Effects of Biophoton treatment, on isolated rat cortical neurons, through the Biophoton device according to J. Boswinkel

Thesis

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presented by

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Graz, October 2010

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I hereby confirm that the contribution to the presented thesis is by myself according to the rules.

Graz, October 2010

Thesis accepted

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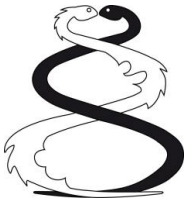
TABLE OF CONTENTS

SUMMARY



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Effects of Biophoton treatment, on isolated rat cortical neurons, through the Biophoton device according to J. Boswinkel

Author: Dietrich Vastenburg
Supervisors: Chris Endler, Mario Merlini

Introduction

This introduction has the purpose to inform in general about the background of the related research that is based on the concepts of Biophotons and its applied technology, and the research findings behind it.

Biophotons are weak emissions of light radiated from the cells of all living things. A photon is a single particle of light. Plants, animals and humans have an intensity of their emission from some hundreds up to one thousand photons/second/cm², and an almost continuous spectrum within the optical range of at least 200 - 800 nm [1]. All organisms, including plants, constantly produce photons as part of their vital activities. The light of the photon is too faint to be seen by the naked eye.
Biophotons create a dynamic, coherent web of light. A system that could be responsible for chemical reactions within the cells, cellular communication throughout the organism, and the overall regulation of the biological system, including embryonic development into a predetermined form. According to Popp in a live interview, to be seen on the internet [2], a chemical reaction in a cell can only happen if the molecule which is reacting, is excited by a photon. So the photon is necessary to stimulate a molecule to a chemical reaction. So every living cell is producing light.

Research Question

The main objective of this study was to see if an effect on isolated rat cortical neurons could be observed upon treatment with through the Biophoton device. To this aim the following questions were asked:

- Will the isolated neurons stay alive longer and/or will they show any differences in growth upon treatment by the Biophoton device, as compared to the non treated control group.
- Will neuronal growth be significantly affected by treatment through the Biophoton device, and if so, what will be the difference when compared with the same untreated / unexposured neurons?

Methods

Design

The research was performed in a completely sterile area under standard culture conditions (temperature (37° ± 0,1°; 7,5% CO2) on isolated neurons from rats (embryonic cortical neurons).
To be able to understand the eventual influences of treatment through the Biophoton device on neuronal viability, neurons were divided into seperate groups: a control group, an untreated part in a separate Petri-dish, and different Petri-dishes treated with several different Biophoton programmes (each Petri-dish contains about 60.000 cells).

Participants

Dietrich Vastenburg
Mario Merlini (University of Zürich, Switzerland)

Materials

Biophoton device according to J. Boswinkel
Rat Cortical neurons → standard dissection protocol *
Coverslips in Petri dishes
Medium → standard neuronal culture medium *
Microscope → Bright field microscope with attached camera (Leica DMI RE2, Heerbrugg, Switzerland)
Incubator → (37°C, 7,5% CO2, 100% rel. humidity)
All treatments were performed in a sterile laminar flow hood

* Almeida A and Medina JM (1998) A rapid method for the isolation of metabolically active mitochondria from rat neurons and astrocytes in primary culture. Brain Res Prot 2(3):209-14

Performance of the Study

The study was performed in the laboratory of the University of Zürich, August Forel-Strasse 1, Zürich.

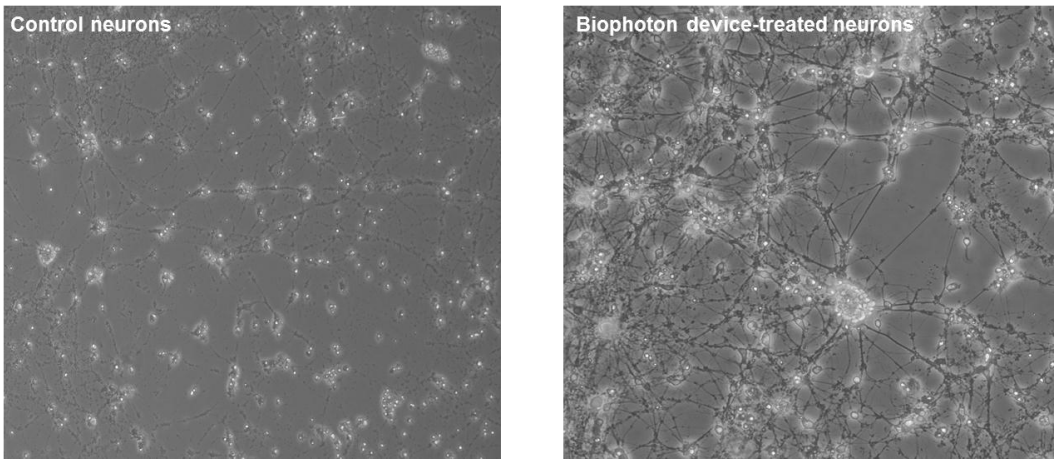
Statistical Analysis

Results were analysed using the imaging software ImageJ. Statistics were approached using Student's t-test and SEM (standard error of the mean = standard deviation (SD) divided by sample size. In our case sample size is the number of repetitions (n) of the respective treatments (n=3). Statistical analysis using SD showed a comparable degree of significance as compared to using SEM).

Results

Survey

In answer to the lead research question – if neuronal growth and survival would be affected by Biophoton device treatment – there was found a significant increase in neuronal growth and synaptic interactions between neurons.



Bright field microscope pictures, magnification = 100 x

Discussion & Conclusions

Interpretation of Results

The key question in this study was if treatment with the Biophoton device would have any effect on neurons in culture.
We found that there was a significant increase in possible neuronal interactions as was shown by an increase in the number of synaptic contacts between treated neurons as compared to the untreated control neurons (see above pictures). The fact that Biophoton device treatment showed such a strong impact on the neurons could be due to the fact that neurons are highly communicating cells.

Self-Critical Remarks

One of the limitations of this study was the fact that the neuronal cultures used were so-called single cell cultures, that is, no presence of other cells which are normally present in the brain. This can influence the effects of Biophoton device treatments. For future studies it is therefore of importance to grow neurons in co-culture with these other cells.

Suggestions for Further Research

What happens on the protein level, more specific, the expression of proteins involved in neuronal survival & synapse formation.

References

[1] *Bischof M.; Das Licht in unseren Zellen 2001*

[2] *Popp F.A., Life interview for Science and Spirituality television station on YouTube, 2008, especially last 8 minutes http://www.youtube.com/watch?v=zcmaMV1TETU*

1. INTRODUCTION

Biophotons are light emissions from biological systems with intensities in the order of a few hundreds of photon / cm^2 surface area, and an almost continuous spectrum within the optical range of at least 200-800nm [1]. Therefore, it is believed that chemical reactions such as oxidation are the source of energy for biophoton emissions in living bodies. Accordingly, all organisms, including plants, constantly produce biophotons as part of their vital activities.
It has been reported that photon emissions vary according to differences in growth processes [2], and are elevated by environmental stresses [3] and disease response included by pathogen attack [4].

This research was performed to obtain the Academic Master Degree of Complementary and Integrated Health Sciences of the Interuniversity College for Health and Development, based in Graz, Austria. This thesis is established and written to report the authors research, including background information and state of knowledge on the associated subjects concerning our research; The Effects of Biophoton treatment, on isolated rat cortical neurons, through the Biophoton device according to J. Boswinkel

1.1 Background and State of Knowledge

This introduction has the purpose to inform in general about the background of the related research that is based on the concepts of Biophotons and its applied technology, and the research findings behind it.
One of the reasons to give some more explanation to this study field is that the focal point of Biophotons is not very common yet within the scientific community. The two groups of students, merely from the Netherlands, have decided together with the staff of the Interuniversity College, to explore this field more as the Biophoton theory in practice was also the study background of the members of these groups.
In the final stage during the research period the students had either one of the two focuses to deal with: Fundamental Research or Clinical Research. And as already mentioned both groups work with the fundamentals of Biophotons. The different subjects of the theses are related to the agricultural aspects (seedlings, tomatoes, milk), animals (neurons) and human beings (clinical).

What Biophotons are and how they work will be explained in the following paragraphs, starting with photons, then the Biophotons and finally some information on the 'Biophoton Therapy Device J. Boswinkel' used.

1.1.1 Photons

In physics, a photon is an elementary particle, the quantum of the electromagnetic interaction and the basic unit of light and all other forms of electromagnetic radiation. It is also the carrier for the electromagnetic force. The effects of this force are easily observable at both the microscopic and macroscopic level, because the photon has no rest mass; this allows for interactions at long distances. Like all elementary particles, photons are governed by quantum mechanics and will exhibit wave particle duality— they exhibit properties of both waves and particles. For example, a single photon

may be refracted by a lens or exhibit wave interference with itself, but also act as a particle giving a definite result when quantitative momentum is measured.

The modern concept of the photon was gradually developed by Albert Einstein to explain experimental observations that did not fit the classical wave model of light. In particular, the photon model accounted for the frequency dependence of the energy of light, and explained the ability of matter and radiation to be in thermal balance [5].

The photon concept has led to momentous advances in experimental and theoretical physics, such as lasers, quantum field theory, and the possible interpretation of quantum mechanics. It has been applied to photochemistry, high- resolution microscopy and measurements of molecular distances. Recently, photons have been studied as elements of quantum computers and for sophisticated applications in optical communication such as quantum cryptography [6].

1.1.2 Biophotons

Biophotons are weak emissions of light radiated from the cells of all living things. A photon is a single particle of light. Plants, animals and humans have an intensity of their emission from some hundreds up to one thousand photons/second/cm², and an almost continuous spectrum within the optical range of at least 200 - 800 nm [7]. All organisms, including plants, constantly produce photons as part of their vital activities. The light of the photon is too faint to be seen by the naked eye. The weakness of its light can be compared to candlelight seen at a distance of 20 km. Photons have been detected and verified without doubt by using a photomultiplier [8]. As they appear from living cells, we call them Biophotons. The study field of Biophotons: Biophotonics, is part of Life Sciences, according to the International Institute of Biophysics, Neuss, Germany.

1.1.3 History and Research

Around 1923 the Russian scientist Professor Alexander Gurwitsch discovered an "ultra weak" photon emission from living systems (onions and yeast), he suggested connections between photon emission and cell division rate. He called this photon emission "mitogenetic radiation" [9]. His experiments indicated the wavelength in the range of around 260 nm [10]. After initial world-wide recognition in the 1920s and 1930s, some claims appeared that the “mitogenetic radiation” did not exist at all. Because of that and the subsequent political cataclysms in Europe and Russia, work on this phenomenon dropped almost to zero level. However in the 1950’s a group of Italian physicists with L. Colli made a very sensitive photomultiplier with which they discovered in the spectrum range from green to red light emitted from seedlings, corn and beans with photons in quantity of 10 to a 100 per second per cm² [7].
It was in 1974 that the German biophysicist Prof. Dr. Fritz-Albert Popp proved the existence of the photons. At that time he was looking for an understanding about the optical properties of the molecule Benzpyrene in relation to carcinogenicity. With Gurwitsch at hand with the mitogenetic radiation research, Popp concluded that if the assumed optical effect of Benzpyrene were correct, then there must be some kind of light source in the cell, and very weak photon ‘signals’ would be able to trigger drastic changes in the behaviour of cells.

With Popp's photomultiplier, it was possible to prove that low-level light emissions are a common property of all living cells. It has different intensities for human, plant or animal cells, for different cell types, and it can vary from one moment to the next. It is not regular, but comes often as "photon explosion" (spikes), especially when the cells are irritated by outside means and in the case of cell death [9].
The results of Popp's research also indicate that Biophotons originate from a coherent (or/and squeezed) photon field within the living organism, its function being intra and intercellular regulation and communication [11]. The Russian scientist A.B. Burlakov repeated the experiments of Gurwitsch in the 1990's and proved that there is Biophoton exchange and influence between fertilized fish eggs that were in optical contact divided by quartz glass filters [12].

1.1.4 Biophotons and DNA

According to Prof. Popp, the leading researcher of Biophotons in the last 35 years, light is constantly being absorbed and remitted by DNA molecules within each cell's nucleus. The DNA-string has the optimal length for receiving and sending electromagnetic frequencies with its information. Beside this, these Biophotons create a dynamic, coherent web of light. A system that could be responsible for chemical reactions within the cells, cellular communication throughout the organism, and the overall regulation of the biological system, including embryonic development into a predetermined form. According to Popp in a live interview, to be seen on the internet [13], a chemical reaction in a cell can only happen if the molecule which is reacting, is excited by a photon. So the photon is necessary to stimulate a molecule to a chemical reaction. So every living cell is producing light.

1.1.5 Coherence

By photosynthesis, where the photons are used to get energy, the coherence is extremely high. Coherence means that the photons can be super positioned, so that the message which is submitted by the photons, gets very clear [11].
The laser-like coherence of the Biophoton field is a significant attribute, making it a prime candidate for exchanging information in a highly functional, efficient and cooperative fashion, lending credence to the idea that it may be the intelligence factor behind biological processes. It is a known fact that the speed of light is faster than any chemical reaction.
Biophoton emissions will vary according to the functional state of the organism. If a disease such as cancer affects certain cells they will radiate a different photonic signature than healthy cells of the same type. In this way Biophotons can be a non-invasive tool for assessing the state of health or vitality. Applications can extend far into other areas like testing food and water quality, checking for chemical or electromagnetic contamination, or agricultural testing for products that improve crop resistance to disease. Biophysicists in many European and Asian countries are currently engaged in such research [14].
Popp's Biophoton theories and concepts provide an intriguing and promising path for more international research, which could lead to major developments in our understanding of life, the mechanisms of healing and health for all living creatures, and the interconnection with the world around us.

1.1.6 'Biophoton Therapy Device J. Boswinkel'

The research linked with these theses uses the 'Biophoton Therapy Device J. Boswinkel' as a medium to transfer information. According to the manufacturer this device operates on the following principles:

- Each cell emits Biophotons and they provoke up to 100.000 chemical reactions per second
- Every living cell emits its own characteristic light pattern
- When a cell is healthy, it emits coherent light, and when a cell is diseased it emits chaotic light
- Every biochemical reaction is preceded by an electromagnetic signal, the Biophoton, that 'steers' the chemistry of the cell with certain information
- When the steering signal within the cell is inadequate, then the biochemistry doesn't work properly and the cell will show certain symptoms of disturbance
- The 'Biophoton Therapy Device J. Boswinkel' corrects the steering signal, which in turn corrects the biochemistry in the cells.

In the described research of these theses the Biophoton device is used to alter the condition of living organisms or cells.
The inventor of the 'Biophoton Therapy Device' is Dr. Johan Boswinkel. Since the early 1980's he has consequently been developing instruments in order to establish a therapeutic application of Biophotons. Together with Dr. R. van Wijk of the University of Utrecht who is also a member of Prof. Popp's team, he e.g. performed four experiments in order to determine the influence of his device on the quality of milk. There was a significant result, though more research work to be done.
Johan Boswinkel uses the statement: 'Light is the language of life, Biophotons hold the keys to the quality of all living beings' [15].

1.2 Neurons

Neurons are large cells with wide arborisations, have an active metabolism generating photons, contain little pigment, and have a prominent cytoskeleton consisting of hollow microtubules.

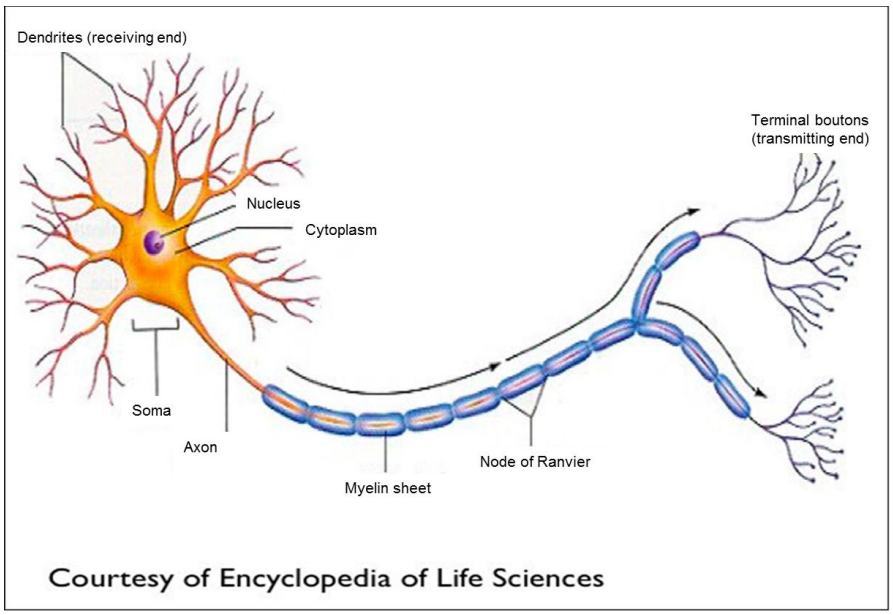


Figure 1. Structure of a typical neuron

Neurons, or nerve cells, carry out the functions of the nervous system by conducting nerve impulses. They are highly specialized and amitotic. This means that if a neuron is destroyed, it cannot be replaced because neurons do not go through mitosis. The image above illustrates the structure of a typical neuron. Each neuron has three basic parts: cell body (soma), one or more dendrites, and a single axon.

1.2.1 Cell Body

In many ways, the cell body is similar to other types of cells. It has a nucleus with at least one nucleolus and contains many of the typical cytoplasmic organelles. It lacks centrioles, however. Because centrioles function in cell division, the fact that neurons lack these organelles is consistent with the amitotic nature of the cell.

1.2.2 Dendrites

Dendrites and axons are cytoplasmic extensions, or processes, that project from the cell body. They are sometimes referred to as fibers. Dendrites are usually, but not always, short and branching, which increases their surface area to receive signals from other neurons. The number of dendrites on a neuron varies. They are called afferent processes because they transmit impulses to the neuron cell body. There is only one axon that projects from each cell body. It is usually elongated and because it carries impulses away from the cell body, it is called an efferent process.

1.2.3 Axon

An axon may have infrequent branches called axon collaterals. Axons and axon collaterals terminate in many short branches or telodendria. The distal ends of the telodendria are slightly enlarged to form

synaptic bulbs. Many axons are surrounded by a segmented, white, fatty substance called myelin or the myelin sheath. Myelinated fibers make up the white matter in the CNS, while cell bodies and unmyelinated fibers make the gray matter. The unmyelinated regions between the myelin segments are called the nodes of Ranvier.

In the peripheral nervous system, the myelin is produced by Schwann cells. The cytoplasm, nucleus, and outer cell membrane of the Schwann cell form a tight covering around the myelin and around the axon itself at the nodes of Ranvier. This covering is the neurilemma, which plays an important role in the regeneration of nerve fibers. In the CNS, oligodendrocytes produce myelin, but there is no neurilemma, which is why fibers within the CNS do not regenerate.

Functionally, neurons are classified as afferent, efferent, or interneurons (association neurons) according to the direction in which they transmit impulses relative to the central nervous system. Afferent, or sensory, neurons carry impulses from peripheral sense receptors to the CNS. They usually have long dendrites and relatively short axons. Efferent, or motor, neurons transmit impulses from the CNS to effector organs such as muscles and glands. Efferent neurons usually have short dendrites and long axons. Interneurons, or association neurons, are located entirely within the CNS in which they form the connecting link between the afferent and efferent neurons. They have short dendrites and may have either a short or long axon.

1.2.4 Neuronal Transmission (communication)

One of the main processes in the brain is neuronal transmission, the process via which neurons communicate with each other. Neuronal communication is at the basis of the brain's capacity to carry out its vital functions.
Neurons communicate with each other via release of so-called neurotransmitters, molecules that are released by one neuron and bind to the other neuron's neurotransmitter-receptors. This binding of neurotransmitters to the respective receptors of the other (receiving) neuron leads to the generation of an electric signal in the receiving neuron, the so-called action potential. It is this electric action potential that is passed on via the axon to the terminal boutons (see figure 1) and leads to release of neurotransmitters. This chemical transmission enables neurons to regulate the strength of the signals they pass on to the other neurons: a weak signal is generated by a small amount of neurotransmitter and a strong one by a larger number of neurotransmitter molecules. A summary of neurotransmitter release is depicted in figure 2.

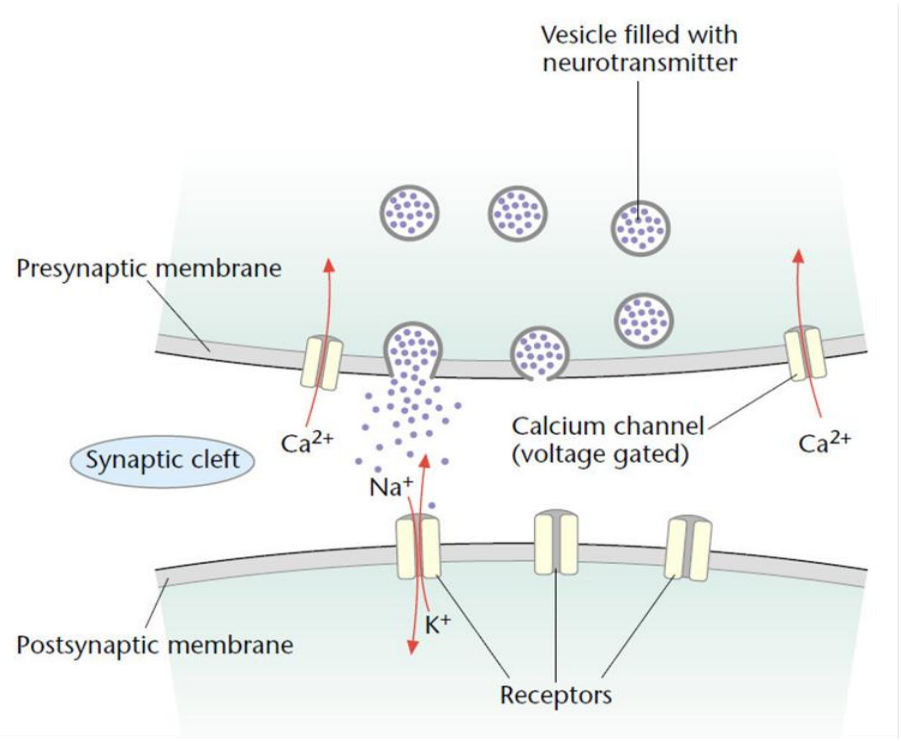


Figure 2.

Schematic representation of a chemical synapse, showing the movement of calcium ions through voltage-gated calcium channels, causing neurotransmitter-containing vesicles to bind to the presynaptic membrane and releasing molecules into the synaptic cleft. These molecules are able to diffuse across the gap and bind to receptors in the postsynaptic membrane. Ligand-gated (fast) receptors are shown in which the movement of ions takes place across the receptor itself once the neurotransmitter attaches to its binding site. It is this neurotransmission process which enables neurons to communicate with one another.

Source: SEER's Training Website.

1.3 Research Question

The main objective of this study was to understand if an effect on isolated rat cortical neurons could be observed upon treatment with biophotons through the Biophoton device. To this aim the following questions were asked:

- Will the isolated neurons stay alive longer and/or will they show any differences in growth upon biophotons treatment / exposure by the Biophoton device, as compared to the non-treated control group.
- Will neuronal growth be significantly affected by biophoton treatment/exposure through the Biophoton device, and if so, what will be the difference as compared to the same untreated / unexposured neurons?

2. METHODS

2.1 Design and execution of the study

The research was performed in a completely sterile area under standard culture conditions (temperature (37º ± 0,1º; 7,5% CO2) on isolated neurons from rats (embryonic cortical neurons). Neurons were kept in a special neurobasal culture medium on cell culture-treated glass cover slips (about 20.000cells on each slip) in small Petri-dishes. Visual analysis of neural morphology and viability was done using a bright field microscope. To be able to understand the eventual influences of Biophoton treatment / exposure by the Biophoton device on neuronal viability, neurons were divided into seperate groups: a control group, an untreated part in a separate Petri-dish, and different Petri-dishes treated with several different programs of the Biophoton device (each Petri-dish contained about 60.000 cells). The growth and viability of the differently treated neurons was measured using the image analysis software program ImageJ. The microscopy pictures represent the average of morphological status of the neurons.

The first step of the project was to find out which Biophoton device programme gives the best reaction on the isolated neurons. The standard programs of the Biophoton device are all based on human beings ranging from babies to adults. Therefore a program had to be developped that would work best for the isolated neurons. As neurons in culture are sensitive to already minor changes in their environment the first tested program was the standard 1 minute program (small degree of Biophoton device generation for human beings). New programms were developed (by trial and error) they were so called (M, N1, N2 etc…) on which there was a higher neuronal viability and a bigger increase in synaptic contacts between neurons. The differences in outcome on synaptic interactions between the different programs were significant (see barchart 1 + 2). In the M program the exposure time was increased with an "all pass" frequency. In the N1...N5 programs the exposure time was reduced and the frequencies were lowered gradualy from 1000Hz till 200Hz. The presence of synaptic contacts was even more increased upon addition of the homeopatic remedy ‘Cerebrum Compositum’ to the used programs with the Biophoton device.

The properties of the homeopatic remedy Cerebrum Compositum:
The total action of Cerebrum compositum is not only directed towards improving the whole cerebral function, including memory, but also towards the prevention or improvement of circulatory disturbances arising from arteriosclerosis with the consecutive lowering of cerebral capacity not only in the aged but also in underdeveloped children showing a poor performance at school, as well as for stress.
The improvement of the cerebral and neural functions which can be achieved by means of Cerebrum compositum is also found to be favourable in the widest variety of organic diseases of the nervous system, especially in association with other antihomotoxic therapeutical measures (Galium-Heel, etc.), as well as for migraine. (ref. Biotherapeutic Index, Ordinatio Antihomotoxica et Materia Medica by Biologische Heilmittel Heel GmbH, Germany)

2.2 Participants

Dietrich Vastenburg
Mario Merlini, PhD (University of Zurich), supervisor and laboratory assistant

2.3 Materials

Biophoton device according to J. Boswinkel
Rat Cortical neurons → standard dissection protocol *
Coverslips in Petri dishes
Medium → standard neuronal culture medium *
Microscope → Bright field microscope with attached camera (Leica DMI RE2, Heerbrugg, Switzerland)
Incubator → (37°C, 7,5% CO2, 100% rel. humidity)
All treatments were performed in a sterile laminar flow hood

* Almeida A and Medina JM (1998) A rapid method for the isolation of metabolically active mitochondria from rat neurons and astrocytes in primary culture. Brain Res Prot 2(3):209-14

2.4 Performance of the Study

The study was performed in the laboratory of the University of Zürich, August Forel-Strasse 1, Zürich.

2.5 Statistical Analysis

Results were analysed using the imaging software ImageJ. Statistics were approached using Student's t-test and SEM (standard error of the mean = standard deviation (SD) divided by sample size. In our case sample size is the number of repetitions (n) of the respective treatments (n=3). Statistical analysis using SD showed a comparable degree of significance as compared to using SEM).

3. RESULTS

The pictures below show the first reactions on the development of the neurons 5 days & 10 days after the biophoton treatment through the Biophoton device.

Study 1

Neuron biophotons study I

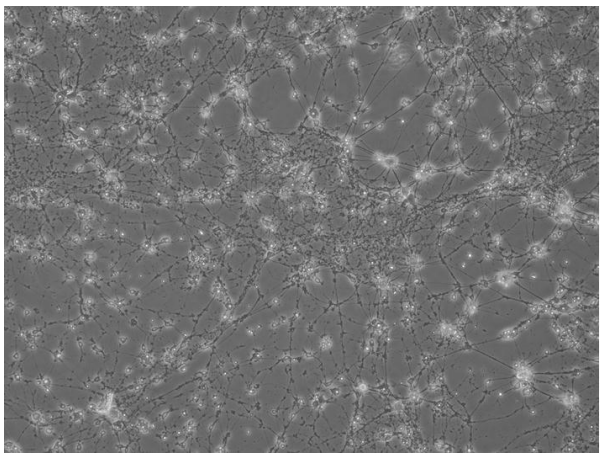


Figure 1 left.

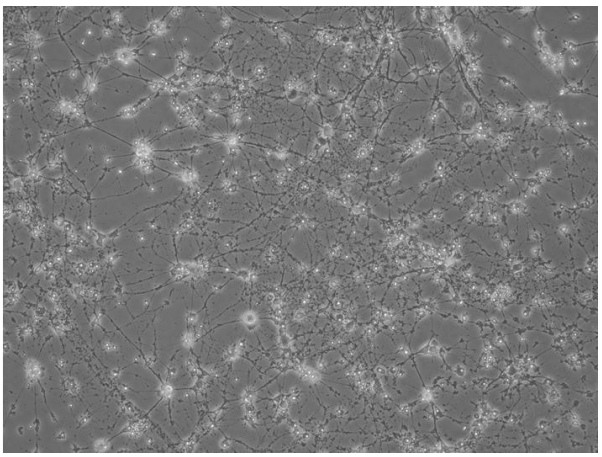


Figure 1 right.

Left: control (untreated) neurons after 5 days and right: after 10 days in culture. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

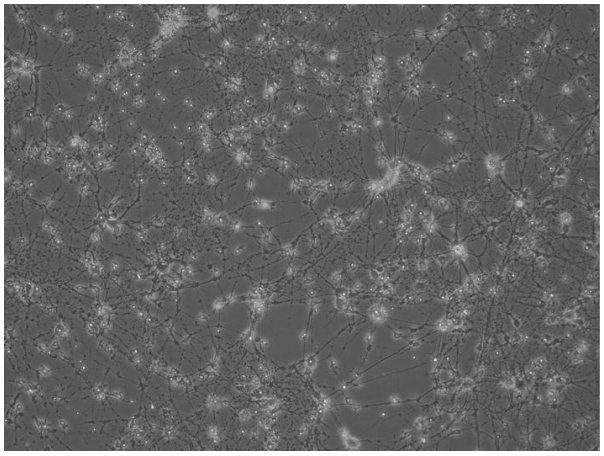


Figure 2 left.

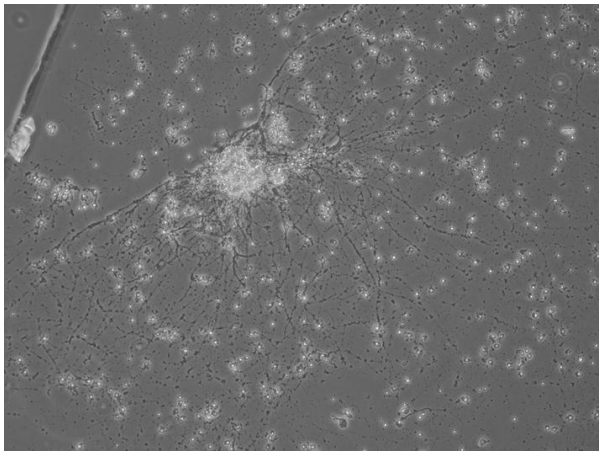


Figure 2 right.

Left: program B_{normal} treated neurons after 5 days and right: after 10 days in culture. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

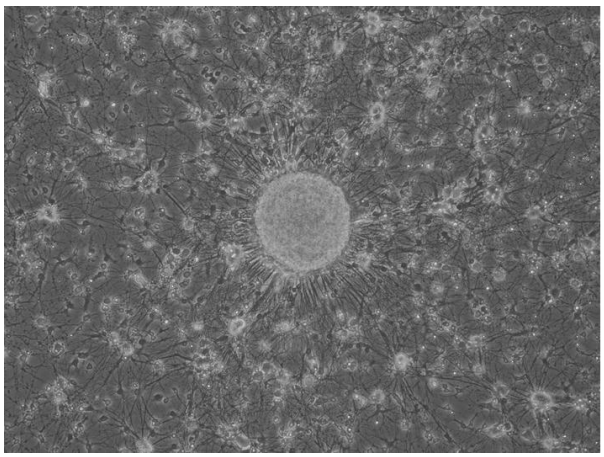


Figure 3 left.

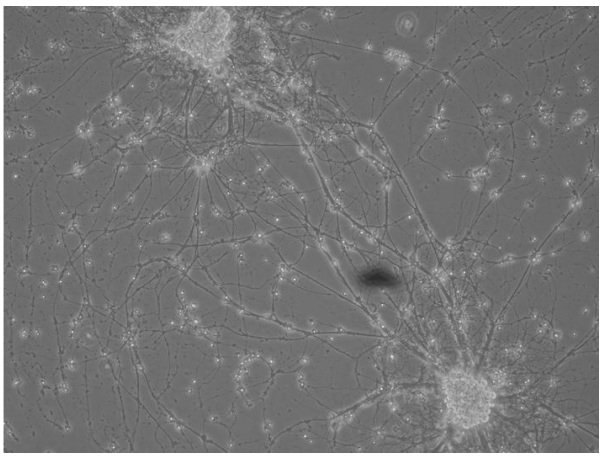


Figure 3 right.

Left: program $B_{normal + cerebrum}$ treated neurons after 5 days and right: after 10 days in culture. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

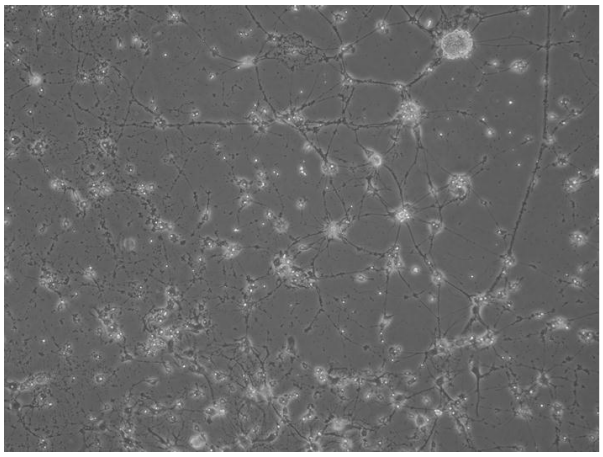


Figure 4 left.

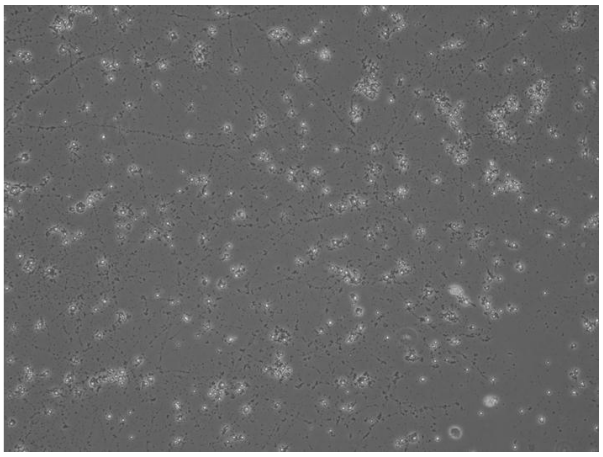
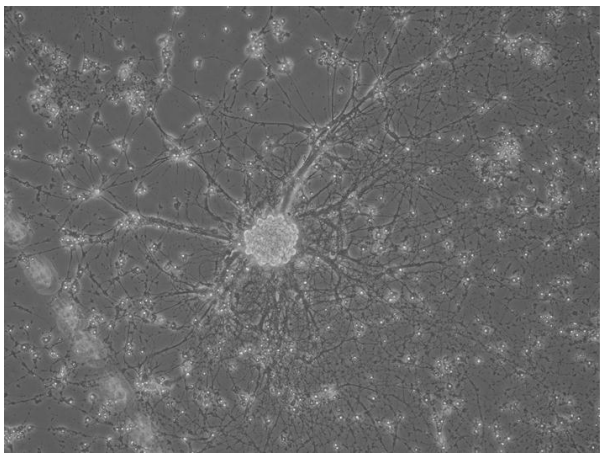


Figure 4 right.

Left: program B_{normal} culture medium treated only, after 5 days and right: after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.



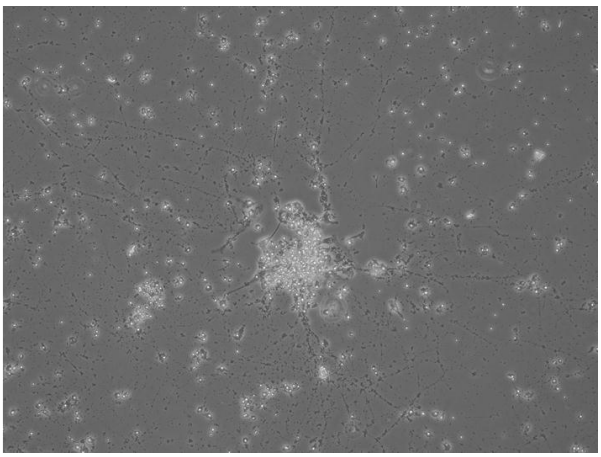


Figure 5 left. ***Figure 5 right.***

Left: program $B_{normal + cerebrum}$ culture medium treated only, after 5 days and right: after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

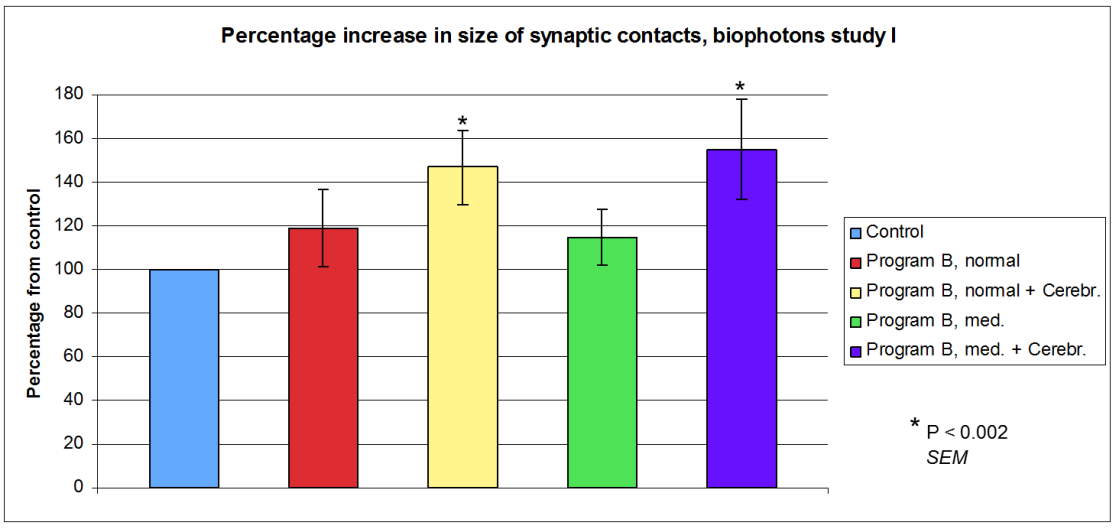


Figure 6.

Bar graph showing the effects of the different Biophoton device treatment programs on size of synaptic contacts after 10 days in culture.

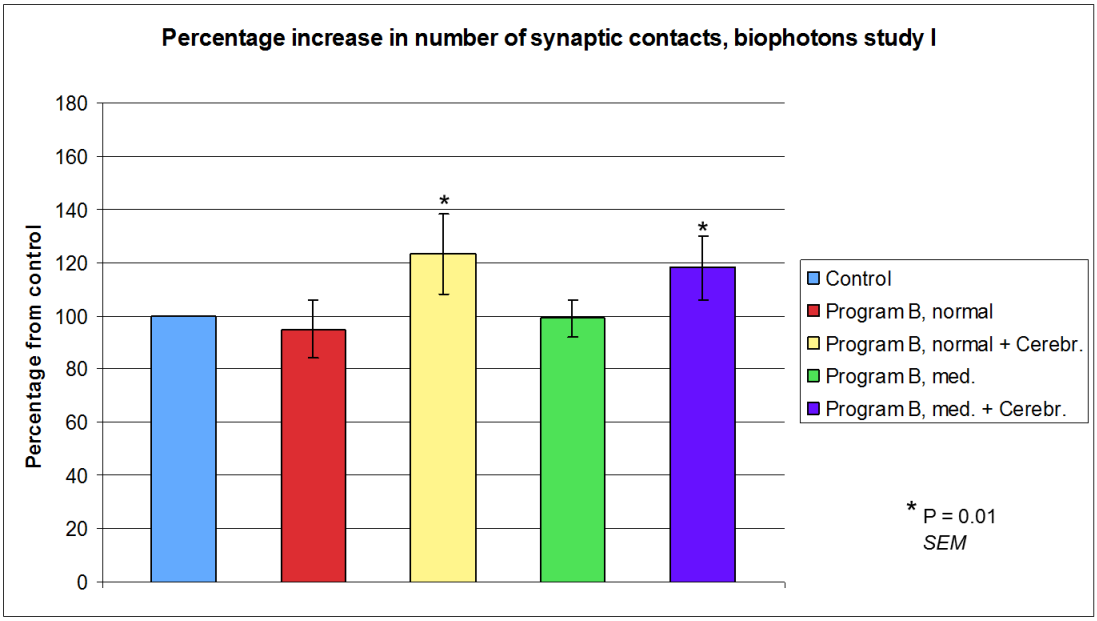


Figure 7.

Bar graph showing the effects of the different Biophoton device treatment programs on number of synaptic contacts after 10 days in culture.

Study 2

Neuron biophotons study II

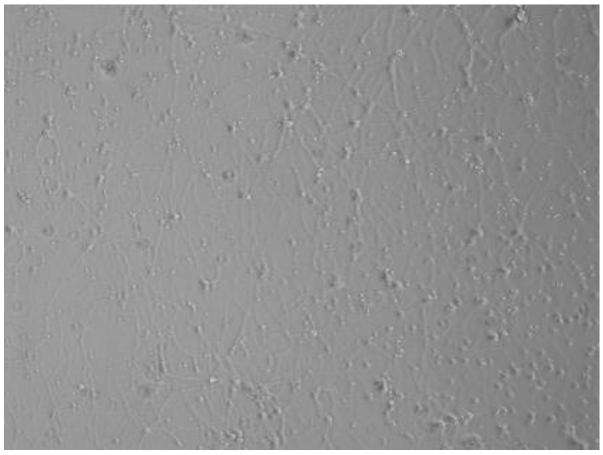


Figure 8 left.

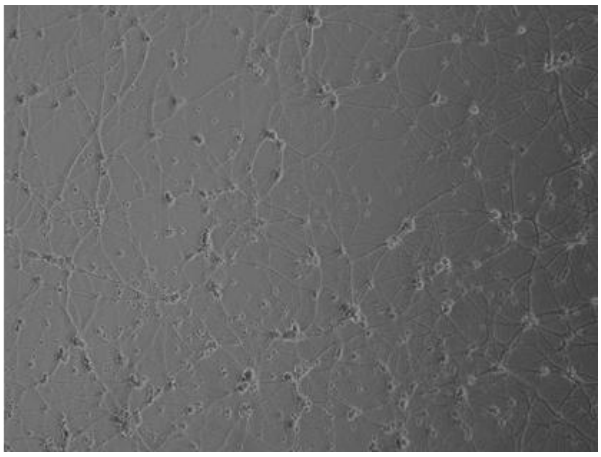


Figure 8 right.

Left: control (untreated) neurons at start of experiment and right: after 10 days in culture (end of experiment). Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

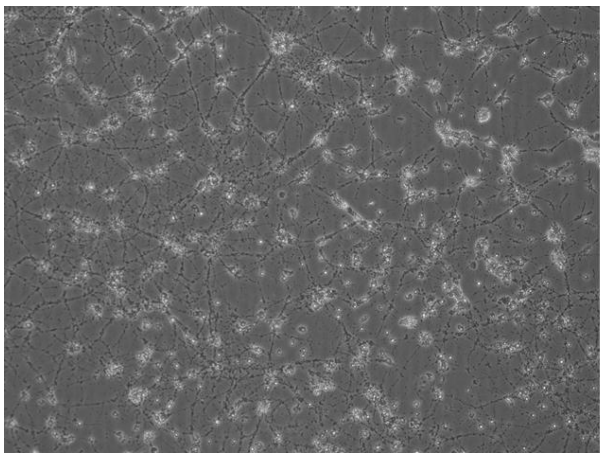


Figure 9 left.

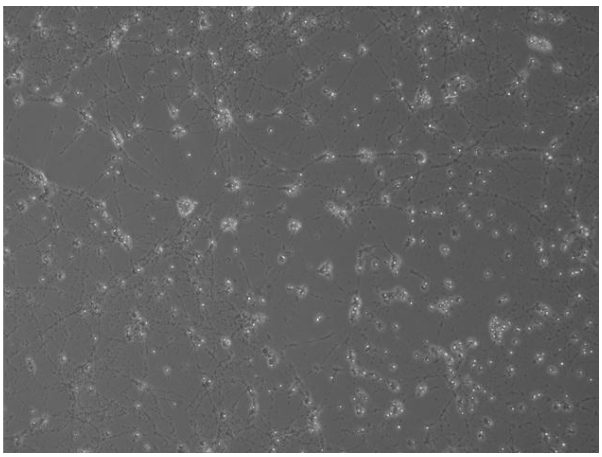


Figure 9 right.

Left: program M_{normal} culture medium treated only, after 10 days and right: M_{normal} treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

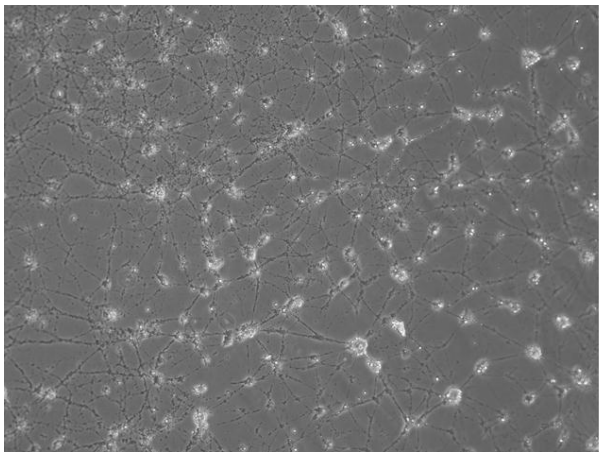


Figure 10 left.

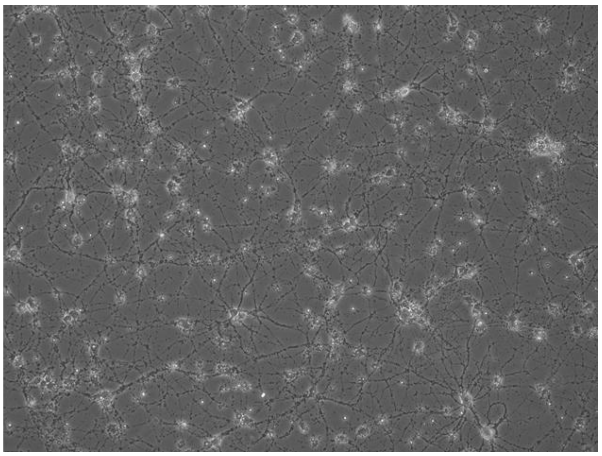


Figure 10 right.

Left: program $M_{normal + cerebrum}$ culture medium treated only, after 10 days and right: $M_{normal + cerebrum}$ treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

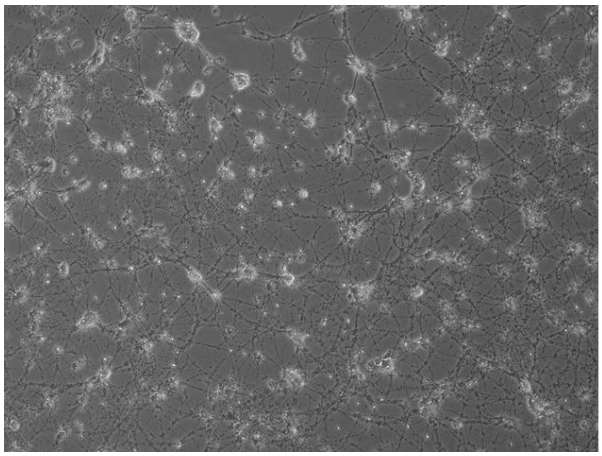


Figure 11 left.

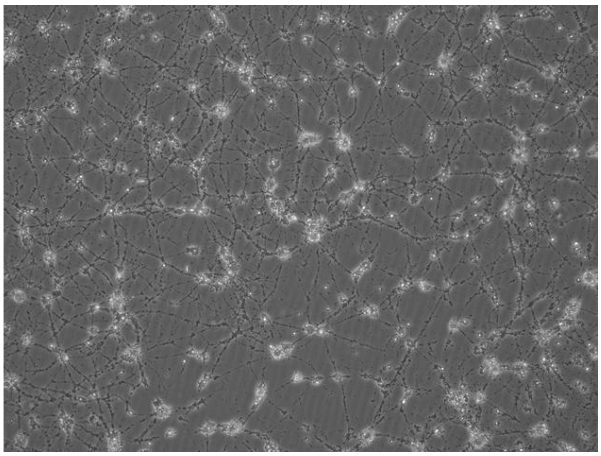


Figure 11 right.

Left: program B_{normal} culture medium treated only, after 10 days and right: B_{normal} treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

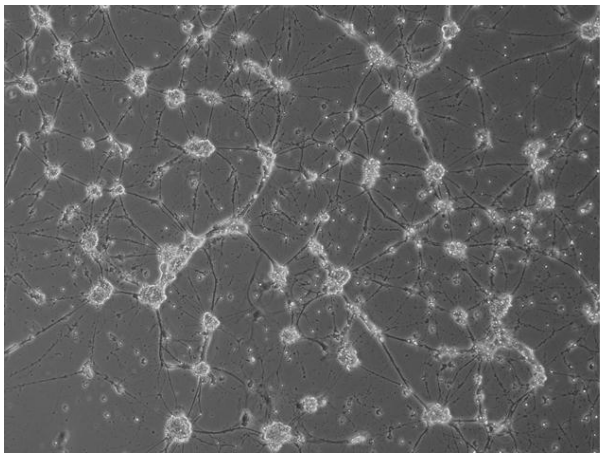


Figure 12 left.

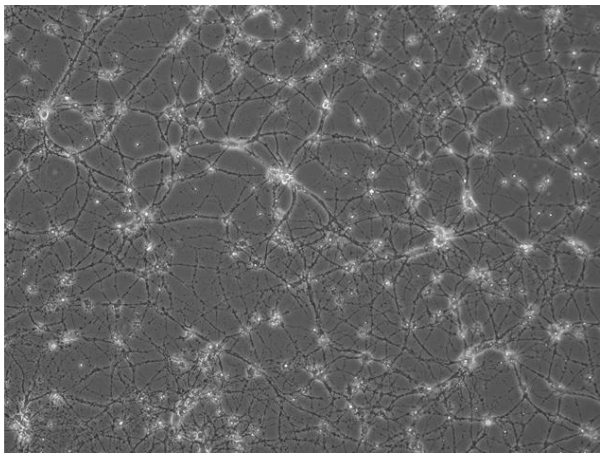


Figure 12 right.

Left: program $B_{normal + cerebrum}$ culture medium treated only, after 10 days and right: $B_{normal + cerebrum}$ treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

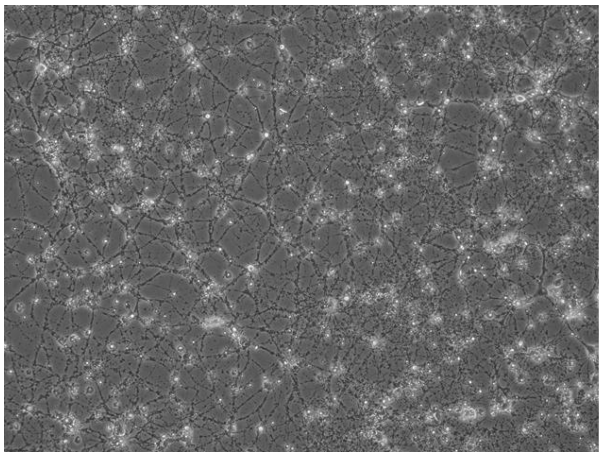


Figure 13 left.

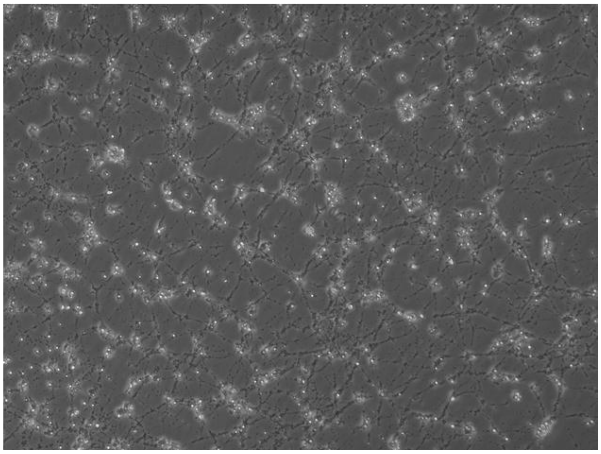


Figure 13 right.

Left: program $N1_{normal}$ culture medium treated only, after 10 days and right: $N1_{normal}$ treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

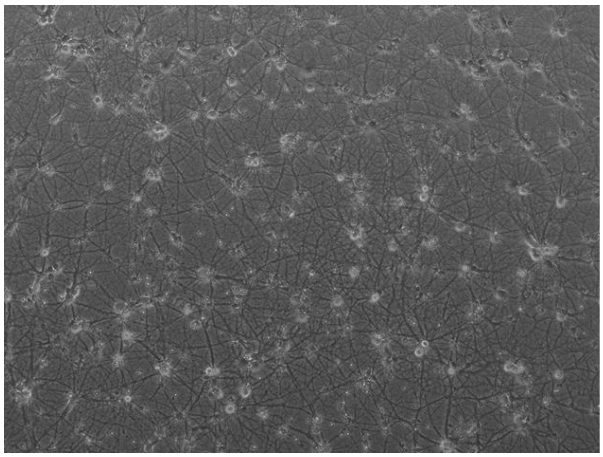


Figure 14 left.

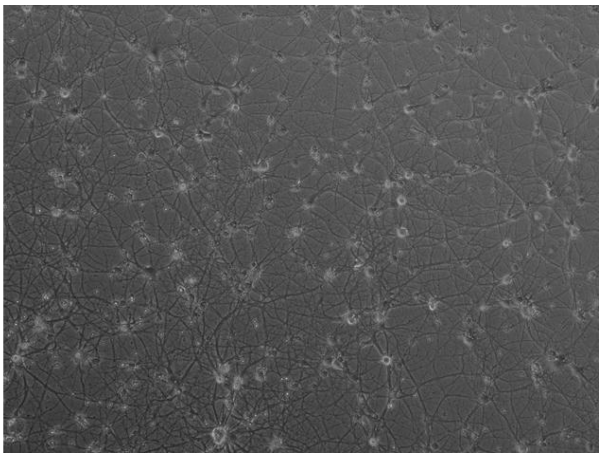
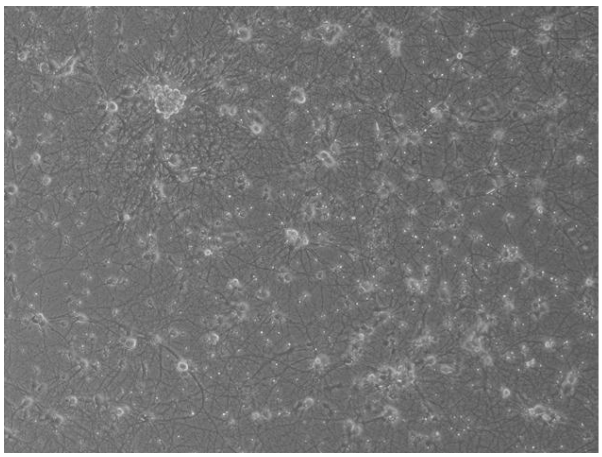


Figure 14 right.

Left: program $N1_{normal + cerebrum}$ culture medium treated only, after 10 days and right: $N1_{normal + cerebrum}$ treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.



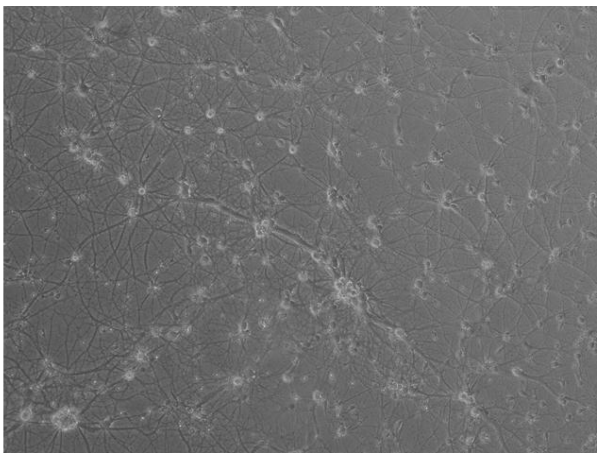
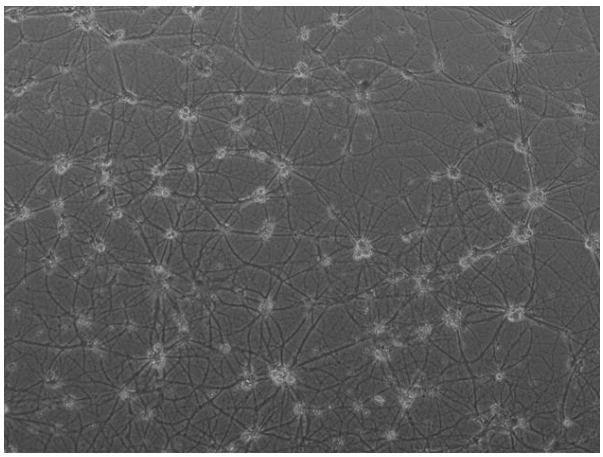


Figure 15 left. ***Figure 15 right.***

Left: program $N2_{normal}$ culture medium treated only, after 10 days and right: $N2_{normal}$ treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.



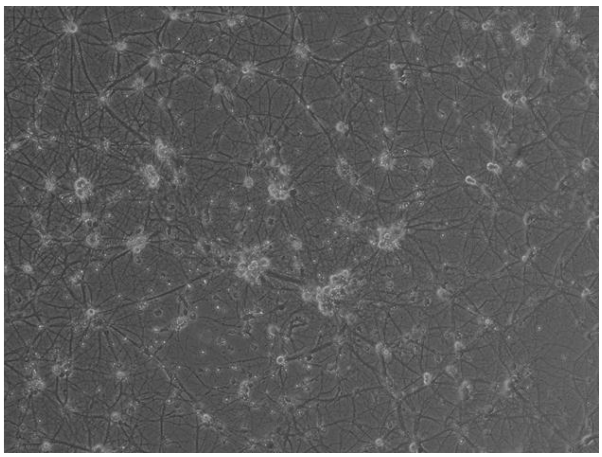


Figure 16 left. ***Figure 16 right.***

Left: program $N2_{normal + cerebrum}$ culture medium treated only, after 10 days and right: $N2_{normal + cerebrum}$ treated neurons after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

Study 3

Neuron biophotons study III

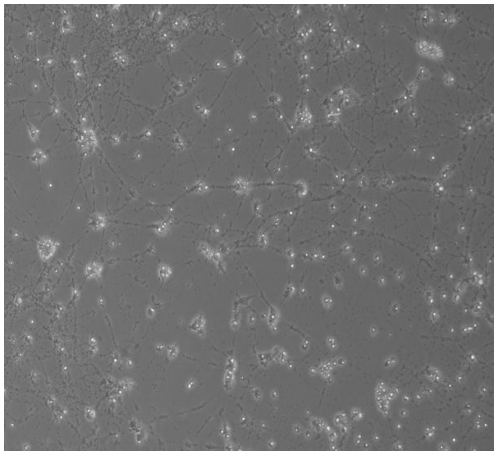


Figure 17.

Control (untreated) neurons after 10 days in culture (end of experiment). Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

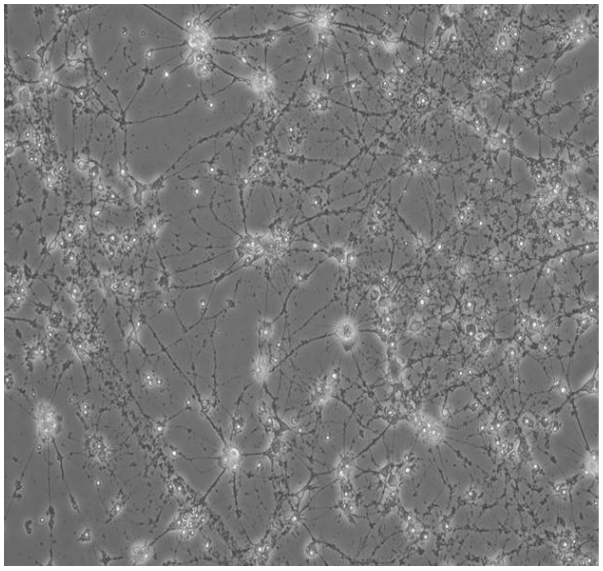


Figure 18 left.

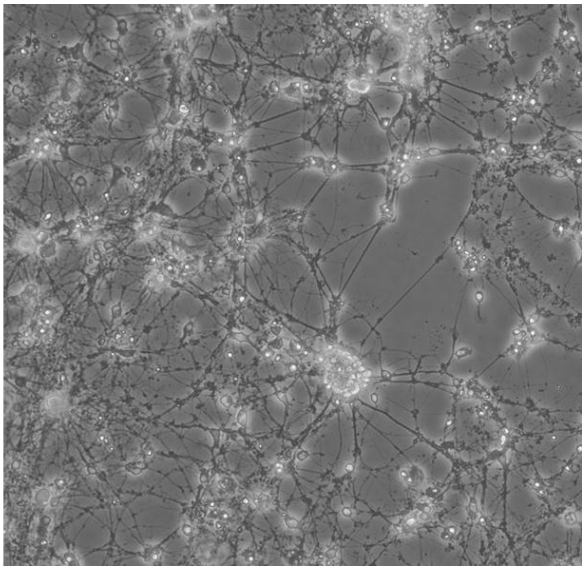


Figure 18 right.

Left: program N3$_{normal}$ culture medium treated only, after 10 days and right: N5$_{normal}$ culture medium treated only, after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

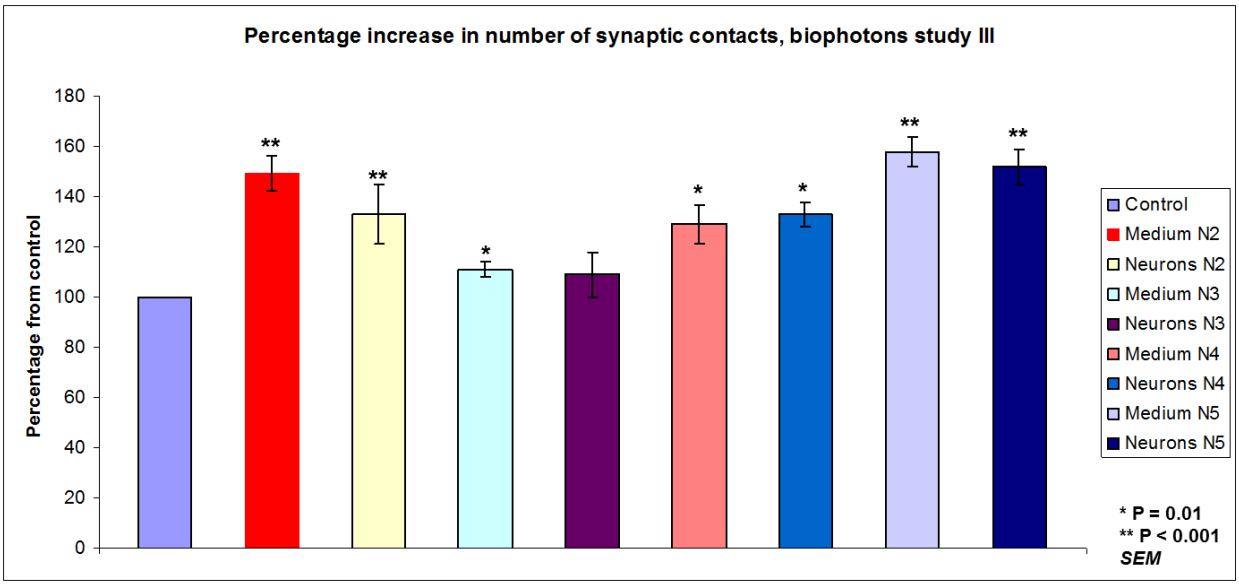


Figure 19.

Bar graph showing the effects of the different Biophoton device treatment programs on number of synaptic contacts after 10 days in culture.

3.1 Survey

In answer to the lead research question – if neuronal growth and survival would be affected by Biophoton device treatment – there was a significant increase in neuronal growth and synaptic interactions between neurons.

3.2 Special Observations

The presence of synaptic contacts was even more increased upon addition of the homeopatic remedy 'Cerebrum Compositum'to the used programs with the Biophoton device.

4. DISCUSSION & CONCLUSIONS

4.1 Interpretation of Results

The key question in this study was if treatment with the Biophoton device would have any effect on neurons in culture. To this aim there was started with a basic program of the Biophoton device. There was a significant increase in possible neuronal interactions as was shown by an increase in the number of synaptic contacts between treated neurons as compared to control, untreated neurons (figures 6 and 7). However, no difference in neuronal survival was observed between the treated and untreated groups. To further understand if Biophoton device treatment in general or if specific characteristics of biophoton treatment programs would lead to such results, follow-up studies were performed in which several different Biophoton device programs were applied to the neurons. The programs differed in exposure time and in exposed frequency. Interestingly, the program with the lowest frequency and exposure time (N5, see figure 19) showed the most significant increase in the number of synaptic contacts between the neurons. Program B – the basic one minute program with the highest frequency – did show a trend towards a negative influence on neuronal viability. This could be due to the fact that isolated neurons in culture are vulnerable to changes in their culture conditions.
However, in every Biophoton device program used – whether the non-beneficial program B or the most beneficial program N5 – in which was added the homeopathic remedy Cerebrum a significant increase on synaptic contacts was visible. This interesting finding could be related to the specific positive effects on cerebral functioning which are known to exist for this remedy.
The fact that Biophoton device treatment showed such a strong impact on the neurons could be due to the fact that neurons are highly communicating cells. As biophotons are involved in cell-cell signaling and communications it could be hypothesized that biophotons play a vital role especially in neuronal cross-talk / connections. A Biophoton device treatment could thus be a way of stimulating or at least affecting neuronal behavior.

4.2 Self-critical Remarks

One of the limitations of this study was the fact that the neuronal cultures used were so-called single cell cultures, that is, no presence of other cells which are normally present in the brain. This can influence the effects of Biophoton device treatments. For future studies it is therefore of importance to grow neurons in co-culture with these other cells.

4.3 Suggestions for further Research

The described studies have shown an real impact of Biophoton device treatments on neurons in culture. To further understand and analyse what exactly is affected by this treatments the following experiments are necessary:

- What happens on the protein level, more specific, the expression of proteins involved in neuronal survival & synapse formation
- Co-culture of neurons with other brain-specific cells

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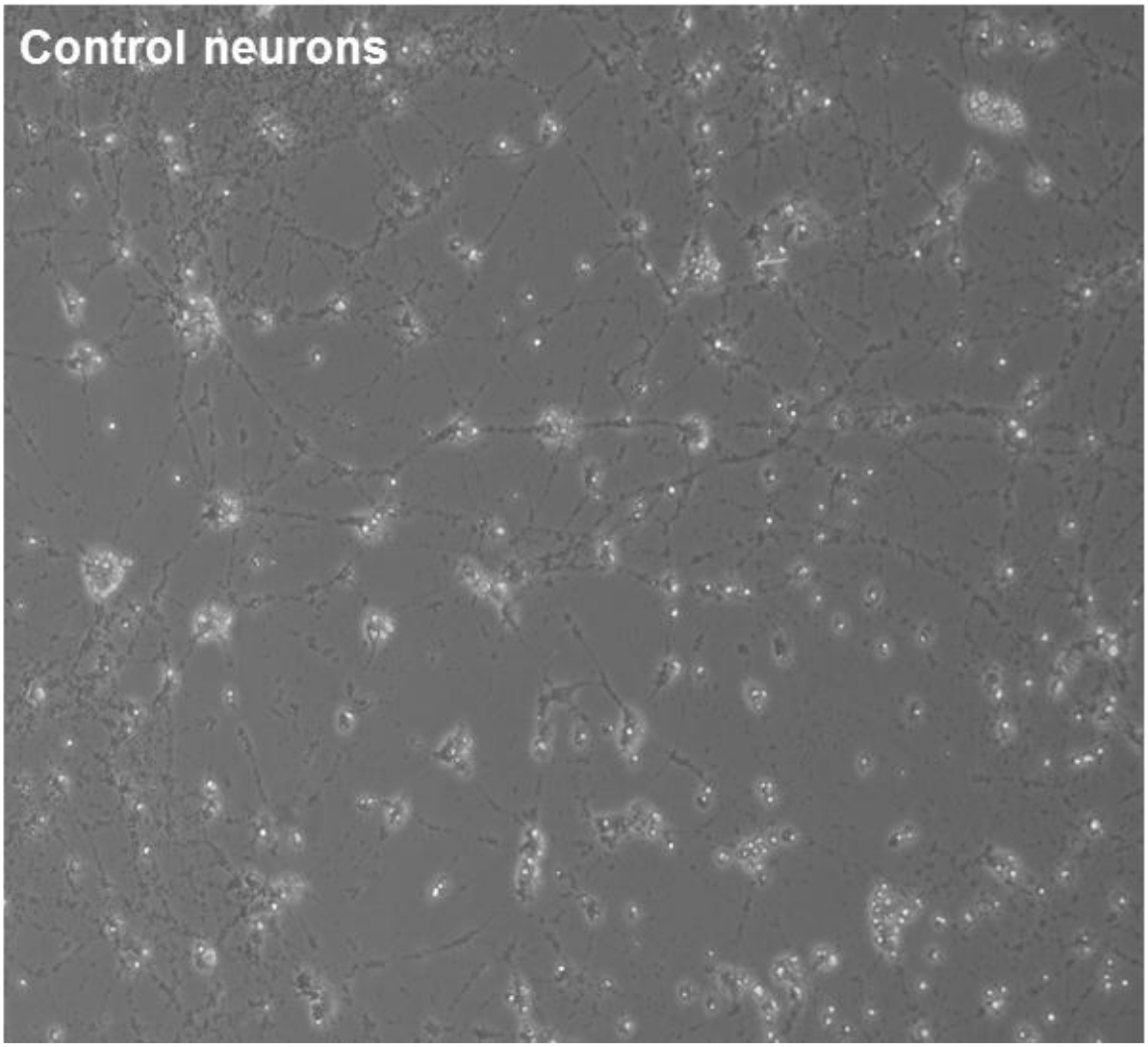
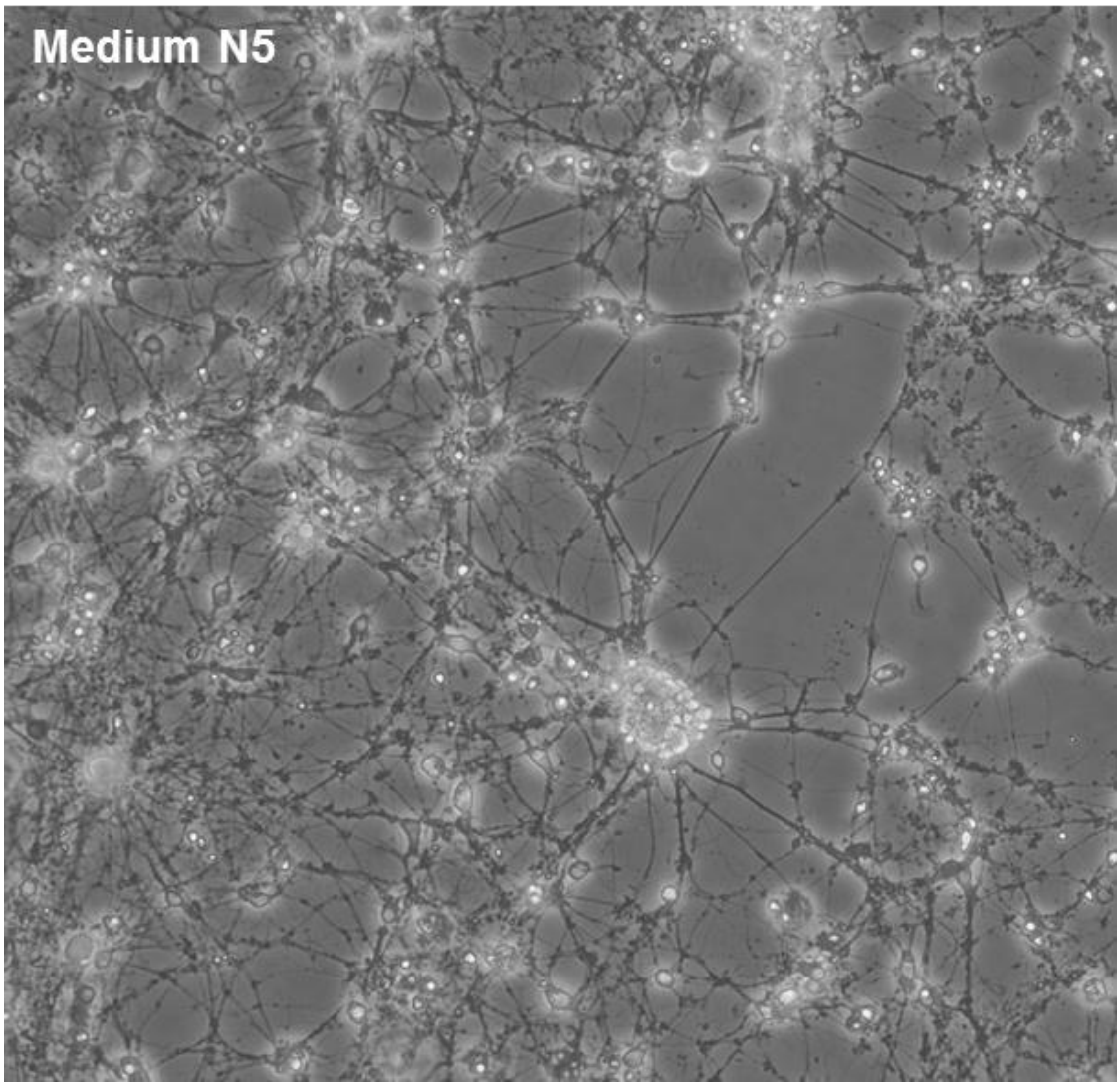
APPENDIX I



Appendix I

Control (untreated) neurons after after 10 days in culture. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.

APPENDIX II



Appendix II

Program $N5_{normal}$ culture medium treated only, after 10 days. Magnification = 100 x and visible area = 1.8 mm^2. Culture area = 190 mm^2 = 20.000 neurons in total. On average 2000 neurons were within the visibility area.