Impact of low intensity millimetre waves on cell functions

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Investigations on the biological impact of low levels of millimetre-wave energy date back to the first experiments on the generation and detection of these high-frequency signals by Sir Jagadis Chunder Bose at the end of the 19th century. Slightly more than a hundred years later, millimetre-wave transmission has become a ubiquitous commercial reality. Despite the widespread use of millimetre-wave transmitters for communications, radar and even non-lethal weapons systems, only a handful of researchers have funded programmes focusing on millimetre-wave interactions with biological systems. As such, there is a growing need for a better understanding of the mechanisms of these interactions and their possible adverse and therapeutic implications. Independent of the health impact of long-term exposure to high doses of millimetre-wave energy on whole organisms, there exists the potential for subtle effects on specific tissues or organs which can best be quantified in studies which examine real-time changes in cellular function as energy is applied. In this Letter, a series of experiments are presented which show changes in cell membrane potential and the action potential firing rate of cortical neurons under short (1 min) exposures to continuous-wave 60 GHz radiation at $\mu W/cm^2$ power levels, more than 1000 times below the US government maximum permissible exposure. The findings have implications for non-contact stimulation and control of neurologic function, and might prove useful in a variety of health applications from suppression of peripheral neuropathic pain to the treatment of central neurological disorders.

Introduction: In 1901 while on a lecture tour in the UK, Sir Jagadis Chunder Bose, the first person to generate, detect and characterise accurately millimetre waves, is quoted as stating [1], 'How lucky we are that the natural eye absorbs this radiation and protects us by veiling our sense against insufferable radiance in these days of space-signaling by Hertzian waves.' In 1883 the first experiments on free space transmission of radio waves for signalling purposes were conducted by Tesla, and in 1884 Bose [2] repeated these experiments using microwaves. In 1887, Marconi [3] made his famous demonstration of wireless transmission for communications applications at Salisbury Plain, UK.

A little more than a hundred years later, we really do find ourselves in a world where we are continuously bathed in low power microwave and millimetre-wave radiation. The widespread deployment of millimetre-wave, and soon submillimetre-wave, generators for wireless telecommunications [4], airport and checkpoint security screening [5], and even non-lethal crowd control weapons [6], has prompted renewed scientific interest in the effects of this wavelength range on biological materials and organisms. Owing to their established role in the telecommunications industry, their potential for greater penetration into tissue, and the availability of commercial generators and detectors, millimetre waves have been more widely studied than submillimetre-wave or terahertz (THz) frequencies for their biological impact. High levels of millimetre-wave power absorption have received much attention from the bioelectromagnetics safety standpoint. However, power levels that fall well below the United States Federal Communications Commissions established maximum permissible exposure (MPE) limits of 1 mW/cm^2 for 6 min in the 30–300 GHz frequency regime [7] have received considerably less investigation.

Neuronal activity is a particularly good marker for gauging stimulus thresholds since the neuronal membrane is optimised for sensing and in conducting electrical impulses with millisecond temporal response. Several research groups [8–18] have noted significant impact on neuronal activity induced *in vivo* by modest levels of millimetre-wave exposure (40–130 GHz, 1–100 mW/cm^2, seconds to minutes) that are not much higher than the MPE. Synchronisation of the firing rate of neurons in the hypothalamus of both rabbit and rat was observed at and below 10 mW/cm^2 [8, 9]. 53 GHz exposure of the sciatic nerve in rats at only 4mW/cm^2 increased the action potential amplitude [10]. Even at 2–3mW/cm^2, at certain frequencies between 40 and 52 GHz, an isolated frog sciatic nerve showed measureable changes in the amplitude and latency of its compound action potential [11]. Higher levels of millimetre-wave power (10–100mW/cm^2), which tend to raise the temperature of the exposed sample, have been shown to produce changes in neuronal activity that sometimes do, and sometimes do not, correlate with broadband radiant heating [12–15]. For example, changes in action potential firing rates in skate skin exposed to 130mW/cm^2 at 54 GHz were anti-correlated with those produced by radiant heating [12]. In another study, however, exposure of snail pacemaker neurons to 75 GHz radiation at levels sufficient to raise the temperature several degrees in a few seconds showed changes in firing rate that matched those produced by radiant heating [13]. Similar results, correlating millimetre-wave exposure (62 and 75 GHz) and direct temperature rise, were also reported for the changes induced in ionic currents in these neurons [14]. Most recently, the electrical response of an exposed frog sural nerve (>45mW/cm^2 at 42 GHz) showed threshold effects and transient behaviour that were not well reproduced by broadband radiant heating [15]. Additional studies have focused on millimetre-wave induced changes in cell membrane permeability. Small increases in current transport across lipid bilayers were seen with both pulsed and continuous-wave (CW) millimetre wave power between 54 and 76 GHz [16]. Similarly, the permeability of phospholipid based liposomes increased after exposure at 130 GHz with 10–17mW/cm^2 [17]. Annexin V, an extracellularly-applied marker, was used to visualise the outward and inward migration of the membrane-forming lipid, phosphatidylserine, during exposure of keratinocytes to 42 GHz at 35mW/cm^2 [18]. Radiant heating of the cells failed to reproduce the effect. In a set of experiments on mouse skin receptors, the tail flick response was observed to decrease with millimetre-wave exposure [19, 20] in contrast to simple heating. A possible explanation for some of these effects may come from recent investigations on the prevalence and role of macromolecule-bound water, particularly inside and adjacent to the cellular membrane, which point to strong specific absorption in the millimetre-wave band [21–24].

In our own studies, we have looked at both millimetre-wave induced apoptosis and transient membrane permeability in epithelial cells *in vitro* [25, 26], as well as real-time changes in the activity and membrane permeability of individual pyramidal neurons in patch-clamp probed cortical slices [27]. The latter experiments were conducted at exposure levels 1000X below the MPE and resulted in at most 3°C increase in the tissue bath temperature at the highest exposure level. We believe these experiments yield the strongest evidence to date for significant impact of low-power millimetre waves on cell function. Independent of any human safety related issues, the ability to modulate neuronal activity via optically focused millimetre-wave beams has use as a basic neuroscience tool, and perhaps clinical implications for suppression of peripheral neuropathic pain and treatment of central neurologic disorders.

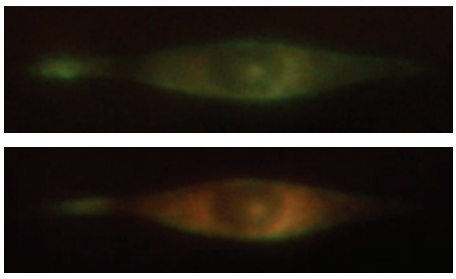

Fig. 1 *Top: photograph of single H1299 cell in oxonol and phosphate buffered saline, expressing lipid bound fGFP at cell membrane (transfected with pEGFP-F vector) before millimetre-wave exposure. Bottom: same cell after exposure. Red colour indicates FRET induced oxonol fluorescence*

Experiments on epithelial cells in vitro: In our first set of experiments we employed an immortalised epithelial cell line, H1299 (a gift from Alex Sigel, Caltech, Pasadena, CA, USA) to look at changes in membrane permeability with exposure to modest levels of millimetre-wave power: ~10mW/cm^2 for 2 min at 50 GHz. To enable optical measurements of the membrane potential, we transfected cells with a plasmid containing farnesylated green fluorescent protein (fGFP) which binds to the inner leaflet of the plasma membrane. Upon exposure to blue light (490 nm) the fGFP fluoresces green (520 nm), lighting up the inner leaflet (Fig. 1 top). A voltage sensitive dye, oxonol, was added to the media which localises at the outer membrane while the membrane is negatively polarised. The membrane depolarisation evoked by millimetre-wave exposure causes the oxonol to migrate towards the inner leaflet and become a quenching agent for the GFP fluorescence through a Förster resonance energy transfer (FRET) process. The

resultant fluorescent signature changes from green to red (620 nm) indicating the degree of membrane depolarisation (Fig. 1 bottom). After the millimetre-wave power was turned off, the fluorescent signature shifted back to pre-exposure wavelengths (green) within a few minutes, indicating the transient effect of the millimetre-wave exposure (Fig. 2). The bath temperature was monitored during the exposure and did not rise more than 3°C. This first set of experiments gave us an indication that millimetre waves can have a significant effect on cell function within a physiologically relevant temperature range.

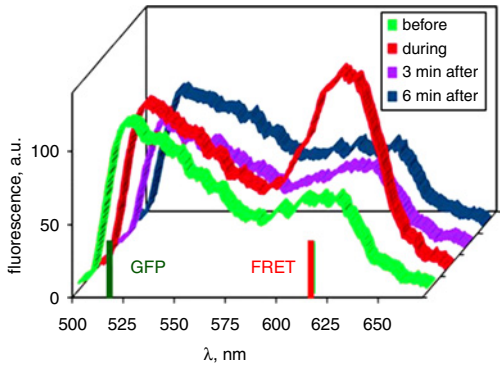

Fig. 2 *H1299 cell spectrum (green GFP and red FRET lines) before, during and after exposure to ~1–3mW/cm² energy at 50 GHz for 2 min. 620 nm red FRET line indicates membrane depolarisation (open). 520 nm green GFP line is the normal membrane signature. Note: membrane depolarises then repolarises after 3 min*

Experiments on neurons in vitro: The epithelial cell studies were extended to a neuronal cell line B104 (a gift from Peter W. Vanderklish, Scripps Institute, San Diego, CA, USA), again transfected with a plasmid expressing fGFP. A similar FRET experiment was performed using 10 mW/cm² at 60 GHz for 2 min, and the resultant shift in fluorescent signal was recorded with a pair of ocular mounted photomultipliers, each with a narrowband filter centred on 520 and 620 nm. The same red shift in the fluorescent signature (indicating membrane depolarisation) was recorded, but despite discontinuation of the millimetre-wave signal, no recovery to the pre-exposure state was observed. Subsequent discussions with the inventors of the fGFP/oxonol FRET technique (Professors Vergara and DiFranco, UCLA, Los Angeles) pointed towards possible enhanced solubility of oxonol in phosphate-buffered saline (PBS) upon millimetre-wave exposure resulting in a permanent increase in the red background signal in the medium, obscuring the changes at the plasma membrane.

Patch clamp experiments on cortical slices: Real-time changes in neural activity were examined through patch-clamp electrophysiology experiments on individually probed pyramidal neurons in cortical slices from 13–16-day-old rat pups. The experimental arrangement is shown in Fig. 3. 300 μm-thick transverse slices containing the cerebral cortex were placed in the tissue chamber (Fig. 3 upper right) and incubated at room temperature in artificial cerebrospinal fluid (ACSF, approximately 3 ml) with bubbled oxygen (95%) and CO_2 (5%) at a pH of 7.4. The glass recording pipette was filled with K-gutonate, KCl and NaCl (140:5:4) plus a buffer agent (HEPEs, 10 mM). A gigaohm seal was formed with a pyramidal neuron and the whole cell current-clamp recording was made while injecting a positive current (75–200 pA) for five seconds out of every 20 seconds (25% duty cycle) to improve the neuronal viability. The intracellular-to-extra-cellular voltage was recorded continuously and the appearance, firing rate, peak voltage, rise and fall times, and input resistance ($\Delta V/\Delta I$) of the action potential spikes upon the positive current cycles provided the baseline and the millimetre-wave induced changes that are the basis for the experiments. Millimetre-wave power (60 GHz, with 7.5, 15, 30, 60, 120 and 185 mW present at the waveguide output port) was introduced in random sequences via a mechanical rotary vane attenuator (no electronic switches were used to prevent recording artifacts), held fixed for 1 min (three current cycles), then turned off. The bath temperature was constantly monitored by an in situ thermocouple. The temperature rise with power level varied from <0.1°C to a maximum of 3°C at the highest power setting. Fig. 4 shows sample recordings taken at four different millimetre-wave power levels from approximately 0.1 to 1 μW/cm² as calculated at the plane of the cortical slice after absorption through 2.2 mm of ACSF. Changes in the firing rate were observable at power levels of ~0.3 μW/cm² and above. Rise and decay slopes of individual action potentials and membrane resistance (Fig. 5) were also strongly correlated with the millimetre-wave power level indicating opening of the membrane ion channels. These experiments are described in greater detail in [27].

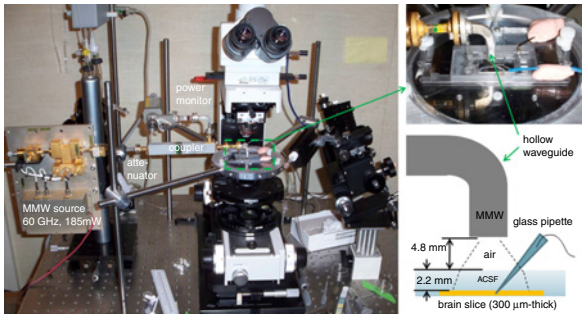

Fig. 3 ***Left****. Photograph of patch clamp measurement setup with vertically coupled RF power source.* ***Above right****: close up showing waveguide and tissue chamber.* ***Below right****: schematic showing probe geometry and distances used for calculating the beam profile and power distribution at the tissue slice. Reprinted with permission from Journal of Neural Engineering (JNE)*

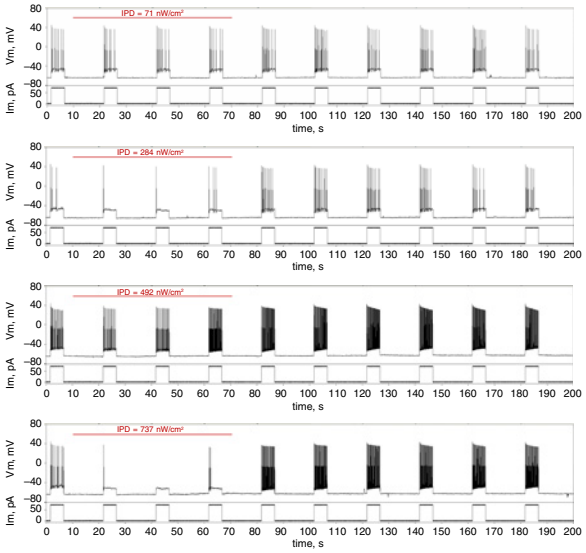

Fig. 4 *Sample recording of neuronal activity (membrane voltage, V_m) and injected membrane current (I_m) against time at four power levels applied for one minute. Incident power density (IPD) was calculated according to beam spread and loss in the 2.2mm of ACSF fluid above the cortical slice. Top to bottom: 71, 284, 492, 737nW/cm². Red bars indicate the time and duration of exposure. For each sequence above the neuron was stimulated for 5 s and allowed to rest for 20 s. As the power is increased strong inhibition of the neuron action potential firing rate is seen during the exposure. Excitation was also observed. In all cases the neuron returned to pre-exposure firing rates within 6 min. Reprinted with permission from JNE*

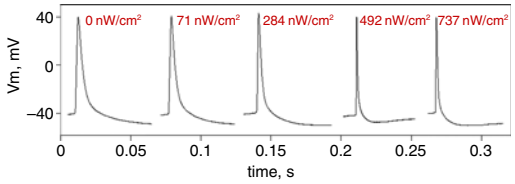

Fig. 5 *Typical action potential shapes against RF power density. Exposure was for 1 min at 60 GHz and corresponds to the four sets of curves in Fig. 4. Reprinted with permission from JNE*

Discussion: Figs. 4 and 5 indicate strong correlation between extremely low millimetre-wave power densities and neuronal activity. All eight patch clamped-probed neurons measured showed a similar increase in

the plasma membrane permeability. At power levels of approximately 300 nW/cm^2 and above, we observed strong inhibition of the action potential firing rate in some of the neurons, and increased firing in others, perhaps indicating the functional heterogeneity in the studied neuronal population. The rise in bath temperature during exposure was $<3^{\circ}C$ and could not fully account for the dramatic changes in the membrane permeability, as the equivalent radiant heating produced a much smaller change in the membrane parameters [27]. These results are believed to be the first positive correlative measurements of real-time changes in neuronal activity with ultra-low-power millimetre-wave exposures. The experiments point to changes in membrane channel opening, which if controllable and proven to have no negative health impact, might be used as a tool for suppression of peripheral pain and neuromodulation for treatment of central neurological disorders. A lack of high accuracy specific absorption rate (SAR) data for each sample puts large error bars on our experimental values for the tested exposure levels, however even given such uncertainty, the effects we have recorded are clearly observable at levels well below the recommended MPE. We have only begun to evaluate the real-time effects of millimetre waves on cellular functions. Further work is certainly needed, and we hope that the results presented in this Letter will catalyse governmental bodies and private foundations overseeing the safety and applications of millimetre-wave technologies.

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