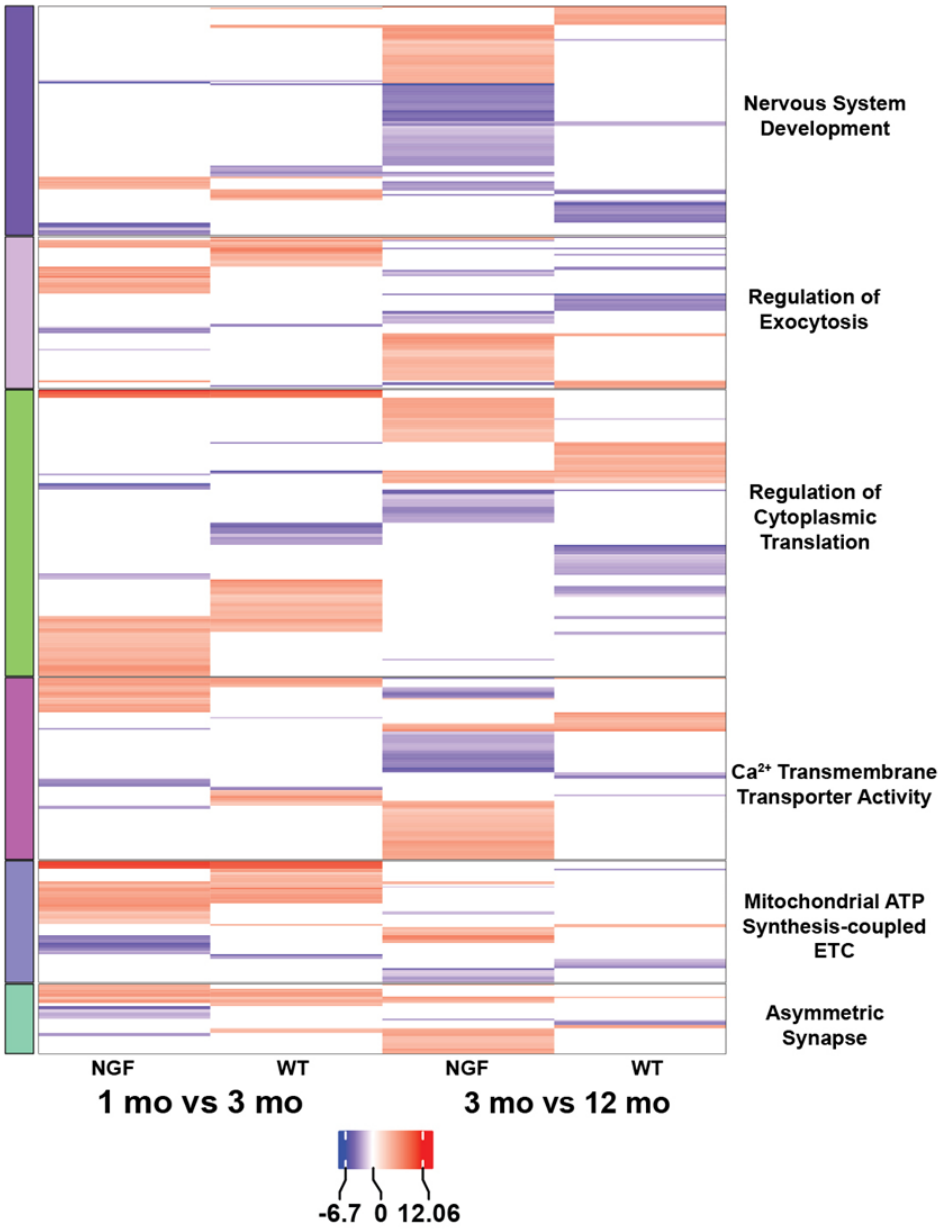

Figure 4: Heatmap showing pathway clusters affected by proNGF accumulation in early stage and late progression of proNGF mediated neurodegeneration. The heatmap displays differential enrichment of individual pathways based on differential gene expression. The pathways were clustered into themes using PAVER. Rows represent individual pathways and columns represent experimental conditions or comparisons. Red and blue indicate positive and negative enrichment, respectively. Highlighted GO themes include Nervous System Development, Regulation of Exocytosis, Calcium Ion Membrane Transport Activity, Mitochondrial ATP Synthesis Coupled Electron Transport, and asymmetric synapse. NGF: TgproNGF#3; WT: Wild Type; ETC: Electron Transport Chain.

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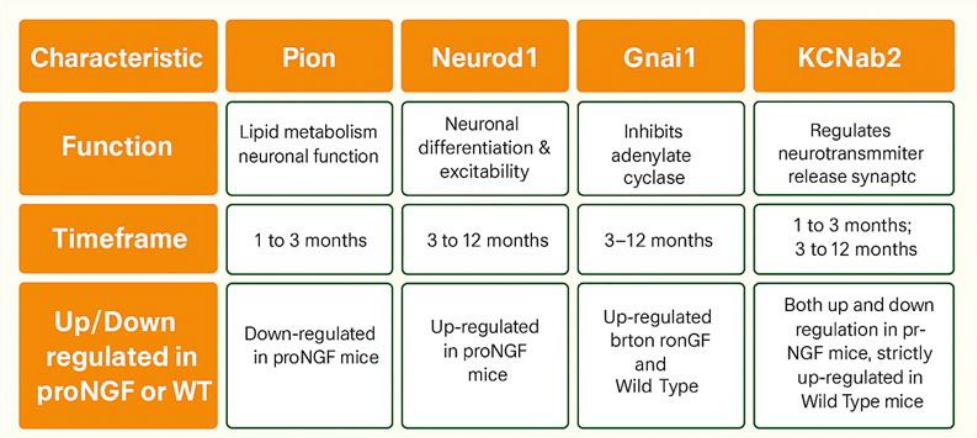

Characteristic	Pion	Neurod1	Gnai1	KCNab2
Function	Lipid metabolism neuronal function	Neuronal differentiation & excitability	Inhibits adenylate cyclase	Regulates neurotransmmiter release synaptc
Timeframe	1 to 3 months	3 to 12 months	3–12 months	1 to 3 months; 3 to 12 months
Up/Down regulated in proNGF or WT	Down-regulated in proNGF mice	Up-regulated in proNGF mice	Up-regulated brton ronGF and Wild Type	Both up and down regulation in pr-NGF mice, strictly up-regulated in Wild Type mice

Figure 5: Summary figure of relevant genes for proNGF-mediated Alzheimer's disease progression. Expression patterns of the 5-HT2A- genes in proNGF mice over time highlight gene function, timeframe, and regulation status in proNGF vs. WT.

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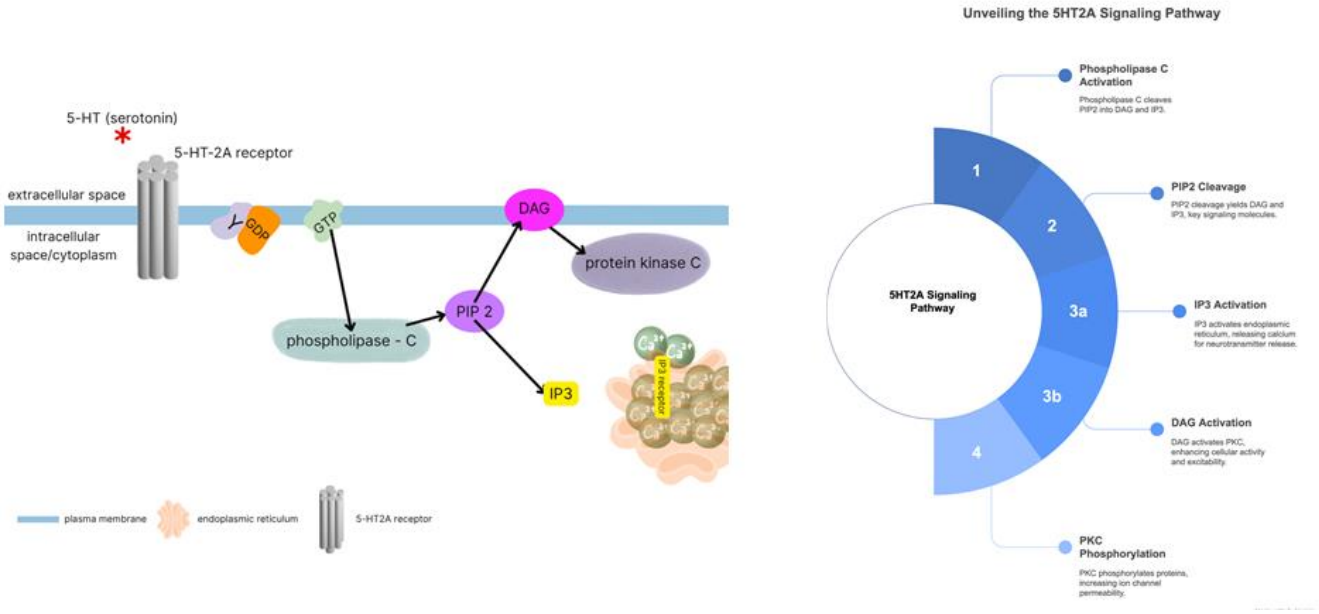

Figure 6: Schematic Overview and Stepwise Breakdown of the 5-HT2A Signaling Pathway. (A) An illustration of the 5-HT2A receptor signaling cascade. Upon serotonin (5-HT) binding to the 5-HT2A receptor on the plasma membrane, the associated G protein directly activates phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into two second messengers: diacylglycerol (DAG) and inositol triphosphate (IP_3). IP_3 stimulates calcium release from the endoplasmic reticulum, while DAG activates protein kinase C (PKC), which mediates downstream phosphorylation events. (Right) A simplified step-by-step diagram summarizing the key stages of the 5-HT2A signaling pathway: (1) activation of phospholipase C, (2) cleavage of PIP_2 into DAG and IP_3, (3a) IP_3-mediated calcium release, (3b) DAG-mediated PKC activation, and (4) PKC-dependent phosphorylation of target proteins affecting cellular excitability and function.

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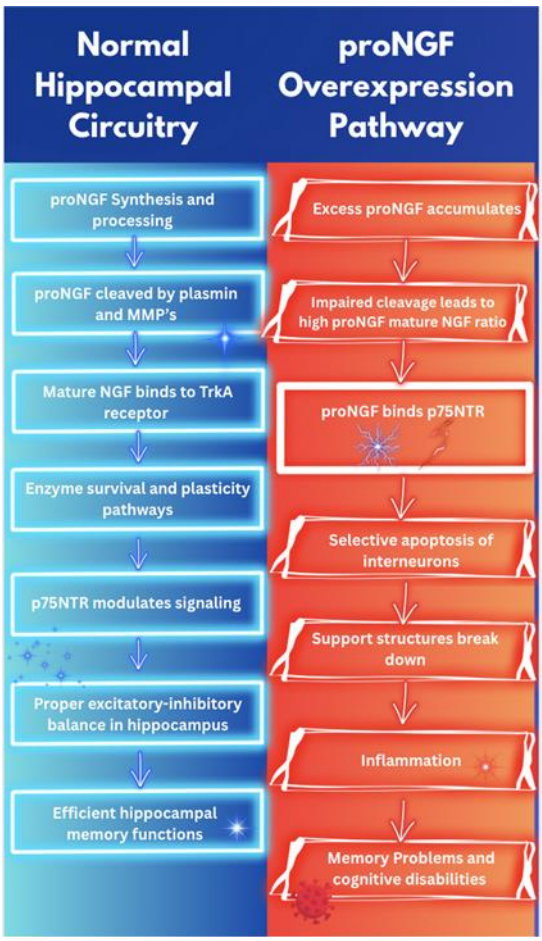

Figure 7. Impact of proNGF Overexpression on Hippocampal Circuitry. Comparison of normal and pathological hippocampal signaling pathways. Under normal conditions (left), proNGF is processed into mature NGF, promoting survival, plasticity, and balanced circuitry via TrkA and p75NTR signaling. In contrast, proNGF overexpression (right) leads to impaired cleavage, excess proNGF binding to p75NTR, which in turn causes loss of cholinergic connections to PV interneurons, structural breakdown, inflammation, and cognitive deficits.

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Genotype	Comparison	Down	Up	NS
Wild Type	1m vs 3m	736	860	14370
	3m vs 12m	541	1597	13828
TgProNGF#3	1m vs 3m	675	602	14689
	3m vs 12m	1344	2255	12367

Table 1: Differential gene expression in WT and TgproNGF#3 mice across age. Using the limma pipeline, differentially expressed genes (DEGs) were analyzed in male WT and TgproNGF#3 mice during early (1–3 months) and late (3–12 months) stages. Genes were classified as upregulated, downregulated, or not significant (NS) based on adjusted p-values, with the corresponding numbers displayed for each group.

Table 1: Differential gene expression in WT and TgproNGF#3 mice across age. Using the limma pipeline, differentially expressed genes (DEGs) were analyzed in male WT and TgproNGF#3 mice during early (1–3 months) and late (3–12 months) stages. Genes were classified as upregulated, downregulated, or not significant (NS) based on adjusted p-values, with the corresponding numbers displayed for each group.