Early brain response to low-dose radiation exposure involves molecular networks and pathways associated with cognitive functions, advanced aging, and Alzheimer's disease

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Understanding the cognitive and behavioral consequences of brain exposures to low-dose ionizing radiation has broad relevance for health risks from medical radiation diagnostic procedures, radiotherapy, environmental nuclear contamination, as well as earth orbit and space missions. Analyses of transcriptome profiles of murine brain tissue after whole-body radiation showed that low-dose exposures (10 cGy) induced genes not affected by high dose (2 Gy), and low-dose genes were associated with unique pathways and functions. The low-dose response had two major components: pathways that are consistently seen across tissues, and pathways that were brain tissue specific. Low-dose genes clustered into a saturated network ($p < 10^{-53}$) containing mostly down-regulated genes involving ion channels, long-term potentiation and depression, vascular damage, etc. We identified 9 neural signaling pathways that showed a high degree of concordance in their transcriptional response in mouse brain tissue after low-dose radiation, in the aging human brain (unirradiated), and in brain tissue from patients with Alzheimer's disease. Mice exposed to high-dose radiation did not show these effects and associations. Our findings indicate that the molecular response of the mouse brain within a few hours after low-dose irradiation involves the down-regulation of neural pathways associated with cognitive dysfunctions that are also down regulated in normal human aging and Alzheimer's disease.

Keywords: low-dose radiation, brain, transcriptome, gene expression, bioinformatics, cognitive function, aging, network and pathway bioinformatics; Alzheimer's disease, human, mouse

Introduction

Ionizing radiation sources are ubiquitous and virtually every one is exposed to low doses from cosmic rays, soil radioactivity, and diverse man-made contaminations. The Department of Energy defines the upper range of low dose to be 10 cGy. The usage of low-dose radiation is also rapidly gaining acceptance in a broad variety of medical diagnostics such at CT scanning (1) with doses of 30-70 mGy per head scan series and 20-50 mGy for each abdominal series (2). Also, during conventional radiotherapy regimens (e.g., cancer), large tissue volumes receive low-dose exposures. In 2007, it was estimated that the annual integrated US population dose from all medical procedures for the first time exceeded the dose from background sources. Thus, understanding the molecular neurological effects of low-dose ionizing radiation has relevance for assessing health risks: (a) from medical radiation diagnostics, (b) from side effects of radiotherapy, (c) for nuclear energy workers, and (d) to astronauts during space travel.

Epidemiological approaches have been disappointingly ineffective for assessing the health risks after low-dose exposures, and linear-no-threshold (LNT) models are applied to estimate the health risks from low-dose exposures by extrapolation from high-dose epidemiological data (3). Several of the major assumptions underlying the relevance of LNT models to low-dose exposures are becoming increasingly controversial: (i) that low-dose radiation elicits the same molecular response mechanisms as high-dose but at lower frequencies, and (ii) that, in aggregate, the cellular responses to radiation damage are linear in the shape in the low-dose range. Recent observations of cellular responses in the low-dose range, such as genomic instability, adaptive response and bystander effects, further put into question the validity of the LNT model (4, 5).

Tissues differ significantly in their sensitivity to ionizing radiation (6) and significant variation in radiation-induced gene expression has been observed among tissues and cell lines, but the significance of low-dose exposures for cell survival, tissue damage, and individual health are poorly understood (7, 8). The adult brain is generally considered insensitive to ionizing radiation due to the relatively radioresistant nature of neurons to cell killing (9), but a small population of neuronal precursor cells are relatively sensitive (10-13). Radiation exposures to human brain, such as external beam radiotherapy, have been associated with neurological damage and cognitive impairment especially in children (14, 15). The neurological deficits of high-dose radiation are progressively detrimental over time and are thought to be due to demyelination and neural loss (16) with associated neural and cognitive deficiencies (17). Some of these cognitive defects have been observed as consequence of impaired neurogenesis following exposure to ionizing radiation (11, 18-20). Radiotherapy contains both low-dose and high-dose components. As the population of long-term survivors of radiotherapy continues to grow, understanding the differential irradiation effects low- and high-dose radiation on cognitive function is emerging as a major neurological health concern (21).

Gene expression is a very sensitive indicator of radiation exposure, and low doses are known to modulate the transcript profiles in irradiated cells and tissues (22, 23). Previous transcription profiling studies of brain tissue have reported significant expression changes following a variety of insults (23-27). We reported that doses of 10 cGy induced changes in gene expression in the mouse brain that were qualitatively different from those at high dose (2 Gy) and involved neural signaling activity (23). A subsequent expression study of rat hippocampal tissue after cranial radiation reported that the major responses were centered around Myc and the tumor suppressor

gene Tp53 (28). However, neither of these studies employed modern bioinformatics tools for pathway and functional analyses. Also, there are rapidly growing numbers of transcriptomic databases of physiological and pathological brain conditions (29-33) that can be queried for discovering relationships between gene expression alterations and phenotypical changes and whether there are common brain responses that are affected by different external insults.

The objectives of our study were to apply advanced bioinformatics analyses of transcript profiles obtained from mouse brain tissue after whole body radiation to: (a) identify and characterize the molecular interaction networks and pathways induced in brain tissue after 10 cGy low-dose exposures (using 2 Gy as a reference dose), and (b) investigate the biological implications of the low-dose brain responses by conducting an unbiased search for associations with genetic, anatomical, and disease knowledge databases, including transcriptional profiles from human brain tissue of aging individuals and Alzheimer's disease (AD) patients.

Materials and Methods

Transcriptome dataset from irradiated mouse brain tissues

The gene expression data of irradiated mouse brain tissue utilized in the current analyses were generated in a previous microarray experiment conducted in our laboratory (23), which was reviewed and approved by the IACUC committee of the Lawrence Livermore National Laboratory. Briefly, eight to ten week-old B6C3F1/HSD male mice were exposed to whole-body gamma-radiation at 0, 10 cGy or 2 Gy using a ^{137}Cs source with a dose-rate of 0.64 Gy min^{-1}. Four hours after treatment, mice were euthanized by CO_2 inhalation and RNA isolated from brain coronal sections (a single section per mouse) and hybridized onto Affymetrix Mgu-74Av2 gene chips as described (23). The coronal sections included the motor cortex, portions of the frontal and parietal lobes, hippocampus, and diencephalons (coordinates 205-305 from the High Resolution Mouse Brain Atlas; http://www.hms.harvard.edu./research/brain/atlas.html). Statistical analyses of the Affymetrix oligonucleotide array output, conducted as described in (23), identified 855 probe sets that were modulated at $\geq|1.5|$ at either 10 cGy or 2 Gy; of these we identified for the current study 529 annotated genes based on Unigene ID from NCBI.

The microarray findings of radiation-induced transcriptional changes were confirmed by RT-PCR for Pcna, Psma4, Csnk2a1 and Sod1 (23). The radiation-induced down-regulation of Grin-1 was confirmed in the present study by qPCR (Applied Biosystems, Foster City, CA) using the Taqman Gene Expression assay #Mm00433785_m1 (Applied Biosystems).

Gene Interaction Networks and Pathway analysis

Several bioinformatics tools were used to characterize the radiation-modulated genes for cellular processes and biomolecular pathways that were differentially affected by low-dose versus high-dose exposures: Gene Ontology (GO) enrichment analysis and Ingenuity Pathway Analysis (IPA 5.0). GO analyses was performed using GO tree machine (34) to generate biological processes, molecular function and cellular component categories that were differentially associated with the low-dose unique, high-dose unique, and the genes that were in common to both doses. GO categories were filtered based upon significance of over-representation of "hits" by using a selected threshold for p-values of hypergeometric distribution ($p \leq 0.001$). The IPA knowledge base includes updated literature information on molecular networks, biological process and an extensive library of well characterized signaling and metabolic pathways to understand the transcriptional networks, phosphorylation cascades and protein-protein interactions. Radiation modulated genes were also analyzed in IPA 5.0 for pathway enrichments with respect to reference chip MGu74Av2, to rank the top statistically significant overrepresented canonical signaling and metabolic pathways and to determine whether there was significant up- or down-regulation. The Fisher's exact t-test was applied to examine the statistical over representation of the pathways, using a threshold p-value of ≤ 0.05.

Differentially modulated genes were also overlaid onto the IPA knowledgebase interactome to identify networks that were significantly enriched. Generated networks were arranged according to IPA score (higher the score, the lower is the probability of finding focus genes in a given network due to chance). These networks were further analyzed to identity the major nodes (genes in each network with the highest number of interactions with other genes) and the functions associated with the genes in the network.

Comparisons with other brain gene expression profile datasets related to cognitive dysfunction.

We used L2L microarray tool (ver 2007.1; (35)) to identify other datasets that shared significant pathway enrichment with our low-dose and high-dose genes. We divided these analyses into: (a) down-regulated low-dose unique genes, (b) up-regulated low-dose unique genes, (c) down-regulated high-dose unique genes, and (d) up-regulated high-dose unique genes. The search using upregulated low-dose genes found no significant hits. The search with the down-regulated low-dose genes identified two datasets: (i) a human aging brain dataset generated from human postmortem tissue samples of the frontal cortex of 30 individuals, whose age ranged from 26 to 106 years (36); and, (ii) a human Alzeheimer's Disease (AD) dataset generated from hippocampal tissues from 22 AD subjects of varying severity (Incipient, n = 7; Moderate, n = 8; Severe, n = 7) and 9 controls subjects (31). HG-U95Av2 and HG-U133A gene chips (Affymetrix) were used for the human aging (36) and AD (31) datasets, respectively. The search using down-regulated high-dose genes identified no hits, while the search with up-regulated high-dose genes identified the AD but not the aging dataset; however, it identified different functions of overlap between the radiation and AD datasets compared to the down-regulated low-dose genes (see results). Gene lists from datasets with significant hits were imported into IPA and compared with our radiation modulated gene set to identify pathways that were in common among the data sets using IPA enrichment analyses, which were conducted as mentioned above.

When presenting the results of the analyses of the mouse low-dose radiation response, we use the mouse gene nomenclature, e.g., Capn3. When we refer to the results of the comparison of the three databases, we use the human nomenclature, e.g., CAPN3.

Results

Comparative gene expression profiling after low-dose versus high-dose exposures

We used transcriptional profiling to contrast the gene functions, gene interaction networks and associated biochemical pathways that were modulated in mouse brain tissue after whole body low-dose exposure (10 cGy) versus high-dose exposure (2 Gy). Oligonucleotide microarray analyses of mRNA collected 4 hours after irradiation identified 396 genes that were modulated after low-dose and 406 genes after high-dose exposures (≥|1.5| fold). Of these, 123, 134, and 272 modulated genes (total 529) were unique to low dose, unique to high dose, and in common between both doses, respectively. These findings confirm that low-dose exposures modulates the expression of a substantial number of genes that were not affected after high doses and suggest that the cellular damage response mechanisms after low-dose radiation are qualitatively different from high-dose mechanisms.

Gene Ontology (GO) analyses (Table 1) showed that low-dose exposure of brain tissue induced significant biological processes, molecular functions and cellular components that were distinctly different from those induced after high doses, and that both doses shared some common functions. High-dose unique genes were preferentially associated with protein biosynthesis and metabolism, negative regulation of neurogenesis, RNA binding and transferase activity, and cellular location in mitochondria and ribosomes. The low-dose unique genes were associated with GTP binding, G-protein mediated signal transduction, Rho protein signal transduction, fatty acid binding, chromatin remodeling and various other functions associated with the activities of neurotransmitters and their receptors. These GO findings indicate that low-dose exposures

induced damage responses in the brain are complex and functionally diverse and significantly different from functions associated with high dose exposures.

To understand better the differences in functions between the low- and high-dose unique genes (i.e., this comparison excluded the genes that were expressed in both dose groups), we performed bioinformatics analyses for differential pathway enrichment (Figure 1), differential gene interaction networks (Supplemental Material: Figures S1 and S2), and, specifically, for differential Tp53 and Myc effector gene interactions (Figure 2).

Analyses of differential pathway enrichment (Figure 1) shows that the high-dose unique gene set was enriched for a few pathways including amyloid processing, oxidative stress response, oxidative phosphorylation, xenobiotic metabolism signaling, and Wnt/ß-catenin signaling (Figure 1). In contrast, the low dose-unique gene set was significantly enriched for 13 pathways (Figure 1) that are well known for their importance in synaptic plasticity, including axon guidance signaling, integrin signaling, synaptic long term depression (LTD), synaptic long term potentiation (LTP), G-protein coupled signaling.

Analyses of gene interaction networks identified six networks for the low-dose unique gene set (Figure S1) and different six networks for the high-dose unique gene set (Figure S2); p-values for individual networks ranged from 10^{-52} to 10^{-13}. Both the low- and high-dose groups of networks contained sub-networks with Myc and Tp53 as major nodes. We applied differential pathway enrichment analyses to examine the relative functions of the genes associated with low-dose Myc-Tp53 networks (Figure 2A, also see Figure S1) versus high-dose Myc-Tp53 networks

(Figure 2B, also see Figure S2). As shown in Figure 2C, the low-dose network was significantly enriched for SAPK/JNK signaling, estrogen receptor signaling, integrin signaling, p38 MAPK signaling, while the high-dose network was significantly enriched for EGF, IL-2, and IGF-1 signaling. Both low-dose and high-dose networks were enriched for G1/S cell cycle checkpoint. These findings show that Tp53 and Myc affect a substantially different set of effector genes after low-dose exposures in contrast to high-dose exposures, and that the special signaling functions of the low-dose Tp53-Myc effector genes are different from the signaling functions induced after high-dose exposures.

Analyses of the low-dose radiation genes.

The full set of genes induced after low-dose exposure clustered into a highly saturated gene interaction network (Figure 3, $p = 10^{-53}$). Inspection of this saturated network identified five down-regulated genes associated with synaptic transmission (Grin1 and Gria3 are glutamate receptors genes; Nsf and Syt1 are transporters; Plat1 is a peptidase). The networks also contained the transcription factor, Jun, and the gene Kif1A that have both been associated with neurodegeneration (37). We also found that the low-dose gene set contained 10 synaptic ion channel genes of which 8 are down regulated (Gabrb1, Gria3, Grik5, Grin1, Kcnq2, Kcna1, Ryr2, Scn8A) and two are up-regulated (Fxyd1 and Scn1A). Synaptic ion channels play important role in synaptic plasticity (38). The transcriptional changes of several low-dose genes were confirmed by PCR, including the down-regulation of Grin-1 by qPCR (0.88 ± 0.07; $p < 0.05$) and the up-regulation of Psma4 (Figure S1, network 3), Pcna, Csnk2a1 and Sod1 by RT-PCR (23).

Pathway enrichment analysis of the low-dose unique genes (Figure 4A) was used to identify the pathways that were predominantly up- or down-regulated after low-dose exposures (based on the proportions of up- and down-regulated component genes). Only two pathways contained significant proportions of up-regulated genes after low-dose exposures: integrin signaling and SAPK/JNK signaling. In contrast, there were 13 pathways (Figure 4A) that contained predominantly down-regulated genes. Six of these contained only down-regulated genes (amyloid processing, amytrophic lateral sclerosis signaling, calcium signaling, chemokine signaling, glutamate receptor signaling, synaptic long term potentiation). Many of these low-dose down-regulated pathways are known to be associated with learning, memory and cognitive functions. Similar analyses of the pathways associated with up- and down-regulated high-dose genes (supplemental figure S3) showed a very different overall picture. Up regulated genes were enriched in the pathways of oxidative phosphorylation, NRF-2 mediated oxidative stress response, Cell cycle checkpoint regulation, protein ubiquitination, and glycine, serine, and threonine metabolism. Down-regulated genes were enriched in keratan sulfate biosynthesis, cysteine metabolism, chondroitin sulfate biosynthesis, and xenobiotic metabolism signaling. Amyloid processing pathway was down regulated after both low-dose (Figure 4a) and high-dose exposures (supplemental figure 3).

Comparative analyses of transcriptional regulation in mouse brain tissue after low-dose irradiation against published datasets.

Based on our pathway enrichment findings (Panel 4A), we hypothesized that low-dose radiation exposure may have effects on cognitive function. To inspect this hypothesis, we conducted an unbiased bioinforamatic survey of a compendium of datasets of transcriptional profiles of

various genetic and disease conditions including those from individuals with diminished cognitive function. Analysis with the set of up-regulated low-dose genes failed to identify any knowledgebase with significant expression overlap. Analyses with the set of down-regulated low-dose genes analyses identified two knowledgebases with significant gene overlap: the aging human brain and Alzheimer's disease (Figure 4B and C). As described in the methods section, IPA was applied to the set of genes that was modulated in postmortem human cortex tissue from individuals of advanced ages up to 106 years-old (n=30, (36)) and to the gene set that was modulated in hippocampal tissue from Alzheimer's patients (n= 22, (31)). Comparison of the relative pathway enrichments for low-dose radiation, human aging and Alzheimer's disease gene expression profiles (Figure 4A, 4B and 4C) showed remarkably consistent findings not only in the direction of expression modulation (predominantly down-regulation), but also in the specific pathways involved. Six pathways were in full agreement among all data sets, and five out of six showed consistent down regulation. Integrin signaling pathway was the only pathway that was consistently up regulated. In addition, three pathways (axonal guidance signaling, actin cytoskeleton signaling and ERK/MAPK signaling) were significantly affected in all three data sets, but they differed somewhat in the direction of their effects (up or down regulation).

We then compared the component genes of the pathways that were consistently affected across all three data sets. Figure 5 identifies each gene of the synaptic long term depression (LTD) pathway whose expression was modulated in mouse brain tissue after radiation. For comparison, the specific genes of the LTD pathway that were affected in the aging human brain and in AD patients are listed in the figure legend. PRKCB1 and GRIA3 were consistently modulated in the LTD pathway in all three data sets. Isoforms of GNAS, a guanine nucleotide binding protein,

were also modulated in all three data sets (GNAS in radiation and Alzheimer's disease; GNAQ in aging). Similar analyses of the down-regulated pathways of Figure 4, identified the following genes that were consistently down regulated across in all three data sets: MEF2C in calcium signaling, SLC1A1 in glutamate receptor signaling, and PRKCB1 in G-protein coupled receptor signaling and in synaptic long term potentiation.

Figure 6 illustrates the component genes of the integrin signaling pathway, the only pathway consistently up regulated in all three data sets, and highlights the genes that were modulated after radiation exposure of the mouse brain. For comparison, the integrin pathway genes that were modulated in the human aging brain and in AD patients are listed in the figure legend. Two integrin pathway genes were affected only in the radiation and aging data sets: TLN1 and ACTA1. Five other integrin-related genes were affected only in the radiation and AD data sets: (CAPN3, CRKL, RALA, RHOQ, and ACTN3). There were no genes that were consistently upregulated in all three data sets.

Comparative analyses of transcriptional regulation in mouse brain tissue after high-dose irradiation against published datasets.

The results of the L2L analysis of the high-dose genes showed a very different pattern than that of low-dose genes. The set of down-regulated high-dose genes failed to identify any expression knowledgebases with significant association with radiation response. However, analyses with the set of up-regulated high-dose genes identified the AD knowledgebase. WNT/β catenin pathway was marginally affected in both the high-dose radiation and AD datasets (Figure 1, Figure 4c) with no significant enrichment of up or down-regulated genes. Amyloid processing pathway was

downregulated after high-dose radiation (supplemental figure 3), and in AD (Figure 4c), as well as after low-dose radiation (Figure 4a), however, it did not show a significant overlap with genes associated with advanced human age (Figure 4b). Finally, xenobiotic metabolism was significantly down-regulated in the irradiated mouse brain (Figure 1 and supplemental figure 3), human aging (Figure 4b) and AD (Figure 4c).

Discussion

Rapidly developed microarray technologies offer rich and abundant transcription profile information from different species, organs, condition and diseases. We have applied recent bioinformatics advances to understand the functions of radiation-modulated genes in the mouse brain, to discriminate between low- and high-dose gene networks and pathways, and to gain insight into the radiation damage response pathways in brain tissue by comparison with the damage response pathways in the aging human brain and in Alzheimer's disease patients.

Our findings demonstrate that the mechanisms of the mouse brain response within a few hours after low-dose exposure involves cellular damage response functions that are inherently complex and differ significantly from high-dose responses. The radiation response of brain tissue involves functions associated with Tp53 and Myc, as previously suggested by Yin et al (23) and Achanta et al (28). However, our bioinformatics analyses demonstrates that the Tp53–Myc associated effector functions after low dose are very different from those after high doses. We found that genes modulated after low-dose exposure are associated with common damage response functions and with brain-tissue specific functions associated with memory, learning and cognition. We also found a high degree of concordance of affected pathways in the brain tissue of mice after low-dose exposure, brain tissue of aging humans, and brain tissue of Alzheimer's patients. High-dose exposures did not elicit any of these pathway responses. These findings strongly suggest that low-dose irradiation modulates the expression of gene pathways that are involved in cognitive function and that low-dose radiation exposure down regulates the same response mechanisms that are defective in aging and Alzheimer's disease. Our analysis demonstrated, for the first time, that 10 cGy irradiation may have effects on cognitive function.

Further studies are warranted to verify the current findings using larger numbers of animals and at various brain regions, specifically in hippocampus, which is important for learning and memory.

Brain radiation and cognitive function

It is well known that high dose irradiation has detrimental effect on cognitive function (18, 28, 39), while the information of the low-dose effect on memory is sparse. At present, the pathogenesis of radiation-induced cognitive injury remains unresolved. It has been speculated that low-dose radiation can lead to cognitive dysfunction without inducing significant morphological changes (40, 41). Molochkina et al (42) found that after single low-dose whole body gamma-irradiation (15 cGy), brain membranes functioning changed significantly as judged by membrane-bound acetylcholinesterase activity. Low-dose exposure is also involved in common-inflammatory reactions (43). Here, we demonstrated that low-dose irradiation at 10 cGy may have effect on cognitive function through GO, network and pathway analyses.

Radiation-induced cognitive impairments are generally more severe in the developing or young brain (44). Rola et al. (45) reported that young mice (21-day-old at the time of irradiation) showed persistent changes in neurogenesis, which were associated with spatial memory retention deficits at three months post treatment. The age of mice, at the time of radiation exposures in current study, was 8-to-10 weeks old, which is more equivalent to adolescent humans. Future studies are needed to understand the biochemical mechanisms that underlie the concordance of pathways we observed among low-dose radiation, aging and AD and whether the low-dose radiation response varies with advanced age.

Our analysis identifies certain cell types and cellular components of the radiation response of the brain. Genes associated with synapse and neuron projections are enriched after low dose whereas genes associated with mitochondria are enriched after high doses, and ribosomes are enriched after both low and high doses. This suggests that synaptic signaling is damaged after low-dose exposures. It also suggests that high-dose damage processing takes place in mitochondria that are known to be involved with apoptosis-initiating events. Unfortunately, microarray analyses based on large tissue volumes does not allow the resolution to determine whether our findings suggest a homogeneous response across all neural cells or whether the responses are specific to certain brain regions such as the neurogenic precursor cells (20). Further studies with microdissection and in situ imaging with gene probes will be required to address this question. For example, Lowe et al (27) employed transcript bioinformatics and in situ RNA hybridization to demonstrate that katamine, a glutamate receptor antagonist, modulated genes in specific cell types of the brain.

The pathway analyses of Figure 1 emphasize the complexity of the low-dose response. Low-dose radiation modulate (i.e., induces or represses) the expression of genes that function in a variety of signaling pathways involved in axon movement and architecture (axon guidance signaling, actin cytoskeletal signaling), blood vessel formation (ephrin signaling), and metabolism (protein ubiquitination pathway, cAMP signaling, insulin receptor signaling). We provide evidence that the mitogen activated ERK/MAPK and stress-activated SAPK/JNK signaling pathways are modulated after low-dose exposures, but in different directions, ERK/MAPK was significantly downregulated (Figure 4A). Rho-GTPases which have an important role in activation of

ERK/MAPK signaling cascade (46) were preferentially and significantly modulated after low-dose but not high-dose exposures (Table 1). SAPK/JNK, a well-known stress activated pathway (47) was upregulated and preferentially associated with the low-dose Tp53-Myc functions (Figure 2). Our findings are consistent with the report of a negative cross-talk relationship between the stress-activated JNK pathway and the mitogen-activated ERK pathway (48).

Our network and pathway analyses showed that low-dose irradiation response involved a set of pathways that function in neural plasticity, memory, and learning: Glutamate receptor signaling, LTP, LTD, G-protein coupled receptor signaling and Integrin signaling. The establishment of memory requires coordinated signaling between presynaptic and postsynaptic terminals in the CNS. Integrins make up a large family of cell adhesion receptors that are known to mediate bidirectional signaling between cells, their external environment, and have also been implicated in control of pathogenesis in several neurodegenerative diseases (49, 50). Recent pharmacological and genetic studies have suggested that beta1-integrins are critical in synaptic plasticity and memory formation. Chan et al. (49) reported that deletion of alpha3-integrin resulted in impaired LTP and the impairment in LTP and working memory is similar to that observed in beta1-integrin conditional knockout mice. In addition, integrins mediate beta-amyloid-induced cell-cycle activation and neuronal death (51). In the current study, we found that the integrin pathway was up-regulated by low-dose exposure.

The striking similarity of pathway changes among the low-dose irradiated mouse brain, human aging brain and AD patient brain

The low-dose damage response in mouse brain involves mainly down regulated genes and pathways, with a few exceptions (Figure 1 and 4A). Among the 15 low-dose pathways, thirteen were significantly down regulated. Six of these contained down-regulated genes only. When these pathways were compared with those obtained in human aging brains and Alzheimer's brains, it was found that six pathways have high degree of concordance (Fig. 4, A,B,C). All of them, except one, were down regulated in all three conditions. In addition, an example pathway map of LTD showed the impressive similarity of down-regulated genes in all three conditions (Fig. 5), with glutamate receptor gene, GRIA3 and G-protein binding gene, GNAO1 modulated in postsynaptic membrane region; while protein kinase C gene was down regulated in cytoplasm region. Haroutunian et al. (30) reported that more genes are down-regulated than up-regulated at any given disease severity stage in a study of 115 postmortem individuals with Alzheimer's disease and dementia using microarray analysis. The disparity between up- and down-regulated genes became more pronounced in the advanced stages of disease. The reasons for this generalized gene downregulation are not obvious. Haroutunian et al. (30) hypothesized that cell loss or compensatory mechanisms can be invoked. Our observation of overwhelming down-regulated network/pathways induced by low-dose exposures are in good agreement with those found in human aging brain and in the brains of AD patients.

Strand et al. (52) compared gene expression profiles in regions of motor cortex, caudate nucleus and cerebellum between the healthy human and mouse using microarray. Contrasting between the two species, they found general similarity in region-specific gene expression patterns in healthy human and mouse brains. Given the fact that region-specific expression patterns of thousands of genes have significant correlation between the healthy human and mouse, Strand et

al. (52) believed that "it is reasonable to expect a high degree of concordance between microarray phenotypes of human neurodegenerative diseases and their mouse models". Our findings are consistent with Haroutunian's observation and Strand's speculation.

Alterations of the glutamate excitation pathway, either through reduced glutamate transport or aberrant expression of glutamate receptors, are known to occur during aging and Alzheimer's disease (53). Our comparative analyses of transcriptional regulation in the mouse irradiated brain and in the human aging and Alzheimer's brain identified 5 genes that were consistently downregulated across all three data sets. It is interesting to note that all five genes, GNAS (a G-protein), GRIA3 (an AMPA glutamate receptor), SLC1A1 (a glutamate transporter), PRKCB1 (a protein kinase C), and MEF2C (a transcription factor), play important roles in the glutamate transmitter pathway, which is the major neuronal excitatory pathway in the CNS (54-56). The first three genes directly interact with glutamate, either as receptors (GNAS and GRIA3) or as transmembrane transporters (SLC1A), while PRKCB1 and MEF2 regulate AMPA receptor function (57) and synaptic numbers (58), respectively. Activation of glutamate receptors is responsible for basal excitatory synaptic transmission and many forms of synaptic plasticity such as synaptic long-term potentiation (LTP) and long-term depression (LTD), which are thought to underlie learning and memory. This raises that tantalizing possibility that low-dose radiation may affect memory and cognitive function through alteration of the glutamate excitatory pathway. These identified genes may also represent targets for pharmaceutical intervention strategies to treat Alzheimer's diseases as in the case of glutamate NMDA-receptor and its antagonist memantine (59).

Genetic studies of several of genes uniformly under-expressed in all three data sets (low-dose, aging, and Alzheimer's Disease) have shown them to be involved in cognitive function. The protein kinase C (PKC) family of enzymes has been implicated in synaptic plasticity and memory in a wide range of species. PRKCB1 one of the isoforms of PKC was under expressed in our low-dose unique set. PKCbeta knockout mice exhibited loss of learning and suffered deficits in both cued and contextual fear conditioning (60). The PKC expression pattern and behavioral phenotype in the PKCbeta knockout animals indicate a critical role for the of PKCbeta isoform in learning and neural signal transduction mechanisms. The AMPA glutamate receptors (AMPAR) GLUR2, and GLUR3 are important for synaptic targeting/stabilization of AMPAR and the expression of hippocampal long-term depression (LTD). Double knock out mice in GLUR2 (Gria2) and GLUR 3 (Gria3) were severely impaired in basal synaptic transmission in vivo, demonstrating that GLUR2/3 is essential to maintain adequate synaptic transmission (61). Mice with homozygote mutations in Slc1a1 displayed decreases locomotor activity and behavioral abnormalities (62). Finally, Mef2c is a member of the Mef2 family of transcription factors (Mef2a-d) that are highly expressed in the brain and play key roles in the development and function of the nervous system (58, 63, 64). Activation of Mef2c promotes the transcription of a set of genes that restrict synapse number (58), while its deletion results in embryonic lethality (65).

In summary, our findings confirm the complex nature of the brain tissue response to low-dose ionizing radiation, and that low-dose exposures affect many different gene pathways that are not utilized after doses more typically experienced in cranial radiotherapy. Our findings suggest that brain tissue exposed to low doses employs two broad categories of radiation damage responses:

(i) conserved damage response pathways such as those controlled by Tp53 and Myc functions, and (ii) brain-tissue specific damage response signaling pathways that are associated with neural function and cognition. The high concordance of affected pathways that we observed between low-dose radiation, aging and Alzheimer's diseases warrants further study to determine whether the down regulation of these pathways after low-dose radiation is simply a transient coincidence of neural damage response mechanisms with no long-term consequence after low-dose exposures, or whether low-dose effects on these pathways portend more serious persistent damage to memory and cognition akin to defects seen in aging and Alzheimer's disease. A better understanding of any long term effects of low-dose exposure on cognitive function will have major implications for assessing neural health risks from medical radiation diagnostics, from side effects of radiotherapy, as well as the occupational risks to nuclear energy workers and to astronauts during space travel.

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Figure Legends

Figure 1. Comparative pathway enrichments for low-dose unique and high-dose unique genes. The low- and high-dose unique genes were evaluated for pathway enrichment using IPA (see methods section). Significantly enriched pathways are listed on the left and their log values of significance are shown by the length of the colored bars (Grey for low dose; black for high dose). The threshold line for enrichment is $p \leq 0.05$.

Figure 2. Merged Tp53-Myc networks from low- and high-dose gene sets and their relative pathway enrichments. The unique low-dose and high-dose gene sets were assigned to individual gene networks (Figures S1 and S2). The two low-dose networks containing Tp53 and Myc were merged (top left panel) and two high-dose networks containing Tp53 and Myc were merged (top right panel). The genes marked with gray boxes were modulated in our microarray experiments after low-dose (top left) or high-dose exposures (top right). These merged networks were compared for relative pathway enrichment (bottom panel). The modulated genes are in black. Pathways that are significantly enriched in the low-dose set are shown on the left (inside the hatched box), while pathways that are unique to high dose are shown on the right inside (solid box). The threshold line for pathway enrichment is $p \leq 0.05$.

Figure 3. Saturated low-dose gene interaction network. Network analyses was applied to the gene set containing both low-dose unique and genes that were in common between low and high dose exposures. The two top networks were merged to create the saturated gene interaction network ($p=10^{-53}$). The majority of the genes in the network, including two of the major nodes, are down-regulated after low-dose exposure.

Figure 4. Pathway enrichment analyses for the up- and down-regulated genes among three transcriptomic data sets: brain tissue from mice exposed to low-dose radiation (current study), brain tissue from aging humans, and brain tissue from Alzheimer's disease patients. In each panel, the significantly enriched pathways are shown. For each pathway, the length of the bars shows whether the pathways were significantly down-regulated (grey bars) or up-regulated (black bars) with the threshold line for significance of $p \leq 0.05$. The agreement of pathways across all three datasets is represented by three types of arrows at the right side of pathway in each panel. An arrow pointing upward indicates that the pathway was significantly up-regulated in all three datasets. An arrow pointing down indicates that the pathway was significantly down-regulated in all three datasets. An up and down combination arrow indicates that the pathway was significantly modulated in all three data sets, but that direction of gene modulation was not the same in all three datasets. **Panel A**: Brain tissue from mice that received 10cGy of whole body radiation (unique low-dose genes, current study). **Panel B**: Human brain tissue from aging brains (36). **Panel C:** Hippocampal brain tissue from Alzheimer's Disease patients (31).

Figure 5. Synaptic long term depression (LTD) cascades in mouse brain tissue after low-dose exposures in vivo. LTD is one of the most significantly down-regulated pathways in all three datasets. In pathway map (modified from IPA), all genes that were modulated in the mouse brain by exposure to ionizing radiation are shown in bold. Each bolded gene that was down-regulated is enclosed in a rectangle, while those that were up-regulated are enclosed in hexagons. For comparison, in the aging human brain dataset, the following genes of this pathway map were down-regulated: AMPAR, ERK1/2, Gα, Gαq, PKC and PLA2. Only RYR was up-regulated in

the aging brain. For comparison, in the Alzheimer's brain dataset, the following genes contained of this pathway map were down-regulated: AMPAR, Gα, , GUCY, IP3R, MEK1/2, PKC, PLA2 and PP2A. Gene in this pathway map that were up-regulated in Alzheimers disease were mGlUR and RAS.

Figure 6. Integrin cascades in mouse brain tissue after low-dose exposures in vivo. Integrin is the only pathway that was signficantly up-regulated in all three datasets. In this pathway map (modified from IPA), the genes that were modulated in the mouse brain by exposure to ionizing radiation are shown in bold. Genes that were down-regulated are enclosed in rectangles, while those that were up-regulated are enclosed in hexagons. For comparison, in the aging human brain dataset, the following genes contained in this pathway map were up-regulated: α-INTEGRIN, β-INTEGRIN, CAVEOLIN, FYN, MRLC, PI3K and TALIN. Gene down-regulated were ACTIN, ERK1/2, MKK4, Pak and RAP. In the Alzheimer's brain dataset, the following genes in this schematic were up-regulated: 7SPAN, CDC42, CRKL, RHO, RHOGAP5, and TALIN. Gene in this schematic that were down-regulated in Alzheimer's disease were: ACK, ACTIN, ACTININ, ARF, CALPAIN, GIT and PIX.

SUPPLEMENTAL FIGURES.

FIGURE S1: Top six low dose networks shown in order of significance. Network 1 ($p=10^{-47}$), Network 2 (10^{-25}), Network 3 (10^{-21}), Network 4 (10^{-16}), Network 5 & Network 6 (10^{-16}). Up-regulated genes are in red, down-regulated genes in green.

FIGURE S2: Top six high dose networks shown in order of significance. Network 1 (10^{-24}), Network 2 (10^{-22}), Network 3, 4, 5 & 6(10^{-20}). Up-regulated genes are in red, down-regulated genes in green.

Figure S3: Pathways with significant enrichment of up-regulated (red) and down-regulated (green) genes from the high-dose unique set.

Table 1. Gene Ontology analyses of biological processes, molecular functions, and cellular components that are significantly enriched (p values are show) in the low-dose unique, high-dose unique and common gene sets.

	High Dose Unique	Low Dose Unique	Common
Biological Process			
protein biosynthesis	0.0001		
protein metabolism	0.004		
negative regulation of neurogenesis	0.0088		
small GTPase mediated signal transduction		0.0059	
Rho protein signal transduction		0.0002	
ribosome biogenesis and assembly			0.0095
amino acid metabolism			0.0079
DNA metabolism			0.0032
oxygen and reactive oxygen species metabolism			0.0049
Molecular Function			
rRNA binding	0.0008		
transferase activity	0.0042		
fatty acid binding		0.0014	
GTP binding		0.0019	
GTPase activity		0.0003	
structural constituent of ribosome			0.0007
actin binding			0.0094
transcription coactivator activity			0.0048
oxidoreductase activity			0.0027
6-phosphofructokinase activity			0.0034
electron transporter activity			0.0014
Cellular Component			
mitochondrion	0.0001		
synaptic vesicle		0.0045	
chromatin remodeling complex(nucleoplasm)		0.0044	0.0047
synapse		0.0011	
ribosome	0.007		0.0024
neuron projection			0.0005
dendrite			0.0047
secretory granule			0.0005

The GO category enrichment p-values for low and high dose unique and common set is shown above.
Genes from chromatin remodeling complex are distributed in two groups low dose unique and in common.

Figure 1

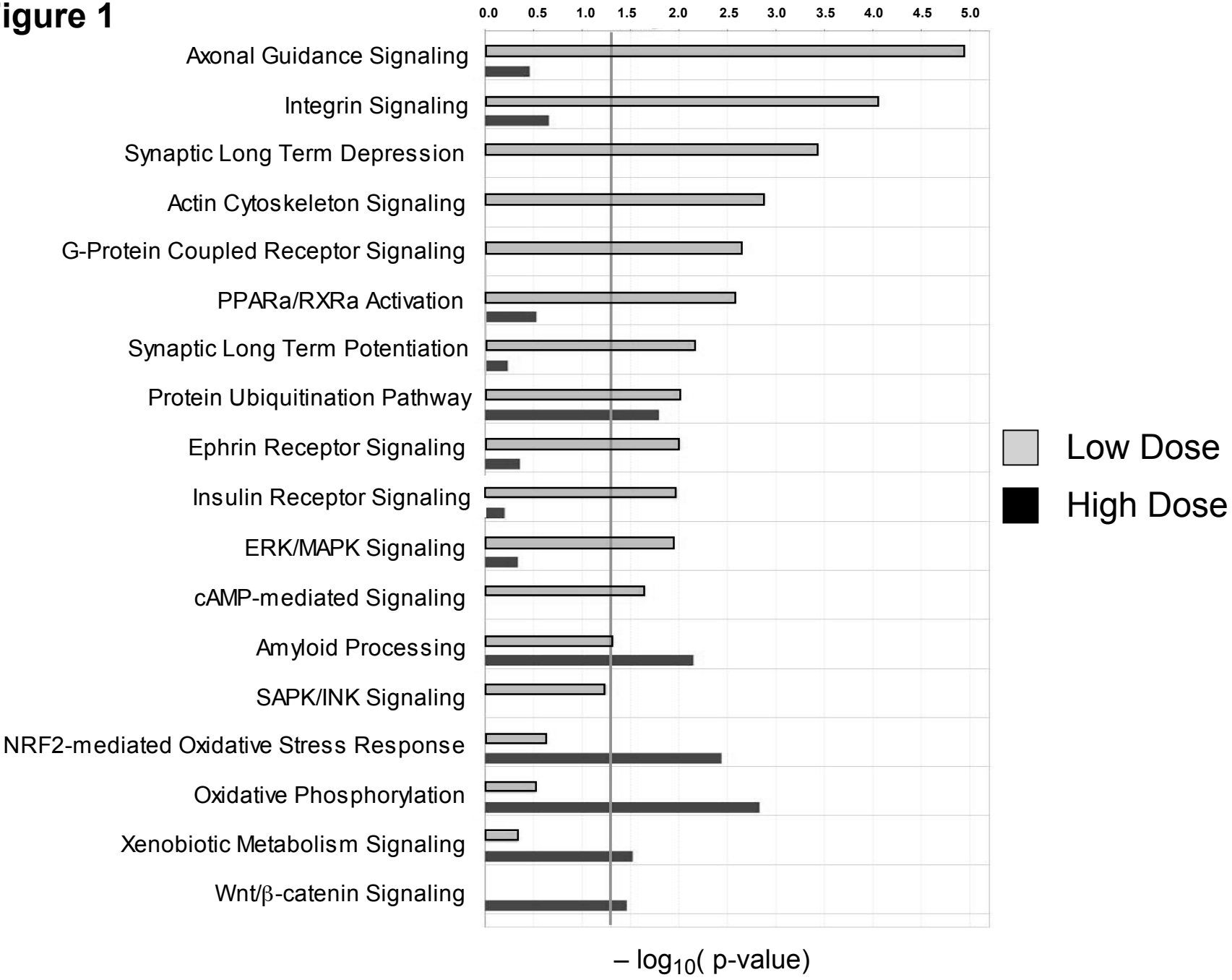
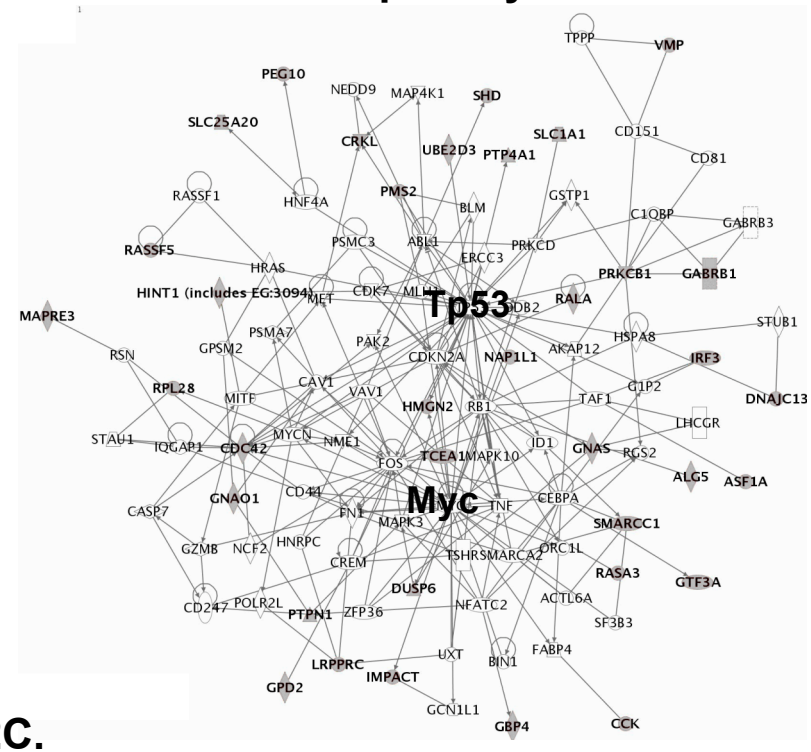
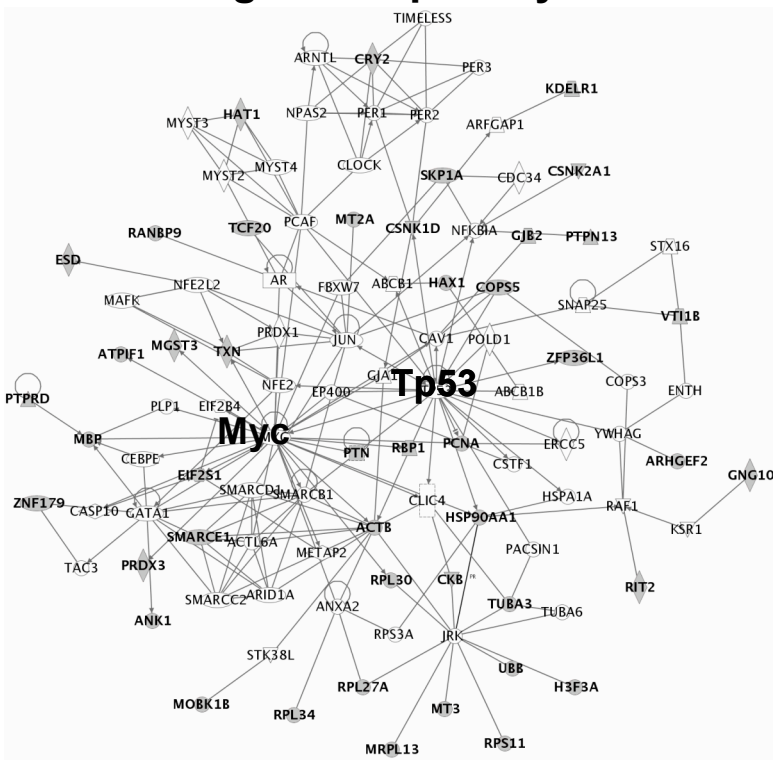

0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Axonal Guidance Signaling
Integrin Signaling
Synaptic Long Term Depression
Actin Cytoskeleton Signaling
G-Protein Coupled Receptor Signaling
PPARa/RXRa Activation
Synaptic Long Term Potentiation
Protein Ubiquitination Pathway
Ephrin Receptor Signaling
Insulin Receptor Signaling
ERK/MAPK Signaling
cAMP-mediated Signaling
Amyloid Processing
SAPK/INK Signaling
NRF2-mediated Oxidative Stress Response
Oxidative Phosphorylation
Xenobiotic Metabolism Signaling
Wnt/β-catenin Signaling
Low Dose
High Dose
– log10(p-value)

Figure 2.

2A. Low-dose p53-myc network



2B. High-dose p53-myc network



2C.

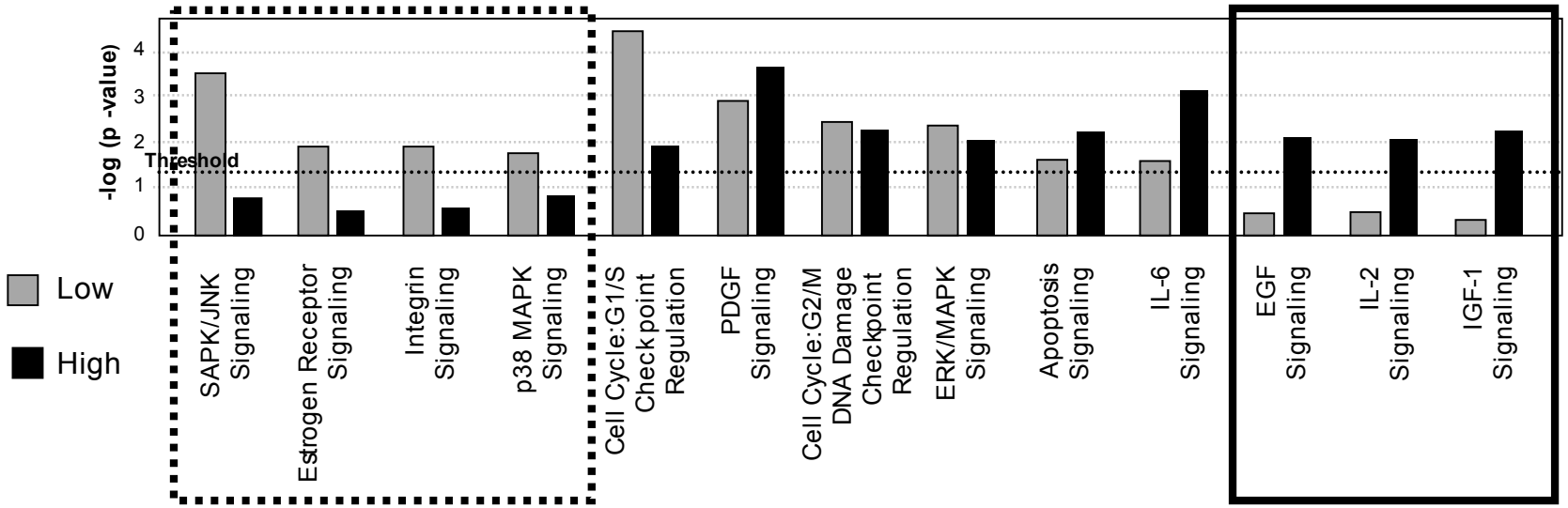
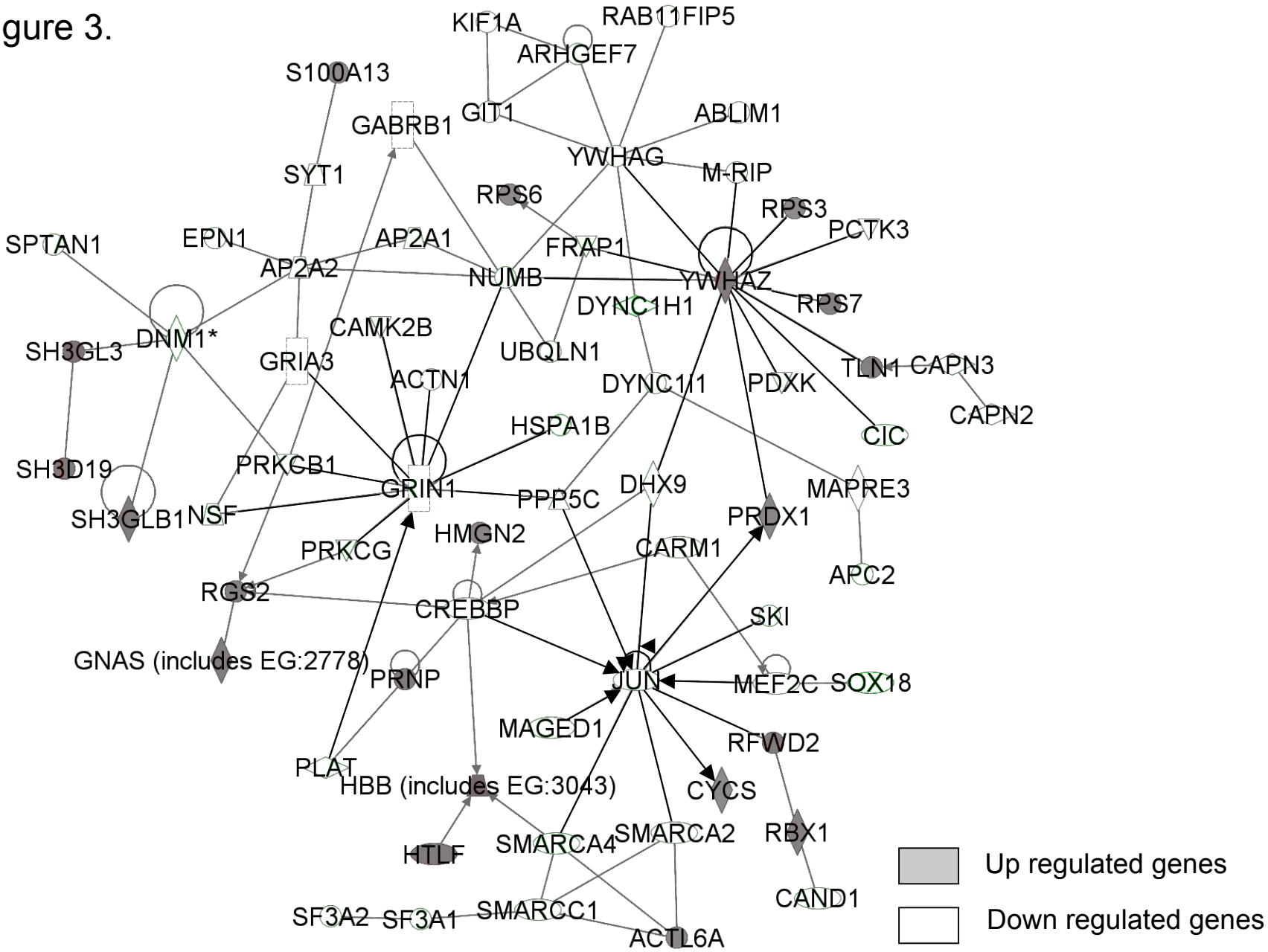


Figure 3.


KIF1A
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ARHGEF7
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GABRB1
GIT1
ABLIM1
YWHAG
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SYT1
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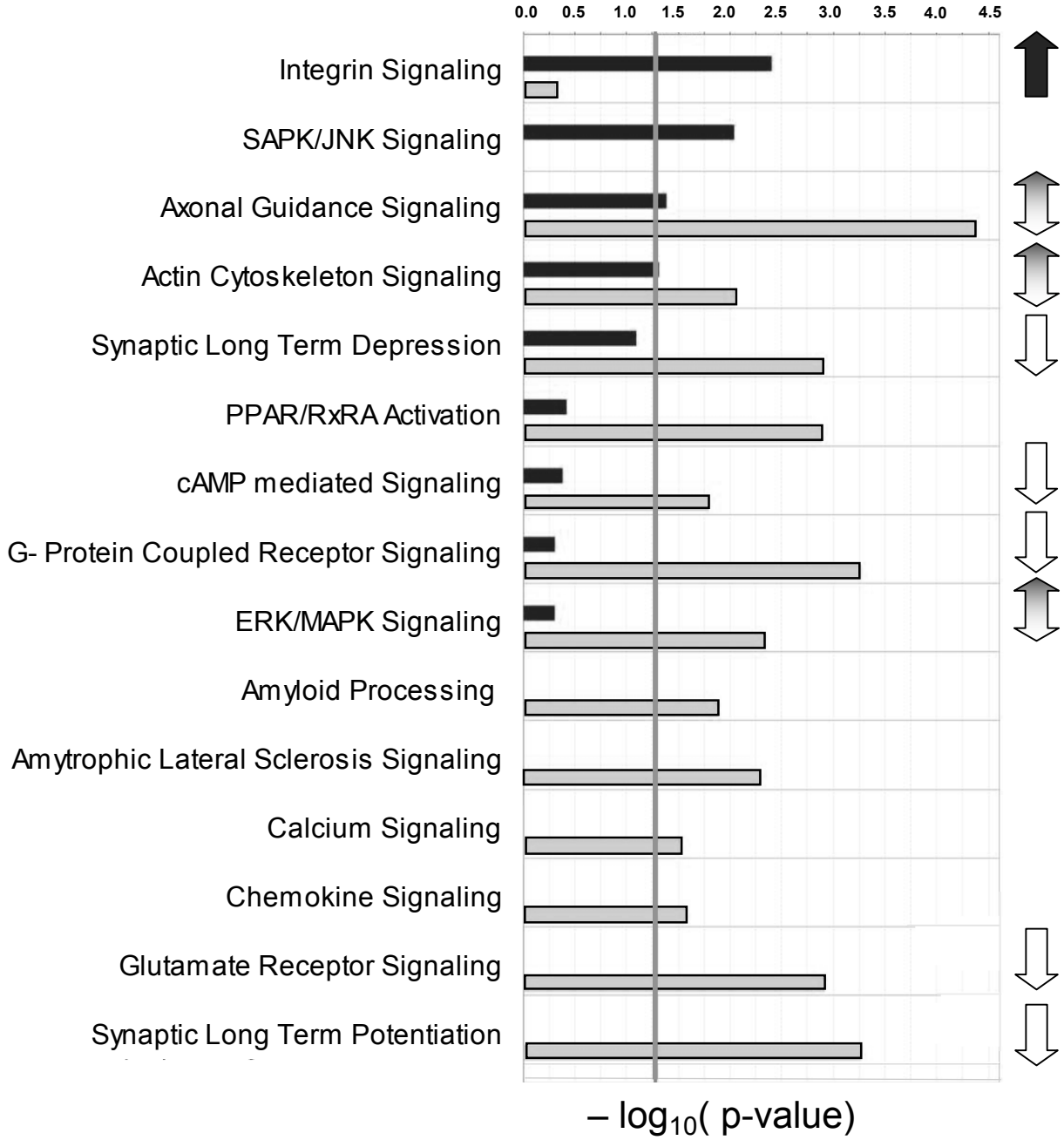
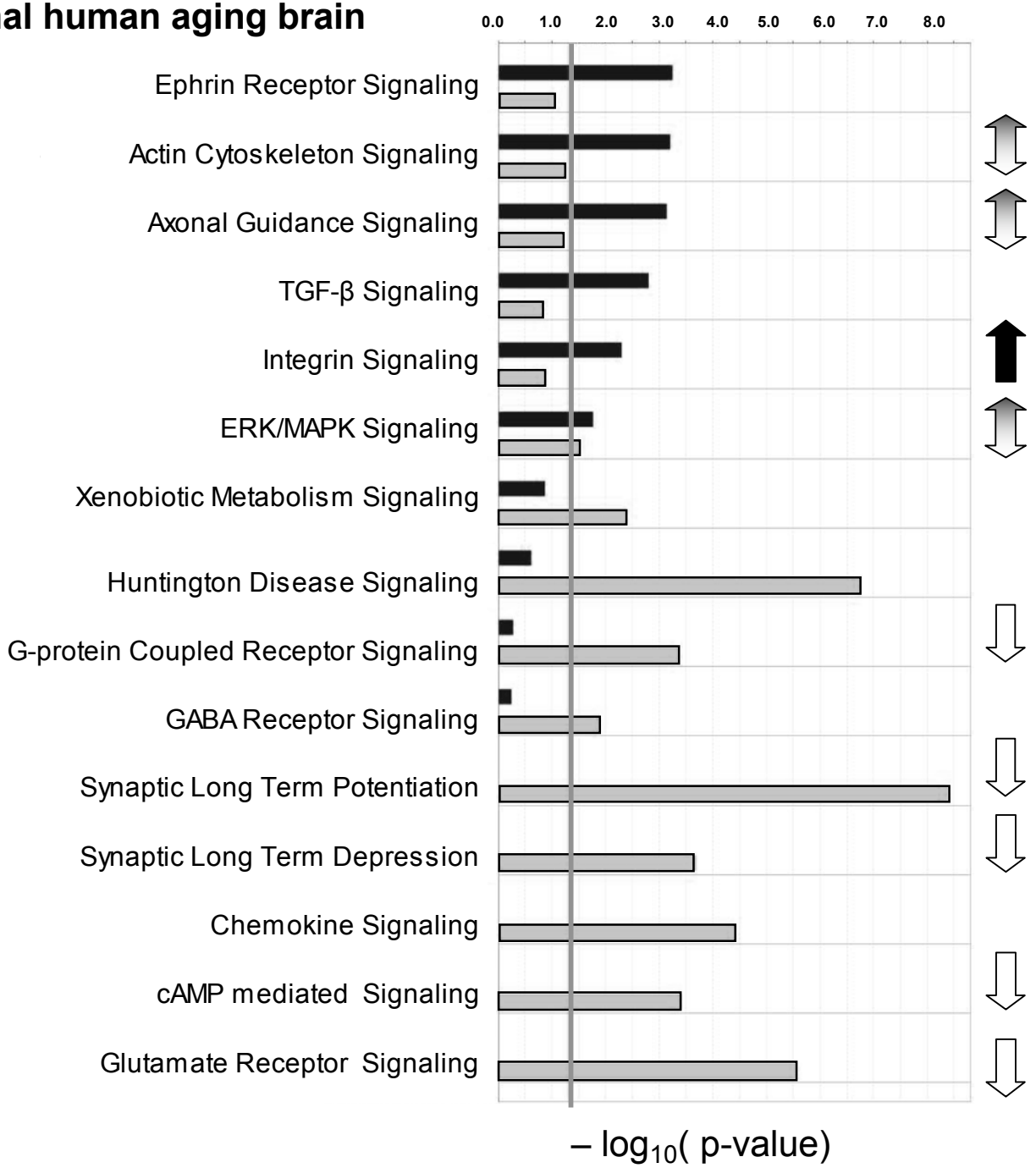
RPS3
PCTK3
SPTAN1
EPN1
AP2A1
FRAP1
AP2A2
NUMB
YWHAZ
DYNC1H1
RPS7
CAMK2B
SH3GL3
DNM1*
GRIA3
UBQLN1
TLN1
CAPN3
ACTN1
DYNC1I1
PDXK
CAPN2
HSPA1B
CIC
SH3D19
PRKCB1
GRIN1
PPP5C
DHX9
MAPRE3
SH3GLB1
NSF
PRDX1
HMGN2
PRKCG
CARM1
APC2
RGS2
CREBBP
SKI
GNAS (includes EG:2778)
PRNP
JUN
MEF2C
SOX18
MAGED1
RFWD2
PLAT
HBB (includes EG:3043)
CYCS
HTLF
SMARCA4
SMARCA2
RBX1
CAND1
SF3A2
SF3A1
SMARCC1
ACTL6A
Up regulated genes
Down regulated genes

Figure 4A. Low-dose irradiated mouse brain



4B. Normal human aging brain



4C. Brain tissue from Alzheimer's Disease Patients

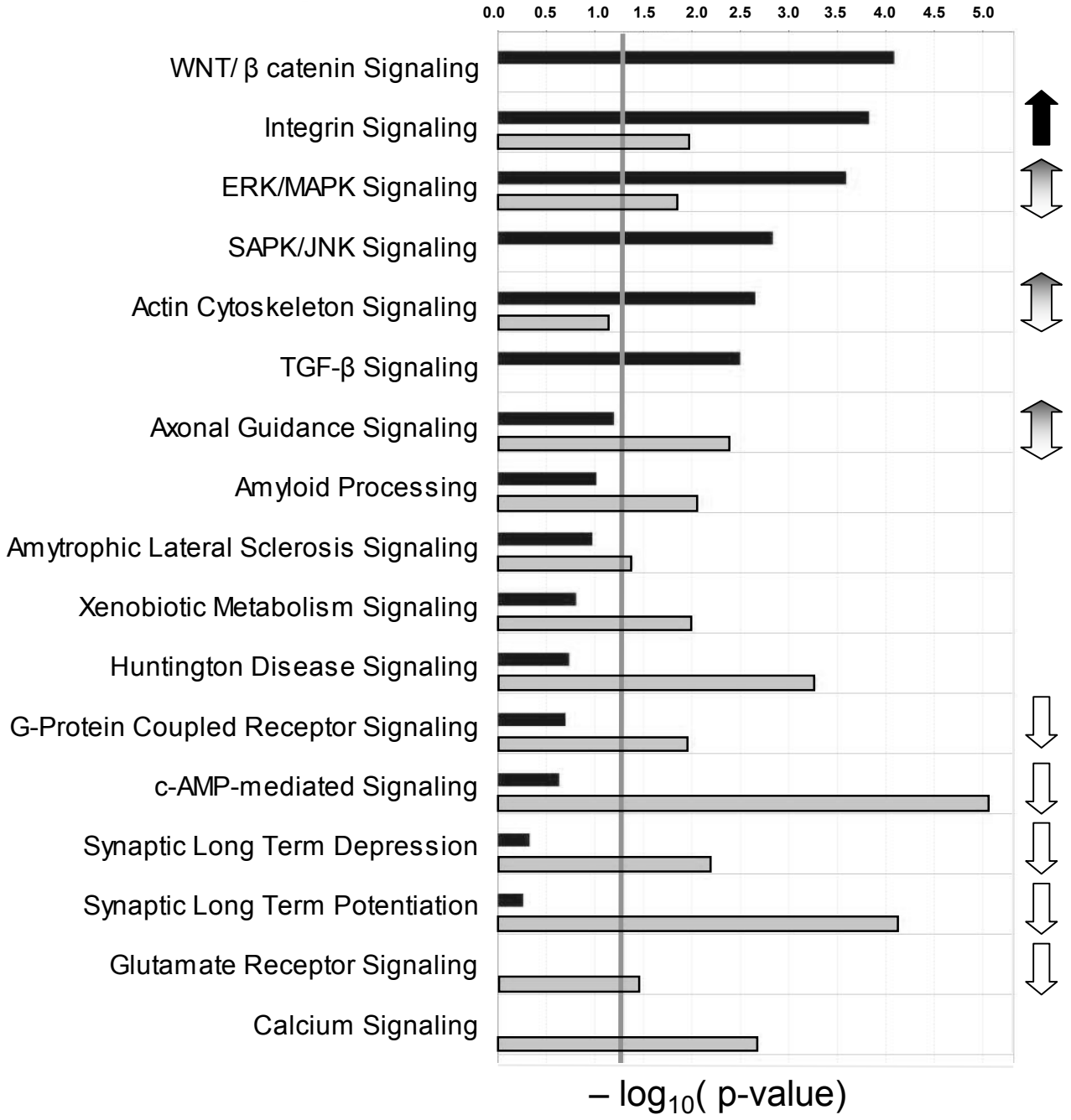


Figure 5 **Mouse Brain: Low Dose Unique LTD pathway**

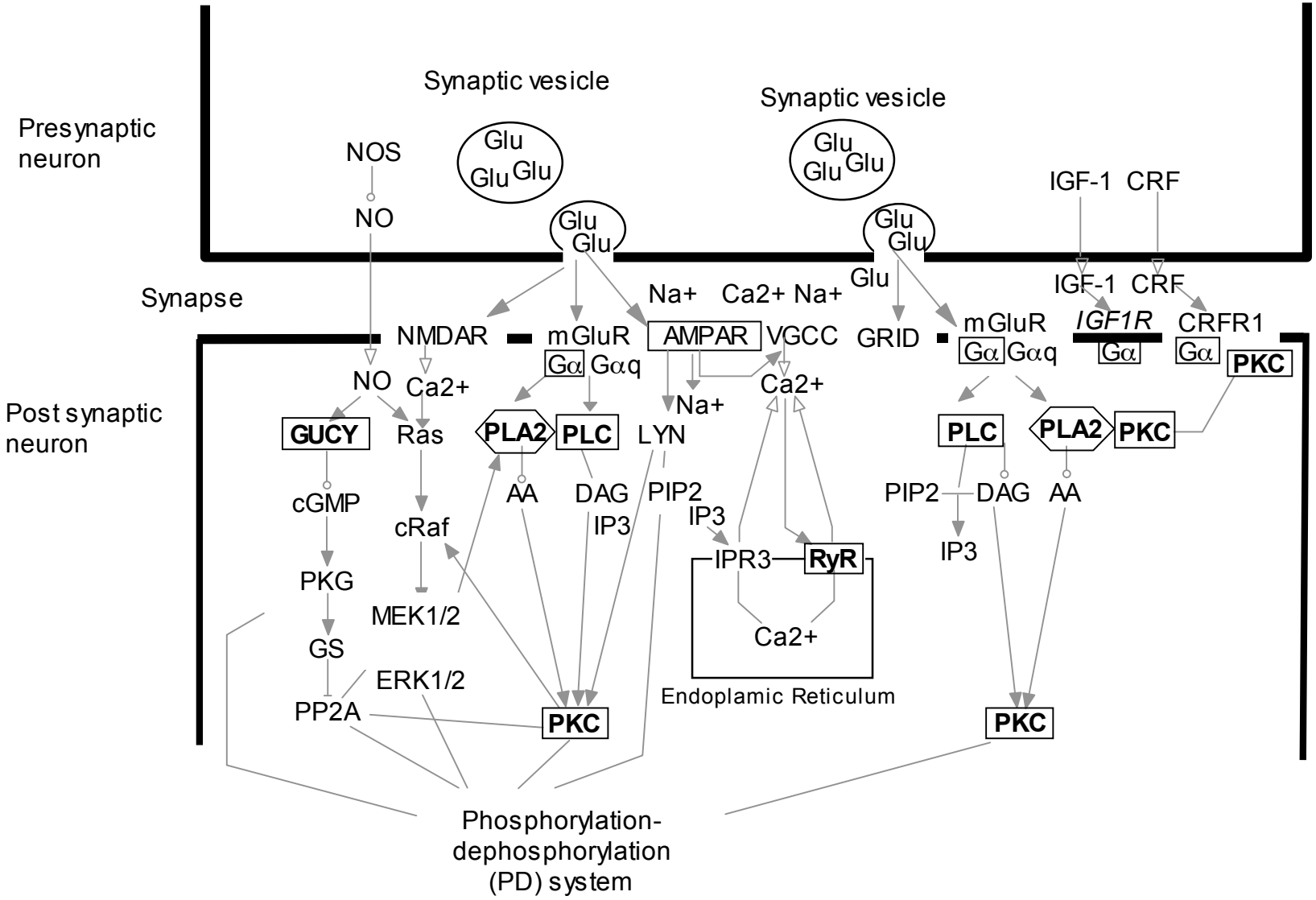
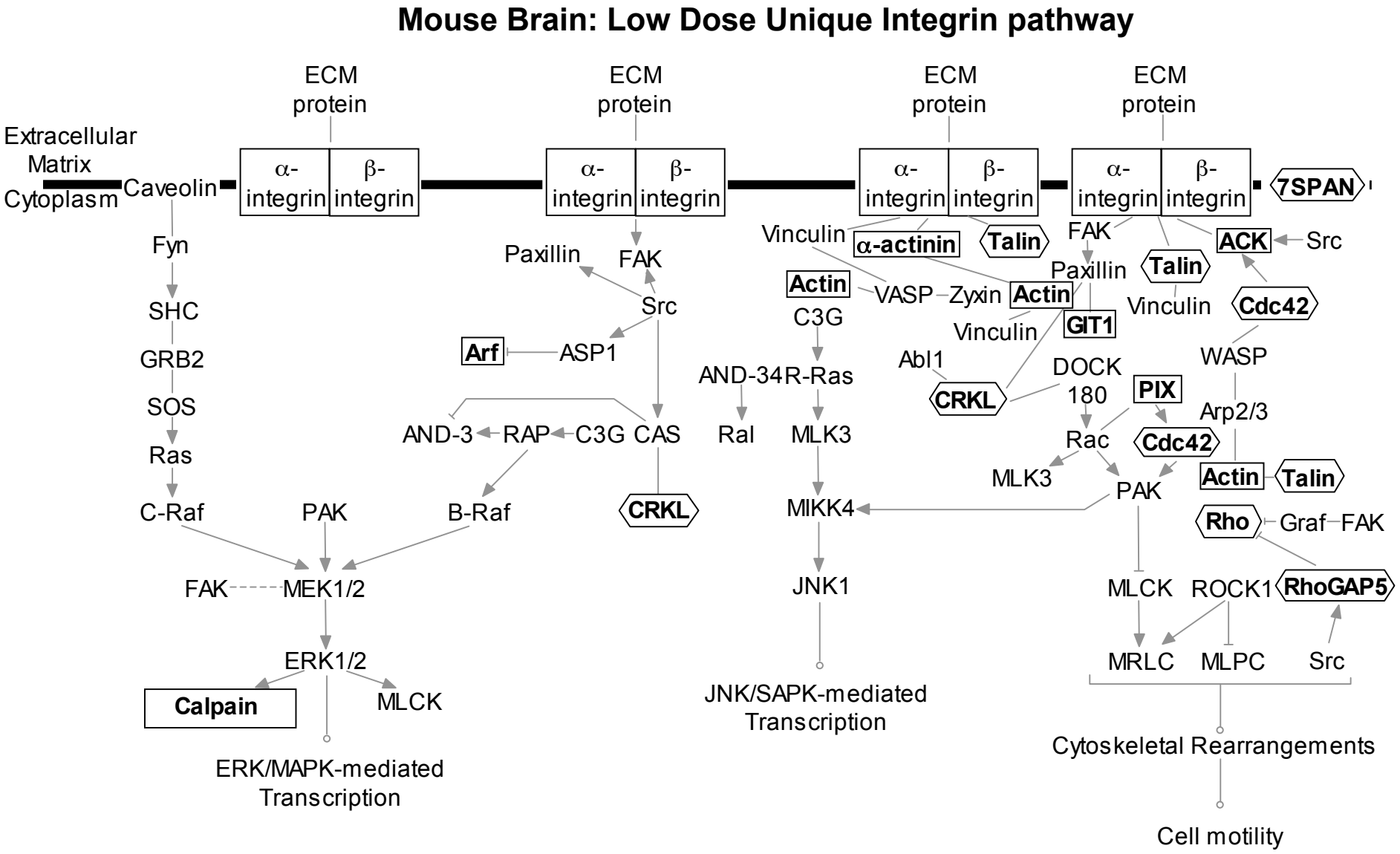


Figure 6.


Mouse Brain: Low Dose Unique Integrin pathway
ECM protein
ECM protein
ECM protein
ECM protein
Extracellular Matrix
Cytoplasm
Caveolin
α-integrin
β-integrin
α-integrin
β-integrin
α-integrin
β-integrin
α-integrin
β-integrin
7SPAN
Fyn
SHC
GRB2
SOS
Ras
C-Raf
PAK
B-Raf
FAK
MEK1/2
ERK1/2
Calpain
MLCK
ERK/MAPK-mediated Transcription
Paxillin
FAK
Src
Arf
ASP1
AND-3
RAP
C3G
CAS
CRKL
AND-34
R-Ras
Ral
MLK3
MIKK4
JNK1
JNK/SAPK-mediated Transcription
Vinculin
α-actinin
Talin
Actin
VASP
Zyxin
Actin
C3G
Vinculin
GIT1
Abl1
CRKL
DOCK 180
PIX
Rac
Cdc42
MLK3
PAK
FAK
Paxillin
Talin
ACK
Src
Vinculin
Cdc42
WASP
Arp2/3
Actin
Talin
Rho
Graf
FAK
MLCK
ROCK1
RhoGAP5
MRLC
MLPC
Src
Cytoskeletal Rearrangements
Cell motility

Supplemental Fig 3.