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Effects of exposure of the ear to GSM microwaves: in vivo and in vitro experimental studies

Efectos de la exposlción del oído a micro-ondas GSM: estudios experimentales in vivo e in vitro

Abstract
The effects of mobile phone (GSM) microwaves on the ears of guinea pigs were investigated in two in vivo experiments and one in vitro experiment. In the first experiment, three groups of eight guinea pigs had their left ear exposed for 1 h/day, 5 days/week, for 2 months, to GSM microwaves (900 MHz, GSM modulated) at specific absorption rates (SARs) of 1, 2 and 4 W/kg respectively, and a fourth group was sham-exposed. Distortion-product otoacoustic emissions (DPOAEs) were measured for each ear before exposure, at the end of the 2-month exposure period, and 2 months later. In the second experiment, the same protocol was applied to eight sham-exposed and 16 exposed guinea pigs at 4 W/kg, but the auditory brainstem response (ABR) thresholds were monitored. Repeated-measures ANOVA showed no difference in DPOAE amplitudes or in ABR thresholds between the exposed and non-exposed ears and between the sham-exposed and exposed groups. In the course of the second experiment, acute effects were also investigated by measuring once, in all animals, ABR thresholds just before and just after the 1-h exposure: no statistically significant difference was observed. In vitro, the two organs of Corti (OCs) of newborn rats ($n = 15$) were isolated and placed in culture. For each animal, one OC was exposed for 24–48 h to 1 W/kg GSM microwaves, and the other was sham-exposed. After 2–3 days of culture, all OCs were observed under light microscopy. They all appeared normal to naive observers at this stage of development. These results provided no evidence that microwave radiation, at the levels produced by mobile phones, caused damage to the inner ear or the auditory pathways in our experimental animals.

Sumario
Se investigaron los efectos de las micro-ondas (GSM) de los teléfonos móviles sobre los oídos de los cobayos en dos experimentos in vivo y en uno in vitro. En el primer experimento, 3 grupos de 8 cobayos (GP) tuvieron su oído izquierdo expuesto durante 1 hora/día, 5 días/semana, por 2 meses, a micro-ondas GSM (900 MHz, con modulación GSM) con Tasas de Absorción Específica (SAR) de 1, 2 y 4 W/kg, respectivamente, y un cuarto grupo tuvo una exposición fingida. Se midieron emisiones otoacústicas por productos de distorsión (DPOAE) antes, al final del período de exposición de 2 meses y 2 meses después. En el segundo experimento, se aplicó el mismo protocolo con 8 exposiciones fingidas y 16 GP expuestos a 4 W/kg, pero se monitorizaron los umbrales de las Respuestas Auditivas del Tallo Cerebral (ABR). Las mediciones repetidas ANOVA no mostraron diferencia en las amplitudes de los DPOAE o en los umbrales de las ABR entre oído expuestos y no expuestos, y entre los grupos con exposición verdadera y fingida. En el curso del segundo experimento, se consideraron los efectos agudos midiendo una vez, en todos los animales, los umbrales ABR inmediatamente antes y una hora después de la exposición: no se observó una diferencia estadísticamente significativa. In vitro, se aislaron y colocaron en cultivo dos órganos de Corti (OC) de ratas recién nacidas. Para cada animal, un OC fue expuesto por 24-48 horas a micro-ondas GSM de 1-W/kg; el otro se sometió a una exposición fingida. Luego de días adicionales en cultivo, todos los OC fueron observados bajo microscopía de luz. Ante observadores independientes, todos lucieron normales en esa etapa de crecimiento. Los resultados no muestran evidencia de que la radiación por micro-ondas, a los niveles producidos por los teléfonos móviles, puedan causar daño en el oído interno o en las vías auditivas de nuestros modelos experimentales.

Because of the rapid development of mobile telephony during recent years, concern has arisen about the potential health hazards associated with the personal use of mobile phones. These fears concern mainly cancer and the brain. However, the inner ear is the organ that is closest to the mobile phone. The effects of radiofrequency radiation (RFR) on the ear and on hearing have rarely been investigated in humans (Kellényi et al, 1999; Ozturan et al, 2002) and only occasionally in animals (Marino et al, 2000). Other studies have dealt with the possible interference of mobile phone radiation with hearing instruments such as hearing aids or cochlear implants (Kompis et al, 2000; Skopec, 1998; Sorri et al, 2001). Within the framework of the COMOBIO project (http://tsi.enst.fr/comobio/), we were assigned the task of investigating the effects of GSM RFR on the ear structures and on auditory function.

Since the brain is also exposed to the microwaves, changes in auditory functions could be caused by action at the level of the inner ear and/or the central auditory pathways. In order to differentiate between these two possible sites of action, experiments were aimed at monitoring separately peripheral and central auditory functions after exposure of the animals to microwave radiation.

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Materials and methods

Peripheral function was monitored at the level of the outer hair cells (OHCs) of the organ of Corti (OC) by recording otoacoustic emissions (OAEs). These OHCs, located in the inner ear, are responsible for the reception and selective amplification of basilar membrane vibrations, after sound waves have been transferred to the inner ear fluids by the ossicular chain. In the course of this active amplification, via contractile properties of the OHCs, new sound vibrations develop which travel backwards along the basilar membrane, and through the ossicular chain and the tympanic membrane, and can be recorded as sound in the external auditory canal by a sensitive microphone. During stimulation with two simultaneous pure tones at different frequencies F_1 and F_2 ($F_2 > F_1$), the microphone in front of the tympanic membrane records not only the two stimulating tones but also combination tones, particularly the cubic difference tones $2F_1 - F_2$. These inner generated sounds in the ear canal are referred to as distortion-product otoacoustic emissions (DPOAEs). The recording and analysis of these distortion products provide an indirect measurement of the basic OHC function.

The vibrations of the basilar membrane also stimulate the inner hair cells (IHCs) of the OC, which stimulate the fibres of the auditory nerve to which they are connected. Activity arising in the auditory nerve in turn triggers activity in the central auditory pathways, which can be recorded as sound-evoked potentials.

It is these peripheral (DPOAEs) and central (auditory brainstem response (ABR)) activities that we recorded in the experimental animal. Thus, comparison of changes at the central and the peripheral levels should allow the localization of the potential site of action of the RFR.

Unfortunately, in these experiments, it was not possible for technical reasons to monitor at the same time, in the same animals, both OAEs and ABR thresholds. Thus, two successive experimental series were conducted. In the first one, we measured DPOAEs in groups of guinea pigs exposed to three different levels of absorbed power (specific absorption rate (SAR) of 1, 2 and 4 W/kg). In the second experimental series, ABR thresholds were monitored in groups of guinea pigs exposed at the highest SAR level of 4 W/kg. In this second experimental series, to investigate the potential acute effects of the RFR exposure, we measured ABR thresholds just before and just after the 2-h exposure, in each guinea pig once during the 2-month exposure period.

In addition, to investigate potential specific effects of RFR directly on the OHCs, we conducted a third experiment in vitro. Here, the developing OC, harvested from newborn rats and maintained in culture, was investigated in both the microwave-exposed and sham-exposed conditions.

Exposure system: in vivo

During exposure or sham exposure, and DPOAE or ABR measurements, the guinea pigs were maintained in a holding device composed of two sliding-yoke PVC tubes, each with a V notch. The notch provided a hole for the guinea pig's neck, so that the head could be kept outside the tube and maintained straight by a sliding ring adjusted over the nose of the animal (Figure 1). A platform was also adjusted inside the tube assembly, on which the guinea pig's body could lay comfortably (Figure 1). The animals quickly adapted to the device and could stay inside it for hours without signs of discomfort. Animal handling throughout these experiments was performed with the authorization of the French Ministry of Agriculture, in accordance with EEC regulations.

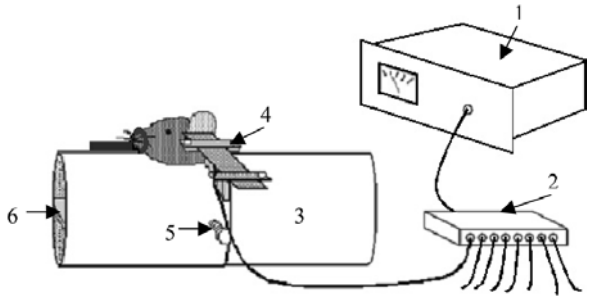

Figure 1. Setup for exposure of guinea-pig left ear to the GSM microwaves. 1, RFR generator; 2, eight-channel divider; 3, holding box; 4, loop antenna; 5, fixation screw (nylon); 6, resting platform for the guinea pig.

The exposure equipment (Figure 1) consisted of a GSM microwave generator (RFS900-60, 900 MHz, with GSM rectangular modulation at 217 Hz (the signal was such that the power was on during one-eighth of the period with a repetition rate of 217/s)) connected to an eight-channel divider, so that eight guinea pigs could be exposed at once. Eight specially developed loop antennas (P. Lévêque, C. Dale, B. Veyret & J. Wiant, unpublished data) were connected to the divider. The antennas were held with a PVC arm fixed to the holding box (experiment 1) or individual PVC stands (experiment 2), so that the antenna was maintained horizontally at the level of the external auditory canal of the left ear, in contact with the edge of the pinna (Figures 1 and 2), thus exposing mainly the peripheral auditory structures (the coiled cochlea and the eighth nerve). An eight-compartment setup was constructed from RFR-absorbing panels so that each guinea pig was placed in one compartment with its own antenna (Figure 2), to avoid interference among antennas.

Dosimetry

Local SAR measurements were done on an anaesthetized guinea pig: the tip of a temperature probe (M600 fiberoptic probe, Luxtron, Santa Clara, CA, USA) was inserted through the round window into the cochlea. It was cemented to the skull to avoid movements of the probe during SAR measurement. High power settings of the generator had to be used to provide large temperature increases and therefore good signal-to-noise ratios. The average of three measurements at 7-, 17- and 34-W input to the loop antenna gave an SAR of 3.5 ± 0.4 W/kg per incident watt in the cochlea.

In vivo protocols for tests and exposure

Exposure and test protocols were the same for all in vivo experiments. The exposure schedule was 1 h/day, 5 days/week, for 2 months. Tests were performed before (T_0), at the end of (T_2) and 2 months after the end of the 2-month exposure (T_4). At the

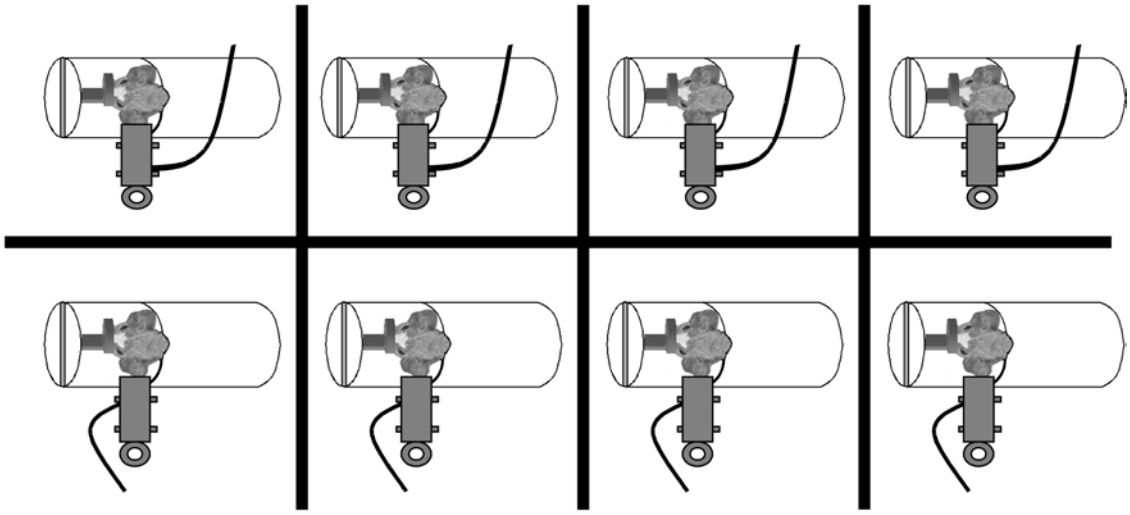

Figure 2. Exposure setup for groups of eight guinea pigs. The black bars represent the RFR-absorbing panels.

end of a given experiment, if a guinea pig showed a significant change in the responses between T_0 and T_4, the status of both ears was functionally and morphologically studied.

In the first experiment (DPOAEs), four groups of eight pigmented female guinea pigs were constituted at random: three groups had their left ear exposed at 1, 2 and 4 W/kg respectively, and the fourth group was sham-exposed. In the second experiment (ABR thresholds), two groups of eight albino female guinea pigs had their left ear exposed to the GSM microwaves at 4 W/kg, and one group of eight guinea pigs was sham-exposed. All guinea pig groups were composed of animals with normal DPOAEs or ABR thresholds at T_0.

In both experiments, for each group, the time of exposure within the day, as well as the position of each guinea pig within the exposure setup (Figure 2), were systematically rotated, to homogenize the overall exposure conditions. During sham exposures, the guinea pigs were placed under the same conditions as the exposed groups, with the GSM generator on (thus maintaining the same 50-dB SPL low-frequency generator fan noise as for the exposed groups), but the output to the eight-channel divider was set at zero, so that the antennae delivered no RFR.

DPOAE measurements

The guinea pigs were lightly anaesthetized with an intramuscular (IM) injection of 1 ml/kg of a solution made of 1 volume of 2% xylazine (8 mg/kg; Rompun, Bayer, Leverkusen, Germany) and 2 volumes of 50 mg/ml ketamine chlorhydrate (33 mg/kg; Ketalar, Parke Davis, Courbevoie, France).

The F_1 and F_2 tones were elicited, and the cubic distortion tone $2F_1 - F_2$ analysed, with the use of a commercial device developed for clinical use: the Virtual M330 software and hardware, with an Etymotic Research acoustical ear probe assembly (composed of one insert microphone and two tubes connected to two separate speakers).

The F_1/F_2 ratio was 1.21 (i.e. $F_2 = 1.21 \times F_1$), with the geometric mean $F_e = \sqrt{(F_1 \times F_2)}$ varying from 0.5 to 8.01 kHz in steps of 1/6th octave (i.e. F_1 varied from 0.45 to 7.28 kHz). F_1 and F_2 levels were both presented at 75 dB SPL. F_1, F_2 and cubic distortion tones at $2F_1 - F_2$ were measured by the calibrated insert microphone inside the ear probe assembly.

The probe, fitted with an ear-tip composed of an 8-mm-long and 5-mm outside diameter silastic tube, was inserted in the ear canal, with great care being taken to obtain a tight seal. The DPgram (amplitude of the $2F_1 - F_2$ DPOAE as a function F_e) was then recorded and repeated until three reproducible tracings were obtained. A mean DPgram was then calculated. The procedure was successively performed for each ear at T_0, T_2 and T_4.

ABR thresholds

The protocol consisted of recording from remote locations (i.e. at the surface of the skull) the electrical activity evoked in the auditory brainstem neural pathways (from the eighth nerve to the inferior colliculus) by transient acoustical stimulations (far-field potentials).

Owing to the very small amplitude of the signals at the electrode sites, compared to the bioelectric ongoing background activity, it was necessary to average a large number of responses (typically 1000) to a repetitive stimulation. Nevertheless, it is still necessary to reduce as much as possible this bioelectric noise. For this purpose, the guinea pigs were maintained in the holding devices under light sedation with one IM injection (0.3 ml) of 10% medetomidine (Domitor) (Pfizer, Orsay, France), and additional noise reduction was obtained by injecting, at each electrode site, a local anaesthetic (2% lidocaine; Xylovet, CEVA, Libourne, France), which significantly reduced the neuromuscular activity close to the electrodes while not affecting the evoked activity in the auditory pathways.

The stimulating and recording equipment was based on PC-controlled TDT (Tucker Davis Technologies, Gainsville, Florida, USA) hardware and software for signal generation (SigGen) and data acquisition and averaging (BioSig), in turn controlled by software (DETAUSA) developed at INRS (Institut National de la Recherche sur la Sécurité, Nancy, France) by Robert Lataye. Altogether, the system synthesized the acoustical stimuli (tone pips of 4-ms duration with 2-ms rise–fall times (no plateau), with six pure-tone frequencies: 1, 2, 4, 8, 16 and

24 kHz). They were presented via a small headphone transducer (Sennheiser HD 480 II, Wedemark, Germany) placed against the tested ear, at a rate of 21 stimuli/s. The levels were varied in steps of 10 dB from 10 to 70 dB SPL.

The electrodes were stainless steel needles. One electrode, for recording activity from the central part of the brain, was placed under the skin at the top of the head (vertex). Two needle electrodes for recording peripheral activity were placed under the skin behind the pinna (mastoid area) of each ear and connected together. A fourth needle electrode was placed at the nose. This electrode served as a ground electrode, while the vertex and mastoid electrodes were connected to the positive and negative inputs of a TDT HS4-DB4 biological amplification system. Amplification was 100 000, with a bandwidth of 0.3–3 kHz. Responses were recorded with a 30-ms window at a sampling rate of 50 μs/point. One thousand responses were averaged. Automatic rejection of artefacts as implemented in the BioSig software was utilized. An initial recording, without acoustical stimulation, was started with the above conditions and repeated until the amplitude of the averaged signal (residual bioelectric noise) was less than 200 nV peak-to-peak. Then, the responses to the tone pips at all frequencies and intensities were automatically recorded. At the end of the recording session, the DETAUSA software automatically calculated the input–output functions (response amplitude versus sound level) for each frequency and extrapolated a 'threshold' level corresponding to a 200-nV response amplitude (Figure 3). Thus an 'ABR audiogram' was constructed. These ABR audiograms were measured twice, during the same week, before the exposure period (T_0 and T_0') to evaluate the variability, and then once at T_2 and once at T_4.

Statistical analysis

A repeated-measures analysis of variance was performed on the data. The dependent variable consisted of the DPOAE amplitudes or ABR thresholds, and the independent variables were the frequency, the group (1 W/kg, 2 W/Kg, 4 W/Kg or sham), the ear (left, right) and the time (T_0, T_2, T_4). The analyses were done using the SAS software (SAS Institute Inc., Cary, NC, USA).

Final controls

The protocol included, at the end of the experiments, a functional and morphological evaluation of both ears in guinea pigs which would have shown significant changes in the responses between T_0 and T_4. However, this was not necessary, since only one guinea pig out of the 56 animals in the two experiments showed significant impairment in one ear. Analysis of the ears in this guinea pig revealed complete cochlear ossification of the exposed (left) ear and a normal right ear (see 'Results').

In vitro experiments

Two pieces of wire-patch cell (WPC) apparatus were used to expose the OCs in Petri dishes at well-characterized SAR levels. The WPC has been described elsewhere (Laval et al, 2000). Briefly, it consists of two parallel square metallic plates, the bottom one being powered with RFR through a coaxial line passing through the top plate. Four poles at the four corners between the plates allow for optimal tuning of the device, which, in these experiments, was set at 900 MHz. Extensive dosimetric measurements showed that the uniformity of the SAR in the culture medium was very good (< 15%) and that the efficiency was 0.6 W/kg per incident watt. Each WPC was placed in its own incubator for true blind operation.

OCs of newborn rats ($n = 15$) were dissected out at postnatal day 3–4.

The OCs were isolated under sterile conditions, and the stria vascularis and spiral ganglion removed. Dissection of the OC was carried out in Hanks' balanced salt solution (Sigma, France) ($CaCl_2$ 1.3 mM, $MgSO_4$ 1 mM, KCl 5.4 mM, NaCl 137 mM, KH_2PO_4 0.4 mM, NaH_2PO_4 3.4 mM, D-glucose 5.5 mM, pH 7.4, 290 mOsm/kg H_2O). The OCs were then placed in a culture

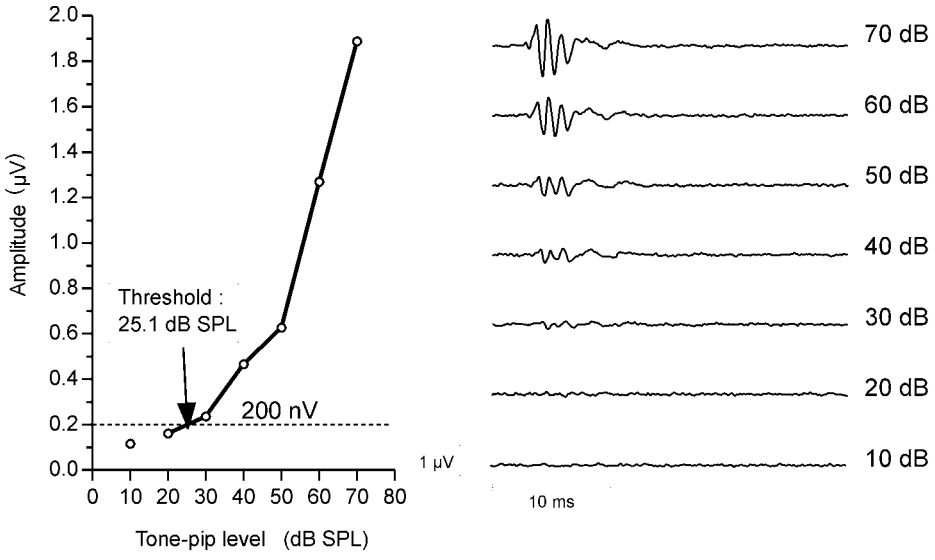

Figure 3. Right: Typical ABR responses to a 16-kHz tone pip from 10 to 70 dB SPL. Left: peak-to-peak amplitude of the responses as a function of tone-pip level and determination of threshold (extrapolated level for a 200-nV response amplitude).

medium composed of Dulbecco's modified Eagle's medium (DMEM) (Sigma) containing 10% fetal bovine serum (Sigma), 25 mM HEPES buffer and penicillin (2500 IU). Cultures were grown at 37°C in 5% CO_2 for 2–3 days.

The OCs were then exposed to the GSM microwaves at an SAR of 1 W/kg for 24 h. For each rat, one OC was exposed, and the other OC was sham-exposed (i.e. subjected to the same treatment but without the GSM signal). Temperature was monitored in the exposed and sham-exposed OCs. After the exposure, the OCs were placed back in the culture chamber and maintained there for 2–3 days. They were then fixed with 4% paraformaldehyde (1 h) and permeabilized using a 0.5% solution of Triton X-100 (15 min). They were rinsed three times in phosphate-buffered saline (PBS) and incubated in 1 μg/ml phalloidine-rhodamine for 45 min at room temperature in darkness. They were rinsed again three times in PBS (2×6 min and 1×4 min). Finally, the OCs were mounted on glass slides and observed under fluorescent microscopy by naive observers uninformed about the exposed or sham-exposed status of the observed OC. Because the OC curls during its in vitro development, it was not possible to make accurate counts of hair cells along the basilar membrane, but general aspects of the hair cell population were evaluated, particularly in terms of the ratio between missing and normal hair cells in the areas that could be easily observed.

Results

Experiment 1: DPOAEs

Of the 32 guinea pigs tested, only one, in the 2 W/kg exposure group, showed significant impairment of its left (exposed) ear, compared to the right ear. The DPOAEs from the left ear had disappeared at T_2, while those from the right ear were normal at T_2 and T_4. Therefore, the ears of this guinea pig were more closely examined. At the time of killing, the left cochlea appeared completely ossified, while the right cochlea was functionally and morphologically normal. This left-ear ossification, as will be discussed below, did not appear to be related to GSM exposure. This guinea pig was removed from the 2 W/kg group. One guinea pig died in the 1 W/kg group, and the data from the remaining 30 guinea pigs were analysed.

Figure 4 presents the mean DPgrams (DPOAE amplitude and standard error of the mean) of each group, for the right (unexposed) and left (exposed) ears, at T_0, T_2 and T_4.

Statistical analysis (repeated-measures ANOVA) showed great variability in the measures for the right (unexposed) ears over time. At T_0, sham-exposed animals tend to have higher DPOAE mean amplitudes than the other groups ($p < 0.03$). At T_2, the 1 and 4 W/kg groups had significantly lower DPOAE amplitudes than the sham-exposed group ($p < 0.006$ and $p < 0.001$, respectively). At T_4, only the 2 W/kg group showed significantly increased amplitudes compared to the sham-exposed group ($p < 0.0004$). Overall, there was no dose–response relationship between DPOAEs and SAR.

For the left (exposed) ears, the four groups did not differ at T_0, even if the amplitudes were higher in the sham-exposed group. At T_2, lower amplitudes were observed in the 1 and 2 W/kg groups than in the sham-exposed group ($p < 0.009$ and $p < 0.04$, respectively). At T_4, no significant difference was observed among groups. No dose–response relationship between DPOAEs and SAR was found.

Experiment 2: ABR thresholds

In this experiment, only the highest SAR level of 4 W/kg was studied. The mean ABR audiograms for each group, for each ear, at T_0, T_0', T_2 and T_4 are presented in Figure 5. It is clear that all thresholds increased between T_0 and T_4, in the exposed and sham-exposed groups, for the left and right ears (Figure 5).

Using the values estimated from the repeated-measures ANOVA, the mean ABR thresholds for the left and right ears and the differences between groups and between ears are presented in Figures 6 and 7.

A significant increase of mean ABR threshold with time for both ears was observed ($p < 0.001$). For the left ears, no significant difference in the thresholds was observed between the two groups ($p < 0.60$). For the right ears, the same pattern was observed: no significant difference in the thresholds between the two groups ($p < 0.60$) (Figure 6). Thus, the only significant increase in mean ABR threshold was observed with time for both ears ($p < 0.001$).

The difference in the ABR thresholds as a function of time between the exposed and the sham-exposed animals is presented in Figure 7 (left side) for the left and right ears. No significant difference was observed between the groups for the left ear or the right ear.

The difference in the ABR thresholds as a function of time between the left and right ears for the exposed and the sham-exposed animals is presented in Figure 7 (right side). No global significant difference was observed between the ears for the exposed ($p < 0.10$) and the sham-exposed ($p < 0.40$) groups. However, at T_4 the threshold is significantly higher in the right ear in the exposed animals ($p = 0.02$) and in the sham-exposed animals ($p = 0.05$).

Mean ABR thresholds were also measured immediately before and after exposure to GSM RFR at some time during the 2-month exposure period: no significant difference was observed ($p < 0.96$).

Experiment 3: in vitro

In none of the 15 newborn rats was it possible to observe a difference between exposed and sham-exposed OCs. Figure 8 shows views of parts of the exposed and sham-exposed OCs of three different rats. The hair cell population and pattern of organization appears completely normal at this stage of development (postnatal day 6–9).

Discussion and conclusions

The analysis of the results is rather straightforward, as there were no statistically significant differences among groups for all variables studied. However, in one guinea pig, in experiment 1, in the 2 W/kg group, complete ossification of the left (exposed) cochlea apparently developed during exposure, while the right ear was not affected. It would be tempting to relate this condition of the left ear to exposure to GSM microwaves. However, this is the only case (out of a total of 40 exposed guinea pigs, and a total of 56 in the overall experiments) that showed such ossification. This occurred at a medium level of exposure (2 W/kg). This situation did not occur in any of the 8 + 16 guinea pigs exposed at 4 W/kg. Such ossification of the cochlea was observed on several occasions in our laboratory during the same period, in guinea pigs from the same provider. In none of the

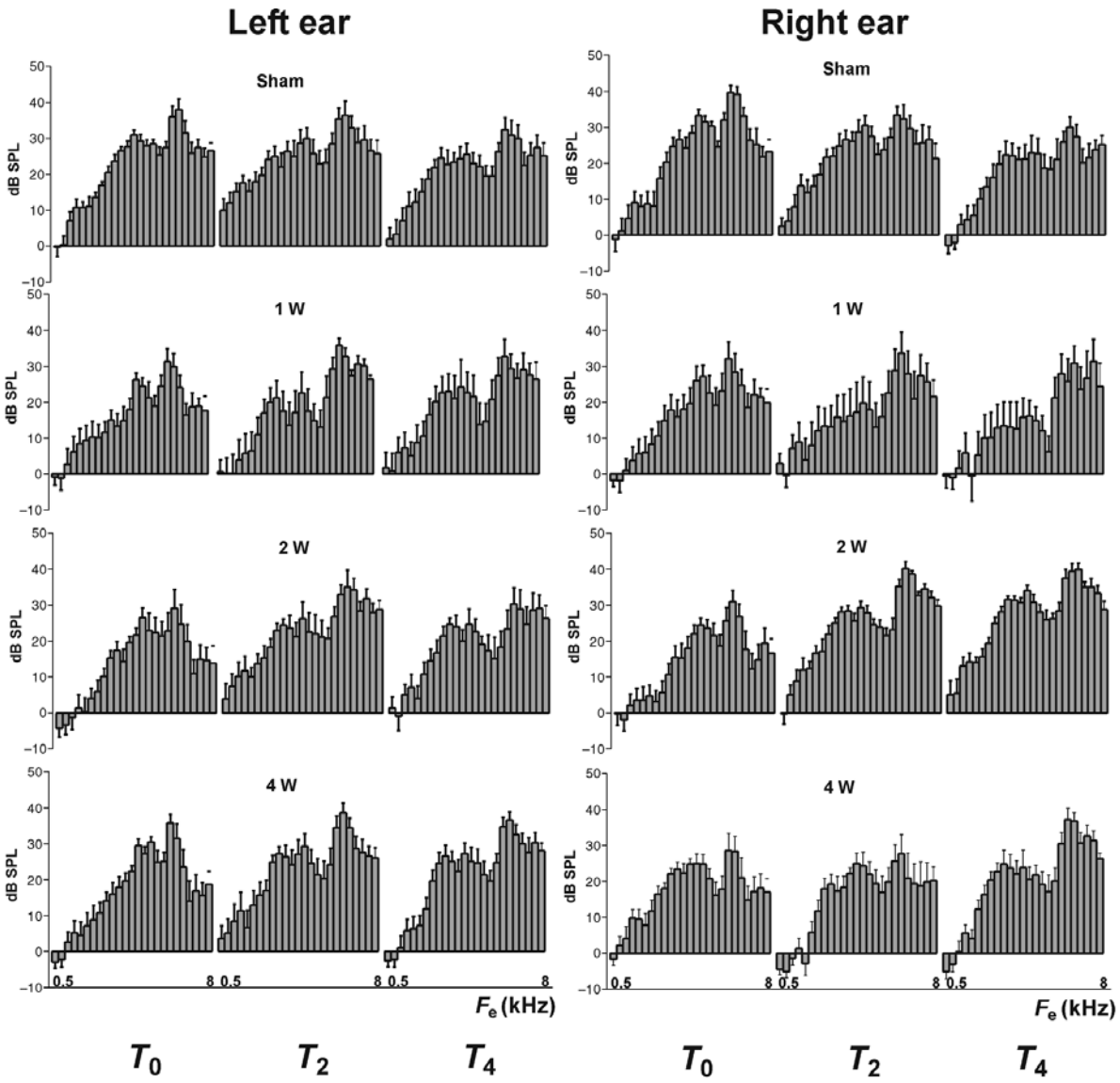

Figure 4. Mean DPOAE amplitude and standard error of the mean at each of the 25 frequencies from 0.5 to 8 kHz, before (T_0), at the end of (T_2) and 2 months after the end of (T_4) the 2-month exposure, for the left and right ears, in the sham-exposed group and in the three groups exposed at SARs of 1 W/kg, 2 W/kg and 4 W/kg (1 h/day, 5 days/week, for 2 months) ($n = 8$ in the sham-exposed and 4 W/kg groups, and $n = 7$ in the 1 W/kg and 2 W/kg groups).

cases could this ossification be related to the particular experiment. Labyrinthitis ossificans, which can be unilateral, as in this case, can be caused by various pathological conditions, the most common being otitis media and pneumococcal meningitis, diseases which the guinea pigs are prone to (Suga & Lindsay, 1975; Brodie et al, 1998). Therefore, this guinea pig was excluded from the statistical analysis.

Interestingly, in all guinea pigs, although DPOAE response amplitudes did not change between the beginning and the end of the experiment in experiment 1, ABR thresholds increased progressively during the course of experiment 2 (Figure 6). The significant progressive increase in ABR thresholds in all guinea pigs, however, is not related to the GSM exposure, since it was the same in the exposed and sham-exposed guinea pigs, and occurred for both ears. Altogether, these observations indicate, whatever the exposure conditions, maintenance of normal peripheral auditory function (experiment 1) and progressive impairment of central auditory function (experiment 2). It is unlikely, however, that this progressive ABR threshold elevation indicated progressive impairment of auditory function, as occurs in ageing. This conclusion is reasonable, since the 4-month experimental period was not sufficiently long to permit presbyacusis to develop. Moreover, presbyacusis, which is due mainly to progressive cochlear alteration, does not seem to be the case here, since DPOAEs were unaffected in the same conditions in experiment 1. Still, the ABR threshold elevation appears to be related to the 4-month experimental period. Between the beginning and the end of both experiments 1 and 2, the mean

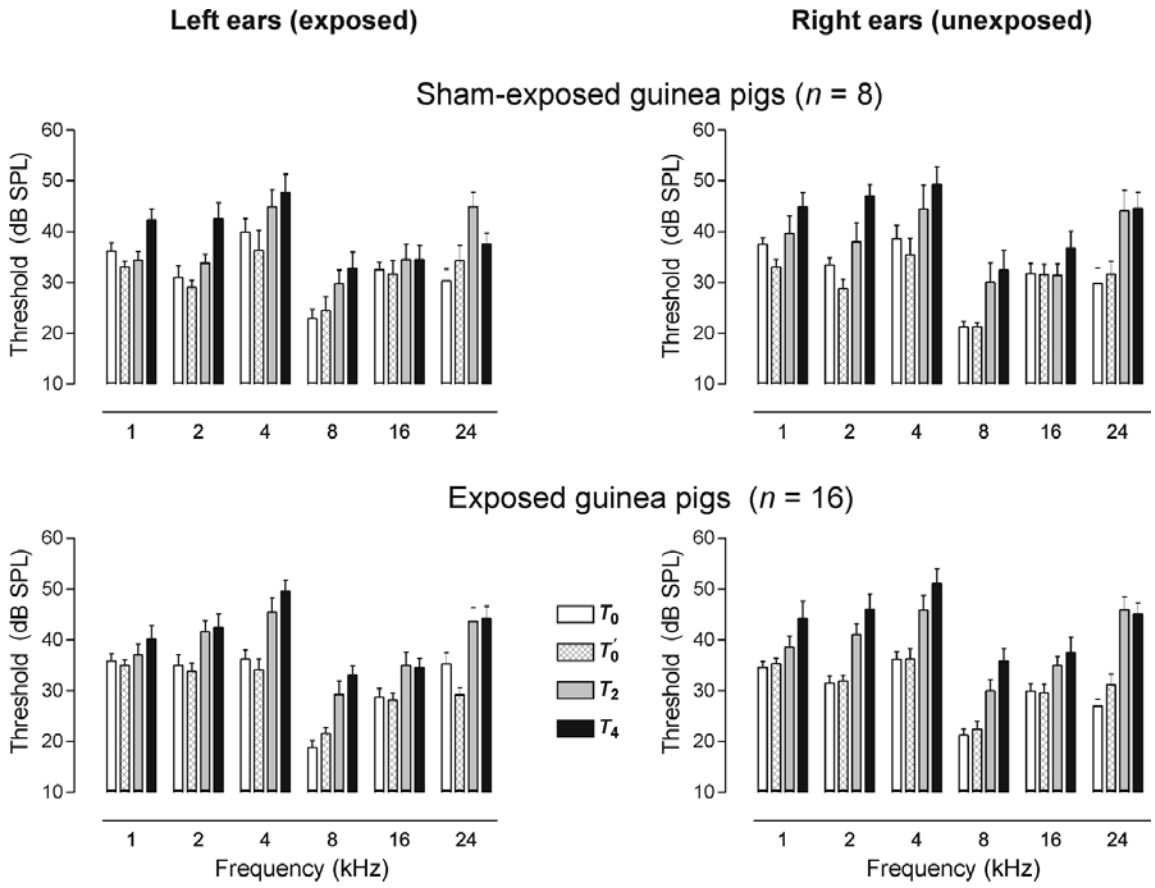

Figure 5. Mean ABR thresholds and standard errors of the mean at each of the six frequencies and at each of the different times (T_0 to T_4) for the left and right ears in the sham-exposed and exposed guinea pigs (SAR 4 W/kg, 1 h/day, 5 days/week, for 2 months) ($n = 8$ and $n = 16$ respectively).

weight of the guinea pigs increased from about 300 g to about 600 g. As a consequence, the size of the head increased also. This would result in an increase in the distance between the central sources of the evoked potentials and the electrode recording sites in older animals. Given similar activity in the central auditory pathways, the amplitude of the signal at the electrode recording sites would be attenuated. Since the threshold amplitude chosen was always the same (200 nV), the sound level needed to reach this criterion amplitude in the older, larger guinea pigs had to be higher than in the younger, smaller guinea pigs. The threshold deteriorated by about 10 dB, which corresponds to a 30% attenuation of the signal between the beginning and the end of the experiment. Interestingly, although the guinea pig's head size changed similarly between the beginning and the end in experiment 1, DPOAE amplitudes did not change, indicating that the head size did not affect the DPOAE acoustical recording conditions.

From the statistical analysis of the ABR thresholds (experiment 2) (repeated-measures ANOVA), the comparison of values at T_0 and T'_0 gives an estimation of the variability of the measures. The comparisons at T_2 and at T_4 show systematic differences between the sham-exposed and exposed groups (Figure 7, left) for both ears, with more threshold elevation in the exposed group, suggesting that the microwave radiation could globally affect the thresholds, whatever the ear. However, this difference is not statistically significant. Between ears, the threshold elevation was statistically significantly lower at T_4 in the left ear (Figure 7, right) in both the exposed and sham-exposed groups. However, it is difficult to explain how the presence of the antenna close to the left ear could have a local effect on that ear, as the antenna either sends or does not send an RFR signal. Moreover, the ANOVA analysis of the data over all frequencies, at the different time intervals, showed that, globally, there was no reliable difference between groups and between ears. Comparison of thresholds just before and just after a single exposure also showed no acute effect of exposure. The non-significant results may be due to insufficient power of the analysis. However, several measures were performed and analysed simultaneously, providing sufficient data. In addition, the differences between groups or ears were small or even opposite to the expected effect, and a lack of statistical power was probably not the cause of the non-significant results.

In addition, the in vitro study demonstrated no effect of GSM exposure on the development of the OC in newborn rats. Indeed, should some changes have occurred, further studies would be necessary to identify the mechanisms of cellular alteration.

The results of experiment 1 confirmed the results of Marino et al (2000), who investigated Sprague-Dawley rats after

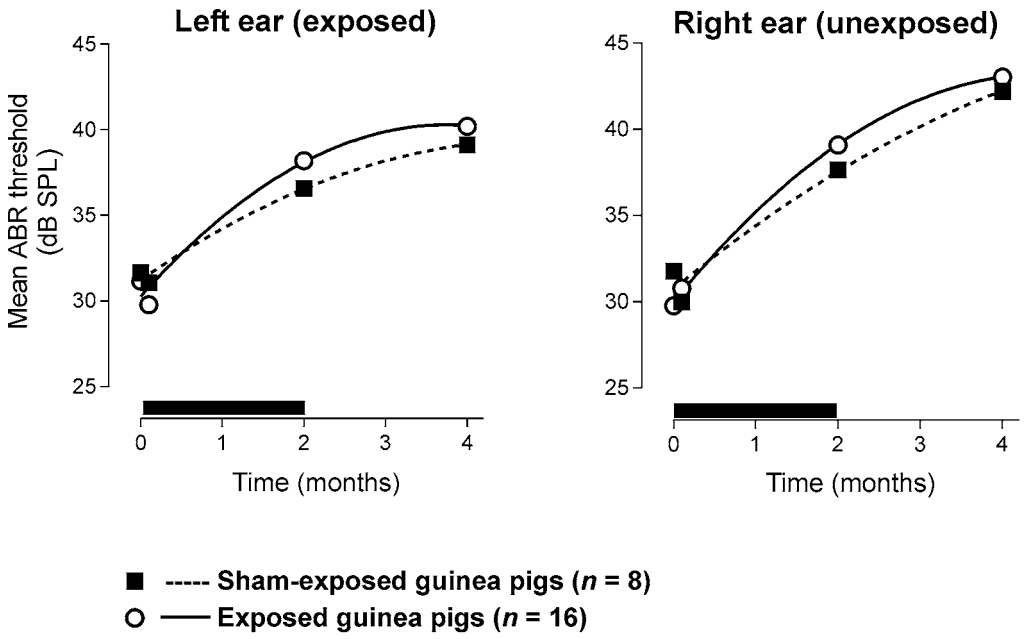

Figure 6. Mean ABR threshold (averaged over the six frequencies) as a function of time for the left and right ears in the sham-exposed and exposed groups (SAR 4 W/kg, 1 h/day, 5 days/week, for 2 months) ($n = 8$ and $n = 16$ respectively). The four grouped symbols around time 0 represent threshold at T_0 and T_0''. Black bars represent duration of exposure period.

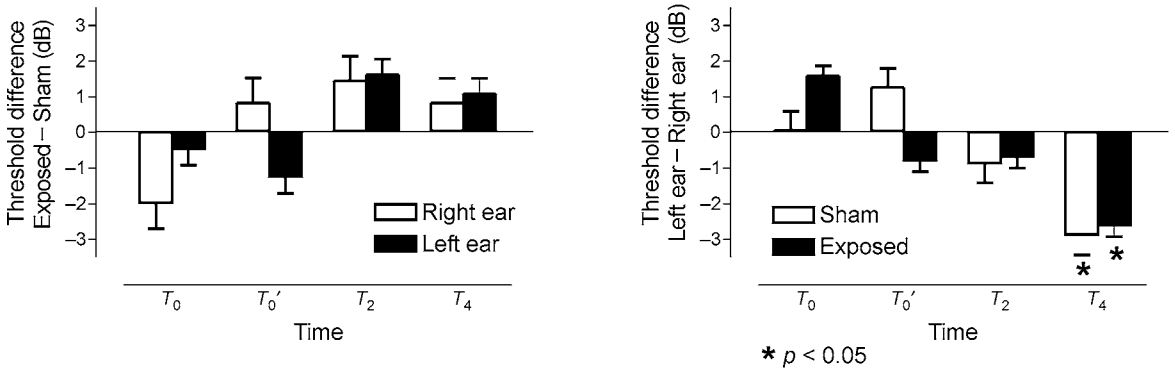

Figure 7. Left: difference between the exposed and the sham-exposed guinea pigs ($n = 16$ and $n = 8$ respectively) and standard errors of the mean of the ABR thresholds (averaged over the six frequencies), for the left and right ears as a function of time. Right: difference between the left and right ears and standard errors of the mean of the ABR thresholds (averaged over the six frequencies), for the exposed and the sham-exposed guinea pigs ($n = 16$ and $n = 8$ respectively) as a function of time.

a 900-MHz microwave exposure (SAR of 2 W/kg, 2 h/day, 5 days/week for 4 weeks). They also measured DPOAEs and found no significant effect. Furthermore, Ozturan et al (2002) found no acute changes in evoked OAEs in human volunteers exposed for 10 min to normal GSM phones. This report is consistent with our observation, in experiment 2, of no immediate changes in ABR thresholds from just before to just after a single 2-h exposure. Changes in ABR wave V latency have been reported in humans (Kellényi et al, 1999): 10 human volunteers were exposed for 15 min to a regular GSM phone. The maximal power was 2 W. The wave V latency increased by 0.207 ms for the exposed side and by only 0.029 ms for the unexposed side. Such a latency increase (although the time interval between wave I and wave V was not documented) is interpreted by the authors as a pure-tone threshold elevation of about 18 dB above 2 kHz. They objectively observed similar temporary threshold shifts in three of the cases that were also audiometrically monitored. However, the details of the technique and statistical analysis were not provided in this preliminary note, which, to our knowledge, was not followed by a full publication. The authors attribute these short-term changes to local thermal effects and/or ionic membrane permeability changes. Another recent publication considered the incidence of vestibular schwannoma and tinnitus in a population of cell phone users in Sweden (Hardell et al, 2003). Cases of

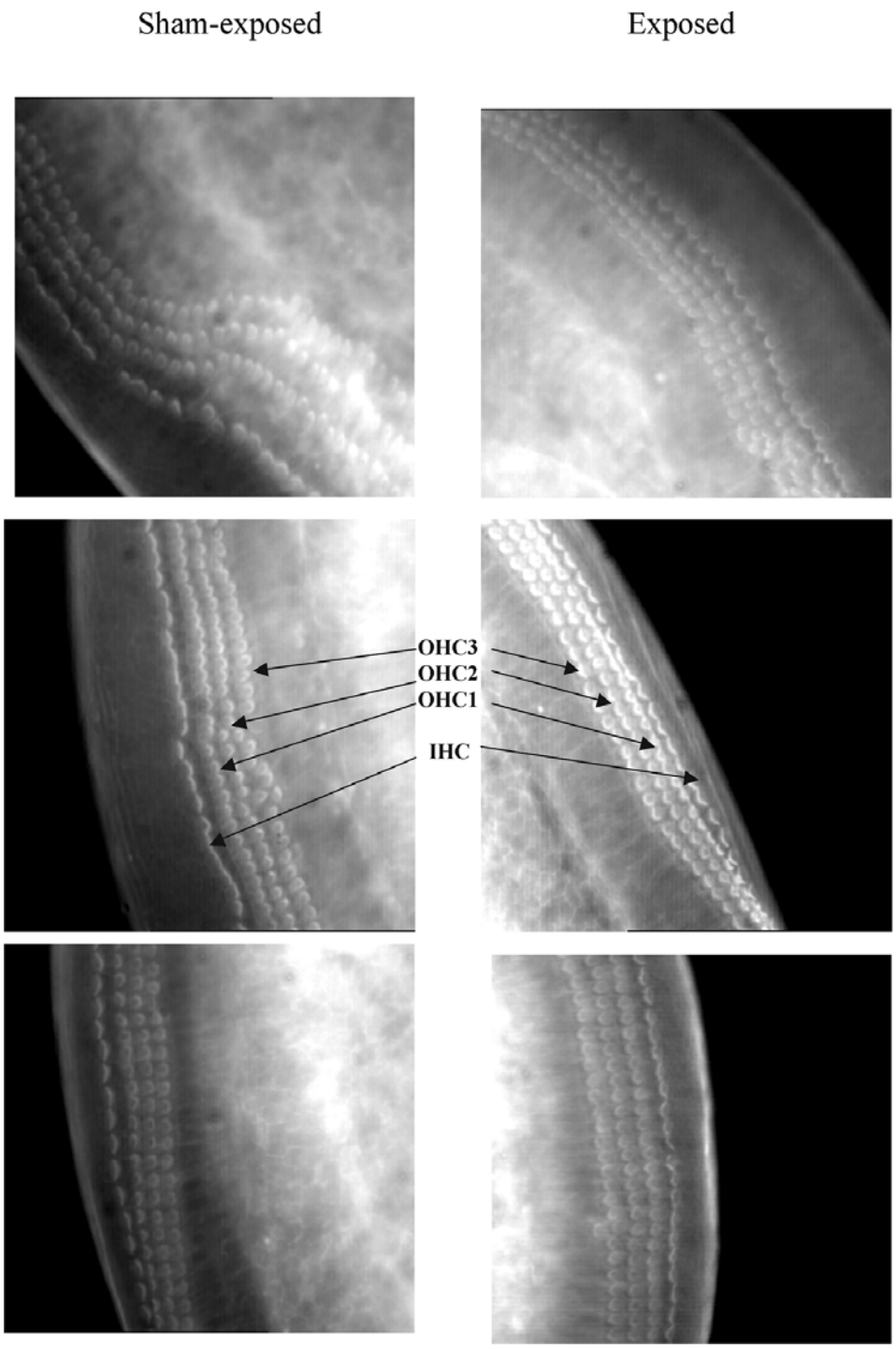

Figure 8. Samples of cultured organ of Corti from three different newborn rats (one rat per row), one having been sham-exposed (left) and the other having been exposed to the GSM microwaves (right). Arrows indicate the three rows of outer hair cells (OHC 1–3) and the single row of inner hair cells (IHC).

unilateral tinnitus in the ear exposed to the mobile phone were reported, without any magnetic resonance imaging evidence of acoustical neurinoma, or hearing loss or other pathology. In the same paper, an increased incidence of vestibular schwannoma in cell phone users was noted, although the small number of cases, and many confounding factors, do not permit statistically based conclusions to be drawn. Discrete changes in cognitive functions, involving hearing, in humans during or after exposure to GSM RFR have been occasionally reported (e.g. Freude et al, 1998; Preece et al, 1999); these changes are probably related to localized

heating, and cannot be attributed to changes in the function of the primary auditory system.

In conclusion, the present paper provided no evidence that microwave radiation, at the levels produced by cell phones, caused damage to the inner ear or central auditory pathways in our experimental animals.

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Notice of Errata

Please note that the article "Supplementary formulas and tables for calculating and interconverting speech recognition scores in transformed arcsine units" (Sherbecoe, R.L. & Studebaker, G.A., 2004; International Journal of Audiology, 43:442-448) contained errors.

The paragraph that extends from pages 443 to 444 should read as follows: Scores on the normalized arcsine unit scale are within about ±0.5 units of scores on the Stevens arcsine unit scale. That is to be expected because the numerical term $\sqrt{1000}$ in the Stevens transform closely approximates the ratio $100/\pi$ in Equation 13. This ratio, which can also be expressed as 100/(2arcsin(1)) or 50/(2arcsin $\sqrt{0.5}$), equals the quotient produced when a score of 50% is divided by the arcsine transform of 50%. If $100/\pi$ is substituted for $\sqrt{1000}$ in Equation 9, the Stevens transform and the normalized arcsine transform provide the same results. However, the normalized arcsine transform still has the advantages that it is a simpler formula than the Stevens transform, that it does not require modification to produce limiting values of exactly 0 and 100, and that it rescales the same numerical range in radians (0 to π) as the rau transform.

Additionally, the third sentence beneath Table 1 on Page 444 should read: To convert the V factors to percentages, use $P=\sqrt{50\pi(e+2\pi)}\arctan((V-50)/\log_e(50)\pi))+50$.