Phosphenes in space: a study on the interaction between carbon ions and rod photoreceptor

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Abstract

Phosphenes, or light flashes, have been reported by astronauts since the first Apollo flights to the Moon. They are usually described as occurring in the dark and typically before falling asleep. The light flashes are thought to originate as an effect of high-energy particles interacting with the visual system. The study presented in this thesis investigates the hypothesis of a direct excitation of the rod photoreceptors inside the retina and in particular the interaction between carbon ions and rods. Also the retinal and cortical electrophysiological response of mice submitted to carbon ions irradiation have been studied.

This thesis has been developed as a section of the 'Anomalous Long Term Effects on Astronauts' (ALTEA) program, and all the experiments concerning this thesis took place on ground. The objective of the ALTEA project is more general, and is aimed to assess the functional risks on the central nervous system due to particle flux in microgravity conditions during long term space mission. ALTEA includes a series of experiments both in orbit and on ground. The experiments on board International Space Station will contribute to define the causes of the anomalous phosphene perception of the astronauts by detecting the incident particles through the head of the astronauts and concurrently monitoring the central nervous system functionality. The experiments on ground include the direct irradiation with carbon ions of the outer segments of the rods, and an animal model to investigate the retina/cortex responses of mice irradiated in controlled laboratory experiments. In these experiments on ground I directly contributed in the preparation, the test and the optimization of the experiments and in the following analysis of the data. The whole project has been supported by the Italian Space Agency.

The outline of this thesis includes an introduction to the interdisciplinary ALTEA

program in the first chapter.

In the second chapter ALTEA-MICE (Mice intermittent Irradiation with Concurrent Electrophysiological monitoring) study is described: the experimental sessions as well as the evidences in the electrophysiological responses of the mouse retina have been described in detail. My role has been chiefly the experimental set up, and I also collaborated on the data analysis. The main results of this investigations have been published [1], [2].

In the third chapter the system of the rod outer segments in suspension and the mechanism of the activation of the photoreceptors are described.

The fourth chapter deals with the radiation effects in the biological tissue, and in the fifth chapter there is particular regard to the effects of radiation on samples containing a suspension of rods extracted from bovine retinae.

The effects on the rod photoreceptor rhodopsin has been studied: measurements have been performed irradiating with ^{12}C ions the outer segments of the rods in suspension. The amount of the isomeric transformations (*bleaching*) of the molecules of chromophore into the photoreceptor gives the extent of the effects of radiation. I collaborated to the experiment and carried out the following data analysis.

Finally, in the sixth chapter the process of the photo-isomerization of rhodopsin is explained by a model: the process starts with the water radiolysis and the formation of hydroxyl radicals OH in order to achieve the lipid peroxidation, then there is the subsequent emission of visible photons which are responsible for the photoreceptor bleaching. Since rhodopsin is surrounded by phospholipids, present in high concentration in the disc membranes, the effect of chemiluminescence is proposed to be the cause of the photo-transduction cascade and the light flash perception as a consequence. In order to test this hypothesis, a series of experiments was aimed at studying the effects of the hydroxyl radicals produced in an enzymatic way. The main results of this work are illustrated in chapter six, and have been published in [3].

Chapter 1

ALTEA: an investigation on heavy ion effects on nervous system

1.1 Visual phosphenes in space

On the historic July 1969 Apollo 11 mission, astronauts in lunar orbit reported an incidental finding of various starlike flashes, streaks and clouds in their visual field. Actually, astronaut Edwin Aldrin was the first to report phosphenes, or light flashes (LF), in eyes during the space flight [4]. Subsequently, more detailed observation were made on Apollo mission 12-17 and on Skylab 4 flights; during the Apollo-Soyuz test project (ASTP) and on board Mir. Presently, on board International Space Station (ISS) orbiting in the Low Earth Orbit (LEO), crew members perceive LF. Although it is clear that the phosphenes are related to high-energy particles in the space radiation environment, many details about LF origins are still unknown.

The flashes get perceivable usually under dark adaptation conditions. The flashes are usually colorless; only in few cases they are colored. Because of these features, the flashes are commonly accepted to be due mostly to scotopic vision. The different shapes, as reported in some graphic descriptions in [5], are commonly illustrated as stars, double stars, 'supernovas', streaks, 'sky of stars', clouds, bright flashes with halo, and so on, observed, on Apollo, Skylab, and on Mir Station. Apparently they differ from the diffuse glow from X-ray exposure or induced from neutron generators [6]. Early human studies in accelerators [7] also suggest a correlation with increased particle flux in the eye. On Skylab 4 two dedicated light flash observing sessions were conducted by one of the crewmen during the mission (on January and on February 1974) [8], [9]. The phosphenes were seen only during crew sleep periods at times when the crewmen were awake in their darkened sleep compartments.

On Mir [12], it is much lower (Sileye-1, $0.18 \pm 0.02\,LF/min$; Sileye-2, $0.13 \pm 0.01\,LF/min$) than on Apollo [13] ($0.23{\pm}0.01\,LF/min$), Skylab [14] ($1.3{\pm}0.1\,LF/min$) and ASTP [15] ($0.46 \pm 0.05\,LF/min$). Frequency occurrence of LF is up to 25 times higher near the magnetic poles than in equatorial latitudes. In general, the suggested explanation for LF was in terms of interaction between cosmic ray particles and the eye.

Reference [16] is a study initiated to collect information from people who have recently flown in space. It is a survey conducted by anonymous questionnaire and was performed among astronauts regarding their experience of sudden LF in space. In all, 98 surveys were distributed to current NASA and ESA astronauts. Among the 59 respondents, 47 noticed them sometime during space flight. Most often they were noted before sleep, and several people even thought the LF disturbed their sleep. There is an increase of occurrence with orbital altitude and inclination, as one would expect from the increased particle fluxes there. The LF predominantly appear white, but other colors are mentioned, pale green, blue and in particular yellow (10%); they have elongated shapes, and most interestingly, often come with a sense of motion. The motion is described as sideways or in-out, but never in the vertical direction.

Interest in the flashes has arisen from the practical and fundamental significance of the phenomenon. The practical aspect embraces the radiation safety, in-flight rest, and capacity for work of astronauts in long-term and distant space flights. Long travels outside the shielding magnetosphere are also being considered, with the Moon and Mars as next plausible targets. Health risks due to microgravity and cosmic radiation should nevertheless be expected to increase.

Almost all manned flights took and take place in LEO, at altitudes varying between 200 and 500 km, with the only exception of the Apollo program, which took man on the Moon and outside Earth's geomagnetic shielding. Most LEO missions (such as Mir, ISS and Shuttle) have a low inclination (51.6° with respect terrestrial axis) to avoid high latitude areas, where the lower geomagnetic cutoff results in a higher cosmic ray flux [17]. Cosmic rays and radiation measurements inside spacecraft have been the subject of intense investigations throughout the course of space exploration. These studies are particularly difficult since they need to take into account the orbit dependence of cosmic ray flux and its propagation inside the varying absorber thicknesses of the spacecraft.

The biophysics of particle action and the visual structures/functions eventually involved in the generation of phosphenes remained undefined. The aims of the SilEye-1, SilEye-2, Alteino experiments were to investigate this phenomenon: they are the precursors of the ALTEA experiments. SilEye-1 and SilEye-2 experiments [18] performed LF observations on board Mir space station in the years 1995-1999; a total of 18 hours of observation in 19 sessions resulted in the observation of 145 LF. SilEye-2 consists of a silicon detector telescope coupled to a 'helmet' with an eye mask, worn by the astronauts to carry out LF observations [10]. ISS data have been taken in 2002 with Alteino device [19] during the Soyuz-34 flight. Alteino was composed of two distinct devices: the cosmic ray advanced silicon telescope and an ElectroEncephaloGrapher (EEG). Alteino project helped set the experimental baseline for the ALTEA experiments, while providing novel information on the radiation environment onboard ISS and on the brain electrophysiology of the astronauts during orbital flights [20] [21].

1.2 The ALTEA program

ALTEA is a multidisciplinary project (including sub-projects such us ALTEA-MICE experiments, ALTEA-biophys) carried on with international collaborations. The project has been financed by the Italian Space Agency (ASI) with the collaboration of the National Institute for Nuclear Physics (INFN) and rated 'Highly recommended' by the European Space Agency (ESA).

The ALTEA facility is available to the international scientific community for human electrophysiological experiments, studies on particle flux, and dosimetry in the ISS; the ALTEA hardware has been operating in the ISS - USLab since August 2006. It improves the particle observation capabilities of its precursors SilEye and Alteino detectors while performing an advanced electrophysiological monitoring of the Central Nervous System (CNS) during long orbital flights.

The ALTEA space particle detector is a natural development of previous SilEye and Alteino detectors, with a much larger solid angle coverage for the particles passing through the head. To obtain objective information about the LF perception we included in the system the possibility of recording electrophysiological signals via a high definition EEG. To monitor the status of the visual system in microgravity a visual stimulator was also added. Correlations between electrophisiological changes and passages of particles through the brain and/or retina in the space-adapted conditions have been studied.

The detector system consists of an helmet shaped mechanical structure holding 6 advanced silicon telescopes to monitor incoming comic rays, an EEG to monitor brain activity and a visual stimulator, to determine the functional status of the visual system and study its dynamics. The silicon particle telescopes will be positioned over the whole cerebral cortex. Each telescope is made of six silicon planes, and each plane contains two contiguous basic sensor; the basic sensor is obtained assembling back to back two chips with ion implanted resistive strips, $8 \times 8\, cm^2$ of sensitive area, $380\, \mu m$ thick, strip pitch of $2.4\, mm$. To allow both x and y coordinate measurement the strips of the two detectors are perpendicular.

The EEG system will measure the concurrent changes in the cortical bioelectrical activity; it is made up of a 32 electrode EEG cap including three floating electrodes for retinogram measurements, and high resolution electronics allow for electrophysiological readings. The visual stimulator, used to deliver standard stimuli, will permit to perform suitable stimulation routines, to determine the status of the visual system. A three-button pushbutton is used to signal the LF perception. At the highest sensitivity, the silicon detector system is able to detect particles from He to relativistic Mo, and protons between 25 and $200\, MeV$ [22]. All information will be stored together via an integrated data handling system that will also allow transmission of the data to ground [23].

The ALTEA program includes ground-based experiments which are a series of experiments based on measurements in particle accelerators. In the following sections 1.5 and 1.4 ALTEA-MICE and ALTEA-biophys are explained in detail.

1.3 The ALTEA facility on board International Space Station

Figure 1.1: 8 March 2007. Astronaut Sunita Williams, Expeditions 14 and 15 Flight Engineer, receives assistance from Astronaut Michael Lopez-Alegria, Expedition 14 Commander, in donning the sensor studded cap as she prepares to calibrate equipment for the Anomalous Long Term Effects in Astronauts' Central Nervous System experiment in the Destiny laboratory module, image NASA ISS014E16195.

ALTEA space is mounted on an express rack in the USLab and can be utilized in two modalities: dosimetry (DOSI) and central nervous system monitoring (CNSM) [9].

In the DOSI unmanned modality, the six detectors on the helmet (silicon detector system, SDS), continuously measure the environmental radiation. Data are

Figure 1.2: 8 March 2007. Astronaut Sunita Williams, Expeditions 14 and 15 Flight Engineer, wears the Anomalous Long Term Effects in Astronauts' Central Nervous System experiment helmet while conducting the experiment in the Destiny laboratory module, image NASA ISS014E16208.

downlinked in real time to the ground, to the User Home Base in the Department of Physics of the University of Rome Tor Vergata. Real time and off line software provides tools to discriminate the kinds of particles, calculate trajectories and energy of the particles, constructing spectra of the measured radiation. ALTEA operated in DOSI mode almost continuously from August 2006 to July 2007.

The manned experimental modality (CNSM) is specifically aimed at the study of the interaction between particle passages and brain electrophysiological dynamics. The detector is extended normal to the rack. The astronaut wears the EEG cap (see figure 1.1), inserts the disposable pregelled electrodes, slides into the SDS helmet and wears the visual stimulator which also permits dark adaptation. In this con-

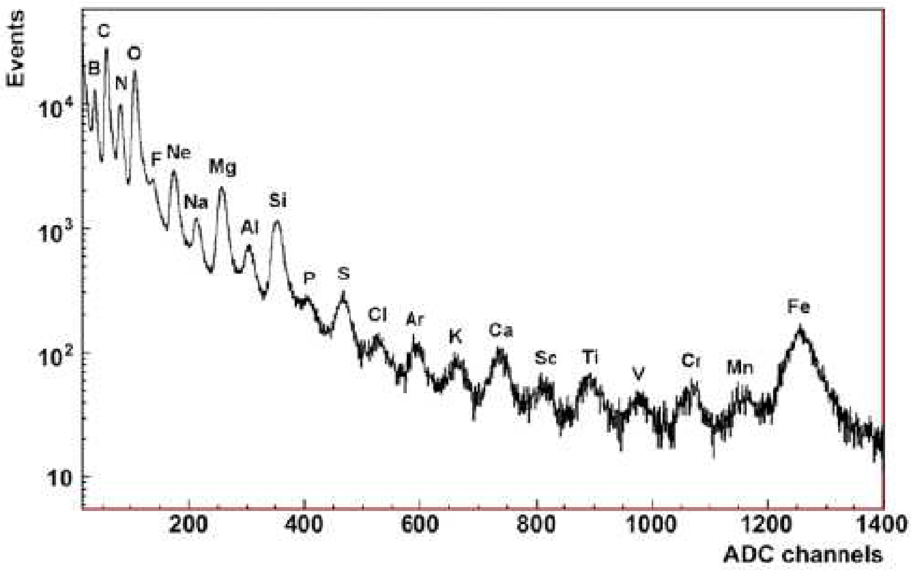

Figure 1.3: A spectrum of the radiation environment into the USLab of the ISS. From [9].

figuration, the SDS measures the particles passing through the astronauts head(see figure 1.2). For about $25\,min$ he/she is presented with a standard set of visual stimuli, then the astronaut relaxes; after about $5-10\,min$ of dark adaptation the observing session starts: each perception of a LF is signaled with the pushbutton. The observing session lasts about one hour. Total measurement time is about $1.5\,h$: one orbit. Seven sessions with three astronauts have been performed. The last one was lengthened to allow a measurement with a full passage in the SAA (the other six did not pass over the SAA during the observing time).

In the figure1.3 a measured spectrum of the radiation environment into the US-Lab is shown. The spectum contains only fast particles, which releases an almost constant energy (that is that the difference in released energy between the two outer planes of the silicon telescope is $< 10\%$). The plotted data refer to about six months acquisition with 10 MIP threshold, where MIP means Minimum Ionizing Particle $1\,MIP = 109\,keV/380\mu m$ of silicon.

1.4 ALTEA-biophys rationale and experiments

The ALTEA-biophys section of the ALTEA program is a set of ground-based experiments direct, in particular, to search for answers to the question: what are the specific (or one of the possible) interactions causing the functional effects of the LF?

As explained in section 2.1, rhodopsin is at the start of the phototransduction cascade in the process of vision. It is one of the best molecular transducers for converting a visible photon into an electric signal. It is therefore the first candidate as the target for the radiationvisual system interaction. For this purpose we started *in vitro* investigations of the behavior of rhodopsin when hit by heavy ions. We have been working to study the possibility that rhodopsin can also be activated by irradiation with ^{12}C nuclei. Intact rod outer segments (ROS) containing rhodopsin were isolated from bovine retina. Suspended ROS were irradiated with ^{12}C ($200\,MeV/n$, well below the Cherenkov threshold, see section 3.2) at GSI. Spectrophotometric measurements investigated the activation (bleaching) of the rhodopsin. With these measurements we were able to show that radiation can induce bleaching.

1.5 ALTEA-MICE rationale and experiments

We set up an animal model using mice, irradiated with very short bursts of heavy ions while concurrently acquiring electrophysiological data from the retina and from the cortex. MICE is the acronym for *Mice intermittent Irradiation with Concurrent Electrophysiological monitoring.*

ALTEA-MICE investigates the effects of heavy ions on the visual system of both normal mice and mice with gene defects affecting retinal sensors. The experimental main goal is to develop an animal model to better understand human risk related to particle exposure. The experiments are expected to provide background information that will supplement the ALTEA project on astronauts' safety. ALTEA-MICE also helps identify reliable laboratory conditions comparable in important respects to those of astronauts in space and suitable for further investigations. Experiments have been performed at the Brookhaven National Laboratories (BNL - Upton, NY, USA), with NASA support, and at the Gesellschaft fur Schwerionenforschung mbH