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In silico Gene Expression and Pathway Analysis of *DEK* in the Human Brain Across the Lifespan

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Abstract

DEK, a chromatin-remodeling phosphoprotein, is associated with various functions and biological pathways in the periphery, including inflammation, oncogenesis, DNA repair, and transcriptional regulation. We recently identified an association between DEK loss and central nervous system diseases, such as Alzheimer's. To understand DEK's potential role in disease, it is critical to characterize DEK in healthy human brain to distinguish between neural DEK expression and function in healthy versus diseased states like dementia. We utilized two public databases, BrainCloud and Human Brain Transcriptome, and analyzed *DEK* mRNA expression across the lifespan in learning and memory relevant brain regions. Since DEK loss induces phenotypes associated with brain aging (e.g., DNA damage, apoptosis), we hypothesized that neural *DEK* expression may be highest during fetal development and lower in elderly individuals. In agreement with this hypothesis, *DEK* was most prominently expressed during fetal development in all queried forebrain areas, relative to other ages. Consistent with its roles in the periphery, pathways related to *DEK* in the brain were associated with cellular proliferation, DNA replication and repair, apoptosis, and inflammation. We also found novel neural development-relevant pathways (e.g., synaptic transmission, neurite outgrowth, myelination) to be enriched from genes correlated with

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Author Contributions

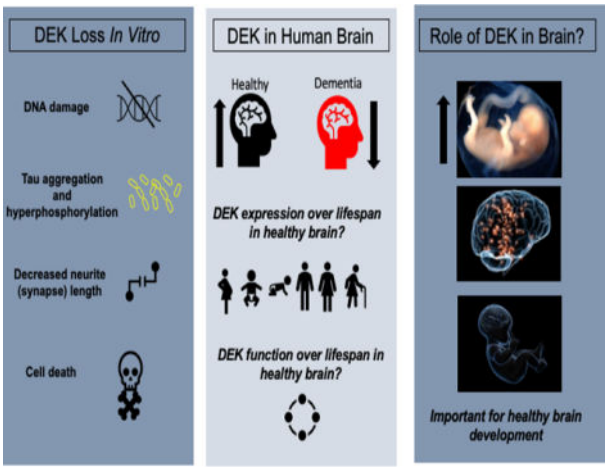
ANG carried out data organization and data analysis. ETN assisted with the two-way ANCOVA analyses, and AL provided feedback on analyses. AP carried out GSEA pathway analyses. ANG wrote the manuscript with support and input from LMPV and MBS. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Conflict of Interest

The authors declare no conflict of interest.

DEK expression. These findings suggest that *DEK* is important for human brain development. Overall, we highlight age-related changes in neural *DEK* expression across the human lifespan and illuminate novel biological pathways associated with DEK that are distinct from normal brain aging. These findings may further our understanding of how DEK impacts brain function and disease susceptibility.

Graphical Abstract



Our lab recently identified a link between DEK loss and Alzheimer's disease. It is difficult to determine the impact of DEK loss in neurodegenerative diseases without characterizing its expression in healthy human brain. We report that DEK expression is highest in several queried forebrain regions during the prenatal vs. postnatal period. Pathway analyses suggest DEK is critical for embryonic brain development. This study provides a framework to further exploit DEK's role in brain function and disease.

Introduction

Our group was the first to associate DEK, a chromatin-associated phosphoprotein, with cognitive-related disorders, such as Alzheimer's disease, using human cells (Greene et al. 2020) and postmortem brain tissue (Ghisays et al. 2018, O'Donovan et al. 2018). Specifically, a gene ontology analysis revealed that DEK loss is associated with age-related dementias and Alzheimer's disease. Furthermore, we demonstrated that DEK loss in a neuronal cell model resulted in phenotypes associated with dementias and Alzheimer's disease, including apoptosis and hyperphosphorylated Tau. Notably, these findings mirror what is observed in peripheral tissues, with DEK loss leading to DNA damage (Smith et al. 2017), cellular senescence (Wise-Draper et al. 2005), and cell death (Kavanaugh et al. 2011, Smith et al. 2017). In 2018 we reported that DEK is expressed throughout the murine brain, including in areas important for learning and memory, such as the hippocampus, amygdala, and prefrontal cortex (Ghisays et al. 2018). Here, we expand upon our previous work by characterizing DEK expression in the healthy human brain across the lifespan.

DEK has previously been established as a proto-oncoprotein that is overexpressed in a majority of solid tumors (Sanchez-Carbayo et al. 2003, Grasemann et al. 2005, Wu et al. 2008, Khodadoust et al. 2009, Liu et al. 2012, Privette Vinnedge et al. 2015). Classically, DEK is known to promote cellular proliferation and DNA repair and inhibit apoptosis (Waldmann et al. 2002, Wise-Draper et al. 2006, Khodadoust et al. 2009, Kavanaugh et al. 2011, Privette Vinnedge et al. 2011, Broxmeyer et al. 2012, Koleva et al. 2012, Waidmann et al. 2014, Privette Vinnedge et al. 2015, Smith et al. 2017). DEK is multifaceted and can impact immune function, as well. For example, DEK secreted from immune cells can act as a chemoattractant (Mor-Vaknin et al. 2006), bind to DEK antibodies in autoimmune disease (Sierakowska et al. 1993, Dong et al. 2000, Mor-Vaknin et al. 2011), and facilitate the formation of neutrophil extracellular traps (NETs; (Mor-Vaknin et al. 2017). Thus, we sought to determine whether DEK is also closely associated with these biological pathways in the brain.

Based on previous studies in the periphery, we posit that DEK may be regulated by steroid hormones in the brain. *DEK* is an estrogen receptor alpha (ERα) target gene and is associated with positive hormone receptor status in breast cancer (Privette Vinnedge et al. 2012). The link between DEK and female-biased diseases, such as Alzheimer's disease, allows us to postulate that there could be a sex difference in DEK protein expression across the human lifespan, which may be brain region dependent. Through our characterization of DEK expression in wild-type C57/Bl6 mice, we observed a sex difference in the number of DEK-positive cells in the CA1 region of the hippocampus; specifically, female mice had more DEK-positive cells in this area than male mice (Ghisays et al. 2018). The CA1 is highly implicated in spatial and contextual learning and memory (Pittenger et al. 2002, Ji and Maren 2008, Bartsch et al. 2011, Jeong et al. 2018). Using human postmortem brain tissue, we found a decrease in DEK expression in the anterior cingulate cortex in women with severe dementia, but not in men (O'Donovan et al. 2018). However, no sex difference in DEK protein expression levels was observed in individuals without cognitive impairment. It is possible, then, that there is a sex difference in neural DEK expression that is limited to a diseased state. Understanding how disease impacts DEK expression or how dysfunction in DEK may contribute to disease susceptibility is important, but there is a need to characterize DEK expression in the healthy human brain in order to ultimately understand DEK's potential role in diseases of the central nervous system. In line with this goal, we sought to determine whether biological factors (i.e., sex and age) affect *DEK* expression in the brain and to present potential biological processes that *DEK* could be involved in through pathway enrichment analysis of *DEK*-correlated genes.

We hypothesize that *DEK* expression will be highest in the human brain during early development, and will decline with age, because normal-to-high DEK expression is associated with cell growth and proliferation. Since *DEK* is an ERα target gene, we also postulated that *DEK* expression will be higher in pre-menopausal females, which have the highest levels of circulating estrogen. We hypothesize that *DEK* expression will be associated with the expression of development-, immune system-, and steroid hormone-related genes, as it is in the periphery. Last, we posit that *DEK* will be associated with biological pathways relevant to neural development during the fetal stage and will be related to different processes, such as hormone signaling, in later stages of life. Given that our

previous findings suggest a prominent role for DEK in learning and memory, we focused our attention on learning and memory relevant forebrain regions including the dorsolateral prefrontal cortex (dlPFC), medial prefrontal cortex (mPFC), ventrolateral prefrontal cortex (vlPFC), hippocampus, and amygdala. The cerebellum was included as a control hindbrain region that is associated with learning and memory. The inclusion of this hindbrain region allows us to determine if any changes across *DEK* across the lifespan is limited to distinct areas of the brain.

Methods

Human data

To confirm the expression of *DEK* in multiple cell types and regions in the brain, we accessed the Brain RNA-Seq and Human Protein Atlas online tools. The former dataset contains RNA-seq of cell types isolated from mouse and human brain (Zhang et al. 2016), https://www.brainrnaseq.org/). The Human Protein Atlas, specifically the brain atlas, contains mRNA expression data of human genes in various areas of the brain (Sjöstedt et al. (2020), https://www.proteinatlas.org/humanproteome/brain), drawing from the FANTOM5 Forrest et al. (2014), https://fantom.gsc.riken.jp/5/) and GTex (Genotype-Tissue Expression Project, https://gtexportal.org/home/, dbGaP accession number phs000424.vN.pN) datasets.

Human postmortem gene expression data from brain tissue was obtained from the BrainCloud (Colantuoni et al. 2011) and Human Brain Transcriptome (HBT; (Johnson et al. 2009, Kang et al. 2011) databases. In the BrainCloud dataset, RNA was isolated from the dorsolateral prefrontal cortex grey matter tissue in 269 individuals with no neuropathological or neuropsychiatric diagnosis. According to Colantuoni et al., total RNA was extracted and amplified, then hybridized to microarrays. Once normalized, log2 intensity ratios were adjusted using surrogate variable analysis to reduce the impact of noise on gene expression levels. For more details on sample collection and data preparation, see Colantuoni et al. (2011).

The HBT dataset provides mRNA expression data from a multitude of brain regions, including forebrain areas such as prefrontal cortices, amygdala, and hippocampus, as well as hindbrain (cerebellum). RNA was collected postmortem from 57 cognitively healthy donors. Authors of the HBT dataset used Partek Genomics Suite version 6.5 (Partek Incorporated, St. Louis, MO, USA) to perform RMA background correction, quantile normalization, mean probe set summarization, and log2 transformation (Kang et al. 2011). For gene set enrichment pathway analysis (GSEA), genes from HBT were filtered using the criteria of a log2-transformed signal intensity of greater than or equal to 6 in at least one sample/subject.

Statistical analysis

To analyze *DEK* expression across the lifespan, two-way ANCOVAs were performed using SPSS Statistics 18.0.0. Because the post-mortem interval (PMI; the time between death and sample collection) of the human samples significantly predicted *DEK* expression via linear regression, PMI was included as a covariate in this analysis. Age and Sex were main factors in the two-way ANCOVAs for each brain region. Sidak post-hoc tests were run after finding

a significant interaction or main effect of age and/or sex. Results were plotted using the means and standard errors from the model adjusted for the covariate, PMI, in GraphPad Prism 8.

To determine what biological pathways are related to *DEK* in the brain, the Pearson correlation coefficient with *DEK* was calculated for each gene that met expression criteria. Pearson correlations were calculated within each age group separately as well as using all samples/subjects from all age groups together. GSEA pathway analysis was performed using the Pearson correlation coefficient as a rank for each gene. To further determine what pathways are uniquely associated with *DEK* in specific age groups, genes with a correlation coefficient greater than or equal to 0.6 were compared across age groups such that lists of genes expressed uniquely in a particular age group were generated using ToppGene to be used for the comparison of DEK-associated pathways in the hippocampus vs. cerebellum (Table 4). To consolidate gene ontology (GO) terms to be visually represented in figures and tables, GO IDs and Odds Ratios were retrieved using Enrichr (Chen et al. 2013, Kuleshov et al. 2016, Xie et al. 2021). The top 500 GO term IDs, ranked by p-value, were input into REVIGO to remove obsolete and redundant GO terms (Supek et al. 2011). Subjects were not separated by sex; we found no overall difference in DEK expression between males and females (p=0.074; data not shown). GSEA and ToppGene analyses yielded results for *DEK*-associated molecular functions, cellular components, biological processes, and human phenotypes, but only biological processes are discussed here to simplify and consolidate the results. The REVIGO interactive graphs were exported into Cytoscape (version 3.9.1) and used to create bubble maps in Figures 3–5.

To identify age-associated pathways that are independent of DEK, a differential gene expression analysis was conducted for each age group versus the remaining age groups using Limma package v3.42.2 in R 3.6.1. Gene expression was compared at each age vs the other two ages (i.e., group 3 vs. Group 1&2). The gene lists were then filtered based on fold change >1.5 or <0.5 (log2FC >0.58 or <−0.58) and p values <0.05. The resulting filtered gene lists were then analyzed for enriched gene ontologies using the ToppFun function of ToppGene (https://toppgene.cchmc.org/enrichment.jsp) with FDR and p<0.05. Gene ontologies were validated, and odds ratios were retrieved using Enrichr. The top 100 ontologies from biological processes were then summarized and filtered for redundant gene ontology (GO) terms using REVIGO (http://revigo.irb.hr) (Tables 5–8).

Results

DEK expression in neural cell types

DEK is expressed in most major cell types in the brain, including astrocytes, microglia, oligodendrocytes, endothelial cells, and neurons (Fig 1A). Fetal astrocytes, oligodendrocytes, and microglia had higher levels of DEK expression compared to other cell types. In addition, it was confirmed that *DEK* is expressed ubiquitously throughout the human brain (Fig 1B).

DEK expression across the lifespan

Two-way ANCOVA revealed an interaction between the effects of age and sex on *DEK* expression in the dorsolateral prefrontal cortex (dlPFC)(HBT; $F(2, 45) = 5.104$, $p = 0.01$), medial prefrontal cortex (mPFC) ($F(2, 45) = 7.259$, $p = 0.002$), and vlPFC ($F(2, 45) = 3.915$, $p = 0.027$). A main effect of age on *DEK* expression was observed in all brain regions queried, including the dlPFC (BrainCloud, Fig. 2A, $F(5, 256) = 34.141$, $p < 0.001$; HBT, Fig 2B, $F(2, 45) = 12.882$, $p < 0.001$), the mPFC (Fig. 2C, $F(2, 46) = 9.634$, $p < 0.001$), the vlPFC (Fig. 2D, $F(2, 44) = 10.382$, $p < 0.001$), the hippocampus (Fig. 2E, $F(2, 44) = 6.499$, $p = 0.003$), the amygdala (Fig. 2F, $F(2, 41) = 24.916$, $p < 0.001$), and cerebellum (Fig. 2G, $F(2, 40) = 3.469$, $p = 0.041$). Specifically, in all forebrain regions, *DEK* expression was highest during fetal development stages and sharply declined during early childhood through adulthood (dlPFC; BC, $p < 0.001$; HBT, $p < 0.001$; mPFC, $p \leq 0.001$; vlPFC, $p \leq 0.001$; Hippocampus, Fetal v. Pre-adult $p = 0.014$, Fetal v. Adult $p = 0.003$; Amygdala, $p < 0.001$). In the cerebellum, *DEK* expression was lower in adulthood (Pre-adult v. Adult $p = 0.037$). A main effect of sex was found in the dlPFC with data from BrainCloud ($F(1, 256) = 7.569$, $p = 0.006$). In this region, males had significantly higher *DEK* expression than females throughout life ($p = 0.006$). However, *DEK* expression in the prefrontal cortices, using data from HBT, was highest in the brains of male fetuses but this was reversed in adulthood, such that *DEK* levels were higher in adult females compared to males (dlPFC, Fig. 2B, $F(2, 45) = 5.104$, $p = 0.01$; mPFC, Fig. 2C, $F(2, 46) = 7.259$, $p = 0.002$; vlPFC, Fig. 2D, $F(2, 44) = 3.915$, $p = 0.027$).

DEK-associated pathway analyses

Across all age groups and brain regions, biological pathways enriched from genes positively correlated with *DEK* expression (Table 1, in red) include DNA replication and repair, RNA processing, cell cycle, gene expression regulation, and ubiquitin activity. Other positively enriched pathways include ribosome biogenesis and metabolism, as well as hormone secretion (not displayed in the table; p=0.0034; OR=4.82). Biological pathways enriched from genes negatively correlated with *DEK* expression (Table 1, in blue) include synaptic signaling and neurotransmitter transport, macrophage activation, glutamate receptor signaling, regulation of neuronal death, memory, and steroid hormone signaling (not displayed in the table; p=0013; OR=7.56).

As seen in Table 2, the top genes harboring a positive association with *DEK* expression include USP1, SRBD1, RMI1, RBL1, and NUP107, which have functions in ubiquitin processing, RNA binding, DNA repair, cell cycle regulation, and nuclear transport, respectively (Stelzer et al. 2016). The top genes with a negative correlation to *DEK* expression include GRIN1, IGSF8, PPP2R2C, IQSEC2, and MROH1, which function in synaptic plasticity via NMDA receptors, response to viral infection, cell growth and division, synaptic organization, and binding, respectively (Stelzer et al. 2016).

When *DEK*-associated biological pathways are separated by age group, distinctions can be made about DEK's possible functions during different age periods. To do this, we explored enriched biological processes associated with *DEK* during different age groups. Table 3 lists the top 20 biological processes positively enriched from *DEK*-correlated

genes in each age group, while Figures 3–4 display the negatively enriched pathways. The pathways from genes negatively correlated with *DEK* tend to offer more novel functions for *DEK* in the central nervous system, as seen in Table 1, so more focus was placed on the negatively enriched pathways. Positively associated pathways remain fairly stable across age groups; *DEK* is associated with functions such as mRNA processing, DNA repair, ribosome biogenesis, and gene expression throughout life (Table 3). Differently, in adulthood, DEK appears to be involved in additional pathways related to translation (Table 3). Pathways enriched from genes negatively correlated with *DEK* expression only during fetal development (6–38 post-conceptional weeks) include neuron projection development, neurotransmitter biosynthesis, and myelination (Figure 3). After birth, specifically in pre-adulthood (0–20 years old), *DEK* is negatively associated with glial cell proliferation, hippocampal neuron apoptosis, and reproductive system processes (Figure 4). One pathway negatively associated with *DEK* only in adulthood (20+ years old), seen in Figure 5, is superoxide metabolism, a function important in aging (Sasaki et al. 2008). Interestingly, there are several biological processes associated with *DEK* after birth that are not highly enriched in fetal development. For example, immune system and inflammatory response, serotonin and adrenergic signaling, response to amyloid-beta, neuron differentiation, and cell migration are all associated with *DEK* in pre-adulthood and adulthood but not during fetal development.

Because of the different expression pattern of *DEK* in the forebrain vs. the hindbrain (cerebellum), we investigated the difference in potential biological functions of *DEK* in these regions across life. We also chose to differentiate between pathways related to *DEK* in the cerebellum vs. hippocampus because these regions are important for learning and memory. In addition, we know that DEK is highly expressed in the hippocampus in the murine brain (Ghisays et al. 2018). We evaluated the difference in DEK-associated pathways in these regions only during adulthood to begin to understand potential variations in DEK function during this age group in which we know DEK expression is important (O'Donovan et al. 2018). In the hippocampus, many pathways positively related to *DEK* expression represent mitochondrial processes, and signaling pathways such as steroid hormone signaling (Table 4, left column). In contrast, *DEK* expression in the cerebellum is associated with synaptic transmission, protein modifications, neuron death, neurotransmitter metabolism, and sensory perception (Table 4, right column). In both the hippocampus and cerebellum, DEK expression in adulthood could be important for the cellular response to oxidative stress and maintaining neuronal projections.

Age-associated pathway analyses

In order to determine if DEK associated expression in genes and gene ontologies (categories) are primarily related to typical changes that occur in the aging brain, we compared the results of the correlation analysis vs pathway analysis using differential gene expression analysis. When we queried age-related pathways in the hippocampus, the fetal stage of development is primarily characterized by an upregulation of genes that are associated with DNA repair, chromosome organization and segregation, and histone modification which are not seen in the preadult and adult stages (Table 5). The pre-adult and adult stages had several overlapping biological pathways that were upregulated including

those associated with neuron projection development, neurotransmitter function, chemical synaptic transmission, mitotic cell cycle process, regulation of membrane potential/action potential, and learning and memory. (Table 5). In contrast, many of the gene ontologies that were upregulated in the fetal stage of development were downregulated in the preadult and adult stages including chromosome organization and segregation, DNA repair and replication, and brain and head development (Table 6). In this vein, the fetal stage is associated with downregulation of gene ontologies that are typically highly expressed in the pre-adult and adult stage including chemical synaptic transmission, regulation of membrane potential, regulation of neuron projection development, and behavior (Table 6).

The cerebellum is characterized by an upregulation of pathways that are associated with hindbrain development, developmental growth, chromosome organization, microtubule-based process that are unique to the fetal stage (Table 7). The pre-adult and adult stages share several upregulated gene ontologies including chemical synaptic transmission, gliogenesis, and myelination. (Table 7). Similar to the hippocampus, many of the biological processes (i.e., chemical synaptic transmission, neurotransmitter transport, regulation of ion transport, myelination, and gliogenesis) that are highly expressed during the pre-adult and adult stages are downregulated in the fetal stage in the cerebellum (Table 8). Within the cerebellum, the preadult and adult periods are characterized by some overlapping pathways including those related to chemotaxis, growth, synaptic organization or signaling, cell-cell adhesion via plasma membrane adhesion molecules, and nervous system development (Table 8). However, there are some distinct pathways in the cerebellum between preadult (small GTPase mediated signal transduction) and adult (Ras protein signal transduction) stages (Table 8). Overall, in both the hippocampus and cerebellum, genes and gene ontologies that are highly expressed in the fetal stage are downregulated in the pre-adult and adult stages. For example, in the cerebellum, gene ontologies that collectively control organismal/developmental growth or growth factor responses are upregulated in the fetal stage and downregulated in the pre-adult or adult stages.

Discussion

The goal of the current study was to characterize *DEK* expression in the healthy human brain. To accomplish this goal, we analyzed *DEK* expression in multiple learning and memory-relevant brain regions across the lifespan using the BrainCloud and Human Brain Transcriptome (HBT) databases. Next, in order to further our understanding of the potential role of DEK in human brain, we evaluated the biological pathways enriched from genes correlated with *DEK* expression in various brain regions. We also compared the results across age groups to discover whether DEK-related functions change throughout the human lifespan.

All forebrain areas analyzed (prefrontal cortex, hippocampus, amygdala) have significantly higher *DEK* expression during fetal development which declined after birth, while *DEK* expression in the hindbrain target region of the cerebellum remained relatively stable until adulthood where it begins to decline. This presents an interesting difference in the potential roles of DEK in the forebrain versus the hindbrain. We hypothesized that there would be a sex difference in *DEK* mRNA expression, with females having higher *DEK* mRNA

expression relative to males in at least some of the queried brain regions. This hypothesis was based on previous data indicating that *DEK* is an ERα target gene (Privette Vinnedge et al. 2012) and our previous report of increased DEK expression in the hippocampus in female mice compared with male mice (Ghisays et al. 2018). However, the findings from the BrainCloud database demonstrated that males had higher *DEK* expression in the dlPFC throughout life relative to females. Even though data from HBT in the dlPFC showed the same age-related effect on *DEK* expression as seen in BrainCloud, we did not observe a similar sex effect as there was no difference in *DEK* expression in males and females. This could be due to differences in sample collection methods, sample size, RNA extraction techniques, post mortem intervals of the donors, or data normalization methods between the two databases. Interestingly, there was an interaction between age and sex in the dlPFC, mPFC, and vlPFC from HBT, with male *DEK* expression being higher during fetal development, and females having higher *DEK* levels after birth. While males have an overall total brain volume that is typically greater than females, females have larger volumes of prefrontal cortices than males and reach their peak volume at an earlier age after puberty (Lenroot and Giedd 2010, Liu et al. 2020). DEK is likely important for neural development, which could account for its expression differences between males and females in the frontal cortices during stages when neural development occurs at a faster rate, i.e., before birth in males and after birth in females. Future *in vitro* and *in vivo* (rodent) studies may investigate the potential significance of these sex differences in neural *DEK* with regards to brain function, physiology, and behavior.

To evaluate possible functions that DEK may have in the brain, we took a non-targeted approach by completing pathway analysis of genes whose expression are correlated with that of *DEK*. Pathways enriched from a set of genes with a positive correlation to *DEK* expression across age groups and brain regions include many functions that DEK is known to carry out in the periphery, such as DNA replication and repair (Kavanaugh et al. 2011, Deutzmann et al. 2015, Smith et al. 2017), RNA processing (McGarvey et al. 2000), cell cycle (Nakashima et al. 2017), gene expression regulation (Fu et al. 1997, Hollenbach et al. 2002, Campillos et al. 2003, Koleva et al. 2012), hormone signaling (Privette Vinnedge et al. 2012), and metabolism (Matrka et al. 2017). In contrast, many of the pathways enriched from genes negatively correlated to *DEK* expression are novel to what is known about DEK's functions. For example, DEK has never before been linked to synaptic structure and transmission, action potential, neurotransmitter transport, or sensory perception. These results help to inform future directions of study of neural DEK by highlighting key biological functions that DEK may be involved in. By understanding DEK's functions in the healthy brain, we can use this to inform and compare to DEK's role in the diseased brain.

To take a closer look at the specific genes that are most strongly correlated with *DEK* throughout the brain, the complete lists of significantly positively and negatively associated genes were sorted by correlation coefficient. The top 50 genes from each list are named in Table 1. As expected, some genes positively correlated with *DEK* are associated with cancer and immunodeficiency, such as RRM1, POLE2, SMC4, MSH6, and SRSF10 (Stelzer et al. 2016). In the healthy brain, many of these genes are involved in processes such as DNA replication and repair, chromatin remodeling, and RNA splicing (Stelzer et al. 2016).

In contrast, some of the genes negatively correlated with *DEK* expression are potential tumor suppressors, such as KCNMA1, BIN1, and NDRG4 (Stelzer et al. 2016). Given *DEK*'s history as an oncogene, it follows that *DEK* would be negatively associated with tumor suppressive genes. Other negatively correlated genes with *DEK*, including MINK1, SYP, SNPH, DNM1, JPH3, and TPPP, are related to learning/memory processes and cognition-related disorders, such as AD, Huntington's disease, epileptic encephalopathy, and intellectual disability (Stelzer et al. 2016). It is notable that our research group and others have identified neurodegenerative diseases, including AD and Huntington's, as potentially related to changes in DEK expression, particularly the loss of DEK expression (Christodoulou et al. 2020, Greene et al. 2020, Miao et al. 2020). Again, this highlights the interesting difference between positively and negatively correlated genes and pathways to *DEK*. However, there are exceptions to each of these observations, such as tumor suppressor genes' expression being positively correlated with DEK, or other positively correlated genes to *DEK* being associated with intellectual disability. Overall, though, there is a general pattern for genes positively correlated with *DEK* to be related to functions previously associated with DEK in the periphery, while negatively correlated genes tend to be related to novel functions for DEK, many of which are linked with learning and memory. Because the effects of posttranslational modifications of DEK on its function are largely unknown, it is difficult at this time to differentiate between the functional significance of DEK expression being positively or negatively correlated with particular pathways. For the purpose of focusing on the unique roles of DEK by age and brain region, the pathways enriched from positively and negatively correlated genes will be discussed jointly here after.

To explore the potential age-specific functions of DEK in the brain, we compared DEK-associated biological processes across age groups, focusing on negatively enriched pathways. During fetal development, the most significantly enriched pathways related to DEK include processes such as nervous system development, apoptosis, biosynthesis, synaptic transmission, and cellular organization. Cellular communication and signaling are crucial for healthy brain development (Basson 2012, Perrimon et al. 2012, Navarro Quiroz et al. 2018) and regulation of cell death is also important to ensure that healthy cells are allowed to proceed with differentiation (Blaschke et al. 1996). DEK is known to regulate apoptosis and cell death in the periphery (Wise-Draper et al. 2005, Wise-Draper et al. 2006), and we now find that this function of DEK may be important during neural development *in utero*. Enriched DEK-associated pathways during fetal development also include microtubule-based processes such as neuron projection and dendritic spine development. We have previously observed a link between DEK loss and Tau hyperphosphorylation and development of neurite processes (Greene et al. 2020). This pathway analysis corroborates our hypothesis that DEK may be important for microtubule stability and neurite outgrowth.

In early life, i.e., both fetal development and pre-adulthood (before 20 years old), DEK-related pathways include immune system development processes and glial cell proliferation. DEK's relationship with immune system functions may shift as humans age, from immune system development to inflammatory responses. After birth, in both pre-adulthood and adulthood, DEK-associated pathways include cytokine production, NF-κB signaling, and neuroinflammation. In general, DEK seems to be important for the cellular response to various stimuli, whether it is a virus, DNA damage catalyst, or sensory stimulus.

DEK's roles in DNA damage and immune responses are recognized outside of the brain (Kavanaugh et al. 2011, Pease et al. 2015, Smith et al. 2017, Pease et al. 2020), but we now have reason to believe that DEK can have these same functions in the central nervous system.

DEK is an estrogen receptor alpha (ERα) target gene and its expression is upregulated *in vitro* in response to estrogen, progesterone, and androgen administration (Privette Vinnedge et al. 2012). Here, we also see that DEK may be related to sex steroid hormone signaling pathways, as pathways enriched from genes correlated with DEK during pre-adulthood and adulthood include reproductive processes and steroid hormone signaling. Neurotransmission is another type of cell communication we found to be associated with *DEK* expression. For example, adrenergic signaling and serotonin receptor signaling were enriched from genes correlated with *DEK* after birth. The adrenergic system is important for cardiac function (Rengo 2014); many enriched pathways from genes correlated with *DEK* were relevant to cardiac function but we did not include them in order to focus on the central nervous system in the current manuscript. Serotonin can have many physiological actions, from regulating sleep/wake cycles, to cardiac function and digestion (Berger et al. 2009). It is difficult to say at this time how DEK is specifically linked to the serotonin system. Other DEK-associated pathways found in our current analysis include neurotransmitter biosynthesis, metabolism, and transport. Future studies may investigate the precise role of DEK in neurotransmission and regulating neurotransmitter levels, such as serotonin. Previous research indicates that altered DEK expression could reprogram cellular metabolism (Matrka et al. 2017), but it is not yet known if DEK is involved specifically in neurotransmitter metabolism.

Aside from age-specific roles for DEK in the brain, it is important to establish if there is a difference in DEK-associated pathways in various brain regions. To begin to answer this question, we compared *DEK* expression and DEK-associated biological pathways in the forebrain and hindbrain. We chose the hippocampus and cerebellum to represent these areas because DEK is highly expressed in these structures and they are known to be important for learning and memory (Thompson and Kim 1996). First, there are several distinct differences in *DEK* expression and related pathways between forebrain regions, including the hippocampus, mPFC, and amygdala, and the cerebellum. *DEK* expression remains relatively high throughout life in the cerebellum, while *DEK* levels sharply decline in the forebrain after birth. Although the lower postnatal mRNA expression levels of *DEK* in the forebrain do not take away from its potential functional significance, it is possible that the higher transcriptional levels of *DEK* in the cerebellum hint at an important function for DEK throughout life in this brain region that may be different than its role(s) in the forebrain. For example, sensory perception is associated with *DEK* in the cerebellum during adulthood, but not in the hippocampus. There is increasing evidence to suggest that the cerebellum plays a role in perceptual processes, such as nociception, visual and auditory processing, and attentional adjustment of perceptual discrimination (Rondi-Reig et al. 2014, Baumann et al. 2015, Breska and Ivry 2021). It is even proposed that the white matter integrity of the cerebellum can affect sensory processing (Narayan et al. 2021). Given DEK's potential involvement in myelination, it is possible that *DEK* expression could be important for maintaining white matter integrity in various brain regions, including the cerebellum. In addition, DNA replication and cell cycle, which are related to DEK in the periphery and are

positively associated with *DEK* expression in the whole brain throughout life, are among the pathways negatively enriched in the cerebellum during adulthood. Interestingly, these processes are not associated with DEK in the adult hippocampus, again suggesting that DEK may have unique roles in the forebrain versus hindbrain which could account for differences in *DEK* expression between these regions across age groups.

An additional goal of this study was to parse out DEK-related pathways across the lifespan from "normal" age-related changes in brain. In doing so, we identified genes and gene ontologies that were more prominently expressed when DEK was considered as a factor vs. when it was not (age-only). For example, biological pathways associated with ribosome biogenesis, mRNA splicing, mRNA transport, DNA metabolic processes, double strand break repair, cellular responses to DNA damage, and mitochondrial function were more prominently associated with DEK. Again, these findings in brain are consistent with its reported role in the periphery. Not surprisingly, there were some shared gene ontologies between DEK dependent vs DEK independent (age-only) pathways including DNA repair and microtubule organization. These findings suggest that changes in DEK expression in brain over the lifespan yields a unique pattern of gene expression that is not simply due to brain aging alone.

Not only can DEK have functions across multiple biological pathways in various brain regions, but it is expressed in different cell types. Because we have previously observed that DEK is co-expressed with neurons, microglia, and astrocytes in the murine brain (Ghisays et al. 2018), we postulate that DEK may have roles in multiple neural cell types in the human brain. Consistent with this hypothesis, in the human brain, DEK is expressed in numerous cell types including fetal and mature astrocytes, neurons, microglia, oligodendrocytes, and endothelial cells (represented in Figure 1; http://www.brainrnaseq.org/; Zhang et al. (2016)). Thus, there is congruency between the localization of DEK in major cell types between the murine and human brain. Notably, its expression pattern in brain is increased in astrocytic tumors in glioblastoma (Feng et al. 2017), which is consistent with its role in the periphery as a proto-oncoprotein. These findings suggest that the expression of DEK in distinct neural cell types in the brain may differ in healthy vs diseased states. Future studies will investigate DEK's roles in different cell types in the brain to better understand its role in the healthy aging vs. aging associated with neuropathological conditions (e.g., Alzheimer's disease).

There are several limitations to this study. First, it is correlative in nature and while the findings suggest potential roles for DEK in the human brain, we cannot fully determine the functional significance of the results. Second, while the pattern of *DEK* expression across ages is similar in the dlPFC across the two databases we used, there was a main effect of sex on *DEK* using BrainCloud data, but from the HBT data we found an interaction between age and sex. This could be due to differences between the two databases in sample size, tissue collection, RNA extraction, and data normalization. Third, the HBT sample size was smaller than BrainCloud; therefore, we could not stratify the samples from HBT into as many age groups. Thus, we combined age groups that would not normally be analyzed within one group. As a consequence, we could have missed subtleties in the effect of age on *DEK* expression. For example, both early childhood and adolescence were included in the pre-adult age group, but those are very distinct developmental periods.

Nonetheless, it is clear that *DEK* expression in the selected forebrain regions (e.g., prefrontal cortex, amygdala, hippocampus) and hindbrain structure is highest during the fetal stage of development (e.g., 6–38 weeks post-conception) and in the forebrain its expression declines after birth, no matter the specific age group. For example, pre-adults (individuals between 0 and 20 years old) and aged individuals (20+ years old) do not have significantly different *DEK* mRNA levels. It is important to note, though, that the relatively lower levels of *DEK* mRNA expression in older individuals vs. fetal development is not necessarily indicative of the fact that DEK is not important in the aged brain. It is worth noting that in the healthy adult human brain, expression of the DEK protein is still easily detectable (O'Donovan et al. 2018). Finally, we focused on a limited number of brain regions in this study. The databases we drew from did not include midbrain structures, so we cannot say if the age-related change in neural *DEK* expression is limited to forebrain areas. However, *DEK* is expressed in midbrain regions (represented in Figure 2; https://www.proteinatlas.org/; Sjöstedt et al. (2020)), therefore this is a discrepancy that could be addressed in future studies.

Further, the gene set enrichment analysis method used here is limited in its nature by analyzing each pathway independently for their enrichment of DEK-associated genes. Because genes overlap between pathways, while one relevant pathway may be enriched in its association to DEK expression, other pathways may also be significantly enriched because of the overlapping genes, resulting in potential false positives. Similarly, if a small number of genes highly correlated to DEK expression are common to many pathways, there could be many irrelevant pathways, i.e., background noise, that are enriched due to the broad functions of those genes. Additionally, genes are assigned to pathways based on their known functions from previous research, and genes can have different levels of experimental evidence from the literature to corroborate their annotation. Therefore, it may be beneficial in future studies to use multiple gene expression pathway analysis methods and multiple databases to determine what pathways overlap between analyses and are indeed likely associated with DEK in the brain.

This is the first study to characterize DEK in the human brain across the lifespan. We have established that *DEK* is highly expressed in the forebrain during fetal development and confirmed that neural DEK is associated with biological pathways that it is known to be related to in the periphery. In addition, we report for the first time that *DEK* may be important for neural development, synaptic transmission, and sensory perception. This is a necessary first step into understanding DEK's roles in the human brain. We know that DEK loss in the aged brain has implications for cognitive impairment and could result in phenotypes of Alzheimer's disease and other neurodegenerative disorders, such as Huntington's disease (Ghisays et al. 2018, O'Donovan et al. 2018, Greene et al. 2020, Miao et al. 2020, Xiang et al. 2020). Taken together, the data suggest an interesting association of DEK expression with neural development and neurodegeneration. Therefore, it could be the case that DEK is important for brain development, but that its precipitous decline during aging in certain individuals (e.g., genetically vulnerable) may uniquely predispose them to certain neurodegenerative diseases. Clearly, future studies should employ a more thorough analysis of brain developmental stages (e.g., before and after the onset of neurogenesis) or neuronal differentiation models to better inform us of the contribution of DEK in various processes at play in the embryonic brain. In addition, future studies will determine whether

there are differences in DEK-associated gene pathways in the aged brain of healthy patients and those with neuropathological conditions to further our understanding of its function in the central nervous system.

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Data Availability Statement

The data that support the findings of this study are openly available in the Human Brain Transcriptome database at https://hbatlas.org/, and by request from BrainCloud at https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000417.v2.p1.

List of Abbreviations

ANCOVA	Analysis of covariance
BIN1	Bridging Integrator 1
CA1	cornu ammonis
dbGaP	database of Genotypes and Phenotypes
DNA	Deoxyribonucleic acid
POLE2	DNA Polymerase Epsilon 2, Accessory Subunit
dlPFC	dorsolateral prefrontal
ERα	Estrogen Receptor alpha
GO	gene ontology
GSEA	gene set enrichment pathway analysis
GTex	Genotype-Tissue Expression Project
GRIN1	Glutamate Ionotropic Receptor NMDA Type Subunit 1
HBT	Human Brain Transcriptome
IGSF8	Immunoglobulin superfamily member 8
IQSEC2	IQ Motif And Sec7 Domain ArfGEF 2
MROH1	Maestro Heat Like Repeat Family Member 1
mPFC	medial prefrontal cortex
MSH6	mutS homolog 6

NMDA	N-methyl-D-aspartate
NDRG4	N-Myc Downstream Regulated Gene 4
NF-κB	Nuclear factor-κB
NUP107	nucleoporin 107
PMI	post-mortem interval
KCNMA1	Potassium Calcium-Activated Channel Subfamily M Alpha 1
PPP2R2C	Protein Phosphatase 2 Regulatory Subunit Bgamma
RBL1	RB Transcriptional Corepressor Like 1
RMI1	RecQ Mediated Genome Instability 1
RNA	Ribonucleic acid
RRM1	Ribonucleotide Reductase Catalytic Subunit M1
RMA	Robust Multichip Average
SRBD1	S1 RNA Binding Domain 1
SRSF10	Serine And Arginine Rich Splicing Factor 10
SMC4	Structural Maintenance Of Chromosomes 4
USP1	ubiquitin-specific protease 1
vlPFC	ventrolateral prefrontal cortex

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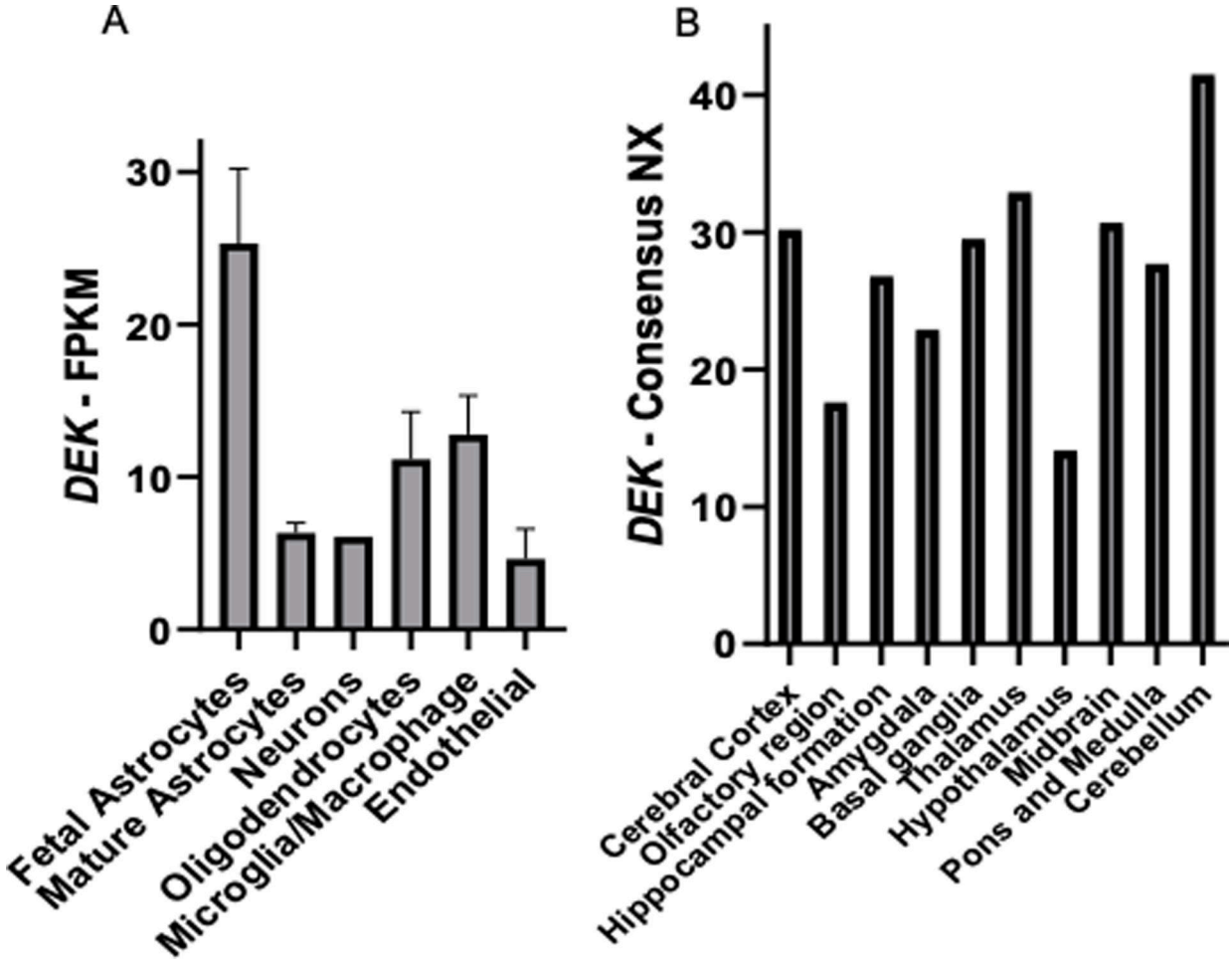


Figure 1.

DEK is expressed in multiple cell types and regions in the human brain. **A)** Data from the Brain RNA-Seq database demonstrates that *DEK* is expressed in multiple cell types in the human brain. FPKM = Fragments Per Kilobase of transcript per Million mapped reads. Original data and graph can be found at https://www.brainrnaseq.org/. **B)** Normalized expression (NX) of *DEK* RNA expression across human brain regions in adults. NX values were determined by combining data from the GTEx Human brain RNA-Seq dataset and FANTOM5 Human brain CAGE dataset. Original data and graphs can be found at https://www.proteinatlas.org/ENSG00000124795-DEK/brain.

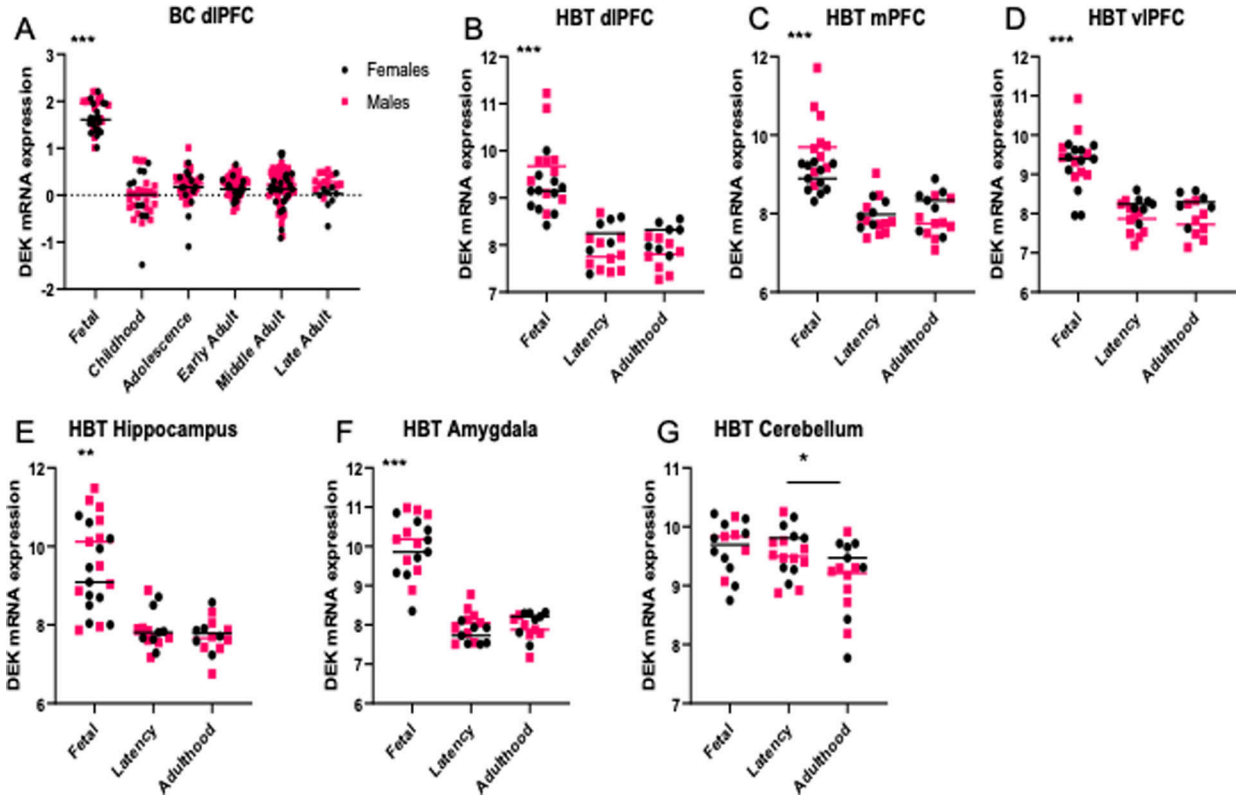
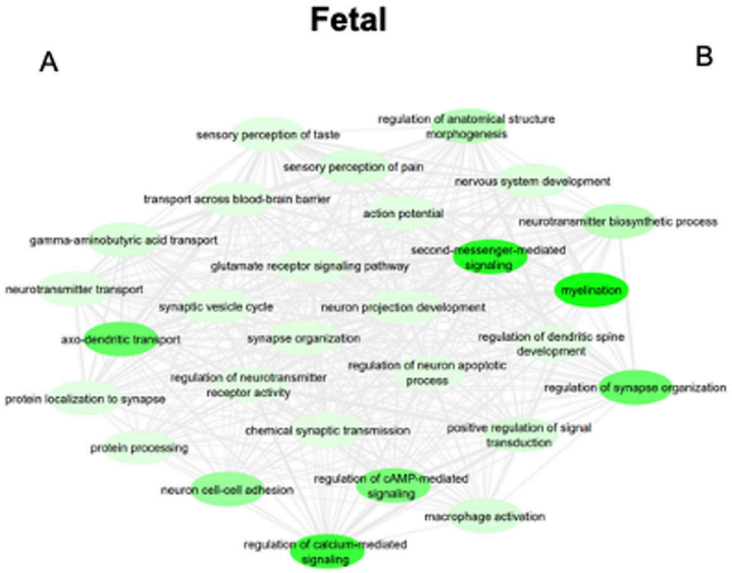


Figure 2.

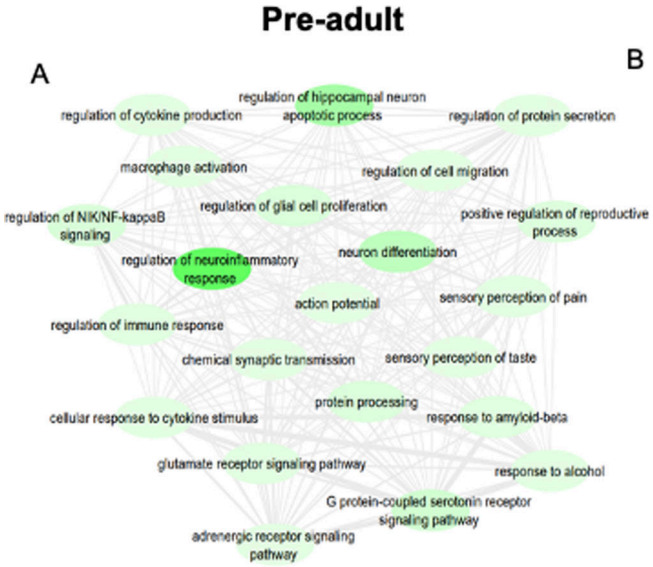
DEK expression across the lifespan. **A)** *DEK* expression is significantly greater during fetal development (6–38 weeks post-conception; n=38) than in childhood (0–10 years old; n=34), adolescence (10–18 years old; n=45), early adulthood (18–39 years old; n=57), middle adulthood (40–59 years old; n=73), and late adulthood (60+ years old; n=22); ***p<0.001. Data from BrainCloud in dorsolateral prefrontal cortex (dlPFC). **B-D)** *DEK* expression is significantly greater during fetal development (6–38 weeks post-conception; n=15–22) than in pre-adults (0–20 years old; n=14–16) or in adulthood (20+ years old; n=14–15) in the dlPFC, medial prefrontal cortex (mPFC), and ventrolateral prefrontal cortex (vlPFC); ***p<0.001. In addition, there is an interaction effect between age and sex on *DEK* expression in these regions (dlPFC, p=0.01; mPFC, p=0.002; vlPFC, p=0.027). Data from Human Brain Transcriptome (HBT). **E-F)** *DEK* expression is significantly greater during fetal development in the hippocampus (**p<0.01) and amygdala (***p<0.001). Data from HBT. **G)** A significant main effect of age on *DEK* expression was found in the cerebellum (p=0.041). Post-hoc analysis revealed that *DEK* expression in adulthood is significantly decreased compared to pre-adults (*p<0.05). Data from HBT.



GO term: Biological Process	Adj P-value	Odds Ratio
chemical synaptic transmission	2.8372E-83	14.525525
regulation of neurotransmitter receptor activity	2.8893E-38	47.500793
neurotransmitter transport	1.4819E-26	18.920932
action potential	1.5632E-17	20.383781
glutamate receptor signaling pathway	2.6558E-16	23.861246
synapse organization	6.3481E-14	6.5611208
synaptic vesicle cycle	1.497E-07	12.3436
transport across blood-brain barrier	5.2868E-07	5.2376438
sensory perception of taste	1.5061E-06	32.167521
regulation of dendritic spine development	2.3824E-06	14.641189
regulation of neuron apoptotic process	4.6575E-06	4.418559
protein processing	1.584E-05	4.0009863
protein localization to synapse	2.7756E-05	22.49684
neuron projection development	4.6527E-05	3.0426803
nervous system development	0.00105548	1.9138683
sensory perception of pain	0.00161537	8.030743
gamma-aminobutyric acid transport	0.00187533	32.061329
macrophage activation	0.00206977	5.3592814
positive regulation of signal transduction	0.00218179	2.1894717
regulation of anatomical structure morphogenesis	0.00828475	2.585377
neurotransmitter biosynthetic process	0.00837655	8.9102018
neuron cell-cell adhesion	0.01535314	7.2893901
regulation of cAMP-mediated signaling	0.02176368	5.0663733
regulation of synapse organization	0.02585612	4.8127986
axo-dendritic transport	0.02634543	16.01617
regulation of calcium-mediated signaling	0.03580437	4.3748061
second-messenger-mediated signaling	0.04062977	2.505402
myelination	0.04361604	3.2105983

Figure 3.

DEK-associated **gene ontologies** in all brain regions during fetal development (6–38 post-conceptional weeks). The most significantly enriched pathways from genes negatively correlated with *DEK* are represented in the map **(A)** and the corresponding table **(B)**. A lighter green bubble color is indicative of a lower p-value. REVIGO was used to consolidate ontology terms and bubble map was generated in Cytoscape.

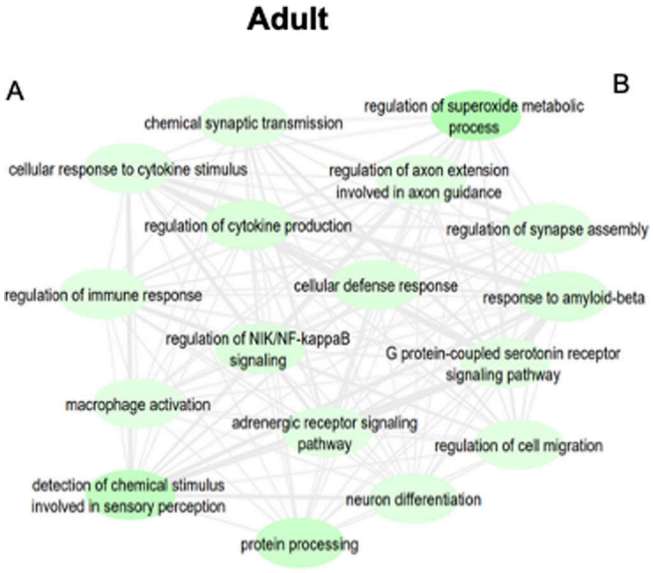


B

GO term: Biological process	Adj P-value	Odds Ratio
cellular response to cytokine stimulus	1.7056E-23	3.6232084
chemical synaptic transmission	6.0333E-22	4.4206932
regulation of cytokine production	4.2473E-10	4.1627674
action potential	4.2333E-08	8.0169812
regulation of cell migration	1.2743E-07	2.3451636
macrophage activation	3.1539E-07	8.7635928
regulation of protein secretion	1.039E-06	3.6306486
sensory perception of taste	1.1603E-06	32.756708
adrenergic receptor signaling pathway	3.5613E-06	13.356882
response to alcohol	5.3479E-05	7.2863401
sensory perception of pain	5.4477E-05	9.9278585
glutamate receptor signaling pathway	0.00010364	5.9217474
regulation of immune response	0.00010888	2.5788845
response to amyloid-beta	0.00016074	5.1031898
positive regulation of reproductive process	0.00027145	5.7007948
protein processing	0.00032748	3.0378616
regulation of glial cell proliferation	0.00036947	21.79881
regulation of NIK/NF-kappaB signaling	0.00041839	3.4105343
G protein-coupled serotonin receptor signaling pathway	0.00077293	16.348214
neuron differentiation	0.00154913	2.2858796
regulation of hippocampal neuron apoptotic process	0.0025589	43.550535
regulation of neuroinflammatory response	0.0055871	6.358646

Figure 4.

DEK-associated **gene ontologies** in all brain regions during pre-adulthood (0–20 years old). The most significantly enriched pathways from genes negatively correlated with *DEK* are represented in the map **(A)** and the corresponding table **(B)**. A lighter green bubble color is indicative of a lower p-value. REVIGO was used to consolidate ontology terms and bubble map was generated in Cytoscape.



B

GO term: Biological process	Adj P-value	Odds Ratio
cellular response to cytokine stimulus	2.5211E-26	4.1472969
regulation of cell migration	3.4416E-09	2.6719651
chemical synaptic transmission	3.9116E-08	2.8330069
regulation of immune response	8.0317E-06	3.0298513
macrophage activation	1.2392E-05	7.5178772
G protein-coupled serotonin receptor signaling pathway	2.0201E-05	30.935806
regulation of axon extension involved in axon guidance	4.8915E-05	9.4848361
regulation of synapse assembly	5.359E-05	5.6437928
adrenergic receptor signaling pathway	6.9247E-05	10.858442
neuron differentiation	0.00020812	2.6733333
cellular defense response	0.00037691	4.7997254
regulation of NIK/NF-kappaB signaling	0.00050723	3.5916804
response to amyloid-beta	0.00053579	4.9818361
regulation of cytokine production	0.00068338	2.6684971
protein processing	0.00170198	2.9072687
detection of chemical stimulus involved in sensory perception	0.00247794	6.238905
regulation of superoxide metabolic process	0.00343979	26.46121

Figure 5.

DEK-associated **gene ontologies** in all brain regions during adulthood (20+ years old). The most significantly enriched pathways from genes negatively correlated with *DEK* are represented in the map **(A)** and the corresponding table **(B)**. A lighter green bubble color is indicative of a lower p-value. REVIGO was used to consolidate ontology terms and bubble map was generated in Cytoscape.

Table 1.

DEK-associated **gene ontologies** in all brain regions and age groups. Enriched pathways from genes positively negatively associated with DEK expression are labeled in red; negatively enriched pathways are in blue. Pathways with the lowest p-value begin at the top of the list for each color/association. Normalized enrichment score (NES) reflects the degree to which a gene set is overrepresented at the top or bottom of a ranked list of genes, accounting for the difference in gene set sizes. Odds ratio represents the relative abundance of DEK-associated genes in each pathway vs. genes not in the pathway.

Association	GO term: Biological Process	Adj. P-value	NES	Odds Ratio
Positive	ribosome biogenesis	3.2218E-68	3.810316	18.009808
	DNA replication	1.6042E-64	3.818609	37.949158
	cellular response to DNA damage stimulus	2.627E-63	3.292782	9.4652765
	regulation of mRNA splicing, via spliceosome	1.1487E-32	2.928372	17.84078
	regulation of gene silencing by RNA	5.7028E-28	3.017145	28.026503
	DNA damage response, signal transduction by p53 class mediator	3.3227E-24	2.869532	15.624064
	chromosome organization	2.1607E-19	3.587029	8.9705635
	DNA synthesis involved in DNA repair	1E-17	2.820032	20.367827
	regulation of ATP metabolic process	1E-17	1.808558	14.691581
	protein localization to chromosome	7.7E-16	2.816571	23.621524
	nucleocytoplasmic transport	1.1227E-11	2.284504	13.203869
	regulation of chromosome segregation	2.7247E-11	3.590709	37.799067
	histone modification	1.3067E-09	2.556048	5.001056
	regulation of cell cycle	1.2124E-08	3.296365	2.928732
	regulation of ubiquitin protein ligase activity	1.3124E-07	2.169337	15.962811
Negative	chemical synaptic transmission	7.54E-72	−2.22474	14.455558
	neurotransmitter transport	4.85E-27	−2.85102	21.703556
	action potential	1.0314E-17	−2.40512	22.98916
	regulation of neuronal synaptic plasticity	1.9162E-14	−2.70312	30.954329
	glutamate receptor signaling pathway	5.3858E-10	−2.32602	14.856009
	response to amyloid-beta	7.9402E-10	−1.31689	12.461061
	long-term memory	1.9565E-09	−2.18819	29.845313
	synapse organization	1.6659E-07	−1.87446	4.889468

Association	GO term: Biological Process	Adj. P-value	NES	Odds Ratio
	macrophage activation	5.3244E-07	−2.18501	10.856086
	regulation of neuron death	3.3848E-06	−1.63992	5.3672935
	sensory perception of taste	7.664E-05	−2.34644	21.585311
	regulation of dendrite extension	0.00091983	−1.78438	10.789266
	receptor metabolic process	0.00200576	−1.89467	4.5074253
	retrograde axonal transport	0.00413632	−2.1452	9.7978658
	gamma-aminobutyric acid metabolic process	0.00622536	−2.03437	32.275338
	regulation of MAPK cascade	0.00872801	−1.42452	2.4750818
	regulation of calcium-mediated signaling	0.00885153	−1.93225	5.8819723

Table 2.

Top 100 genes correlated with *DEK* expression in all brain regions and age groups. Genes with the strongest correlation to *DEK* expression start at the top of each list (negatively or positively correlated with *DEK*).

Positive		Negative	
Gene	**Correlation Coefficient**	**Gene**	**Correlation Coefficient**
USP1	0.94	GRIN1	−0.85
SRBD1	0.92	KCNMA1	−0.84
RMI1	0.92	IGSF8	−0.83
LIN9	0.92	PTPRN	−0.82
RBL1	0.91	PDE4A	−0.82
NUP107	0.91	PPP2R2C	−0.82
TMPO	0.91	RAPGEFL1	−0.82
KlF20B	0.91	IQSEC2	−0.82
NUP54	0.91	ARHGEF4	−0.82
NUP205	0.91	BIN1	−0.82
RRM1	0.91	MROH1	−0.82
PRPF40A	0.91	CEP170B	−0.81
MCM6	0.9	NDRG4	−0.81
POLE2	0.9	MINK1	−0.81
ERI1	0.9	ABLIM2	−0.81
SMC4	0.9	CASKIN1	−0.81
MSH6	0.9	TUBG2	−0.8
HAUS6	0.9	PITPNM2	−0.8
SRSF10	0.9	TMEM59L	−0.8
WDR76	0.89	DNM1	−0.8
POLA1	0.89	CAMTA2	−0.8
UGDH	0.89	UNC13A	−0.8
STAG1	0.89	FBXO44	−0.8
CPSF3	0.89	ARF3	−0.8
NUP43	0.89	LYPD5	−0.8
STIL	0.89	TPPP	−0.8
ACTL6A	0.89	CPNE6	−0.79
DNA2	0.89	SYP	−0.79
C18orf54	0.89	RAB11FlP4	−0.79
SUZ12	0.89	KIAA0513	−0.79
KNTC1	0.89	MAPK8IP3	−0.79
STAG2	0.88	THY1	−0.79
GABPB1	0.88	JPH3	−0.79
DHX15	0.88	FBXL18	−0.79
CASP8AP2	0.88	DMTN	−0.79

Positive		Negative	
Gene	**Correlation Coefficient**	**Gene**	**Correlation Coefficient**
PCNA	0.88	HSPA12A	−0.79
CNOT9	0.88	TOM1L2	−0.79
SPATA5	0.88	CYP46A1	−0.78
CDKAL1	0.88	CAMK2B	−0.78
CHD1	0.88	TNK2	−0.78
PHF6	0.88	ABHD12	−0.78
SASS6	0.88	SNPH	−0.78
RFC4	0.88	ZBTB47	−0.78
ATAD5	0.88	AGAP2	−0.78
ZMYM1	0.88	RAB40B	−0.78
WDHD1	0.88	AZIN2	−0.78
SPDL1	0.87	PLEKHA6	−0.78
CNTLN	0.87	TTBK1	−0.78
NDC1	0.87	GPR61	−0.78
SLC25A24	0.87	CACNA1D	−0.78

Table 3.

Top 20 positively enriched *DEK*-associated **gene ontologies** within each age groups in all brain regions. Pathways are listed in order by lowest adjusted p-value, then by highest odds ratio to display the most highly enriched pathways at the top of each list. Enrichr was used to retrieve the **gene ontologies** (GO terms) enriched from positively correlated genes and the associated statistics.

Fetal Development			Pre-adulthood			Adulthood		
GO term: Biological Process	**Adj P-value**	**Odds Ratio**	**GO term: Biological Process**	**Adj P-value**	**Odds Ratio**	**GO term: Biological Process**	**Adj P-value**	**Odds Ratio**
mRNA processing	2.567E-146	28.258433	mRNA processing	3.281E-106	18.589207	mRNA processing	1.723E-131	23.384215
mRNA splicing, via spliceosome	1.318E-141	30.671828	mRNA splicing, via spliceosome	2.0004E-95	18.05645	mRNA splicing, via spliceosome	1.783E-128	25.610369
RNA splicing	3.664E-131	30.97823	RNA splicing	5.7125E-90	18.547828	RNA splicing	3.193E-117	25.269163
DNA repair	8.2448E-90	15.078876	gene expression	1.7439E-87	12.601939	gene expression	1.0481E-94	12.852176
DNA metabolic process	2.2702E-87	15.765298	RNA processing	3.2451E-68	19.624996	ribosome biogenesis	7.8587E-72	18.327958
double-strand break repair	2.4508E-70	22.47355	nuclear-transcribed mRNA catabolic process	6.697E-62	18.379107	ncRNA processing	9.8052E-68	16.146435
mitotic spindle organization	1.3356E-63	20.769036	DNA metabolic process	1.6458E-53	9.9259487	rRNA processing	7.2205E-66	18.635317
RNA processing	8.6536E-63	17.410882	ncRNA processing	1.1198E-52	12.978199	RNA processing	3.8599E-65	17.60567
cellular response to DNA damage stimulus	1.1702E-62	9.2185648	DNA repair	7.6505E-51	8.9556035	cellular macromolecule biosynthesis	1.1534E-62	9.6612988
DNA replication	2.3212E-61	34.476404	ribosome biogenesis	2.3765E-49	12.690657	translation	1.8412E-58	12.808307
gene expression	3.1434E-54	8.0800181	cellular macromolecule biosynthesis	3.1708E-49	8.3734627	translational elongation	2.1686E-57	32.216584
ncRNA processing	2.1412E-53	12.882187	regulation of translation	3.3982E-47	13.035326	mitochondrial translation	1.8936E-55	29.770635
microtubule cytoskeleton organization	4.5666E-53	21.193448	mRNA export from nucleus	6.1379E-46	22.447106	rRNA metabolic process	6.5297E-55	16.056575
mRNA export from nucleus	4.59E-53	27.328143	rRNA processing	4.0714E-45	12.795513	translational termination	1.6863E-52	31.588519
ribosome biogenesis	2.9806E-51	12.90128	RNA metabolic process	5.5813E-44	16.307516	mitochondrial translational elongation	1.4217E-51	35.160785
RNA export from nucleus	1.065E-50	25.807262	mRNA-containing ribonucleoprotein complex export from nucleus	3.3218E-42	22.15944	DNA metabolic process	7.9233E-51	8.9019139
mRNA transport	3.8339E-50	26.096154	mRNA transport	8.5464E-42	20.498498	mitochondrial translational termination	4.24E-50	33.209937

Fetal Development			Pre-adulthood			Adulthood		
GO term: Biological Process	**Adj P-value**	**Odds Ratio**	**GO term: Biological Process**	**Adj P-value**	**Odds Ratio**	**GO term: Biological Process**	**Adj P-value**	**Odds Ratio**
mRNA-containing ribonucleoprotein complex export from nucleus	1.9574E-49	27.512852	cellular response to DNA damage stimulus	1.3949E-41	6.8062948	transcription by RNA polymerase II	9.6503E-49	7.6908903
rRNA processing	2.1589E-49	13.74466	RNA splicing	2.6719E-41	21.766331	transcription, DNA-templated	1.4255E-48	10.332887

Table 4.

A comparison of *DEK*-associated **gene ontologies** uniquely enriched in adulthood in the forebrain (hippocampus) vs. hindbrain (cerebellum). Enriched pathways from genes positively associated with DEK expression are labeled in red; negatively enriched pathways are in blue. These results were rendered with ToppGene to determine genes correlated to *DEK* only in distinct age groups and Enrichr to retrieve the biological processes associated with these genes. REVIGO was used to consolidate ontology terms.

Association	Hippocampus			Cerebellum		
	GO term: Biological Process	P-value	Odds Ratio	GO term: Biological Process	P-value	Odds Ratio
Positive	mitochondrion organization	0.0000138	4.073500561	neuron projection maintenance	0.00026539	18.45337159
	mitochondrial transport	4.03639E-05	7.171081678	dephosphorylation	0.000321767	3.036031183
	nucleotide metabolic process	0.001742217	8.992283773	protein ubiquitination	0.000467178	1.952604146
	ribosomal large subunit biogenesis	0.001949861	5.038914027	post-translational protein modification	0.000525664	2.193394046
	regulation of mitochondrial membrane potential	0.002408125	5.940241228	neuron cell-cell adhesion	0.001944462	9.223816356
	negative regulation of lipid transport	0.005062474	28.37472767	neuron projection organization	0.001944462	9.223816356
	cellular response to oxidative stress	0.008456816	2.931567329	cellular response to reactive oxygen species	0.006362487	3.465958213
	regulation of organelle assembly	0.008679242	3.61578703	neuron migration	0.007771372	3.777959451
	protein stabilization	0.008765581	2.541361078	transport across blood-brain barrier	0.01052808	2.844710845
	intermembrane lipid transfer	0.010310514	17.02309368	gamma-aminobutyric acid metabolic process	0.011432611	18.40343348
	membrane lipid biosynthetic process	0.010712995	4.031363787	sensory perception of sour taste	0.011432611	18.40343348
	ribosome disassembly	0.013539029	14.18518519	chemical synaptic transmission	0.022996688	1.73965959
	energy derivation by oxidation of organic compounds	0.019206567	5.81093688	positive regulation of neuron death	0.023779258	3.293821839
	myelin maintenance	0.021106579	10.63779956	synapse organization	0.033296143	2.132281014
	regulation of integrin-mediated signaling pathway	0.04017501	7.090413943	calcium-mediated signaling using intracellular calcium source	0.035643088	4.603868195
	steroid hormone mediated signaling pathway	0.045662986	6.544662309	associative learning	0.037501141	7.885550787
				modulation of chemical synaptic transmission	0.037818355	2.194276571
				MAPK cascade	0.039065538	1.652256369
				glutamate receptor signaling pathway	0.039607783	3.350463023
Negative	antibacterial humoral response	0.007909055	4.41613153	neurotransmitter biosynthetic process	0.007094776	6.503626943
	positive regulation of synaptic transmission, GABAergic	0.01537952	13.72932862	DNA replication checkpoint signaling	0.014680245	5.001992826
	negative regulation of cell projection organization	0.049969331	3.055476753	regulation of chromosome segregation	0.01802418	4.644460893

Association	Hippocampus			Cerebellum		
	GO term: Biological Process	P-value	Odds Ratio	GO term: Biological Process	P-value	Odds Ratio
				cell cycle checkpoint signaling	0.028997209	5.415300546
				positive regulation of low-density lipoprotein receptor activity	0.029983069	10.82471264
				mitotic DNA integrity checkpoint signaling	0.03610366	4.873511648
				extracellular structure organization	0.047244335	1.572933459

Table 5.

Top 10 positively enriched age-associated gene ontologies in the hippocampus and five select gene ontologies of interest. Gene ontologies are listed in order by lowest p-value, to display the most highly enriched ones at the top of each list. These results were rendered with the Limma package in R to determine genes that are differentially expressed in distinct age groups. The ToppFun function in the ToppGene suite and Enrichr were used to identify the 100 top gene ontologies of biological processes of the differentially expressed genes. REVIGO was used to consolidate ontology terms.

Hippocampus Upregulated Gene Ontologies								
Fetal			Pre-adult			Adult		
GO term	p value	Odds Ratio	GO term	p value	Odds Ratio	GO term	p value	Odds Ratio
chromosome organization	2.01E-58	0.41542118	chemical synaptic transmission	1.16E-43	0.674345326	chemical synaptic transmission	3.00E-32	0.592972918
cell cycle process	2.76E-42	0.203456613	modulation of chemical synaptic transmission	4.46E-29	0.470511559	ion transmembrane transport	2.74E-26	0.395176726
cell division	8.09E-37	0.747710754	regulation of membrane potential	2.28E-21	3.158672799	modulation of chemical synaptic transmission	1.41E-21	0.92270779
negative regulation of cellular macromolecule biosynthetic process	4.31E-29	0.588053036	neuron projection development	3.08E-21	0.602625883	regulation of ion transport	2.65E-20	0.348431597
DNA repair	1.88E-28	0.106522018	ion transmembrane transport	9.31E-20	0.742212259	regulation of membrane potential	3.82E-20	1.681851048
chromosome segregation	1.89E-24	1.518573922	regulation of ion transport	6.65E-19	0.654853042	gliogenesis	4.34E-17	4.147826087
head development	3.40E-19	0.885592399	neurotransmitter transport	3.08E-16	1.684012265	locomotory behavior	1.54E-16	0.447963365
positive regulation of transcription, DNA-templated	6.20E-18	0.633676804	import into cell	6.90E-16	2.165918268	response to metal ion	9.46E-14	0.191571119
central nervous system development	9.09E-18	1.772992814	regulation of neuron projection development	2.33E-14	1.815103671	regulation of transporter activity	2.55E-12	1.12099301
protein localization to chromosome	1.40E-15	0.321663405	learning or memory	6.46E-14	1.805451128	cell junction organization	5.24E-12	0.583918396
signal transduction by p53 class mediator	2.36E-13	1.900458142	regulation of nervous system process	2.26E-13	1.837548387	learning or memory	5.70E-11	2.696301668
regulation of chromosome segregation	7.01E-12	1.518573922	regulation of neurogenesis	1.72E-11	1.284852951	blood circulation	2.15E-10	0.395176726
response to ionizing radiation	1.35E-12	0.225675492	central nervous system myelination	4.96E-11	0.536556043	regulation of neurotransmitter transport	2.72E-10	0.896649795
peptidyl-lysine modification	8.68E-12	0.308344312	brain development	4.66E-10	2.419898064	regulation of neuron projection development	3.89E-10	2.198580166
histone modification	2.01E-11	0.589005575	action potential	6.31E-10	2.198512586	oligodendrocyte differentiation	8.01E-10	0.707729193

Table 6.

Top 10 negatively enriched age-associated gene ontologies in the hippocampus and five select gene ontologies of interest. Gene ontologies are listed in order by lowest p-value, to display the most highly enriched ones at the top of each list. These results were rendered with the Limma package in R to determine genes that are differentially expressed in distinct age groups. The ToppFun function in the ToppGene suite and Enrichr were used to identify the 100 top gene ontologies of biological processes of the differentially expressed genes. REVIGO was used to consolidate ontology terms.

Hippocampus Downregulated Gene Ontologies								
Fetal			**Pre-adult**			**Adult**		
GO term	**p value**	**Odds Ratio**	**GO term**	**p value**	**Odds Ratio**	**GO term**	**p value**	**Odds Ratio**
cation transport	4.12E-38	2.612946958	mitotic cell cycle	4.43E-46	2.349890359	chromosome organization	2.65E-44	2.230512366
modulation of chemical synaptic transmission	5.16E-37	3.901537552	chromosome organization	7.46E-46	4.225031996	mitotic cell cycle process	9.41E-38	1.519985359
chemical synaptic transmission	4.06E-33	3.243656908	cell division	2.68E-27	2.17324263	organelle fission	2.39E-35	0.840448625
regulation of membrane potential	5.87E-33	3.692982062	chromosome segregation	2.93E-23	5.065349144	cell division	1.53E-27	2.792411981
regulation of ion transport	3.10E-32	3.883237604	regulation of cell cycle	1.19E-19	2.700191142	chromatin assembly	3.27E-23	3.808146096
behavior	2.15E-30	1.180983776	negative regulation of transcription, DNA-templated	6.36E-15	1.766189266	chromosome segregation	8.64E-20	5.188542422
neurotransmitter transport	8.91E-28	3.77004812	cell cycle checkpoint signaling	1.49E-14	12.68469657	Head (and brain) development	3.78E-19	2.333058003
vesicle-mediated transport in synapse	2.18E-23	2.467956251	chromatin remodeling	2.72E-14	2.137986877	negative regulation of transcription, DNA-templated	3.47E-18	1.612272621
regulation of system process	1.01E-21	2.657940663	protein localization to chromosome, centromeric region	3.78E-14	13.87229437	DNA replication	6.44E-17	5.0219347
cell junction organization	2.59E-21	1.145134492	cellular response to DNA damage stimulus (DNA repair)	4.01E-13	1.974011064	regulation of cell cycle	1.05E-16	1.528470364
import into cell	8.03E-19	1.477486233	microtubule-based process	1.60E-10	1.486377709	neuron projection development	3.97E-15	1.201478451
regulation of transporter activity	1.75E-18	1.933432109	signal transduction by p53 class mediator	1.38E-09	1.630475382	DNA repair	1.23E-14	2.117709487
organelle fusion	7.22E-18	0.505803485	megakaryocyte differentiation	4.17E-09	2.296858201	axon guidance	6.66E-13	2.456917444
response to metal ion	5.44E-13	1.74662065	brain development	4.24E-09	2.419898064	neural precursor cell proliferation (regulation of neurogenesis)	4.70E-11	2.837070938
regulation of neuron projection development	3.77E-10	2.586608702	growth	7.05E-09	1.404720023	microtubule-based process	7.36E-10	1.223290106

Table 7.

Top 10 positively enriched age-associated gene ontologies in the cerebellum and five select gene ontologies of interest. Gene ontologies are listed in order by lowest p-value, to display the most highly enriched ones at the top of each list. These results were rendered with the Limma package in R to determine genes that are differentially expressed in distinct age groups. The ToppFun function in the ToppGene suite and Enrichr were used to identify the 100 top gene ontologies of biological processes of the differentially expressed genes. REVIGO was used to consolidate ontology terms.

Cerebellum Upregulated Gene Ontologies								
Fetal			**Pre-adult**			**Adult**		
GO term	**p value**	**Odds Ratio**	**GO term**	**p value**	**Odds Ratio**	**GO term**	**p value**	**Odds Ratio**
cell morphogenesis	3.21E-39	0.357650551	chemical synaptic transmission	3.22E-21	2.296701	chemical synaptic transmission	1.02E-22	1.325412176
neuron projection development	3.25E-41	1.647426744	modulation of chemical synaptic transmission	3.10E-14	1.156861966	inorganic ion transmembrane transport	2.76E-18	1.093730539
synapse organization	3.49E-23	1.169569261	behavior	4.66E-14	4.545306313	regulation of membrane potential	1.09E-14	2.439391503
developmental growth	1.27E-21	1.189545455	ion homeostasis	1.13E-13	1.409166486	modulation of chemical synaptic transmission	4.93E-14	1.914227531
cell division	1.29E-21	0.615693013	cation transport	7.89E-13	1.464437046	neurotransmitter transport	1.27E-13	1.388766584
mitotic cell cycle process	2.17E-21	0.452033283	regulation of system process	9.98E-11	2.405352078	regulation of ion transport	3.12E-12	1.005942173
homophilic cell adhesion via plasma membrane adhesion molecules	1.24E-19	1.467915358	regulation of synaptic plasticity	1.87E-10	30.81789474	central nervous system development	3.59E-12	2.419729407
regulation of synapse organization	3.96E-17	0.443631961	myelination	7.48E-09	1.77895506	synaptic vesicle cycle	5.13E-11	0.650030849
cell adhesion	5.22E-17	1.859751122	neuron death	3.76E-08	1.479188332	behavior	9.11E-11	2.096752816
chemotaxis	9.53E-16	1.14846395	oligodendrocyte development	9.72E-08	2.271604938	neuron development	1.31E-09	1.422312049
hindbrain development	1.70E-15	5.560642814	cell junction organization	1.89E-07	3.747303544	myelination	2.22E-09	2.199090192
central nervous system neuron differentiation	2.14E-15	2.383424408	actin filament-based process	4.12E-07	5.09512275	regulation of transporter activity	1.62E-08	1.572316103
microtubule-based process	1.22E-13	0.652474436	regulation of Ras protein signal transduction	1.54E-06	2.53712839	gliogenesis	5.71E-08	5.822532403
chromosome organization	2.16E-13	0.433893388	gliogenesis	1.63E-06	2.55581761	cell junction organization	8.25E-08	3.427677925
neural precursor cell proliferation	8.68E-13	0.693515436	response to metal ion	3.31E-06	0.582569632	regulation of cell projection organization	6.86E-07	4.443024312

Table 8.

Top 10 negatively enriched age-associated gene ontologies in the cerebellum and five select gene ontologies of interest. Gene ontologies are listed in order by lowest p-value, to display the most highly enriched ones at the top of each list. These results were rendered with the Limma package in R to determine genes that are differentially expressed in distinct age groups. The ToppFun function in the ToppGene suite and Enrichr were used to identify the 100 top gene ontologies of biological processes of the differentially expressed genes. REVIGO was used to consolidate ontology terms.

Cerebellum Downregulated Gene Ontologies								
Fetal			**Pre-adult**			**Adult**		
GO term	**p value**	**Odds Ratio**	**GO term**	**p value**	**Odds Ratio**	**GO term**	**p value**	**Odds Ratio**
chemical synaptic transmission	4.06E-33	2.653812317	neuron projection development	1.76E-21	1.75425923	cell morphogenesis	6.28E-37	1.953977195
modulation of chemical synaptic transmission	2.15E-21	3.516443154	central nervous system development	7.14E-20	2.288827043	regulation of nervous system development	2.47E-26	2.556508604
neurotransmitter transport	1.03E-20	3.136246236	cell division	1.97E-13	2.895959265	cell adhesion	2.32E-24	2.236123452
regulation of neurotransmitter levels	4.35E-19	3.564158055	chemotaxis	8.62E-11	2.729971989	regulation of plasma membrane bounded cell projection organization	6.17E-24	0.698254915
neuron projection development	3.92E-17	1.647426744	mitotic cell cycle process	4.56E-11	1.182282565	cell-cell adhesion via plasma-membrane adhesion molecules	1.49E-21	4.663199388
regulation of ion transport	1.98E-16	4.057524087	cell-cell adhesion via plasma-membrane adhesion molecules	2.64E-10	4.355540563	synapse organization	3.86E-20	4.056598985
secretion	3.06E-13	0.85369715	organelle fission	1.37E-09	2.559086134	regulation of synapse structure or activity	8.52E-20	4.056598985
response to metal ion	5.44E-13	1.824531295	synaptic signaling	1.06E-08	1.201627821	cell junction organization	1.90E-19	3.427677925
regulation of system process	5.64E-13	2.775877724	animal organ morphogenesis	1.76E-08	0.949685535	chemotaxis	1.94E-19	2.701182168
regulation of transporter activity	5.98E-13	2.220581114	growth	7.39E-08	1.682682048	growth	9.58E-18	1.620351077
cellular homeostasis	4.63E-12	1.811767044	protein localization to chromosome	9.36E-08	7.4526356	cell division	9.59E-17	2.228699288
protein localization to synapse	1.58E-11	5.560642814	microtubule-based process	9.49E-08	2.408304498	mitotic cell cycle process	3.36E-12	1.367827087
amino acid transport	8.07E-11	2.119554205	chromosome segregation	1.00E-07	5.12237395	stem cell development	3.37E-12	4.050725669
myelination	1.47E-10	6.721834862	small GTPase mediated signal transduction	1.05E-07	3.373686496	Ras protein signal transduction	1.37E-11	2.417567633
gliogenesis	6.15E-10	1.448537549	hindbrain development	2.97E-07	7.4526356	regulation of locomotion	1.39E-11	0.62833002