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## Upregulation of Specific mRNA Levels in Rat Brain After Cell Phone Exposure

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*Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.*

**Keywords** Cell phones; mRNA; Rat brain.

### Introduction

Cell phones are major emitters of microwave radiation. Effects of this radiation on humans have been studied but the results are contradictory. Finnie et al. (2002) and Kuribayashi et al. (2005) found no change in the permeability of the blood-brain barrier (BBB) using a rat model. In contrast, Salford et al. (2003) found, after a 2 h exposure, significant leakage across the rat BBB. Finnie (2005) reported there was no change in the proto-oncogene c-fos protein level in the mouse brain after a short-term exposure. Hata et al. (2005), studying the secretion of melatonin and serotonin from the rat pineal gland, found no changes either. One long-term study exposed rats for 90 min per day, 5 days per week for 104 weeks; this chronic exposure did not induce tumor growth in the rat central nervous system (Shirai et al., 2005). This study, although long term, did not evaluate early or subtle effects of exposure including changes in gene expression. Very little data has been published on this topic. The change in mRNA levels of apoptotic, heat shock, and general cellular proteins after exposure has been evaluated. Human HeLa-60 and Mono-Mac-6 cells exposed to intermittent non-thermalizing levels of 1.9 GHz

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pulse-modulated RF fields did not cause any detectable change in stress-related gene expression (Chauhan et al., 2006). No statistically significant effects on murine fibroblasts gene expression after 24 h exposure at 5 W/kg were found either (Whitehead et al., 2006). Up-regulation of the heat shock protein Hsp70 in human lens epithelial cells was found after RF exposure but this might be involved in protecting these cells from DNA damage and maintaining the cellular capacity for proliferation (Lixia et al., 2006). A short-term exposure of 2 h showed up-regulation of apoptotic proteins caspase-2, caspase-6 in murine neurons and astrocytes (Zhao et al., 2007). There has not been enough data generated on changing mRNA levels after long-term cell phone exposure using a whole animal model. Using these criteria, subtle neural or vascular injuries over prolonged exposure times could be found by measuring the mRNA level changes of injury associated proteins that are necessary for cellular repair and known to respond to cellular injury. These proteins include: Calcium ATP-ase ( $\text{Ca}^{2+}$ -ATPase) (Chen et al., 2002; Kurnellas et al., 2005), Neural Cell Adhesion Molecule 1 (NCAM-1) (Skaper, 2005; Skibo et al., 2005; Thornton et al., 2005), Neural Growth Factor B (NGF-B) (Blesch et al., 2005; Moore et al., 2006), and Vascular Endothelial Growth Factor A (VEGF-A) (Greenberg and Jin, 2005; Zachary, 2005). We hypothesize that cellular damage to the rat brain; leading to increased mRNA transcription of these injury-associated proteins will be caused by chronic exposure to cell phone radiation leading to gradual degenerative and functional loss.

### **Materials and Methods**

Fourteen 3-month-old male Sprague-Dawley rats, weighing 250–300 g, were the subjects of this research. (Note: For the care and use of laboratory animals, this study used the guidelines of the Biomedical Resource Center of the Medical College of Wisconsin. The Institutional Animal Care and Use Committee of the Medical College of Wisconsin approved the protocol.) The rats were divided into two groups of eight rats each. One group received cell phone radiation exposure, and the other group acted as a control group.

The four cell phones used in the study were Nokia 3588i (Keilalahdentie, Finland), which have a personal communications service code division multiple access (PCS CDMA) frequency band of 1.9 GHz (800 MHz digital and 800 MHz analog). These cell phones have three different modes: AMPS mode, CELL mode, and PCS mode. The various modes can be used based on signal reception, antenna use, and other factors associated with reception of different types of radiofrequency signals. In AMPS mode, the specific absorbance rate (SAR) at a distance of 2.2 cm was measured to be 1.80 W/kg, and the power range was 0.0063–0.607 W. The SAR at a distance of 2.2 cm in CELL mode was 0.9 W/kg, and the power range was 0.00001–0.487 W. The SAR at 2.2 cm away in PCS mode was 1.18 W/kg, and the power range was 0.00001–0.335 W. The frequencies and specific modes of this phone fall within the cell phone radiation parameters set by the U.S. Federal Communications Commission (FCC) (19). Each cell phone was positioned 1 cm from the head of the rats, at equal distances between two rats in holding chambers.

Customized holding units (Fig. 1) and cell phone platforms were constructed for this study. The holding units for the rats consisted of 5.1 cm × 15.2 cm PVC tubes with holes for circulation, fitted with 0.59-liter clear plastic bottle tops on one end and common 7.6-cm bolts with nuts at the other end. As the rats grew larger during this study, new tubes were fashioned using 8.9 cm-diameter PVC pipes with holes for air circulation, 1-liter bottles, and 10.2 cm bolts with nuts. The holding units were plastic, because metal can absorb radiation energy.

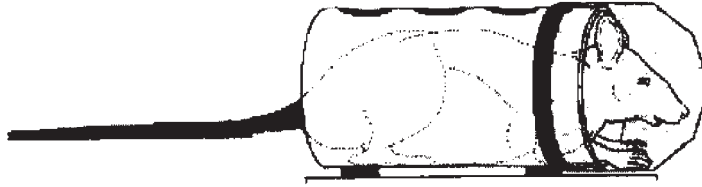


**Figure 1.** Rat in plastic PVC holding tube. The tube has a clear plastic bottle top and numerous air holes for adequate ventilation.

The rats were acclimated to these holding units for 1 week before the beginning of testing by placing the units in the rat cages to allow the rats to become familiar with their smell and feel. After less than one day, the rats voluntarily entered the units to rest and sleep in them. By the end of the week, the rats would enter the holding units as soon as they saw them. Owing to this acclimation process, anesthesia was not required during the exposure time. Therefore, the rats did not have any ill effects or altered physiology from anesthesia, rendering the comparison to humans more relevant.

The experimental rat group was exposed to 3 h of cell phone radiation, followed by a 30 min rest period outside of the tubes and a second exposure for 3 more hours per day. During the 30 min rest period, the rats were removed from the tubes and were free to walk around, eat, and drink. The rats received this daily cell phone exposure for 18 weeks. The 8 rats in the control group were placed in identical tubes for the same amount of time as the experimental rats but without cell phone exposure.

To address the concern that the harmful effects of cell phones are due to heat given off by the phone rather than the radiation emitted, we took temperatures from both groups during a standard day of exposure. Temperatures of the rats were taken at the side of the face surface nearest the phone, using a Mini-Alarm thermometer with a probe (Fisher Scientific, Hampton, NH). These readings were taken approximately every 12 min during both of the 3-h exposures. Final rectal temperatures were taken at the end of each of the two exposure times with a Big-Digit thermometer (Fisher Scientific). Because the rectal measurements irritated the animals, repeated measurements were not practical.

After week 18, the rats were sacrificed. Brain tissue was harvested and flash frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Total RNA was extracted using a SV Total RNA Isolation Kit (Promega, USA). The RNA was quantified by 260/280 ratio using a Beckman Spectrophotometer (Beckman, USA). RT-PCR was performed using a SuperScript III One Step RT-PCR Kit (Invitrogen, USA). Each reaction had 50 ng RNA with 0.5  $\mu\text{l}$  of each specific forward and reverse primer (10  $\mu\text{M}$ ). The primers (PE Applied Biosystems, USA) used were as follows:

Beta Actin (BA) forward 3'-AGCCATGTACGTAGCCATCC-5', reverse 3'-CTCTCAGCTGTGGTGGTGA-5',  $\text{Ca}^{2+}$ ATPase forward 3'-CTGTCCA TGTCCCTCCACTT-5', reverse 3'-GGGTGGTTATCCCTCCAGAT-5', NGF-B forward 3' CTGTGTGCAGGAGAGATGGA-5', reverse 3'-ATTGAGACCAGGGGACTGTG-5', NCAM-1 forward 3'-AAAGGATGG GGAACCCATAG-5', reverse 3'-TAGGTGATTTTGGGCTTTGC-5' and VEGF-A forward 3'-CAATGATGAAGCCCTGGAGT-5' and reverse 3'-TTTCTTGCGCTTTCGTTTTT-5'.

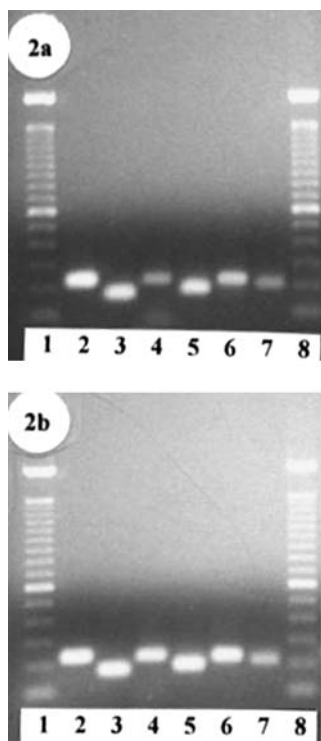


**Figure 2.** (a, b) Examples of fluorescent pictures of the agarose gels used in the quantitation of the RT-PCR band intensity. (a) Control and (b) Experimental. Lanes are as follows: 1-MWM (100Bp), 2-BA, 3-CaATPase, 5-NCAM-1, 6-NGF-B, 7-VEGF-A, and 8-MWM (100Bp).

The reactions ran at 55°C for 30 min, 94°C for 2 min and 15 sec, 55°C for 30 sec, and 72°C for 1 min for 40 cycles. The DNA was run on a 1% Agarose gel containing ethidium bromide in TAE buffer. A photo of the gel was taken on a Fotodyne 21 UV box (Fotodyne, USA) with an Electrophoresis Photo Documentation Camera and Hood (Fisher Scientific, USA) (Fig. 2). The photo was then quantitated in a MultiImage Light Cabinet with AlphaImager 2000 software (Alpha Innotech Corporation, USA).

### Results

The intensity of the bands of interest was read by the AlphaImager 2000 software and was assigned a unitless number. The greater the amount of specific cDNA amplified the more intense the agarose gel band was and a higher number was generated by the software. These numbers will now be identified in relative intensity units (RIU).

The mRNA levels of the four injury-associated proteins (Ca<sup>2+</sup>ATPase, NCAM-1, NGF-B, and VEGF-F) were examined. The Beta Actin mRNA level was used as the internal control since it is a housekeeping protein and its level of expression does not change. The mean Beta Actin intensity of the 14 rats in this study was 60916 (data not shown). This value was used along with the individual Beta Actin intensities to obtain a normalization coefficient for each value (data not shown). The final values in Table 1 were obtained by multiplying the specific protein RIU with the corresponding normalization coefficient. Normalization of the data is a routine and accepted practice necessary to standardize the experimental variations inherent in any type of PCR.

**Table 1**

Normalized RT-PCR Data. The RT-PCR agarose gel band intensity measured in RIU of each protein was normalized by multiplying the measured levels with the normalization coefficients calculated using each specific Beta Actin RIU as controls of RNA integrity. Included are the averages (AVE) and standard deviations (SD). These data are in Fig. 3

| Rat          | Ca-ATPase | NCAM  | NGF   | VEGF  |
|--------------|-----------|-------|-------|-------|
| Experimental |           |       |       |       |
| 1            | 56706     | 64634 | 66846 | 55515 |
| 2            | 57430     | 55601 | 32073 | 53240 |
| 3            | 62401     | 59774 | 36596 | 41464 |
| 4            | 58590     | 59039 | 55911 | 46599 |
| 5            | 60948     | 55179 | 54698 | 49964 |
| 6            | 56973     | 57389 | 59043 | 53192 |
| 7            | 60462     | 58463 | 44401 | 47375 |
| Ave          | 59073     | 58583 | 49938 | 49621 |
| SD           | 2216      | 3167  | 12616 | 4855  |
| Control      |           |       |       |       |
| 8            | 56074     | 59378 | 32245 | 40788 |
| 9            | 55485     | 45441 | 36068 | 35378 |
| 10           | 44934     | 44519 | 43644 | 41152 |
| 11           | 54502     | 54560 | 53210 | 51302 |
| 12           | 44383     | 38860 | 16697 | 23838 |
| 13           | 51001     | 51291 | 48746 | 51811 |
| 14           | 51497     | 50128 | 36621 | 41979 |
| Ave          | 51125     | 49168 | 38176 | 40893 |
| SD           | 4810      | 6838  | 12049 | 9575  |

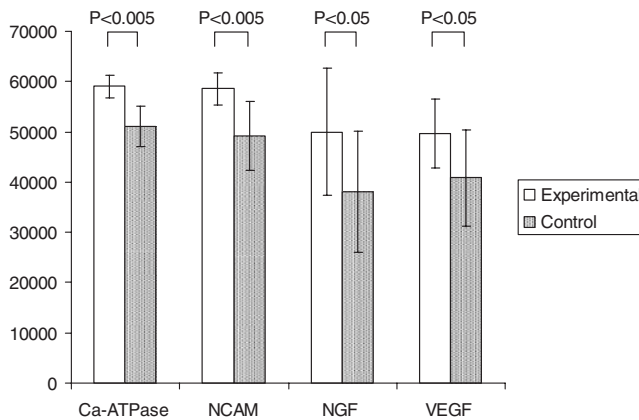


**Figure 3.** This bar graph shows the RIU (y axis) differences between the control and experimental rats with regard to the levels of the four mRNAs tested. The standard deviations are noted on the tops of the bars as well as the *T* test *p* values. These data are from Table 1.

Meaningful and accurate comparisons between mRNA levels of each protein could now be made.

As seen in Table 1, the normalized RIU of the four specific proteins were as follows.

- The control group mean values were:  $51125 \pm 4801$  for  $\text{Ca}^{2+}$ -ATPase;  $49168 \pm 6838$  for NCAM-1;  $38176 \pm 12049$  for NGF-B; and  $40893 \pm 9575$  for VEGF-A.
- The experimental group mean values were:  $59073 \pm 2216$  for  $\text{Ca}^{2+}$ -ATPase;  $58583 \pm 3167$  for NCAM-1;  $49938 \pm 12616$  for NGF-B; and  $49621 \pm 4855$  for VEGF-A.

The RIU difference of each protein between two groups was all statistically significant. (Using a standard independent *T*-test. See Table 1:  $P < 0.005$  for  $\text{Ca}^{2+}$ -ATPase;  $P < 0.005$  for NCAM-1;  $P < 0.05$  for NGF-B; and  $P < 0.05$  for VEGF-A).

Data were gathered to show whether the heat given off by phone exposure might increase facial surface or internal temperatures of the rats. In our previous study (Yan et al., 2007), the mean surface temperature of the head after 3 h of radiation exposure was  $33.3 \pm 0.5^{\circ}\text{C}$  for the experimental group and  $33.5^{\circ}\text{C} \pm 1.1^{\circ}\text{C}$  for the control group. Similarly, after the full 6 h of exposure, the mean surface temperature was  $33 \pm 0.6^{\circ}\text{C}$  for the experimental group and  $32 \pm 0.8^{\circ}\text{C}$  for the control group. The mean rectal temperature after 3 h of exposure was  $35.6 \pm 0.3^{\circ}\text{C}$  in the experimental group and  $36.4 \pm 0.5^{\circ}\text{C}$  in the control group. The mean rectal temperature after 6 h of exposure was  $35.9 \pm 0.6^{\circ}\text{C}$  in the experimental group and  $35.9 \pm 0.5^{\circ}\text{C}$  in the control group. The differences in temperatures measured by a standard independent *T*-test were not statistically significant ( $p > 0.5$ ).

## Discussion

Long-term, high-dose, whole-body exposures are needed to accurately assess the effects of cell phone microwave radiation. Short to moderate exposure times using animal models, cell cultures, or questionnaires filled out by human subjects have been the methodologies of choice. These methods have not yielded satisfactory results to settle the controversy involving cell phones. To generate accurate data, whole-body exposure time of a sufficient length is needed to correspond to that of the average person's use of a cell phone. An intensive long term exposure with a total time of 756 h was therefore performed. Given the expected lifespan of rats are 1–2 years (8,760–17,520 h), this total exposure time translates into 4–9% of their lifespan. This is comparable to human subjects who use cell phones for between 0.96–2.16 h per day for over 30 years. Irreparable cellular injury can occur and subsequently lead to tissue and system damage without reaching the stage of causing cancerous growth. Various proteins that are up or down regulated after injuries have been investigated. For this reason, four injury-associated proteins, which serve as indicators of acute cellular injuries, were chosen.  $\text{Ca}^{2+}$ -ATPase is the major enzyme responsible for the concentration gradient in healthy nerves. If an injury occurs, calcium influx causes edema and cell death. This protein is needed in increased amounts to restore the gradient prior to nerve healing. NCAM-1 contributes to nerve regeneration by increasing the ability of Schwann cells to adhere to the axon. An increase in synaptogenesis, fasciculation, and general nerve growth are also functions of this protein.

NGF-B stimulates nerve sprouting and neurite growth. The increased production of this protein under conditions of tissue injury or inflammation also aids in angiogenesis using a VEGF mediated pathway. VEGF-A in concert with NGF-B aids in nerve regeneration and wound healing. Injuries stimulate the neurotropic and neuroprotective properties of this protein. These proteins showed mRNA up-regulation in the exposed group compared to the controls. These increases were all statistically significant. These up-regulations, we believe, were stimulated by cellular damage caused by cell phone radiation. Although these proteins aid in cellular repair after acute injury, if some injuries are only partially repaired, repetitive insults over a long period of time may result in cumulative cellular injuries, leading to cellular dysfunction. Even though the inherent plasticity of the brain may compensate partially or completely for the cellular dysfunction, the plasticity and the self-repair ability of the brain decrease over time. At some point, when the compensatory mechanisms of the brain are overwhelmed, the effects of cellular dysfunction will then manifest as clinically significant functional disabilities. The exact mechanism of cell phone exposure induced injury is still unknown; perhaps the electromagnetic effect does play an important role. It would seem prudent for people to keep their cell phone exposure to a minimum in case there are small but irreversible injuries taking place.

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