Frank Gollnick

Why do we need research

on mobile radio fields in cells and tissues?

The results of two projects promoted by the Research Association for Radio Applications (Forschungsgemeinschaft Funk, FGF) performed at the Humboldt University Berlin

Research on the potential influence of electromagnetic fields (EMF) on living organisms and humans is performed at all different organisation levels of organisms: from population studies (epidemiology) via studies on individuals (humans and animals), cells and tissues (of humans, animals and plants as well as unicellular organisms and bacteria) to the lowest, i.e. the molecular level where the components of living substances (molecules, ions) and the material of living cells (for example membranes) are observed.

Scientists at all levels (fig. 1) are searching for evidence proving or disproving strain, impairments or even health dangers supposedly resulting from the nowadays increasing EMF exposure. Only a combination of a great number of studies undertaken in different areas of scientific research one day will allow us to draw overall reliable conclusions on potential hazards of mobile radio fields.

Until now, science has not yet succeeded in reaching this aim; the objective observer might be confused when confronted with the numerous seemingly contradictory positive and negative findings often inhibiting rather than promoting an evaluation of results. The task to relate the many single findings to one another concerning the relevance for our health has still to be accomplished. Further, there is a lack of studies drawing a connection between the above mentioned study levels, i.e. showing whether a certain relevant effect found at one level is portable across across other levels – a highly controversial issue. As long as interaction mechanisms are not clearly identified that could help to explain where fields potentially might attack living material, the evaluation of single findings' health relevance will remain unsatisfactory. Interdisciplinary research simultaneously covering sev-

Population	**Epidemiological studies** search for effects of high frequency electromagnetic fields based upon a large number of persons
Individual	**Animal studies** can controlled application of a certain factor (f.e. of an electromagnetic field) trigger a measurable response from the body?
Cells and tissues	**Cellbiological studies** can electromagnetic fields have an influence on cell behavior?
Molecules	**Biochemical and biomolecular studies** can electromagnetic fields have an impact on biochemical response or molecular structures?
Medical appliances	**studies on interference shielding** do electromagnetic fields have an impact on medical appliances and medical body aids?

Abb. 1: Study levels

eral life organisation levels is rare, in many areas not even possible, or – if possible after all – rather costly.

All this leads to the question whether and/or where deeper research is worthwile. After all, 40,000 scientific publications on the topic are already available, though at closer inspection a great part of the studies shows considerable weaknesses in conduction.

So, an improved research quality is highly desirable and nowadays is increasingly achieved. Of utmost importance are interaction mechanisms fit to derive models for explaining possible EMF targets in biological material. Which study levels may help us to identify these mechanisms?

Study level of cells and tissues

Experiments performed at the study level of cells and tissues often refer to the lowest level - the level of molecular components of life. The two study levels overlap. For studies on cells and tissues scientists mainly take samples from experiment animals tried and tested in pharmaceutical research, by biopsy (tissue sample taken by means of a cannula) or blood samples from humans. Further, cultured human, animal and plant cells as well as bacteria cultures are applied.

This study level is particularly advantageous insofar as disturbing influences of the whole organism coming from the brain and the nervous system do not occur. During behavioral experiments on animals, in sleep laboratories using participants, or questionnaire interviews as a part of population studies emotions, illusions or subconscious behavior always play an important role and should be carefully separated from the actual influence-free states, behaviors or statements. This is a huge problem for all studies at the higher levels of individuals and population. Generally, scientists try to neutralize so-called confounders applying comprehensive statistical methods to identify as many confounders as possible and exclude them. However, literature shows that this often enough is not achieved.

Thus, many studies suffer from insufficient consideration of disturbing influences suggesting wrong results and inhibiting detection and evaluation of potential health dangers. This results in the above mentioned dilemma that practically more than one half (some even speak of 80%) of the findings published in scientific literature on the issue of electromagnetic compatibility should be taken with caution when aiming for a reasonable assessment of potential health risks. However, it is doubtful whether this rule always is observed.

Cells and tissues have no confounding thoughts and nervous impulses, thus allowing us to observe completely unadulterated biological responses. Of course, also during experiments based on cells and tissues we have to do with a huge number of possible confounders. However, these are much more easily and effectively controlled by appropriate technical means than those of whole organisms. Here, the share of useful literature (in the above mentioned sense) is higher.

On the other side, it is more difficult to derive health relevance for humans from studies on cells und tissues. An increased division rate of brain cells in a petri dish exposed to a simulated mobile radio field does not necessarily imply that at corresponding exposure an increased division rate also occurs in the human head, in other words, that there is a potential tumorigenesis. For such a connection to be drawn one would have to know the above mentioned interaction mechanisms responsible for the effect and develop respective theoretical models. In most cases, this has not been achieved. But ultimately, the living cells each organism is composed of are the key to deeper knowledge of the issue.

Targets within the cell

In short, living cells principally have the same structure: a bilayered, seemingly half-liquid membrane consisting of flat strung-together phospholipids (phosphate molecules bonded to fatty molecules) surrounds a liquid-filled space. Within this space there are soluble salts (ions), aminosoureas, nucleic acids (genetic substance: DNA, RNA), proteins, fats and a number of different little spaces again encased by one- or bilayered membranes with special functions, so-called organelles (or cell compartments). Proteins form a so-called cytoskeleton for support and cell movement; the cell nucleus contains the genotype being the largest compartment. Not particularly specialised cells have an average size of approximately 1/30 mm. As is known, based upon this fundamental structure (nearly identical even in plants and animals) in the course of evolution a large number of specialised cells has developed.

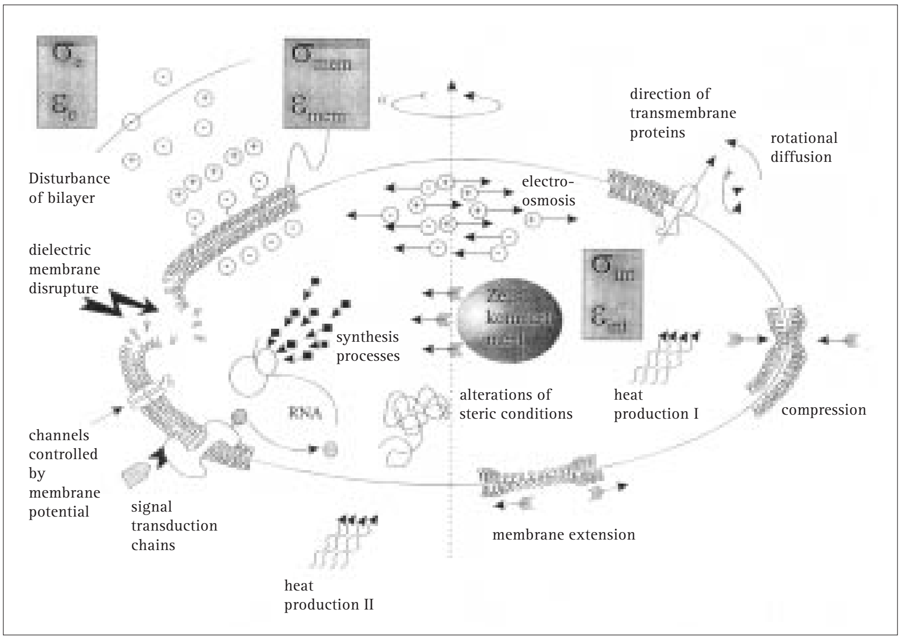

Fig. 2: Diagram of an oval biological cell. Emphasized are structures, processes and power effects that principally may be influenced by external electric fields. (Drawing: H. Glasser)

Of great importance is the question which cells structures or contents may serve as a target for an impact caused by external EMF. Here, it is necessary to differentiate between structures and processes within and at cells.

Figure 2 shows an oval cell. Emphasized are the components and power effects in question. As we can see, potential effects are focused on membranes, cell compartments, proteins and the related synthesis, regulation and transduction processes, nucleic acids as well as electrically charged soluted particles (f.e. ions) marked by plus (+) and minus (-).

It is general consensus that the demonstrated effects can be caused by external EMF. For example, 'dielectric membrane disrupture' only occurs at extremely high field strengths in experiment; it never occurs under normal mobile phoning conditions.

In the discussion on potential detritous influences of mobile radio fields two questions are crucial: At which extent can shown effects at cells be health relevant concerning our body; in which size field strengths have to occur in order to initiate the shown effects on structures and processes, subsequently causing alterations within the cell? For some time now, science deals with these questions, and only the answers to these questions will confirm, disprove or explain suggestions that disturbances found at higher organism levels actually are caused by effects of electromagnetic fields. Without knowledge of cellular interaction mechanisms having an impact on the body, a disturbance observed in the body remains a mere phenomenon und might as well be caused by other influences possibly overlooked in the course of a given study. This should be cleared up beforehand, though it proves more difficult with each higher organisation level of the examined object, that is, most difficult in studies on humans where psychologic factors and subjective perception never can be completely excluded. So, does this mean that research is at a dead end?

Both approaches – at the lower level of cells and at the higher level of the whole organism – make sense when aiming for serious research on potential health dangers caused by EMF. Ultimately, only a combination of results from both research areas and stable proof of interaction channels between cause and/or targets of EMF effects within cells as well as valid measurements of an impact on the organism will provide the evidence necessary to decide whether we should expect health damages caused by mobile radio fields.

Accordingly, in December 2000 during the international workshop 'Portability of research results ...' organised by the FGF experts from different scientific disciplines, among others, addressed the here discussed topic (see the report in this issue). As was shown, there still is a lot to do to gain the desirable comprehensive insight into the matter.

Pointing in the right direction are two research projects promoted by the FGF and realised at the Humboldt University in Berlin. In his final report prepared for the FGF, Prof. Günter Fuhr declares: "Claiming that field impacts on animals and particularly humans can be explained solely based on the organism level is a simplification. (...) According to all scientific knowlegde, an impact occurs at the nuclear and molecular level at any field type whatsoever. Therefore, it is necessary to

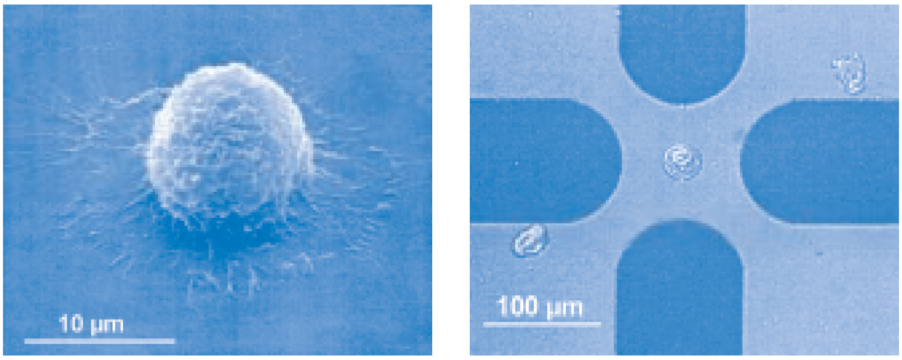

Fig. 3: (a) rounded cell taken from the connective tissue of the mouse (b) cell (protoplasm) removed from cell wall taken from immature (embryonic) tissue of the larch set between four electrodes at a semiconductor electrode chip (see also fig. 5)

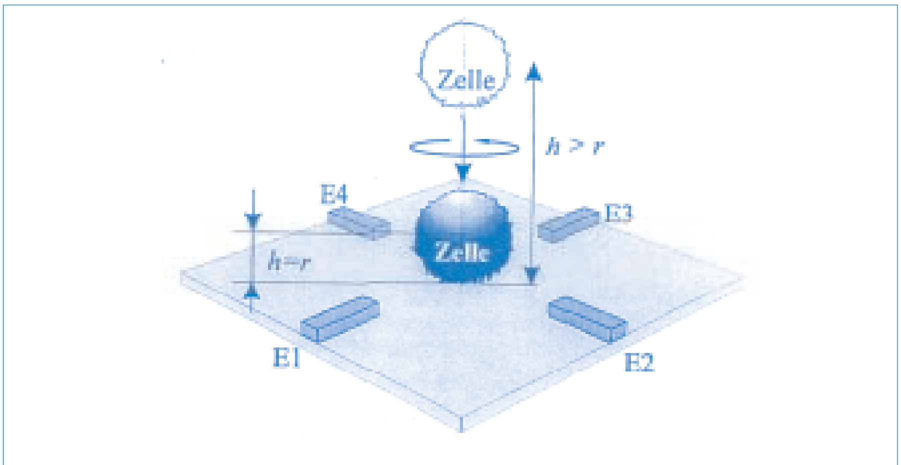

Fig. 4: Set-up for measurements of field powers and subsequent calculation of occurring field strengths within a cell at a semiconductor electrode chip. Cell between four electrodes (E1 - E4) in elevation and rotation caused by the powers of the rotating field. For calibration the cell is replaced by a latex ball of the same size.

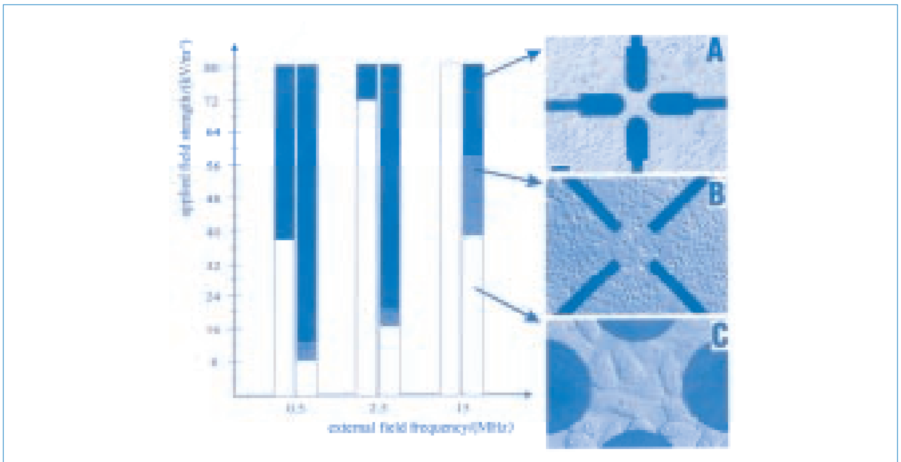

Fig. 5: Responses to a short-term (20 seconds, left columns) and a long-term (3 days, right columns) exposure of cell cultures (cells from connective tissue of the mouse) at three different frequencies (0.5, 2.5 and 15 MHz, unmodulated) and electric field strengths ranging from 1 to 80 kV/m. Meaning of the hatching: see text. As shown in A, B and C, microscopic screenings were evaluated.

experiment on the smallest possible living unit, namely isolated cells. We think that our research project just brought to a conclusion contributes to this endeavor.“

Semiconductor electrode chips as carriers of cell cultures

The project directed by Prof. Günter Fuhr at the Institute of Biology of the Humboldt University Berlin for the first time applied semiconductor material as a background for culturing cells in special chambers designed for direct field exposure. Semiconductors nowadays are generally known from electronics and computer technology as a carrier and basic material for electrical wiring and microchips. This material makes it possible to considerably miniaturize electrical conductors and to bring them nearer to the carrier material of cultured cells than previously possible. This means that the electrodes used in experiment – as a sort of transmission antenna for the input of high frequency test fields – have a very similar size to that of the tested cells (fig. 3).

In view of exact field calculations in the tested cells and of the strength of appliable fields (nearly without disturbing heat production) whole new dimensions open up. Here, a system was developed that provides reliable basic data – of previously unknown precision concerning field strength assessement within and at the cell – on cell alterations under exposure to extremely strong field. However, to field strengths below 100 V/m the system does not apply. This value lies between monitor transmission (approximately 10 V/m) and the strength of the geomagnetic field (200 V/m). In summary, with the aid of the semiconductor electrode chip primarily rather strong field effects can be measured in experiment.

The project used a whole new strategy for providing precise calculations of the actual field strengths and temperature values within the cells, too. Living round cells or round latex test particles in a saline solution were elevated by the rotating field and themselves rotated between four electrodes (so-called quadrupole design). The time of set-off and the rotation velocity of the cell in the constantly rotating field allowed to draw precise conclusions on the fields strengths occurring at the cellular level (fig. 4).

Here, a wide frequency and field strength range could be tested. The inaccuracy factor lay only at ± 10% - a calculation previously impossible at the given size of objects (approximately 1/100 mm) using traditional computerised field simulations.

Field values showing within the cells as a rise in temperature were measured by a as well sophisticated method: The division rate of cells cultured at the electrode chip served as an indicator of the input of 'thermal energy' (= temperature) produced by the respective electromagnetic test field. Generally, the cells of our body only divide optimally, that is with the highest possible velocity, within a quite narrow temperature range. Above and below this temperature window doubling periods are considerably longer. For the test, cells of mice were cultured at semiconductor material at a temperature between 30(and 40° C (± 0.1°). At each set temperature (steps of 0.5° C) cell division velocity was measured and a division rate optimum was identified. When at constant temperature of the background (and the surroundings of the measurement chamber) an additional field was produced, the optimum went to lower temperatures. The discrepancy in comparison to the previously identified optimal temperature therefore had to result from the energy input of the field. In both cases, the cells 'experienced' the same temperature range. The effect particularly showed at the lower temperature produced by heating the electrode chips. Of course, this 'thermal' effect of the EMF only occurred under exposure to relatively strong fields lying considerably below the frequency range of mobile radio. However, this method enables us to measure actually experienced temperature values – not only temperature of the immediate environment (as is normally the case during measurements at cells) – by means of clearly observable cell response. This approach is also of use at higher frequencies helping to differentiate between heat effects and possible other effects caused by the added fields.

The impact on cell growth, survival rate and mobility (deformation and wandering) across a wide frequency and field strength range (500 kHz - 15 MHz, 1 - 80 kV/m) was examined using four crossed electrodes mounted to semiconductor material (fig. 5).

(A, B, C) Exemplary presentation of different field impact levels on the cells positioned at the centre of the four electrodes: (A) no surviving cells at the centre; (B) surviving, but damaged cells at the centre, identifiable by the thinned-out cell layer with rounded cells; (C) healthy cells. Measurement points in (C) are equivalent to 1/13 mm.

Here, at short-term field exposure of 20 seconds the range of exposure leading to serious damages at the cell membrane (black areas in respective left columns of the diagram) was identified. The long-term constant exposure (three days; right columns) differentiates between no damages at all (white areas), deterioration of at least one of the above mentioned parameters (gray areas; mainly decreased growth rate) and no surviving cells at the centre of the electrodes (black areas). As is seen, surprisingly there was no measurable impact on cells at 15 MHz field exposure over three days at a field strength of up to 40 kV/m (fig. 5C).

Slot-conductor systems designed for higher frequencies

In his efforts to cover the frequency range of mobile radio in experiment, in the course of the FGF project Prof. Fuhr developed a new semiconductor electrode chip especially designed for field exposure in the GHz range. The so-called 'planar slot-conductor resonator' consists of a thin circuit board (glass wafer) upon which a very thin meandric slot-conductor is mounted by laser technology (fig. 6).

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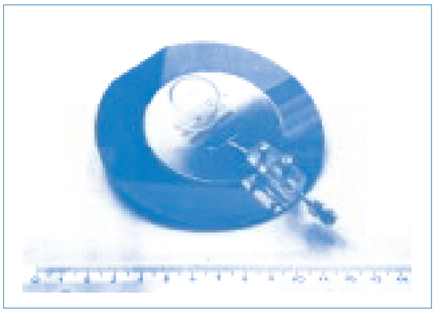

Fig. 6: View of a slot-conducting system for cultivation and exposure of cells in the GHz range mounted to the object holder (black plate) of a microscope. To the lower right the high frequency conductor, at the centre the cultivation chamber (mounted glass ring). The meandric slot-conductor can be seen. Measurement range in cm.

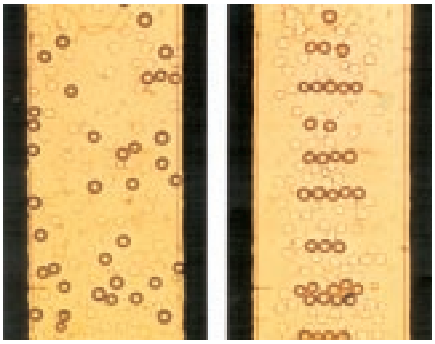

Fig. 7: A look into the channel (yellow area) of a slot-conducting system with cultured white blood cells attached to the transparent ground (clear) and round latex test particles (dark). Illustration to the left: prior to field activation. Illustration to the right: 10 seconds after field activation. Explanation: see text. Channel width: 1/5 mm.

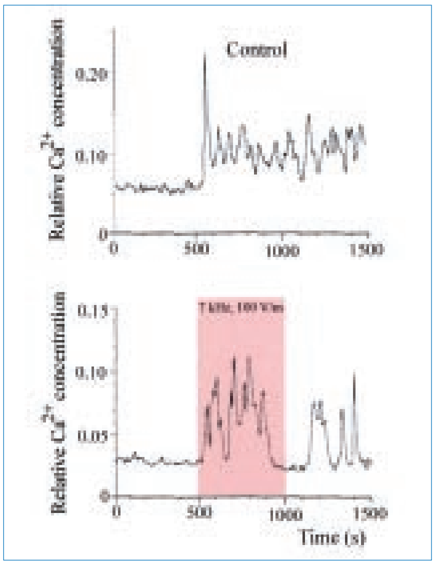

Fig. 8: Measurement of the internal calcium contents of two single cells (lymphocytes) over a time period of 1500 seconds. Calcium releases from the internal calcium reservoirs are seen as measurement curve peaks. The regular baseline within the first 500 seconds shows the basic calcium contents of normally approximately 100 nmol/l. Upper graphic: Control experiment without electric field. Lower graphic: Exposure of the cell in marked time period to a 7 kHz electric field of 100 V/m field strength. Left: calcium contents in relative units.

nel of the slot-conductor cells can be cultured and observed through the microscope. After applying a high frequency electric signal, f.e. 900 MHz, at the cellular level inside the slot-conductor well-defined high frequency fields of very high field strengths may develop. Certain limited areas within the slot-conductor can be differentiated showing either a purely magnetic field or a purely electric field. Similar to the quadrupole electrode chips depicted above, field and temperature definition with the help of test particles and cells also is possible in the slot-conductor.

Figure 7 shows human lymphocytes (white blood cells, approximately 1/100 mm) are cultured in a nutrient solution attached to the ground within the channel of a slot-conducting system. Next to them, latex test particles of nearly the same size, but somewhat darker swim freely in the solution without field. At exposure to a 900 MHz field (fig. 7, picture to the right) the particles arrange in the form of a string of beads because of the repulsion powers of the electric field also affecting the lymphocytes lying below. By means of arranging velocity the field strengths in the GHz range actually affecting the cellular level can be measured with hence unknown precision; not only – as previously was the case – calculation but also an exact assessment is possible.

This new designed system opens up additional possibilities to examine single cells in high frequency fields of the lower GHz range.

Keeping track of calcium

The project directed by Prof. Roland Glaser at the Institute of Biology of the Humboldt University Berlin examined the most important neurotransmitter within the cells – calcium – at exposure to the electric field component of EMF. Study object were cell cultures of human lymphocytes (see above). Each living cell was injected with a dye indicating under the microscope which amount of calcium is released from the reservoirs contained in the cell. Such calcium releases (or also external influx into the cells) essentially serve as a signal for a large number of life processes, for example as a starting signal for cell division. Since lymphocytes often show seemingly spontaneous (i.e. without recognisable external reason) spurts of calcium release, this project required a high statistical level to separate the measured 'spontaneous' from possibly 'involuntary' releases caused by the experimental electric field. Should an electric field be able to cause such 'involuntary' calcium releases, this naturally could unbalance the whole, subtly tuned intracellular signal system. Related to this complex is, among others, the much discussed potentially increased leukemia risk caused by electromagnetic fields. Leukemia (blood cancer) is an uncontrolled increase of lymhocytes normally strictly regulated in the body with the help of calcium.

UVA light interferes with calcium measurements

It is methodically necessary to radiate the living lymphocytes during measurement of their calcium contents with UVA light. Fluorescent dyes nowadays in use to indicate the respective calcium contents are sufficiently sensitive only when brought to luminiscence by UVA light.

The FGF project showed that cells produce more 'spontaneous' (i.e. in this case: independent of the EMF) calcium releases with higher doses of UVA light irradition. This insight provides valuable clues for the evaluation of already published positive findings of research studies having applied the same examination methods without paying attention to the problem of UVA light.

In his experiments, Prof. Glaser radiated lymphocytes with UVA light at a half-life dose of 10 ±3 kJ/m². These cells proved particularly susceptible compared to other test cell types (bone cells, cancer nerve cells).

Thorough evaluation of data

Two experiments on a single cell demonstrated in fig. 8 exemplarily show how difficult it is to differentiate between 'spontaneous' calcium releases and those possibly caused by the field.

In such cases, research generally has to select a particular large base of study objects for statistical methods being able to contribute to solving the problem. Since Prof. Glaser's study related to a wide frequency spectrum (1 - 200 kHZ, 50 and 150 MHz) being closely examined in its impact on cells, correspondingly a large number of single registrations (approximately 100 to 200 per frequency) had to be statistically evaluated. As a rule, electric field strengths of 100 V/m were produced at cellular level during the second third of an experiment. The total evaluation of calcium response (increased values within the cells at least twofold) at field exposure in the low frequency range of 1 - 200 kHZ (fig. 9) compared to controls (without field exposure; light blue marking line at 49%) showed no statistically significant deviations of average values (t-test, error probability p=0.05).

Because of the strong scattering of single values combined in each column of fig. 9 also purely optical devations from controls were not statistically significant (for example at 2 kHz, 7 kHz and 150 kHz).

By means of the so-called Kolmogoroff/Smirnoff test data were subjected to another statistical evaluation method of higher selectivity. This test divides cells into classes of a certain value of the measured calcium release at field exposure. Here, the result showed highly significant deviations occurring at frequencies of 20 kHz, 50 kHz and 100 kHZ (error probability p=0.001). However, the method of testing led to the conclusion that only a small number of cells in each tested group had contributed to the result. But as long as only a small part of the cells shows a response to field exposure, according to Prof. Glaser there is no validated field effect. We can only speak of hints of potential field effects. A more thorough differentia-

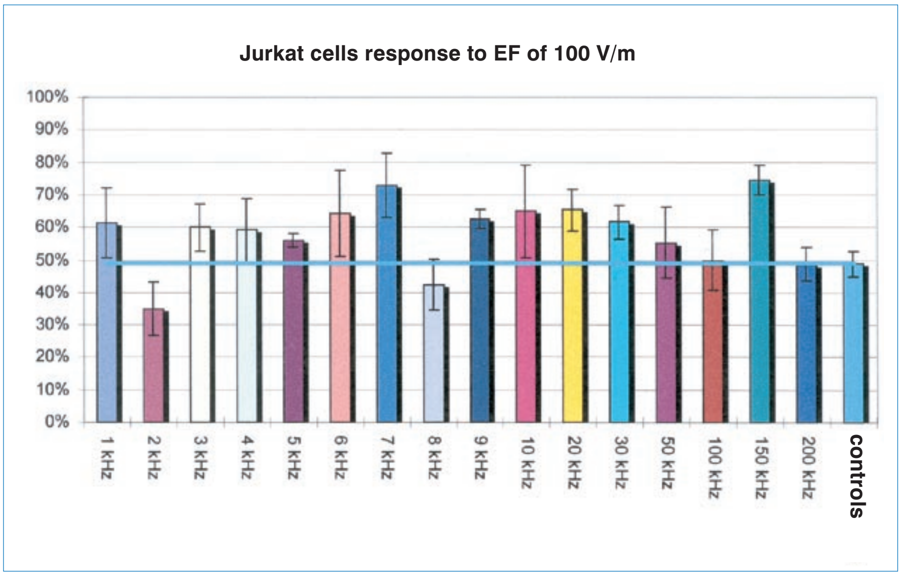

Fig. 9: Evaluation of cell response (at least twofold increased calcium values during field exposure phase) of lyphocytes to electric fields of 100 V/m at 16 different frequencies. In control experiments without field exposure, (outer right column and horizontal blue marking line) approximately 49% of the cells showed increased values during the critical period (see also fig. 8). Each column combines the values of 100 - 200 single cells (average values with standard deviation stratified by number of cells and tests).

tion between responsive and non-responsive cells could not be achieved.

During corresponding experiments on the same cells - though at a different exposure set-up - in the high frequency field (50 MHz and 150 MHz) the t-test neither did identify significant deviations of average values between the tested groups during different treatments (30-seconds field or sham exposure, 7-seconds field or sham exposure). The Kolmogoroff/Smirnoff test again indicated - at least in one case - a highly significant deviation at 50-MHz exposure (7-seconds field exposure versus 30-seconds sham exposition). Here, the previously mentioned limitations due to the type of the statistical test have to be considered, too.

No alarming results

Both depicted studies showed no results giving cause for serious concern regarding the effects of low and high frequency EMF on cells. High field strengths quite late resulted in measurable effects pointing to certain damaging tendencies. Potential types of damage could be demonstrated by grades. Of course, only small sectors of the highly complex processes occurring in living cells as well as of the frequency field spectrum could be observed. Regarding the observed lymphocytes, it would certainly be of use to more precisely examine phenomena concerning proof methods and cell condition (f.e. position in division cycle). Here, possibly targets of field impact and interaction mechanisms could be discerned.

It was shown that it is worthwhile to pay close attention to all details of cell examination before drawing conclusions of potential consequence. It is just now that we gradually achieve a certain methodical standard allowing us to collect reliable basic data and to identify/adjust measurement inaccuracies. In this context, the two presented FGF projects do contribute valuable new insight and knowledge which may help open up new possibilities for the required reliable research on possible field effects at the level of cells and tissues.

Dr. Frank Gollnick, biologist, long-time staff member at the Physiological Institute II of the University Bonn, now is scientific advisor of the FGF

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