A neurophysiological approach to radiation-induced 'phosphene' phenomenon: Studies in awake and anaesthetized mice.

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Abstract

This experiment was designed to study mechanisms of 'phosphene vision'. In human physiology 'phosphenes' refer to luminous sensations produced by stimuli other than light. Experiments performed to reproduce the phenomenon, refer to phosphenes induced by electrical stimulation of retina and by accelerated particles during space flights. In order to study these mechanisms we programmed a series of experiments: in a preliminary experiment, visual evoked responses (VEPs), electroretinograms (ERGs) and oscillatory potentials (OPs) to flash stimulations were recorded in unanaesthetized and anaesthetized mice bearing chronically implanted electrodes. In the unanaesthetized animals, the responses displayed stimulus-depended increase in amplitude and decrease in latency, up to a plateau at about 2.082-2.383 phy. In particular, OPs at 1 Hz stimulus showed a maximal amplitude effect starting at about 2.684 phy intensity. In the anaesthetized animals, urethane produced a latency increase and an amplitude decrease of all evoked responses. Finally, to study the 'phosphene phenomenon' a beam of accelerated carbon particles (energy 100 and 300 MeV/n) was delivered into the retina and sensorimotor cortex of anaesthetized mice. Cycles of light flashes were also administered. Following accelerated particles stimulation cycles to the retina, 500 to 5000 per stimulus, 30 stimuli per cycle, stimulus time-related waveforms in mice cortex were recorded. Their amplitude and latency appeared to be related to energy delivered. The short waveform latency, and the lack of retinal potentials and oscillatory potentials following particles stimuli suggest that the impact of accelerated particles does not produce activation of retinal chemical structures. This appears as the first objective demonstration of radiation-induced 'phosphenes'.

Introduction

In human physiology 'phosphene' refers to luminous sensations produced by stimuli other than light. Experiments performed to reproduce the phenomenon in laboratory conditions, refer mainly to phosphenes induced by electrical and magnetic stimulation of retina or cerebral cortex [1] and by accelerated particles during space flights [2].

Phosphenes were also described following accelerated particles directed to the eyes of voluntary scientists in laboratory conditions [3]. In order to study these mechanisms, cerebral and retinal responses to flash stimuli were evaluated by visual evoked potentials (VEPs), electroretinogram (ERG) and oscillatory potentials (OPs) in accord to the following protocol:

- Study of visual responses (VEPs, ERG, OPs) evoked by flashes in awake mice in laboratory conditions
- Study of visual responses (VEPs, ERG, OPs) evoked by flashes in anaesthetized mice in laboratory conditions
- Study of the same parameters evoked by flashes in anaesthetized mice in the laboratory for accelerated particles studies (GSI-Darmstad, Germany)
- Study of visual responses evoked by accelerated particles in anaestehtized mice (GSI)

Since the advent of transgenic technology, the characterization of neurophysiological parameters and the study of chronic recordings of visual evoked responses in animals are auspicious. Visual evoked potentials, retinal evoked potentials and oscillatory potentials provide an objective functional measurement of the visual pathways and related neural systems, both in humans and in animals [4, 5, 6]. In particular, flash stimulation has been used both in clinical and experimental research; because of its feasibility in non-cooperative, non-sedated subjects.

Thus, the characterization of responses to visual stimuli at low intensity and fixed frequencies was an essential step to study the 'phosphene phenomenon'. Finally, we attempted to reproduce neurophysiological correlates of phosphenes, i.e., objective recording of stimulus-locked electrical waveforms. Beams of accelerated particles directed to the eyes of laboratory mice were used as triggers for evoked potentials, and we recorded the physiological signals from the retina and from the cortex.

Methods

In the first part of the experiment evoked responses were recorded in eight male outbred CD1 mice obtained by Charles River - Italy at the age of 90 days. The animals were housed in a transparent plastic cage under standard animal conditions with free access to food and water, a 12 hrs light/dark cycle, and an ambient temperature of 21 °C. The animal care and use followed the directives of the Council of the European Communities. The Bioethical Committee of the Italian National Institute of Health approved the experimental protocol.

Under xylazine (Rompun, Bayer AG, Leverkusen-Germania, 20 mg/kg i.p.) and ketamine (Ketavet 100, Gellini Farmaceutici Spa, Peschira Borromea-MI, 32 mg/kg i.p.) and locally injected lidocaine (Lidocaina 2%, Azienda Terapeutica Italiana A.T.I. srl, Ozzano Emilia-BO, 0.1 ml s.c.) anesthesia, the animals were chronically implanted with 4 cortical electrodes for VEPs and 2 tungsten teflon-coated electrodes, in contact with the dorsal ocular bulb and under the nose skin, for ERG recording. For details see [7, 8]. The implants were fixed with dental cement. During the surgery, a heated table maintained the body temperature at 37 °C. A recovery period of 1 week was allowed before the EEG recording.

A Grass Instrument PS 22 photic stimulator model supplied the flash stimulation. During the recording, 90 successive light flashes (stimulus rate: 1 flash per second, duration: 10 μs) were administered. The stimulus intensity range (11 steps from 0.097 to 3.289 phy) was chosen according to the results obtained in preliminary sessions, with the lowest intensity eliciting no response in most animals. Phy was used to estimate the number of photoisomerizations per rod per second expressed in log units, based on previous work by Pugh et al. [9], that gives a conversion factor from light into nervous energy by means of rodopsin activation. For example, Grass PS22 maximal intensity (x16) corresponds to 3.286 phy, and to 945.6 cd/m^2*s.

Each mouse was recorded alone while housed in a cage with mirrored walls, and the ceiling corresponding to the flash stimulator positioned outside the soundproof cage to avoid acoustic interferences. The EEG and ERG signals were amplified through a DC powered preamplifier, with a gain of 1000X and an analog filter at 1 Hz highpass (HP) and a 500 Hz lowpass (LP) 6dB/octave. The acquisition was performed at 2 kHz sampling rate. An InstruNet A/D 16 bit conversion board delivered the signals to the acquisition system (Superscope- GW Instruments, Somerville, Massa-chusetts, USA) on a personal computer (Macintosh 8150 Power PC) customized and adapted by Analysa (Villafalletto CU, Italy). Brain and retinal activity and trigger were continuously acquired and saved as raw data to be analyzed off-line. At 1 Hz frequency stimulation acquisition was performed over 90 s. The protocol of the experiment was the following: recording of evoked responses in the awake animals, and in the same animals after administration of urethane (0.6 g/Kg). The animals were not scotopically adapted. The amplitude and latency of the VEPs and ERG responses were measured after baseline normalization. The latency was calculated as the absolute value between stimulus onset and the peak of the first negative response, whereas amplitude was taken both as an absolute value of negative peak amplitude and as peak-to-peak value between the first negative peak and the following most prominent positive peak. Oscillatory visual and retinal potentials (cOPs and rOPs) were extracted through the application of a digital 50 Hz HP filter (linear phase FIR, 6 dB/ octave) to the raw data [9]. The latency of the oscillation was measured in ms from the trigger to the response peak. The amplitude was calculated as the peak-to-peak value between the first negative and the first positive peaks.

In the second part of the experiment another group of mice (n = 8) were chronically implanted in a similar manner as previously reported. After recovery, the animals were transferred to the Gesellschaft fur Schwerionenforschung MBH (GSI) in Darmstadt (Germany).

Five days later the animals were again anaesthetized (urethane), and their eyes were positioned with the help of a laser pointer, in line with the trajectory of an accelerated carbon particle burst (energy 100 and 300 MeV/n, delivered through a brass collimator of 3 mm calibre) directed to the left eye and in some runs 5 mm caudal to the eye, (sensorimotor cortex), for control purposes. Approximate estimates of the maximum energy delivered by each burst, also taking into account the dimension of the beam, suggest a figure of about 2 GeV at retinal level. After several preliminary trials, the final protocol of stimulation cycles included 30 stimuli (bursts) per cycle; each stimulus consisting of bursts of 500 or 3000-5000 particles, the number being constant for each cycle, with particles delivered within a temporal window of 5 ms, with 20 s interstimulus interval. The impact of the particles on a pair of scintillators placed in line with mice eyes provided the trigger for off-line averaging [10].

The three traces, i.e., cortical, retinal and trigger were recorded on a STAC real- time analyzer after amplification (x1000) of biologic signals, and all were oversampled at 2.9 MHz, with 20 bit A/D conversion, followed by resampling at 2.56 kHz, in order to avoid aliasing phenomena. Also responses to flash cycles were recorded both at the beginning and at the end of the particles session, and averaged in real time.

The differences in latency and peak-to-peak amplitude of VEPs and ERGs before and after anaesthesia were evaluated using Student's *t* test. Differences were considered to be significant when P<0.05.

## Results

Visual potentials (VEPs, ERGs and OPs) evoked by flashes before and after anaesthesia with urethane

The morphology of VEPs in all conditions was characterized by a first negative peak (N1) at a latency between 26-29 ms in awake animals and a first positive peak at about 50 ms latency at the stronger intensities. The amplitudes of the responses were quite variable: at minimal intensities the first negative peak varied between 5 and 50 V amplitude or more in the eighth animal in each group. In an overall view, it appears that responses decreased in latency and increased in amplitude with the increase of the stimulus intensity. After anaesthesia with urethane, N1 increased in latency especially at lower intensities of luminous stimulation (30.1±0.6 vs 41.3 ±2.3 ms) and decreased in amplitude.

The morphology of the ERG was characterized by a first positive peak followed by three negative peaks labeled b1, b2 and b3. b1 appeared at a mean latency of 24 ms at the lowest intensity of luminous stimulation and showed a

similar trend to N1 of VEPs because of decrease in latency with increase in stimulus intensity. After anaesthesia, we observed an increase of b1 mean latency (from 24 ±1 ms to 26.5 ±0.9 ms, at that intensity) and no substantial variations in amplitude (data not shown).
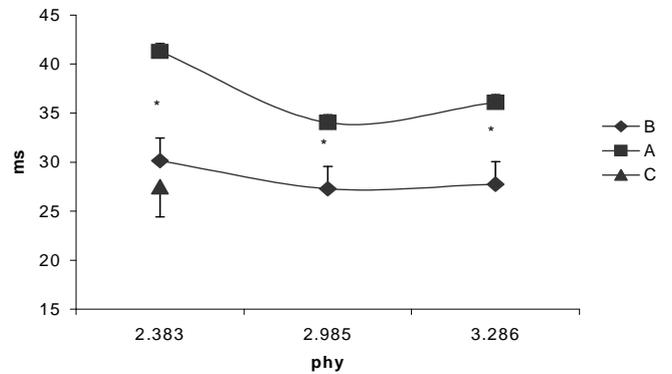
Figure 1. *Latency-intensity curves of VEPs in mice. B awake, A after urethane anaesthesia C after accelerated particles during anaesthesia. Abscissa: luminous intensity in phy; ordinate: latency in ms. *=P<0.01.*
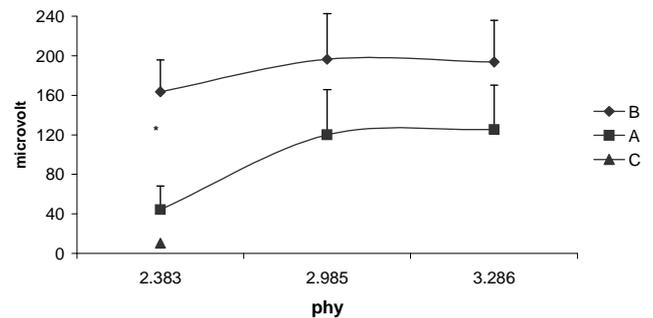
Figure 2. *Amplitude-intensity curves of VEPs in mice. B awake, A after urethane anaesthesia C after accelerated particles during anaesthesia. Abscissa: luminous intensity in phy; ordinate: amplitude in μV *=P<0.01.*
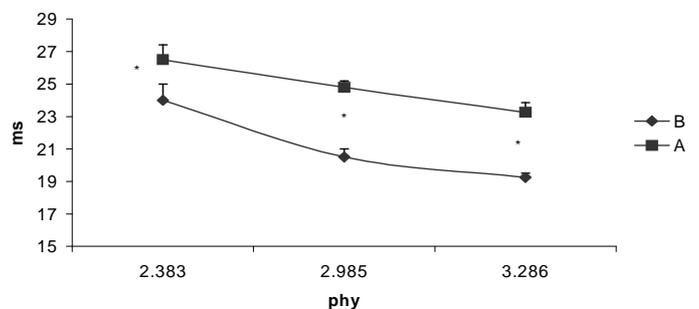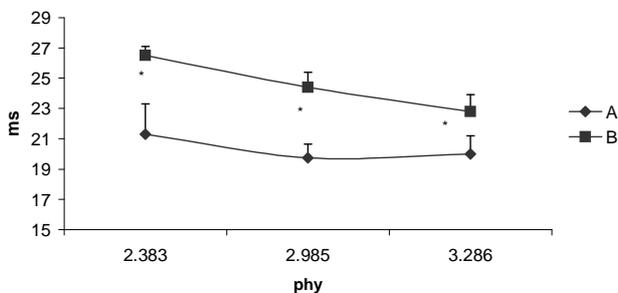

Figure 3. *ERG latency-intensity curves before (B) and after (A) anaesthesia. Abscissa: luminous intensity in phy; ordinate: latency in ms. * =P<0.01*

50 and 55 ms at stronger intensities. Latency- and amplitude-intensity curves display a trend very similar to the one showed by VEPs. However, the duration of the total oscillatory event varied between 25 and 30 ms. After anaesthesia with urethane we observed a significant increase of latency, and a decrease of amplitude. Figure 4 shows the data of retinal OPs latency before and after anaesthesia.

Visual potentials evoked by flashes after anaesthesia with urethane in GSI

Cortical and retinal evoked potentials following flash stimuli were recorded in the eight mice, with characteristics similar to those recorded in anaesthetised mice and rats previously published from our and other's laboratories [7, 11]. In our anaesthetized mice, VEPs had an average of 42 µV amplitude and 58.2 ms latency; ERG b1 wave maximal amplitude had an average of 19 µV, and 77.4 ms latency.

Figure 4. *Retinal Ops latency-intensity curves before (B) and after (A) anaesthesia. Abscissa: luminous intensity in phy; ordinate: latency in ms. * =P<0.05*

Visual potentials evoked by accelerated particles in GSI

A total of 39 trials (burst cycles) were administered to mice, but the 17 preliminary trials administered to three mice, served for establishing final recording conditions, were discarded from the final study. This report is relevant to the five mice, which underwent a total of 22 trials, each consisting of 30 beam stimuli of 500 or 3000-5000 particles for each stimulus. In fourteen of these 22 trials, the beam was directed to the retina of the left eye, while in eight trials the beam was directed towards the sensorimotor cortex of the animals, 3-5 mm caudally to the retina, in order to have a series of control recordings.

An average of responses following the 30-bursts cycles of accelerated carbon particle beam, and standard error, were gathered from electroencephalographic signals recorded at the visual cortex level. Statistical analysis performed by computer programs allowed clustering of averaged waveforms into three categories: i.e., waveforms showing the maximum negative values between 0 to 50 ms latency after trigger, or 51 to 100, or above 100 ms. The first temporal lag included 5 averaged potentials with mean amplitude of 10.2 ± 2.5 µV, and a mean latency of 30.3 ± 3.1 ms. (**Figs. 1 and 2**) No signals above the background noise were ever recorded from the retinal electrode. During the second temporal lag no potentials with the highest negative peak were recorded. The third temporal lag included 9 averaged potentials with maximum amplitudes varying from 2.1 to 21 µV. However, these potentials did not show any consistent
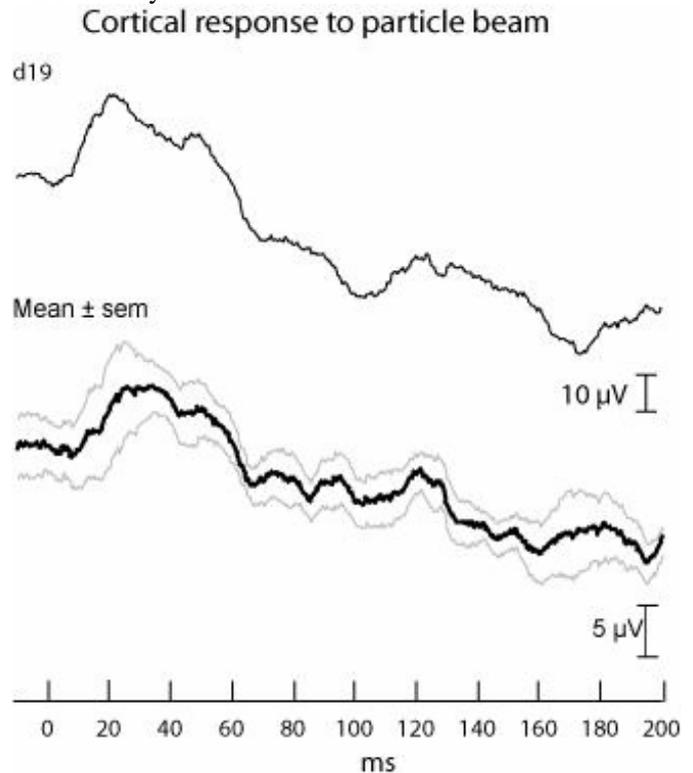
Figure 5. *Example of one potential recorded in the cortex of a mouse administered with 3000-5000 particles at 300 MeV/n. (upper part). Grand average of the 5 evoked potentials with highest negative (upward) peak falling within the first 50 ms after trigger, recorded in the cortex. Standard error of the mean is shown in grey (lower part). Abscissa: time in ms; ordinate: amplitude in µV.*

temporal relation to the trigger, since they were scattered on a range of latency from 130 to 280 ms following the trigger. The grand average did not show any consistent difference from background noise. Potentials recorded from the retinal electrode never showed any consistent signal above the background noise. No rapid oscillatory potential with a reliable signal-to-noise ratio could be extracted from either the cortex or retinal channels, in any experiment, (Fig 5).

Discussion

In our experimental conditions, VEPs, ERG and OPs as a function of stimulus intensity displayed an increase in amplitude and a slight decrease in latency up to a plateau

at about 2.082-2.383 phy. In particular, OPs showed a plateau intensity effect starting at about 2.684 phy.

These results provide the VEP, ERG and OPs amplitude and latency curves as a function of varying luminance band. Based on our results, it is possible to choose the optimal intensity between 2.082 and 2.985 phy to elicit good responses. In our experimental conditions, urethane produced quantitative alterations in the mouse VEPs. We observed a latency increase and an amplitude decrease of all evoked responses. The latency increase, a characteristic of many anesthetics, is due to a depression of conduction in the visual pathways. This effect is not unique to urethane. In general, substances that appear to increase GABAergic inhibition tend to increase the amplitude of the second positive peak evoked in cats by step increments in luminance, whereas substances that tend to decrease GABAergic inhibition tend to decrease the amplitude of this peak [12].

Nevertheless, the results obtained in this first part of the study can also serve as reference in the low intensity range; which is more likely to occur in response to particles in accelerator beams oriented either directly or indirectly towards the visual system. [2].

The results obtained in the second part of the study are in line with previous investigations. Reports published in the '70s describe that dark-adapted human eye can recognise the passage of a single fast KZE ion through the retina [13], while a total of 900 stopping nitrogen particles directed in nine positions of various depths of the occipital lobe did not induce subjective visual phenomena. [3]. These findings suggest that cerebral cortex has a much higher threshold for detecting stimuli induced by "anomalous" energy sources (as accelerated particles are) than retina. Yet, light sensation was reported by some patients on irradiation of the temporal lobe, and olfactory sensations were reported by patients receiving helium-ion irradiation therapy for tumors of the head and neck [13]. These conditions suggest that the cerebral cortex needs to be hit by particles endowed with much higher energy than the retina to become aware of the "sensation" and that the subjective sensation described by a human being is a tool much more sensitive then the objective recording of an electric stimulus-related waveform, as we observed in our mice. In fact, in our mice beams directed only to the retina (and only after at least 500 particles administration) were able to evoke visual electrical signals, while particles (500 to about 5000) directed to the brain did not. This hypothesis also confirms previous investigations [3] that showed that nitrogen beam paths (266 MeV/n) were able to induce phosphenes only when the beam was directed to the retina of the volunteering scientists. Beams directed either to the vitreous body, or to the optic nerve however, produced no sensation. Another finding to be discussed in our experiment is the short latency between the trigger and the responses recorded in the cortical electrodes. Letelier JC [14] showed that synaptic activation produced by the electrical and visual (flash) stimulation of retinal inputs to the optic tectum of a bird (Columba Livia) follow different patterns. Flash stimuli induce a complex pattern of current sinks, which are recorded with a 25 to 30 ms delay, while electrical stimuli generate sinks confined to retinorecipient layers, and can be recorded in the optic tectum as soon as 3-5 ms after stimuli.

In conclusion our data suggest that the responses recorded in the cortical electrodes in scotopic conditions in mice indicate that these responses are produced by direct beam stimulation. The retina is a better subject than the visual pathway or the cerebral cortex, and presumably the physiological effect is produced through impact on structures between the receptor's inner segment and the ganglion cell body, as suggested by Potts [15] and not by stimulation through chemical mechanisms of receptors. Further experiments are needed to verify the relationship between risk and benefit of such phenomena, in view of prolonged space flights programmed by international space agencies, and the possible risk of the visual system. This experiment appears as the first objective demonstration of accelerated particle-induced "phosphenes" in living organisms.

Acknowledgements

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