NASA/TP-2020-220443

An Adverse Outcome Pathway for Potential Space Radiation Induced Neurological Diseases

Kaitlyn Mi
Brown University, Providence, Rhode Island

Ryan B. Norman
Langley Research Center, Hampton, Virginia

February 2020

The NASA STI Program Office . . . in Profile

Since its founding, NASA has been dedicated to the advancement of aeronautics and space science. The NASA Scientific and Technical Information (STI) Program Office plays a key part in helping NASA maintain this important role.

The NASA STI Program Office is operated by Langley Research Center, the lead center for NASA's scientific and technical information. The NASA STI Program Office provides access to the NASA STI Database, the largest collection of aeronautical and space science STI in the world. The Program Office is also NASA's institutional mechanism for disseminating the results of its research and development activities. These results are published by NASA in the NASA STI Report Series, which includes the following report types:

- TECHNICAL PUBLICATION. Reports of completed research or a major significant phase of research that present the results of NASA programs and include extensive data or theoretical analysis. Includes compilations of significant scientific and technical data and information deemed to be of continuing reference value. NASA counterpart of peer-reviewed formal professional papers, but having less stringent limitations on manuscript length and extent of graphic presentations.

- TECHNICAL MEMORANDUM. Scientific and technical findings that are preliminary or of specialized interest, e.g., quick release reports, working papers, and bibliographies that contain minimal annotation. Does not contain extensive analysis.

- CONTRACTOR REPORT. Scientific and technical findings by NASA-sponsored contractors and grantees.

- CONFERENCE PUBLICATION. Collected papers from scientific and technical conferences, symposia, seminars, or other meetings sponsored or co-sponsored by NASA.

- SPECIAL PUBLICATION. Scientific, technical, or historical information from NASA programs, projects, and missions, often concerned with subjects having substantial public interest.

- TECHNICAL TRANSLATION. English-language translations of foreign scientific and technical material pertinent to NASA's mission.

Specialized services that complement the STI Program Office's diverse offerings include creating custom thesauri, building customized databases, organizing and publishing research results ... even providing videos.

For more information about the NASA STI Program Office, see the following:

- Access the NASA STI Program Home Page at ***http://www.sti.nasa.gov***

- E-mail your question via the Internet to help@sti.nasa.gov

- Fax your question to the NASA STI Help Desk at (301) 621-0134

- Phone the NASA STI Help Desk at (301) 621-0390

- Write to:
  NASA STI Help Desk
  NASA Center for AeroSpace Information
  7115 Standard Drive
  Hanover, MD 21076-1320

NASA/TP-2020-220443

An Adverse Outcome Pathway for Potential Space Radiation Induced Neurological Diseases

Kaitlyn Mi
Brown University, Providence, Rhode Island

Ryan B. Norman
Langley Research Center, Hampton, Virginia

National Aeronautics and
Space Administration

Langley Research Center
Hampton, Virginia 23681-2199

February 2020

Acknowledgments

This work was supported by the NASA Internships, Fellowships, and Scholarships Program and the NASA Human Research Program.

Available from:

NASA STI Program / Mail Stop 148
NASA Langley Research Center
Hampton, VA 23681-2199
Fax: 757-864-6500

Contents

Figures

Tables

Abstract

Astronauts have begun to spend increasingly longer periods in space, putting themselves in foreign environments in order to explore the unknown. Space radiation is one of the largest health risks faced by astronauts on their missions. The space radiation environment has the ability to cause high levels of irreversible damage. Multiple sources of charged particle radiation exist in the space environment that may increase risk of carcinogenesis, degeneration of bodily tissue (e.g. gastrointestinal, cardiovascular, or pulmonary), acute radiation syndromes, and acute and late central nervous system (CNS) disorders. In order to help inform an understanding of the risk of degenerative CNS disease due to radiation exposure, an initial step is presented here to develop an adverse outcome pathway from radiation exposure focused on Alzheimer's disease.

Introduction

Astronauts, even within the safety of a spacecraft, are subject to many health risks associated with spaceflight. Of primary interest to this work is the space radiation environment. Outside the spacecraft, a low intensity background of high charge and energy particles called galactic cosmic rays (GCR) permeates the Solar System. GCR are known to originate in supernovae throughout our galaxy (Ackermann et al., 2013). The GCR environment consists of fully ionized nuclei with ions covering the entire periodic table. Ions with lower atomic number tend to be more abundant in the GCR environment, however details of the nuclear physics provide some exceptions (Simpson, 1983). Because GCR are comprised of charged particles, the magnetic field of the sun modulate the low energy portion (below approximately 3 GeV/n) of the GCR spectrum. Both the complex nuclear structure and the high energy characteristics of the GCR environment make standard radiation shielding practices ineffective and astronauts have elevated radiation exposure within the interior of spacecraft.

In addition to GCR, the radiation environment of space can be enhanced by solar energetic particles (SEP). SEP, as the name suggest, originate from the sun during either solar flares or coronal mass ejections (Reames, 2013). SEP, which are primary composed of protons, are sporadic events that can significantly increase the intensity of the radiation environment of space. Fortunately, SEP tend to have an energy spectrum which is dominated at energies for which moderate amounts of spacecraft shielding significantly attenuate the event within a spacecraft (Townsend et al., 2018).

The health risks associated with the space radiation environment are of concern for both the in-flight mission success and the long-term health of astronauts. A major risk for the long term health of astronauts is the increase in mortality and morbidity from carcinogenesis due to space radiation exposure (Huff et al., 2016). The risk for cancer in terrestrial radiation-exposed cohorts is well documented and the risk is thought to also apply to the radiation environment of space (Cucinotta et al., 2013). Acute radiation syndromes are also well documented in terrestrial radiation exposure events (Hall & Garcia, 2006). In addition, space radiation has been shown to increase the incidence of cataracts in astronauts (Cucinotta et al., 2001). The evidence for other space radiation-induced health decrements comes mainly from animal studies. These health decrements include acute and late central nervous system (CNS) disorders, and other degenerative effects in human tissues such as cardiovascular disease or gastrointestinal dysfunction (Nelson et al., 2016; Patel et al., 2016).

The acute CNS consequences resulting from space radiation are somewhat understood, as previous neuroscientific studies have demonstrated evidence of cognitive deficits in domains such as learning and association, facial emotional recognition, logical reasoning, executive function, and risk assessment, lasting up to six months after space radiation exposure (Garrett-Bakelman et al., 2019). These acute neurocognitive deficits have been demonstrated not only in rat models, but also in human ones, from data gathered from both Chernobyl residents (World Health Organization, 2006) and tests done with astronauts (Garrett-Bakelman, et al., 2019) after their times in space. While consistent with previous findings, confounding factors for both Chernobyl residents and Astronauts may be occurring. In the case of correlation between mortality due to cerebrovascular events and radiation exposure, results have been mixed. The study by Azizova et al. (2011) of Mayak workers showed correlation between radiation exposure and mortality due to cerebrovascular events, while the meta-study by Little (2016) of terrestrial radiation exposures found that the excess relative risk for cerebrovascular disease per Sievert was significant but highly heterogeneous.

On the other hand, the long-term effects of extended exposure to space radiation are not as well studied. A small viable population size in addition to a lack of understanding about the development of neurodegenerative disorders in general have contributed to the small amount of data available for true analyses of the possibility for neurodegenerative disorders as a result of space radiation. It is, however, fundamental that these risks are thoroughly evaluated and understood for the future of space travel and exploration. Therefore, this report is a summary of some of the neurophysiological changes that could lead to Alzheimer's disease as a result of low dose ionizing radiation. Included is an Adverse Outcome Pathway (AOP), a structured diagram used in environmental science and toxicology to map out events that occur after exposure to a foreign agent. An introduction to AOP can be found at aopwiki.org.

Neuroanatomy and Neuropathology of Alzheimer's Disease (AD)

According to the Center for Disease Control, Alzheimer's disease (AD) is the most common type of dementia worldwide (CDC, 2019). The Alzheimer's Association states that more than 5.8 million Americans live with the disease-- this number is projected to rise to 14 million by 2050 (Alzheimer's Association, 2019). The neurodegenerative disorder is characterized by memory loss, problems with language, and spatial disorientation. The progression of the condition leads to gradual worsening of the aforementioned cognitive deficits and the emergence of other symptoms: decrease in attention span, decrease in executive function, and loss of working memory. Patients have a post-diagnosis life expectancy of anywhere between two and twenty years, depending on both the individual and the stage of AD at the time of diagnosis. Associated with the process of aging, AD's onset is usually after 65 years of age. (This is with the exception of patients with early-onset AD, whose dementia-like symptoms begin at around 40-50 years of age. Early-onset AD patients account for a minority (5-10%) of AD diagnoses (Campion et al., 1999).)

The cause of AD is unknown, but one of the most widely accepted theories for the syndrome is known as the Beta-Amyloid (Aβ) hypothesis (Bear et.al, 2009). Aβ proteins are formed as a byproduct of the amyloid precursor protein (APP), and though Aβ proteins' roles remain mostly unidentified, their formation and the plaques they leave behind begin a mechanism that leads to the cognitive and behavioral deficits that are diagnosed as AD. However, this theory of AD onset is incomplete due to its inability to account for the relation between Aβ plaques and neurofibrillary tangles, the latter of which are created due to hyperphosphorylation of tau proteins.

In post-mortem examinations, AD patients exhibit excess buildup of Aβ plaques, tau tangles, and gross atrophy of hippocampal neurons. However, there exists no known relationship between Aβ protein and development of tau tangles. Moreover, there remains no proven causative link between Aβ plaque buildup, neurofibrillary tangle formation, and AD progression, as medications and interventions designed to block Aβ accumulation and tangle creation have been unsuccessful in halting or even slowing the progression of the disorder (Kametani & Hasegawa, 2018).

The hippocampus, found bilaterally in the medial temporal lobe, is the center for episodic memories and spatial awareness. Of all the parts of the brain, it is the first to be affected by AD, and its atrophy leads to the hallmark symptoms of AD. Lesions to the hippocampus cause locational disorientation and memory loss, specifically in the module of episodic memory. These aforementioned memories are a type of long-term memory that require associations to time and place of the event, often requiring personal or emotional connection. Conscious recollection is a sign of proper retrieval of the episodic memory, and loss of this ability exhibits itself behaviorally and symptomatically as dementia.

The hippocampus is also important for a few other reasons. For one, it contains the dentate gyrus, one of the only sites of neurogenesis in the human brain (the other area designated for neuron formation is around the olfactory bulb). The formation of neurons in the human brain is mostly complete by birth. Generation rate is dependent on lifestyle choices such as physical exercise, (which increases neurogenesis), and smoking, (which decreases neuron regeneration), as well as neural processes like learning. It should also be noted that neurogenesis rate decreases as a result of stress and aging.

Besides neurogenesis, long term potentiation (LTP) also occurs within the hippocampus. LTP occurs as a result of learning and is a process that physically changes the shape of neurons due to strong activation of NMDA receptors. LTP's partner, long term depression (LTD), weakens synapses post-LTP. The synergistic relationship between LTD and LTP leads to successful long-term encoding of information that can last for years. In AD rat models, Aβ-infusion has been linked to impairment of LTP, possibly contributing to the learning and memory problems found in AD patients (Itoh et al., 1999).

Oxidative Stress

Besides the Aβ hypothesis, other theories have also arisen regarding the onset of AD. Though nothing is definitive, oxidative stress has been found to be linked to the progression of AD. Oxidative stress refers to the amalgamation of damage done by reactive oxygen species (ROS) and other free radicals to a biological system. The most common examples of ROS are superoxide (O_2^-) and hydrogen peroxide (H_2O_2). ROS are formed naturally as byproducts of cellular metabolism. They are critical to the homeostatic environment—lower levels of ROS can disrupt cellular processes such as cell division, and on the opposite side of the spectrum, raising levels past equilibrium can cause significant amounts of cellular and tissue damage (Finkel et al., 2000). In a homeostatic environment, intracellular antioxidants such as Vitamins A, C, and E are created by mitochondrial enzymes and hold the role of reducing reactive species, thus eliminating the negative effects of oxidative stress.

ROS, particularly superoxide (O_2^-), will also react with nitric oxide (NO) to form peroxynitrite ($ONOO^-$). Produced by nitric oxide synthase (NOS), NO plays an important role in cellular signaling, vasodilation, and immune response (Drew & Leeuwenburgh, 2002), as well as in acting as a neurotransmitter. Combined with a free radical, however, the product, $ONOO^-$, is a

strong oxidant and reactive nitrogen species (RNS). In high amounts, peroxynitrite is classified as a neurotoxin, leading to apoptotic or necrotic neuronal cell damage (Bonfoco et al., 1995).

The free radical theory of aging states that biological aging is a result of ROS and RNS induced oxidative stress. The theory hypothesizes that ROS/RNS cause damage that accumulates and leads to aging and age-dependent diseases. ROS and RNS cause deletion in mitochondrial DNA (mtDNA), leading not only to apoptosis but also to inefficiencies in mitochondria that leads to further generation of ROS (Indo et al., 2007). ROS and RNS can also cause damage to cellular signaling pathways, lipids, and proteins, possibly leading to an increase in rate of onset for ageing and age-dependent disorders (Finkel & Holbrook, 2000).

Neurons appear especially susceptible to the perils of oxidative stress for a few reasons. Firstly, free radicals wreak havoc on poly-unsaturated fatty acids (PUFAs) and other redox-sensitive proteins. Neuronal membranes contain a high percentage of PUFAs, specifically arachidonic and docosahexaenoic acid, and these lipids' peroxidation is a biomarker for AD development. Patients with mild cognitive impairment (MCI), a stage of cognitive decline between normal aging and the dementia symptom of AD, show an increase of lipid peroxidation levels in the temporal lobe (Pratico & Sung, 2004). The same increase in lipid peroxidation is found in AD patient brains (Azzam et al., 2012).

Neuronal PUFAs aside, the brain has another inherent quality that leads to its weakness to ROS and RNS. Due to its complexity, the brain and its normal operations require high levels of cellular metabolism due to its high demand for energy. This particular ATP-bound stipulation calls for high levels of oxygen and leads to higher production of ROS within neuronal mitochondria. High levels of cellular respiration, dependent almost solely on the electron transport chain (ETC), can contribute to production of free radicals (Liu et al., 2002). It is believed that inefficiencies within the electron transport chain in neuronal mitochondria is what leads to overproduction of free radicals, specifically the activity of cytochrome-c oxidase and its relationship with complex 4 (Yves, 2000).

Lastly, the brain lacks the antioxidant enzymes in neuronal mitochondria, leading to higher vulnerability to the effects of ROS. Neurons possess lower levels of catalase when compared to other body tissues, which could contribute to sensitivity to oxidative stress (Cobley et al., 2018).

Recent studies have found significant correlation between increased levels of ROS and development of neurodegenerative disorders such as AD. It is hypothesized that the free hydroxyl radical, hydrogen peroxide, peroxynitrite, and nitric oxide are involved in AD (Yves, 2000).

Neuroinflammation

Neuroinflammation results from infection, brain injuries, and general damage to the brain. Chronically, it is the continuous activation of glial cells and other immune responses in the brain. In recent years, chronic neuroinflammation has become the subject of studies related to Alzheimer's due to its presence in the CNS in AD patients (Kinney et al, 2018). Central to the process of neuroinflammation are microglia and astrocytes, glial cells that support neuronal function and protect neuronal processes.

Microglial cells support and protect neurons in the CNS and are part of the endogenous immune response in the brain. These neural macrophages scavenge for molecules, undergo phagocytosis, and participate in extracellular signaling that triggers activation of other macrophages both inside and outside the brain. Microglia will actively move through their areas regularly, searching for foreign material. This "foreign material" includes Aβ plaques, and microglia will surround and engulf said plaques upon contact, leading to clumps of microglia

around sites of Aβ deposition (Tuppo & Arias, 2005). It should also be noted that, given the large amounts of plaques in certain areas, it has been hypothesized that microglia remove Aβ plaques and travel to ventricular areas in order to deposit the plaques (Tuppo & Arias, 2005).

Besides removing external molecules, microglia also participate in extracellular signaling by releasing molecules such as cytokines in order to induce cascade reactions. Cytokines are a broad class of proteins that primarily signal between cells. IFN- γ (interferon gamma) and TNF-α (tumor necrosis factor alpha) are two such molecules, responsible for rapid activation of nearby microglia and neuronal apoptosis, respectively. In addition to cytokines, macrophage inflammatory proteins are also produced by microglia, signaling a site of cleanup to macrophages outside of the nervous system, which can cross the blood brain barrier and aid in phagocytosis. Most importantly, chronic inflammation causes microglial generation of ROS, contributing to oxidative stress damage in the brain (Fialkow, Wang, & Downey, 2006).

Astrocytes are the most commonly found cell in the brain. Much like microglial cells, they play an important role in supporting and protecting neuronal function. Seen as the main support cell for neurons, these star-shaped glial cells modulate synaptic strength, provide metabolic support, release and uptake neurotransmitters, and promote myelination of oligodendrocytes, among other important tasks. Astrocytes release cytokines and neurotrophins which trigger immune cell entry into the central nervous system. The role of astrocytes is not fully understood, and future investigation is required to precisely pinpoint their role in AD development.

Radiation Effects on the CNS

It is important to note that the mechanism through which radiation can induce apoptosis in the developed CNS is not well understood (Belka et al., 2001). However, it has been determined that ionizing radiation's high energy particles can cause damage to the CNS in a few different ways. The first is through the process of neuroinflammation. As stated in previous sections, chronic neuroinflammation is the long-term activation of microglial cells and astrocytes. The increase in activity of glial cells due to radiation parallels the neuroinflammation seen in the normal aging process, and, much like normal senescence, causes an increase in production of ROS and RNS (Huang, Zou, & Corniola, 2012).These free radicals go on to react with intracellular proteins, lipids found in neuronal membranes, as well as DNA and mtDNA. Perforation of neuronal membranes, if severe enough, can lead to apoptosis. The deletion and mutation of mtDNA in some cases can also cause cell death, but damaged mtDNA can, more importantly, create inefficiencies in mitochondrial function that leads to production of more ROS/RNS (Finkel & Holbrook, 2000). This mtDNA-driven oxidative stress induced in neurons is not only able to spread to nearby unaffected cells, but also has the ability inhibit mtDNA repair pathways (Azzam, Jay-Gerin, & Pain, 2012). ROS and RNS can also cause DNA damage through depurinations and depyrimidations, as well as through base pair oxidations (Azzam et. al, 2012). These DNA mutations can essentially lead to apoptosis.

Aside from the processes of neuroinflammation and oxidative stress, tau hyperphosphorylation (Li et. al, 2014), Aβ plaque accumulation (Cherry et al., 2012), dendritic spine thinning (Raber et. al, 2016), and demyelination (Panagiotakos et al., 2007) can also occur as a result of radiation. Changes in gene expression have been demonstrated in rat models as a result of low-dose ionizing radiation, with evidence pointing towards altered signaling pathways in axon architecture, blood vessel formation, metabolism, neuroplasticity, memory, learning, and LTP/LTD (Lowe et al., 2009). At 0.1 Gy exposure to gamma rays, affected genes had roles in

synaptic signaling and glutamate transmission, though it remains to be seen whether there are long-term implications for such changes (Yin et al., 2003). Increased Aβ plaque deposition levels and tau tangle formation, too, are significant given the two proteins' neuropathological relevance in AD.

Dendritic spines have been found to be affected by low-dose ionizing radiation. Found on dendrites in the CNS, dendritic spines are crucial to the processes of learning and memory and are responsible for excitatory synapses. Sparsity of dendritic spines is seen in patients with intellectual impairment. Studies have found that murine dendritic complexity decreases as a result of 1 Gy of gamma radiation, a trend that continues at least one-month post-irradiation (Parihar & Limoli, 2013). Both the number and the density of dendritic spines show a dose-dependent reduction one month after radiation. These morphological changes could lead to deficits in learning and memory and decreased synaptic strength and plasticity even long after irradiation has occurred.

Late onset demyelination, too, has been seen as a result of high doses of x-ray radiation (Hodges et al., 1998; Panagiotakos et al., 2007). The mechanism of this process is unknown, but a hypothesis is that oligodendrocytes (which make up the myelin sheath in neurons in the CNS), though not outwardly damaged by ionizing radiation, inherently carry genetic damage that leads to instability in oligodendrocyte progeny, leading to demyelination of CNS neurons that occurs significantly after irradiation (Panagiotakos et al., 2007). Demyelination has been linked to MCI, AD, and is associated with deficits in associative and categorical learning and memory encoding and retrieval (Bouhrara et al., 2018).

Radiation has also been implicated in decreased neurogenesis. Adult neurogenesis occurs in two places: the sub-granular zone (SGZ) of the dentate gyrus and the sub-ventricular zone (SVZ) found near the olfactory bulb. The former area is more frequently studied due to its location in the hippocampus, the center for learning and memory. As radiation tends to affect proliferating cells more adversely, these two areas of the brain, which actively produce neurons, are extremely sensitive to the effects of radiation (Huang et al., 2012). The role of neurogenesis in AD onset and progression is unclear, with some papers concluding that there is a deficit in neurogenesis, while others find that neurogenesis contrarily increases. To resolve these opposing findings, it has been postulated that different stages of AD correspond with different rates of neurogenesis: whereas early stage AD patients exhibit decreased levels of neurogenesis, mid to late stage AD patients experience increased rates of neurogenesis to compensate for mass neuronal death (Mu and Gage, 2011). Regardless, it is clear that neurogenesis rates are affected by ionizing radiation and that change in neurogenesis is, in one way or another, associated with AD.

Adverse Outcome Pathway (AOP)

Given the aforementioned events that occur as a result of ionizing radiation, the following AOP is proposed. Figure 1 encompasses the three main hypothesizes backing AD development (Beta Amyloid, neuroinflammation, and oxidative stress) as well as the other processes and changes that occur as a result of exposure to ionizing radiation at low doses. The pathway is, of course, incomplete given the complexity of AD and neurodegeneration and the many unknowns surrounding the effects of ionizing radiation. It is, however, a good starting point in unraveling one of many risks that underlie the future of space travel.

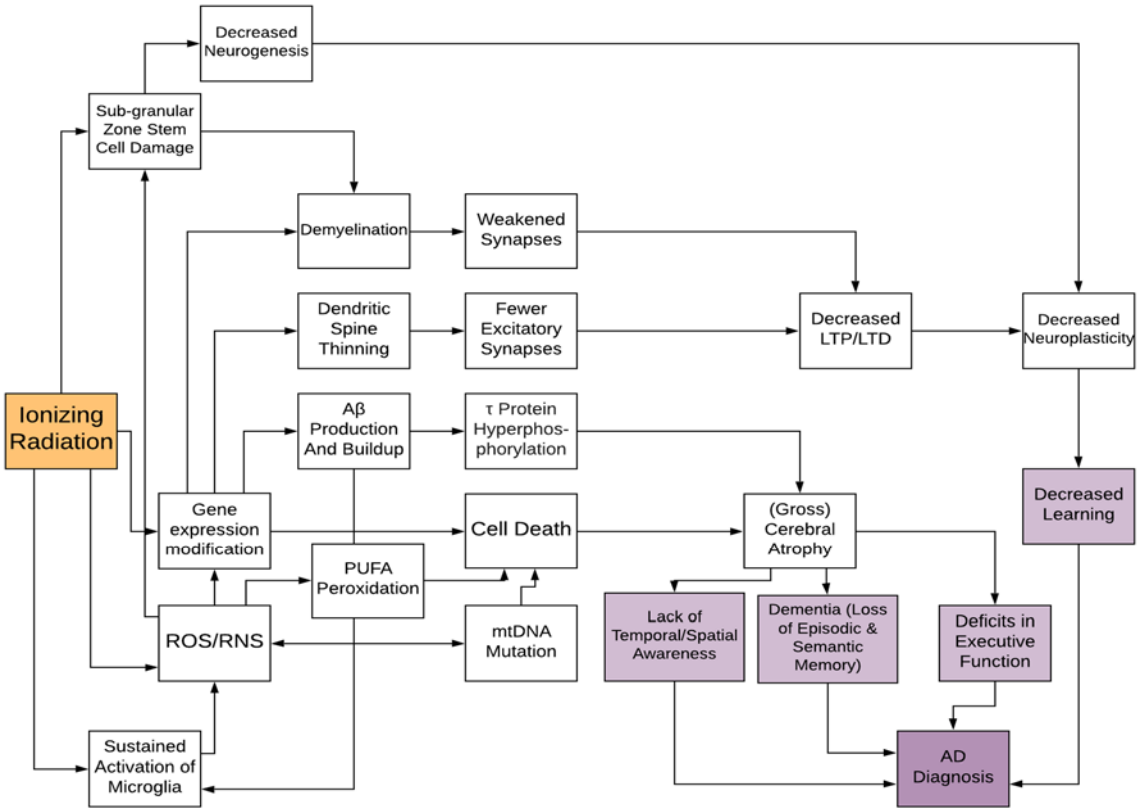

Figure 1. AOP for Space Radiation Induced Alzheimer's Disease

An adverse outcome pathway is a structured representation of a series of biological events beginning with a molecular initiating event and ending with an adverse outcome. Causally connected key events are linked together with key event relationships. Key events progress from simple to complex in biological organization, for example from sub-cellular to cellular to tissue to organ system to an adverse outcome to the organism. The adverse outcome is typically defined by a regulatory requirement in either individuals or populations. Key events are evaluated for essentiality which relates whether the downstream key events or adverse outcome are prevented if an upstream key event is blocked. Key event relationships are evaluated for biological plausibility and how well the mechanistic relationships of the pathway are understood. That is to say, how well one can predict what changes when the pathway is disturbed. (Bal-Price & Meek, 2017)

Figure 1 presents the adverse outcome pathways that have been identified which link ionizing radiation exposure to Alzheimer's disease. As multiple pathways may contribute to the advancement from radiation insult to disease, the full scope of the current understanding regarding the key events and key event relationships are shown in single figure.

Table 1 presents the level of evidence for each key event relationship for the adverse outcome pathway shown in Figure 1, along with comments, and references for the specific evidence.

Table 1. Key Event Relationships for Adverse Outcome Pathway for Space Radiation Induced Alzheimer's Disease

Key Event relationships	**Evidence Level**	**Notes**	**Papers**
Ionizing Radiation to Subgranular Zone damage	[A] *in vivo* rat [B] *in vivo* rat, *in vitro* human [C] *in vivo* rat, *in vivo* mouse, *in vivo* human (high-dose)	---	Hodges et al., 1998[A]; Panagiotakos et al., 2007[B]; Huang et al., 2012[C]
Ionizing Radiation to Gene expression modification	[D] *in vivo* mouse [E] *in vivo* mouse	altered signaling pathways in axon architecture, blood vessel formation, metabolism, neuroplasticity, memory, learning, and LTP/LTD	Lowe et al., 2009 [D]; Yin et al., 2003[E]
Ionizing Radiation to ROS/RNS	*in vivo* rat, *in vivo* mouse, *in vivo* human (high-dose)	---	Huang, et al., 2012
Ionizing Radiation to sustained activation of microglia	[C] *in vivo* rat, *in vivo* mouse, *in vivo* human (high-dose) [F] *in vitro* human, *in vivo* human (high dose)	---	Huang, et al., 2012[C]; Azzam, et al., 2012[F]
Subgranular Zone damage to decreased neurogenesis	*in vivo* rats, *in vivo* mouse, *in vivo* human (high-dose)	---	Huang et al., 2012
Subgranular Zone damage to demyelination	*In vivo* rat, *in vitro* human	---	Panagiotakos et al., 2007
Gene expression modification to Aβ plaque production and buildup	---	Aβ production is triggered by the Amyloid Precursor Protein	Bear et. al, 2009
Gene expression modification to cell death	---	Assumption that DNA mutations will trigger preprogrammed apoptosis	---
ROS/RNS to Gene expression modification	*in vitro* human, *in vivo* human (high dose)	---	Azzam et. al, 2012
ROS/RNS to PUFA peroxidation	*In vitro* human, *in vivo* mice	---	Finkel & Holbrook, 2000

ROS/RNS to mtDNA modification	[G] *in vivo* human [F] *in vitro* human, *in vivo* human (high dose)	---	Indo et al., 2007[G]; Azzam, et al., 2012 [F]
Sustained activation of microglia to ROS/RNS	[C] *in vivo* rat, *in vivo* mice, *in vivo* human (high-dose) [H] *in vitro* human	---	Huang, et al., 2012[C] Fialkow, et al., 2006 [H]
Demyelination to weakened synapses	---	Neurobiology basis; Demyelination leads to reduced conduction velocity	Bear et al., 2009
Dendritic spine thinning to fewer excitatory synapses	---	Neurobiology basis; Demyelination leads to reduced conduction velocity due to protective qualities of fat	Bear et al., 2009
Aβ production and buildup to tau protein hyperphosphorylation	---	Neurobiology basis; Aβ/Tau tangle theory of AD, Aβ plaques do not lead to tau tangle formation, but the two events are highly correlated, and are considered to be the hallmarks of AD.	Bear et al., 2009
Aβ production and buildup to sustained activation of microglia	*In vitro* human, *in vivo* rats	---	Tuppo & Arias, 2005
PUFA peroxidation to cell death	[I] *in vivo* human [F]*in vitro* human, *in vivo* human (high dose)	---	Pratico & Sung, 2004[I] Azzam et al., 2012[F]
Weakened synapses to decreased LTP/LTD	---	Neurobiology basis; LTP/LTD depends on synaptic strength at NMDA and AMPA receptors	Bear et al., 2009
Fewer excitatory synapses to decreased LTP/LTD	---	Neurobiology basis; LTP/LTD depends on synaptic strength at NMDA and AMPA receptors	Bear et al., 2009

Tau protein hyperphosphorylation to cerebral atrophy	---	Neurobiology basis; Aβ/Tau tangle theory of AD, which states that tau tangles are directly correlated with neuronal death due to structural damage	Bear et al., 2009
Cell death to cerebral atrophy	---	Definition of atrophy is mass cell death	---
mtDNA mutation to cell death	---	Assumption that mtDNA mutations will trigger preprogrammed apoptosis	---
mtDNA mutation to ROS/RNS (and vice versa)	*in vivo* human	---	Indo et al., 2007
Decreased LTP/LTD to decreased neuroplasticity	---	Neurobiology basis; Based on current theory that LTP/LTD are fundamental to learning processes and synaptic modulation processes fundamental to the concept of neuroplasticity.	Bear et al., 2009
Cerebral Atrophy to Lack of temporal/spatial awareness	---	Loss of neurons in temporal lobe and hippocampus lead to hippocampus dysfunction; hippocampus is responsible for temporal and spatial awareness	---
Cerebral Atrophy to dementia	---	Loss of neurons and neuronal connection leads to cerebellar dysfunction.	---
Cerebral Atrophy to deficits to executive function	---	Loss of neurons in prefrontal cortex and frontal lobe lead to malfunction of frontal lobe, which is responsible for higher-level function	---

Decreased neuroplasticity to decreased learning	---	Neurobiology basis; based on current theory that neuroplasticity underlies our learning processes	Bear et al., 2009

Discussion

Much of the proposed adverse outcome pathway is based on *in vivo* rat and mouse data. It should be acknowledged that these models, though reliable, should be carefully considered in terms of translation to human expression. Secondly, note that many of the events in the pathway have neurobiological or neurocognitive foundations. It is fair to assume that the delicate balance of the neural environment can be disrupted by events such as imbalance in neurovascular structure, protein misfolding, and cellular atrophy. More complicated cognitive processes in the brain such as learning and memory, which we are still only beginning to understand, outwardly rely on neurobiological functions such as LTP and LTD. Though it is generally agreed upon in the fields of neuroscience and cognitive sciences how such processes underlie critical neurological function, these neurobiological underpinnings remain hypothetical in nature and require further study.

Cognitively speaking, it is well established that loss of grey and white matter leads to dysfunction of the brain area affected by the atrophy. Although specific cognitive functions cannot be completely isolated to just one portion of the brain, it has been identified that certain neural connections in the brain are more relevant for certain cognitive functions than others. The hippocampus, for example, is a local site of cognitive functions such as episodic memory and spatial awareness, and lesions to this bilateral structure found in the temporal lobe lead to marked deficits on tasks that require such functions. Another example of this is the frontal lobe, where the cognitive skills of executive function have been found to be localized. Damage to this region has been inexplicably linked to deficits on tasks such as the Wisconsin Card Sorting Task and the Stroop task. The linkage of such deficits due to low-dose radiation to a diagnosis of Alzheimer's is useful in its implications for radiation sources on Earth, as well as in its pathology, which could hold more clues to the treatment of Alzheimer's.

Summary

It should be noted that there are a few caveats for an adverse outcome pathway relating low-dose ionizing radiation to AD development. Firstly, it is important to note that although some arrows in the diagram are causal, others are only correlational. This can be attributed to the fact that there remains no answer to the question of what causes AD. Though it is hypothesized that factors such as Aβ plaque production, neuroinflammation, and oxidative stress all contribute to the neurodegenerative disorder, efforts to inhibit the aforementioned processes have not stopped, or even slowed, the progression of the disease. A concrete example of a correlational (rather than causal) relationship can be seen in the middle of the pathway, which shows the module of "Aβ production and buildup" leading to "tau protein hyperphosphorylation". Aβ protein accumulation does not cause tau protein hyperphosphorylation, but the two are very clearly correlated, not only with each other, but also with the progression of AD as a whole. AD, currently, does not have a

cure, nor does it have a definitive cause. The modules on the left side of the pathway certainly contribute to the changes seen on the right side of the pathway, and it is true that these functional changes lead to the cognitive deficits seen in AD, but efforts to halt both earlier and later processes have not proven to be beneficial to AD patients.

Secondly, rat models, non-human primate (NHP) models, and human *in vitro* models are not wholly accurate representations of the *in vivo* cellular responses seen in humans as a result of ionizing radiation. Though trends in these models may accurately reflect the cellular responses of radiation on humans, they may not be entirely correct and require careful extrapolation and analysis. Neurological changes observed in irradiated rats is only a semi-comparable way of analyzing cognition, as behavioral neuroscience is not a replacement for cognitive neuroscience.

Along the same vein, it is important to note the differences in environments between rat models and human astronauts. Space radiation is made of many different particles for sustained periods of time, with different amounts of energy associated with different types of exposure to radiation. The barrage of naturally occurring high energy particles of space radiation have been modeled through ^{56}Fe, ^{137}Cs, and other particles within the lab, as it is almost impossible to recreate a true space-radiation environment in a lab. Recent efforts at the NASA Space Radiation Laboratory (NSRL) to develop the Galactic Cosmic Ray Simulator (GCRsim) were done to address some of these concerns (Norbury et al., 2016). The NSRL GCRsim facility conducted the first full exposures to animals in the Fall of 2018, so results are anticipated in the near future. That being said, the assumption that a lab created environment accurately mirrors the true space environment could lead to possible erroneous conclusions.

Extensive research done on rats, NHP, and *in vitro* levels within laboratories remains fundamental in beginning to uncover the effects of low-dose ionizing radiation on the nervous system as a whole. The theories, pathways, and models created as a result of these studies are highly necessary in understanding the risks astronauts are exposed to as they undergo missions to space. Increasing the amount of data is the only way to accurately predict radiation thresholds for safe space travel. Acute nervous system damage is significantly better understood than long-term CNS damage, but the latter should certainly be accounted for when planning further space missions.

In addition, on the pure neuroscience front, additional research on the effects of ionizing radiation should continue. Neuroinflammation, oxidative stress, and neurogenesis are processes affected by both aging and radiation, and the related biological and cellular factors of the events are still not well known. These deeply complicated processes are interlocked in ways that remain occluded to researchers in every discipline and require further investigation. The glutamatergic system, as well as the cerebrovascular system, have been found to be altered in AD patients, as well as in dementia patients. There remains little to no literature regarding the effects of low-dose ionizing radiation on the two aforementioned systems, and future studies should seek to correlate changes in the blood brain barrier, microvascular system, and glutamate receptors with radiation at <1 Gy.

Lastly, an understanding of Alzheimer's (as well as other neurodegenerative diseases) on behavioral, cognitive, and neuropathological levels remains insufficient to create causal linkages between symptomatic and underlying biological events. More studies are needed to fully uncover details regarding Alzheimer's, including but not limited to topics such as the glutamatergic system, vascular changes, and possible inhibitory processes to reduce these neuropathological damages from occurring in the first place. Interdisciplinary research is required for true quantitative analysis.

References

Ackermann, M., et al. (2013). Detection of The Characteristic Pion-decay Signature in Supernova Remnants. *Science*, 339, 807-811. doi: 10.1126/science.1231160.

Alzheimer's Association (2019). 2019 Alzheimer's Disease Facts and Figures. https://www.alz.org/media/documents/alzheimers-facts-and-figures-2019-r.pdf

Azzam, E. I., Jay-Gerin, J., & Pain, D. (2012). Ionizing Radiation-induced Metabolic Oxidative Stress And Prolonged Cell Injury. *Cancer Letters, 327*(1-2), 48-60. doi:10.1016/j.canlet.2011.12.012.

Azizova, T.V., et al. (2011). Cerebrovascular Diseases in Nuclear Workers First Employed At The Mayak PA in 1948–1972. *Radiation and Environmental Biophysics,* 50: 539. doi:10.1007/s00411-011-0377-6.

Bal-Price, A., & Meek, M. E. (2017). Adverse Outcome Pathways: Application to Enhance Mechanistic Understanding of Neurotoxicity. *Pharmacology & Therapeutics* 179, 84-95. doi:10.1016/j.pharmthera.2017.05.006.

Bear, M. F., Connors, B. W., & Paradiso, M. A. (2009). *Neuroscience: Exploring The Brain*. (Lippincot Williams & Wilkins, Philadelphia, PA, USA).

Belka, C., Budach, W., Kortmann, R. D., & Bamberg, M. (2001). Radiation Induced CNS Toxicity – Molecular And Cellular Mechanisms. *British Journal of Cancer,85*(9), 1233-1239. doi:10.1054/bjoc.2001.2100.

Blasko, I., Stampfer-Kountchev, M., Robatscher, P., Veerhuis, R., Eikelenboom, P. & Grubeck-Loebenstein, B. (2004). How Chronic Inflammation Can Affect The Brain And Support The Development of Alzheimer's Disease in Old Age: The Role of Microglia And Astrocytes. *Aging Cell*, 3: 169-176. doi:10.1111/j.1474-9728.2004.00101.x.

Bonfoco, E., Krainc, D., Ankarcrona, M., Nicotera, P., & Lipton, S. A. (1995). Apoptosis And Necrosis: Two Distinct Events Induced, Respectively, By Mild And Intense Insults with N-methyl-D-aspartate Or Nitric Oxide/Superoxide in Cortical Cell Cultures. *Proceedings of the National Academy of Sciences*, 92(16), 7162-7166. doi:10.1073/pnas.92.16.7162.

Bouhrara, M., Reiter, D. A., Bergeron, C. M., Zukley, L. M., Ferrucci, L., Resnick, S. M., & Spencer, R. G. (2018). Evidence of Demyelination in Mild Cognitive Impairment And Dementia Using A Direct And Specific Magnetic Resonance Imaging Measure of Myelin Content. *Alzheimers & Dementia,*14(8), 998-1004. doi:10.1016/j.jalz.2018.03.007.

Campion D., et al. (1999). Early-Onset Autosomal Dominant Alzheimer Disease: prevalence, Genetic Heterogeneity, and Mutation Spectrum. *The American Journal of Human Genetics*, 65(3), 664-670. doi: 10.1086/302553.

CDC (2019). “What Is Alzheimer's Disease?” Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 20 Sept. 2019, https://www.cdc.gov/aging/aginginfo/alzheimers.htm.

Cherry, J. D., Liu, B., Frost, J. L., Lemere, C. A., Williams, J. P., Olschowka, J. A., & O’Banion, M. K. (2012). Galactic Cosmic Radiation Leads to Cognitive Impairment and Increased Aβ Plaque Accumulation in a Mouse Model of Alzheimer’s Disease. *PLoS ONE,7*(12), e53275. doi:10.1371/journal.pone.0053275.

Cobley, J. N., Fiorello, M. L., & Bailey, D. M. (2018). 13 Reasons Why The Brain Is Susceptible to Oxidative Stress. *Redox Biology, 15*, 490-503. doi:10.1016/j.redox.2018.01.008.

Cucinotta, F. A., et al. (2001). Space Radiation And Cataracts in Astronauts. *Rad. Res.*, 156, 460-466.

Cucinotta F. A., Kim M. H., Chappell L. J., & Huff J. L. (2013). How Safe Is Safe Enough? Radiation Risk for A Human Mission to Mars. *PLOS One*, 8(10), e74988.

Drew, B., & Leeuwenburgh, C. (2002). Aging and the Role of Reactive Nitrogen Species. *Annals of the New York Academy of Sciences*, 959(1), 66-81. doi:10.1111/j.1749-6632.2002.tb02084.x.

Fialkow, L., Wang, Y., & Downey, G. P. (2007). Reactive Oxygen And Nitrogen Species As Signaling Molecules Regulating Neutrophil Function. *Free Radical Biology and Medicine, 42*(2), 153-164. doi:10.1016/j.freeradbiomed.2006.09.030.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, Oxidative Stress And The Biology of Ageing. *Nature*, 408(6809), 239-247. doi:10.1038/35041687.

Garrett-Bakelman E. F., et al. (2019). The NASA Twins Study: A Multidimensional Analysis of A Year-long Human Spaceflight. *Science*, 364(6436), 1-20. doi: 10.1126/science.aau8650.

Hall, E. J., & Garcia, A. J. (2006). *Radiobiology for the Radiologist.* (Lippincot Williams & Wilkins, Philadelphia, PA, USA).

Hodges, H., Katzung, N., Sowinski, P., Hopewell, J. W., Wilkinson, J. H., Bywaters, T., & Rezvani, M. (1998). Late Behavioural And Neuropathological Effects of Local Brain Irradiation in The Rat. *Behavioural Brain Research, 91*(1-2), 99-114. doi:10.1016/s0166-4328(97)00108-3.

Huang, T., Zou, Y., & Corniola, R. (2012). Oxidative Stress And Adult Neurogenesis—Effects of Radiation And Superoxide Dismutase Deficiency. *Seminars in Cell & Developmental Biology, 23*(7), 738-744. doi:10.1016/j.semcdb.2012.04.003.

Huff, J., et al. (2016). Evidence Report: Risk of Radiation Carcinogensis. National Aeronautics and Space Administration, Houston, TX USA.

Indo, H. P., et al. (2007). Evidence of ROS Generation By Mitochondria in Cells with Impaired Electron Transport Chain And Mitochondrial DNA Damage. *Mitochondrion*, 7(1-2), 106-118. doi:10.1016/j.mito.2006.11.026.

Itoh, A., et al. (1999). Impairments of Long-term Potentiation in Hippocampal Slices of β-amyloid-infused Rats. *European Journal of Pharmacology, 382*(3), 167-175. doi:10.1016/s0014-2999(99)00601-9.

Kametani, F., & Hasegawa, M. (2018). Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease. *Frontiers in Neuroscience, 12*. doi:10.3389/fnins.2018.00025.

Kinney, J. W., et al. (2018). Inflammation As A Central Mechanism in Alzheimer's Disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, 4, 575-590. doi: 10.1016/j.trci 2018.06.014.

Li, L., Wang, W., Welford, S., Zhang, T., Wang, X., & Zhu, X. (2014). Ionizing Radiation Causes Increased Tau Phosphorylation in Primary Neurons. *Journal of Neurochemistry, 131*(1), 86-93. doi:10.1111/jnc.12769.

Liu, Y., Fiskum, G., & Schubert, D. (2002). Generation of Reactive Oxygen Species by The Mitochondrial Electron Transport Chain. *Journal of Neurochemistry, 80*(5), 780-787. doi:10.1046/j.0022-3042.2002.00744.x.

Lowe, X. R., Bhattacharya, S., Marchetti, F., & Wyrobek, A. J. (2009). Early Brain Response to Low-Dose Radiation Exposure Involves Molecular Networks and Pathways Associated with Cognitive Functions, Advanced Aging And Alzheimers Disease. *Radiation Research, 171*(1), 53-65. doi:10.1667/rr1389.1.

Markesbery W. R., & Lovell M. A. (YEAR) Damage to Lipids, Proteins, DNA, and RNA in Mild Cognitive Impairment. *Archives of Neurology*, 2007; 64(7), 954–956. doi:10.1001/archneur.64.7.954.

Mikkelsen, R., & Wardman, P. (2003) Biological Chemistry of Reactive Oxygen And Nitrogen And Radiation-induced Signal Transduction Mechanisms. *Oncogene*, *22*, 5734-5754. doi:10.1038/sj.onc.1206663.

Mu, Y., & Gage, F. H. (2011). Adult Hippocampal Neurogenesis And Its Role in Alzheimers Disease. *Molecular Neurodegeneration, 6*(1), 85. doi:10.1186/1750-1326-6-85

Nelson, G. A., Simonsen, L., & Huff, J. L. (2016). Evidence Report: Risk of Acute and Late Central Nervous System Effects from Radiation Exposure. National Aeronautics and Space Administration, Houston, TX USA.

Norbury, J. W. (2016). Galactic Cosmic Ray Simulation at The NASA Space Radiation Laboratory. *Life Sciences in Space Research*, 8, 38-51. doi:10.1016/j.lssr.2016.02.001

Packer, L., Fuehr, K. (1977). Low Oxygen Concentration Extends The Lifespan of Cultured Human Diploid Cells. *Nature*, 267, 423-425.

Panagiotakos, G, et al. (2007). Long-Term Impact of Radiation on The Stem Cell and Oligodendrocyte Precursors in The Brain. *PLOS ONE, 2*(7). doi:10.1371/journal.pone.0000588

Parihar, V. K., & Limoli, C. L. (2013). Cranial Irradiation Compromises Neuronal Architecture in The Hippocampus. *Proceedings of the National Academy of Sciences,110*(31), 12822-12827. doi:10.1073/pnas.1307301110.

Patel, Z., et al. (2016). Evidence Report: Risk of Cardiovascular Disease and Other Degenerative Tissue Effects from Radiation Exposure. National Aeronautics and Space Administration, Houston, TX USA.

Raber, J., Allen, A. R., Weber, S., Chakraborti, A., Sharma, S., & Fike, J. R. (2016). Effect of Behavioral Testing on Spine Density of Basal Dendrites in The CA1 Region of The Hippocampus Modulated By 56Fe Irradiation. *Behavioural Brain Research, 302*, 263-268. doi:10.1016/j.bbr.2016.01.035.

Reames, D. V. (2013). The Two Sources of Solar Energetic Particles. *Space Science Reviews,* 175(1–4), 53–92.

Simpson, J. A. (1983). Elemental And Isotopic Composition of Galactic Cosmic Rays. *Annual Review of Nuclear and Particle Science,* 33, 232-381.

Townsend, L. W., et al. (2018). Solar Particle Event Storm Shelter Requirements for Missions Beyond Low Earth Orbit. *Life Sciences in Space Research*, 17, 32-39. doi:10.1016/j.lssr.2018.02.002.

Tuppo, E. E., & Arias, H. R. (2005). The Role of Inflammation in Alzheimer's Disease. *The International Journal of Biochemistry & Cell Biology*, 37(2), 289-305. doi:10.1016/j.biocel.2004.07.009.

World Health Organization (2006). *Health Effects of the Chernobyl Accident And Special Health Care Programmes*. B. Bennet, M. Repacholi, Z. Carr, eds. (Geneva, Switzerland).

Yin E., Nelson, D. O., Coleman M. A., Peterson L. E., & Wyrobek A.J. (2003). Gene Expression Changes in Mouse Brain After Exposure to Low-dose Ionizing Radiation. *The International Journal of Radiation Biology*, 79 (10). October 2003, 759-775. doi: 10.1080/09553000310001610961.

Yves C. (2000). Oxidative Stress And Alzheimer Disease, *The American Journal of Clinical Nutrition*, 71 (2). February 2000, 621-629. doi: 10.1093/ajcn/71.2.621s.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*
1-02-2020

2. REPORT TYPE
Technical Publication

3. DATES COVERED *(From - To)*

4. TITLE AND SUBTITLE

An Adverse Outcome Pathway for Potential Space Radiation Induced Neurological Diseases

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Mi, Kaitlyn; Norman Ryan B.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
651549.02.07.01

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

NASA Langley Research Center
Hampton, VA 23681-2199

8. PERFORMING ORGANIZATION REPORT NUMBER
L-21110

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

National Aeronautics and Space Administration
Washington, DC 20546-0001

10. SPONSOR/MONITOR'S ACRONYM(S)
NASA

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
NASA-TP-2020-220443

12. DISTRIBUTION/AVAILABILITY STATEMENT

Unclassified-
Subject Category 93
Availability: NASA STI Program (757) 864-9658

13. SUPPLEMENTARY NOTES

14. ABSTRACT Astronauts have begun to spend increasingly longer periods in space, putting themselves in foreign environments in order to explore the unknown. Space radiation is one of the largest health risks faced by astronauts on their missions. The space radiation environment has the ability to cause high levels of irreversible damage. Multiple sources of charged particle radiation exist in the space environment that may increase risk of carcinogenesis, degeneration of bodily tissue (e.g. gastrointestinal, cardiovascular, or pulmonary), acute radiation syndromes, and acute and late central nervous system (CNS) disorders. In order to help inform an understanding of the risk of degenerative CNS disease due to radiation exposure, an initial step is presented here to develop an adverse outcome pathway from radiation exposure focused on Alzheimer's disease.

15. SUBJECT TERMS

Alzheimer; Carcinogenesis; Degeneration; Environment; Space radiation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			STI Help Desk (email: help@sti.nasa.gov)
U	U	U	UU	23	19b. TELEPHONE NUMBER *(Include area code)* (757) 864-9658