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FULL ARTICLE

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Acceleration of newborn rats' development with the use of photobiomodulation and the near possibility of application in human premature babies

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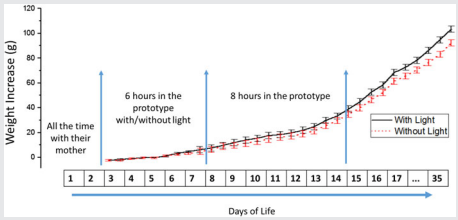
Abstract



Photobiomodulation was explored to find evidence of stimulation during the development of newborn rats. A light chamber device was used, and rat pups were divided into groups after birth. Investigation of the process' security was performed before the full experiment. Following a protocol of alternating illumination and mother's presence during the first 13 days, we observed that, in the group that received photobiomodulation, the pups opened their eyes faster, indicating earlier achievement of maturity. The rate of weight gain also indicates faster metabolic activity in the group that was photostimulated. This study is the first step toward the use of photobiomodulation for premature newborn human babies.

KEYWORDS

growth, human, newborn, photobiomodulation, phototherapy

1 | INTRODUCTION

Photobiomodulation (PBM) is the application of light for biostimulation in a process that is already performed by the cell but can be stimulated by light [1]. It was also known as low-level laser therapy until a demonstration that light-emitting diodes (LEDs) were as efficient as lasers in this application, and it has been termed low-level light therapy. Several studies show that, at low irradiation intensities, the cells undergo several alterations, such as cell growth and increase in the number of some organelles, as well as a change in the adherence of cellular culture of fibroblasts and stimulation of RNA and DNA synthesis [2–4].

At high irradiation, however, light can damage cells and inhibit growth, which indicates that the optimal light dose is an important parameter to achieve the expected effect [5]. These opposite effects show the importance of understanding the mechanisms and parameters involved in the application of light in biological samples.

Red and near-infrared light have been most often used for biostimulation due to absorption of the photon by the mitochondria and units of cytochrome c oxidase, which show absorption peaks in these regions [6–8]. However, there are other endogenous chromophores that could be used for PBM, which show absorption in other wavelengths [9].

The light can be used in the treatment of diseases, with purposes other than PBM. The light application near the blue region of spectrum is, for example, very well established in the treatment of jaundice in newborns in a process known as phototherapy [10]. Blue light illumination helps to metabolize bilirubin, which is excreted by the liver. The child is placed naked in a cradle equipped with light sources, with the eyes covered with a protective mask [11].

As light can accelerate natural biological processes and is already used to treat jaundice in children's intensive care

units (ICUs), understanding light's influence on newborn rats' development and the acceleration of this process can directly help premature children in these ICUs and meets the needs of the global healthcare systems, making the incubation process more efficient.

The possibility of therapies with the light application is also important for the treatment of human newborns with incomplete formation. The correct application of PBM can be a great step to guarantee the healthy growing of newborns, resulting in a decrease of fatalities or complications [12].

This situation has fundamental importance in ICUs of hospitals as premature infants are part of the constant reality in health facilities in the world. In Brazil, 340 000 babies were born premature in 2012, with an average of 930 premature babies a day, according to the System of Live Births Information, the Brazilian Unified Health System and Ministry of Health [13].

After red light was applied to chicken eggs with an impressive result regarding the acceleration of the formation of the embryos [14], in addition to the results of rats demonstrated in the present study, we are closer to the goal of the treatment of premature human newborns. This report aimed to discuss this important possible application and to initiate the studies in this direction.

2 | MATERIAL AND METHODS

2.1 | Light parameters

The prototypes for newborn rats were developed to guarantee the same conditions in the groups with constant illumination and without light. Both cages had a heating system to keep the temperature of the pups similar to that of the contact with the mother, and there was a feedback control to stop the heat at 37°C. A ventilation system was installed to distribute the heat homogeneously. For the cage with light, LEDs emitting in 630 nm were pasted above and below of the cage to illuminate the pups' entire bodies. For the cage without light, there were no LEDs; however, in both groups, there were small holes for air circulation. Figure 1 shows the prototype with the wires for the illumination installation, the ventilation system and the system with the lights on.

Light intensity exposure to the animal was adequately measured, and the power meter was placed on the platform where the rats walked and slept, resulting in 4 mW/cm^2.

2.2 | Experimental groups

The pregnant Wistar rat was maintained in the cage until the birth of the pups. Considering that each gestation results in between 4 and 14 pups, the same litter was divided into illuminated and nonilluminated groups. From the birth, the pups were with their mother for two whole days to guarantee feeding with colostrum and their survival. The control group stayed with the mother all the time during the first 21 days to compare natural growing until weaning.

One group was chosen for the verification of the safety of illumination and the possibility of side effects on the motor coordination and vision of the adult animal. From the eighth day of life, the pups started to be removed from the mother and remained in the respective cages of each group. The pups were kept for 4 hours in the prototype of the light chamber under the conditions with and without light; they

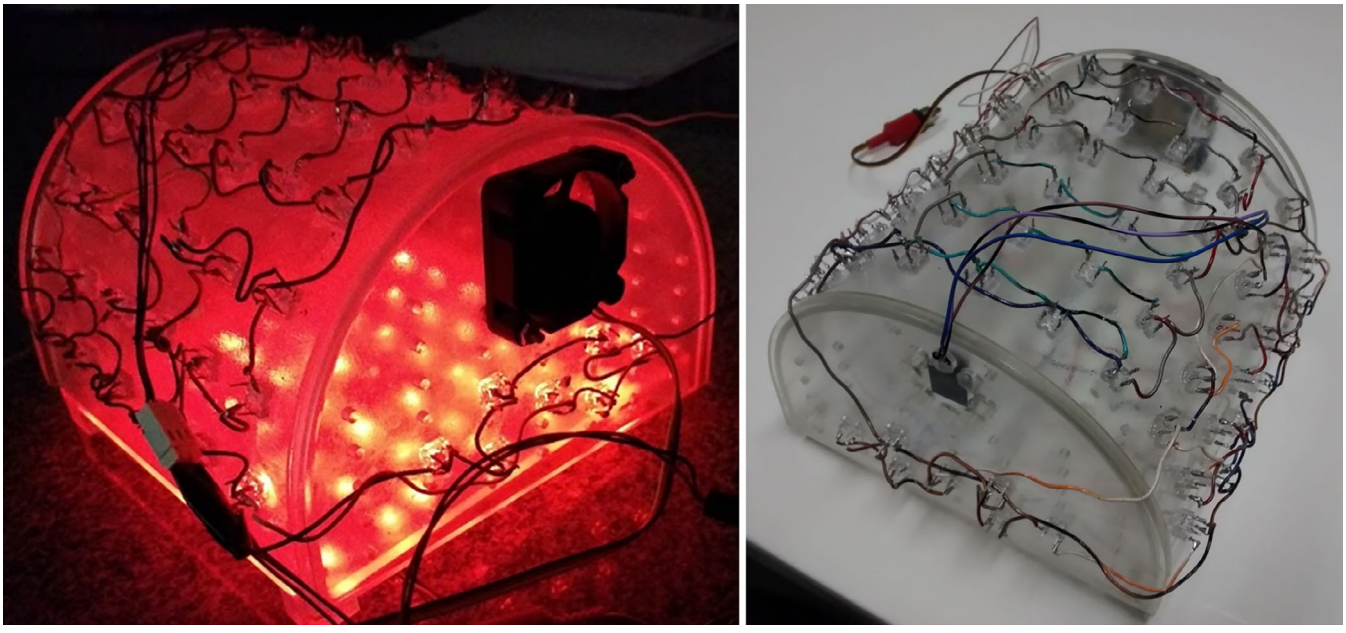


FIGURE 1 Illumination prototype for the light chamber of newborn rats with heating and ventilation systems

were brought back to the mother's cage for feeding for 2 hours and then stayed for more 4 hours separated in their respective prototypes. During the night, to avoid differences in the light–dark cycle, all animals stayed with the mother. They were weighted every day, and this procedure was repeated until 21st day of life or more, when weaning happens.

In the security group, the pups were separated of their mother in the second day after the birth. Until 1 week of life, they stayed separated for 3 hours (with or without red light according to the group), then spent 3 hours with the mother for feeding and were then separated again for 3 hours in their respective groups. After the eighth day of life, they stayed for two periods of 4 hours in the illumination chamber prototypes with an interval of 2 hours with their mothers, considering that, during the whole night, they stayed under maternal cares. Each period of feeding with the mother was established for the guarantee of feeding and survival of animals. The illumination with the prototype was performed until the appearance of fur on skin interfered with adequate light penetration (until 14th day approximately), and the rats were definitively separated from the mother on the 21st day, at weaning. A total of five pregnant Wistar rats were used.

A summary of all groups is showed on Table 1.

2.3 | Data Analysis

After the birth, all animals were weighted every day to compare the groups. Among different litters, a comparison was made using the ratio between the weight of each day and the weight of the first experiment day. A blood sample of the safety group was taken to verify the variation of basic blood count between illuminated and nonilluminated groups. Images were captured each day, and signals of development were analyzed: the day the eyes opened and the appearance of fur. A paired Student t test was used to compare the weight of each group (control, nonilluminated and illuminated), and the significance level was 5%, which means the P value $< .05$.

3 | RESULTS AND DISCUSSION

First, it was necessary to guarantee the security of the influence of light for the rats, and to evaluate this, a group was formed with illumination initiated 8 days after the birth. Even with fur, the illumination was maintained until the adult life of rats (21 days after the birth) to verify motor activities and the influence of light on the eyes. All rats of both groups (with and without light) demonstrated the same behavior without any damage or noticeable modification. To confirm these results, a blood count was performed of all animals, and no significant alterations in the counting of the blood cells series were observed.

Although there are different applications of the present study, it is important to know the adverse effects of the application of light on newborns. An application of light widely used in human neonates as a gold standard, according to the American Academy of Pediatrics since 1958, is for the treatment of jaundice, a disease related to excess bilirubin in the blood [10]. Some studies show the possibility of inducing oxidative stress with the use of these wavelengths, producing free radicals and possible DNA damage, enabling the development of skin diseases, such as melanocyte nevus. However, due to inconclusive studies relating these two factors [11], it is considered that the benefits of light application outweigh the side effects.

A comparison between high and low doses of light, which means the amount of energy delivered to a specific surface/volume, to newborns with low weight showed no significant differences among the groups [15]. This result of literature shows that blue light application (around 450 nm) did not result in side effects for the newborns and can be indicative of a possibility of light application in other wavelengths. Specifically, red or near-infrared light, which correspond to wavelengths longer than 600 nm, should cause fewer side effects as the wavelength is inversely proportional to the amount of energy [2, 4] in concordance with our results for 630 nm.

TABLE 1 List of groups and procedures

Groups	Initial day of mother separation	Days of life	Hours in the prototype/cycle (x2 cycle)	Total hours in the prototype/day	Hours of feeding with mother between the cycles
Control	-	0–21	-	-	24
Safety with light	8	8–21	4	8	2
Safety without light		8–21	4	8	2
With light	2	2–7	3	6	3
		7–14	4	8	2
Without light		2–7	3	6	3
		7–14	4	8	2

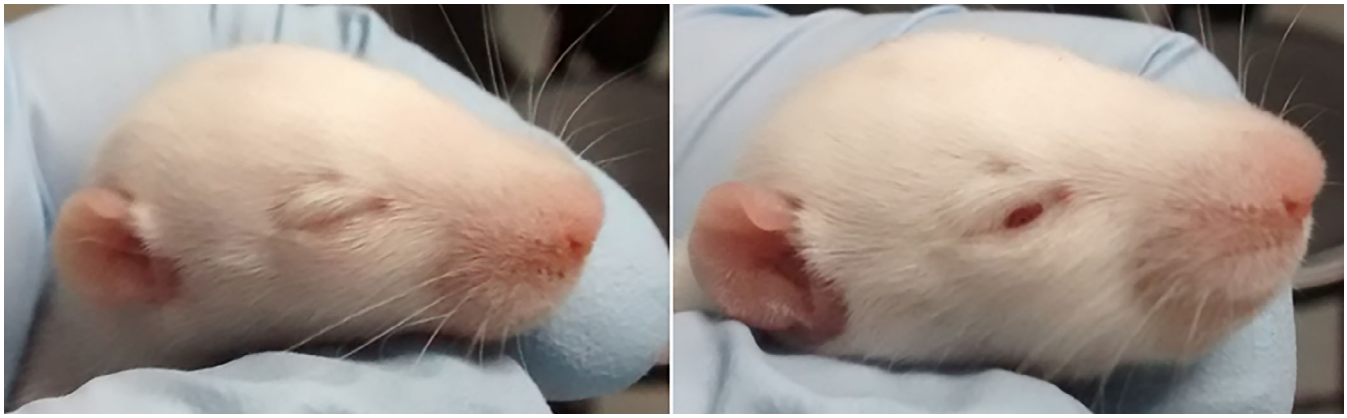


FIGURE 2 Difference between closed and opened eyes, showing one aspect of pup development

From these results, it can be observed that the illumination was performed earlier, started 2 days after the birth and until the fur disrupted the light penetration. At all times, the same litter was divided into two groups (illuminated and nonilluminated) and were subjected to the same conditions of mother separation and feeding to compare the results. The day when the eyes of rats start to open is one of the characteristics that indicate the development of rodents, and therefore, it was observed for illuminated and nonilluminated groups.

On the 13th day of the experiment (and, therefore, on the 15th day of life), in the light group, 71.4% of pups opened their eyes, in contrast to 16.7% of the nonlight group. Figure 2 shows the difference between the open eyes and closed eyes of the nonlight and light groups.

The rats were weighed everyday to verify the growth curve. Differences of weight between the illuminated and nonilluminated group from the same breed as a function of the day are showed on the graph of Figure 3. Two important days were highlighted in the graph: the day that the light was stopped because of the size of fur and the day that there was the separation of mother as their feeding had been done by the mother rat until this specific day.

It is clear that the illuminated group had a growth curve systematically higher than the non-illuminated group. However, this difference is prominent after the weaning, indicating that the illumination in the first 10 days of life can cause significant increase of weight during the development of rats. After stopping feeding by the mother, the illuminated group showed an increasing weight rate 20% higher than the nonilluminated group.

It seems that the illumination in the first stage of life (first period) established a metabolic rate that became noticeable after stopping mother's milk. The fast metabolic activity after mother's feeding results in better and higher food processing, with final results in the rate of weight increase.

A ratio of growth of each group was calculated to compare the difference among the growth curve of the rats that are in the experiment and the natural growing of control group. This ratio was calculated because the rats are from different litters, and they were born with different weights. Using the graph in Figure 4, it is possible to verify that the group with natural growth, which corresponded to the control group where pups were with their mother all the time, has had a higher ratio of weight increase in the first days. However, the illuminated group was able to achieve the same growth of the control group and exceed the increase of weight, mainly after the separation from the mother.

The group without light (but with mother separation during the time in the prototype without light) was always below the control groups but exceeded it exactly at the point after separation from the mother, which may suggest a hypothesis of maternal independence of the pups that were away from the mother for a few hours over the days. The statistical analysis for weight showed that the illuminated group was significantly different (P-value $<.05$) when

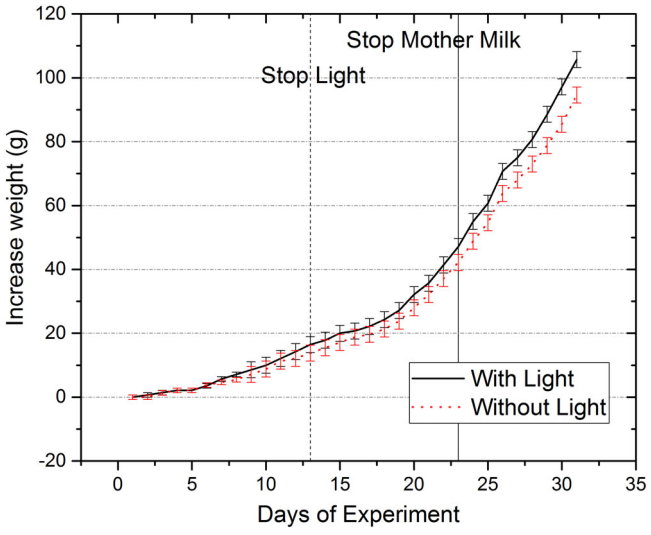


FIGURE 3 The growth curve showing the difference between illuminated and nonilluminated groups with indication of the day that the light was stopped and the day of weaning

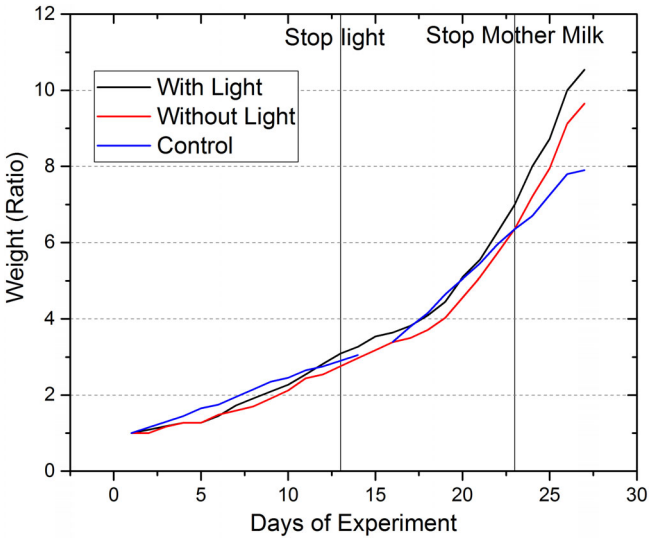


FIGURE 4 Growth curve showing the differences among illuminated groups, nonilluminated group and natural growing with the mother

compared to both the control and nonilluminated groups. However, when the nonilluminated group was compared to the control, there was no statistical difference ($P = .48$), and this analysis corroborates with the results that light could influence the growth of newborns.

Some effects of PBM can be short or long term, including increase of blood flow and antioxidant defenses, improved ATP production and alteration of growth factor expression [16]. These alterations could be an explanation for the different curves between the illuminated and nonilluminated groups.

The application of light has demonstrated the growth of cellular activity, such as phagocytes and leukocytes, in addition to increasing the amount of calcium ions in the cytoplasm. In some cells, activation of cytosine and protein synthesis, enhancement of cell growth and division and improvement of blood circulation due to the relaxation of vessel walls, also called vasodilation, occurs [1].

Further growth with the use of light has previously been observed in birds and embryos of chicken, with light focused on the eggs [14], similar to the results of the present study. Many of mechanisms of PBM are common in different organisms, including nonmammalians forms of life, such as worms, fish and birds [16]. All of them, with dose adjustments, could influence the growth factors and metabolism and, therefore, illumination also can influence the development of rat pups.

With these results and a better understanding of how light influences the development of living beings, there is an opportunity for new ways of the application of PBM in newborn human babies, especially premature babies.

The introduction of a safe and effective technology in the development of these newborns meets the needs of health systems. Because light can accelerate the natural biological processes and is already used to treat some newborn diseases, such as jaundice, its use in embryonic development and newborns can directly help these premature human babies in hospitals. This is clearly an interesting and promising field for investigations and is certainly a public health problem with a great deal of room for technological improvements. Future studies with other wavelengths (as in near-infrared region) and with premature rats are being considered in the near future. In the case of premature infants, the natural demand for metabolic activity may result in a larger influence of the light.

This study has demonstrated the potential use of PBM on the development of newborn rats using illumination at 630 nm and an established protocol. The process is observed to be secure for the babies, with a positive indication. Certainly, the protocol must to be adjusted for optimization. The results demonstrated some possibilities for the study of premature babies.

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Please see Supporting Information online.

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