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Review article

Microglia: Ally and Enemy in Deep Space

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ABSTRACT

In 2024 the first female astronaut will land on the moon, advancing our preparations for human missions to Mars. While on Earth we are protected from space radiation by our planet's magnetic field, on such deep space voyages astronauts will be exposed to high energy particles from solar flares and galactic cosmic rays (GCR). This exposure carries risks to the central nervous system (CNS) that could jeopardize the mission and astronaut health. Earth-bound studies have employed a variety of single-beam and sequential radiation exposures to simulate the effects of GCR exposure in rodents. Multiple studies have shown that GCR simulation induces a maladaptive activation of microglia – the brain-resident immune cells. GCR simulation also induced synaptic changes resulting in lasting cognitive and behavioral defects. Female and male mice show different susceptibilities to GCR exposure, and evidence suggests this sexually dimorphic response is linked to microglia. Manipulating microglia can prevent the development of cognitive deficits in male mice exposed to components of GCR. This discovery may provide clues towards how to protect astronauts' cognitive and behavioral health both during deep space missions and upon return to Earth.

1. Introduction

In 2024, astronauts will begin an extraordinary journey that will take them back to the Moon in preparation for the first human voyage to Mars. During space travel, mission success depends upon astronauts' intact cognitive function. A potential threat to neurological health is that astronauts will experience far higher radiation loads on the way to Mars than those incurred on the International Space Station (Nelson, 2016). As we wander farther from Earth's protective magnetic field, galactic cosmic rays (GCR) are added to the chronic stressors of space travel. To understand the impact of GCR exposure on astronaut neurological function, neuroscientists use preclinical rodent models. For rodent studies, simulated GCR mediated changes or alterations are defined as differences observed from unirradiated animals of the same biological sex. Simulated GCR exposure impacts cognitive and behavioral functions, synaptic integrity, and microglial activation (reviewed in (Cekanaviciute et al., 2018; Cucinotta and Cacao, 2019)). As the innate immune cells of the central nervous system (CNS), microglia serve as the first line of defense against insult to the brain, initiating neuroinflammation via cytokine and chemokine release (Prinz et al., 2019). However, neuroinflammation can have deleterious consequences if the release of proinflammatory factors does not resolve (Clayton et al., 2017; Hemonnot et al., 2019). Given the impact of GCR simulation on maladaptive microglia activation and cognitive function, targeting this cell population is a promising avenue for mitigating the consequences of GCR exposure on astronaut performance. Microglial depletion has proven to be an effective countermeasure able to reset maladaptive microglial activation and prevent cognitive and behavioral impairments in male mice (Krukowski et al., 2018a; Allen et al., 2020). Accumulating evidence indicates that female and male mice have different responses after GCR exposure, and that this may be related to sex-specific differences that drive microglia response. It is crucial to understand the sex-specific effects of GCR exposure on brain function to predict and treat adverse pathology that could result from prolonged spaceflight. Here we review the most recent work on the physiological and pathological roles of microglia and their relationship with biological sex and

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cognition under GCR exposure.

2. Microglial homeostasis, synaptic remodeling, and sexual dimorphism on earth

While our interests lie in the functionality of the brain and microglia among the stars, their biology evolved in the context of Earth's magnetic field and gravity. We therefore briefly preface this review with the known functions of microglia in the healthy and unhealthy brain. Microglia are the immune cells in the central nervous system (CNS), and represent approximately 10 % of CNS cells (Prinz et al., 2019; Salter and Stevens, 2017). These cells originate from erythromyeloid precursors that arise in the yolk sac between E8.5 and E9.5 and migrate into the CNS via blood circulation by E10.5 (Ginhoux et al., 2010; Saijo and Glass, 2011). Upon entry into the CNS, microglia remain isolated from peripheral immune cells (Saijo and Glass, 2011) and maintain their homeostasis entirely by self-renewal (Elmore et al., 2015; Li and Barres, 2018). Microglia present different morphological and transcriptomic features during different phases of development (Dubbelaar et al., 2018; Lenz and Nelson, 2018). During embryonic and early postnatal stages, microglia display a "generic macrophage"-like amoeboid morphology and expression profile, indicative of activation and high proliferation (Bennett et al., 2016; Prinz et al., 2019). Microglia actively participate in the formation and wiring of the developing neonatal brain circuitries by regulating synaptic pruning (removal of synapses, the site of cell-cell interaction) as well as spinogenesis (formation of new spines, protrusion on dendritic shaft where excitatory synapses are located) (Lehrman et al., 2018; Paolicelli et al., 2011; Stevens et al., 2007).

In the mature brain, homeostatic microglia exhibit a characteristic morphology with ramified and dynamic processes that can cover areas up to 10x their soma (Prinz et al., 2019) and continuously scan the environment while interacting with neighboring cells (Colonna and Butovsky, 2017). Microglia are crucial in maintaining homeostasis and regulating physiological processes in the CNS. One of the key features of microglia is their ability to quickly respond to pathological conditions (such as mechanical tissue disruption, infection, protein aggregation, oxidative stress etc.). Microglial morphology, along with their transcriptome and secretome, can quickly change in response to environmental perturbations. A repertoire of pattern recognition receptors (PRRs) allow microglia to detect "danger signals" such as materials containing pathogen associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) including ATP or DNA released from neurons (Akira et al., 2006; Hirsiger et al., 2012; Kigerl et al., 2014). After detecting these signals, microglia migrate to the damage site and adopt an amoeboid-like "reactive" morphology to engulf those materials (Fan et al., 2017; Nimmerjahn et al., 2005). Alongside phagocytosis, microglia respond to CNS changes by secreting proinflammatory cytokines such as IL-1β, IL-6, and TNF-α as well as reactive oxygen species (ROS) that mediate wound healing and inflammatory responses (Labzin et al., 2018; Prinz et al., 2019). Interestingly, microglia-secreted ROS impact not only the surrounding neurons but also microglia themselves, prompting the production of proinflammatory substances and thus creating a self-propagating neurotoxic cycle (Rojo et al., 2014; Spencer et al., 2016).

2.1. Microglia remodel synapses as part of cognitive processes

In addition to engulfing metabolites and debris from apoptotic cells and secreting trophic factors to support neurons, microglia are involved in remodeling synapses (for review see: (Colonna and Butovsky, 2017; Kierdorf and Prinz, 2017; Tay et al., 2019; Tremblay et al., 2011)). Indeed, microglia continue to refine synaptic connections through phagocytosis across adolescence and adulthood (Galloway et al., 2019; Schafer et al., 2013; Tremblay et al., 2011). The complement system – a group of proteins that takes part in the immune response by binding and targeting cells and cellular debris for rapid elimination – 'tags' inactive or inappropriate synapses, thus promoting microglia-mediated synapse elimination (Presumey et al., 2017; Schafer et al., 2012; Stephan et al., 2012). Signaling between complement receptor 3 (CR3), a phagocytotic receptor on microglia, and its ligand complement component C3, are a key mechanism underlying the microglial engulfment of synapses (Schafer et al., 2012). Importantly, synapse remodeling by microglia is believed to be critical in cognitive processes such as learning and memory (Morris et al., 2013; Parkhurst et al., 2013).

2.2. Sex-specific features of microglia

Microglial cells display biological sex differences during development and in the adult rodent brain (Villa et al., 2019). Biological sex-specific distinctions are observed in microglial numbers, morphology, and brain distribution as well as at the functional and transcriptomic level (Crain et al., 2013; Guneykaya et al., 2018; McCarthy et al., 2015; Mouton et al., 2002; VanRyzin et al., 2019; Verkhratsky et al., 2019; Weinhard et al., 2018). Importantly, microglial sex dimorphism in rodents is also observed in response to a challenge. Insults to the female rodent's brain (such as stroke, liposaccharide stimulation and brain injury) display a dampened inflammatory response when compared to the male counterparts (Acaz-Fonseca et al., 2015; Bodhankar et al., 2015; Loram et al., 2012; Villa et al., 2018). Transcriptomic analyses have shown sex-dependent differential patterns that correspond with a neuroprotective state of female microglia (Villa et al., 2018). Moreover, the differential morphology and expression profile of rat female microglia after stress exposure could explain women's susceptibility to stress-linked psychopathology (Bollinger et al., 2016). It can be hypothesized that sex-dimorphic microglia features could contribute to the sex differences in the incidence and therapeutic responses of most CNS disorders. Nevertheless, more evidence is needed to elucidate the relevance of microglial sex-specific features in specific CNS pathologies.

3. Microglia, Biological Sex, and cognition after exposure to simulated Galactic Cosmic Rays (GCR)

On a three-year mission to Mars, astronauts will be exposed to an estimated cumulative dose of 24.5–36 cGy of galactic cosmic rays (GCR); 10 fold higher than on the ISS (Cekanaviciute et al., 2018; Nelson, 2016). The GCR spectrum is composed of 90 % protons, 9% He ions, and 1% heavy ions, or HZE particles (high charge Z and high energy E), with energy ranging between 0.1–1 GeV/n (Cekanaviciute et al., 2018; Mewaldt, 1994). The fluence and energy of this spectrum is modulated by the 11-year solar cycle (Nelson, 2016). To understand the long-term impact of radiation exposure on brain function, neuroscientists have relied on rodent models. Rodent studies using simulated GCR and HZE irradiation have identified a number of cellular and molecular changes summarized in (Cekanaviciute et al., 2018). Here we will discuss the current understanding of GCR exposure on microglia activation and its sex-specific impacts on cognitive output.

3.1. Single beam exposure

Initial rodent studies investigating the impact of GCR exposure on cognitive output focused on individual beam exposure. Proton radiation comprises the majority of *in situ* GCR exposure. Three months after irradiation with a 10 cGy dose of protons in male mice Raber and colleagues found a negative correlation between recognition memory and the number of newly born activated microglia (BrdU/CD68 positive) in the dentate gyrus (Raber et al., 2016), suggesting a link between increased microglia numbers and impaired memory functions. On the other hand, another study reported that 200 cGy proton irradiation in male mice did not affect contextual or auditory fear conditioning, nor microglia activation ($MHCII^{+}$) or numbers ($Iba\text{-}1^{+}$) in the hippocampus (Sweet et al., 2014). These discrepancies might be due to the different

doses, as it is common to see a bell curve response to GCR, where changes are detected after lower, but not higher, dose exposure. While many studies of proton irradiation and the brain exist in the literature, few also examine microglial responses, and the literature could benefit from expansion here.

^{4}He is the second most abundant component of the GCR spectrum. Radiation with 5 and 30 cGy ^{4}He increased ED1^{+} activated microglia in the male perirhinal cortex (PRC) (Parihar et al., 2018), but 50 cGy ^{4}He did not alter microglial abundance (CD11b^{+}CD45^{low}) in the male whole brain (Krukowski et al., 2018a). Proinflammatory cytokines were elevated in the male whole brain (Parihar et al., 2018). Furthermore, irradiated male mice displayed disrupted functional connectivity between CA1 of the hippocampus and the PRC (Parihar et al., 2018), which likely contributed to impairments in spatial, episodic, and recognition memory, deficits in cognitive flexibility, and reduced rates of fear extinction (Krukowski et al., 2018a; Parihar et al., 2018). Depression-like behaviors and anxiety effects were reported in (Parihar et al., 2018), but no effects on anxiety were seen in (Krukowski et al., 2018a). It is possible microglia-mediated synaptic engulfment plays a role in the disrupted functional connectivity and cognitive deficits induced by ^{4}He. However, 50 cGy ^{4}He increased neither complement receptor nor phagocytotic markers in microglia (Krukowski et al., 2018a). Nevertheless, microglia that repopulated the brain after temporary depletion (discussed in Section 4) had reduced C5aR (complement receptor) and LAMP1 (phagocytotic marker) levels that corresponded with rescue of ^{4}He induced cognitive deficits and increased pre-synaptic and decreased post-synaptic markers (Krukowski et al., 2018a). This suggests that although elevated microglial activation and cognitive deficits induced by ^{4}He irradiation may not be mediated by enhanced microglial synaptic engulfment, reduced phagocytotic activity may be protective.

Other studies investigate heavy ions less abundant in the GCR spectrum, including ^{56}Fe, ^{48}Ti, and ^{16}O. A negative correlation between recognition memory and the number of newly born activated microglia was found in the hippocampus of male mice 3 months after ^{56}Fe exposure (Raber et al., 2016). Both ^{48}Ti and ^{16}O radiation individually increased ED1^{+} activated microglia in the medial prefrontal cortex (mPFC) 15 and 27 weeks post-exposure (Parihar et al., 2016). The increases in activated microglia were accompanied by reductions in spine density, altered spine morphology, and increased PSD95 puncta in mPFC neurons. Furthermore, spine density and PSD95 puncta were significantly correlated (positively and negatively respectively) with recognition memory in male mice (Parihar et al., 2016). While these data do not directly show microglia interacting with synapses, they once again suggest synaptic remodeling as a mechanism by which microglia mediate neuronal and cognitive health.

Other single ion exposure investigations have used the transgenic APPswe/PSEN1dE9 (APP/PS1) mouse model of Alzheimer's disease, which develops accelerated Aβ plaque pathology. In Liu 2019, APP non-irradiated mice had higher levels of CD68^{+} activated microglia than WT mice, where CD68 was undetectable. Furthermore, female APP mice had higher CD68 levels than males. Irradiation with 10 cGy and 50 cGy ^{56}Fe reduced CD68 levels in female but not male APP mice 2 months later. However, irradiation did not change microglial abundance measured by Iba1, nor neuroinflammation assessed by stable binding of a translocator protein ligand in WT or Tg mice. Furthermore, there were no significant changes in presynaptic (SYP, VGluT2) or postsynaptic (PSD95, Homer-1) markers in any group. Nevertheless, radiation resulted in sex-specific and exacerbated transgenic behavioral phenotypes in locomotor activity (male and female), contextual fear conditioning (male), and motor learning (female) (Liu et al., 2019). Similar to Liu et al. Cherry et al. reported that ^{56}Fe irradiation in APP/PS1 male mice accelerated Aβ plaque pathology in male mice and impaired contextual fear (male) and recognition memory (female and male). However, no changes were measured in either CD68 or Iba-1 staining around hippocampus plaques (Cherry et al., 2012). In these two studies, Tg mice, in whom microglia were already highly activated, showed enhanced and sex-specific behavioral phenotypes after irradiation, compared to nonirradiated Tg counterparts (Cherry et al., 2012; Liu et al., 2019) and irradiated WT mice (Liu et al., 2019). This model suggests an interaction between microglial activation and irradiation which impacts behavior. However, pre-existing pathology in APP/PS1 mice obscures a clear interpretation of these results. It is possible that additional mechanisms beyond changes in microglial activation state mediate the effects of radiation on cognition. Liu et al. suggested behavioral changes in Tg mice could also be mediated by reactive oxygen species, mitochondrial dysfunction, and alternative modes of synaptic dysfunction, such as alterations in neurotransmitter release (Liu et al., 2019). Dose also matters, as the microglia activation and abundance in the wildtype controls in Liu 2019 was muted after 10 and 50 cGy exposure, but enhanced after 500 cGy ^{56}Fe exposure (Raber et al., 2016; Rola et al., 2008). Nevertheless, wildtypes male but not female controls showed changes in locomotor activity in response to ^{56}Fe exposure, further emphasizing sex-specific responses to irradiation (Liu et al., 2019).

A few caveats arise with single ion irradiation studies when attempting to relate their findings to the experience of deep-space travel. First, many ions used for irradiation represent only a small fraction of the GCR spectrum astronauts will experience. Thus, the radiation doses of these single ion studies may be far greater than realistic, even if the total radiation dose is comparable to cumulative GCR dose. For instance, 50 cGy ^{56}Fe is a far larger dose than the cumulative 30−40 mGy estimated for particles with $Z > 10$ on a 3 year trip to Mars (Nelson, 2016). Second, combinations of GCR components may have synergistic or competing effects (Raber et al., 2016). It is therefore important to test combined exposures of several GCR components, and to standardize simulated GCR doses to enhance inter-study comparability.

3.2. Multi-beam exposure

Expanding irradiation to three or six sequential beam components more closely simulates GCR and affects microglial activation, synapses, and behavior. Simulated GCR containing protons (60 %), helium ions (20 %), and oxygen ions (20 %) induced deficits in anxiety, social interaction, and recognition memory in male mice, concurrent with increased microgliosis (abundance as measured by Iba-1 staining in the hippocampus). These microglial increases corresponded with synaptic loss in the male hippocampus at 100 days post exposure. In contrast, irradiated females did not show cognitive or behavioral impairments, changes in microgliosis, or synaptic composition (Krukowski et al., 2018b). In another combination study, sequential irradiation of protons (60 %), ^{16}O (20 %), and ^{28}Si (20 %) induced higher home cage activity in the dark phase (males, 50 cGy), increased depressive behavior (males and females, 50 cGy), and impaired object recognition (males and females, 50 cGy and 200 cGy) 2 months post exposure (Raber et al., 2019). Here, cortical levels of activated microglia (CD68^{+}) increased following irradiation in female but not male mice, in contrast with the proton, helium, and oxygen irradiation study (Krukowski et al., 2018b). Importantly, microglia activation was measured in a different brain region (cortical vs hippocampus) and at a higher GCR total dose (200 vs 50 cGy). In a third model of multi-ion GCR exposure, rapidly delivered, sequential 6 ion irradiation of protons (50 %), ^{4}He ions (20 %), ^{16}O ions (7.5 %), ^{28}Si (7.5 %), ^{48}Ti ions (7.5 %), and ^{56}Fe ions (7.5 %) resulted in elevated anxiety (female), impaired recognition memory (female; male shams failed to perform, obstructing interpretation of irradiated males), and decreased memory retention in passive avoidance (male and female) measured 2 months post exposure. In the 6 ion study (Raber et al., 2020), there was a trend toward higher microglial activation (CD68^{+}) in the cortex of irradiated male mice (50 cGy), but this did not reach significance. Conversely, there was no microglia activation measured in female mice exposed to any dose (25, 50, 200 cGy) of the six ion GCR. Differences in microglial activation in male and female between the Raber 2019 and Raber 2020 studies could be the result of GCR ion

percentages differences. Despite behavioral and cognitive deficits in male mice, both studies from the Kronenberg group (Raber et al., 2020, 2019) found a positive correlation between microglia activation and brain derived neurotrophic factor (BDNF), a protein fundamental in neuronal and memory function (Miranda et al., 2019; Piepmeier and Etnier, 2015; Rashid et al., 2020). This puzzling finding suggests that microglial activation offers some neuronal health benefits not translated to cognitive performance. Overall, the activation of microglia by GCR exposure is associated with elevation of neuroinflammatory markers, synaptic changes, and behavioral disruption. Such effects may differ as a result of radiation dosage, brain region examined, and sexual dimorphism. Nevertheless, microglia have emerged as a potential therapeutic target to reduce the pathologies associated with GCR.

4. Countermeasures targeting microglia rescue cognitive deficits

In parallel with identifying the potential hazards of space travel, we need to identify countermeasures to avoid or mitigate risk. Evidence suggests temporary microglial depletion may be one way to mitigate GCR-induced pathologies. PLX5622 (PLX) is a CSF1-R inhibitor that induces depletion of microglia (90 %) within 3 days of beginning treatment and maintains the depleted state throughout the duration of treatment (Dagher et al., 2015; Elmore et al., 2014). Microglia repopulate from precursors in the CNS within seven days of ending PLX treatment (Dagher et al., 2015; Elmore et al., 2015, 2014). Brief microglia depletion and fast repopulation prevents the development of long term recognition memory deficits (novel object recognition), normally seen after whole body GCR helium irradiation in male mice (Krukowski et al., 2018a). Sustained treatment with PLX is also effective at recovering temporal order, novel place recognition, and fear extinction deficits found in males 4–6 weeks after irradiation (Allen et al., 2020). Microglia that repopulate after withdrawal of PLX demonstrate reduced markers of inflammatory and phagocytotic function (Krukowski et al., 2018a). Furthermore, the treatment increases pre-synaptic markers (synapsin-1) (Krukowski et al., 2018a) and decreases post-synaptic makers (PSD95) compared to irradiation alone (Allen et al., 2020; Krukowski et al., 2018a). These results suggests that microglial depletion prevents synaptic loss induced by irradiation, as microglia primarily phagocytose presynaptic terminals (Schafer et al., 2012), and GCR exposure alone increases PSD95 (Parihar et al., 2016). Interestingly, sustained PLX treatment, a condition that causes persistent lack of microglia, is unable to rescue changes in intrinsic and synaptic excitability induced by helium irradiation, and disruptions in regional connectivity may remain, despite recovery of behavior (Allen et al., 2020). It is important to note that during PLX treatment microglia in the brain are depleted by 90 %. Thus while many effects of GCR are ameliorated (Allen et al., 2020), homeostatic functions of microglia can't be studied while PLX is sustained. In contrast, short term PLX treatment is effective against GCR-induced pathology, and allows microglia to repopulate with reduced inflammatory and phagoctyotic function (Krukowski et al., 2018a).

Both PLX5622 studies above were conducted using a single radiation exposure, but the timing and duration requirements of such a treatment may differ under the prolonged radiation of long-duration space flight. Fortunately, PLX provides a broad and flexible administration window, as it was effective when administered 1–2 weeks following irradiation and showed long term efficacy whether it was withdrawn after 15 days or sustained throughout testing (Allen et al., 2020; Krukowski et al., 2018a). It is important to consider that systemically delivered CSF1R inhibitors affect other cells of the peripheral immune system. Indeed, 3 weeks of PLX treatment leads to changes in $CCR2^{+}$ monocyte progenitor cells, $CX3CR1^{+}$ bone marrow-derived macrophages, $CD117^{+}$ hematopoietic progenitor cells, and $CD34^{+}$ stem cells. Furthermore, many of these effects persist an additional 3 weeks after cessation of the inhibitor (Lei et al., 2020). Previous publications have reported 14 days of PLX treatment has no impact on the percentage of $Ly6C^{hi}$ monocytes in circulation (McKim et al., 2018; Sawicki et al., 2019). Understanding how these peripheral cell populations would be impacted by GCR exposure and potential CSF1R inhibitor treatment remains to be determined. Finally, the necessity and efficacy of this countermeasure may differ by sex, given the possibility that female mice are resilient to microglial activation (Krukowski et al., 2018b; Raber et al., 2020) and have a different profile of behavioral impairments compared to males in response to multi-ion simulated GCR. Thus far, PLX treatment has not been tested as a countermeasure to GCR–type exposures in female mice.

5. Summary

Microglia play an important role in the CNS, scavenging cellular debris, remodeling synapses, and producing cytokines and chemokines. Despite the variety of deep space radiation paradigms (individual vs multi-ion) employed in initial rodent studies, it is clear microglial activation regulates maladaptive cognitive and behavioral dysfunction associated with GCR exposure. Rodent analogues of GCR exposure allow neuroscientists to report significant changes in CNS health and behavior and make informed translational models of the risk astronauts will face in deep space. From these models, NASA may set thresholds at which biological effects of radiation are unacceptable risks, given concerns about mission integrity and astronaut health. An important difference between experimental paradigms and long duration missions is the use of single exposures or sequential events in a short window. While radiation loads in experimental paradigms are calculated to approximate loads experienced over three years of travel to Mars, in reality, this load will be distributed across that period. Microglial and CNS responses to acute and chronic irradiation may differ as repair mechanisms and other homeostatic processes transpire concurrently with chronic irradiation. Chronic irradiation may constantly promote microglial activation and neuroinflammation, changing the effective parameters of countermeasure administration. While temporary microglial depletion emerges as a promising countermeasure to space-radiation insult, so far it has only been tested in male mice. In fact, the majority of studies on simulated GCR irradiation to date have been done only in male rodents, and our only data on the human health effects of deep space come from the all-male Apollo missions. Of the total people who have traveled to space, only 11.5 % are female. NASA's most recently graduated astronaut class, Group 22, is 45 % female. Preclinical studies report sex-dimorphic differences in response to GCR; thus, understanding these differences in females, including the need for and effects of countermeasures such as PLX5622, is critical to the integrity of future deep space missions to the moon and Mars. Further work exploring the cycle of space-radiation insult, microglial activation, and cognition should also take into account the role of biological sex.

Declaration of Competing Interest

The authors report no declarations of interest.

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