Study of the efficacy of PSIO glasses for the inhibition of melatonin

Comparative analysis of the inhibition of melatonin: between continuous blue light stimulation (470 nm) and blue flickering light (470 nm) combined with an audio stimulation program at variable frequency.

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Abstract: Several studies have showed the influence of the inhibition of melatonin by blue light (470 nm) (1) In the following report we have tested the effect of continuous blue light - emitted by a PSIO device without sound stimulations (the PSIO is a pair of glasses equipped with eye-glasses emitting colored lights and with an integrated MP3 player) - on the production of melatonin at 10 PM and the difference with an audio-visual stimulation (emitted with a PSIO device) using same wavelength range of blue light (470 nm). The study has considered four groups of students aged from 18 to 26 y.o. and the experiment was conducted at 10 PM when the curve of melatonin production has already begun to increase for one hour approximately. The four groups were proposed to rest on a comfortable clinic table with or without PSIO glasses, rest time being for all groups of 30 min; Group 1 was a control group doing just a rest without PSIO glasses in a room with controlled light at about 75 watts; Group 2 was designed as placebo group with a pair of PSIO glasses but emitting only a continuous (670 nm) red light (without any sound stimulations). Group 2 was considered as a "placebo" group, as red light being known by several studies to have no effect on the inhibition of melatonin; Group 3 was the group practicing a similar session with same PSiO glasses but emitting only blue light (470 nm) on the continuous mode (without any sound stimulations) and Group 4 was a group practicing a normal session of audio-visual stimulation with a range of colored blue light (wavelength around 470 nm). Results have already showed that the control group (1) had a normal increase of melatonin production; the Placebo group (2) had a normal increase of melatonin production and was responding similarly to the control group thus proving that PSIO glasses has no placebo effect concerning the inhibition of melatonin. Group 3, the PSIO continuous blue light (470 nm) stimulation group was responding with a strong inhibition of melatonin, and a small decrease in melatonin level was actually recorded. Group 4, the PSIO audio-visual stimulation group obtained also a strong similar inhibition of melatonin. Preliminary conclusions are that PSIO glasses, using a continuous blue light stimulation (470 nm) or a range of pulsated emitting blue light stimulations combined with audio stimulations both have a strong inhibition effect on the melatonin production at 10 PM.

Keywords: melatonin inhibition; blue colored light; audio-visual stimulation; AVS; PSIO glasses device; IQ;

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Summary

Introduction

During the 20th century, our society has evolved into a society where more and more people live inside rather than outside. This new lifestyle exposes individuals to artificial light which can generate a permanent jet lag leading to fatigue, depression and burnouts, fairly common nowadays. Gradually, it is necessary to find relaxation tools to rest the brain from intellectual work and at the same time regulate jetlag (due to the intense activity under artificial light day and night). For example, the quality of light during evening study will determine the production (or not) of several hormones called in a global process of biological rhythm.

Several tools exist today that use light and sound stimulation to improve mind functioning capacity. We have specifically tested the effects of the PSIO device (an audio visual stimulation device in the form of a pair of glasses equipped with eye-glasses emitting colored lights and with an integrated MP3 player) to check its power to regulate jet lag and particularly the inhibition of the hormone associated with sleep, the melatonin.

The PSIO device is manufactured by PSYCHOMED.COM SA, a Belgian company which has 25 years' experience in this field of research and development and which has already manufactured several other pre-models. The device uses flickering colored light and audio recording (voices, music or beat and music) on several specific rhythms to induce alpha and theta waves, a state of consciousness at the border of sleep. The colored lights can be programmed especially to enable an easier awakening or on the contrary, to induce a short power nap or a good sleep allowing a faster recovery.

The previous models (Dreamer, Mind Booster, etc…) used red diodes and the sessions were eyes closed. For the PSIO, PSYCHOMED has introduced RGB diodes and has developed patented optical eye-glasses allowing to do a session with eyes partially opened. This has led to a significant improvement of this audiovisual stimulation technique by adding the field of light therapy to the method. Indeed, using the combined effect of certain wavelengths in relation to the issued colors and frequencies of stimulation, it was possible to induce different states of consciousness (such as wakefulness or sleep instead). Studies have indeed shown that blue light (470 nm) has a strong influence on melatonin, the hormone associated with sleep, as others have shown that red light has no effect on the secretion of melatonin.

PSIO device characteristics

The technology used in the PSiO is a combination of colored stimulation and pulsating beats at very precise frequencies, sometimes combined with binaural beats and with relaxing music and suggestive voices that at times enter sequentially and at other times in multi-evocation (that is, simultaneously) using the method developed by Dr Milton Erickson, the creative genius behind indirect suggestion. The texts of the recorded messages are written by doctors specialized in psychosomatic medicine. The speakers are professionals experienced in "sophrology" and suggestion. The music is developed in collaboration with musicology and relaxation specialists. In the present study, only the effects of music and light combined has been analyzed (Experimental-GR 4).

Dominant wave length of 3 RGB LEDs used in PSIO

Values			Unit
Red	**Green**	**Blue**	
625	**528**	**470**	**nanometer (nm)**

The PSIO Light stimulations can be either continuous or pulsed. The study has applied a series of continuous or pulsed stimulations depending on each specific group.

A brief history of light stimulation

The fact that flashing light can create beautiful images and a strong distraction effect to the attention has been known by humans since the discovery of fire. The phenomenon also intrigued the scientists of the ancient world who explored its practical applications.

In 125 A.D., Apuleius did an experiment with a flashing light stimulus created by the rotation of a potter's wheel. In +/- 200 A.D., Ptolemy observed that by placing a spoked wheel between an observer and the sun, the flickering of the sun through the spokes of the wheel could create patterns and colors in the observer's eyes and produce a feeling of euphoria.

Closer to our time, in the 19th century, Joseph Plateau, a Belgian scientist, famous for his research on retinal persistence and an originator of the cinema, used the flashing of light through a wheel to study the phenomenon of oscillation fusion. By making the light oscillate faster and faster, he discovered that, at a given point, the oscillations seemed to "melt" into a single, stable light scheme. Plateau also discovered that healthy people were able to see separate light flashes at a much higher blinking speed than sick people could. (Over the past years, studies using light sources such as the “tachistoscope” to create rapid light flashes have revealed that experienced meditators are able to see distinct light flashes at a much higher blinking speed than non-meditators). At the end of the last century, the French psychologist Pierre Janet discovered that when his patients at the “Salpêtrière hospital” in Paris were exposed to flickering lights, their symptoms of disequilibrium decreased and they became more relaxed. Modern scientific research into the effects of rhythmic light started in the mid-1930s when scientists discovered that the brain's electrical rhythms tended to take on the same rhythm as a light stimulus, a process known as FFR = Frequency Following Response.

Research increased significantly at the end of the 1940s when the great British neuroscientist W. Grey Walter, the pioneer of the EEG and discoverer of theta waves, used an electronic stroboscope and advanced EEG equipment to study what he called "the flicker phenomenon". He discovered that rhythmically flashing light quickly modified the activity of the brain waves that are found throughout the encephalon and show its activity type. The flashes literally lead to very deep states of relaxation and produce very distracting, vivid and colorful mental images. Brion Gysin can be considered one of the great precursors of AVS systems. He was a British-Canadian artist, poet, writer and painter. He was born on 19 January 1916 in Taplow, Buckinghamshire and died on 13 July 1986 in Paris. An experience he had in 1958 led him to create the "Dreamachine”. In 1960, Brion Gysin spoke to his friend the scientist Ian Sommerville about the possibility of reproducing the phenomenon that had led to his visions. On 15 February 1960, Ian Sommerville answered him and told him that he had built a simple machine to create light pulses with a perforated paper cylinder and a plate spinning at 78 rpm. They experimented with several different cuts for the machine which Gysin then named the Dreamachine. The results of their experiments were published in the second issue of Olympia magazine, in January 1962.

The Dreamachine was a rotating cylinder with slits and a bulb in the center. The rotating cylinder causes the light emitted by the bulb to pass through the slits at a certain frequency. This has the property of plunging the brain into a state of relaxation and of producing visions in the user when they look at the Dreamachine with closed eyes, through their eyelids.

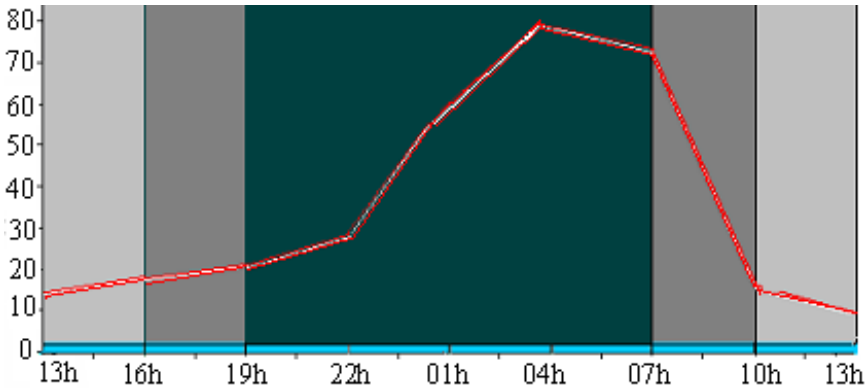
In its original form, a Dreamachine is made from a cylinder with slits cut in the sides. The Dreamachine cylinder is placed on a record turntable and rotated at 78 or 45 revolutions per minute. A light bulb is suspended in the cylinder and the speed of rotation and the number of slits causes the light emitted to pass through the slits at a constant frequency between 8 and 13 impulses per second. Gysin's research attracted the attention of several artists including the American writer William Burroughs, with whom he developed a simple flickering light system called the "Dream machine". Burroughs described it in

1960: "Subjects report dazzling lights and unearthly brilliance and color... Elaborate geometric constructions of incredible intricacy build up from multidimensional mosaic into living fireballs...or resolve momentarily into apparently individual images and powerfully dramatic scenes like brightly colored dreams." A series of scientific studies in the 1960s and 1970s demonstrated that the effects of flashing at certain frequencies appeared to have amazing powers. Different scientists found that this photic stimulation could have a number of beneficial effects, such as increasing the state of suggestibility, improving certain intellectual functions such as memory, and creating harmony in the mind. Some even talked at this time of a form of hemispheric synchronization. In France, starting in the 1950s and for over 30 years, Dr Lefebure carried out research on the impact of repeated light stimulation on the brain and, particularly, on the use of "phosphenism" in pedagogy and for psychic development. "Phosphenes" are subjective light sensations, that is, they are not produced directly by excitement of the retina by light, but by the brain itself after a period of exposure to a stable light source. Dr Lefebure was a true pioneer in the field.

Influence of the light on biologic rhythms is knowed since a century. The influence of flickering light is less known on biological rhythms. The purpose of this study was to analyze the influence of the PSIO flickering blue light (470 nm) on the secretion of melatonin, the associated sleep hormone.

Melatonin production curve

Linked to the daylight, the melatonin production curve begins around 9 – 10 PM, reaches its peak at around 3 AM and decreases radically its production from 7 AM to 10 AM. The study has tested the inhibition of the melatonin production just when the production curve is growing radically, thus at 10 PM.



Daily curve of melatonin secretion

Melanopsin Discovery

In 2002, David Berson at Brown University discovered the missing link: the ganglion cells in the inner retina (in front of the rods and cones) contained a photopigment called *melanopsin*. This newly discovered receptor matched perfectly with the 'action spectrum' of blue light. Since the discovery of melanopsin, the scientists have been able to trace its projections via the retinohypothalamic tract to the SCN in the hypothalamus and other areas in the brain. (1)

This discovery meant that the eyes also transmit light through a non-visual system, and studies comparing the visual against the non-visual system show that the blue-light melanopsin pathway was not only responsible for regulating our circadian system, but the alerting system as well.

Method

The purpose of this study was first to check the capacity of certain colored lights (emitted by the PSIO) to inhibit melatonin.

Groups

4 groups were proposed for the experimentation:

1. **GR 1** was a control group
2. **GR 2** was a placebo group
3. **GR 3** was an experimental group with blue continuous light
4. **GR 4** was a second experimental group with blue flickering light and synchronized sound stimulations.

Types of light stimulations per group

1. **GR 1** was a control group that was not exposed to any light nor sound stimuli.
2. **GR 2** was a placebo group that was exposed to continuous red light (670 nm) emitted by the PSIO.
3. **GR 3** was an experimental group that was exposed to continuous blue light (470 nm) emitted by the PSIO.
4. **GR 4** was a second experimental group that was exposed to a pulsed blue light (470 nm) and synchronized sound stimulations.

Gender Distribution

	Male Population	**Female Population**
Control Group	7	13
Placebo Group	8	12
Blue (continuous) Group	10	11
Experimental (pulsed blue) Group	10	10
Total	**35**	**46**

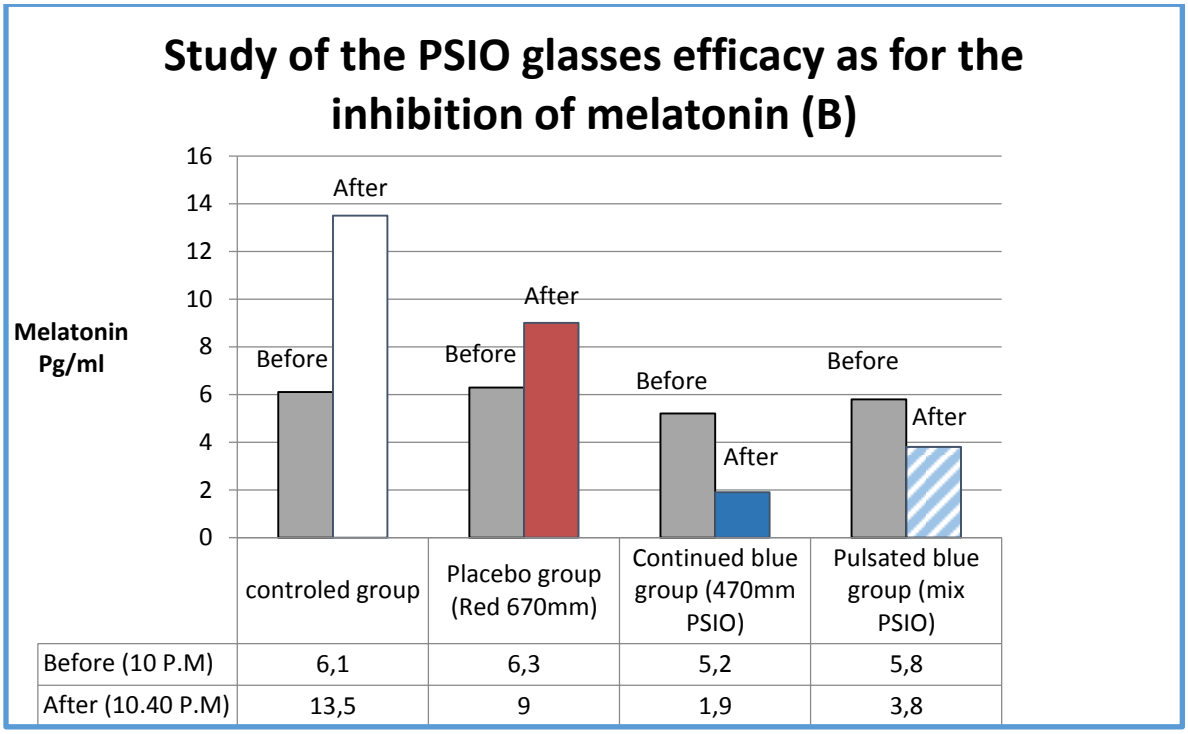
Timing

In collaboration with the laboratory of analysis we have designed the experimentation to take place at 10 PM in order to analyze the effects of light on the beginning of the ascendant curve of the secretion of melatonin. It has been established that a visible effect could be more easely observed if the participants are tested when the curve of melatonin is in the ascendant phase thus at 10 PM.

The participants usually arrived at the clinic at 9.45 PM. The first take of saliva began at 10 PM (T1). After a variable time of salivation depending on the participant, the experimentation would begin for a total time of 30 min. After the 30 min session, a second sample of saliva was taken (T2).

Results

1. A first analysis of the calculated averages shows these patterns of measures before experimentation (T1) and after experimentation (T2).



The above figure shows a normal increase in melatonin level for the control and placebo group from time 1 (before) to time 2 (after) but reveals a strong inhibition of the melatonin production for the continued blue and flickering blue groups from time 1 (before) to time 2 (after).

However, it was found during the study that the duration of salivation was very variable depending on the individual. It was therefore necessary to make an adjustment of the results taking into account the salivation time of each individual. In order to do this, the salivation time difference (End Test - Test Start - 30) was divided in two and we assumed that for the same subject, the two periods of salivation is the same.

The level of melatonin in the first measurement (baseline) is thus fixed up and the level of melatonin in the form of the second measurement is adjusted downward to adjust the duration of salivation (Var A = Var B). The figure below shows this adjustment.

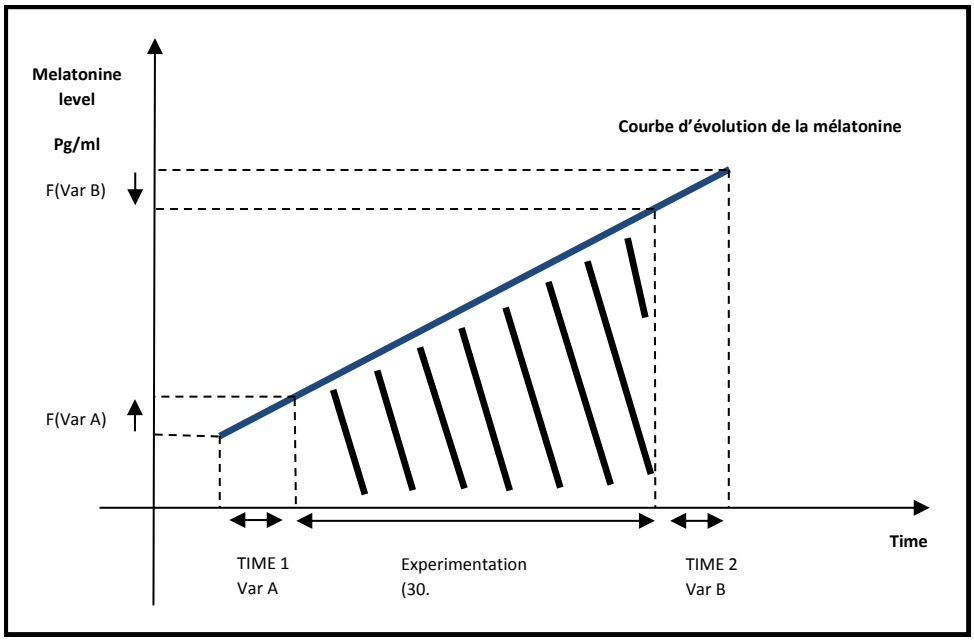
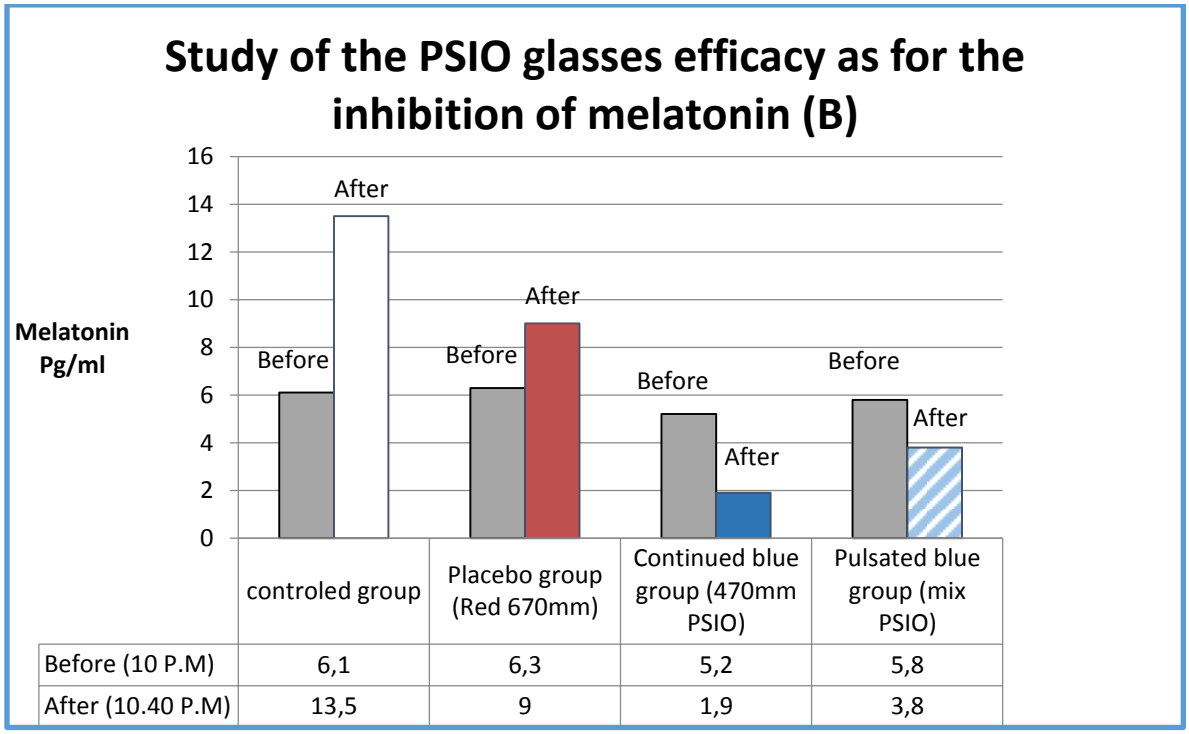


Diagram of the evolution of the curve of melatonin with variable salivation time

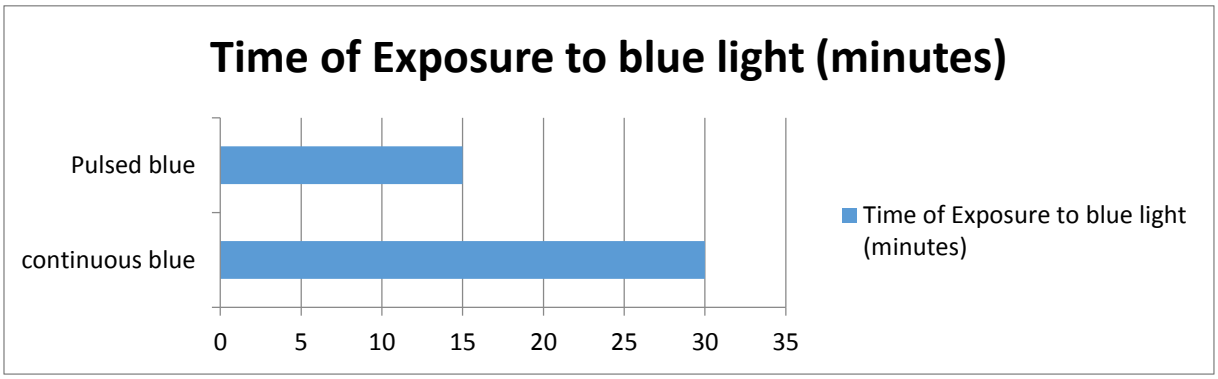
2. A second analysis has permitted to correct the results according to the salivation time which is slightly different among the test population.



Comments: Taking into account the results of the second analysis, we find that for the continuous Blue group (G3), the inhibition percentage of melatonin level from T1 to T2 is 63.4% and for the pulsed blue light group (G4), the inhibition percentage of melatonin level from T1 to T2 is 34.5%.

Group	Progression of Melatonin level from T1 to T2 for corrected values (%)
Control	121%
Placebo	43%
Continuous blue	**-63%**
Pulsed blue	**-34%**

However, despite the slightly lower inhibition of the blue pulsed light group compared to the group with continuous blue light, it should be noted that the actual time of exposure to blue light is halved during the pulsed blue stimulation as shown in the graph below.



Statistical analysis

Methodology

The objective is to compare the levels of melatonin after experimentation between the different groups namely the control group (G1), the placebo (G2), the experimental "continuous blue" group (G3) and the experimental "pulsed blue» group (G4). After statistical analysis, we observed that there is no real difference between melatonin levels at time 1 between the different groups. The results observed at time 1 are similar and comparable between the groups.

However, there is a heterogeneity in the distribution of the results around the observed mean for each group (shown below by the variance), but the statistical model takes into account the difference in variance.

Results

Descriptive statistics

The first table presents the mean levels of melatonin at baseline (T1), melatonin after experimentation (T2) and presents the mean levels of melatonin for the corrected values (salivation time), together with the variance on the measurement.

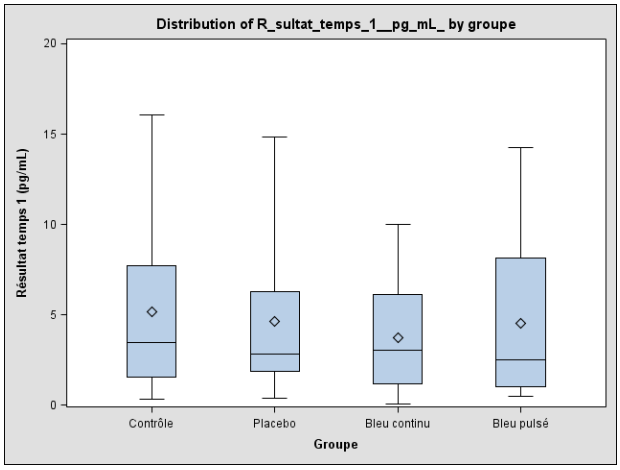
Group	NObs	Results Time 1 (pg/mL) Mean (Variance)	Results Time 2 (pg/mL) Mean (Variance)	Results Time 1 corrected (pg/mL) Mean (Variance)	Results Time 2 corrected (pg/mL) Mean (Variance)
Control	20	5.2 (25.55)	14.4 (146.10)	6.1 (25.79)	13.5 (150.96)
Placebo	20	4.7 (17.66)	10.7 (68.22)	6.3 (17.79)	9.0 (68.20)
Continuous blue	21	3.7 (8.99)	3.3 (9.49)	5.2 (10.34)	1.9 (8.44)

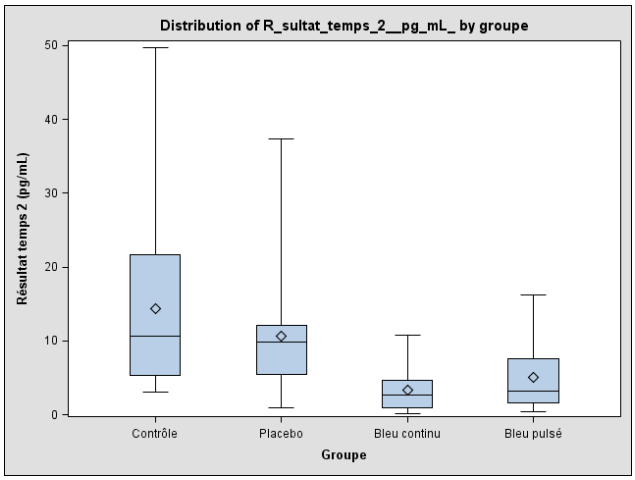
Group	NObs	Results Time 1 (pg/mL) Mean (Variance)	Results Time 2 (pg/mL) Mean (Variance)	Results Time 1 corrected (pg/mL) Mean (Variance)	Results Time 2 corrected (pg/mL) Mean (Variance)
Pulsed blue	20	4.5 (18.84)	5.1 (22.51)	5.8 (18.89)	3.8 (23.11)

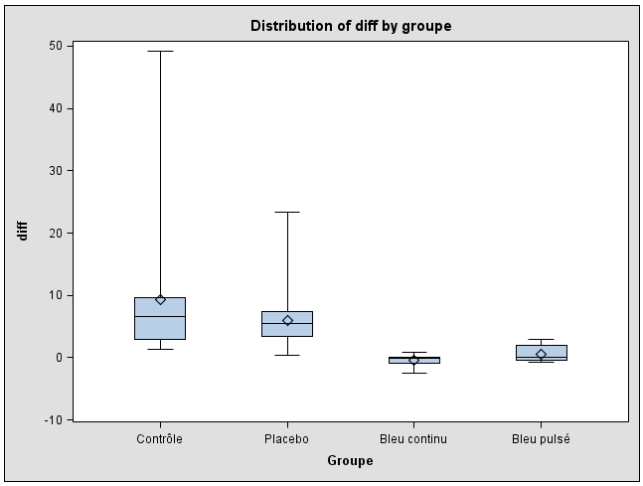
The boxplots below show the distribution of values collected for each group, and the calculated average (represented by a diamond-shaped dot) and median (represented by a line which cuts the set of values into two equal groups) for both non-corrected and corrected values of melatonin.

The first three diagrams show for each group

- the distribution data at time 1 (T1) (pg/mL)
- the distribution data at Time 2 (T2) (pg/mL)
- the difference in the level of melatonin between time 2 and time 1. The difference is calculated for each individual as follows: level of melatonin time 2 - level of melatonin time 1 (pg/mL)

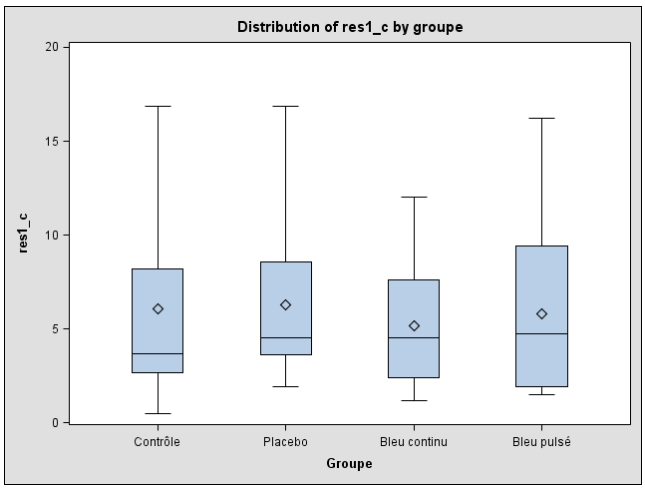


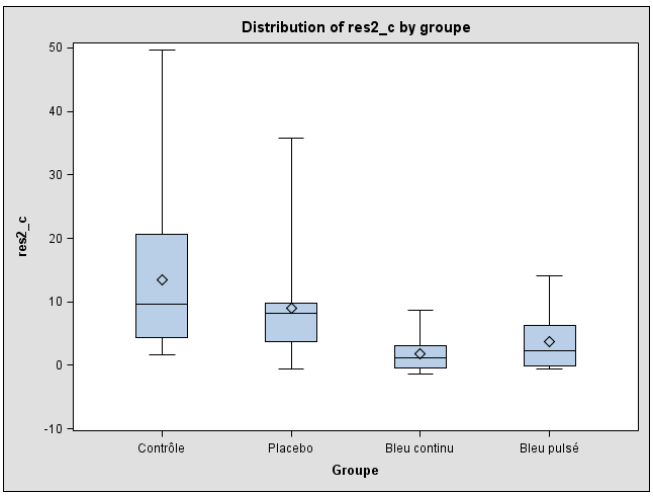


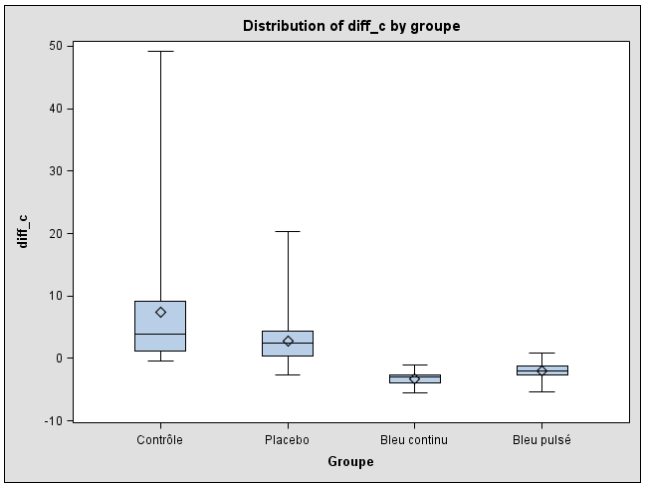


The three diagrams below show for each group

- the distribution data of the corrected results time 1 (pg/mL)
- the distribution data of the corrected results time 2 (pg/mL)
- the difference of melatonin levels between Time 2 and Time 1. The difference is calculated for each person as follows: the corrected level of melatonin time 2 - corrected level of melatonin time 1 (pg/mL)







Comparison of melatonin levels (including corrected level) between groups

The comparison of melatonin levels will focus on three aspects:

1. Overall comparison of the results of time 1 with each group
2. Overall comparison of the results of time 2 with each group
3. Overall comparison of the differences between melatonin levels of time 2 and time 1 with each group

The results are compared to observe whether the difference found between the melatonin levels of each group is significant or if it is simply due to chance. We thus conducted a statistical test that allows us to obtain the p-value, which is the probability of getting the same value of the test if the null hypothesis were true (that is to say, if there is no real difference) and in this case, allows us to determine whether the observed difference is

1. due to chance alone and therefore does not exist in reality.

   Or

2. the direct result of a significant difference between groups.

The table shows the p-values of the overall comparison of different answers (see table) between the different groups.

	Result T1 (pg/mL) Mean (Variance)	**Result T2 (pg/mL) Mean (Variance)**	**Difference (temps2 – temps1) (pg/mL)**	**Result T1 corrected (pg/mL) Mean (Variance)**	**Result T2 corrected (pg/mL) Mean (Variance)**	**Corrected difference (temps2 – temps1) (pg/mL)**
P-value (overall comparison between groups)	0.6745	<0.0001	<0.0001	0.7859	<0.0001	<0.0001

Traditionally, if the p- value is less than the value of the pre-defined threshold (usually 5% or 1%), we reject the hypothesis that the difference is due to chance alone.

The table above shows that, for an overall comparison between the groups and a comparison in time:

1. The P-value of the column ‘Result T1’ is 0.6745 and it is 0.7859 for ‘Result T1 corrected’. This means that there is no significant difference between melatonin levels at baseline (time1) between groups (nor for the corrected nor for the uncorrected values of melatonin).
2. The P-value of the column ' Result T2’ is less than 0.0001, and is less than 0.0001 for ‘Result T2 corrected’. This therefore means that there is a significant difference (significance level of 5%) between melatonin levels after experiment (Time 2) between the different groups (for both the corrected and not corrected values of melatonin). However, further in the analysis, we will see that there is no significant difference between certain groups such as the placebo group (G2) and control (G3).

3. There is a significant overall difference between the difference in melatonin levels between T1 and T2 (changes over time) for the different groups.

The following graphs show the levels of melatonin in each group at time T1 (before) and at time T2 (after) for both the corrected and not corrected values of melatonin. It therefore represents the evolution of melatonin over time knowing that the testing time is 30 min.

When there is a significant 'p -value' for the overall comparison (value revealing if there is a real difference between the groups in levels of melatonin or the difference in melatonin level), it is interesting to see between which specific groups a significant difference exists.

Comparison of Results of Time 2 (T2) between each group

For the comparison of melatonin levels after the experimental period (both corrected and uncorrected values), there are significant differences between the following groups:

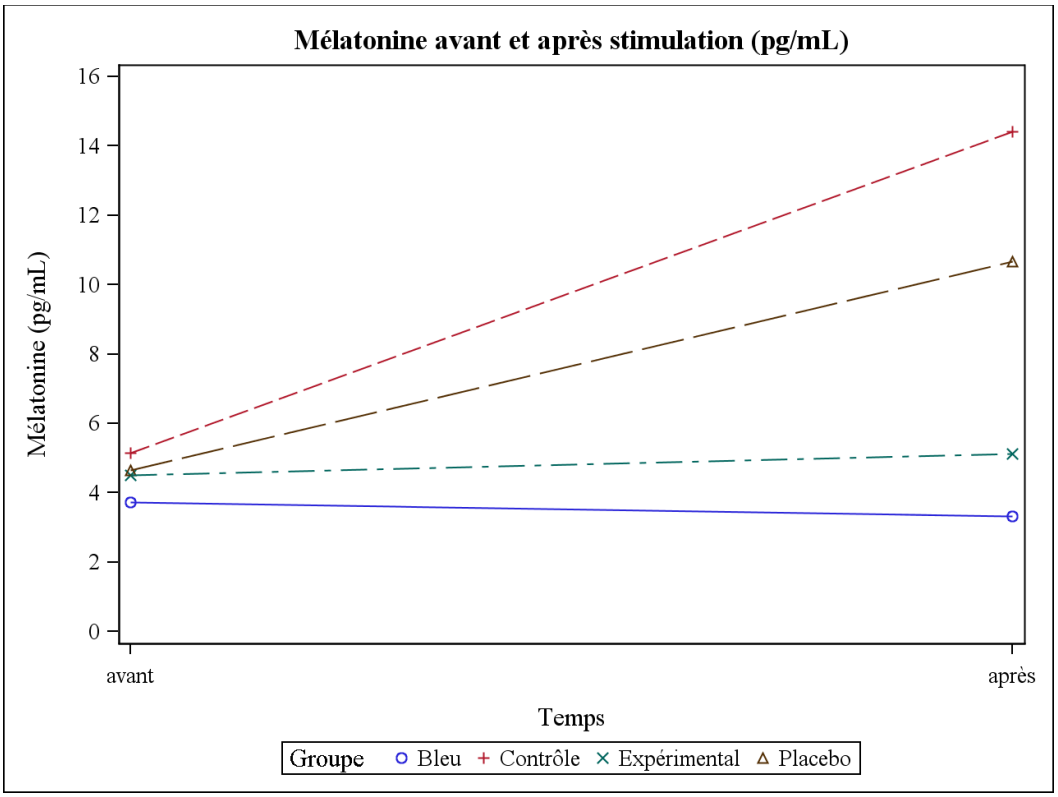
1. Continuous Blue Group (G3) and Control Group (G1)
2. Continuous Blue Group (G3) and Placebo Group (G2)
3. Pulsed Blue Group (G4) and the Control Group (G1)

Comparing results from time 1 to the time 2 between each group

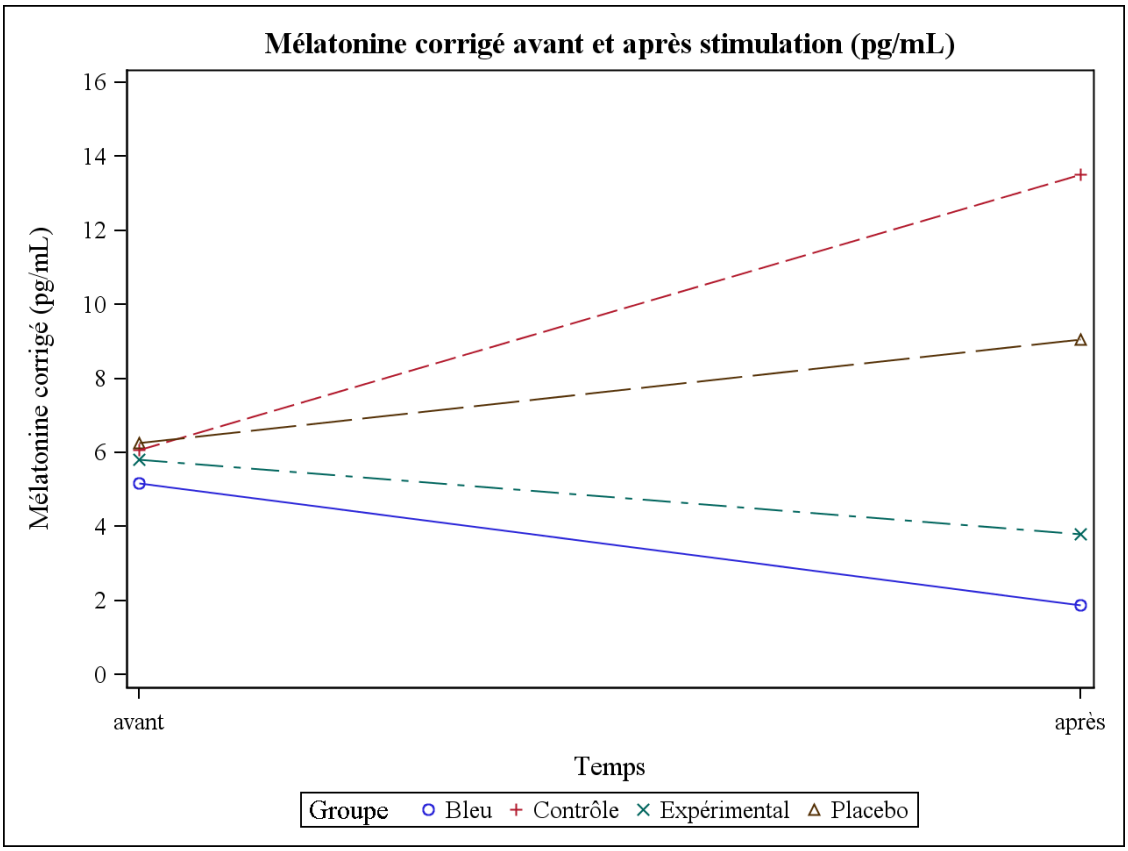
When comparing the difference in melatonin levels between T2 and T1 for each group (both corrected and uncorrected values) between them, significant differences between the following groups are highlighted

1. Continuous Blue Group (G3) and Control Group (G1)
2. Continuous Blue Group (G3) and Placebo Group (G2)
3. Control group (G1) and the Pulsed Blue Group (G4)
4. Pulsed Blue Group (G4) and the Placebo Group (G2)

Melatonin level before (T1) and after experimentation (T2) (Pg/ml)



Adjusted Melatonin level before (T1) and after experimentation (T2) (Pg/ml)



It can be seen from the above graphs that the corrected difference decreases for both the continuous blue group (G3) and the pulsed blue group (G4) while there is an increase in the level of melatonin for both control group (G1) and placebo group (G2).

In terms of percentage, the production of melatonin varies over time T1 → T2 for each group (the corrected values) as follows:

Group	**Melatonin progression from T1 to T2 for corrected values (%)**
Control	121%
Placebo	43%
Continuous blue	**-63%**
Pulsed blue	**-34%**

Linear regression analysis

Model with melatonin levels not corrected for duration of test

A regression model was created to study the effects of different variables on the outcomes of melatonin levels after experimentation (T2). These variables are:

1. Groups together
2. The reference level of melatonin which is the result T1
3. The interaction between a group and the reference level of melatonin in the same group

To assess levels of melatonin after experimental period (Result T2) between groups, the regression model is built where melatonin after experimentation is regressed on the three variables mentioned above.

After analyzing the results, a significant effect of the three variables on the melatonin levels after experimental period (Result T2) was observed, and shows that the differences are not due to chance.

The table below shows the results obtained by the regression model.

Least Squares Means

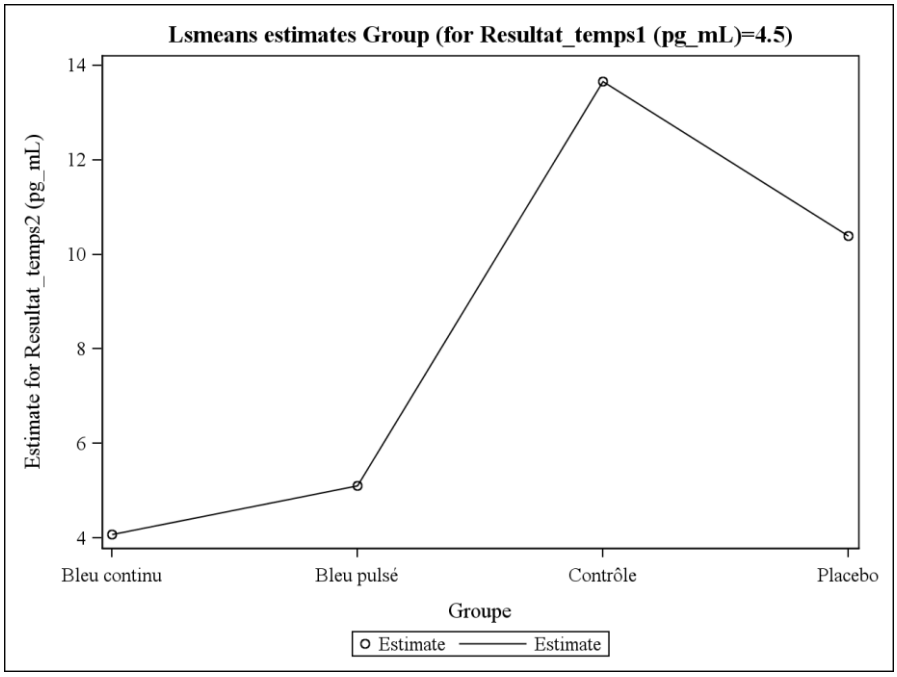
Effect	Group	R_sult_time_1__pg_mL_	Estimate	Standard Error	DF	t Value	Pr > |t|
Group	**Control**	4.50	13.6668	2.4692	73	5.53	<.0001
Group	**Placebo**	4.50	10.3926	0.8649	73	12.02	<.0001
Group	**Continuous blue**	4.50	4.0698	0.2198	73	18.51	<.0001
Group	**Pulsed blue**	4.50	5.1100	0.2635	73	19.40	<.0001

It should be noted that the level of melatonin at baseline (T1) is set at the global average concentration of 4.5.

For the control group, the average level of melatonin after experimental period is estimated to be equal to 13.67. For the placebo group, the average level of melatonin after experimental period is estimated to be equal to 10.39. For the 'Continuous blue' group (G3) the average is estimated at 4.07 while it is estimated to be at 5.11 for the 'Pulsed blue' group (G4).

The above results are very close to the observed average and are a confirmation of the validity of the model. The model is a good representation of reality.

Estimates of the average rates of melatonin groups are also presented in graphical form and show the difference of average melatonin T2 between each group.



In the table 'Differences of Least Squares Means', the estimated model differences between mean melatonin levels after stimulation in the different groups is calculated. The adjusted p-value (last column) indicates whether the difference between two groups is significant or not (when P-value <0.05, there is a significant difference between two groups)

Differences of Least Squares Means										
Effect	**Group**	**Group**	**R_sult_time_1__pg_mL_**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
Groupe	**Continuous Blue**	**Control**	4.50	-9.5970	2.4789	73	-3.87	0.0002	Tukey-Kramer	0.0013
Groupe	**Continuous Blue**	**Pulsed Blue**	4.50	-1.0402	0.3431	73	-3.03	0.0034	Tukey-Kramer	0.0173
Groupe	**Continuous Blue**	**Placebo**	4.50	-6.3228	0.8924	73	-7.09	<.0001	Tukey-Kramer	<.0001
Groupe	**Control**	**Pulsed Blue**	4.50	8.5568	2.4832	73	3.45	0.0009	Tukey-Kramer	0.0051
Groupe	**Control**	**Placebo**	4.50	3.2742	2.6163	73	1.25	0.2148	Tukey-Kramer	0.5965
Groupe	**Pulsed Blue**	**Placebo**	4.50	-5.2826	0.9041	73	-5.84	<.0001	Tukey-Kramer	<.0001

In the column 'Estimate', the estimated difference is presented. For example, for the first line, the average difference in the melatonin group after experimentation between 'continuous blue' group and 'control' group is equal to -9.60 and the adjusted P-value (Adj P – last column) indicates 0.0013. This therefore means that the difference between the "continuous blue" group and the control group is real considering that the adjusted P-value is close to 0.

It is easy to see that all differences are significant, except from the difference in the level of melatonin after experimental period between the control group and the placebo group which is not significantly different, adjusted P = 0.6. Melatonin level reference is attached to the overall average concentration of 4.5.

Model with melatonin levels adjusted for duration of test

In the adjusted regression model, the salivation variables are taken into account. It is assumed that for a same participant, the 2 variables of salivation duration are equal.

After taking into account of the salivation variables in the regression model (Var A = Var B), a significant effect of the studied variables on corrected melatonin levels after experimentation (T2 corrected results) was observed and shows that the differences are not due to chance.

The table below shows the results obtained by the regression model and shows a slight difference in melatonin levels observed compared with the 'uncorrected' regression model.

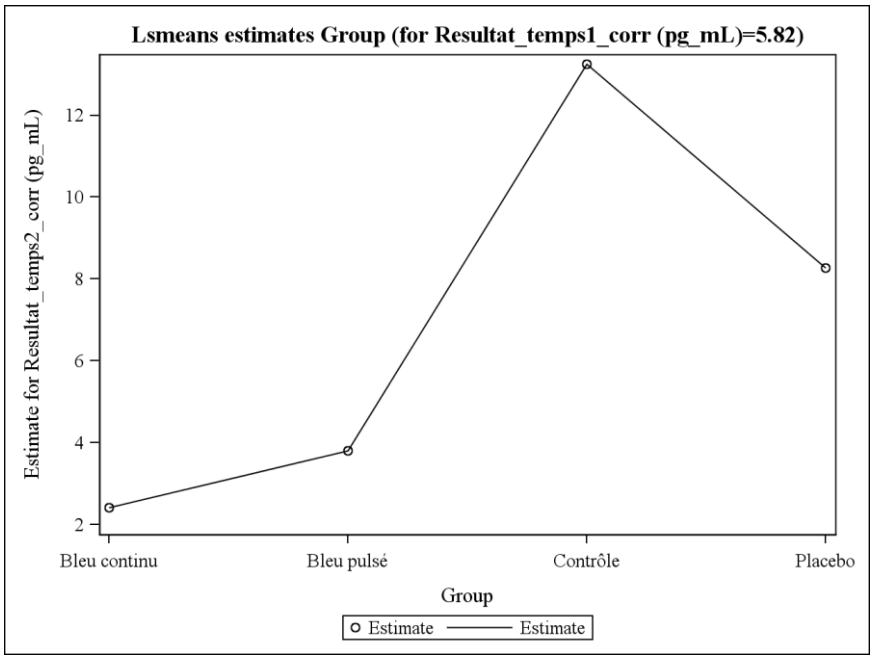
Least Squares Means

Least Squares Means							
Effect	**Group**	**res1_c**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**
Group	**Control**	5.82	13.2509	2.5626	73	5.17	<.0001
Group	**Placebo**	5.82	8.2685	0.8863	73	9.33	<.0001
Group	**Continuous Blue**	5.82	2.4152	0.2531	73	9.54	<.0001
Group	**Pulsed Blue**	5.82	3.8060	0.3745	73	10.16	<.0001

It should be noted that corrected levels of melatonin at baseline (T1 corrected) are set at the overall average concentration of 5.82 (sum of the average time 1 divided by 4).

For the control group, the average corrected level of melatonin after experimental period is estimated to be equal to 13.25 (against 13.67 for the uncorrected model). For the placebo group, the average adjusted melatonin level after experimental period equals 8.27 (against 10.39 for the uncorrected model). For the continuous blue group (G3) this average is estimated at 2.42 (against 4.07 for the uncorrected model) while it is estimated at 3.81 (against 5.11 for the uncorrected model) for the pulsed blue group (G4).

Estimates of the corrected average rates of melatonin groups are also presented in graphical form and show the difference of average melatonin T2 between each group.



In the table 'Differences of Least Squares Means', the estimated model differences between corrected mean melatonin levels after experiment period in the different groups is calculated. The adjusted p-value (last column) indicates whether the difference between two groups is significant or not (when P-value <0.05, there is a significant difference between two groups)

Differences of Least Squares Means										
Effect	**Group**	**Group**	**res1_c**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adjustment**	**Adj P**
Group	**Continuous Blue**	**Control**	5.82	-10.8357	2.5751	73	-4.21	<.0001	Tukey-Kramer	0.0004
Group	**Continuous Blue**	**Pulsed Blue**	5.82	-1.3908	0.4520	73	-3.08	0.0029	Tukey-Kramer	0.0153
Group	**Continuous Blue**	**Placebo**	5.82	-5.8534	0.9217	73	-6.35	<.0001	Tukey-Kramer	<.0001
Group	**Control**	**Pulsed Blue**	5.82	9.4449	2.5898	73	3.65	0.0005	Tukey-Kramer	0.0027
Group	**Control**	**Placebo**	5.82	4.9824	2.7116	73	1.84	0.0702	Tukey-Kramer	0.2644
Group	**Pulsed Blue**	**Placebo**	5.82	-4.4625	0.9622	73	-4.64	<.0001	Tukey-Kramer	<.0001

In the column 'Estimate', the estimated difference is presented. For example, for the first line, the corrected average difference in the melatonin group after experimental period between ‘continuous blue' and 'control' group is equal to -10.84 and the adjusted P-value (Adj P – last column) indicates 0.0004. This therefore means that the difference between the "continuous blue" group and the control group is real considering that the adjusted P-value is close to 0.

It is easy to see that all differences are significant, except from the difference in the level of melatonin after experimental period between the control group and the placebo group which is not significantly different, adjusted P = 0.26. Melatonin level reference is attached to the overall average concentration of 5.82.

Statistical Conclusions

1. There is hardly any difference between the results obtained on the melatonin levels as reported and the melatonin levels corrected for test duration.

2. For both analyzes (models before and after adjustment) differences in melatonin levels after experimentation (at time T2) between the different groups (overall comparison) are significant and are therefore not due to chance, with the exception of differences between the groups below which are statistically not significant and are therefore due to chance:

    a. the placebo group (G2) and the control group (G1)
    b. the " continuous blue " group (G3) and the " pulsed blue " group (G4)

3. It should be noted that the continuous blue group (G3) and the pulsed blue group (G4) both react with a strong inhibition of melatonin secretion. However, while the continuous blue group (G3) recorded a decrease in melatonin levels over a period of 30 minutes, the pulsed blue group (G4) recorded a similar decline for an exposure time to blue light divided by two (LED is “on” 50% of the time and “off” 50 % of the time).

Final Conclusions

In summary, we can conclude that the PSIO strongly inhibits the secretion of melatonin, with both stimulation of blue continuous light and pulsed blue light. There was no placebo effect in this experiment.

In conclusion, the results show that stimulation with pulsed blue light obtained a similar inhibition as stimulation with continuous blue light. However, the time of exposure to blue light was reduced by half (at the **minimum)** with pulsed blue light stimulation (light pulsation implies that diodes are "on" 50% of the time and "off" 50% of the time). Furthermore, part of the session (probably 50 %) took place with eyes closed due to a major 'letting go' from the participant that appears automatically with light pulsation. Therefore, inhibition of the melatonin level with pulsed blue light could be similar to that of blue continuous light, taking into account that exposure time to blue light could be of only 25% compared with blue continuous light. A new study comparing a group receiving the stimulation session with eyes closed during the time of the test should be the next experiment.

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