Homeopathy (2008) 97, 3–9

doi:10.1016/j.homp.2007.11.002, available online at http://www.sciencedirect.com

ORIGINAL PAPER

The effect of homeopathically prepared thyroxine on highland frogs: influence of electromagnetic fields

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***Background*: Previous experiments show that amphibian larvae are responsive to homeopathically prepared thyroxine.**
***Methods*: We studied the effect of a highly diluted and agitated thyroxine solution exposed to various electromagnetic fields on metamorphosis in highland *Rana temporaria*. The devices tested were: microwave oven, mobile phone, airport X-ray, and a red light barcode scanner. Animals were treated either with homeopathically prepared thyroxine (10^{-30} parts by weight, 10^{-35} in the water in which the animals were kept), or analogously prepared blank solution, or analogously prepared thyroxine exposed to the electromagnetic field of one of the devices tested. Solutions were administered at 48 h intervals according to a standardized protocol.**
***Results*: Animals treated with the standard test solution thyroxine 10^{-30} metamorphosed more slowly than the control animals, ie the effect of the homeopathically prepared thyroxine was opposed to the usual physiological effect of molecular thyroxine. The cumulative number of test animals that had reached the four-legged stage at defined points in time was smaller in the group treated with homeopathically prepared thyroxine at most of the points in time. This was found independently by all three research teams involved.**
In contrast, this effect did not occur when the thyroxine solution had been exposed to the field of the early model microwave oven, or mobile phone. There was no difference between aqueous or alcoholic solutions were used, and there was, if any, only a small protective effect from aluminum foil. Airport X-ray and red light barcode scanning did not diminish the effect of the homeopathic solution. *Homeopathy* (2008) **97**, 3–9.

Keywords: amphibian; metamorphosis; thyroxine; homeopathic solution; homeopathic solution; inverse effect; curative effect; electromagnetic fields

Introduction

The amphibian model appears to be a useful tool for explaining certain phenomena encountered in homeopathy and homeopathy research.[1–4]

One of the bases of homeopathy, namely *the principle of similars*, can be demonstrated by first hyperstimulating *Rana temporaria* by immersion in an aqueous molecular thyroxine solution (10^{-8} parts by weight, not submitted to an agitation process) and then inducing an inverse 'curative' effect by a homeopathically prepared solution (10^{-13}) of the same hormone.[5] There appears to be a relationship between the effect of homeopathically prepared thyroxine and a naturally or artificially elevated thyroxine level in the animals

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Received 13 December 2006; revised 14 November 2007; accepted 14 November 2007

during metamorphosis. This is in some respects analogous to intoxication/detoxication studies, where organisms are first treated with a high dose of a toxin and then with a sequentially diluted and agitated solution of the same toxin.[6,7] Significant antidotal effects have been observed, for example with highly diluted Cd on Cd intoxicated frog spawn.[8] Artificial acceleration of metamorphosis can also be achieved by raising temperature of the water in which the animals live. This might be considered a 'curative' effect in the light of the homeopathic principle of similiars.

Researchers based at the Zoological Institute of Graz University, the Department of Molecular Cell Biology of Utrecht University, the Boltzmann Institute in Graz, the Zoological Institute of Vienna University and the Federal Institute for Veterinary Medical Investigation Graz have all found that ultramolecular thyroxine solution (10^{-30}) led to a significant inhibition of rate of metamorphosis.[9,10] The intervals between time points vary, but were *equal* within any one experiment. Figure 1 shows pooled data from these five laboratories (Figure 1) ($N = 1620$ per group). Differences between groups are statistically significant with $P<0.01$ (Chi-square) at most of the measurement points. Significance was confirmed with logistic regression and multiple hazards model.[10]

Another characteristic, namely that of a *drug proving effect*, is illustrated by the finding that very frequent application of the solution, causes first a slowing and subsequently an acceleration of metamorphosis. Here, the effect of the solution is at first inverse to ('curative phase') and later concurrent with that of molecular hyperstimulation ('drug proving').[4]

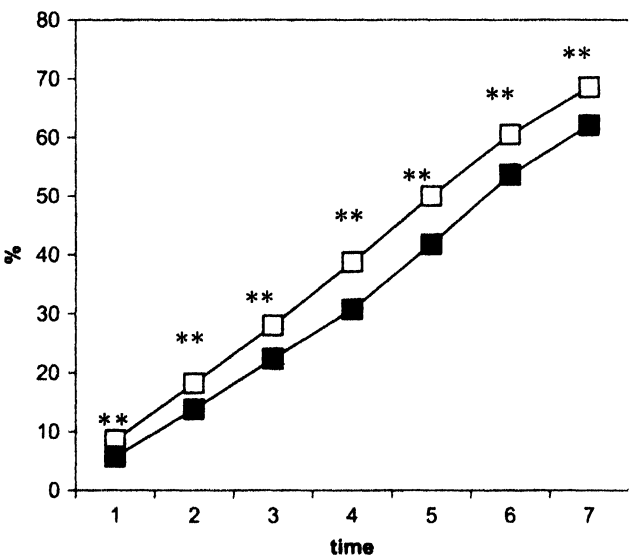


Figure 1 The influence of highly diluted thyroxine *vs* analogously prepared water, on highland *Rana temporaria*. Results from five researchers in five laboratories, pooled data from.[2,10] Ordinate—cumulative frequency of 4-legged tadpoles (*N*); abscissa—points in time; black squares—cumulative frequencies of animals treated with homeopathically prepared thyroxine; white squares—animals treated with analogously prepared water; and **—$P<0.01$. For further details, see text.

This 'frog's perspective' on homeopathy can be useful in demonstrating and illustrating certain phenomena of homeopathy from the biologist's point of view, but it does not cover the whole field of homeopathy in humans.

One characteristic of homeopathy research has been the difficulty of *inter researcher reproducibility* of experiments. For 17 years, we have been engaged in finding, describing and varying the parameters that are crucial for the amphibian experiment.[11] Initially, we trained independent colleagues at our own laboratory. Our basic amphibian experiment (with highland tadpoles) produces broadly consistent results independent of experimenter. Recently, an independent group has successfully performed a similar experiment in Brazil.[12] Nevertheless, we have also done 'research on the researcher'. This line of investigation can be illuminating when people with 'green thumbs' or 'healing capacities' produce seemingly inexplicable results.

Information storage in the carrier substance is a crucial point in research on and practice of homeopathy. In the study presented here, we investigated the usability of the amphibian model to test the effect of various electromagnetic fields on a homeopathically prepared thyroxine solution. Starting with an early model microwave oven (the electromagnetic fields of which exceed those generated by modern microwave ovens), fields more commonly found in our technical environment were used, namely those of a mobile phone, an X-ray luggage inspection device at an airport and a red light barcode scanner (660 nm).

Our hypothesis was that the amphibian model could be useful to test such influences. We assumed that the early microwave oven might be destructive to the homeopathically prepared solutions, with regard to the other devices, no hypotheses were formulated.

For control purposes and in continuation of our main line of research we also included repetitions of the basic experiment, with no electromagnetic field.

Materials and methods

Researchers and blinding

The experiments were carried out independently by Scherer, Suanjak and the Weber/Welles team in the laboratory of the Interuniversity College. All experiments, including application of test and control substances 10^{-30} as well as scoring of the stage of the animals, were performed blind. An external observer who came to the laboratory, the veterinarian M. Wurm, was responsible for the blinding procedures. The same blinding method was used in each case. Substances used for treatment (see below) were prepared in sets each consisting of the test solutions and the control solution. All substances were prepared in glass vials identifiable by the plaintext designation on the pull-off label. All solutions were left in their

glass vials to avoid any extraneous influences through decanting. The plaintext labels were then removed by the person responsible for blinding and replaced with labels bearing encoded designations. The code was not made known until after the presentation of the results. For reasons of laboratory convenience (danger of cross-contamination) we did not use more than one vial per substance. The project was organized by Endler.

Animals, staging, water and further laboratory conditions

Rana temporaria tadpoles were taken from highland pools in the Austrian Alps at ca. 1500 m above sea level. The starting stage was defined as the point at which the hindlegs of the 2-legged tadpoles are straddled such that one can only just see through the triangle formed by thigh, shank, and tail. This point of development occurs during Gosner's stage 31.[13] The tadpoles were observed until the forelegs, which are preformed under the skin, broke through and the animals had thus entered the 4-legged stage. In previous experiments with Scherer, different authorities from the Zoology Institute of Graz University as well as from the Environmental Agency of the County of Styria carried out parallel counts to assess the counting method for reliability and obtained identical results. For the same purpose, one researcher had documented some of her counting results photographically. The animals were kept in Basins containing 8 l of well water each.

Twenty animals were allotted to white plastic basins according to a random procedure. This was done in the same way by each of the teams: 20–30 basins were used by each team. One by one, animals were fished out of the main tub and distributed over the basins so that there was one in each. This was repeated twenty times. The purpose of this procedure was to ensure that the animals were distributed homogenously in terms of their level of activity and swimming behaviour in the main tub. The basins were arranged in rows of 3–5 according to the number of substances used in the experiment. The spatial arrangement of treatment groups within rows rotated from row to the next, ie basins with identical treatment groups were arranged in diagonals, and was left unchanged throughout the experiment to avoid the danger of cross-contamination through splashing. Indirect natural light was used. Room temperature was 21 ± 1 °C. The tadpoles were fed with blanched greens (lettuce) ad libitum. Experiments were carried out in the laboratory of the Interuniversity College in September and October 2000–2005. Further details are given in.[14–18]

Data set and experimental design

A total of 2980 animals was used: 149 basins each containing 20 animals. These formed a total of 21 groups according to treatment.

Six different experiments were performed (rows in Figure 2). In each experiment, one group (100–200 animals) was treated with control substance and one group (100–200 animals) with standard test solution (left column in Figure 2). Thus, 860 animals were treated with control solution and 860 with test solution. This part of the study can be understood as a modified repetition of the studies described above[1,2,9,10] (see Figure 1): a large scale experiment to demonstrate the effect of a homeopathically prepared ultramolecular dilution of thyroxine. But these six experiments had the additional purpose of investigating the influence of a certain type of electromagnetic fields on the test solution. This part of the study is pilot research on environmental factors that could influence the homeopathically prepared high solution of thyroxine. Due to the relatively small number of animals in each of the different groups, results are not definitive, but allow planning of further study protocols.

Preparation and administration of hyperstimulation and test solutions; exposure to electromagnetic fields and shielding

First, all animals were exposed to the stock solution of tetra-iodo-thyronine sodium pentahydrate (T_4, Sigma, 10^{-4} parts by weight in double distilled water, diluted in the basin water to a final concentration of 10^{-8}) (immersion in thyroxine 10^{-8}, hyperstimulation). In previous experiments with highland *Rana temporaria*, this treatment accelerated metamorphosis by around 5%. Depending on the experiment, one group was then treated with the homeopathically prepared standard test solution (thyroxine 10^{-30}, 'TD30'), another with the analogously prepared solvent water (solvent 10^{-30}, 'WD30') and, depending on the protocol, one or two groups were treated with test solution that had been exposed to an electromagnetic field.

In the preparation of the test solution thyroxine 10^{-30} the stock solution (10^{-4}) was diluted with pure double distilled water in 26 steps of 1:10, and agitated after each step of solution according to standardized instructions. Using disposable pipettes, 1 ml of the precedent solution was added to 9 ml of water in a 20 ml glass vial. Agitation was performed by hand, with an amplitude of approximately 20 cm: the vial was banged 30 times against a rubber impediment at intervals of approximately 0.5 s to create mechanical shocks. The solution thyroxine 10^{-30} ('TD30') is nominally 0-molar. For control, double distilled water was prepared analogously ('WD30').

Microwave oven, aqueous solutions: Measurements of electromagnetic fields in the immediate vicinity of the microwave oven (an early model) showed 50 Hz magnetic and electric fields of 110 μT and 5 V/m, respectively, and radiofrequency electromagnetic fields with an intensity of 2.45 GHz at 10 mW/m^2. The

measured levels of 50 Hz magnetic flux density, generated by a transformer coil, and radiofrequency power density exceeded those generated by modern microwave ovens. The microwave oven was used for irradiating two samples of thyroxine solution TD30; the phials were placed immediately next to the microwave oven and irradiated for 100 s. One of these samples was left unprotected, the other was wrapped in two layers of commercial aluminum foil. A third sample of TD30 and the water control WD30 were in a separate room and not exposed to the microwave oven.

Microwave oven, ethanol solutions: For this experiment, thyroxine samples and the water control were prepared with 42% v/v ethanol instead of water. One sample was exposed in the same manner as described above, one sample was not exposed. There was no sample wrapped in aluminum foil involved.

Mobile phone, 0 cm: Two samples of TD30 were exposed to a commercial mobile phone with a near field equivalent, measured over a 100 m^2 sensor area, of 300 nW/m^2 in standby and 339 nW m^2 during call set-up. This was done by placing the phials immediately next to the mobile phone and then performing five successive call set-ups with intermediate return to standby. One sample was left unprotected while the phial containing the other was wrapped in aluminum foil.[14,15]

Mobile phone, 25 cm: One sample of TD30 was exposed to the mobile phone in the same manner as described above, the only difference being that it was placed at a distance of 25 cm from the mobile phone.[15,16]

X-ray: One sample of TD30 was exposed to an X-ray luggage inspection device at an airport during routine operation. The device had a nominal dose equivalent of 0.7 μSv (0.07 mrem or 0.7 mGy). Phials were passed four times through the device at a conveyor speed of 0.2 m/s.[17]

Red light scan: One sample of TD30, bearing a paper label with a barcode, was exposed once to a red light barcode scanner (660 nm).[18]

An estimate of how much of the applied electromagnetic energy possibly was converted into thermal energy in the solutions leads to the conclusion that rise of temperature can be neglected with regard to room temperatures that have not influenced to test solution's activity in previous experiments.

Probe solution of 3 μl (WD30 or TD30) was added per animal and per 300 ml of basin water at intervals of 48 h.

Comparison and evaluation of data

The cumulative frequency of animals treated with control or test substances having reached the 4-legged stage was aggregated for each day. Seven points in time were considered in each case to allow comparison between experiments. The interval between two successive points in time were equal within one experiment, but varied from 1 to 3 days depending on the overall duration of the experiment.[11,15,17,18]

Chi-square tests were used to compare groups. Different statistical methods had been discussed in connection with the amphibian model previously, including variance analysis, *t*-test, survival analysis, proportional hazards model, logistic regression.[1,5,10] These usually give comparable results but (1) need larger numbers of basins in one experiment. Furthermore (2), depending on differences in the overall duration of experiments, S.D. is usually variable when experiments from different laboratories are pooled. Interestingly (3), hyperstimulation (immersion in thyroxine 10^{-8}), as used in the experiments described here, leads to an increase of S.D. from about ± 1.0 to ± 1.5 for control groups to slightly higher values for the hyperstimulated groups.[5]

In order to obtain results comparable to those of previous publications, we restricted ourselves to the Chi-square test. Being aware of the problem of dependent data, however, we calculated *P*-values for each of the seven time points used to describe every experiment.

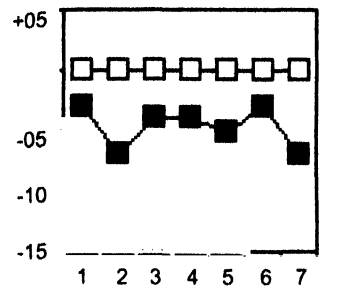
Within each experiment, control solution was compared with standard test as well as with test solutions treated with the different electromagnetic fields. Results from standard test solution and control solution were pooled over all experiments; results from the test solutions treated with the different electromagnetic fields were not pooled over all experiments.

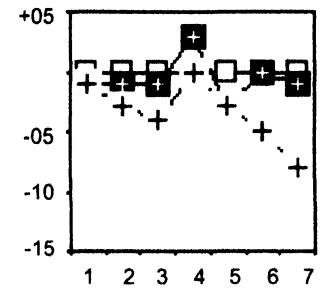
The absolute cumulative frequencies of four-legged animals have been published previously.[11,15,17,18] In order to give an overview and to allow comparison between these findings, the present article deals only with frequency ratios. These have been depicted by normalizing the values of the control group to give a horizontal line in each chart which is arbitrarily defined as the 50% level (see Figure 2). Each chart shows the difference between the % value of the control group and that of the relevant test group at seven points in time.

Results

860 animals were treated with homeopathically prepared thyroxine 10^{-30} (standard test solution) and 860 animals were treated with standard control solution. In addition, further groups of animals (ie a total of 1160 animals) were treated with additional solutions according to the different protocols.

Figure 2 The influence of highly diluted homeopathically prepared thyroxine, exposed to different electromagnetic fields. White squares—values of 4-legged control animals, normalized; abscissa—differences of percentage of 4-legged animals; TD30—agitated thyroxine 10^{-30}; WD30—analogously prepared water; *—$P < 0.05$; and **—$P < 0.01$. For further details, see legend to Figure 1 and text.

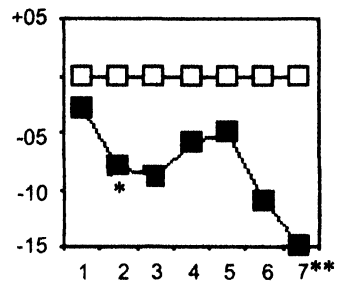


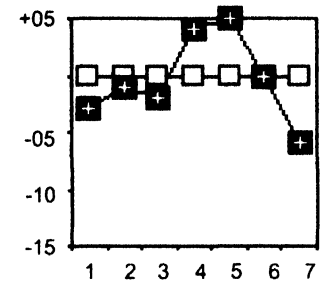


Scherer 2000 — **microwave, aqueous solutions**

left:
white = WD30 (N = 100)
black = T D30 (N = 100)

right:
white squares = WD30 (N, see left)
white crosses = TD30 (microwave) (N = 100)
black crosses = WD30 (microwave + protection) (N = 100)

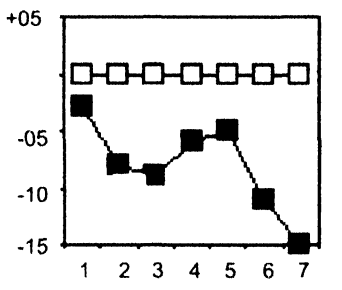


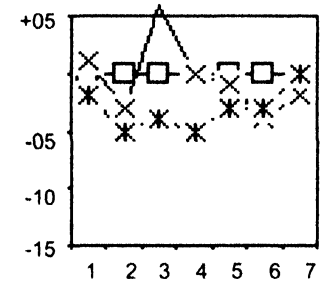


Scherer 2000 — **mircowave, ethanol solutions**

left:
white = WD30 (N = 100)
black = T D30 (N = 100)

right:
white squares = WD30 (N, see left)
white crosses = TD30 (microwave) (N = 100)

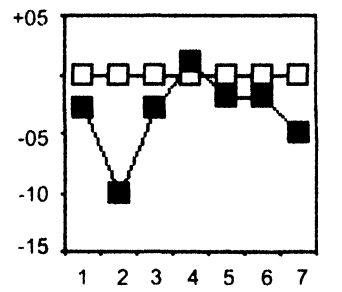


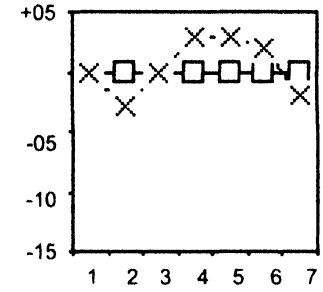


Suanjak 2004 — **mobile phone, 0 cm**

left:
white = WD30 (N = 100)
black = T D30 (N = 100)

right:
white squares = WD30 (N, see left)
X = TD30 (mobile) (N = 100)
X! = TD30 (mobile + protection) N = 100)

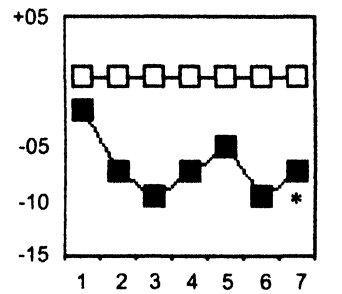


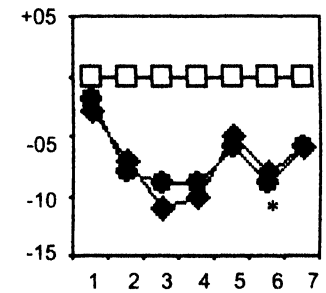


Weber/Welles 2005 — **mobile phone, 25 cm**

left:
white = WD30 (N = 160)
black = T D30 (N = 160)

right:
white squares = WD30 (N, see left)
X = TD30 (mobile) (N = 160)

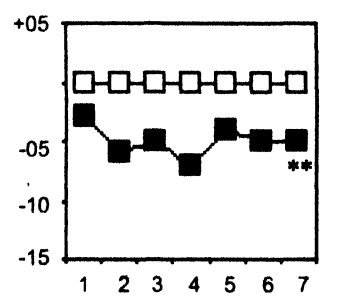


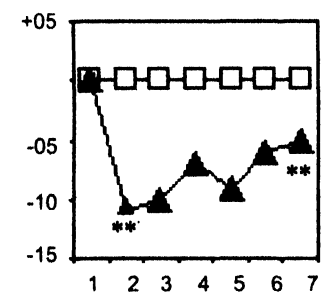


Scherer 2003 — **X-ray**

left:
white = WD30 (N = 200)
black = T D30 (N = 200)

right:
white squares = WD30 (N, see left)
black rhombs = TD30 (X-ray) (N = 100)
black stars = TD30 (X-ray + protection) (N = 200)





Scherer 2002 — **red light scan**

left:
white = WD30 (N = 200)
black = T D30 (N = 200)

right:
white squares = WD30 (N, see left)
black triangles = TD30 (scan) (N = 200)

Control solution versus standard test solution: As can be seen in Figure 2, left row, animals treated with the test solution (black squares) metamorphosed more slowly (5–10%) than control animals (white squares). The number of test animals that reached the four-legged stage at defined points in time was smaller in the group treated with homeopathically prepared thyroxine at most of the points in time. This trend was found independently by all researchers in all six experiments. When experiments are considered separately, this trend is statistically significant only at a few points in time (see Figure 2). When data from all six experiments are pooled, differences are statistically significant at some but not all (3 out of 7) points in time: $P(2)<0.05$, $P(3)<0.05$, $P(7)<0.01$. In other words, the effect of the homeopathically prepared thyroxine was opposed to the usual effect of molecular thyroxine. This result has been discussed in more detail in.[11]

Control solution versus test solution exposed to an electromagnetic field: In contrast (see Figure 2, right row), this effect did not occur when the thyroxine solution had been exposed to the fields of the early model microwave oven, or the mobile phone. There was no difference whether aqueous or alcoholic solutions were used, and there was, if any, only a small protective effect from aluminum foil. Practically no trends or statistical significances were found. On the other hand, airport X-raying and red light barcode scanning did not diminish the effect of the homeopathic solution. For result of statistic analysis of separate experiments, see Figure 2. Data of test substances exposed to the different electromagnetic fields were not pooled.

Discussion

These data confirm that a special preparation process of stepwise solution and agitation (final concentration in the basin water 10^{-35} parts by weight) can lead to an inversion of the well-known stimulatory effect of thyroxine on amphibian metamorphosis.

Furthermore, these data suggest that the effect exerted by the thyroxine solution in the basic experiment is blocked when it is exposed to the field of an early model microwave oven, or to that of a commercial mobile phone. In the experiment where an aqueous and an alcoholic solution were compared, there was no difference with regard to their stability towards the fields of the microwave oven. There was, if any, only a small protective effect from the metal foil which some of the samples were wrapped in. On the other hand, in these pilot experiments airport X-ray and red light barcode scanning did not diminish the effect of the homeopathic solution. Before these experiments have been subject to further in depth studies, it seems too early to attempt an explanation of why some of the used fields do not, whereas the fields generated by other devices do affect the informational content of a homeopathic solution.

The preliminary finding of a destructive influence of certain electromagnetic fields on homeopathic solutions is in agreement with the assumptions of many manufacturers and therapists as well as with the instructions they give to patients on how to store homeopathic preparations. Our findings suggest that great care is warranted in handling homeopathic preparations.

Further experiments with the amphibian model suggest that globules provide a more robust means of information storage. This notion finds support in established knowledge among homeopathy users. One must consider that most homeopathic remedies on the market today are sold in the form of globules rather than of solutions. Experiments with globules are ongoing.

Biophysical theories have evolved which support the possibility of such findings. Physics research has revealed that water dipoles may develop phase coherent oscillations through radiation coupling.[19] It was proposed that these could be modulated as a time-ordered pattern of signals. We believe that the theoretical explanation of homeopathy – similarly to the explanation of a wide range of other phenomena in physiology, psychology and epistemology – could be broadened by the application of de Broglie's concept of the wave nature of particles and the particle nature of waves.[20] Biophysical questions have been addressed with the help of the amphibian model.[21]

A possible methodological refinement for further studies would be to use unsuccussed solvent as an additional control, apart from the succussed solvent used in the experiments presented here. Furthermore, blank solutions exposed to the electromagnetic fields should serve as additional controls.

Summing up a side finding presented in the results section of this paper and discussed in,[11] further experiments on highland amphibian should be performed without pre-treatment by molecular thyroxine as, in contrast to our previous assumption derived from lowland amphibian,[5,22] such pre-treatment does not seem to enhance the effect of the homeopathically prepared thyroxine solution 10^{-30} in highland animals. It may be hypothesized, however, that the increase in water temperature from the highland biotope (eg 15 °C) to laboratory room temperature (21 ± 1 °C) is an important stimulus that makes highland amphibian sensitive to homeopathically prepared thyroxine. This remains to be further investigated.

With differences of 5–10% between groups, results with the amphibian model are statistically significant only when an adequate number of animals are included in the study, ie observed effects are modest with regard to patient's expectations.

A comprehensive overview on basic questions and problems and the state of the art in research on homeopathy has been given in.[4]

Acknowledgements

We thank Peithner Inc.,Vienna, for their expertise and help in electronic and technical issues. Thanks are due to the editor of *Homeopathy* for his suggestion that our team should compile main findings and publications on the amphibian model since 1991 when the model was first presented in that journal.

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