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VIsual System Activation by Ionizing Radiation: Modelling the irradiation setup for whole rabbit eye exposures

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Summary. — The earliest evidence of space radiation effects on brain functions has been the perception of phosphenes (flashes of light), reported by astronauts since the Apollo missions. The recently reported effects of radiation on sensory channels other than visual suggest, besides the largely investigated activation of retinal processes, a direct action of radiation on neurons or neural networks. In particular, ionizing radiation may influence the biological signaling mainly due to calcium ion homeostasis. In this context, within the VISAIR project we are conducting an experimental study with *ex vivo* whole rabbit eyes, maintained bio-active in a suitable medium, to investigate the activation of the visual system and analyze possible modulation of the effects with different LET/Z. Modelling is needed to interpret the results and extrapolate findings to radiation rates relevant for space exploration. We here present representative results obtained with the code PHITS, after implementation of a software replica of the experimental setup, to verify dosimetric quantities and characterize the radiation field in the target eye.

1. – Introduction

Cosmic radiation represents a major issue for human space exploration and several aspects of the risk assessment are under continuous scrutiny, in order to determine specific countermeasures and minimize the damaging effects on astronauts [1, 2].

The idea that ionizing radiation interacting in the eye can produce “flash-like light sensations” arose well before the time of space missions [3, 4]. The perception of phosphenes, *i.e.*, flashes of light (hereinafter light flashes or LF) in absence of real light, was reported for the first time by Edwin Aldrin, on his return from the Apollo mission, and thenceforth confirmed by almost all astronauts [5]. LF were immediately recognised as a phenomenon of radiobiological interest, and they have been largely investigated during the 70s and from the 90s on, by dedicated observations in space, during the following missions, and ground-based experiments in accelerators [2]. These studies have pointed out the single ion hit as responsible for the phosphene [2, 6], whose perception is associated to an electrophysiological mass response in the retina, similar to the response to light. This kind of effect has been studied on mice at GSI (Darmstadt, Germany): bursts ($\leq 2\,\mathrm{ms}$) of ^{12}C ions are able to evoke retinal processes, activating the visual cortex, as well as interacting directly with neuron dynamics [6, 7].

Positive visual anomalies analogous to phosphenes have been reported also by patients undergoing hadron therapy, such as ^{12}C ion therapy at GSI (Darmstadt, Germany) [8, 9] or proton-therapy at ICPO (Orsay, France) [10, 11] and at PTRC (Loma Linda, California, USA) [12]. In particular, these two last clinical studies have shown how also low-charged and low-LET radiation can be responsible for the LF perceptions. A possible modulation of the effects with charge (Z) and LET of the incident particles is still to be investigated.

Particular effort has been dedicated to the study of the interaction mechanisms underling the LF phenomenon. The differences in the LF characteristics, *e.g.*, shapes and colours, may suggest a variety of interaction processes. Cherenkov effect has been indicated as a possible candidate in different works [11, 13]. In general, LF production seems to be connected to the interaction of the ionizing radiation with ocular structures, mainly the retina. A model for LF generation was proposed in [14] and tested *in vitro* [15, 16]: chemiluminescence from radical-driven lipid peroxidation in the retina is able to bleach rhodopsin, hence activating the phototrasduction cascade, responsible for the anomalous perceptions.

A recent clinical study, conducted at PTRC (Loma Linda, California, USA) [12] on patients treated with proton therapy for brain tumors, has revealed that radiation can evoke illusory perceptions in four different sensory sistems (auditory, taste and smell beside visual). Together with previous results on mice [6, 7], this also seems to suggest the presence of a more general mechanism of radiation interaction with cortical neural networks. Ionizing radiation may indeed affect the biological signaling, mainly due to calcium homeostasis, being therefore responsible for the activation of all the sensory systems.

Obtaining a clear picture of all possible interaction processes behind the activation of LF would be important for a better knowledge of response mechanisms activated by radiation and to be able to quantify possible biological damage and to define effective countermeasures for risk mitigation and health protection.

In this paper we present the VISAIR project, which is conducting an experimental study on *ex vivo* whole rabbit eyes to try to shed light on radiation-induced activation of the visual system. In particular we focus on the simulation work, needed to verify the experimental setup for future irradiations and characterize the radiation field in the target eye, to be able to further correlate the characteristics of the field to the results of biochemical measurements.

2. – The VISAIR project

The VISAIR (*VIsual System Activation by Ionizing Radiation*) project, funded by the Italian Space Agency, has the aim of further investigating the radiation-induced activation of the visual system of astronauts, as a continuation of previous works mainly focusing on rhodopsin [14-16].

In this context, the VISAIR Collaboration is running an experimental study of the modulation of induced effects with LET and Z of the incident particles. The goal is as well to study more generally the activation of other sensory systems, hence the choice to investigate possible direct effects of radiation on calcium signalling.

The chosen experimental model is the whole eye, since other structures of the visual system beside the retina may be most likely directly involved in the activation process [17]. The experiments are thus performed on *ex vivo* whole rabbit eyes cultured in a suitable medium to maintain the bio-activity. Irradiation runs with different beams (*e.g.*, X-rays, ^{12}C, ^{56}Fe) and different energies are foreseen starting from 2021.

Preliminary biochemical tests on the experimental model have been successfully conducted. In particular, the optimal conditions/parameters to preserve the eye vitality have been identified and the model has been characterized for its response to visible light: the Ca^{2+} ion concentration in vitreous humor has been measured by means of spectrophotometric techniques, for eyes kept in conditions of dark and after different times of eye exposure to visible light. The measured increase of Ca^{2+} concentration for exposure times higher than 1 minute provides a preliminary indication of the effect of visible light on Ca^{2+} signaling. This offers the validation of the experimental model, and represents the premise for the characterization of the eye response to ionizing radiation.

It should be noted that all the experimental measures are necessarily performed at radiation rate levels much higher than those compatible with the space environment. For this reason, a simulation work is required to interpret the experimental data and extrapolate results to radiation rates relevant for space exploration [17].

3. – Simulation results and discussion

The adoption of the rabbit eye as experimental model requires to implement radiation transport simulations at a macroscopic scale, to characterize the radiation field in the entire organ. Simulations have been performed using the general purpose Monte Carlo code PHITS (Particle and Heavy Ion Transport code System, v.3.20), able to deal with the transport of a variety of particles, via continuous energy loss, over wide energy ranges [18].

Firstly, the geometry of the system has been reproduced, as shown in the left panel of fig. 1. A representative rabbit eye, whose radius is estimated to be $\sim$0.7 cm, is placed in a polyethylene Falcon tube of 50 ml in the suitable solution, necessary to preserve its vitality. Both the eye and the solution are modelled as water-equivalent. Such setup will be used during the beam measurements. The beam parameters need then to be defined. For the present work, a Fe ion beam at the energy of 1 GeV/u has been considered, with a beam cross-section of 5 cm^2 to cover the whole eye. Fe indeed represents a candidate beam for VISAIR measurements, of interest for the space environment. In the right panel of fig. 1, the simulated fluence of all particles (primary Fe ions and secondary particles, generated by the beam interactions with the target) is shown.

This configuration for the simulation allows the necessary check of dosimetric quantities as, in particular, the dose homogeneity within the target organ. To this aim, the rabbit eye volume has been divided into 14 parallel layers of thickness 0.1 cm each (see the inset of fig. 2, left panel), and the dose deposited by the beam as well as selected secondary ions in each layer has been calculated. This is illustrated in fig. 2, where the dose per particle deposited is shown as a function of depth. More specifically, the total

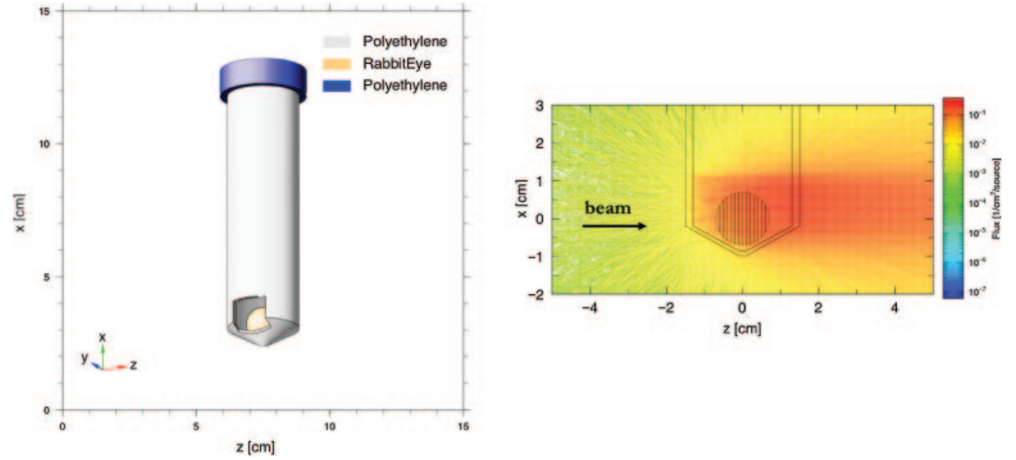

Fig. 1. – Left panel: simulated geometry of the experimental setup, the rabbit eye in a test Falcon tube (50 ml). Right panel: simulated fluence of all particles (primary Fe ions and particles generated by the beam interactions with the experimental setup). Simulations were performed with the code PHITS v.3.20.

dose and the dose contribution due to Fe ions are displayed in the left panel, while the secondary ion contributions are reported in the right panel. As one can note the dose deposited is almost entirely due to Fe ions, and the dose dishomogeneity over the whole organ is $\approx 6\%$. Data on the relative contribution to the total dose by different species are reported in table I, which offers a further characterization of the mixed field induced by primary Fe interactions.

To complete the set of dosimetric information on the radiation field, the dose distributions of the Linear Energy Transfer (LET) for both primary and secondary particles have been used to calculate the corresponding dose average LET values ($\langle \text{LET} \rangle_D$). $\langle \text{LET} \rangle_D$

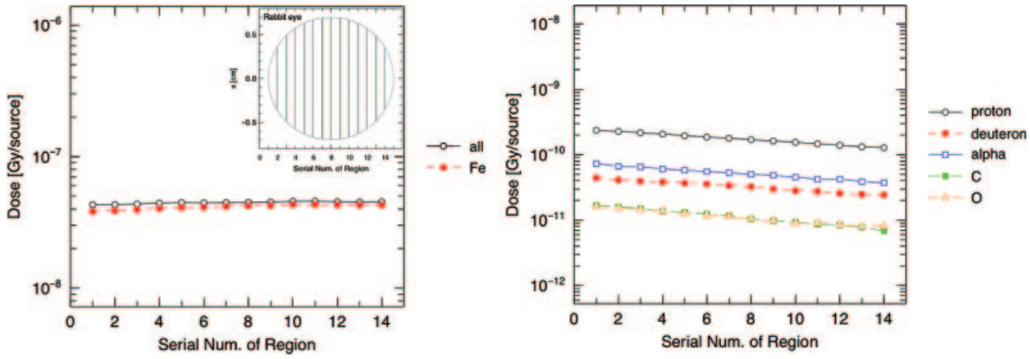

Fig. 2. – Simulation of the dose per particle deposited in the eye as a function of depth. The eye volume is divided into 14 regions (inset). Left panel: total dose (in black) and Fe ion contribution (in red). Right panel: contributions to dose from selected secondary particles: protons (in black), deuterons (in red), alpha particles (in blue), C ions (in green) and O ions (in orange). Simulations were performed with the code PHITS v.3.20.

Table I. – *Relative dose values (%), values of dose avarege LET ($\langle LET \rangle_D$) and dose mean lineal energy $\bar{y}_D$ in keV/μm for different contributions to the total dose irradiating the eye with 1 GeV/u Fe ions in the chosen experimental setup. Simulations were performed with the code PHITS v.3.20.*

	Relative dose (%)	$\langle \mathrm{LET} \rangle_D$ (keV/μm)	$\bar{y}_D$ (keV/μm)
Fe ion	93.38	154.8	80.9
proton	0.39	2.8	3.4
deuton	0.07	7.5	8.1
α-particle	0.11	39.6	40.3
C ion	0.02	134.4	134.8
O ion	0.02	38.4	65.4

values are reported in table I. By definition, LET is calculated on a macroscopic scale. To obtain a characterization of the stochastic energy deposition at microscopic (cellular/subcellular) level, microdosimetric functions are implemented in PHITS. In this work, the dose distribution of the lineal energy y, calculated in a sensitive site of 1 μm diameter, has been considered. The lineal energy is defined as the ratio of the deposited energy in a sensitive site to the mean chord length of the site [19]. The first moment of the dose distribution of y, *i.e.*, the dose mean lineal energy ($\bar{y}_D$), has thus been calculated for primary and secondary particles and the corresponding values are reported in table I. A high LET value of ≈ 155 keV/μm is associated to the 1 GeV/u Fe beam, while, interestingly, the corresponding dose mean lineal energy is lower, due to the weight in dose of secondary electrons when sampling Fe tracks with the 1 μm sensitive site.

All results reported in this work are obtained with a statistics of at least 10^5 per run (10^3 particles per batch per 100 simulation batches) to keep relative errors on dosimetric quantities under few percent.

In summary, a software replica of the experimental setup has been developed, which will be used to reproduce the experimental conditions for the future VISAIR beam measurements. In this way, the external beam parameters, such as Z and energy, could be linked to precise dose, LET or lineal energy distributions, to be further correlated with measured biological effects. In particular, the calculation of relative dose contributions by different species in a mixed field and associated microdosimetric quantities will also allow coupling between the radiation field in the macroscopic organ and effects at the cellular/sub-cellular level [20]. Simulations will also be necessary to try to extrapolate the results obtained in ground-based accelerators to dose-rate levels characteristics of the space radiation environment.

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