ORIGINAL ARTICLE

Effect of chronic exposure to a GSM-like signal (mobile phone) on survival of female Sprague-Dawley rats: Modulatory effects by month of birth and possibly stage of the solar cycle

Hella Bartsch [1,4], Heinz Küpper [1], Ulf Scheurlen [2], Fritz Deerberg [5†], Eckard Seebald [1], Klaus Dietz [3], Dieter Mecke [4], Hansgeorg Probst [4], Thilo Stehle [4], Christian Bartsch [1,4]

1 Center for Research in Medical and Natural Sciences, University of Tübingen, Tübingen, Germany
2 Unit of Animal Protection and Laboratory Animals, University of Tübingen, Tübingen, Germany
3 Department of Medical Biometry, University of Tübingen, Tübingen, Germany
4 Interfaculty Institute of Biochemistry, University of Tübingen, Tübingen, Germany
5 Former Central Institute for Laboratory Animal Breeding, Hannover, Germany

†This work is dedicated to the memory of Dr. Fritz Deerberg who died October 27, 2002. He was an outstanding veterinarian pathologist, without whom we had never dared to delve so deeply into research on experimental animals.

Correspondence to: PD Dr. Christian Bartsch,
Center for Research in Medical and Natural Sciences, University of Tübingen,
Ob dem Himmelreich 7, 72074 Tübingen, Germany.
e-mail: christian.bartsch@uni-tuebingen.de

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Abstract

During 1997–2008 two long-term (I and II) and two life-long (III and IV) experiments were performed analyzing the effect of chronic exposure to a low-intensity GSM-like signal (900 MHz pulsed with 217 Hz, 100 μW/cm² average power flux density, 38–80 mW/kg mean specific absorption rate for whole body) on health and survival of unrestrained female Sprague-Dawley rats kept under identical conditions. Radiofrequency (RF)-exposure was started at 52–70 days of age and continued for 24 (I), 17 (II) and up to 36 and 37 months, respectively (III/IV).

In the first two experiments (1997–2000) 12 exposed and 12 sham-exposed animals each were observed until they were maximally 770 or 580 days old. In experiment I no adverse health effects of chronic RF-exposure were detectable, neither by macroscopic nor detailed microscopic pathological examinations. Also in experiment II no apparent macroscopic pathological changes due to treatment were apparent. Median survival time could not be estimated since in none of the groups more than 50% of the animals had died.

In the course of two complete survival experiments (2002–2005; 2005–2008) 30 RF- and 30 sham-exposed animals each were followed up until their natural end or when they became moribund and had to be euthanized. A synoptical data analysis was performed. Survival data of all four groups could be fitted well by the Weibull distribution. According to this analysis median survival was significantly shortened under RF-exposure in both experiments by 9.06% (95% CI 2.7 to 15.0%) (p=0.0064); i.e by 72 days in experiment III and 77 days in experiment

IV as compared to the corresponding sham-treated animals (III: 799 days; IV: 852 days). Both groups of animals of experiment III showed reduced median survival times by 6.25% (95% CI –0.3 to 12.4%) (p=0.0604) compared to the corresponding groups of experiment IV (53 days: sham-exposed animals, 48 days: RF-exposed animals) which may be due to the fact that animals of experiment III were born in October and animals of experiment IV in May indicating that the month of birth affects life span.

From the results of the last two experiments it has to be concluded that chronic exposure to a low-intensity GSM-like signal may exert negative health effects and shorten survival if treatment is applied sufficiently long and the observational period covers the full life span of the animals concerned. The current data show that survival of rats kept under controlled laboratory conditions varies within certain limits depending on the month of birth. In view of our previous observations regarding an inhibitory or no effect of RF-exposure on DMBA-induced mammary cancer during the 1997–2000 period, an additional modulatory influence on a year-to-year basis should be considered which might be related to changing solar activity during the the 11-years' sunspot cycle. These potentially complex influences of the natural environment modulating the effects of anthropogenic RF-signals on health and survival require a systematic continuation of such experiments throughout solar cycle 24 which started in 2009.

INTRODUCTION & RATIONALE

The aim of the current *in vivo* rodent experiments was to simulate permanent exposure to a GSM-like signal (GSM: Global System for Mobile Communications) and to explore possible physiological and pathophysiological consequences since only limited information exists regarding potential chronic effects of low-dose radiofrequency (RF)-exposure in the vicinity of mobile phone base stations. Due to considerable methodological problems no reliable epidemiological studies have been published until now addressing the immediate question whether life within urban base-station networks used for mobile telecommunication may bear any substantial health risk as compared to remote areas with no or little RF-exposure. Recent publications dealing with the effects of TV transmitters, operating at frequencies comparable to mobile phone base-stations, gave no indications for negative chronic effects on human health including childhood leukemia, even though these transmitters operate at much higher intensities than base-stations (Merzenich *et al.* 2008). The same applies to epidemiological studies on the use of at-home cordless phones according to the DECT (Digital Enhanced Cordless Telecommunications) standard operating at low intensities (Schüz *et al.* 2006). Epidemiological studies on the effects of mobile phone signals, including the ongoing INTERPHONE study, focus on the question whether long-term use of hand-held mobile phones may be harmful or not (Cardis *et al.* 2007; IARC 2008). Here it appears that regular use of less than a decade does not lead to an elevated risk to develop brain tumors. However, for extended daily phone calls on a regular basis over a period of more than ten years the question is still open and additional investigations are needed to obtain firm conclusions. In order to simulate such long-term exposure the still ongoing Perform A study on experimental animals was initiated, the results of which until now do not indicate substantial negative effects (Tillmann *et al.* 2007; Smith *et al.* 2007; Oberto *et al.* 2007; Hruby *et al.* 2007).

Most experimental *in vivo* studies were designed to simulate local effects of mobile phones to the human head. For this reason, animals were restrained in many experiments (e.g. in so-called Ferris-wheels; Faraone *et al.* 2006) to ensure precise local absorption rates in the head region. These experiments as well as those where animals were allowed to move freely have given little evidence for negative effects on health including tumor growth and development (for reviews see: Elder 2003; Moulder *et al.* 2005). In such experiments, however, animals were not followed up until their natural end, with the exception of a few studies, such as those of Sommer *et al.* (2004, 2007) on AKR mice with limited life expectancy due to the genomic presence of a leukemogenic virus.

When addressing the situation of exposure to RF-signals emitted by mobile phone communication networks and corresponding simulations under *in vivo* experimental conditions it has to be considered that typical 24-hours patterns of energy absorption follow the activity-/rest cycle: at home or when working indoors only a relatively small signal strength variability exists, whereas when working outdoors and moving to/from work considerable variations have to be encountered (Bornkessel *et al.* 2007). In our investigations presented here experimental animals were able to move about freely in their cages throughout and could thus follow their typical day/night pattern of rest and activity so that corresponding smaller resp. bigger variabilities of RF-exposure throughout a 24-hours period resulted. Under our experimental conditions relatively wide variations of energy absorption by the different animals at *each* time-point existed due to their positioning, similar to the human situation within urban base-station networks. On a daily, let alone weekly or even monthly time-scale, such inter-individual differences are, however, negligible due to comparable behavioural patterns. Therefore short-term variations of energy absorption may be of minor importance if exposure is applied life-long.

Our first approach to explore potential health hazards of chronic RF-field exposure was to analyze whether a commonly used model system for human breast cancer (Welsch 1985) would be affected. In three

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identical experiments with DMBA-induced mammary cancer (1997/8, 1998/9 and 1999/2000), in which female Sprague Dawley rats were born not only on the same calendar day of corresponding years, but DMBA-intubation as well as RF-exposure were given/started at the same age of the animals, incongruent results were obtained. In the first experiment chronic RF-exposure led to a significant *delay* of tumor latency whereas absolutely no effect could be found in the two subsequent experiments (Bartsch *et al.* 2002). We were unable to render plausible explanations for these discrepancies but assumed that year-to-year variations may exist. This assumption was further nourished by the results of two long-term studies performed earlier with inbred BDII/Han-rats (1991–1994 and 1996–1999) testing the effect of light as well as melatonin on survival (Deerberg *et al.* 1997; Bartsch & Bartsch 2007) and where also no reproducible effects were found.

Parallel to our DMBA-experiments, studies on healthy female Sprague-Dawley rats were initiated to monitor the health status of long-term RF-exposed animals together with the longitudinal profiles of melatonin and catecholamines. To avoid stress and disturbance of the animals, hormone measurements were performed from urine samples, which were collected once per month overnight as long as animals were apparently healthy, but not beyond 27 months of age. Blood samples for health monitoring were taken from in-cage sentinels and from experimental animals shortly before euthanasia. All experiments were carried out under utmost standardized conditions (e.g. same animal rooms, RF exposure system, breeder, day of year when treatments were started; for more details see *Materials and Methods*) so that no or little response variability towards the applied field had to be encountered according to common experience. To obtain more information regarding presumable year-to-year variations between experiments of identical design within the same surroundings we aimed at repeating the same type of experiment as often as possible to see which type of overall response pattern would emerge. For this purpose we systematically continued these studies on healthy female Sprague Dawley rats, which were initiated in 1997 until 2008. To realize four long-term experiments over a total period of more than a decade maintaining stringent standardization throughout it was unavoidable for the two principal investigators (C.B. and H.B.) to get involved in all aspects of these experiments. It was logistically impossible and unaffordable to perform effective blinding throughout all these four studies. Blinding was taken into consideration during the last experiment of the present series (2005–2008) but was discarded again since it had meant to change positioning and wiring of the exposure chambers (so that the principal investigators would be unaware of the respective treatment). It was felt that this might lead to unknown modifications due to changes of the environmental electric and/or magnetic fields. Alternatively, to put into effect blinding and to maintain positioning and wiring of the exposure chambers simultaneously, it had been necessary to have a second independent team available all of the time (including weekends and holidays) so that animals could be handed over to the principal investigators in a blinded way for daily health inspections as well as for all other experimental procedures. This theoretically conceivable approach was, however, beyond our means to be realized throughout a single long-term experiment, apart from all studies over more than a decade. Blinding was applied at necropsy and for histopathological examinations of tissue samples as well as for hormone determinations. To underline the objectivity of the "open" experimental approach chosen (knowing which animals were RF- or sham-exposed) it is pointed out that during our DMBA-studies (Bartsch *et al.* 2002) non-blinding (experiment 2) and blinding (experiment 3) led to the same result of a zero-effect.

MATERIALS AND METHODS

Animal facility

The two adjacent animal rooms of equal size and proportions used throughout the current studies are located in the Interfaculty Institute of Biochemistry of the University of Tübingen. During all experiments room 1 was used for RF-exposure whereas room 2 contained the control groups. These rooms without windows are located close to the center of a three-storied building in which mobile phone communication, even after repeated upgrading of the surrounding base-stations over the years, is impossible. This well-shielded situation is one reason why, at the beginning of the experiments in the mid-90s, these two rooms were chosen by us.

The lighting regimen comprised 12 h of illumination per 24 h, with lights turned on at 0700 h Central European Summer Time (CEST) throughout the year. The intensity of light at the bottom of the animal cages was 28–35 Lux. It was generated by four energy saving bulbs of 8W each (Energysaver E27, 420 Lumen, Philips) placed at the top of the exposure chambers outside of the wire netting. A figure of the animal cages within the sham/exposure chamber is given in Bartsch *et al.* (2002). Air temperature (average values ± standard deviations) was very similar in the two animal rooms containing the chambers for RF- or sham-exposure. (Exp. I: 22.4 ± 0.6/21.8 ± 0.4 °C; exp. II: 22.9 ± 1.1/22.4 ± 0.6°C; exp. III: 22.0 ± 0.9/21.8 ± 0.7°C; exp. IV: 22.1 ± 0.6/22.2 ± 0.5 °C). Relative air humidity showed closely parallel variations in both rooms with an average of 60% ± 8% standard deviation.

Animals

The studies were approved by the Animal Care Committee of the regional government and are in compliance with the animal welfare requirements rec-

ommended by Portaluppi *et al.* (2008). A total of 168 female Sprague-Dawley rats (i.e. CD-rats) aged 38 days (I: n=24, born on April 27/28, 1997), 35 days (II: n=24, born on April 28, 1999), 22 days (III: n=60, born on October 9, 2002), and 30 days (IV: n=60, born on May 4, 2005) were purchased from Charles River Wiga (Sulzfeld, Germany). In each experiment animals were equally and randomly sub-divided among the sham- and RF-exposed group kept in the two adjacent rooms mentioned above. Twelve animals were housed per cage receiving tap water and food *ad libitum* (pellets, ssniff from RIMH, Soest, Germany). Each animal cage was located within an exposure resp. sham-exposure chamber. Animals were transferred to metabolic cages, located within sham/exposure chambers, once per month for 12–16 hours during the scotophase starting from 7 (I, II) respectively 9 weeks of age (III, IV) until the age of 23 (I), 18 (II), 27 (III), or 24 (IV) months.

Radiofrequency electromagnetic field exposure

Throughout the four experiments animals were kept in the same two rooms described above. The setup built by the former Technology Center of the Deutsche Telekom AG (Darmstadt, Germany) was designed for continuous low-level RF-exposure of unrestrained animals within separate exposure chambers containing either a single animal cage for housing or four metabolic cages for urine collection. They were located in the far field of a flat spiral antenna emitting a clockwise circularly polarized GSM-like RF signal (900 MHz pulsed with 217 Hz, pulse width 577 μs; for a more detailed description see Bartsch *et al.* 2002). A circularly polarized field was used to achieve independence from the orientation of the animals in case of biological interactions. The mean power flux density at the bottom of the cage was 100 $\mu W/cm^2$, with a variation of ± 3 dB between the center (200 $\mu W/cm^2$) and the corners of the cage (50 $\mu W/cm^2$). The specific absorption rate of the whole body (SAR_{WB}) was determined by computer simulations with the MAFIA software program of CST GmbH (Darmstadt, Germany) using an anatomically correct model of the body of a rat (Hombach 1997). These calculations were performed with the help of a standard rat model. At the beginning of the experiments, when animals were two months old (170–220 g), mean $SARs_{WB}$ were 80 mW/kg (range: 32.5–130 mW/kg), which, in case of humans, would be identical to the permissible limit for the general public (ICNIRP: www.icnirp.de/; Otto & von Mühlendahl 2007). At 5–6 months of age, adult rats (300 g) showed a mean SAR_{WB} of 44m W/kg (range: 17.5–70 mW/kg), and older animals (11–12 months; 400 g) had a mean SAR_{WB} of 38 mW/kg (range: 15–60 mW/kg) indicating that $SARs_{WB}$ of animals beyond 5–6 months of age are rather stable. Young rats show higher $SARs_{WB}$ than older animals because of a resonance phenomenon. Their body length (excluding the tail) equals approximately half the wavelength of the 900 MHz signal so that maximal power absorption occurs.

Chronic RF-field exposure (900 MHz pulsed with 217 Hz, 100 $\mu W/cm^2$ average power flux density) was started at 52 (I), 53 (II), 70 (III), and 63 days of age (IV). In the first two experiments exposure was continued until all surviving animals were sacrificed at 25 (I) resp. 19 months of age (II). In experiments III and IV all animals were exposed and observed until their natural end or when they had to be euthanized due to a serious deterioration of their health. Maximal survival time in these experiments was 36–37 months. Exposure in all experiments was practically permanent, interrupted once daily (for 15 min) for feeding and inspection, once weekly for weighing and detailed health inspection (1–2 hours), thrice weekly (1–2 hours) for cage cleaning, and 4–5 hours per month (I, II) resp. every three months (III, IV) for servicing of the exposure devices. All activities were performed during daytime.

Health monitoring

At the beginning of each experiment, when animals were small and fast growing, they were weighed weekly, subsequently every fortnight and, when fully grown, monthly. As the animals became older, mainly after 540 days of age, many started developing different types of tumors. They grew in size and weight, ultimately at the cost of the host's weight. After detection of the first mammary tumor, detailed examinations were carried out weekly.

Typical for this strain of rats, older females develop mammary tumors (Russo & Russo 1996) which are mostly benign (fibroadenoma, fibroma) growing subcutaneously in a non-invasive manner so that they are tolerated for a long time due to lack of pain and restricted mobility. Fast growing mammary tumors sooner or later become ulcerative or even necrotic so that animals have to be euthanized. Tumors growing in the inguinal or uppermost thoracic regions often inhibit the mobility of the hind legs, of the head respectively, so that euthanasia becomes necessary at a relatively early time. In some cases mammary tumor growth is extremely slow so that the animals' well being is affected rather by other diseases (e.g. benign pituitary tumors, cancers within the abdominal or thoracic cavity, renal diseases), often leading to sudden death. Adenohypophyseal tumors are frequent in older female CD-rats: at the age of two years more than 70% of all animals can develop adenomas of the *pars distalis* (Charles River Deutschland 1992). Their development mostly leads to a typical pattern of symptoms with pronounced initial obesity, followed by subsequent cachexia associated with lethargy and in many cases neurological symptoms, such as disturbed equilibrioception or paralysis of the hind legs. Cachexia as well as neurological symptoms represent serious health impairments necessitating euthanasia, which was performed by transferring the animals to an airtight chamber filled with carbon dioxide. Symptoms of extreme weakness and weight loss were also connected with severe kidney diseases leading to progressive renal failure.

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To exclude pathogenic infections of bacterial and viral origin, bacteriological and serological tests were performed by the animal breeder and continued by us throughout the experiments from in-cage sentinels as well as from experimental animals prior to euthanasia. In addition, routine fur and fecal samples were taken for helminthic tests.

Necropsy and histopathological investigations

Necropsy was performed on practically all animals. Only in a few cases macroscopic pathological investigations were impossible due to prior cannibalism or progressive autolysis. In experiment I, in which animals were necropsied at 770 days of age, more than 20 organs per animal were examined both macroscopically and microscopically. Histopathological investigations were carried out on coded sections by a veterinarian pathologist (F.D.) so that he was unaware of the type of treatment. The organs examined included different parts of the brain (cerebral cortex, adenohypophysis, and pineal gland); within the thoracic cavity heart and lung, within the abdominal cavity organs of the gastrointestinal (stomach, pancreas, small and large intestine, liver) as well as of the urogenital tract (kidneys including adrenals, urinary bladder, and uterus, collum, ovary, vagina) were analysed. In addition, axillary as well as inguinal lymph nodes and spleen were examined as well as mammary tumors, if present. In experiment II the above-mentioned tissues were examined macroscopically, fixed and prepared for histopathology but could not be analyzed microscopically due to the death of F.D. and subsequent shortage of funds. In the survival experiments (experiments III and IV) principally the same spectrum of organs was examined as in the preceding two experiments, however, histopathological investigations were carried out only on those organs, which showed apparent macroscopic pathological changes. Complete histopathological investigations comparable to experiment I could not be performed since about ¼ of all animals in experiments III and IV died spontaneously so that autolysis in several cases prevented reliable microscopic examinations.

Determination of the pattern of life-limiting diseases

The diseases detected upon macroscopic pathological investigations of animals in experiments III and IV and judged to be life-limiting were sub-divided into five main categories: mammary tumors (MT), pituitary tumors (PT), tumors in other organs (OT), different types of other (non-tumorous) diseases (OD); a fifth category existed where some animals had died spontaneously for unclear reasons and could no longer be examined adequately due to cannibalism or autolysis (UD).

Incidence of pituitary tumors and comparisons among groups

Pituitary tumors were initially detected macroscopically during dissection by an apparent enlargement and/or modified coloring of the hypophysis. In this case, the gland was removed and hematoxylin-eosin stained sections were judged microscopically in a blinded fashion by a veterinarian pathologist. If the macroscopically visible changes of the hypophysis were confirmed to be neoplastic in nature the presence of a pituitary tumor, i.e. the respective incidence, was accepted. In almost all cases adenomas, i.e. benign tumors, of the *pars distalis* (i.e. anterior pituitary, adenohypophysis) were present. The incidence of pituitary tumors among the different groups was compared statistically taking into consideration the corresponding survival time of the animal concerned. The model takes into account that all observations are either left or right censored. We assumed an exponential distribution for the onset of pituitary tumors. Parameters were estimated by maximum likelihood.

Statistical analysis of survival

In experiments I and II surviving animals were sacrificed at 770 or 580 days of life. In all groups at least 50% of all animals were still alive at these times so that it was not possible to estimate median survival time. In case of experiments III and IV (complete survival studies) the survival times of all animals were plotted according to Kaplan and Meier (Klein & Moeschberger 1997), using the programme Sigmaplot Verson 8.0 (SYSTAT Software Inc., Chicago, Il.). One animal in the RF-exposed group of experiment IV had to be censored which died accidentally during anaesthesia for treatment of overgrowth of frontal teeth (incisors). The four Kaplan-Meier curves of both experiments could be fitted to a Weibull distribution. The fit by a lognormal distribution would also have been appropriate according to the Kolmogorov-Smirnov-test, but with considerably smaller *p*-values. For each group median survival time as well as the times for 75% and 25% survival were calculated. The parameters of the Weibull distributions were estimated by maximum-likelihood. According to the Akaike information criterion (Akaike 1974) the best model does not involve an interaction effect between experiment and RF exposure but an experiment effect though not significant at the 5% level in addition to the RF effect. The goodness of fit of the model was assessed by the Kolmogorov-Smirnov-test. This synoptic analysis of the experiment III and IV is justified because the experiments were carried out according to the same protocol. It has the advantage that model parameters can be estimated with higher precision due to the larger sample size compared to a separate analysis of the two experiments.

RESULTS

Weight development

Chronic RF-field exposure did not affect weight development in any of the four experiments during the first year of life when all animals were still healthy compared

to sham-treated controls; maximal difference of average values between corresponding groups at a given time within an experiment did not exceed ± 4% during this period. This indicates that no immediate metabolic effects of chronic RF-field exposure existed in female Sprague-Dawley rats. Beyond one year of age divergent weight changes gradually manifested among the different animals of both groups depending on the type of disease which occurred. Pituitary tumor development was in many cases connected with initial weight increase followed by a subsequent decline leading to cachexia. Drastic weight reductions were also found in case of renal diseases, whereas mammary tumor growth led to progressive weight increase.

Survival times

In experiments I and II, designed as so-called "stop experiments", surviving animals were sacrificed at 770 and 580 days, respectively. In the first experiment 8/12 sham-exposed control animals (67%) were still living at 770 days as compared to 6/12 RF-treated animals (50%). Since at least 50% of all animals within a group were alive it was impossible to estimate median survival. The same applied to the second experiment where even 11/12 RF- as well as sham-treated animals (92%) were living at 580 days of age.

The Kaplan-Meier plots of the survival times of experiments III and IV are shown in Figure 1. In both experiments animals were observed for a maximal period of about three years. From Figure 1a it is apparent that RF-exposed animals of experiment III show consistently lower survival probabilities than their sham-exposed controls. In experiment IV survival probabilities among the two groups are initially almost indistinguishable but beyond 750 and particularly 850 days of age RF-exposed animals show reduced survival probabilities compared to controls. The observed 25% quantile for survival is shortened by 112 days (see Figure 1b).

It was found that all four survival curves were well described by the Weibull distribution according to the Kolmogorov-Smirnov-test resulting in the following *p*-values: p=0.8651 (sham-treated controls of experiment III), p=0.6420 (RF-field exposed), p=0.6538 (sham-treated controls of experiment IV), and p=0.6711 (RF-field exposed). (The corresponding *p*-values for the lognormal model are only 0.3564, 0.1443, 0.7460 and 0.3145, respectively.) The exponent of the power of time describing the increase of the hazard function is estimated at 5.42 (95% CI 4.68 to 6.20). Using the Akaike information criterion the best model turned out to involve only the main effects "experiment" and "exposure" but no interaction of the two effects. (The Akaike information criterion (AIC) adds the number of parameters and the value of the loglikelihood function. The model with interaction contains 5 parameters and has an AIC value of –6.41. The model without interaction (4 parameters) is to be preferred also to the model without seasonal effect (3 parameters) because AIC is –6.93 compared with –6.17.) The *p*-value for the interaction term is 0.3289. According to the Weibull model survival is significantly shortened in the RF-exposed group in experiments III as well as IV compared to the corresponding controls by 9.06% (95% CI 2.7 to 15.0%) (p=0.0064; 72 days at the medians of experiment III, 62 days at the 75% survival rates and 82 days at the 25% survival rates; 77 days at the medians of experiment IV, 66 days at the 75% survival rates and 88 days at the 25% survival rates; see Table 1). In addition, survival times of both groups of experiment III are shortened compared to the corresponding groups of experiment IV by 6.25% (95% CI –0.3 to 12.4%) (p=0.0604) i.e. 53 days at the medians of the sham-treated animals and 48 days at the medians of the RF-exposed groups (see Table 1). Animals of experiment III were born in autumn, whereas those of experiment IV were born in spring indicating that survival may depend on the month of birth whereas RF-field exposure in these two experiments led to consistent effects independent of season.

Tab. 1. Details of the Weibull fitted survival curves in experiments III and IV.

Experiment	Groups	Median Survival [days]	75% Survival [days]	25% Survival [days]
III:	Sham	799	679	908
(Born in *autumn* 2002)	RF	727	617	826
IV:	Sham	852	724	969
(Born in *spring* 2005)	RF	775	658	881

Pathology: Experiments I and II

Overall pathology: Detailed histopathological investigations performed in animals of experiment I (sacrificed at 770 days) comprising more than 20 organs (for details see *Materials and Methods*) gave no indications for any specific negative effects of permanent RF-field exposure applied for maximally two years. The observed pathologies in both groups, RF- and sham-exposed, were judged to be typical for aging female rats of the Sprague-Dawley strain being in agreement with previously published data (Jones *et al.* 1983–1989). Also in experiment II where animals were sacrificed at 580 days no apparent macroscopic pathological changes were observed due to RF-field exposure compared to sham-exposed animals.

Pituitary tumor incidence: Among RF-exposed animals of experiment I less adenohypophyseal tumors (5/12=41.7%) were detected compared to sham-exposed controls (9/12=75%). Similar observations were made in experiment II where animals were sacri-

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ficed at 580 days (i.e. six months earlier than in experiment I): only 4/12 (33.3%) of RF-exposed animals showed adenohypophyseal tumors compared to 6/12 (50%) among controls. A detailed statistical analysis, however, considering the corresponding survival time of each animal, revealed no statistical significance due to treatment.

Pathology: Experiments III and IV

Life-limiting diseases: For experiments III and IV in which complete survival times were monitored over a maximal period of 3 years, combined macroscopic and histopathological investigations were used to determine which disease(s) were life-limiting (either leading to spontaneous death or necessitating euthanasia due to serious deterioration of general health). These diseases were sub-divided into five main categories (see Table 2). The overwhelming number of life-limiting diseases was neoplastic in nature affecting particularly mammary (MT) and pituitary gland (PT), to a lesser extent other organs of the body (other tumors: OT). The fourth category comprised different types of non-tumorous diseases (OD). A fifth, a very minor category existed in experiment IV where one animal died for unclear reasons (group: unknown disease, UD). Some animals of the different groups had to be censored. They died spontaneously and were detected too late so that progressive autolysis and/or cannibalism had taken place thus preventing reliable pathological investigations. In some cases the animals' lives were not limited by just one type of the above-mentioned four categories of diseases (MT, PT, OT, OD), but by combinations of the same (e.g. MT+PT or PT+OT).

Among the controls of experiment III, the dominant life-limiting disease were mammary tumors (MT: 12/28=42.9%), followed by pituitary tumors (PT: 6/28=21.4%) which in one additional case occurred together with a tumor in another organ (PT+OT: 1/28=3.6%). The third most frequent type were other non-tumorous diseases (OD: 6/28=21.4%), which included e.g. nephropathies. In sham-exposed animals of experiment IV no clear predominance of either life-limiting mammary or pituitary tumors existed (MT: 10/30=33.3%; PT: 11/30=36.7%). It was, however, apparent that considerably more tumors developed at other sites that were life-limiting (OT) compared to experiment III (20% vs. 10.7%) leading to a higher proportion of animals succumbing/euthanized to neoplastic pathologies (90.0% vs. 78.6% in experiment III). Comparable observations were made in the corresponding RF-exposed groups (89.5% in experiment IV vs. 79.2% in experiment III).

The RF-exposed groups of both experiments showed modified distribution patterns among the different types of life-limiting diseases compared to sham-exposed controls and were characterized by a diminished proportion of MT (experiment III: 31.0% vs. 42.9%; experiment IV: 24.1% vs. 33.3%) which was mainly due to a relative increase of PT. In addition, RF-exposed animals showed a tendency to develop multiple life-limiting diseases (polypathologies), particularly in experiment IV (10.3%; see Table 2).

Tab. 2. Number of animals with one type of life-limiting disease or a combination of diseases (polypathology) in sham- and RF-exposed groups (in ***bold italics*** percentage of uncensored animals of corresponding whole group) of experiments III and IV (2002–2008).

	Experiment III				Experiment IV			
Life-limiting Diseases	**Sham**		**RF**		**Sham**		**RF**	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
MT	12	***42.9***	9	***31.0***	10	***33.3***	7	***24.1***
MT+PT	0	***0.0***	0	***0.0***	0	***0.0***	2	***6.9***
PT	6	***21.4***	8	***27.6***	11	***36.7***	11	***37.9***
PT+OT	1	***3.6***	1	***3.4***	0	***0.0***	1	***3.4***
PT+OD	0	***0.0***	1	***3.4***	0	***0.0***	0	***0.0***
OT	3	***10.7***	4	***13.8***	6	***20.0***	5	***17.2***
OD	6	***21.4***	6	***20.7***	3	***10.0***	2	***6.9***
UD	0	***0.0***	0	***0.0***	0	***0.0***	1	***3.4***
Polypathology	1	***3.6***	2	***6.9***	0	***0.0***	3	***10.3***
Total Number	30		30		30		30	
Censored	2		1		0		1	
Uncensored	28	***100.0***	29	***100.0***	30	***100.0***	29	***100.0***

Abbreviations: Mammary Tumors (MT), Pituitary Tumors (PT), Other Tumors (OT), Other Non-tumorous Diseases (OD), Unknown Disease (UD)

Pituitary tumor incidence: In experiment III only 28.6% of all sham-exposed animals (8/28) showed pituitary tumors as compared to 41.4% within the RF-group (12/29) which, however, was not statistically significant taking into account corresponding individual survival times. In experiment IV the incidence of pituitary tumors was considerably higher and practically indistinguishable between the control and experimental group (60.0% vs. 58.6%).

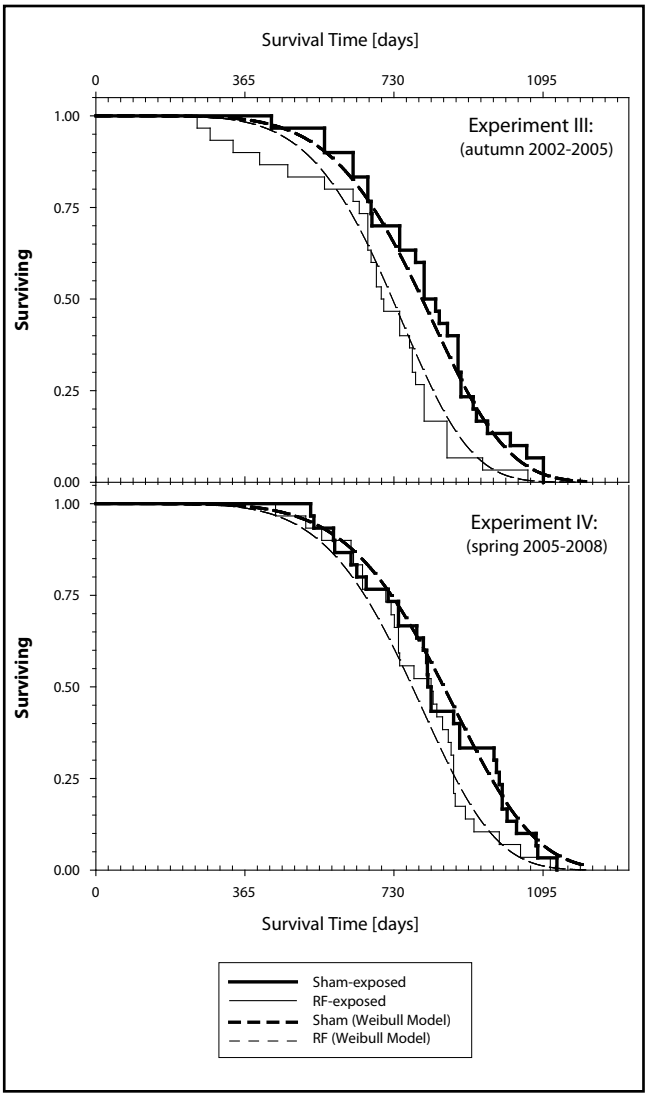

Fig. 1. Kaplan Meier survival plots of sham-exposed (thick line) and radiofrequency(RF)-exposed (thin line) animals of experiments III (Figure 1a: upper panel) and IV (Figure 1b: lower panel). The Weibull fitted survival curves are given as dashed lines (thick: RF-exposed, thin: sham-exposed). The horizontal axis represents the animals' survival times and the vertical axis the estimated probability of survival, i.e. the cumulative survival rate. RF-exposed animals of both experiments showed significantly shortened survival compared to sham-exposed controls (p=0.0064) and the groups of experiment IV lived longer than those of experiment III (p=0.0604).

Pituitary tumor incidence among the four experiments

Comparison of experiments I, II, and IV:
The animals of these three experiments were born in spring (April and May) of different years. The incidence of pituitary tumors in sham-exposed animals was somewhat comparable (experiment I: 75.0%; II: 50.0%; IV: 60.0%) but the effects of chronic RF-field exposure varied leading to a reduced incidence of pituitary tumors in experiments I and II (I: – 44% ; II – 33%, with controls set as 100%), whereas practically no difference was found in experiment IV (– 2.3%). It therefore appears that the effects of chronic RF-field exposure on pituitary development in female Sprague-Dawley rats, which are born in spring, might depend on the years when these animals are born; inhibitory effects are indicated during the 1997–2000 period but not between 2005 and 2008. It has to be stressed, however, again that no statistical significance was found when taking into consideration individual survival times so that the observed phenomenon has to be viewed with caution and within the context of the overall pathophysiolological scenario determining the animals' life span.

Comparison of experiments III and IV:
Animals were born either in October (experiment III) or in May (experiment IV). The sham-exposed animals of experiment III showed very few pituitary tumors (28.6%) whereas controls of experiment IV developed such tumors in 60% of all animals, which was statistically significant (P=0.0496), even if individual survival times were taken into consideration. It therefore appears that pituitary tumor development depends on the month of birth being lower if animals are born in autumn than in spring. RF-exposed animals showed a nominally higher incidence of pituitary tumors in experiment III (41.4%), which, however, was not significant. As mentioned above, pituitary tumor incidence was practically indistinguishable between RF- and sham-exposed animals of experiment IV (58.6% vs. 60%).

DISCUSSION

Present results

In the course of two long-term and two life-long experiments performed during 1997–2008 the effect of chronic exposure to a low-intensity GSM-like signal on health resp. survival of female Sprague-Dawley rats was tested. In the first two consecutive "stop-experiments", carried out between 1997 and 2000 over a period of 750 resp. 580 days, permanent RF-field exposure did not exert any adverse affects on survival and health as judged from macroscopic and/or microscopic pathological examinations. Contrary to that, in the second series of two complete survival experiments (over maximally three years each; 2002–2008) with animals of the same strain and sex, kept under identical experimental conditions as in the preceding two experiments, chronic RF-field exposure significantly

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shortened survival-times compared to corresponding sham-exposed controls. The results of these four studies indicate that negative effects of RF-field exposure on life span apparently take considerable time to develop and may thus be detectable only if complete survival studies are performed. As can be seen from the survival curves of experiments III and IV (see Figs. 1a and 1b) it is apparent that life-shortening effects manifest particularly beyond two years of age of the animals which more or less corresponds to the median survival time of sham-exposed female Sprague-Dawley rats. Such a delayed effect of RF-field exposure on health and survival appeared to be more pronounced in experiment IV where animals were born in spring and which, according to the Weibull model, showed significantly extended life spans for both groups compared to autumn-born animals of experiment III. This indicates a modulatory effect of the month of birth on the life span of female Sprague-Dawley rats whereas the negative impact of chronic RF-field exposure on health and survival appears to be independent of that.

A central question in this context is whether chronic low-intensity RF-field exposure to a GSM-like signal may provoke any specific type of disease to elicit the observed life-shortening effects. According to our current results, this does not seem to be the case (see Table 1); RF-field exposure apparently accelerates the overall species-specific disease pattern which typically arises among aging female rats and which is mainly characterized by different types of benign and malignant tumors, foremost of the mammary and pituitary glands. This can lead to the development of multiple life-limiting diseases (cf. experiment IV). Depending on the month of birth the ratio between mammary and pituitary tumors appears to be shifted; the incidence of pituitary tumors was significantly reduced among controls of experiment III (born in autumn) compared to those of experiment IV (but also experiment I and II), which were born in spring.

It has been observed before that season can affect laboratory rodents, which are typically bred and kept under standardized laboratory conditions throughout the year. Löscher *et al.* (1997) as well as we (Bartsch & Bartsch 2007) reported that the development of DMBA-induced mammary tumors in both outbred Sprague-Dawley rats as well as inbred F344 Fischer rats depends on the month of application. Similar differences were reported for identical doses of anti-convulsant drugs given at different times of the year (Löscher *et al.* 2000). Seasonally divergent responsiveness to pharmacological treatments of the same dose could be caused by profound neuroendocrine and immunological as well as metabolic variations over the year. We observed that female rats under standardized laboratory conditions (including constant light/dark cycle) still showed seasonal rhythms of nocturnal melatonin production with higher levels in summer than in winter (Bartsch *et al.* 1994, 2001) and hypothesized that, in the absence of seasonally modulated photoperiods, omnipresent and typical variations of the geomagnetic field throughout the year may be involved (Bartsch *et al.* 1994). Evidence is growing that, same as in birds, mammals are able to perceive natural magnetic fields (Wiltschko & Wiltschko 2005), via e.g. retinal melanopsin which is involved in non-photopic regulation of the suprachiasmatic nuclei (SCN: i.e. central circadian oscillator; Johnston 2005) controlling the nocturnal production of melatonin by the pineal gland (Pévet *et al.* 2006). Between 1990 and 1992 we analyzed monthly batches of 70 days-old male rats and found that they were always heavier during winter than summer, which in turn was inversely related to peak nocturnal melatonin concentrations in blood (Bartsch & Bartsch 2007) indicating that pronounced seasonal metabolic changes occur in rodents as well, even under constant photoperiodic conditions.

Published data on the effects of RF-field exposure on survival and cancer in rodents

Studies on mice kept under chronic radiofrequency exposure in the 900 MHz-range (800–902.5 MHz) with either continuous (CW) or pulsed waves (PW) including GSM-like signals gave little evidence that they may reduce survival time and enhance tumor development or growth (Moulder *et al.* 2005; Tillmann *et al.* 2007). The applied fields in the different studies showed considerable differences in whole body absorption rates ($SARs_{WB}$) ranging from 0.008–12.9 W/kg, with the highest levels lying much beyond the limit permissible even for human occupational exposure (i.e. 0.4 W/kg). The time during which animals were exposed varied from 35 to 104 weeks. Animals were restrained in several experiments; daily exposure time therefore could not exceed 1–2 hours and was often given for 5 days/week only. Therefore the actual total exposure time (daily exposure x weekly exposure x weeks of exposure) was much lower ranging from 2 to 35 weeks. One study indicated a possible negative impact on health (Repacholi *et al.* 1997) detecting a significantly elevated lymphoma incidence, which, however, was not confirmed subsequently (Utteridge *et al.* 2002; Oberto *et al.* 2007).

Corresponding studies in rats using RF-signals in the 900 MHz-range (835.6–970 MHz) were mostly not found to affect survival or cancer development (Moulder *et al.* 2005; Shirai *et al.* 2007; Smith *et al.* 2007), except for the report by Zook and Simmens (2001) who at very high doses of ethylnitrosourea (applied for the induction of brain tumors) observed more malignancies. Very recently Adang (2008) in his doctoral thesis observed a trend towards shorter survival in female Sprague-Dawley rats at 25 months of age exposed to either a pulsed or unpulsed 970 MHz-signal for a period of 21 months (2 hours per day, every day). Interestingly, this study was performed with a relatively weak field leading to a whole body absorption rate of only 0.08 W/kg being in a range similar to our experi-

ments with around 0.04 W/kg, whereas other studies in which no effect on survival was found, SAR_{WB} was considerably higher (0.30–4 W/kg; Moulder *et al.* 2005). In our experiments III and IV by far the longest exposure times were applied so far published, 147 weeks compared to 2–104 weeks in other studies. It is conceivable that these comparatively short exposure times used in other studies were insufficient to affect the animals' health. It is also very likely according to our experience with experiment I (terminated at 770 days and showing no negative effects on health due to chronic RF-field exposure) that most experiments published to date were terminated too soon so that chronic effects on health did not have enough time to manifest. According to our experiments III and IV adverse effects of RF-field exposure become evident mainly beyond two years of age but most studies were terminated at such time or even earlier. Since in our experiments negative effects were observed at very low SAR_{WB} it appears that the effect of exposure time and subsequent additional time for neagtive health effects to become evident are of greater importance than high energy absorption rates applied over short periods. These considerations stress the necessity to perform complete survival studies so that long-term low-dose effects can be monitored and chronic effects are allowed to develop adequately. Such types of long-term studies on rodents will probably represent the optimal form of *in vivo* experiments to estimate potential long-term low-dose effects of RF-field exposure on humans, simulating situations of permanent exposure by e.g. urban mobile phone base-stations.

Survival studies in mice and rats where RF-signals in the low GHz-range (1.62–2.45 GHz) were applied, being relevant for both GSM- and UMTS-communication, indicate that they may have more pronouced biological effects than 900 MHz signals: Szmigielski *et al.* (1982) and Szudzinski *et al.* (1982) reported a higher incidence of spontaneous mammary as well as benzopyrene-induced skin cancers and reduced survival times in mice when applying 2.45 GHz CW with SAR_{WB} as high as 2.5–7 W/kg. It has been argued that these older experiments were not performed in the same standardized fashion as present studies and that $SARs_{WB}$ were too high. But also Chou et al (1992) showed that 2.45 GHz-fields, leading to only 0.2 W/kg $SAR_{WB,}$ if applied for 109 weeks (21.5 weeks actual total exposure time) accelerated primary tumor development in rats.

Divergent effects of chronic RF-field exposure on health and potentially underlying causes

From the above survey of the literature and mainly our own survival studies (experiments III and IV) it has to be concluded that long-term low dose RF-field exposure of experimental animals may exert predominantly adverse effects on health. It, however, has to be stressed that in our three consecutive DMBA-experiments published previously (Bartsch *et al.* 2002) no negative health effects were observed due to chronic RF-field exposure; on the opposite, a significantly delayed mammary tumor development was found in the first experiment (1997/8) and tumor growth remained unaffected in the subsequent two replication studies (1998/9, 1999/2000). It is not clear why these three experiments yielded incongruent results under identical experimental conditions since all animals were born on the same calendar days of subsequent years (April 27/8 of 1997, 1998, and 1999) so that seasonal modulatory effects can be ruled out. It therefore appears that RF-signals could elicit differential biological effects depending on the year when animals are born and an experiment is carried out.

Against this assumption it may be argued that an outbred strain of rats (e.g. Sprague-Dawley) may undergo uncontrollable and inadvertable genomic changes over the years thus causing differences in responsiveness. Fedrowitz *et al.* (2004) indeed observed that both magneto-sensitive and -insensitive sub-strains can exist within the breeding colony of Sprague-Dawley rats of Charles-River at Sulzfeld, Germany (being our animal supplier as well) and found that DMBA-induced mammary tumor development is stimulated by extremely low frequency (ELF)-fields (50/60 Hz) only if rats originate from sub-area 12 (as opposed to e.g. no. 3). In our second DMBA-experiment (1998/9: Bartsch *et al.* 2002) animals originated from area 12 but mammary tumor development remained unaffected by chronic RF-exposure. This may imply that either the sensitivity of rats to RF- and ELF-fields underlies different molecular mechanisms or that genetic drifts are of minor relevance. We tend to assume that longitudinal modulatory effects on the responsiveness of rodents under standardized laboratory conditions, (e.g. on a year-to-year basis) do exist. This view is supported by the results of previous repetitive survival studies on *inbred* female BDII/Han rats which develop spontaneous, metastasizing endometrial carcinomas in more than 90% of all virgins (Deerberg *et al.* 1995).

Apparent year-to-year variations of biological responsiveness in an inbred strain of rats imply the involvement of exogenous modulatory signals

In the course of two major survival experiments with virgin BDII/Han rats we tested to what extent pineal melatonin may be able to influence the development of hormone-dependent endometrial cancers. Melatonin was either administered throughout life or its endogenous production was permanently suppressed by constant light (LL). In the first set of experiments (1991–1994) melatonin led to statistically significantly extended survival (median: +33 days) whereas LL clearly shortened survival by almost three months compared to controls under L:D =12:12 (Bartsch & Bartsch 2007; Deerberg *et al.* 1997). To our great surprise, neither melatonin nor LL affected survival during replication experiments performed during 1996–1999 with

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animals born at comparable times of the year as in the first experiments. The controls of these experiments showed a drastically extended median survival of more than *five* months compared to those of the 1991–1994 experiments (Bartsch & Bartsch 2007). With the help of detailed macroscopic and microscopic examinations it could be ruled out that the primary tumor underwent histopathological changes or that the qualitative dissemination pattern differed among both series of experiments. It was concluded that endogenous tumor defense processes substantially differed among these two series of experiments being *low* during 1991–1994 and *high* during 1996–1999 explaining extended survival of untreated animals of the second series. Endogenous defense was apparently so strong during the 1996–1999 period that neither LL in a negative nor melatonin in a positive sense were able to affect the animals' health and life span. These results on *inbred*, i.e. genetically identical, animals clearly indicate that *endo*genous defense processes against cancer are modulated by unknown environmental factors which are changing over the years.

In this connection, it is pertinent to mention that the second series of BDII/Han-experiments during 1996–1999 (with up-regulated endogenous cancer defence) coincided with the above-mentioned DMBA-studies on Sprague Dawley rats (1997–2000) where chronic RF-field exposure was able to *inhibit* chemically induced mammary tumors in one out of the three studies. Since the experiments on BDII/Han and Sprague-Dawley rats were performed in different animal facilities within Tübingen we assume that a common *environmental* factor must have been present leading to a fostering of endogenous defense mechanisms against cancer during those years. As opposed to that, the results of the BDII/Han experiments performed during 1991–1994 gave indications for a clearly *reduced* endogenous defense against cancer (shortened survival among controls, further shortening of survival by LL). Therefore it could be that the postulated environmental cue may lead to a reversal of the direction of biological responsiveness within a period of approximately 5 years (mid-point of the 1991–1994 series: 1992.5; mid-point of the 1996–1999 series: 1997.5). This may also explain why in our mobile phone-related experiments rather positive effects were found during 1997–2000 in the DMBA-studies and clearly negative effects for the survival studies between 2002 and 2008. If the results of all these experiments are taken together it thus appears that the unknown environmental cue which regulates endogenous defence mechanisms against cancer may possess a phase-length of approximately 10 years.

Hypothesis: responsiveness of animals to RF-fields and other treatments are modulated by changing solar activity during the 11-years' sunspot cycle

A rhythmic environmental phenomenon fitting into a time-structure of about one decade is the sunspot cycle, which possesses an average length of around 11 years. This cycle is an integral part of changing solar activity which profoundly affects terrestrial life, as can best be seen from the corresponding repetitive sequence of tree-rings (Kromer *et al.* 2001). Figure 2 depicts the course of solar cycles 22 (1986–1996) and 23 (1996–2008) on the basis of the number of recorded sunspots.

If the current two series of RF-experiments, I and II (1997–2000) as well as III and IV (2002–2008) are projected into Figure 2a (see shaded areas) it is apparent that the first two experiments coincided with the ascending limb of solar cycle 23 and the other two with its descending limb. Clearly negative effects of RF-field exposure were observed during the second series of experiments (2002–2008) parallel to *declining* solar activity. In case of experiments I and II, which coincide with *increasing* solar activity, no definite conclusions can be drawn regarding a possible modulatory effect of solar activity on the effects of RF-field exposure due to the limited observational period. In case of the three DMBA-experiments, however, performed during 1997–2000 parallel to *increasing* solar activity (see Figure 2b) positive resp. neutral effects of chronic RF-exposure were detectable. This dichotomy between positive and negative systemic effects of increasing and decreasing solar activity seems to apply particulary well to the above-mentioned survival studies with BDII/Han-rats where during *declining* solar activity (1991–1994) untreated controls showed clearly shortened survival compared to controls of the replication study (1996–1999) performed during *increasing* solar activity (Figure 2b) due to down- resp. up-regulation of endogenous defence mechanisms against cancer. This also explains why animals of the first series (with weakened endogenous defence) did respond towards the administration/suppression of melatonin and animals of the second series (with fostered defence) were not affected by these experimental manipulations.

If one combines and integrates the results of the different studies on both Sprague-Dawley and BDII/Han-rats it is indeed suggestive that changing solar activity during the 11-years' sunspot cycle (Foukal *et al.* 2006) may exert profound modulatory effects on endogenous defense against cancer, even under controlled laboratory conditions (i.e. without changes in luminosity, UV-contents as well as photoperiod). To verify these currently hypothetical but potentially far-reaching interactions between the physical activity of the central star of our planetary system and terrestrial (patho-) physiological processes it will be necessary to further monitor such effects during the new solar cycle (no. 24) which began in 2009. For this purpose, survival studies with female Sprague Dawley rats are continued systematically to test whether chronic RF-field exposure may indeed show no or even positive effects on health during the coming years of anticipated increasing solar activity (2010–2013) whereas predominantly adverse

effects may exist during 2014–2019 when solar activity is expected to decline again.

Three central questions arising from the hypothesis regarding a modulatory effect of the solar cycle on the responsiveness of experimental *in vivo* tumor systems towards RF-field exposure are:

1. *Why is there a physiological need for endogenous cancer defense processes to be modulated by solar activity?*
2. *How could phases of low or high solar activity be "sensed" by animals and which general purpose could it serve?*
3. *What are the molecular correlates of endogenous cancer defense and which mechanisms contribute to their up- or down-regulation?*

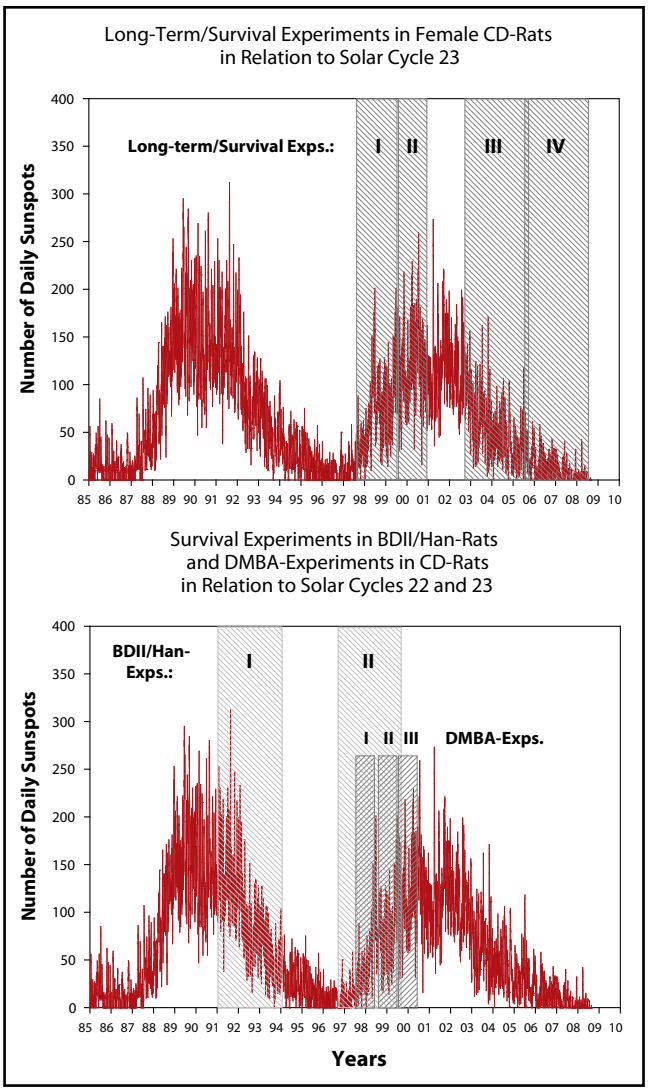

Fig. 2. Number of daily "American" sunspots (solar cycle 22: 1986–1996, and solar cycle 23: 1996–2008; solid lines) as published by the National Geophysical Data Center (NGDC: http://www.ngdc.noaa.gov/stp/SOLAR/ftpsunspotnumber.html#american) and timing of present long-term/survival experiments (shaded areas) in Sprague-Dawley (CD) rats (I–IV: Figure 2a: upper panel) as well of earlier survival experiments in BDII/Han (I,II), and Sprague-Dawley rats with DMBA-induced mammary tumors (DMBA I–III; Bartsch *et al.* 2002) (Figure 2b: lower panel).

Ad 1: Why is there a physiological need for endogenous cancer defense processes to be modulated by solar activity?

It may be assumed that "evolutionary memory" exists that certain phases within the solar cycle require systemic up-regulation to counter potentially life-threatening impacts of e.g. mutagenic or carcinogenic nature. The immediate question arising from this consideration is which phase of the solar cycle might be potentially harmful for life. It could be assumed that preferentially high solar activity around the peak of the 11-years' sunspot cycle may constitute the greatest threat since more mutagenic x-rays are emitted during this phase (Fry 2002). They are, however, mostly absorbed by the atmosphere (Prölss 2004) and therefore cannot substantially harm terrestrial life. The same applies to high-energy charged particles being an integral part of the solar wind. They are dispersed by the geomagnetic field. Only during periods with a drastically diminished geomagnetic field (which repeatedly occurred in pre-historic times; Valet *et al.* 2005) such particles reach the surface of the earth to a greater extent. It, however, appears questionable whether such relatively rare, though potentially dangerous, events may have indeed become part of "evolutionary memory" (Halberg *et al.* 2004a) leading to the development of solar cycle-mediated mechanisms to activate endogenous defense processes against cancer. A more plausible mechanistic scenario, on the other hand, may be the fact that an inverse correlation exists between mutagenic cosmic rays (possessing higher energies than solar particles) which reach the surface of the earth and solar activity (Wissmann 2006): more high-energy charged particles from deep space (e.g. protons, alpha particles and electrons) are able to penetrate the earth's atmosphere during solar minimum because of a concomitant weakening of the solar magnetic field which usually leads to their deflection after having entered the solar system from deep space. For this reason, a repetitive sequence of 5–6 years exists for more or less cosmic rays/particles to reach the surface of the earth. It would therefore be conceivable that this ever-repeating rhythm could have necessitated the development of mechanisms to transiently up-regulate anti-mutagenic processes. Due to a relatively small variability of just 10% for more or less cosmic rays to reach the surface of the earth between solar maximum and minimum (Wissmann 2006) it may, however, be disputed whether a substantially elevated mutagenic threat may indeed exist for terrestrial life at ground-level during solar minimum. This means it is currently unclear why endogenous defense mechanisms against cancer should be modulated by solar activity.

Ad 2: How could phases of low or high solar activity be "sensed" by animals and which general purpose could it serve?

Although our laboratory animals had been reared and kept under standardized laboratory conditions for many generations they still showed pathophysiologi-

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cal response patterns, which followed the solar cycle. The question arises which solar/solar-dependent signal could have reached them in their rooms?

In 1994 we published the hypothesis that natural geomagnetic changes may lead to seasonal melatonin rhythms under standardized laboratory conditions (Bartsch *et al.* 1994). Measurements of the horizontal component H of the geomagnetic field were found to be omnipresent (including our past and present animal rooms) and to be comparable to reference data recorded for South Germany at the Geophysical Institute of the University of Munich at Fürstenfeldbruck (http://www.geophysik.uni-muenchen.de/observatory/geomagnetism/daily-magnetograms). The analysis of these reference data showed that H follows a 24-hour profile, which is typical for each month of the year. Generally, H shows highest values in the morning around sunrise and a trough at noon so that its daily pattern undergoes seasonal modulations. For this analysis all days of a month were averaged irrespective of the fact whether they were so-called "quiet" or "disturbed" depending on absent/low or high solar activity. "Quiet days" show more regular 24-hours profiles of H than "disturbed days". Geomagnetic daily profiles therefore not only contain information regarding season but also about the state of solar activity within the 11-years' sunspot cycle (Cornélissen *et al.* 1998). It is therefore conceivable that animals perceive information about the stage of the solar cycle via the shape of the 24-hour profile of H. In a general way, it can be said that during years of low solar activity quiet days will be more frequent whereas during years of high solar activity disturbed days with more or less irregular daily profiles of H will dominate (Cornélissen *et al.* 1998). Therefore our initial hypothesis regarding a regulatory role of H for seasonal physiological processes can be extended to include hypermodulations by the solar cycle.

An indispensable pre-requisite for geomagnetic changes to affect physiological processes is that they are perceived by an organism. Wiltschko and Wiltschko (2005) elegantly demonstrated by their experiments with birds that a geomagnetic sense exists which is used for spatial, compass-like orientation. Nowadays, it is known that a wide range of species, including mammals, perceive and respond to natural changes of the geomagnetic field (Wiltschko & Wiltschko 2006) and which even seem to include man (Carruba *et al.* 2007; Thoss & Bartsch 2007). The underlying receptor-mediated mechanisms are incompletely understood but involve magnetite-containing cells in the brain as well as specialized photoreceptors in the eye such as cryptochrome as well as melanopsin (Wiltschko & Wiltschko 2006). It therefore appears that a complex interaction exists between non-visual/visual light-mediated signaling and magnetoreception. Currently, it is still unclear how even very small geomagnetic variations in the nT-range, being typical for daily changes of H, may be discriminated by any of the known receptor molecules. Since magnetic orientation of birds is disturbed by a 1.315MHz radiofrequency signal (Thalau *et al.* 2005) it is assumed that a radical pair mechanism is involved at the level of the retinal magneto receptor (Ritz *et al.* 2004). This very observation and hypothesis would clearly support our findings and assumptions regarding solar cycle-mediated effects on health, which could be modulated by RF-fields. Things, however, appear to be more complex since orientation of a mammalian species was not found to be disturbed by a 1.315 MHz-signal (Thalau *et al.* 2006). In addition, the light sensitivity of the human eye which according to Thoss & Bartsch (2003) is co-regulated by the geomagnetic field is not affected by a 902 MHz RF-signal (Irlenbusch *et al.* 2007). Therefore it has to be assumed that different/additional magnetoreceptive mechanisms are involved in mammals, apart from e.g. retinal melanopsin, which seems to include the *superior colliculus*, an integral part of the optic tectum (Nemec *et al.* (2001), as well as magnetite-mediated perception (Wiltschko & Wiltschko 2005).

Since radiofrequency signals may affect living systems via geomagnetic perceptive mechanisms it is conceivable that a general electromagnetic sense exists for both static and alternating fields over a wide range of frequencies. One may wonder why even radiofrequency signals could be part of this sensory system. Radio signals are, however, emitted by galaxies and stars including our own sun and reach the surface of the earth via the so-called radio window of the atmosphere (100m – 1mm wavelength = 5 MHz–300 GHz: http://www2.jpl.nasa.gov/radioastronomy/Chapter4.pdf). Solar radio signals undergo typical changes in their intensity parallel to the 11-years' sunspot cycle (http://www2.jpl.nasa.gov/radioastronomy/Chapter6.pdf). Since solar activity exerts profound effects on terrestrial climate (Arnold 2002), which has always been a decisive determinant for the availability of nourishment it could be evolutionarily advantageous to possess the capacity to "sense" future climatic changes or, on a shorter time-scale, to "feel" the advent of colder or warmer years. Efficient adjustment to such environmental changes implies that animals are able to successfully migrate towards those places where favorable nutritional conditions exist. For this purpose, an effective sense of orientation is indispensable which in turn utilizes geomagnetic informations. Such type of strategy is followed by migratory birds which, however, due to the high predictability of seasonal changes of both weather and concomitant availability of food at the geographic antipodes of their travel became a persistent routine and over time even got integrated into their genome.

These lines of thoughts indicate that perception of the geomagnetic field, perhaps in conjunction with natural radiosignals, allows a complex and physiologically logical integration of different sets of inter-related environmental informations: the specific pattern of the geomagnetic field at a certain place of the earth facilitates local orientation, its daily profile serves as seasonal

cue (in addition to light) and the amount/pattern of disturbances (perhaps in conjunction with corresponding solar radiofrequency signals) renders information about the state of the solar cycle (Johnsen & Lohmann 2005). From this point of view, it is understandable why anthropogenic radiofrequency signals, such as radio-, TV-, as well as mobile phone signals, may be perceived by living organisms which, from the very beginning of evolution, have been familiar with similar solar and cosmic radiosignals.

Although most physiological details of electromagnetic sense perception are still enigmatic it appears that the pineal gland with its hormone melatonin is an integral part of it. Melatonin was found to be crucial for migratory orientation in birds and pinealectomy abolished directional preferences (Schneider *et al.* 1994). Reduced melatonin secretion due to increased pineal calcification was connected with a defective sense of orientation in pigeons and even humans (Bayliss *et al.* 1985). Both artificial manipulations of the geomagnetic field as well as their natural changes during prominent phases of solar activity have been reported to influence melatonin secretion as well as pineal activity in experimental animals as well as in humans (Burch *et al.* 1999, 2008; Semm *et al.* 1980; Stehle *et al.* 1988; Weydahl *et al.* 2001). Since melatonin is centrally involved in photoperiodically controlled seasonal reproduction (Pévet 1988) via neuroendocrine mechanisms (Reiter 1983), it is clear that modulations of pineal secretory activity by solar activity will automatically affect these processes. Such regulatory mechanisms appear to be evolutionarily logical and advantageous since reproduction can be adjusted to changing climatic conditions which are essential determinants for the availability of nourishment and thus for survival of the animals' offsprings.

Ad 3: What are the molecular correlates of endogenous cancer defense processes, and which mechanisms may contribute to their up- or down-regulation?

Finally, it remains to be discussed what may be the molecular components of endogenous defense processes against cancer and in which way they may be modulated by geomagnetic changes/perturbations as well as solar or anthropogenic radiofrequency signals. It is very likely that the pineal gland is centrally involved in this scenario as well. Melatonin inhibits not only hormone-dependent tumor growth (Bartsch & Bartsch 2006; Blask *et al.* 2002) and the formation of free radicals (Reiter *et al.* 2008) but the endogenous production of melatonin is in turn affected by tumor growth in both man and experimental animals (Bartsch & Bartsch 1999). This means that there is a mutual and dynamic link between the pineal gland and malignant disease. Constant light-mediated suppression of melatonin can stimulate development and growth of many tumors (Bartsch & Bartsch 2006). Light to regulate melatonin secretion via the suprachiasmatic nuclei (SCN; seat of the central circadian oscillator in the brain) is perceived by retinal melanopsin, which is also a candidate receptor molecule for geomagnetic perception (Johnsen *et al.* 2007). It therefore appears that at the level of this retinal blue-light receptor to regulate pineal melatonin secretion a molecular convergence may exist with the perception of geomagnetic as well as radiofrequency signals of natural and artificial origin. Central circadian oscillation in the brain including the pineal gland would thus be modulated not only by multiple natural signals depending on time of day, season as well as progression of the solar cycle but also by artificial electromagnetic signals, including RF-signals used for mobile telecommunication. In this way both environmental and artificial cues could affect cancer processes via central neuroimmunoendocrine mechanisms, which are controlled by the pineal gland and other central mechanisms.

If these potential and far-reaching interactions will indeed prove to exist nocturnal melatonin production can be expected to be influenced not only by season (Bartsch *et al.* 1994, 2001) but also by solar activity. This has been indicated by previous studies (Burch *et al.* 1999, 2008; Weydahl *et al.* 2001) and has been postulated by Halberg *et al.* (2004b) for many years. It is also a central aspect of our parallel endocrine studies with animals of experiments I–IV where the main metabolite of melatonin, 6-sulfatoxymelatonin, was determined in its nocturnal secretion pattern over at least 18 months in each of the four experiments (Bartsch *et al.* in preparation). According to our present evaluation, the nocturnal production of melatonin in rats follows the course of solar cycle 23 with increasing quantities parallel to rising solar activity (1997–2000) and lower levels during declining solar activity (2002–2008). Since chronic RF-field exposure shortened survival during 2002–2008 (solar activity was declining and melatonin falling) as opposed to 1997–2000 (melatonin was rising parallel to increasing solar activity) when RF-treatment was even able to inhibit DMBA-induced tumor growth, it is conceivable that the pineal hormone melatonin may be functionally and centrally involved in solar cycle-modulated effects of chronic RF-field exposure on cancer growth and survival.

CONCLUSION

Although the results of our survival studies performed during 2002–2008 clearly show that chronic, practically life-long exposure to a low-intensity GSM-like signal significantly shortened survival of female Sprague-Dawley rats it is, in view of our previously published report on neutral or even inhibitory effects on DMBA-induced mammary tumor growth, conceivable that RF-field exposure underlies additional longitudinal modulatory influences. On the basis of our repetitive survival studies with inbred BDII/Han-rats relating to the role of the pineal hormone melatonin in the control of spontaneous endometrial carcinomas and the divergent results obtained therein we tend to assume that systematic year-

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to-year modulatory influences may exist. We assume that they follow the course of solar activity within the 11-years' sunspot cycle which, according to our recent observations, seems to affect pineal melatonin secretion which is an integral part of endogenous defence against cancer. The activity of the sun may influence laboratory animals via changes in the geomagnetic field, which is omnipresent and perceived by specific receptors, e.g. retinal melanopsin, also involved in the light-mediated synchronization of the SCN (central circadian clock of the brain) and controlling the circadian secretion of pineal melatonin. Reports regarding a disturbance of geomagnetic orientation of birds by RF-fields and experimental manipulations of environmental magnetic fields modifying pineal melatonin secretion support the concept that solar cycle-mediated modulations of RF-effects on cancer and survival may involve the pineal gland. It therefore appears that the current series of *in vivo* experiments designed to test the chronic effects of low intensity GSM-like signals on health and survival may also pave the way for a substantially better understanding of biological variability observed in *in vivo* experiments performed under standardized laboratory conditions due to fundamental environmental influences of natural origin being part and parcel of heliophysical effects on the earth. Anthropogenic RF-signals, including those used for mobile telecommunication, will therefore have to be viewed in the context of these environmental phenomena to adequately define their adverse as well as possibly favorable effects on health. To further prove these complex interactions and processes it is necessary to systematically continue the experiments performed by us during the last decade also throughout the new solar cycle, which began in 2009.

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