Comp Clin Pathol (2015) 24:1271–1276
DOI 10.1007/s00580-015-2113-0

ORIGINAL ARTICLE

Histopathological and immunohistochemical study of rat brain tissue after exposure to mobile phone radiation

Morteza Kafaee Razavi[1] · **Ahmad Reza Raji**[1] · **Mohsen Maleki**[1] · **Hesam Dehghani**[1] · **Alireza Haghpeima**[2]

Received: 8 December 2014 / Accepted: 20 April 2015 / Published online: 10 May 2015
© Springer-Verlag London 2015

Abstract The health effects of cell phone radiation exposure are a growing public health concern. The aim of this study was to investigate the effects of mobile phone exposure at 940-MHz frequency magnetic field on temporal cortex. The study was carried out on 30 Wistar adult male rats (15–20 weeks, weighing 290 g). Thirty male rats were divided into five groups. Rats in the first group were exposed to 940-MHz frequency magnetic field for 30 min every day for a period of 30 days. The second, third, and fourth groups were exposed to 940-MHz frequency for 1, 2, and 3 h every day for a period of 30 days, respectively. The control group did not receive any exposure. Histopathological and immunohistochemical studies were used to determine the possible damages to brain tissue and blood-brain barrier (BBB). The findings of this study revealed that exposure to electromagnetic fields with a 940-MHz frequency has increased the permeability of BBB in all exposed groups. The increased period of exposure caused increased instances of histopathological changes in brain tissue.

Keywords Mobile phone radiation · Blood-brain barrier · Albumin · Temporal cortex

Introduction

Today, mobile phones are one of the most commonly used devices that emit electromagnetic waves (Lonn et al. 2004). These devices are readily available for almost half the world's population in different age groups. Use of the electromagnetic field (EMF) has been increased to extent that many people even from early age of 3 years are constantly exposed to this kind of radiation (Cardis et al. 2007). Mobile technology acts according to electromagnetic radiation in the microwave frequency ranges of 300 MHz to 300 GHz (Andersen 2002; Kundi et al. 2004). Cell phones transmit and receive electromagnetic waves, mainly at frequencies of 800–1900 MHz (Barnett et al. 2007). Most microwaves in the frequency range of 800–1000 MHz can penetrate the cranium, and near 40 % of these frequencies are able to reach the deep layers of brain tissue (Barnett et al. 2007; Stilgoe 2007; Elder et al. 2007; Kuo et al. 2007; Khalatbari et al. 2006). Therefore, it is conceivable that microwave radiation from mobile phones could affect brain functions. Mobile phone usage is a public health concern because of the potential risk of chronic exposure to the low levels of radiofrequency and microwave (RF/MW) radiation that pulse from the phone antenna, in close proximity to the user's head. Part of the head mobile phone is held in its vicinity affected most of the other areas (Griffiths 1999). Some investigations showed that exposure to the radiation from a Global System for Mobile Communications (GSM)-900 MHz mobile phone increased blood-brain barrier (BBB) permeability in mammalian brain (Henrietta et al. 2009). Other studies revealed that a 2-h mobile phone radiation with SAR 0/12, 12, 120 mw/kg, increases albumin levels in neurons after 14 days of exposure and, the same after 28 days of radiation, would damage the neurons (Henrietta et al. 2009; Eberhardt et al. 2008). Also, some investigations showed that exposure to 2-h mobile phone radiation would upregulate the transcription of apoptosis genes in primary cultures of neurons and astrocytes (Zhao et al. 2007). Since mobile phones are usually held close to the head when they are in use, part of the microwave that they emit is absorbed by the brain. For example, most

✉ Morteza Kafaee Razavi
morteza.kafaii@gmail.com

[1] Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Faculty of Science, Islamic Azad University, Mashhad Branch, Mashhad, Iran

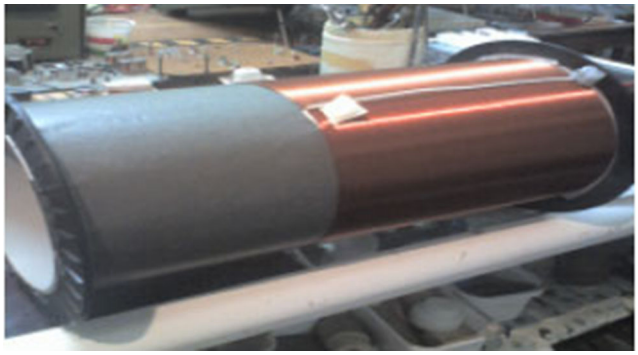

Fig. 1 Electromagnetic field chamber with ability to produce mobile phone radiation (940-MHz frequency)

microwaves in the frequency range of 800–1000 MHz can penetrate the cranium and near 40 % of them can reach the deep brain (Barnett et al. 2007; Stilgoe 2007). Therefore, it is conceivable that microwave from mobile phones could affect brain functions especially temporal cortex. Considering the importance of this subject, the aim of this study was to determine and analyze the effects of electromagnetic radiation emitted in the frequency range of mobile phone (940 MHz) on temporal cortex of rat brain tissue.

Materials and methods

Animals Thirty male Wistar rats (15–20 weeks, weighing 290 g) were used in this study. After 7 days of adaptation to the laboratory environment, rats were divided into five groups. The control and experimental groups 1, 2, 3, and 4 were exposed to a daily EMF of 940 MHz for 0 and 30 min and 1, 2, and 3 h, respectively, for a period of 30 days.

EMF generator

Mobile phone radiation was generated with signal generator that was connected to solenoid (coil). Sinusoidal pulses were received from a 940-MHz signal generator and were delivered to a 600-W amplifier with output current of 5 A. The amplifier output was applied to coil to create a uniform magnetic field. These coils included a circular coil, which were made of coated copper wire with a diameter of 0.78 mm and 287 rounds. The diameter of each coil was 20 cm, and the space between them was equal to the coil radius. Magnetic field chamber consisted of a cylindrical cage from fiber glass (2 mm thick) and was 6.5-cm internal a coil of 1760 turns from electrically insulated 0.75-mm copper diameter and 70-cm-long wire were wound around the outer cylinder at equal distance (Fig. 1).

Histopathological method

After exposure to mobile phone radiofrequency, rats in control and all experimental groups were euthanized by CO_2 gas. Brain tissue was fixed by 10 % formaldehyde for 24 h. Then, right temporal lobe was separated. Tissue preparation for

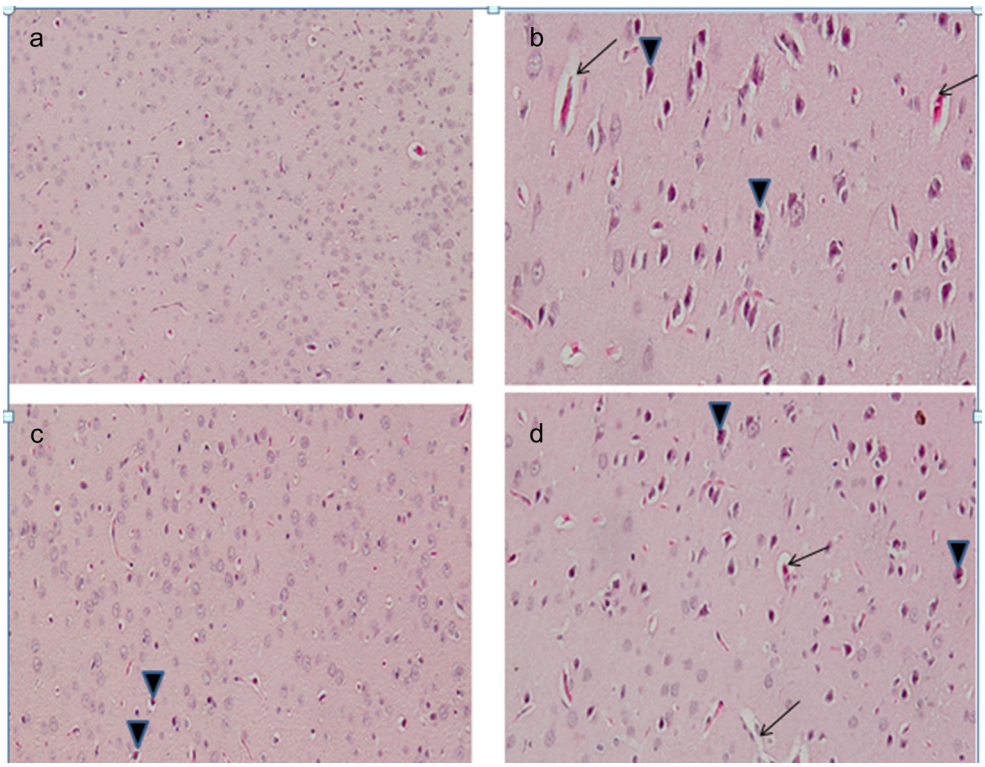

Fig. 2 Control and experimental groups, normal control group (**a**), histomorphological changes such as edema (*arrow*) and apoptotic cell (*arrow head*) in experimental groups after exposure mobile phone radiation (**b-d**), H&E staining, ×20

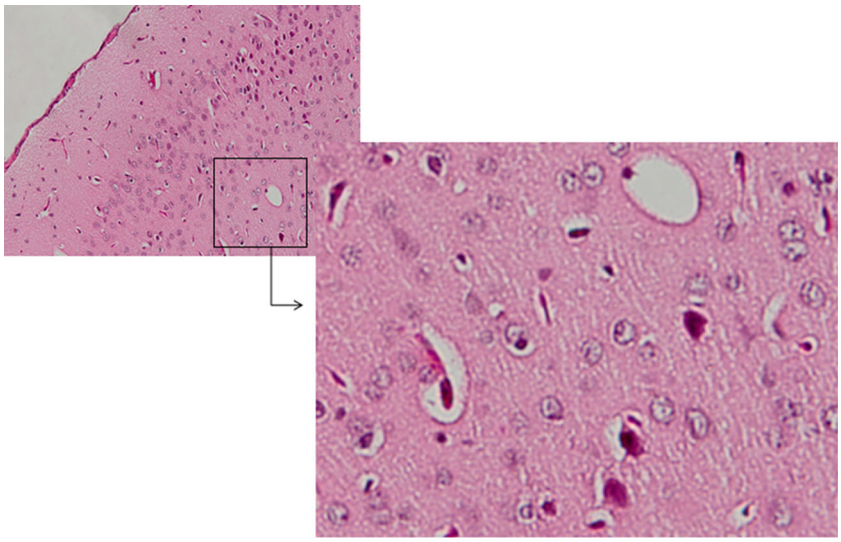

Fig. 3 Temporal cortex after exposure to mobile phone radiation, BBB space, H&E staining, ×20

H&E staining was performed in three stages, deparaffinization, hydration, and staining. Sections were stained with the filtered Harris's hematoxylin for 1 min, rinsed with tap water, immersed in eosin stain for 1–2 min, and rinsed with tap water again. Then, sections (with 6-μm thickness) were subjected to dehydration in ascending concentration of alcohol solutions (50, 70, 80, 95 %×2, 100 %×2).

Immunohistochemistry method

To study the albumin leakage and also changes of BBB permeability, anti-albumin kit was purchased from Abcam company, and according to the company protocol (Abcam), first, sections were deparaffinized with xylene and hydrated with ethanol from 99, 95 to 70 %. Then, the sections were boiled in 10 mM sodium citrate buffer, pH 6.0, and maintained at a sub-boiling temperature about 10 min for antigen unmasking; then, the sections were exposed to air for 10 min. The sections were incubated for 10 min in 5 % hydrogen peroxide and then washed in dH2O. We added 100–400 μl primary antibody (1:200) to each section and incubated overnight at 4 °C; then, we removed antibody solution and the sections were washed with Tris-buffered saline (TBS) (1×), incubated for 45 min with secondary antibody (goat anti-chicken IgY H&L HRP ab97135 Abcam) (1:1000), and washed with TBS three times for 5 min each. We applied 100–400 μl DAB to each section and monitored closely.

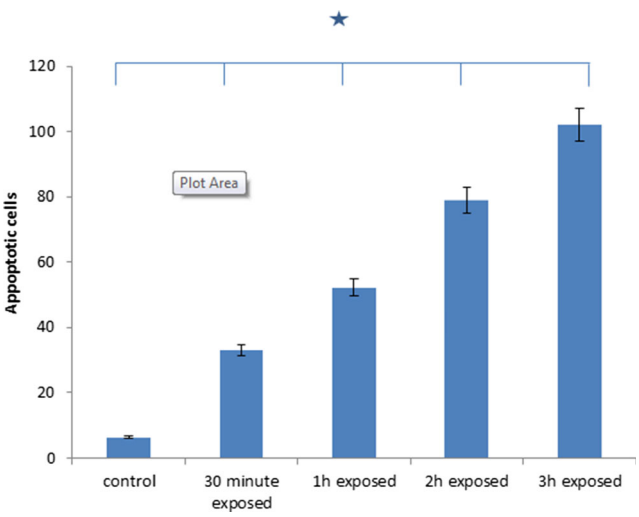

Fig. 4 Determination of apoptotic cells in rats that exposed to mobile phone radiation. After mobile phone exposure, the numbers of positive cell for apoptotic cells were significantly higher in rat experimental groups than the control group ($P<0.05$)

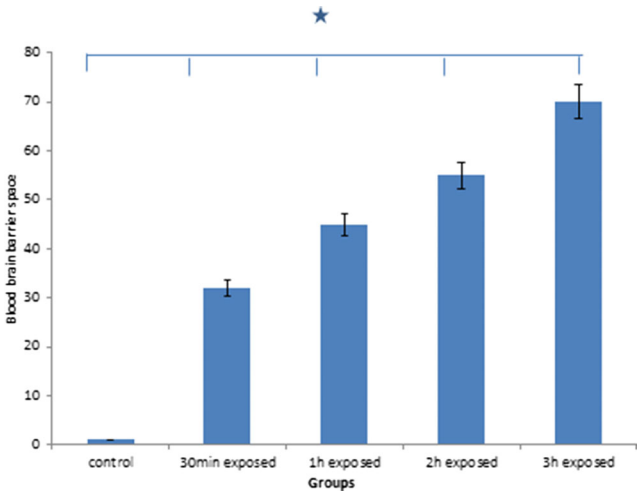

Fig. 5 Morphometric study effects of microwave emitted from mobile phones on experimental groups after 30 days. Mobile phone exposure at 940-MHz frequency magneticfield for 30 min and 1, 2, and 3 h led to significant increase in blood-brain barrier space in comparison with control group ($p<0.05$)

One to ten minutes generally provides an acceptable staining intensity with hematoxylin, and we finally repeated it in 100 % ethanol, incubated the sections two times for 10 s each, and mounted the sections with coverslips.

Morphometric method

The microscopic images revealed that the BBB has made remarkable changes. In order to study BBB morphometrically, in each group, the sections in various groups were imaged with magnification ×40. Then, 40 blood vessels that have diameters of about 8–10 μms were selected (N1) and space around the vessel was measured (N2), Thence, vessel space was subtracted from blood diameter (N2−N1), and results between different groups were evaluated (Fig. 2).

Statistical analysis

To determine significant differences between groups, one-way analysis of variance (ANOVA) test was performed (SPSS version 20). Each of the exposure conditions was made with the Mann-Whitney nonparametric test for independent samples. All differences were considered significant at the $p<0.05$.

Results

Histopathological results

Microscopic and morphometric studies showed an increased in BBB space in all experimental groups when compared with control group (Fig. 3). These differences were more considerable in exposure groups compared to control (Fig. 4). In the control group, brain cells and, also, vessels were normal and apoptotic cell was not observed (Fig. 3), but in all of the experimental groups, apoptotic cell was observed (Fig. 3, BD).

Immunohistochemistry results

Immunohistochemistry study against albumin showed that albumin leakage occurred between experimental groups while in the control group, it was not observed (Fig. 5). According to IHC study, few cells were positive for albumin antibody in first experimental groups; however, positive test slightly increased in another groups, and it shows that with increasing time exposure, albumin leakage is greatly increased and it is statically significant between experimental groups in comparison with control group ($p<0.05$). In addition to the leakage of albumin in brain tissue, albumin uptake by neurons was observed in the experimental groups.

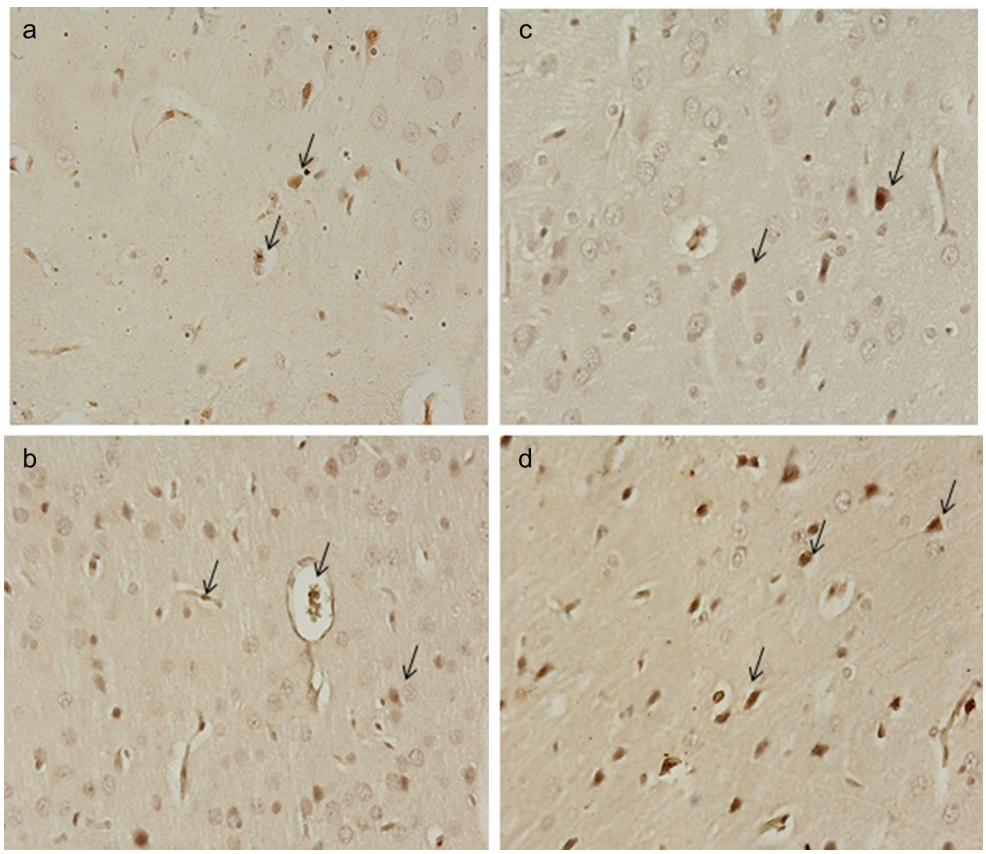

Fig. 6 Albumin leakage and albumin uptake after exposure to mobile phone radiation (AD). Albumin leakage from blood-brain barrier and albumin uptake from neurons after 30-min (**a**), 1-h (**b**), 2-h (**c**), and 3-h (**d**) exposure to mobile phone radiation (*arrow* indicates antibody reaction with albumin in both neuron cell and blood-brain barrier),immunohistochemistry staining, anti-albumin antibody, ×20

Discussion

The present study showed that EMF at 940-MHz frequency (mobile phone radiation) can cause changes in brain tissue. These changes include the following: increase apoptotic cells (Fig. 3), increase BBB permeability (albumin leakage from BBB) (Fig. 5), and increase BBB space (Fig. 6), after 30 min and more at the period of 30 days. According to morphometric study, EMF causes changes in the structure of BBB (edema). The BBB is responsible for the maintenance of the neuronal microenvironment. This is accomplished by isolation of the brain from the blood by the tight junctions that join endothelial cells in cerebral microvessels and by selective transport and metabolism of substances from the blood or brain by the endothelial cells (Zhao et al. 2007; Farrell and Risau 1994). Thus, with changes in this structure, neurons life hazarded and finally neuron damaged. Albumin leakage from BBB can uptake with neurons. Albumin uptake from cells can cause neuronal death. We and others (Salford et al. 2003) have pointed out that when such a relatively large molecule as albumin can pass the BBB, so too can many other smaller molecules, including toxic ones, which may escape into the brain because of exposure to mobile phone radiation. We have not concluded that such leakage is harmful for the brain. However, Hassel et al. (1994) have shown that autologous albumin injected into the brain tissue of rats leads to damage to neurons at the injection site when the concentration of albumin in the injected solution is at least 25 % of that in blood. In the present study, we investigated whether leakage across the BBB might cause damage to the neurons. According to immunohistochemistry study (anti-albumin antibody), we showed albumin uptake with neurons. This study confirms with Henrietta Nittby and partners in research; they showed that EMF (GSM-900 mobile phone) increased BBB permeability in mammalian brain 7 days after exposure to mobile phone radiation (Henrietta et al. 2009). Also, some investigations showed that microwave leads to significant cell death in culture and more in vivo brain neuronal cells were stained positive for TUNEL assay (Zhao et al. 2007). On the other hand, our results showed that EMF in high frequency (mobile phone radiation) changed nerve cell program and induced apoptosis. Although apoptosis is a natural process, increased apoptosis is a pathological process and should be paid more attention. Results revealed that even among the experimental groups, there are significant differences and this shows the importance of time for mobile users. Thirty minutes of mobile use every day of 30-day duration can change BBB permeability and also nerve cell apoptosis. This time can contribute damage to the tissue and threshold time using for mobile users is extremely important for mobile users. Thirty-minute exposure to mobile phone radiation increased BBB permeability, so it is important for us restrict mobile use to decrease this harmful changes.

Conclusion

Although the mechanism of these changes by EMF is not completely clear, it is clear that mobile phone radiation can cause irreversible harm, and we must limit mobile use. The possible risk by radiofrequency EMF exposure of the human body is a major concern for the society. A new, third generation of mobile communication is becoming increasingly important, but the health impact of this radiation modality is largely unknown. Epidemiological studies will not be able to answer this question until after 10–15 years of exposure. It is therefore of greatest importance to study, in the laboratory, biological effects that can lead to health impairment. It is of great importance both to quantify the leakage of albumin through the BBB and to study the toxicological effects of this leakage. This new knowledge can be used as a foundation for new exposure limits that take into account nonthermal biological effects of microwave radiation from mobile telephones and base stations.

References

Andersen J, Bach P, Mogensen P, Pedersen F (2002) Possible exposures from future mobile communications systems. Rev. Radio Sci 935–941

Barnett JL, Timotijevic R, Senior V (2007) Public responses to precautionary information from the Department of Health (UK) about possible health risks from mobile phones. Health Policy 82:240–250

Cardis E, Richardson L, Deltour I (2007) The Interphone study design, epidemiological methods, and description of the study population. Eur J Epidemiol 22(9):647–664

Eberhardt JL, Persson BR, Brun AE, Salford LG, Malmgren LO (2008) Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones. J Electromagn Biol Med 27(3):215–229

Elder AJ, Chou CK, Morrissey J (2007) Radiofrequency exposure and human health. PIERS Online 3:149–153

Farrell CL, Risau W (1994) Normal and abnormal development of the blood- brain barrier. Microsc Res Tech 27(6):495–506

Griffiths DJ (1999) Introduction to electrodynamics. J Benjammin Coummings 3(1):47–49

Hassel B, Iversen E, Fonnum F (1994) Neurotoxicity of albumin in-vivo. J Neurosci Lett 167:29–32

Henrietta N, Arne B, Jacob E, Lars M, Bertil RR, Leif G, Salford LG (2009) Increased blood brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. J Pathophysiol 16(2–3):103–12

Khalatbari S, Sardari D, Mirzaee AA, Sadafi HA (2006) Calculating SAR in two models of the human head exposed to mobile phones radiations at 900 and 1800 MHz. J PIERS Online 2:104–109

Kundi M, Mild KJ, Hardell L (2004) Mobile telephones and cancer: a review of epidemiological evidence. J Toxicol Environ Health 7(5): 351–384

Kuo LC, Kan YC, Chuang HR (2007) Analysis of a 900/1800-MHz dual-band gap loop antenna on a handset with proximate head and hand model. J Electromagn Wave 21:107–122

Lonn S, Ahlbom A, Hall P, Feychting M (2004) Mobile phone use and the risk of acoustic neuroma. J Epidemiol 15:653–659

Salford LG, Brun AE, Eberhardt JL, Malmgren L, Persson BR (2003) Nerve cell damage in mammalian brain after exposure to microwaves from GSM mobile phones. J Elsei Kagaku 111: 881–883

Stilgoe J (2007) The (co-)production of public uncertainty: UK scientific advice on mobile phone health risks. Public Underst Sci 16:45–61

Zhao TY, Zou SP, Knapp PE (2007) Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett 412(1):34–38