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NEUROBIOLOGICAL PROBLEMS IN LONG-TERM DEEP SPACE FLIGHTS

M. E. Vazquez

Biology Department, Brookhaven National Laboratory, Upton, New York, 11973 U.S.A.

ABSTRACT

Future missions in space may involve long-term travel beyond the magnetic field of the Earth, subjecting astronauts to radiation hazards posed by solar flares and galactic cosmic rays, altered gravitation fields and physiological stress. Thus, it is critical to determine if there will be any reversible or irreversible, detrimental neurological effects from this prolonged exposure to space. A question of particular importance focuses on the long-term effects of the space environment on the central nervous system (CNS) neuroplasticity, with the potential acute and/or delayed effects that such perturbations might entail. Although the short-term effects of microgravity on neural control were studied on previous low earth orbit missions, the late consequences of stress in space, microgravity and space radiation have not been addressed sufficiently at the molecular, cellular and tissue levels. The possibility that space flight factors can interact influencing the neuroplastic response in the CNS looms critical issue not only to understand the ontogeny of the CNS and its functional integrity, but also, ultimately the performance of astronauts in extended space forays. The purpose of this paper is to review the neurobiological modifications that occur in the CNS exposed to the space environment, and its potential consequences for extended deep space flight. Published by Elsevier Science Ltd on behalf of COSPAR

INTRODUCTION

Manned space exploration plans for the next century include a piloted mission to Mars. However, for such a mission, humans must be protected against the harsh environment of space, in particular, against the hazards of ionizing radiation and microgravity. In long-term deep manned space flights, the main goal is effective functioning of a crew enclosed in a confined environment, and subjected to continuous operational and environmental stress. When humans are exposed to the conditions of space, a number of neurologic disorders emerge. These pathological changes affect a variety of neural systems ranging from motor to sensory functions, and the effects can be long lasting. However, the nature of the functional and structural mechanisms underlying these changes is unknown. Nevertheless, it is now accepted that these changes are the manifestation of an active process of neural plasticity. Only a few *in vivo* and *in vitro* studies have tested this possibility, suggesting that the space environment (mainly altered gravitational fields) can affect adult and developmental neural plasticity processes. Furthermore, several studies suggest that chronic exposures to space radiation might produce effects similar to aging and neurodegeneration. Thus, it is critical to determine if neural cell populations can function properly under the complex multi-stressor environment encountered in long-term deep space flights.

In this review, I attempt to identify and assess the cellular and molecular problems related to the CNS plasticity that might be associated with long-term space flight. This paper is not intended to be a comprehensive review of the very broad topics of the gravitational biology and radiobiology. Rather, I focus on emerging experimental data from ground-based and space flight experiments on cellular, molecular and tissue effects relevant to neural cells in mammalians. It should also be recognized that other brain cell populations (glial and epithelial cells) and structures (blood brain barrier) are likely, or have been observed to be involved, at some level, in the brain responses to the space environment.

The Space Environment

During extended deep space flights, astronauts will be exposed to a complex environment composed of multiple acute and chronic stressors such as microgravity; closed environmental life support systems, and chronic low-dose exposure of

ionizing radiation. Moreover, isolation, confinement, and sensory deprivation will characterize this environment, and is expected to put a heavy demand on astronauts' physiology and psychology (Newberg, 1994). In addition, other interacting factors such as the absence of cues for circadian rhythms and gravity-dependent alterations in fluid transport/mixing processes can affect basic biological functions (Hughes-Fulford, 1991).

Space radiation environment. Space radiation is one of the primary environmental hazards associated with interplanetary space flight (Blakely and Fry, 1995). Exposure to the exo-magnetospheric space environment is characterized by the presence of complex mixed radiation fields. The major sources of radiation are solar disturbances and galactic cosmic rays (GCR). The components of this radiation are energetic charged particles, protons as well as fully ionized nuclei of all elements. Of particular concern are the high-Z and -energy (HZE) particles, with broad energy spectra at low fluence rates (Badwar, *et al.*, 1994). HZE particles especially Fe and its secondary fragmentation products, are of particular concern due to their high charge and energy deposition. Although, in deep space travel the GCR will be attenuated and fragmented by electromagnetic and nuclear interactions in shielding material, crewmembers will still be exposed to considerable radiation from both primary and secondary nuclei (Wilson, *et al.*, 1995). The importance of heavy ions stems from the inability to effectively shield against them and the potentially greater biological effect they have on post-mitotic cells. Finally, the Sun presents a major radiation hazard for astronauts traveling outside of Earth's protective magnetosphere. Periodic events such as solar flares and coronal mass ejection could prove deadly without proper protective measures.

Microgravity: Neurological Reactions. Short-term exposure to microgravity produces several neurologic changes in which the Space Adaptation Syndrome (SAS) is by far, the most studied. This syndrome was experienced by two-thirds of space travelers, and it is characterized by symptoms ranging from headache and stomach awareness to nausea and vomiting, beginning shortly after entry into orbit (Fujii and Patten, 1992). Typically, symptoms abate within 1 to 14 days into the flight, although the rate of recovery, degree of adaptation, and specific symptoms vary widely between individuals. However, it is unknown if the absence of symptoms reflects a complete adjustment to microgravity. It is possible that astronaut's ability to perform sophisticated tasks remains impaired long after the acute space sickness recedes. Moreover, other sensory-motor alterations, cognitive deficits, vegetative disorders, bone decalcification, muscular atrophy as well as changes in sleep-wake regulation occur during long-term space flight affecting human performance (Newberg, 1994).

Once returned to Earth, another adaptive period resulted. The time required to fully readapt to preflight levels after very long periods of exposure to microgravity is unknown. Missions up to 400 days showed that after landing, ataxia, perceptual illusions, neuromuscular weakness, and fatigue play critical roles in astronaut health and re-adaptation to a 1g environment (Fujii and Patten, 1992). Explanations of these phenomena include central changes due to learning of new perceptual and motor skills and/or effects of spatial disorientation (Gerstenbrand and Muigg, 1993). Overall, this indicates that such changes are largely due to lasting effects of adaptation of both central motor programs and the proprioceptive system. The suggested mechanism responsible for these changes is an active process of neural adaptation or neuralplasticity, a phenomenon in which neurons react to changed conditions by making new connections or strengthening existing ones.

DEVELOPMENTAL AND ADULT NEURALPLASTICITY

The CNS consists of an intricate cellular network of several billion neurons with an extraordinary ability to receive, store, and process information. Much of its function is ultimately dependent on the proper wiring for intercellular communication. During its development, each neuron migrates to a specific location where it synapses with the appropriate target cell(s). After an early period of synaptogenesis followed by the elimination of excess nexi and neuronal death, neural connections, whether normal or abnormal, become more permanent but not static (Jacobson, 1993). Until only recently, most neuroscientists thought much of the brain was "hard-wired" from shortly after birth, with all the information-carrying pathways firmly and immutably formed. However, recent studies conjure up a radically different concept of the brain, as a network that is continually remodeling itself. The intrinsic capability of the brain to change its synaptic connections, or to form entirely new ones, is referred to as neural or synaptic plasticity (Purves and Litchman, 1985). Developmental and adult synaptic plasticity is necessary for determining and maintaining normal brain function but it is also critical for the restoration of function in neural tissues after trauma or disease (Jacobson, 1993). Furthermore, studies suggest that mechanisms encountered during neuronal development are often the same that regulate plasticity and repair in the adult CNS (Finger and Almli, 1985).

Synaptic plastic mechanisms in the adult CNS can be divided into two broad groups, those associated with normal function (synaptic remodeling) and those associated with lesion-induced synaptic loss (reactive synaptogenesis). Synaptic remodeling is involved in learning, memory, and adaptation to new environments (Neill, 1995). Behavioral and cellular studies in humans and animals have revealed two phases of memory: a short-term phase lasting minutes and a long-term

phase lasting days or years. Short-term memories employ rapid and transient changes in synaptic efficacy produced by modulation of synthesis and release of neurotransmitters, receptor synthesis and changes in signaling pathways (Hawkins *et al.*, 1993). Also, new findings postulated that the formation of short-term memory requires integrin-mediated mechanisms to regulate the dynamic of synapse structure or signaling events between synapses and the microenvironment (Jones and Grooms, 1997). In contrast, long-term memory is associated with a cellular program of gene expression, altered protein synthesis, and the growth of new synaptic connections. The best known and studied mechanism for synaptic remodeling in the mature CNS is long-term potentiation (LPT) in the hippocampus. The maintenance of LPT involves both functional and structural changes, including the formation of new synapses using mechanisms similar to those involved in brain development (Bailey *et al.*, 1993).

Reactive synaptogenesis is characterized by the sprouting of intact axons to compensate for synaptic sites vacated by other axons that are degenerating or dying, accompanied by a rapid increase of microgrial cells and astrocytes (Neill, 1994). This neuroplastic reaction requires the formation of new processes (axonal or dendritic), elongation, branch and formation of synaptic contacts in a highly regulated and specific manner. It correlates with the expression of defined genes, including proteins involved in signaling (e.g. *src*, NCAM, integrins), transcription factors (e.g. c-jun, *CREB*), enzymes, trophic factors (BDNF and its receptors) and structural proteins (e.g. actin and tubulin isoforms) (Caroni, P, 1997, Lyford, *et al.*, 1995). The formation and growth of local branches (sprouting) is controlled by mechanisms in the target region. In addition, the expression of growth-associated proteins such as GAP-43 and CAP-23 in neurons alters the threshold for nerve sprouting and potentiates its efficacy (Link *et al.*, 1995). It is important to stress that glial cells are involved in both synaptic remodeling and reactive synaptogenesis responses. The role for glial cells in plastic changes is based on the modulation of transmitter uptake or on regulation of the extracellular ion composition (Muller, 1992). Typical glial responses in synaptoplastic responses is the increase in number after injury, aging and neurodegeneration (Neill, 1995).

In the normal brain, regeneration and plasticity work as compensatory mechanisms, improving neuritic sprouting and facilitating synaptic re-establishment in response to learning and cell loss due to minor injuries or aging (Montague, 1993). The aging brain increasingly depends on its plastic quality (Agnati, *et al.*, 1992). The aged brain is subject to greater challenge (e.g. cell loss, environmental toxic agents, metabolic disorders, etc.), which, by being cumulative, elevates the risk of dysfunctional expression and age-related neurodegenerative disorders (Alzheimer's, Parkinson's, etc.). In addition, the aging brain is characterized by a decreased ability to respond adaptively to physiological-environmental stimuli and cell loss on both a cellular and system level (Walsh and Opello, 1992). Accumulated data supports the idea that extensive plastic changes occur in the adult CNS, and that these changes may be regulated by similar factors that are operative during early neural development (Finger and Almli, 1985). From this perspective, "development" may be an uninterrupted life-long process, and distinctions between early development, injury-induced and natural synaptic turnover may be unnecessarily arbitrary if all share a common mode of regulation.

It is well recognized that the developing CNS is extremely sensitive to environmental toxicants (radiation, chemicals, etc.). This sensitivity is attributed to the particular vulnerability of neuroblast to toxicants (radiation) and its easy involvement in cell death. Several factors determine its high sensitivity to toxicity-induced cell death (by necrosis or apoptosis) such as mitotic state and an initial phase of chemical cytodifferentiation prior to their actual morphological differentiation (Kameyama, *et al.*, 1994). Similar cytochemical changes are also encountered during neuronal plastic changes in adult neuroplasticity. Therefore, it is critical to determine if the mature CNS during periods of sustained plastic changes recapitulates its developmental sensitivity to environmental toxicants such as radiation. Indeed, a clear example is the hippocampus, where extensive neuroplastic changes occur during adult learning and memory and is a very vulnerable brain structure to insults such as trauma, ischemia, radiation, seizures, aging, and severe stress (McEwen, 1994). Thus, understanding how space flight stressors influence neuroplastic processes including its limits and conditions, will be an important step in evaluating the neurological risks of extended missions.

Neuralplasticity and Space Flight Adaptation

The current bibliography shows that with the exception of its importance to space motion sickness, the role of the CNS in the development of space flight-associated changes in health and performance has been generally overlooked. It should be considered, for instance, that the overt symptoms of the SAS, above-mentioned, might be accompanied by subtler but more pervasive readjustments of CNS adaptive changes in functional and structural plasticity. It was suggested that exposure to microgravity might have significant effects on the neuroplasticity of those nervous system structures and systems that sense or respond to gravitational forces (Newberg, 1994). These would include the sensory organs that respond to gravity (i.e. vestibular system), those parts of the brain that process gravity information, and the neural control of weight-bearing

muscles that act against gravity. However, it is unknown if microgravity exerts an effect on the CNS areas not directly involved with either sensing or responding to gravity.

Only a handful of *in vivo* studies has studied the effects of the space flight on the CNS plasticity. Nonetheless, results from the few animal studies conducted in space and ground-based experiments using simulated microgravity or hypergravity, suggest that exposure to gravity alterations does cause changes in the developing and mature nervous system (Krasnov, 1994). Changes observed in space flight experiments can be divided into two groups: first, morphological and biochemical changes that appeared during microgravity, and the second involved the changes occurred after landing. This division is the result of methodological limitations imposed by mission operations and flight hardware, determining that in the past, all brain tissues collected were obtained at different times after landing. This situation imposed an additional confounding factor in the study of microgravity changes during space flight. Other factors such as adequate controls, small sample size and short mission duration, limited our capacity to study potential long-term changes in brain areas induced by microgravity. Nevertheless, data collected from the Biosatellite program and Space Shuttle missions (SLS-1), revealed that young and adult rodents showed important changes in CNS areas that receive extero-, proprioceptive and vestibular inputs (cerebellar nodulus, somatosensory and visual cortex and caudate nucleus) (Krasnov, 1994). Other areas such as the reticular formation, hypothalamus, striatum, posterior cortex and pons medulla were also affected (Krasnov, 1994).

The data obtained suggests that microgravity indirectly induces changes in brain areas a result of a decrease of afferent input inducing a reduction in activity in pyramidal cells in the motor cortex, and a increase of activity in the visual cortex. Re-adaptation to Earth's 1-g level after landing is shown to induce changes in neuronal structures resulting from the increase in afferent inputs to the vestibular system and sensory-motor cortex, with a hypersensitivity to further stimuli (Krasnov, 1994). In general, the decrease in afferent input in the somatosensory and cerebellar nodulus cortex, induce two typical plastic neuroplastic responses:

a) Reactive synaptogenesis: In order to compensate the lack of afferent flow, neural cells respond with the creation of new synapses and neuronal circuits re-arrangements. This neural reaction is a typical regenerative response characterized by an increase of growth cones, dendrite spines and changes in the geometry and orientation of dendrites (Krasnov, 1994, Belichenko *et al.*, 1990).
b) Decrease of functional levels: The lack of afferent input generates a hypofunctional state in the target populations revealed by ultrastructural and neurochemical alterations such as decrease of total protein content, mRNA levels, reduction nucleoli size, decreased synthesis of neurotransmitters and neuropeptides (Krasnov, 1994)

Perhaps, the most studied system is the peripheral and central vestibular systems, since its putative functional alterations were involved in the ethiology of SAS. Several flight and ground-based studies using different species revealed important functional and structural adaptative changes (Ross, 1993). In general, the cellular modifications were characterized by changes in the number of synapses in rats exposed to altered gravity fields. It is thought that this synaptic response represents an adjustment of the CNS to the altered sensory input encountered in microgravity and hypergravity, and likely plays a role in the functional changes that occur in the vestibular system in microgravity.

Considerable attention has been directed to the suggestion of whether if the neural development can be affected by changes in gravity fields. Because these processes are regulated by both chemical and mechanical factors, gravity may play a crucial role as a stimulus for proper development of the nervous system. Therefore, during the Cosmos 1514 flight the brains fetuses, and pups exposed to space flight in utero, were examined morphologically and histochemically (Alberts *et al.*, 1985). Quantitative analysis of the cytoarchitecture of the neocortex showed signs of delayed migration of neuronal elements. In addition, fetuses showed a decrease of metabolic transport from vascular elements to nerve tissues in the rhombencephalon. An increase in the number of capillaries in fetal striatum was reported, suggesting a compensatory response to hypoxia due to reduced size and weight of the placenta of the flight mothers. Ultrastructural studies revealed some delay in neuroblastic differentiation as well as in developmental cytoskeletal changes in unmyelinated fibers, and growth cones of axons and dendrites in the hypothalamic supraoptic nuclei and its terminals in the eminence media. In contrast, ground-based studies showed that hypergravity can impair the normal histological, biochemical, and cytological organization of the brain and neural retina (Murakami and Fuller, 1985).

At the present time, the precise nature and sequence of the basic neurobiological changes induced by the space environment are not clear. However, it is possible to speculate about a general plastic response based on the limited current database. Microgravity and the stress response evoked by space flight conditions might induce directly (cellular level) or indirectly (tissue and system level) plastic modifications in response to de-conditioning, sensory-motor perturbations, cephalic-fluid shifts, metabolic and hormonal changes. It is unlikely that radiation will play an important role in the CNS adaptation to short-term fights. Nevertheless, its systemic influence could be important in the case of an acute high-dose exposure due to

a solar particle event. Animal studies suggest that the adaptative cellular changes observed during and after short-term space flight appear not to be different from those observed in normal brain synaptic plastic responses to deafferentation, trauma, or learning in 1-g environment (Krasnov, 1994). Typical neuroplastic molecular and cellular responses involve gene activation and expression leading to changes in cytoskeletal dynamics, trophic interactions and neurotransmitter levels. These mechanisms might induce neuritogenesis, sprouting-pruning, and reactive synaptogenesis. These changes are oriented to obtain an adaptative functional compensation in response to a decrease of afferent input and neuron activity induced by a radically different environment, with ultimate goal of maintain the functional integrity of the system (Figure 1).

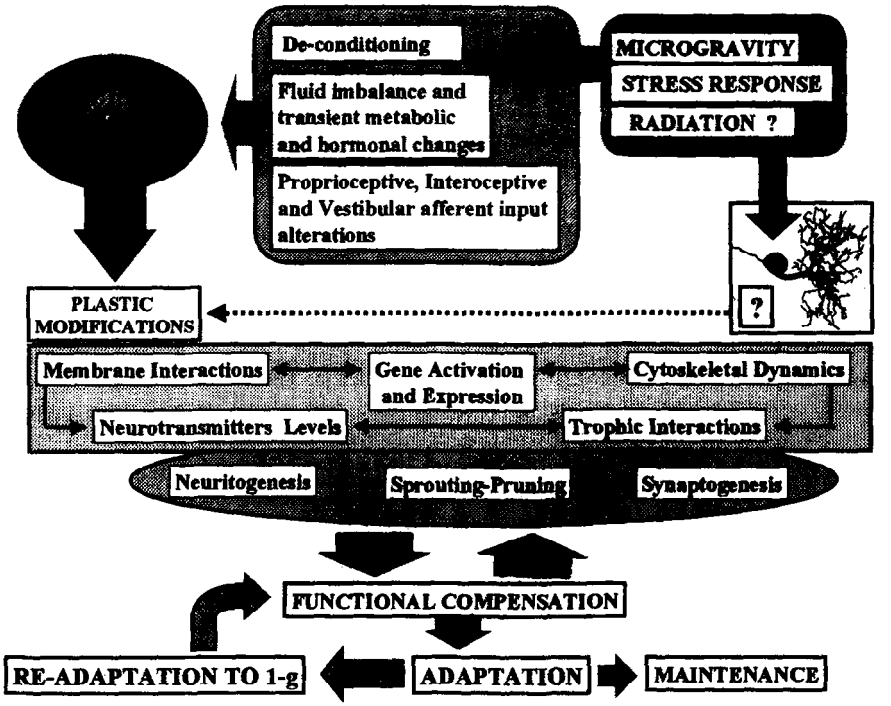

Fig. 1. Possible neuroplastic response to short-term exposure to space flight factors in the CNS.

These plastic changes appear to be effective in short-term space flight adaptation. However, it is unknown if these mechanisms can be sustained over long periods, or if these plastic changes can be transformed in maladaptative responses, impairing the CNS capacity to function in long-term missions. Furthermore, efforts to assess the neurobiological response to the space environment have been complicated by the considerable unknowns regarding the basic biological effects of space radiation and microgravity, particularly in relation to basic cellular functions and structures in eukariotes.

CELLULAR AND MOLECULAR EFFECTS INDUCED BY GRAVITY FORCES

Studies from space flights over the past three decades have demonstrated that there are extensive physiological changes in humans during space flight. The cause of most of these manifestations is not known but the general approach has been to investigate systemic and hormonal changes. However, data from the Biosatellite program, Skylab and Shuttle missions, support the idea that microgravity can influence basic biological mechanisms at the tissue, cellular, and molecular level.

Cellular and Molecular Effects

It is beyond the scope of this review, to provide a complete description of the cellular effects induced by the space environment (Hughes-Fulford, 1991, Moore and Cogoli, 1996). Nevertheless, ground-based and flight experiments using a variety of mammalian and non-mammalian cell types, have demonstrated that a variety of cell functions, are affected by gravitational alterations (Hughes-Fulford, 1991). Analyses of these results suggest that the chemical microenvironment and molecular transport could be affected by microgravity (Albrecht-Buheler, 1991). The majority of the gravitational studies to date indicate that cell regulatory pathways may be influenced by gravitational environment changes. Still, few cell

biology experiments have been performed in space flight and even fewer experiments have been repeated on subsequent flights. Nonetheless, the results indicate significant alterations in cell proliferation, cell growth, differentiation, metabolism, membrane properties, secretory capacity, electrolyte concentration, cytoplasmic streaming, and growth factor signal transduction (Moore and Cogoli, 1996). Suggested mechanisms include alterations in cell to cell communication, calcium levels, and transport of transmitters, receptor interactions, and the cell cytoskeleton dynamics (Albrecht-Buheler, 1991).

It is well know that altered gravity fields can modulate several cellular functions such as cell proliferation and differentiation. Possible molecular mechanisms that can explain these effects have been obtained in recent years indicating that gravity exerts its effect by modulating the expression of proto-oncogenes. Recent studies suggest that gravity may affect the intracellular signaling pathway activated by mitogenic stimuli such as growth factors, resulting in the modulation of proto-oncogene expression (Moore and Cogoli, 1996). Moreover, these results support the notion that microgravity can affect growth factor receptor mediated signal transduction, possibly because of a gravity-sensitive component in the cellular cytoskeleton and or PKC-mediated pathways (Cogoli, 1997). In addition, it has been postulated that the plasma membrane, which contains receptor and signal transduction proteins, is the interface between the proposed gravity sensitive intracellular cytoskeletal compartment and extracellular matrix compartment (Spooner, 1994).

Modern theories of cellular gravisensing mechanism suggest a unifying model based on the concept that cells use tensegrity architecture to organize their cytoskeleton and stabilize their form (Ingber, 1997a). The postulated cell tensegrity architecture is composed of discrete cytoskeletal filamental networks that mechanically link specific cell surface receptors (integrins) to nuclear matrix scaffolds and to potential transducing molecules related to the cytoskeleton (Ingber, 1997b). It has been postulated that cells use tensegrity to respond to environmental stress (gravity changes) affecting cellular cytoskeleton, and thereby control cellular biochemistry and gene expression. These models of gravity sensing could have important neurobiological consequences since the cytoskeleton and cell signal transduction pathways have been demonstrated to play a basic role in neural plasticity during development and maturity (Chambray and Deakin, 1991). Information about tensegrity models is largely lacking for neurons. However, evidence for a direct connection between the cytoskeletal network with the extra cellular milieu mediated by integrins at adhesion sites has been reported (Jones, 1996). In the developing and mature CNS, the extracellular matrix provides a source of extrinsic cues to guide determination of cell fate, neuroblast migration, axon outgrowth and synapse formation (Jacobson, 1993). Modulation of the function of the cytoskeleton by gravity might therefore, influence fundamental neural processes, and consequently have a high impact on neuronal functional and structural integrity.

Neurobiological Effects

Cellular effects. Based on the data presented above, the possibility that the gravity changes may produce a variety of changes in the function, morphology, biochemistry, and metabolism of neural cells, is likely. However, thus far little attention has been given to the space flight-induced effects on basic neurobiology processes. Therefore, it is unknown if neurons can sense and respond to gravity at the molecular, genetic, and cellular levels. The lack of adequate flight hardware to sustain neurons in culture, and the complexity of study neurons in such a complex situation are some of the causes of our extremely limited knowledge in the effects of microgravity on neurons. Nonetheless, two space flight *in vitro* studies, and one *in vitro* study using neural tissue developed under microgravity conditions have been reported. In one case Husson *et al.*, (in this volume) studied neuronal differentiation of co-cultures of neurons and myocites taken from amphibian (*pleurodeles laevi*), exposed to microgravity for 15 days during the BION 9 mission. Also, Vens *et al.* (1996) using a primary mouse cells from neonatal cerebellum tested several culture techniques and evaluated its morphological differentiation during the IML-2 mission. Finally, Viktorov *et al.*, (1988) studied the neural growth and differentiation in explant cultures taken from cerebellums of rats embryos developed in microgravity during the Cosmos 1514 mission. The analysis of these limited experiments suggested an apparent normal structural differentiation when neural cells were exposed for a short time to microgravity. Only Husson *et al.* (this volume) reported some neurite morphological (neurite swellings) abnormalities without apparent functional implications. A preliminary conclusion of these results suggests that isolated neural cells exposed to short-term space flight are insensitive to microgravity. However, the lack of cellular and molecular studies from space flight conditions, in addition to the well-know effects of microgravity on eukariotic cells, plus some limited ground-based studies, makes it difficult to confirm or exclude such an effect at the neuronal level.

As mentioned above, studies indicate that because of the effects of gravity on membrane sites and cytoskeleton, we can expect significant changes in the adjacent cortical cytoskeletal elements, particularly cortical actin, which, secondarily, may affect the entire cytoskeleton. Therefore, major cell functions that depend on specific cytoskeletal dynamics and properties may change also undergo corresponding alterations (Albrecht-Bueler, 1991). The cytoskeleton is the major internal structure defining the morphology of neurons, and that translational and post-translational events which modulate

cytoskeletal dynamics, account for the overall intracellular control of neuronal growth and some basic cellular functions (Cambray-Deakin, 1991). Changes in neuron differentiation mediated by interference with cytoskeletal-related mechanisms induced by simulated microgravity have been described (Gruener and Hoeger, 1991). These changes were expressed by altered neuron structure, neurite morphology, and growth kinetics, collectively resulting in abnormal synaptogenic capacity (Gruener and Hoeger, 1990).

It has been speculated that cytoskeletal abnormalities might increase the susceptibility of the neuron to other injuries (e.g., excitotoxicity) so that multiple stressor factors could culminate in production of disease (Brady, 1993). Xu *et al.* (1993) using a transgenic mice model with a defined abnormality of neurofilament synthesis, demonstrated that primary alterations in neurofilament production can lead to structural changes (axonal swellings) typical of several neurodegenerative diseases. Interestingly, space flight and ground-based *in vitro* studies showed similar neuronal morphological alterations (Husson *et al.*, this volume, Gruener and Hoeger, 1991). However, these findings need to be confirmed to discard artifactual phenomena produced by specimen processing. Nevertheless, it will be critical to confirm whether microgravity can disrupt neuronal structures (cytoskeleton, membrane, etc.) and/or metabolic pathways, changing the neuronal capacity to respond to a secondary stress factor such as radiation.

Gene expression in neurons. Neurons are characterized by tremendous physiological activity, which includes a high level of gene expression. About 30% of the neuron genomic DNA is transcribed as against 5 to 10% in other tissues (Rao, 1993). Likewise, transcriptional activity is two to three times more elevated in neurons as compared to other cells (Tobin and Khrestchatisky, 1989). Several studies indicated the importance of the role of the gene expression as a target of stimulus transduction in neural cells (Tully, 1997). The comprehension of the role of receptor-mediated generation of intracellular messengers and events in the coupling of cell stimulation to gene activation is a critical area of research, especially in order to understand how the cells respond to the space environment.

Current modern molecular hypothesis, the neuronal substrates of information processing and storage and plastic responses, requires biochemical events at the membrane level that are transduced through second-and third messenger systems in changes of gene expression, and finally in DNA plasticity (Tully, 1997). These events may result in the synthesis of proteins for ion channels, cytoskeleton, receptor molecules, enzymes and membrane components. In addition, the transcriptional regulation of gene expression is modulated by the arrangement of several transcriptional factors, such as the heterogeneous class of immediate early genes (IEGs), like *c-fos* and *c-jun* (Morgan and Curran, 1995). It has been shown that IEGs can be rapidly induced under conditions such as a) seizures, b) electrical stimulation, c) sensory stimulation, d) LPT, e) learning, f) circadian cycles, g) DNA damage and h) stress (Papa *et al.*, 1995). Moreover, IEGs expression changes were evident in several tissues and cells after the exposure to altered gravity (Cogoli, 1997, Ohnishi *et al.*, 1996) and ionizing radiation (Keyse, 1993, McLaughlin, *et al.*, 1993).

Ground-based studies showed that simulated microgravity can modulate neural gene expression *in vitro*. Lelkes *et al.*, (1996) using PC12 cells cultured under simulated microgravity (bioreactor), showed a specific enhancement in the expression of phenylethanolamine-N-methyl transferase, the enzyme responsible for the conversion of norepinephrine to epinephrine. Furthermore, the same investigators employing quantitative RT-PCR demonstrated a unique elevation of collagen type IV, an extracellular matrix protein (Galvan *et al.*, 1995). Moreover, new data shows that simulated microgravity alters the pattern of tyrosine phosphorylation in PC12 cells both for short-term (less than one hours) and for prolonged periods of time (10 days). These findings suggest that protein phosphorylation-dependent cellular signaling may be an important candidate for microgravity-sensitive sensing in neural cells (Lelkes *et al*, 1996). In sum, IEGs are rapidly induced by several extracellular stimuli and they can act as "third messengers" to regulate the expression of target genes that may be involved in the neurobiological response and adaptation to microgravity.

RADIATION EFFECTS

For decades it was thought that one of the more serious hazards of long-duration deep space flights might be the possible deleterious effect of HZE particle traversals or hits on the brain and eyes of astronauts (National Academy of Scences report, 1973, Gauger *et al.*, 1986). A new estimate (Curtis *et al*, this volume) predicts that on a three year journey to Mars at solar minimum, between 13 to 46% of neural cells will be traversed by a particle with a Z greater than 16 in certain CNS areas (retina, hippocampus, thalamus). This clearly demands that we define the damage, which may be incurred by heavy ion exposure to be anticipated ultimately with the added effects of microgravity. However, efforts to assess the radiation risks in space have been complicated by the considerable unknowns regarding the biological effects of heavy ions on neural cells, despite the fact that some *in vivo* studies suggest that chronic low-dose exposure to HZE particles might

produce effects similar to aging and neurodegeneration (Joseph *et al.*, 1992, Rabin *et al.* 1989, Lett *et al.* 1987). However, the basic mechanisms of HZE particle neurotoxicity remain to be elucidated.

Usually, studies on the effects of HZE particles on biological systems have addressed radiation-induced changes in mitotic cells (Kraft, 1988). The early responses to charged particle exposures include activation of DNA repair machinery induced by DNA damage, changes in gene expression, the initiation of programmed cellular responses such as apoptosis, and alterations in the tissue microenvironment (Goodhead, 1995). However, it should be taken into account that in general, *in vivo* and *in vitro* studies were carried out with single high-dose exposures that are very different from the "real" space environment. Furthermore, biological effects of these particles cannot be extrapolated in a straightforward manner from available data on x-rays. Although a significant amount of data on biological effects of gamma rays and neutrons has been obtained from atomic bomb survivors and animal studies, there is very little human epidemiology and basic data on neurobiological effects of heavy ions.

Cell survival and/or morphological studies have generated the characterization of the CNS as radiation tolerant to gamma and x-rays exposures (Walden and Farzaneh, 1991). However, little is known regarding its sensitivity to HZE particulate radiation even for these "classic," if incomplete, parameters, nor is there a great deal of information on the "functional" sensitivity of the CNS. Because of the highly differentiated, non-cycling nature of neurons, which is thought to confer radio resistance, relatively little research has addressed the effects of HZE exposure on neurons at the cellular and molecular levels. Nevertheless, *in vivo* studies indicate that low doses of HZE particles such as Fe and Ar, are capable of producing morphological, neurochemical and behavioral alterations (Hunt *et al.*, 1989, Joseph *et al.*, 1992, Rabin *et al.*, 1989, Philpott *et al.*, 1985, D'Amelio *et al.*, 1983). Also, several studies have shown alterations in dopaminergic functions in the CNS and correlated motor behavior of rats after exposure to Fe particles with doses as lows as 0.1 Gy (Joseph *et al.* this volume). Furthermore, data suggest that rats exposed to Fe ions showed important alterations in neuronal signal transduction in the striatum, and in motor behavior parameters known to be affected by age (Joseph *et al.*, 1992). These deficits were characterized by losses in muscarinic receptor sensitivity to agonist stimulation and motor behavior. It appears that for Fe exposures, the mechanisms of damage are composed of cell loss and deficits in signal transduction in the striatum, ultimately expressed as decrements in motor behavior. The mechanisms of this alteration are attributed to changes in membrane signal transduction parameters such as G protein-mAChR coupling/uncoupling (Joseph *et al.*, 1994).

In vitro studies using non-neuronal cells showed that HZE particles are very effective at inducing clustered damage in DNA, a lesion thought to be less repairable and therefore to dominate the biological consequences. It has now been shown that very low doses of charged particles induce double strand breaks (Sutherland *et al.*, 1996), and rejoining in cells is decreased after high-energy particle exposures (Goodhead, 1995). Williams and Lett (1996) have studied age dependant changes in retinal DNA of rabbits exposed to HZE particles. With heavy ions, especially Fe, the evidence suggests that the initial DNA damage is repaired but later, DNA breakdown occurs with age. Nevertheless, these studies employed high single doses of Fe ions and the model tested, retinal photoreceptors, is not considered a typical neural cell. Thus, the interpretation of these studies must be kept in perspective. Although, if these findings can be repeated with relevant doses in fractionated dose experiments, and using a typical neural cell, their significance is that they open the possibility that DNA breakdown occurs many month-years after exposure to radiation. Furthermore, damaged neurons may accumulate from heavy-ion injury, leading the cell to reduce its life span and temporary or permanently impair cell functions.

New techniques are needed to demonstrate the effect of HZE particles in fully differentiated neural cells. Our laboratory is working on the study of the regenerative capacity of retinal ganglion cells exposed to low doses of Fe particles using neural explants (Vazquez *et al.*, 1994). This includes morphometric studies of neurite outgrowth and cell viability assays with fluorescent dyes. Using image-analysis techniques, we have observed a decrease of regenerative neuritogenesis in retinal explants following single low doses of Fe ions (Vazquez, 1997). These results suggest that low fluences of heavy ions can impair the functional integrity (plasticity) and viability of retinal ganglion cells with doses close to those encountered in outer space. However, the mechanisms underlying these effects are not well understood and are under intensive investigation.

The damage by heavy ions has been postulated to be singular, not in the primary effect of the traverse of the ion of individual cell constituents, but in their potential to cause damage to a column of highly structured communicating cells. This indicates that, of the tissues that are potentially vulnerable, most worrisome would be those of the CNS. In the past, ground-based and space flight experiments claimed the existence of microlesions expressed as morphological detectable "holes" in the cell surface, as well as tracks in tissues (Nelson *et al.*, 1981, Philpott *et al.*, 1978). It was suggested that these lesions were originated by the passage of HZE particles with a charge of 20 or more, and with a LET of 200 keV/μm or greater (Todd, 1989). This purported lesion was considered one of the most harmful for the CNS. The neural retina, as

an extension of the CNS, has been used in several studies to first corroborate and later reject the microlesion concept (Nelson *et al.*, 1981). While the evidence for tunnel lesions has been shown to be inconclusive (Krebs, *et al.*, 1990, Kraft, *et al.*, 1979), the data does not exclude the possibility of functional expressions of discrete particle traverses or "microlesions". A "microlesion" is now generally envisioned as a discrete injury, which need not be reflected by morphological evidence of damage. It could simply represent transient or chronic molecular or cellular changes that may alter the cellular/tissue integrity. In the case of neurons, this may in turn impair the neural functions at the integrative level (Worgul *et al.*, 1989). Nevertheless, the concern about such microlesions in the CNS induced by HZE particles, such as Fe, has not been eliminated nor proved (National Research Council Report, 1997).

The critical problem at the present time is that still we do not know the site of the target in particle-induced effect on neurons, notwithstanding that the nucleus has been traditionally considered to be the main cellular target. The above described results suggest that it is important to consider the whole neural cell as a target, since damage to the DNA and membrane sites can apparently impair the neuronal functional and structural integrity. Thus, it is critical to determine whether a single nuclear hit is required for neuronal inactivation, or whether a hit to some cytoplasmic and/or membrane locus can initiate the cascade of events leading to cell death or functional impairment. In sum, dose-response data are lacking for a well-defined damage at the molecular, cellular and tissue levels in neural structures, and specific brain areas. Furthermore, the question of how chronic small doses of cosmic rays under physiological stress conditions might affect cell functions, remains unanswered (Curtis *et al.*, this volume).

ENVIROMENTAL STRESS AND NEURON SURVIVAL

It is unlikely that HZE particle exposure would cause rapid loss of large tissue volumes in the brain. However, subtle but chronic changes may occur such as a decrease in neuroplastic capabilities induced by a direct neuronal effect and/or impairment of supporting cells (glial cells) and vascular damage (capillaries). It is well known that several areas in the CNS have a tremendous capacity to compensate following an insult, due to its substantial structural redundancy, and the ability to regrown and generate new connections (Jacobson, 1993). Nevertheless, chronic neuronal cell loss accompanied by a decrease in the capacity of reorganization, even in populations with great redundancy would be expected to result in progressive functional deficits. Neuronal cell loss is a characteristic feature of many pathological conditions in the CNS such as ischemic insults, seizure syndromes, and neurodegenerative diseases (Walsh and Opello, 1992). In some cases, cell death occurs rapidly, while in others it accumulates slowly over many years. Regardless of its time course, neuronal loss will lead to changes in local structure involving both intrinsic and afferent systems, as well as to distant effects resulting from loss of neurotrophic support for afferent neurons and denervation of target neurons. Generally, neuronal cell loss must be substantial before functional deficits become manifest, suggesting that there is considerable plasticity in response to neuronal death. Nevertheless, the process of reorganization in neural systems following neuronal loss is poorly understood.

In most neuropathological conditions, the precise mechanisms underlying neuronal cell death are not known, but excitotoxicity and oxidative damage have been implicated in many cases (Lees, 1993). Several reports suggested that low-level neuronal impairment in the brain, due to genetic or physiological perturbations, can mortally predispose neurons to demise by insults that normally would not cause cell death (Isacson, 1993). Several studies of the degeneration, neuroprotection and regeneration of CNS neurons have departed from previous simplistic descriptions of neurons as either dead or alive (Isacson, 1993). According to new theories of neuronal health, neurons exist in a dynamic equilibrium constantly influenced by both extracellular physiological changes and intracellular mechanisms designed to react to external stimuli while maintaining structural integrity. On the contrary, low-level neuronal damage induced by genetic or physiological perturbations can induce neurons to being injured by influences, which normally are not lethal. Terms used to describe reversible neuronal cell damage are neuronal shock, stress or insult. Such cellular states, not necessarily associated with morphological change, can be induced by a number of factors and conditions such as those encountered in the space environment.

There is little doubt that space flight is a tremendous stressor for the human body. However, little information has been gathered on how this multi-stressor environment impacts the CNS at the cellular and molecular level. It is know that either psychological or physical stress appears to increase the levels of oxidative stress in the brain inducing biochemical and functional alterations (Ames et. al., 1993, Liu *et al.*, 1996). The cellular and molecular mechanisms of these reactions are not clear. However, considerable evidence exists in that oxidative stress damage to cellular molecules such as lipids, proteins, and DNA, is a major contributor to aging, brain dysfunction and neurodegeneration (Reiter, 1995). Because of the low levels of glutathione a major antioxidant that is responsible for removal of cytosolic peroxides, neural cells are especially vulnerable to oxidative stress and free radical damage (Bains and Shaw, 1997). Moreover, neuronal membranes

contain a high levels of free-radical-susceptible polyunsaturated fatty acids compared with normal plasma membrane, making them more susceptible to peroxidative damage (Halliwell, 1989). Stress-induced oxidative damage also may be mediated by the mechanism of excitoxicity, a common pathway in many neuropathological conditions. Evidence is now emerging that activation of glutamate channels may be an important source of oxidative stress and the two mechanisms may act in a sequential as well as a reinforcing manner, leading to selective neuronal toxicity (Dawson *et al.*, 1995).

INTERACTIONS

In space, the assumption of a possible combined effect of space flight factors, and cosmic radiation, cannot be discarded a priori (Planel *et al.*, 1989). An interaction between these factors and cosmic radiation, could explain the high relative biological effectiveness (RBE) for space radiation, which is suggested by the results of various flight experiments (Nelson, 1996). Furthermore, results from several space flight experiments suggest that the effectiveness of cosmic radiation in the induction of developmental alterations is enhanced under microgravity conditions (Bucker *et al.*, 1986). However, the mechanisms of these interactions are completely unknown. It was suggested that cellular repair of DNA damage is impaired by the microgravity environment (Horneck, 1997). Recent space flight experiments failed to validate this hypothesis, suggesting that other mechanisms than genetic repair deficiency are responsible for the reported synergism of radiation and microgravity (Horneck *et al.*, 1997). However, it is unknown if the probability of misrepair may be increased. Alternative mechanisms have been suggested such as:

1. changes of intracellular diffusion-convective mixing controlled processes that can affect the gradients of nutrients, oxygen and the removal of waste products (free radicals) at the molecular level.
2. modulation of signal transduction pathways, metabolic state and chromatin structure at the cellular level.
3. modification of cell-cell communication, cell migration, pattern formation and differentiation at the tissue level.
4. redistribution of fluids, alterations in hormonal levels and modification of circadian rhythms at the system level.

Therefore, a variety of direct and indirect effects of microgravity could, in theory, modulate the cellular response to radiation. At present, we have no data showing the neurobiological effects of high-energy particles in a microgravity environment. Aside from the physical characteristics of ionizing radiation, the metabolic state of the CNS during the exposure and the details of the physico-chemical environment in which this system is subsequently placed may substantially modify the radiobiological response. There is ample evidence for alteration of the metabolic state of human tissues and their cells under microgravity conditions (Huges-Fullford, 1991). Thus, it is conceivable that stressed neurons under microgravity conditions can be more sensitive to HZE particles and its penumbra, altering completely our established concept of neuronal radioresistance.

CONCLUSIONS

Pivotal to the functional integrity and ultimate survival of astronauts in extended space activities are the strategies to be employed in dealing with the space environment. An indisputable requirement for policy decisions and mission planning is an appreciation, to the level of predictability, of the acute and long-term neurobiological consequences of exposure of humans to the space environment. In extended space flights, cumulative impact of the combination of microgravity radiation, sensory-motor alterations, and chronodysynchrony may produce qualitative and/or quantitative alterations in this response, impairing the CNS' adaptative capacity. The evidence from short-duration space missions indicates that functional capabilities can be maintained despite small decreases in work capacity early in flight. There is no an extensive database for long-duration missions but some reports suggested that important motor and cognitive decrements are present after one year stay in low-Earth orbit, (Newberg, 1994).

The proposed neuroplastic response to short-term missions (Figure 1) involves several developmental and adult molecular and cellular processes that might be altered by internal and environmental factors during extended space flights. It is unlikely that space radiation plays an important role in neuroplasticity in short-term low earth orbital flights. However, it is necessary to consider the long-term consequences of a chronic low dose exposure to GCRs and microgravity interaction under a physiological stress which would necessarily attend a Mars mission (Figure 2).

Adaptability in the CNS is a given, but the limits of adaptability and the issue of irreversibility to adaptive changes (neuroplasticity) are major concerns. The CNS chronically exposed to the space environment may become subject of multiple adaptative mechanisms, injuries and compensations, all of which take some toll and may trigger molecular cascades like those encountered in adaptative or pathological plasticity. Some of these are beneficial, but others may lead to enhanced risk of dysfunction. At this stage of our knowledge, we do not know if the CNS's neuroplastic response can be maintained over a long period, or if the normal pattern of response can be transformed in mal-adaptative initiating a

cascade of events leading to neuropathological states. At the end, the outcome may depend on a delicate balance between neuronal loss (and functional impairment), mal-adaptative responses and a sustained normal neuroplasticity response.

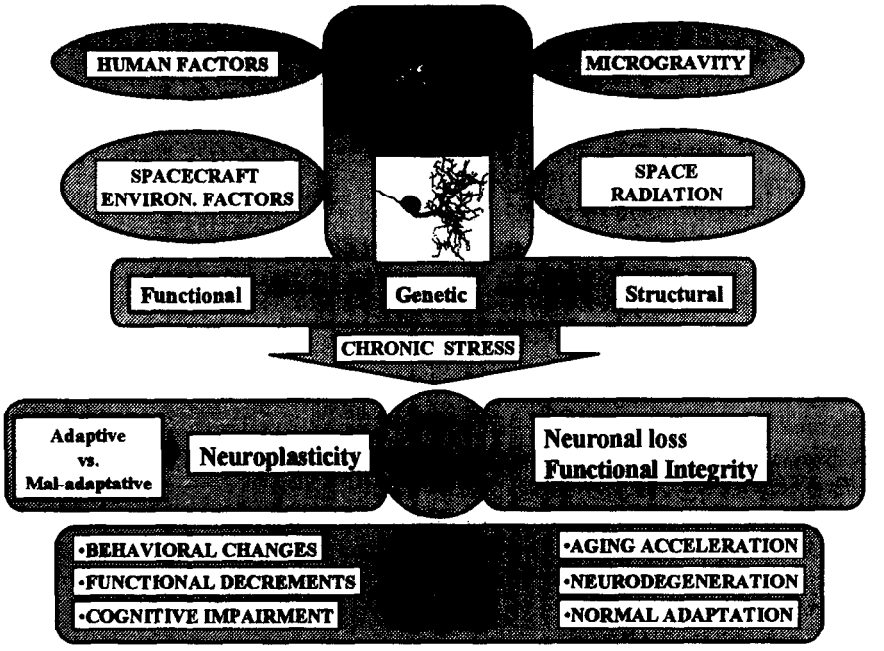

Fig. 2. Possible neuroplastic response to long-term exposure to space flight factors in the CNS.

However, efforts to assess the neurobiological risks in space have been complicated by the considerable unknowns regarding the basic neurobiological effects of space radiation and microgravity beyond the protection of the Earth's magnetic field. Without such information, it is impossible to determine the potential neurobiological risk to the CNS during long-term deep missions.

In order to determine whether exposure to the space environment during a deep space flight is likely to compromise man's ability to function effectively throughout an extended mission, the cellular and molecular effects on the nervous system need to be determined and quantified. Therefore, a rational and efficient strategy for neurotoxicity testing is needed to reduce the uncertainties about the neurobiological risk possessed by space environmental factors. The resulting information should provide the basis for the assessment of risk factors, and thus assist in establishing health safety standards and countermeasures for long-term manned space flights. Research support and facilities must be provided in these areas to validate any plans for human missions to Mars or efforts to establish self-sufficient bases there.

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