

# Does the new Salford study deserve all the attention

**Frank Gollnick,  
Helmut Franke,  
Sheila Johnston**

**Leif Salford and his working group in Lund (Sweden) have recently published an alarming new study, which has gotten a lot of attention, in which the effects of mobile radio emissions on the development of “dark neurons” (DN) in conjunction with the occurrence of microscopic leaks allowing protein to enter the brain are described. Two reputable scientists closely examined the publication, which has only been published online and offered their opinions of it to the FGF. The following article is the joint opinion of Dr. Sheila Johnston (neuro-science expert, in London) and Dr. Helmut Franke (Clinic and Policlinic for Neurology, Münster)**

In the publication “Nerve cell damage in the mammalian brain after exposure to microwaves from GSM mobile phones”, Salford et al., 2003, the reported nerve cell damage was brought about by the effects of mobile radio fields of unknown frequencies (only the GSM-standard was given) at a field strength of 0.24-2.4 W/m<sup>2</sup>, corresponding to 2-200 mW/kg.

The publication is based on a single experiment, which has not been done again or reproduced. For such an experiment a very small test sample was used; 32 rats (altogether there were 4 groups, 8 in each group). Not earlier than 50 days after being exposed to a mobile radio field brain slides were first prepared with two different stains. The following serves as proof for Salford et al. that brain damage occurred:

- firstly, the staining of protein which had permeated the blood brain barrier in an unwanted (pathological) way; staining was aided by a protein specific antibody. Normally the blood brain barrier in a healthy state does not allow harmful substances and proteins to cross over to the brain into actual brain tissue, see Stögbauer, 2002.
- secondly, with a second staining an additional pathological finding was examined: namely, the development of the mentioned “dark neurons” DN. In the literature they are described as proof of diverse damage to nerve cells, caused by various kinds of mechanical effects and harmful metabolic processes (Vohra et al. 2002). A direct quote taken from the arti-

# study it is getting in the media?

cle in question reads as follows; “DNs develop under so many various conditions that the reason why they develop will remain a mystery”.

Generally, DN to be more exact are described as “argyrophile” neurons, whereas the medical term “argyrophilie” merely means the dark tissue staining brought about by silver coating with a ammoniacal silver nitrate solution followed by a reduction caused by formol, tannin etc. (Roche Lexikon Medizin, Ver.3.5). Physicians usually use this staining procedure for particular proof of something e.g. in special tissue regions or in special cells. The staining method used by Salford et al. differed from the one described above. On one side the genetic makeup of the cells (DNA, RNA) is stained and on the other side another stain colour (cresyl violet) is used. On the other hand, this is a normal method of distinguishing damaged cells, (damaged, i.e. cell coverings pitted with holes) from undamaged cells. However, the results in this case are also called dark neurons.

Gallyas et al. (1992) described numerous causes for the development of DN. Moreover, in his publication the appearance of DN is independent of their cause. Even after death, severe shaking of a brain that has not yet been dissected could be responsible for the appearance of DN. These are however not distinguishable from DN which previously occurred when the animal was alive.

Basically various other causes should be considered for the development of DN,

however the development of DN from mobile radio fields has to be considered since it cannot be completely ruled out. With the exception of a concussion occurring after death, other causes were not taken into account and discussed nor were any corresponding control experiments conducted by Salford and his colleagues.

It is hard to understand why the rats, after undergoing 2 hours of field exposure, were first examined 50 days later. It is especially true in view of the fact that Vohra et al. (2002) described the development of DN as an aging phenomena that to date has not been explained. So without any outside influences rats that are already 6 months old show almost double the number of DN when compared to 3-month old rats. The rats at the beginning of the Salford et al. experiments had exactly this age difference.

Moreover, for a publication with high academic standards, among other things, the specific absorption rate was not adequately standardized. The variation in SAR-distribution was given at 6 dB, (SAR = “specific absorption rate”). This indicated a four-fold range of variation. It is amazing that a publication that receives international recognition in the field of mobile radio research only stated that GSM-field irradiation was applied but did not even once mention the frequency used in the investigation. Apart from that a great deal of the usual information for such a study was lacking.

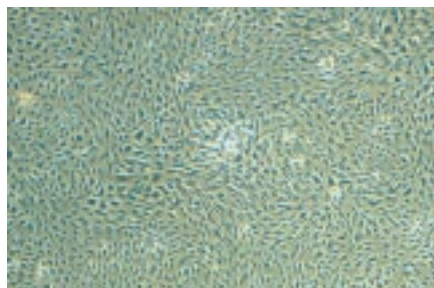
## Statistics

In order to be able to make a conclusive statement with such a small sample, “exact tests” have to be conducted. Nevertheless, this could apply to the type of statistics used, however, if this was really the case it was not mentioned. It is questionable why no numerical recording of the DN was done, but only a ranking was done. The experiment would have been more meaningful if a numerical recording had been done and not a ranking test. Finally a critical reader would ask, are pathologists’ assessments reliable with regard to assigning individual rankings. Can these assessments be reproduced by other pathologists?

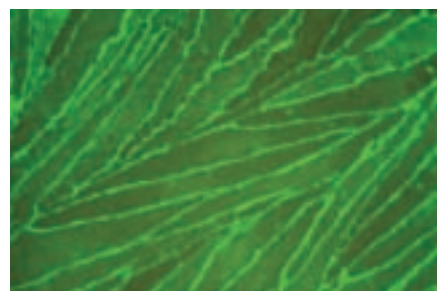
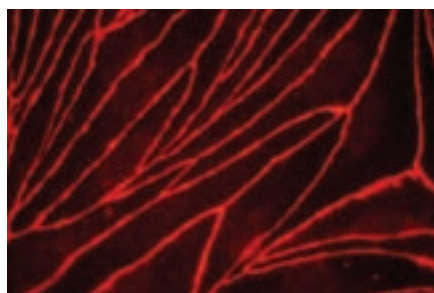
## Results and the Discussion Section of the Publication

Remarks and comments made on the results in the discussion section of the article are very superficial. Salford did completely without a comprehensible numerical description of the exhibited transport of protein out of the vascular system (see above). A clearer correlation between the transport of protein and the development of DN was not demonstrated.

In the rather short presentation of the results concerning protein staining, Salford et al. pointed out that the staining in the control group, displayed more often a dubious positive reaction (a reaction that really should not occur in a control group). Consequently a reliable assignment of the three ranks that he de-



A microscopic image of endothelial cells (see picture page 25) in a cell culture dish, the spirally formed shape of the cells which are tightly packed together is typical for the blood brain barrier.



With the help of staining one can see in a culture dish how the special protein has been made the tight seals of the endothelial cells visible.

terminated for assessing protein staining were obviously not given.

(“Quote: the control rats exhibited no positive reaction or only infrequent reactions and often they exhibited a questionable positive reaction to protein in the hypothalamus”).

In one of the pictures in Salford’s publication, two images of brain slides taken from an irradiated rat are compared to a slide taken from a rat in the control group. To which group the rat was assigned to was later indicated in a corrected version of the paper. By looking at the external form of the brain slides one can determine that they were not taken from the same area of the brain, a comparison is, therefore, only partially valid. Another image shows DN staining exclusively of rats which were exposed to mobile radio signals. Negative control images, in other words the images taken from the brains of rats that were not irradiated were completely lacking in the study. The field strengths assigned to these images were also not given. In a scientific publication this is a matter of course that this information is given.

Moreover, what is very unusual for a scientific publication is that Salford et al., in the discussion of their results, did not cite any other references to substantiate their findings. Another point to consider concerns other similar studies conducted by the authors, where their results could not be reproduced. This was also not discussed or analysed in this publication. The hypothesis concerning a secondary opening in the blood-brain-barrier, which is supposedly caused by the observed flow of protein was mentioned in the discussion but this was not dealt with in detail nor was the reason for it discussed and therefore its existence is rather questionable. Ascertaining that 12-26 week old rats

in their development stage are especially suitable for a comparison to teenagers addicted to mobile phones, is really a very daring statement, especially when there is no data to support it. Further statements regarding the special susceptibility of adolescents in this part of the discussion can only be considered as assumptions. For a scientific publication the facts and statements made in the discussion part of the paper seem to be rather sketchy.

### Inadequate Dosimetry and questionable significance of DN

Sheila Johnston especially emphasizes in her opinion on Salford’s publication that little attention was given to dosimetry and she questions the medical significance of the discovered “dark nerve cells”.

Regarding the given SAR-values, they obviously deal with *estimated* total body mean values, where the SAR-distribution in the brain was determined by a computer simulation. For this kind of study this does not fulfil the criteria of the World Health Organization (WHO), because the SAR-values for the brain are not measured but are only indirectly calculated. Moreover, Johnston noticed since 1998 in a whole series of Salford’s investigations on the blood brain barrier that it is not possible to draw a conclusion from the actual SAR-values he employed because of defective dosimetry. Since these SAR-values were derived from the electrical field (E-field) that existed in the exposure chamber that was employed (a so-called TEM cell) these E-fields *cannot* correspond to the E-fields in the brains of the rats, it can be deduced that the SAR-evaluations are false.

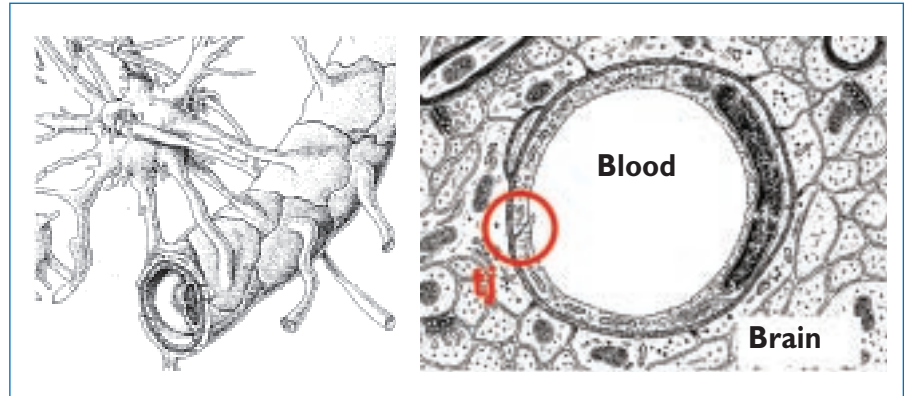
Sheila Johnston has disputed the rather far-reaching interpretations in Salford’s et al. discussion with regard to the possible

negative effects DN could have on human memory during the course of the aging process with the following arguments:

Neuro-degenerative illnesses, (e.g. illnesses with memory loss) occur when the brain's main switching circuits are destroyed, which is caused by nerve cell death and the loss of synapses. This degeneration is selective, i.e. there are nerve cells that are more or less susceptible to such processes. The occurring symptoms of such an illness are dependent on the particular switching circuits which are detrimentally affected and hence give a picture of the selective vulnerability of the nerve cells. In the normal aging process, a reduction in the number of brain cells, caused by the dying of cells, is not a crucial process, at least not in the area of the brain that Salford et al. referred to. With regard to the counting of nerve cells, it is generally very difficult to make a functional association, because the regional differences and the dissimilarities among the cells of the cerebral cortex are enormous. Therefore, the studies which give the most insight into the aging process are those where a certain area of the brain is investigated which is correlated with bodily functions that are well understood (Morrison & Hof, 1997).

However, the "dark nerve cells" (DN) that Salford et al. reported on were randomly distributed everywhere in the rats' brains and therefore it is *not* possible to make an association with particular switching circuits. Hence it is going too far, to try and derive some kind of neurological significance from the results or to interpret them as being indications of possible symptoms of an illness.

Currently many investigations are being conducted on the possible effects to the blood brain barrier from electromagnetic fields by internationally recognized research groups in France, Japan, Germa-



*The blood brain barrier forms a special wall covering within the microscopic blood vessels (capillaries, illustration on the left) in the brain. Larger molecules (e.g. protein) and toxins have no excess to the nerve cells in the brain. In the cross-section illustration of a capillary (on the right) one can see how the endothelial cells are tightly layered and packed together, they cover the inside of the capillaries and are sealed together by the so-called tight junctions (tj). All around them on the outside are the star shaped astrocytes and of course the nerve cells can also be seen.*

ny, and in the U.S.A. The first results are expected to be published this year and one can hope that more solid results will come to light than what was discussed here. Obviously Salford's et al. work is more of a speculative nature than anything else.

*Dr. Frank Gollnick is a biologist and worked for a long time at the Physiological Institute II at the University of Bonn: he is currently working as a scientific expert at the FGF.*

*Dr. Helmut Franke is a biologist and is currently working at the Clinic and Polyclinic for Neurology at the University of Münster. He is a specialist on the blood brain barrier and actively does research in this field.*

*Dr. Sheila Johnston works as an independent expert and consultant, among other things, in the field of neuroscience, she also works for a number of committees and organizations. She is internationally recognized and has her own information service.*

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