Forschungsgemeinschaft Funk e.V.

NEWS letter

1·02

G 14514 ● 10. volume ● No. 1 ● April 2002

The melatonin hypothesis – a survey

by Alexander Lerchl

Introduction

The pineal gland (epiphysis cerebri)[1] produces the hormone melatonin whose level generally depends on the light-dark cycle of the environment (photoperiod). Its main task is to transform the physical parameters of daytime and season into a hormonal signal that may be interpreted by the organism as an endocrine "timer." Thus it is possible to achieve reasonable coordination of daytime (diurnal) as well as seasonal physiological processes with the external world. As melatonin is almost completely produced during the night, it is often called the "hormone of darkness" [1].

The ecological background of seasonal adjustment is the fact that offspring survival is provided for only if birth takes place during a certain time of year ("seasonal reproduction"). On the other hand, diurnal synchronisation is important for quite a number of physiological adjustment processes (circulation, digestion, sleep, etc).

Possibly, other processes are affected to a certain extent by the pineal gland or melatonin, too. So, for example, there is speculation on aging and cancer development being slowed down or even inhibited by the hormone.

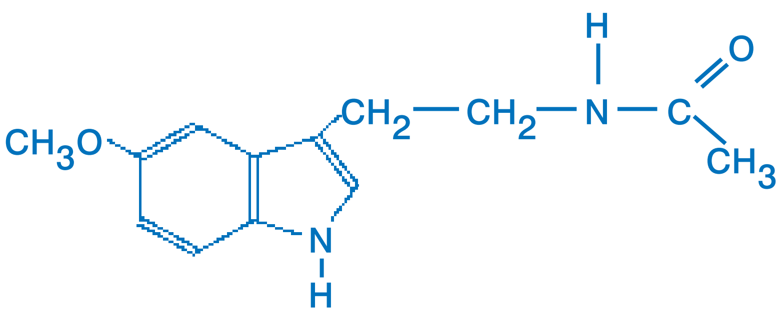

Fig. 1: Melatonin's structural formula (N-acetyl-5-methoxytryptamine)

Forschungsgemeinschaft Funk

Inhalt

RESEARCH
The melatonin hypothesis – a survey p. 1

STUDIES
Long-term exposure with a GSM-like signal (mobile phone) does not stimulate the development of DMBA-induced mammary tumours in rats: results of three consecutive studies p. 8

RESEARCH
Effects of electromagnetic fields (EMF) on the function of the blood-brain barrier p. 13

Embryonic development at exposure to low-frequency and radio-frequency electromagnetic fields p. 18

News from Science p. 21

INTERN
Members Assembly p. 26

INTERNATIONAL
Presenting a new COST-Action: COST 281 p. 28

NEWS p. 32

IMPRINT p. 32

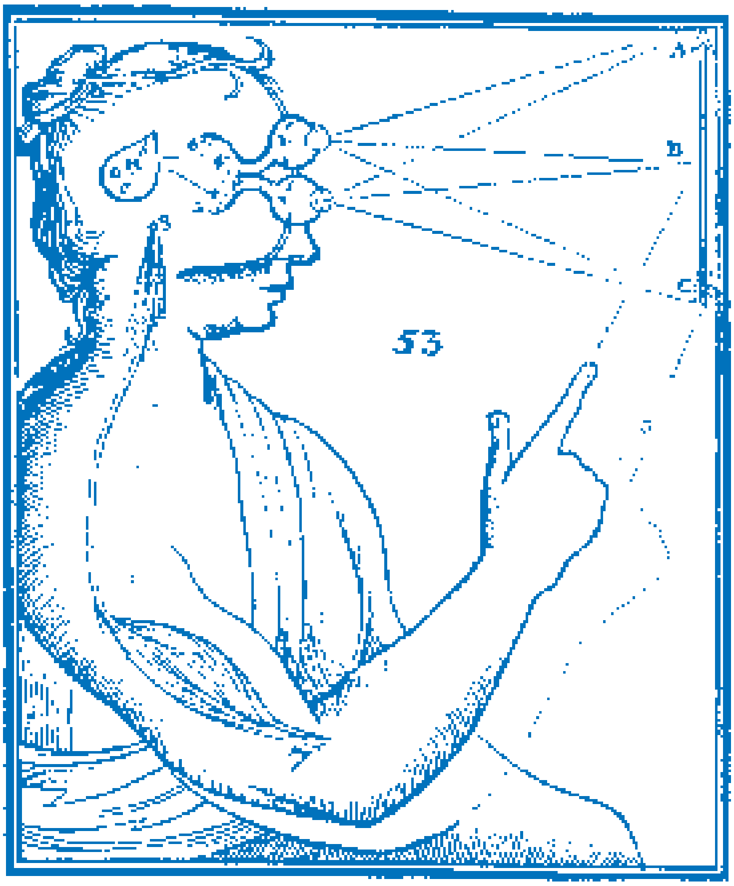

Fig. 2: The pineal gland, as seen and depicted by René Descartes (1596-1650), is where the "rational soul" is located, thus linking soul and body. In this illustration, we can recognise the cone shape of the pineal gland as well as the link between the "chiasma opticum" (optical nerve crossing) and the hormonal gland – an amazing anticipation of up-to-date knowledge.

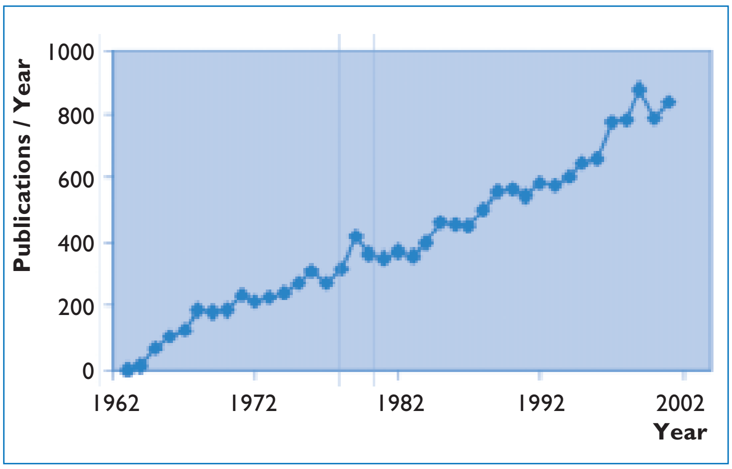

Fig. 3: Increase in the number of publications with the keyword "melatonin" or "pineal" between 1963 and 2001. Data from database MedLine.

The present paper will give a survey of melatonin synthesis, of the effects of this hormone and of non-photoperiodic quantities which can inhibit melatonin secretion. In particular, we will focus on possible effects of weak electric, magnetic and electromagnetic fields. However, due to the continually-increasing amount of information on this topic, this survey obviously makes no claim to be exhaustive. For example, the database MedLine alone lists 16,000 original studies. On the other hand, the main part of the information presented in the following is imperative to a better understanding of the current discussion about the "melatonin hypothesis" as a possible theoretical explanation of available epidemiological data.

Historical survey

In the early fifties, Aaron B. Lerner took an interest in the substance potentially causing the effects of cattle pineal glands on tadpoles (brightening), which had already been observed in 1917. In cooperation with Y. Takahashi, from 1955 on a so-called bioassay for melatonin determination based on the quantification of frog skin brightening was developed. In 1956, J.D. Case joined the group. The final deadline was one week away when Lerner suddenly found out that the searched-for substance was a methoxy-derivative of serotonin (the name "melatonin" goes back to its effects on melanophores and to the fact that it is a serotonin derivative). Very quickly melatonin was synthesized and its hypothesized chemical structure was confirmed (review in [2]).

After elucidating the enzymatic cascade leading to melatonin development, Hoffman and Reiter in 1965 proved that the decrease in gonadal weights of hamsters induced by short photoperiods (duration of daily light exposure) is completely blocked off by removal of the pineal gland [3]. Finally, Wurtman and Axelrod presented two hypotheses of considerable importance for pineal research. They characterised the pineal gland as a "neuroendocrine transducer," that is, an organ with neuronal input and endocrine (hormonal) output. Secondly, melatonin was seen as a hormone mediating photoperiod effects via the blood.

Since these pioneering studies were presented much knowledge has been gained about the effects of melatonin as a hormone, which will be dealt with in what follows. An unexpected development began in the eighties and in the early nineties when melatonin was shown to be an "old invention" of evolution not only occurring in animals, but also in plants and single-cell organisms, being a potent scavenger of damaging oxygen-based free radicals.

The growing knowledge about this substance is also reflected in the steadily increasing number of publications (Fig. 3).

The rhythms of melatonin synthesis

Melatonin synthesis is suppressed by light. Thus, at night high levels of melatonin synthesis are reached, whereas during the day hardly any measurable values are found. This difference is responsible for the diurnal physiological parameters being controlled by melatonin.

However, as the duration of daily light exposure depends on the season (except in the equatorial region), the time period over which melatonin synthesis occurs changes accordingly. This general dependence is substantial for seasonal variations of physiological processes [4-10]. In humans, a dependence of the melatonin synthesis on the season is observed also [11].

Light in the dark

Under natural conditions there will hardly ever be significant light exposure during a normal night. The reactions to an artificial light pulse or to prolonged light exposure during the night result in the suppression or inhibition of melatonin synthesis. However, the light intensities required for this are hugely different for different species. So, rodents active mainly at night are most sensitive in this respect (about 1 lux [12; 13]). In humans, considerably higher light strengths are required (depending on the study, 200 up to about 2000 lux; survey in [14]).

A study in humans may serve as an example of the effects of nocturnal light exposure (see Fig. 4). Different light intensities cause pronounced suppression, but with different variations, of melatonin synthesis.

A particular feature of melatonin synthesis suppression was observed in Djungarian hamsters: When exposed to a 1-min light pulse during the night, the animals responded by a collapse of melatonin synthesis already observed in other studies. However, if animals are exposed to the same light pulse but melatonin values are examined only during the following night, there is an almost identical curve of measured levels: The collapse of melatonin synthesis occurred at the same time [16].

From this result follows the significant insight that the melatonin generating system obviously has a "memory," a fact being of importance for further discussion, since effects caused by artificial light exposure could also possibly have long-term consequences.

Hormonal relevance of melatonin

As shown above, as the "hormone of darkness" melatonin is the endocrine correlated with the photoperiod. This signal is used by many animals for adjusting physiological and other endocrine systems to daytime and/or to season. In other words, by interpretation of the photoperiod or the melatonin signal, the synchronisation of reproduction with the "appropriate" season of the year is realised.

Pro- and anti-gonadotrophic effects: In analysing photoperiodic effects we have to distinguish between two types of seasonal reproduction: in the first case, the

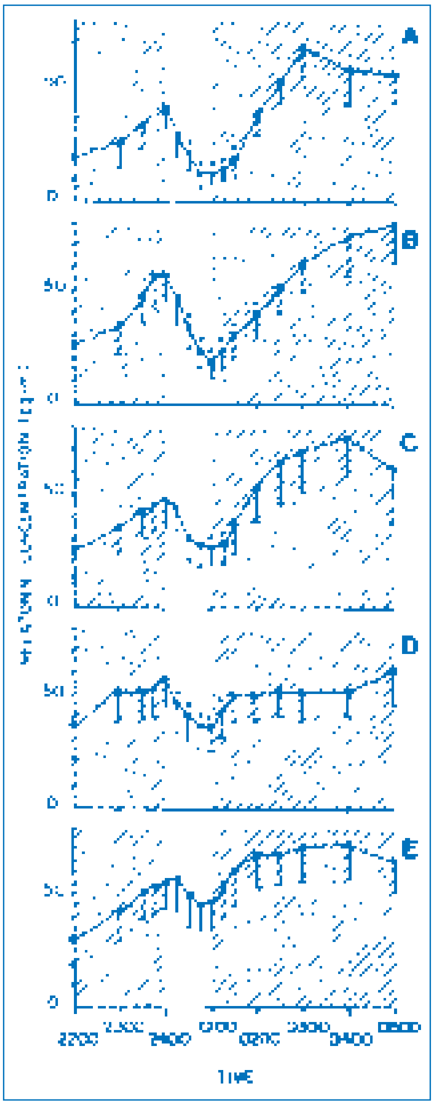

Fig. 4: Suppression of melatonin synthesis in humans caused by light exposures of different strengths during the night. The clear areas stand for the times that the light sources are switched on. Tests were separated by 2 weeks each. A: 3000 lux; B: 1000 lux; C: 500 lux; D: 350 lux; E: 200 lux. Already at 350 lux there is a significant decrease of melatonin development. See [15].

animals respond to photoperiod shortening by an increased readiness to mate (so-called "short-day breeder"), whereas in case two a prolongation leads to the same effect ("long-day breeder")[2].

Nevertheless, both these diametral effects are caused by melatonin synthesis alterations. A survey can be found in [17]. The target site of melatonin probably is a structure in hypophysis, the *pars tuberalis*.

Melatonin and hormone levels in adults: There is some evidence for melatonin potentially affecting the development or functions of other hormones in humans. To these belong steroids (estrogens, testosterone, progesterone), prolactin, gonadotropins (LH and FSH), and growth hormone (GH) (following different authors [18-36]). In particular, there seems to be a phase relationship between melatonin and the synthesis of prolactin and growth hormone. But at present, we do not know to what extent this relationship is endocrinologically relevant.

Physiological relevance of melatonin

Temperature regulation: The role melatonin has in thermoregulation has been known for quite some time [37-40]. Also in humans, melatonin has a distinct impact on body temperature. This is true for natural conditions under which melatonin synthesis is inversely correlated to temperature changes, as well as when melatonin is supplied exogenously. Thereby, a significant, reproducible decrease of body temperature is obtained [41-45].

Diurnal rhythms: "Diurnal" rhythms mean cyclic alterations showing 24-hour periodicity under conditions of a fixed periodic timer, for example the sleep-wake rhythm during the normal 24-hour day. Generally known are the experiments of Aschoff and Weber during which volunteers spent longer time periods in bunkers shielded off from all external timers (for a survey see [46; 47]). Under such test conditions, most people develop a circadian rhythm of about 25 hours. Here, not only activity rhythms but also body temperature and melatonin secretion rhythms show a circadian periodicity [48].

The essential role of melatonin in developing diurnal rhythms can be seen particularly well by the example of blind humans. When blindness is complete, that is, not even light-dark changes can be distinguished, these people develop a free-run rhythm which considerably impairs their well-being because their own rhythm in regular intervals is different in phase to the external conditions. In practice, this means that these individuals often suffer from serious sleep disorders or are often overtired when expected to be awake. Long-term experiments could prove that also melatonin rhythms of such individuals are free-running and thus may be the cause of the disorders.

The basis for this assumption is that experiments have shown that melatonin is able to resynchronise freely-running rhythms of rats ([49-51]). This finding can be explained by melatonin being capable to affect the rhythm of its own synthesis: As mentioned above, the synthesis of the hormone normally is controlled by external light. Without a timer such as this, circadian rhythms develop. Spontaneous rhythms are caused by the activity of the nucleus suprachiasmaticus (SCN), an accumulation of certain nerve cells where also the biological clock is located. These nerve cells have specific binding sites for melatonin and thus may be affected by it concerning their rhythm. In recent years, melatonin often has been used to soften the consequences of time differences during flights across several time zones ("jet-lag") [44; 52-59]. It is hypothesized that by taking melatonin the biological clock in the nucleus suprachiasmaticus is readjusted thus being more quickly adapted to the new "correct" time [60; 61].

Melatonin as an oncostatic agent

For quite some time the pineal gland *in vivo* is known to have an inhibiting effect on the growth of cancer tumours, that is, an oncostatic effect (reviews in [62; 63]). Apart from the frequently-examined breast cancer and melanoma, this concerns a number of other cancer types too, among them colon cancer [64], lung cancer [63] and leukemia [65]. For a long time the cause for these findings was unknown. However, now it is hypothesized that the properties of melatonin as a potent scavenger of free radicals are crucial (see below). Peptides stemming from the pineal gland are also discussed [66] having a role in this.

These effects do not only occur *in vivo*, but also when cancer cells are treated *in vitro* with melatonin [67]. The effects on cancer growth with up to 80% growth inhibition are very clear.

Interestingly, here often it has been found that melatonin has an effect only within a concentration window of about the same amount as the concentration of the hormone in the blood during the night (about 5 x 10^{-10} M). Further, there is evidence for a continuous presence of melatonin being less effective than one imitating physiological variations [68]. At present, we still do not know to which extent the hormone plays a part in the struggle against cancerous diseases.

Melatonin as a scavenger of free radicals

An unexpected insight was that melatonin is a natural potent scavenger of oxygen-based free radicals [69; 70]. Radicals are molecules, made highly reactive by unpaired electrons and binding easily to other molecules, such as the highly-damaging hydroxyl radical (HO). Thereby, genetic alterations can occur, in turn being the cause of malign tissue growth.

Melatonin is capable of destroying these radicals. The concentrations of the hormone required for this are considerably smaller than those of known antioxidants, such as, for example, gluthation. Apart from artificially-induced effects of relatively high doses of melatonin, it was able to be shown that physiological melatonin concentrations also are capable of considerably decreasing the damaging effects of certain HO-promoting substances [69; 71-74]. For the antioxidative effects of melatonin it is of considerable importance that the hormone as an extremely lipophile substance smoothly passes the blood-brain barrier and accumulates in nervous-system cells. In summary, it is hypothesized that melatonin is a substance especially important for the protection of the nervous system [75].

Of particular interest is that melatonin also is produced in single-cell organisms as was initially reported in 1991 [76]. However, in the dino-flagellates *gonyaulax polyedra* examined, not only melatonin was found, but also a distinct diurnal rhythm with high levels was identified during the night. From the findings quoted, it is concluded that melatonin was possibly "invented" very early in evolution. It seems possible that the antioxidative properties of the molecule were then a crucial selection advantage.

Effect of fields on the pineal gland

Magnetic fields: The first report on the effects of weak magnetic fields was presented by Semm and colleagues [77]. Neurophysiological tests in pineal glands had shown that activity (fire rate) decreased when an artificial static magnetic field was switched on. The flux density applied was in the order of that of the geomagnetic field (about 35 µTesla). It has been claimed that this effect could be part of the biological direction compass, for the existence of which there is quite some evidence; however no biophysical explanation as yet exists. As the pineal gland is of relevance for the temporal organisation of an organism, the effects of magnetic fields could indicate the existence of a spatial/temporal orientation.

Unfortunately, the exposure conditions here, as in many other experiments, were badly characterised and insufficiently described. In particular, we can not rule out the possibility that it was not the magnetic field as such, but rather induction currents caused by fast magnetic field alterations, that are responsible for the observed effects. Stimulated by the study of Semm, many experiments were performed dealing with the effects of weak magnetic fields on the function of the pineal gland, particularly concerning melatonin synthesis [78-92]. The main results were that decreases of melatonin synthesis and diminished activity of N-acetyltransferase were found.

A study of Khoory [93] demonstrated that a local compensation of the geomagnetic field had no effect on melatonin synthesis whatsoever. This finding is of crucial interest in so far as a biological "pineal compass system" should register such alterations, too, and should respond accordingly; or that not the magnetic field as such, but its temporal alterations, would be responsible for the published findings.

This assumption was confirmed by studies on mice and rats under exposure to quickly-alternating magnetic fields [94; 95]. Only quick changes led to a decrease of melatonin synthesis whereas slow alterations had no effect. Reviews of the topic may be found in [96-99].

More recent studies have dealt with the possibility of magnetic fields having a direct impact on melatonin synthesis in isolated pineal glands. To this end, pineal glands of Djungarian hamsters were brought into a special chamber flushed with buffer solution (ill. 5).

To date, presented results suggest that weak magnetic fields (16 2/3 or 50 Hz, 86 µTesla, respectively) have an inhibiting impact on melatonin synthesis [100]. However, there were significant results only when the individual results (4 experiments each per frequency) were taken together. This outcome is of relevance with respect to the general role of the variations among experiments, which is still under discussion.

The results on the effects of low-frequency magnetic fields are generally heterogeneous: tests on magnetic fields (mostly 60 Hz) at the work place ("occupational exposure") showed decreased melatonin values [101-104] as well as no effects at all [105]. Investigations by Pflüger [106] showed that the secretion of 6-hydroxymelatonin-sulphate in engine drivers of electric locomotives was significantly decreased as compared to controls. The American scientific community has taken great interest in studies on possible melatonin effects caused by electric blankets. Most of the studies performed on this topic showed no negative effects (see [107; 108]). A recently-published study of many test subjects on the potential impact of domestic exposure ("residential exposure") provided no indication of any decrease of melatonin synthesis [109].

Electromagn

Tests under well-defined laboratory conditions in the low-frequency range also failed to show consistency, since both decreased and unchanged melatonin values were found [110-120].

Electric fields: The potential effects of magnetic fields on the pineal gland have been considered in scientific studies for some time now [98; 121-133]. As for the case of magnetic fields, there is a lack of convincing explanations of the observed effects of exposure to electric fields. In this context, we must point to the many organisms showing considerable sensitivity towards electric fields in the order of about 10^{-7} Vm^{-1}, mainly aimed to locate other living organisms (search for mating partners and/or prey). Insofar a biological effect of much higher field strengths is generally not surprising. However, this insight of course does not suffice as a

Fig. 5: Test chambers designed for direct exposure of pineal glands to weak magnetic fields. In each chamber pineal glands of Djungarian hamsters are flushed with a buffer (perifusion). Then, in the eluate, melatonin is determined. To produce melatonin, pharmacological stimulation of the glands is required. Taken from [100].

basis for risk assessments.

Electromagnetic fields: At present, for electromagnetic fields[3] there are relatively few published studies available on their effects on melatonin synthesis. This may be mainly for technical reasons since a well-defined and controlled exposure to radio-frequency fields is not only very demanding with respect to the apparatus used, but above all regarding the level of theoretical electrical engineering necessary. Field distributions and related absorbed energy values can be determined only by means of sophisticated calculations, which almost rules out investigations autonomously performed by one laboratory by itself. Here, interdisciplinary cooperations are absolutely necessary. However, the studies published to the present have not provided any convincing indication of a suppression of melatonin synthesis [134-137].

It is clear that in view of the uncertainties and fears in the population on one hand, and the available epidemiological data on the other (for example [138]), in-depth research into this topic is urgently needed.

The melatonin hypothesis

The term "melatonin hypothesis" goes back to a study of Stevens [139], in which it was suggested that weak magnetic fields could lead to a decrease of melatonin synthesis which again resulted in increased estrogen production in the ovaries or of prolactin in the pituitary gland. This in turn leads to a heightened cell division rate of certain cells in the breast tissue and to altered responses of these cells to carcinogenesis. Ultimately, these processes lead to an increased risk of developing breast cancer (survey in [140]).

In the meantime, this theory has been refined and revised. It was able to be shown that not only estrogen-dependent tumours are affected by magnetic fields [surveys in [127; 140-145]). A book published in 1997 comprehensively deals with the topic [146]. Other surveys can be found in [147; 148].

Epidemiological data: A series of studies dealt with the possible health effects of artificial fields (for example [99; 138; 139; 144; 145; 149-154]). In most cases low-frequency fields (50 and 60 Hz, respectively) were considered.

In his useful survey, Erren sums up the available data on breast cancer risk concluding that there is overall a slightly increased risk, which is of statistical significance, for women and for men [155].

A long-standing difficulty of retrospective epidemiological studies is that the actual exposure to electromagnetic fields can be estimated only indirectly, for example by means of comparative measurements, by examining cable arrangement in the house or in the environment ("wiring code"), or by considering the participants' occupational status. Further, sociological (for example income, housing conditions) and medically-relevant factors (for example smoking, alcohol consumption) often are related to electromagnetic exposure ("confounding factors") and it is difficult to clearly separate their effects. Of use could be prospective studies with ongoing actual readings of exposure measurements. Appropriate devices permitting measurement of actual exposure, at least in the low-frequency range, are already on the market.

Melatonin as an explanation?

The melatonin hypothesis can be tested by experiment. In recent years the group of Löscher investigated whether magnetic fields (50 Hz, 1 – 100 µTesla) led to DMBA (7.12-dimethylbenz(a)-anthracene)-induced breast cancer in rats occurring more often or spreading more quickly [140; 142; 156-160]. In this model, female rats over time were treated with a dose of DMBA which gave rise to tumours in the breast gland tissue in about 50% of the animals. Additionally, the animals were exposed or sham-exposed. During exposure palpation was performed; after 3 months the animals were sacrificed. An exact pathological examination followed. As the animals were killed during the night, melatonin could be determined in the pineal glands and in the serum.

The results obtained to the present show that the suppression of melatonin synthesis is dependent on the flux density, with a nearly linear relation between flux density and increased tumour occurrence [161]. These findings are seen as the first real evidence that weak magnetic fields in an appropriate animal model can increase the occurrence of induced breast cancer (promotion) [140; 157; 162; 163]. In the United States, these studies were repeated involving considerable effort, however obtaining different results [164]. The differences may have been caused by the tested animals (different breeding line), by different nutrition and different cancer development rates, etc. More recent studies show that replication tests on the effects on melatonin in general lead to overall heterogeneous results [100; 162; 165].

Summary and outlook

The intention of this short survey has been to show that the hormone melatonin plays a part in a number of physiological functions and possibly can prevent damage caused by free radicals. Secondly, it has been repeatedly demonstrated that magnetic or electric fields, respectively, can suppress melatonin synthesis. Finally, a series of epidemiological data has provided evidence for the assumption that exposure to magnetic, electric and electromagnetic fields can cause damage to health, although absolute effects may be interpreted differently.

The melatonin hypothesis supports a cause-and-effect relationship among these three themes. Though this theory to date remains unproven, it is obviously sufficiently well-thought-out to serve as a basis for further research projects.

One of the biggest problems regarding this issue is the lack of any biological ex-

Long-te

with a GSM-
of DMBA-induced

planation of weak field effects to this date. The amounts of energy transmitted are on thermodynamic grounds far too small to cause any significant alteration of the already-present thermal noise (E µ kT). Because of this, nonlinear systems increasingly are considered for finding possible explanations [166-167].

The energies emitted by mobile phones are partially absorbed (up to 50%, sometimes even more) by the surrounding biological tissue. Here, the head and the hand holding the device act as unintentional absorbers. Transmitted powers can be sufficient to cause a measurable effect (heating) of the tissue. However, this heating is slight and highly dependent on antenna type and direction as well as on the nature of the construction of the mobile phone. Nowadays, devices are on the market leading to only a small amount radiation onto the head.

From the situation depicted above it could well be imagined that the function of the pineal gland, which is located at the centre of the brain, could be impaired by electromagnetic fields emanating from mobile phones. At least such an association may be hypothesized and will probably lead to further fears on the part of mobile communication users. Therefore, preventive research is necessary in order to discover or to rule out any possible effects of radio-frequency fields on melatonin synthesis. This would enable the use of this relatively new, highly-attractive technology unimpeded by unfounded fears, and operators could react to warnings about real dangers early on.

Professor Dr. rer. nat. Alexander Lerchl,
International University Bremen,
School of Engineering and Science

The complete study containing an extensive reference index will be published soon by the Research Association for Radio Applications in "Edition Wissenschaft," issue 16/2002.

[1]The pineal gland of vertebrates, also called *epiphysis cerebri*, ontogenetically stems from the diencephalon (therefore *cerebri*) and anatomically is located above this part of the brain (*epiphysis*). Its shape is reminiscent of a pine cone (hence *pinealis*).

[2]Neither of these English-language terms has a generally-accepted equivalent in German.

[3]Electromagnetic fields are radio-frequency fields where the magnetic and electric components can no longer be regarded as separate (about >30 kHz).

Introduction

To the end of year 2000, there were more than 200 million mobile phone users worldwide. The resulting increase in emission of radio-frequency electromagnetic fields (EMF) has led to concerns about possible hazards to human health could exist as a result. In particular, a publication of Repacholi et al. (1) led to misgivings about the safety of modern telecommunication. The authors found an enhanced development of experimental lymphomas in transgenic mice exposed to GSM-like radiofrequency fields (900 MHz, pulse rate 217 Hz). In contrast, other studies found little or no evidence that the weak electromagnetic fields from mobile telecommunication lead to any cancer-initiating or -promoting effect (review by Moulder et al. (2)). Since mobile communication is nevertheless a relatively new technology, even a low-probability cancer-promotion effect could affect thousands of people. Therefore, the WHO has initiated the International EMF Project within which further experimental and epidemiological studies are in progress. Included in such studies are investigations using animals with tumours chemically induced in the laboratory. The current paper reports on such studies.

Materials and methods

- *Animals and initiation of cancer:*

The experiments were approved by the Animal Care Commission of the Regional