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Modification of Polychromatic Linear Polarized Light by Nanophotonic Fullerene and Graphene Filter Creates a New Therapeutic Opportunities

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Abstract: Application of the linear polarized polychromatic and monochromatic low-intensity light on the locus of pain or acupuncture point effectively reduces pain. The purpose of this article is to describe what changes in the analgesic effectiveness of polarized light modified by fullerene or graphene filter will occur. The essence of such a change consists in optimizing the distribution of quanta in the light flux (hyperpolarization). In the model of inflammatory somatic pain (formalin test on mice), we investigated the influence of the BIOPTRON device light, which passed through a fullerene or graphene filter, on the intensity of the pain reaction (licking of the affected limb) and on the painless behavior (sleeping, washing, eating, running) of the examined animals. It was established that a 10-minute application to the inflammation locus or to E-36 acupuncture point of fullerene light caused a significant reduction of pain. Analgesia was 43.5% and 38.5%, respectively. All non-painful behavioral reactions (sleeping, washing, running, eating) increased their duration. Duration of sleep compared with the control increased twice (application of light to the locus of inflammation) and 3 times (application to acupuncture point E-36). Light modified by a layer of graphene, when applied to the point of acupuncture E-36, weakened the pain more than if applied to the inflammation locus. Analgesia was 58.9% and 49.5%, respectively. Among the non-painful behavioral reactions, the most significant changes have undergone sleeping and eating behavior. Duration of sleep compared with the control increased 4 times (locus of inflammation) or 2.35 times (acupuncture point E-36). Comparison of these data with the results obtained for monochromatic polarized light ranges showed that they could be attributed to the most effective group of analgesic light factors. Both fullerene and graphene light, along with analgesic, have a powerful sedative effect, surpassing effects in comparison with all other ranges of polarized light. Polarized polychromatic light of the BIOPTRON device, which has passed through a nanophotonic fullerene or graphene filter, acting onto the pain or acupuncture point E-36, significantly reduces pain and has a sedative effect.

Key words: Fullerene, graphene, polarized light, hyperpolarization, Bioptron device, pain, formalin test, analgesia, acupuncture point.

1. Introduction

The biological effect of light depends on its wave range and power (frequency and amplitude of electromagnetic waves), type of polarization, exposure and total radiation dose. Different tissues have a different response threshold. Therefore, a well-known fact about the preventive or curative effect of sunlight at close examination cannot always be considered proven and not applicable for all pathological processes. In this regard, attempts are continuing to search for such variants of visible electromagnetic radiation (light), which would have a reliable biological effect and was methodologically simple for medical use.

The positive effect of natural sunlight (diffuse light) during the healing of wounds is known even from antiquity. Artificial analogue of sunlight (electric arc lamp), applied by N.R. Finsen [1, 2], turned out to be effective in the treatment of skin diseases, such as lupus. The use of individual components of the solar palette (for example, red or blue light in combination with

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infrared radiation) has revealed the possibility of treating colds and inflammatory diseases of the nose, throat and ear. The use of linear polarized light, which became possible after the creation of laser devices (LASER—Light Amplification by Stimulated Emission of Radiation), proved to be effective in the treatment of diseases and injuries of the skin, mucous membranes [3-5]. At present, in the therapeutic practice, applications of low-intensity monochromatic laser light of various wave ranges from visible to infrared have become popular [6-10].

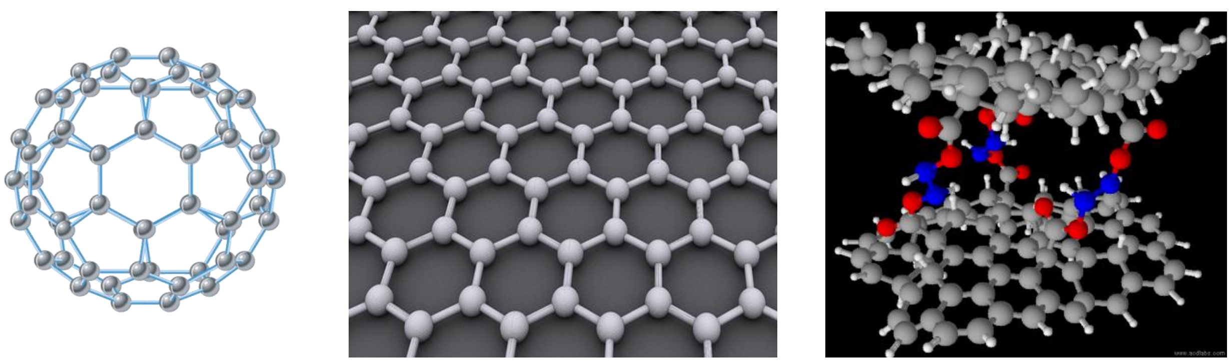
The next step in the development of light therapy was the use of BIOPTRON devices that produce polychromatic linear polarized light: PILER (Polarized Incoherent Low Energy Radiation) [11]. Studies of this version of light have shown that it has a pronounced biological activity, and this property can be successfully applied in medical practice [12].

To ensure the adequacy and reliability of the detection of biological effects caused by exposure to light, we used pain syndrome and response behavioral reactions obtained in animals. This approach leveled the psycho emotional reactions inherent in man. In experiments on the model of formalin-induced pain in animals, it was proved that when applied to the locus of painful inflammation or to the acupuncture point (AP), PILER-light (PL) caused analgesia [13-21]. At equal exposure, the effect significantly depended on the wave range of the light radiation. The flow of polychromatic light could be modified by a filter installed at the output of the BIOPTRON device, resulting in the production of light with a certain wavelength, both polychromatic and monochromatic. We experimentally established the fact of PL anti pain influence, passing through red, orange, yellow, green, blue, dark blue and violet filters. It was found that "warm" colors, especially red, more effectively suppress the inflammatory pain reaction in comparison with the "cold" [20, 21].

In recent years, new materials have appeared that can change, at the nano level, the properties of light passing through them. Of particular interest are the allotropic forms of carbon discovered in the late 20th century: fullerene [22] and graphene [23]. The fullerene molecule (Fig. 1A) has the form of a spherical polyhedron (truncated icosahedron). Carbon atoms in the amount from 20 to thousands combine into molecules, the most stable of which is the C_{60} form. Graphene has a flat network structure (Fig. 1B), consisting of 1-2 layers of interconnected carbon atoms.

One of the properties of the materials containing fullerene or graphene is the ability to influence the light flux. Accordingly, such light can acquire additional properties [24-26].

There is experimental proof that molecule C_{60} transforms incoming light into out coming light [27] and it is shown [28, 29] that the configuration of the quantum-wave structure of light passing through organized carbon molecules (fullerene, C_{60}) in layers can vary. Preliminary preparation of light consists in its vertical linear polarization. It is achieved by reflecting the scattered diffuse light at the Brewster angle from



A B-1 B-2

Fig. 1 Structure of the fullerene C_{60} molecule (A) and graphene molecule as single- and two-layer versions (B-1 and B-2).

the multilayered glass (Bioptron device). Obtained in this way the diffuse polychromatic (halogen) light is transformed into vertically linearly polarized light (PILER). Then it passes through a layer of organic nanophotonic glass (with defined thickness), which is composed of well-ordered fullerene nanostructures. As a result, an additional modulation of photons angular momentum and order of photons electromagnetic fields, the light flux becomes hyperpolarized. Vertically linear polarized light sequentially is changed into horizontal linear polarized light by Fibonacci order—"sunflower" (Fig. 2). It is happened because 20 hexagons (paramagnetic) of C_{60} generate rotation of polarized photons plane, while 12 pentagons (diamagnetic) that rotation make sequentially by Fibonacci law [29]. This is due to the fact that the carbon atoms entering into the C_{60} molecule can affect the trajectory of light quanta by its own electromagnetic oscillations, subjected to the influence of the icosahedral symmetry of the shell. Rotating at a speed of 1.8×10^{10} sec^{-1}, the spatial network of carbon atoms, entering the C_{60} molecule, in accordance with the Fibonacci-Tesla distribution law ($\Phi^2+\phi^2=3$), redistributes the light electromagnetic flux. (This redistribution is described by a numerical sequence in which each successive member of the sequence is equal to the sum of the two previous ones, that is: 1, 1, 2, 3, 5, 8, 13, 21, 34, 55, 89, 144, 233, 377, 610, 987 ... As a result, as the number increases, the ratio of the two neighboring members of this sequence asymptotically approaches the exact proportion of the Golden Section, 1:1.61803).When light passes through a 2-mm fullerene material (10^6 layers of C_{60} molecules—each with 20 hexagons and 12 pentagons), there occur 10^{18} changes of quant motion, making perfect randomization of photons with probability 1 to generate sunflower shape (1/0.61803.. = 1.61803…). With consideration of the rotation of C_{60} molecule, at the end it is created a new more sequenced spiral configuration of the flow. The symmetry obtained in this way symmetry of quanta flux is approaching symmetrical characteristics of biomolecules. It creates mirror resonant effect with opportunities for more efficient use (absorption) of each extending quantum—"the structural lights meets the structural matter" [30].

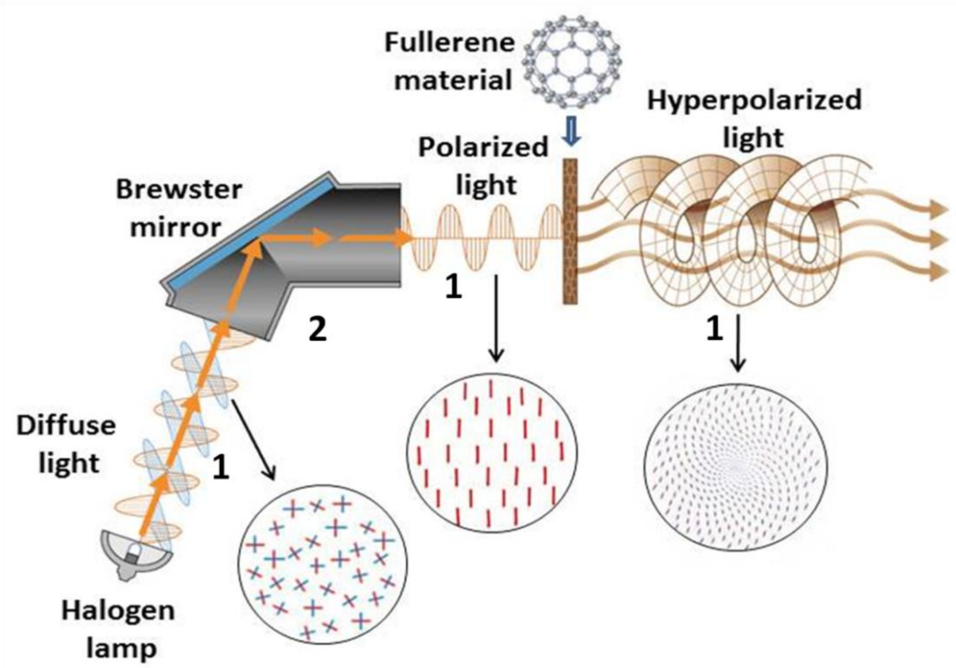


Fig. 2 Scheme of converting diffuse light into vertically linearly polarized and hyperpolarized (in accordance with [28, 29]): (1) scheme of oscillations of vectors of light electromagnetic waves, (2) polarizer of the BIOPTRON device. Differences in the vector structure of the light fluxes are shown separately (circled).

Peculiarities of the interaction of graphene with transmitted light are that graphene almost does not absorb visible light, its transmittance exceeds 97%. Graphene is characterized by a giant Faraday rotation (Fig. 3).

It is known that the Faraday angle (the angle to which rotates plane of polarization of the electromagnetic wave [31]) is proportional to the thickness of the material through which the light passes. As is known, graphene has thickness of only one carbon atom. It is experimentally proved [32] that the effect of rotation of plane of the light polarization in this material reaches 0.1 radian (i.e. approximately 6 degrees). It was expected to see the described effect in practice, but it was assumed that this would be no more than 0.01 radian. The discovered ability of rotation of polarization surprised the authors, because such a large angle of Faraday is usually characterized for thicker materials. Presumably this is due to the free carriers of current in grapheme (hexagons), whose behavior is similar to relativistic particles that do not have mass at rest. When "including" an external electric field in such material, the electrons behave differently than in other substances, which lead to enlargement of the Faraday angle.

Since there is evidence that the light of different wave ranges creates a different biological effect, it is of interest to determine how the light flux modified by the fullerene (composed of dynamical two-layer: hexagons and pentagons) [28] or static two-layer graphene (hexagons) [30] filters will act. To evaluate the biological effectiveness of the PL, which passed through the carbon nano layers (fullerene and graphene), we selected an experimental model of tonic pain caused by subcutaneous administration of formalin.

The purpose of this work was to establish in experiments on laboratory animals whether the PL has an analgesic effect, passing through a fullerene or graphene filter. On the model of chemically induced inflammatory pain (formalin test), we examined how changes the painful and non-painful behavior of animals after application to the focus of pain or to AP E-36 of carbon-modified light of the BIOPTRON device. The result was compared with the effects of red light of the same device, as it was previously observed that it caused the most significant analgesic effect. To determine the general analgesic rating position of the

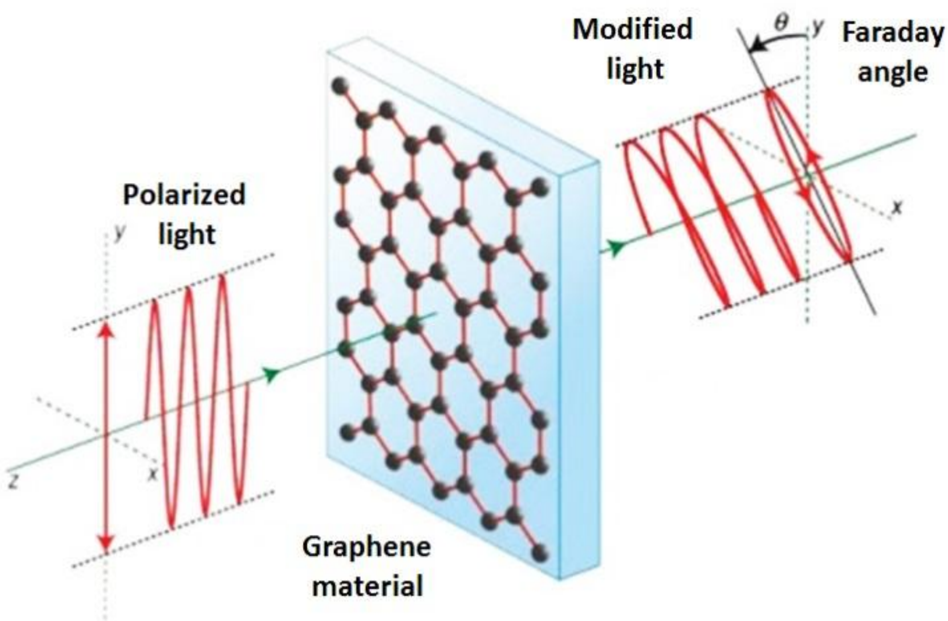


Fig. 3 Schematic representation of the vertically linearly polarized light transformation into a rotating one, while passing through a graphene layer [29].

"carbon" light, we compared the intensity of biological reactions with the results of other monochromatic (orange, yellow, green, blue, dark blue and violet) polarized light ranges obtained by a similar method.

2. Materials and Methods

2.1 Animals

The study was performed on adult white mice weighing 27-33 g, kept in the vivarium of Bogomoletz Institute of Physiology at the National Academy of Sciences of Ukraine and adapted to the conditions of the experiment. The mice were housed in individual plastic cages (36 × 24 × 5 cm) and had easy access to water and food. The animals were kept under controlled temperature conditions (18-20℃) and a 12-hour light day. The day before the experiment, the cells were introduced into the experimental room for adaptation of animals. It is known that nociceptive sensitivity in mice varies within a day [33], therefore all experiments were conducted at the same time of daylight (between 10:00 and 13:00). Each mouse was used only in one experiment. After the experiment, the mouse received a lethal dose of urethane (intraperitoneally). The experiments were carried out in accordance with the ethical guidelines recommended by the International Association for the Study of Pain.

The use of the same type of experimental conditions and the absence of psychological factors that occur in human studies have made it possible to quantify the pain intensity before and after light applications and to perform a correct statistical comparison of the results of different series. Each series included 10-15 animals.

2.2 Creating a Locus of Pain

The locus of inflammation was created by subcutaneous injection of 30 μl of a 5% formalin solution (in 0.9% NaCl solution) to the rear surface of the left hind paw (formalin test). At the injection site, there formed a locus of inflammation, which was a source of pain for several hours, especially intense in the first 60 minutes. Formalin-induced tonic somatic pain is well described in the literature and is widely used to determine the effectiveness of analgesic action of various substances or physiotherapeutic factors [34-36]. The intensity of pain was judged by the duration of licking of the affected leg for consecutive 10-minute intervals after light application during 60 minutes of observation. Pain reaction to formalin consists of two phases. The early phase is acute pain (it lasts no more than 10 minutes) and the late phase is tonic pain (it lasts more than one hour). In our experiments, immediately after the injection of formalin solution, a 10-minute application of light was made, during which the animal was in a special chamber, with partially limited motor activity. Therefore, the first phase of the pain reaction could not been observed. In the future, we will only talk about the second (tonic) phase of the pain. Non-painful behavioral reactions (sleeping, washing, running and eating) were recorded as well.

2.3 Light Applications

Immediately after injection of formalin, experimental animals received on the locus of inflammation or on AP E-36 application of BIOPTRON device light, which passed through a fullerene or graphene filter. AP E-36 is one of the most commonly used to suppress pain in acupuncture. Because of the small size of the animal, a special light-tight nozzle was used, which provided a 5 mm diameter of the light spot. The exposure to light in all experiments was 10 min. The distance from the filter was 5 cm. During the exposure to PL, the animals were in a round plastic chamber with a hole for the left hind paw.

The BIOPTRON-Compact device was a source of halogen radiation converted into a polarized flow by means of a Stoletov glass pile located at a Brewster angle. This device produces polychromatic linearly polarized, incoherent light with wavelengths from 480 to 3400 nm (320-3400 nm without UV filter) with a power density of 40 mW/cm^2 (for a distance of 10 cm). We also used seven color filters from Bioptron Color Therapy Set. The fullerene filter is a 2 mm thick of polymethylmetacrylate organic glass in which, using a special technology that guarantees uniform distribution, a fullerene powder was introduced in concentration of 0.3‰ (C_{60}@PMMA). The power density of the BIOPTRON-Compact light, which passed through the fullerene filter, was 55-65 mW/cm^2 (for a distance of 5 cm). The fullerene filter in due to the presence of opaque particles weakened the passage of the violet-blue part of the light range (Fig. 4). A graphene layer of 2 molecules thick was deposited on the plate of 0.2 mm thick and did not affect the power of the transmitted light flux.

Control (placebo) was a series of experiments on animals with pain reaction to formalin without exposure to light, but all other conditions being equal. After the end of the light application (experimental groups) or simulation of light application (control group), the animals returned to their cells to observe painful and non-painful behavioral parameters within 60 minutes.

2.4 Statistic Analysis

With the help of a special computer program, the duration of pain and non-painful behavioral responses were calculated for every consecutive 10 min and for the entire observation period (60 min). Data are presented as mean ± SEM. To determine the statistical significance of the results, the Student t-test was used. The difference between the groups was considered statistically significant at $P < 0.05$.

3. Results

3.1 Effects of PILER Light Passing Through a Fullerene Filter

The biological efficiency of light, which passed through the nanophotonic fullerene filter, was tested on a model of formalin-induced inflammatory pain in mice. We determined how painful and non-painful behavioral reactions changed after local application of light to the acupuncture point E-36 or directly to the inflammation locus.

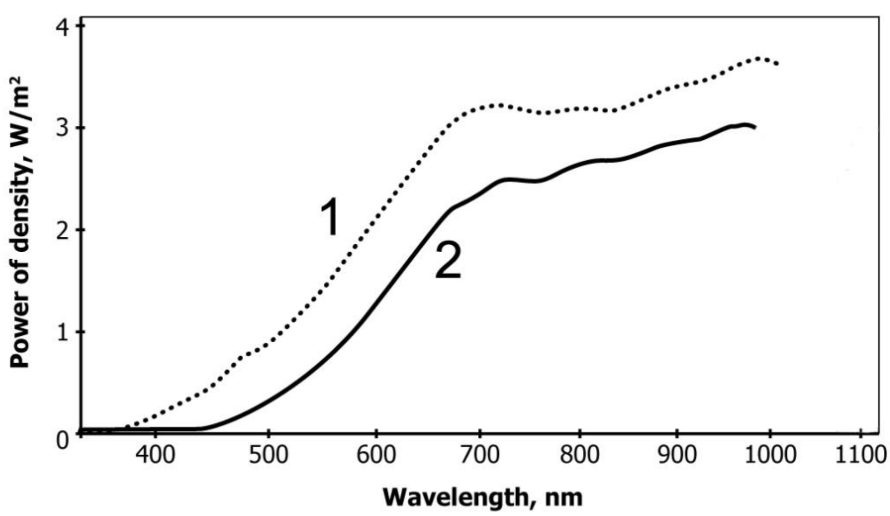


Fig. 4 Spectrum of light (BIOPTRON device) transmitted through a 2 mm thick polymethylmetacrylate layer containing 0.3‰ of fullerene (C_{60}@PMMA), measured by BLUE-Wave Spectrometer VIS-25 (measuring range 300-1100 nm): Horizontally—the wavelength in nanometers, vertical—radiation power of density in W/m^2; (1) without filter, (2) after passing through a fullerene filter.

3.1.1 Pain Responses

We have shown that the light of the BIOPTRON-Compact device with a fullerene filter weakens the pain. The dynamics of the development of the pain response in two experimental groups in comparison with the control group is shown in Fig. 5A. It can be seen that during application of light which passed through the nanophotonic fullerene filter to both the inflammation locus and AP-36, the pain response was weaker during the entire follow-up period than in the control group (without the use of light).

A comparison of total (for 60 min. of observation) duration of the pain reaction in the experimental and control groups (in 60 min of observation) (Fig. 5B, Table 1) showed that the nanophotonic fullerene light statistically significant reduces pain. For 60 minutes of

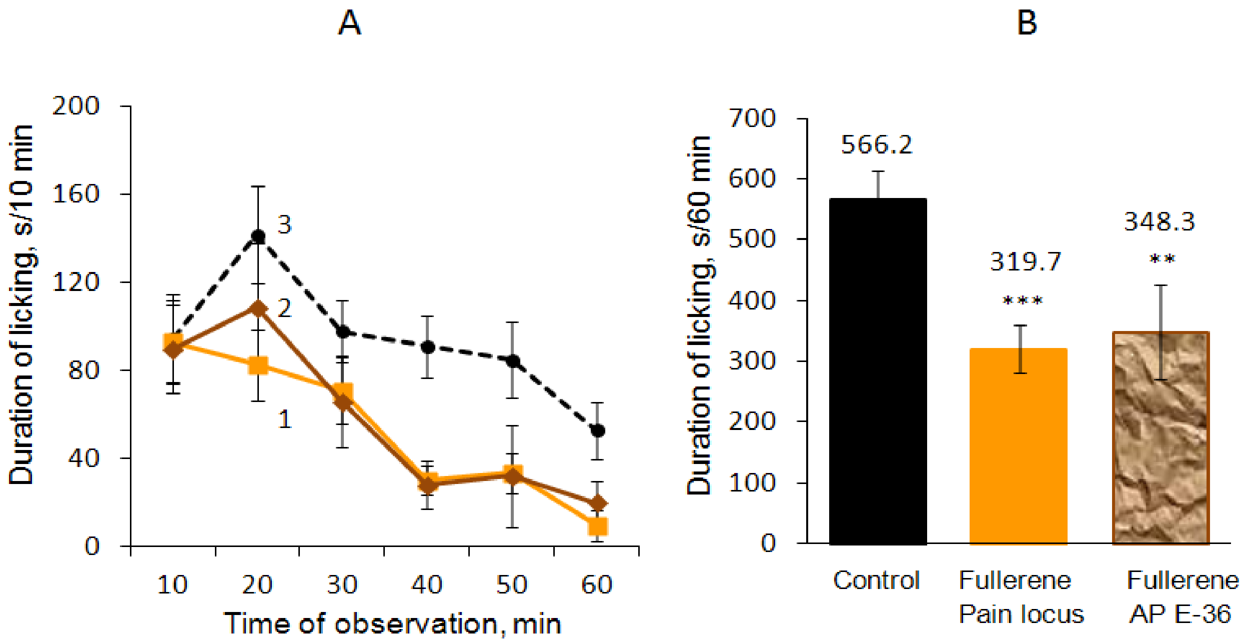


Fig. 5 Pain reactions (licking of the staggered hind limb) after 10 minutes application of the BIOPTRON device light, which passed through the fullerene filter, to the pain locus (1) or to AP E-36 (2) in comparison with the control (3): (A): Dynamics of the reactions in the three groups, (B) Total duration of pain during 60 minutes of observation in the three groups. The bars represent mean ± S.E.M. Numbers above the bars—the duration of pain reaction in seconds.

Significance of differences with the control: ***P< 0.001; **P< 0.05.

Table 1 Average values of the duration (s and % from control) of pain and non-painful behavioral reactions during 60 minutes in the control group (without application of light) and in two experimental groups in which the light of the BIOPTRON device, passing through the nanophotonic fullerene filter, was applied. Light influenced on the pain locus or on AP E-36 within 10 minutes from the distance of 5 cm.

Behavioral responses	Control (placebo)	BIOPTRON device with fullerene filter	
		Pain locus	AP E-36
Licking	566.2 ± 47.1 s 100%	319.7 ± 39.4 s *** 56.5%	348 ± 78 s** 61.5%
Sleeping	386.3 ± 79.3 s 100%	781.2 ± 214.6 s* 202.2%	1228 ± 178 s*** 317.9%
Washing	137.9±32.5 s 100%	231.7 ± 23.2 s** 168%	138 ± 36 s 100.3%
Running	65.5 ± 13 s 100%	114.7 ± 37.8 s* 175.2%	43 ± 13 s* 65.2%
Eating	1.1 ± 0.4 s	29.1 ± 16.4 s*	3.7 ± 3.7 s*

Significance of differences with the control group: ***P < 0.001; **P < 0.05; *P < 0.5.

(The rest is not significant).

follow-up, the duration of pain response in the control group (without the use of light) was on average 566.2 ± 47 sec. After application to AP E-36 or to the inflammation site of PL, which passed through the nanophotonic fullerene filter, the duration of pain was shortened to 348 ± 78 and 319.7 ± 39.4 sec, respectively. If the pain in the control group is taken as 100%, then in the experimental groups it was 61.5% (AP E-36) and 56.5% (locus of pain) from the control.

The anti-pain effect of the light, which passed through the nanophotonic fullerene filter, when applied to AP E-36, was slightly weaker than from a similar application to the locus of pain. Analgesia was 38.5 and 43.5%, respectively. However, these differences are not supported by statistical significance.

The anti-pain effect of nanophotonic fullerene light was comparable with the effect of the BIOPTRON-Compact device with a red standard filter. As has been shown in our previous studies [20, 21], red light also reliably weakened the pain response to formalin. The duration of pain was 54.3% of the control value (analgesia 45.3%).

3.1.2 Non-painful Reactions

Non-painful behavioral reactions (sleeping, washing, running, eating) after application of fullerene light also underwent significant changes (Table 1, Fig. 6). In the group in which the light was applied to the locus of pain, the duration of all non-painful reactions within 60 min of observation significantly increased in comparison with the control. After the action of light on AP E-36, the duration of washing did not change, and the running time was even shortened.

The most significant changes in both experimental groups showed sleeping and eating behavior. The total duration of sleep after application of light to the locus of pain increased twice, and for AP E-36-3 times. The duration of food intake increased from 1.1 sec in the control to 3.4 and 26 sec in the experimental groups. All this testifies not only to the easing of pain, but also to the significant sedative effect of the light, which passed through the nanophotonic fullerene filter.

3.2 Effects of PILER Light, Which Passed Through a Graphene Filter

In both groups, in which the polarized light passed through the graphene filter, all five behavioral responses differed significantly from the corresponding responses in the control group. We observed shortening

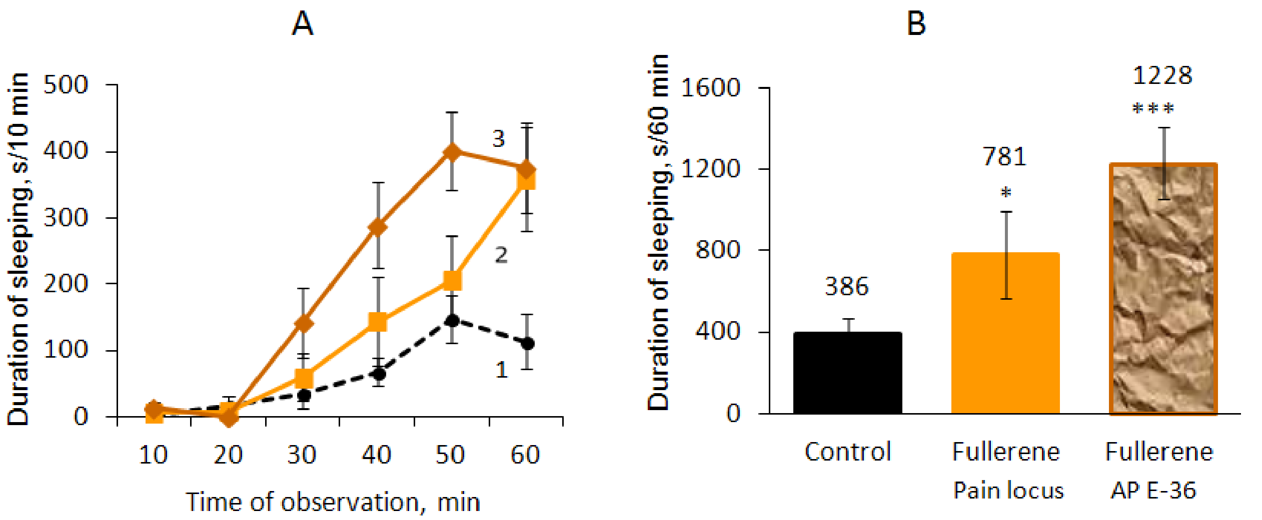


Fig. 6 Duration of sleeping after 10 minutes application of the BIOPTRON device light, passing through the nanophotonic fullerene filter, on the pain locus (2) or on the AP E-36 (3) in comparison with the control (1): (A): Dynamics of sleeping in the three groups. (B): Total duration of sleeping during 60 minutes of observation in the three groups. The bars represent mean ± S.E.M. Numbers above the bars—the duration of the sleeping in seconds.

Significance of differences with the control: ***$P < 0.001$; **$P < 0.05$.

of the duration of the pain reaction (licking of the locus of pain) and an increase in the time of non-painful reactions (sleeping, washing, running, eating).

3.2.1 Pain Responses

Throughout the follow-up period, the pain response in groups receiving applications to the locus of pain or AP-36 of graphene light was weaker than in the control group (without the use of the light). The total duration of pain in 60 minutes of observation was also significantly different from the control (Table 2, Fig. 7).

Experimental data showed that the application of graphene light both to AP E-36 and directly to the inflammation site, statistically significant ($P < 0.001$) weakens the pain. During 60 minutes of follow-up, the duration of pain reaction was, on average, 286 ± 46.8 s

Table 2 Average values of the duration (s and % from control) of pain and non-painful behavioral reactions during 60 minutes in the control group (without application of light) and in two experimental groups in which the light of the BIOPTRON device, which passed through the graphene filter, was applied. Light influenced on the pain locus or on AP E-36 within 10 minutes from the distance of 5 cm.

Behavioral responses	Control (placebo)	BIOPTRON device with graphene filter	
		Pain locus	AP E-36
Licking	566.2 ± 47.1 s 100%	286 ± 46.8 s*** 50.5%	232.5 ± 38.7 s*** 41.1%
Sleeping	386.3 ± 79.3 s 100%	1536.8 ± 151.8 s*** 397.9%	909.1 ± 191 s** 235.3%
Washing	137.9 ± 32.5 s 100%	240.4 ± 37.8 s* 167%	138.7 ± 23.4 s 100.6%
Running	65.5 ± 13 s 100%	24 ± 4.2 s** 36.7%	76.2 ± 12.9 s 116.3%
Eating	1.1 ± 0.4 s	21.3 ± 14.2 s*	33.3 ± 23.4 s*

Significance of differences with the control group: ***$P < 0.001$; **$P < 0.05$; *$P < 0.5$.
(The rest is not significant).

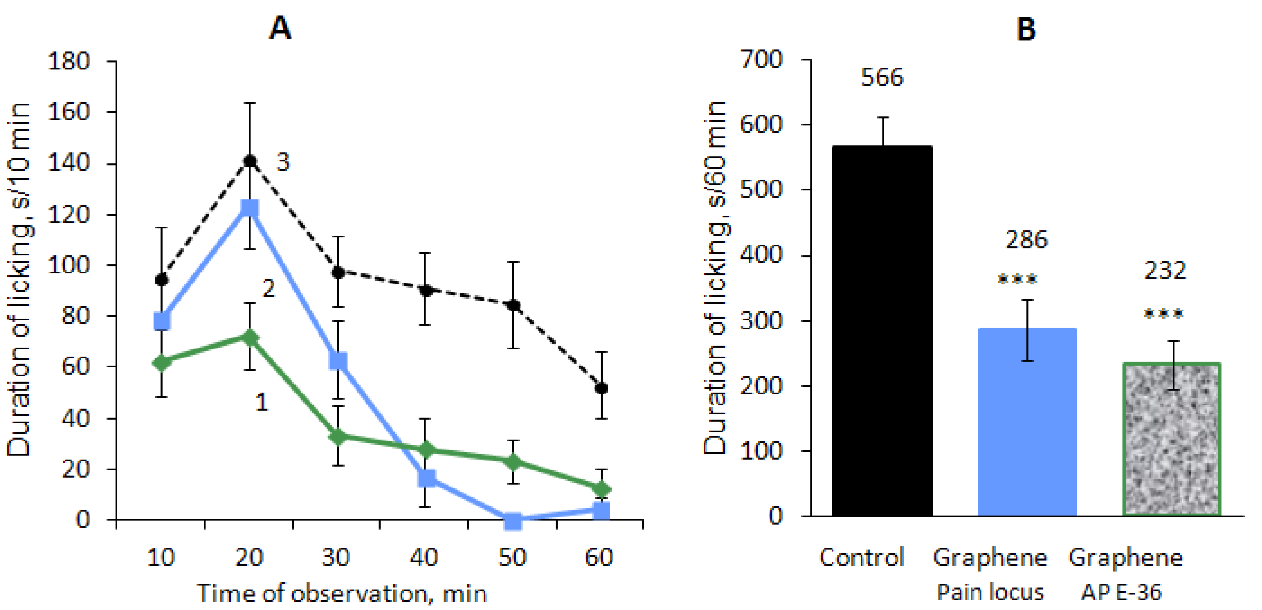


Fig. 7 Pain reactions (licking of the staggered hind limb) after 10 minutes application of the BIOPTRON device light, which passed through the graphene filter, to the AP E-36 (1) or to the pain locus (2) in comparison with the control (3): (A) Dynamics of the reactions in the three groups, (B) Total duration of pain during 60 minutes of observation in the three groups. The bars represent mean ± S.E.M. Numbers above the bars—the duration of pain reaction in seconds.

Significance of differences with the control: ***$P < 0.001$.

(locus of pain) and 232.5 ± 38.7 s (AP E-36), which was 50.5 and 41.1% of the control (566.2 ± 47 s = 100%). The analgesic effect of graphene light turned out stronger compared to the effect of the red standard and fullerene filter (Table 1).

3.2.2 Non-painful Reactions

Non-painful behavioral reactions (sleeping, washing, running, eating) after local application to AP E-36, or to the inflammation locus of polarized graphene light also underwent significant changes. The duration of these reactions within 60 min of observation increased in comparison with the control. The most significant changes demonstrated sleeping and eating behavior (Table 2, Fig. 8). The total duration of sleep increased by 2.35 times (application to AP E-36) and by 3.98 (to the locus of pain). The duration of food intake increased from 1.1 sec in the control to 21.3-33.3 s in the experimental groups. All this testifies to a significant sedative effect of light, which passed through the graphene filter.

The sedative effect of light which passed through the graphene filter turned out to be even more powerful than from fullerene light. In the latter case, the time of sleep was reduced only 2-3 times (with a graphene filter—a maximum 4 times).

3.3 Temperature Effects of Light with Carbon Filters

To check if the temperature factor influences the biological effects of light, we measured how the temperature of the surface affected by the light changed during 10 minutes of observation (the time of the light session). Using a light-tight nozzle with a hole of 5 mm, which is usually used in experiments, we sent the light spot to the thermometer. The temperature was registered every 2 minutes from switching on the light (Fig. 9).

Measurements made by a mercury laboratory thermometer showed that after 10 minutes of influence of the polarized light, which passed through the graphene filter, the temperature raised by 3.3°C. The light passing through the fullerene filter raised the temperature by 4.6°C. For comparison, when using the standard red filter, a recorded temperature went up by 5.6°C. The most intense heating occurred when illumination was done with BIOPTRON-compact without a filter: during 10 minutes of exposure to light,

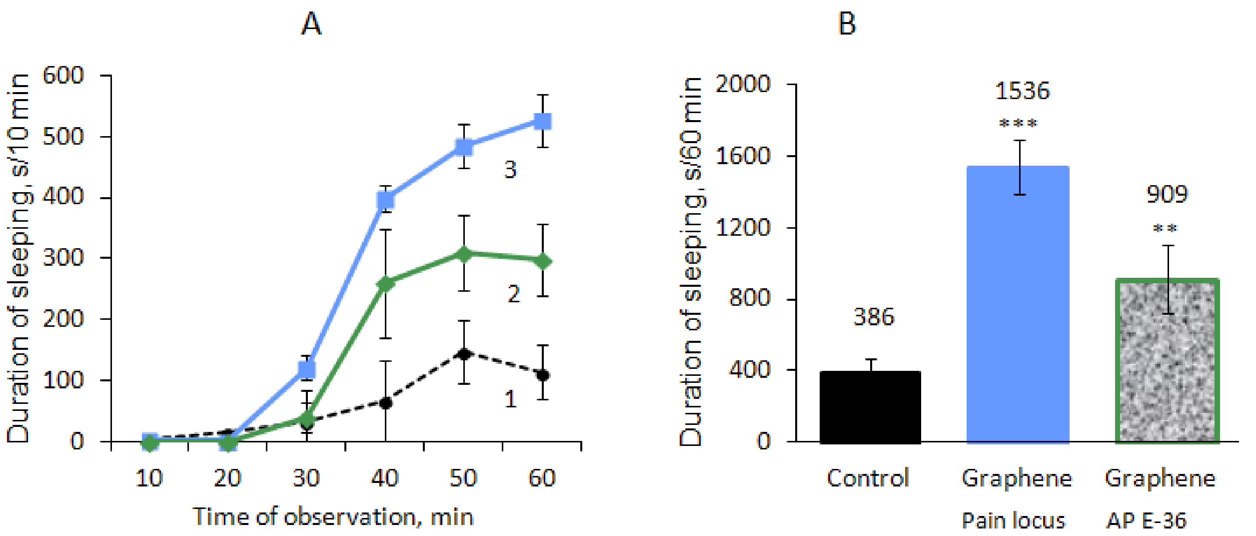


Fig. 8 Duration of sleep after 10 minutes of PILER light application, using the graphene filter, to the pain locus (3) or to AP E-36 (2) in comparison with the control (1): (A) Sleeping dynamics in the three groups, (B) Total duration of sleep during 60 min of observation in the three groups. The bars represent mean ± S.E.M. Numbers above the bars—the duration of sleeping in seconds.

Significance of differences with the control: ***$P < 0.001$; **$P < 0.05$.

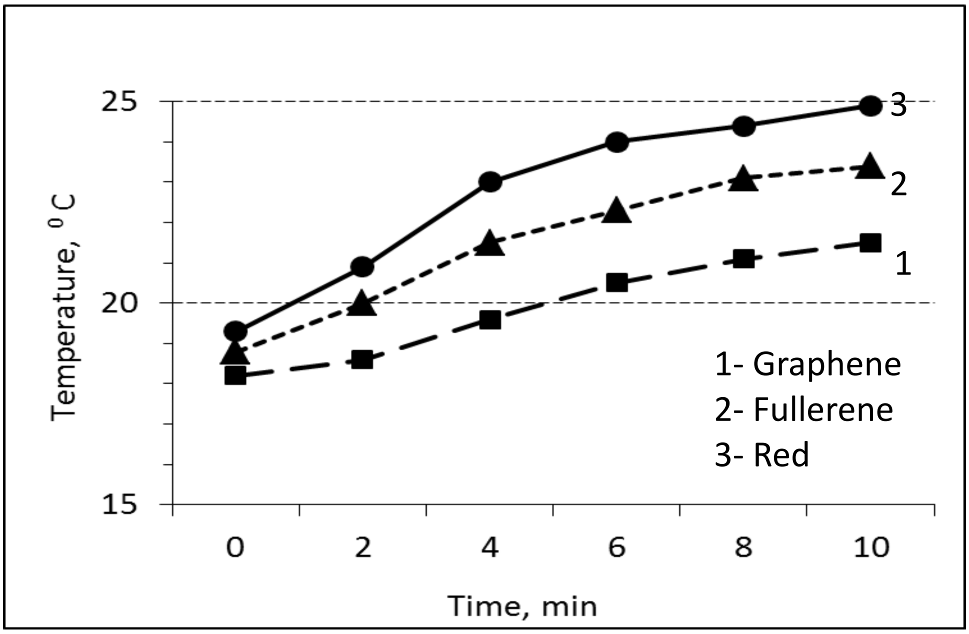


Fig. 9 Temperature change under the influence of the BIOPTRON device light with graphene filter, fullerene filter, or red standard (BIOPTRON Color set) filter. Exposure to light 10 min. The diameter of the light spot is 5 mm. The distance from the light filter to the thermometer is 5 cm.

the temperature rose by 12.5℃ (Not shown in the figure).

3.4 Comparison of Biological Effects of Fullerene, Graphene and Monochromatic Polarized Light

So, on the model of the experimentally caused pain, we found that the light of the Bioptron device, which passed through a fullerene or graphene filter, has reliable biological efficiency. The main indicators of such effectiveness can serve: weakening of the pain reaction (analgesic effect) and increasing of the sleep duration (sedative effect). Previously, we observed similar effects when studying the action of PILER-light, which passed through standard color filters [8, 9]. It was shown that when the PL was applied to the locus of pain, the warm colors of the spectrum (red, yellow, orange) shortened the pain response down to 50.1%-64.1%, while cold colors (blue, green, violet)—only to 31.5%-44.3%. The red light was the most effective in suppressing pain.

It was interesting to determine the location of the fullerene and graphene filters (to the effectiveness of the action) among all the studied light variants. Experiments with monochromatic filters were carried out in a unified manner, but in different years, therefore we cannot compare the absolute values of behavioral responses. However, if we take the pain duration in the control groups as 100% and express the reactions registered after applying the PL, which passed through a certain filter, in percentages (%) of the corresponding control, then the comparison will be completely correct (Table 3).

For a more vivid comparison of the analgesic effect of PL, we present analgesic effects from different filters in the sequence from maximum to minimum (Fig. 10).

As can be seen from Fig. 10, the graphene filter at the output of BIOPTRON device gives a more powerful analgesic effect than the fullerene one. When applied to AP E-36, it provides the greatest analgesia (55.9%) as compared to all tested filters. It surpasses even the most effective of the standard color filters—the red (54.4%). However, statistical analysis has shown that the difference between the two groups is not reliable. When applied to the locus of pain, the light which passed through the graphene filter gave a significantly lower analgesic effect (49.5%) compared with red light (64.1%).

As to PL, which passed through the fullerene filter,

Table 3 Average values of pain response duration during 60 minutes (% from control) and analgesia (%) in nine experimental groups in which was applied the color light of the BIOPTRON device, which passed through different filters.

Light filters	Duration of pain and analgesia after 10 minutes of light application			
	Influence on the locus of pain		Influence on AP E-36	
	Duration of pain (%)	Analgesia (%)	Duration of pain (%)	Analgesia (%)
Control (without filter)	100	-	100	-
White	64.2	35.8	50	50
Red	35.9	64.1	45.6	54.4
Orange	49.9	50.1	62.3	37.7
Yellow	44.6	55.4	62.8	37.2
Green	63.6	36.4	63.9	36.1
Blue	68.5	31.5	60.2	39.8
Purple	55.7	44.3	53.7	46.3
Fullerene	56.5	43.5	61.5	38.5
Graphene	50.5	49.5	41.1	55.9

analgesia was 38.5% (AP E-36) and 43.5% (the locus of pain). As to the effectiveness of pain suppression, the fullerene filter occupied an average position among other filters.

As shown in this paper, the second most prominent manifestation of the biological effectiveness of the PL, which has passed through a fullerene or graphene filter, is the powerful sedative effect of such light. Its criterion is change of sleep duration. A comparison of the average sleep duration in the groups, which received PILER-light applications equipped with different filters, is shown in Table 4 and in Fig. 11.

It was found that in animals with a locus of inflammatory pain (formalin test) who received PL applications, the duration of sleep increased significantly. This testifies to the easing of pain and the sedative effect of the light. When applying the PL to AP E-36 of all the studied light variants, fullerene and graphene had the greatest influence on the duration of sleep. Sleep time increased 3.2 and 2.4 times compared with the control group.

At application of the PL, which passed through the graphene filter directly to the locus of pain, the sleep time increased 4 times, while the other filters extended the sleep only 1.4-2.3 times. Consequently, on the model of formalin-induced pain PL, which passed through a graphene filter, gave the maximum sedative effect.

4. Discussion

The present study proved, for the first time, that the PILER-light of the BIOPTRON device, passing through a nanophotonic fullerene or graphene filter, has a statistically significant analgesic and sedative effect. According to our knowledge, this is the first study in this field.

The fact that the new state of carbon, in particular fullerene, can be promising not only in a technical but also in a biological sense, became clear simultaneously with its discovery (1985), characterized with atomic resolution by scanning tunneling microscopy [25] and the award of the Nobel Prize in Chemistry in 1996 [22]. The spatial spherical structure of the fullerene molecule and the presence of an atom-free cavity in it, primarily aroused interest in terms of application in engineering (new materials, lubricant mixtures, etc.) Pharmacy is discussing a possibility of creating anticancer drugs based on “stuffed” fullerenes, inside of which are radioactive isotopes [35]. Physicians are well aware that practically any disease is accompanied by excessive formation of free radicals in the body. Their accumulation generates an avalanche of pathological processes, oxidative stress. It turned out that hydroxylated fullerene $C_{60}(OH)_{26}$, which is highly soluble in water, has a higher antioxidant activity than vitamins “E” and “A”. Unlike these single-use antioxidants (one molecule of the vitamin neutralizes

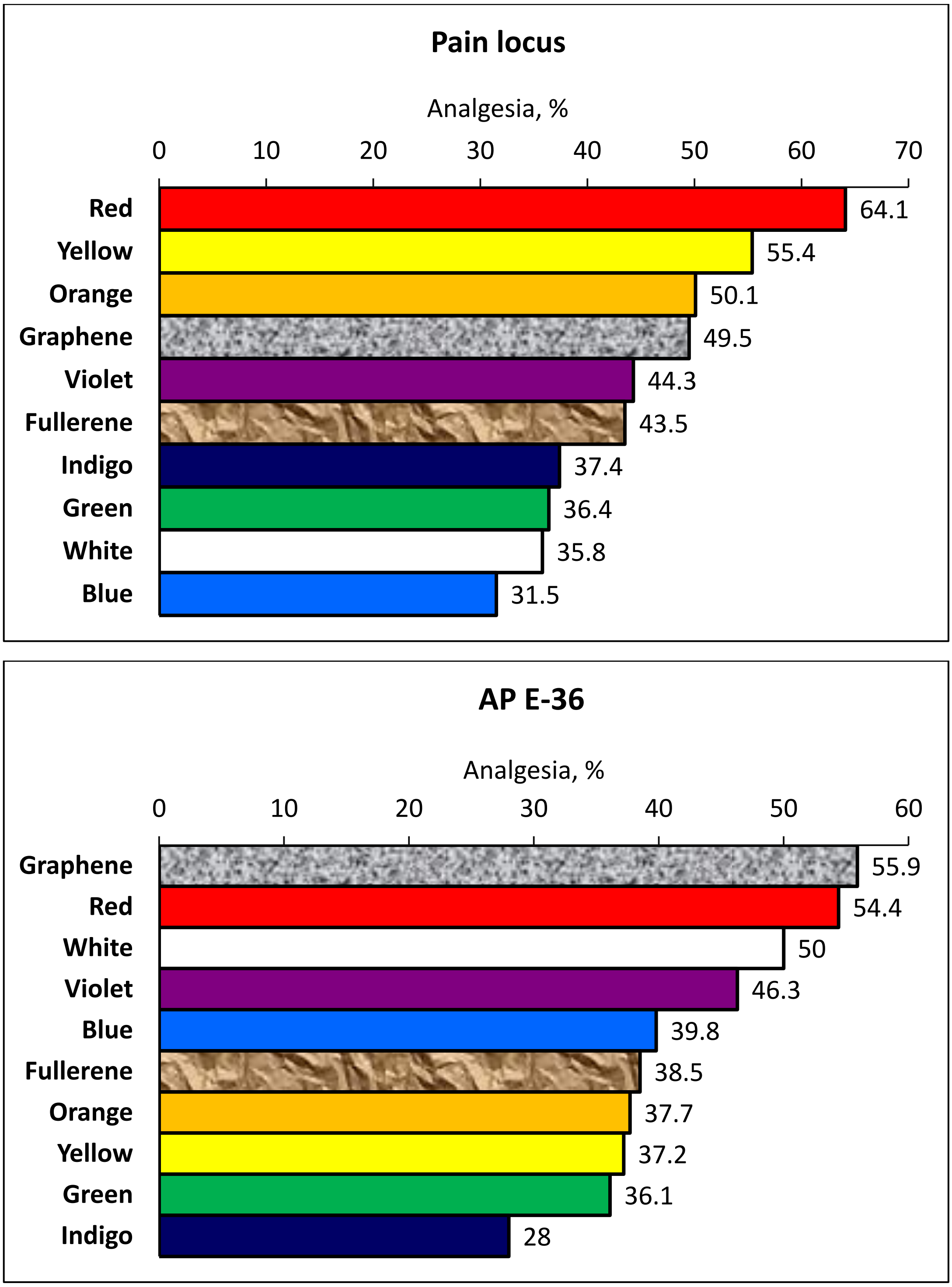


Fig. 10 Dependence of the analgesic effect of polarized light of BIOPTRON device on a filter: Numbers above the bars—mean analgesia in different groups.

Table 4 Changes in the duration of sleep during 60 minutes of observation in mice with formalin-induced pain after application PILER-light of the BIOPTRON device equipped with different filters to AP E-36 or pain locus.

Light filters	Duration of sleep (% of control) after a 10-minute light application	
	Influence to AP E-36	Influence to the locus of pain
Control (without filter)	100	100
White	232.4	228.1
Red	195.1	213
Orange	185.7	191.3
Yellow	181.9	173
Green	154.9	164.9
Blue	108	157.1
Purple	103.7	135.9
Fullerene	317.9	202.2
Graphene	235.3	397.9

only one free radical), fullerenes are reusable: they collect on their surface several radicals that self-destruct. Thus, fullerenes can be considered as an effective trap for free radicals [35]. Accordingly, due to antioxidant properties, fullerenes can influence aging processes. It was experimentally shown (2012) that feeding rats fullerene C_{60} (solution in olive oil, *per os*) resulted in significant slowing down of aging and an almost twice increase of the rats' life-time [36]. The study of the effect of polarized light passing through the nanophotonic fullerene filter on the biophysical indices of the skin (on 32 volunteers) also produced promising results [27]. The authors demonstrated that hyperpolarized light improves the epidermis structure, increasing the amount of collagen fibers, restores the original skin condition. Also, it has been shown that NHS (nanoharmonized substance) based on $C_{60}(OH)_{24}$ and 18.2 MΩ water [39], has significant influence on healing wounds [40].

We have noticed that fullerene hyperpolarization is a consequence of the turn or rotation of a linearly polarized electromagnetic flow, sequentially from vertical to horizontal state, whose waves oscillated in the Fibonacci ordered planes. For the biological object, the spatial matching of its polarization sensitive elements and the polarization plane of radiation is of great importance. The more biological structures that respond to radiation are covered, the greater chance to obtain a reliable final reaction. To increase their number is possible by adding the number of planes of polarization, i.e. to control spatial (azimuthal) position of the plane of polarization. We solved this problem earlier by applying a modulator creating the Faraday Effect [32] mounted on a polarized light beam [41]. In this case, the additional electromagnetic factor made it possible to dose-change the number of azimuthal positions of the polarization planes. The variant of changing the polarized flow considered in this article is based on the use of nuclear forces of crystal lattices of carbon molecules. Due to multiple interaction of light quanta with numerous fullerene molecules [27], the final distribution of quanta passing through the fullerene layer will occur according to Fibonacci-Tesla law [28, 29] (Fig. 2). For a biological object, ultimately, the technique of preparing an electromagnetic flow is not so important as the availability of additional possibilities for a more complete development of resonance processes and a reduction in the mosaic pattern associated with the unevenness of the wave flow. The evidence of the essentiality of carbon hyperpolarization is the authentically occurring biological reactions, in this case the experimentally observed analgesia and sedation, described in this article.

In our experiments with an experimentally evoked locus of chemical inflammation, we obtained evidence of the analgesic and sedative effect of PILER light which, passed through the fullerene filter. After 10

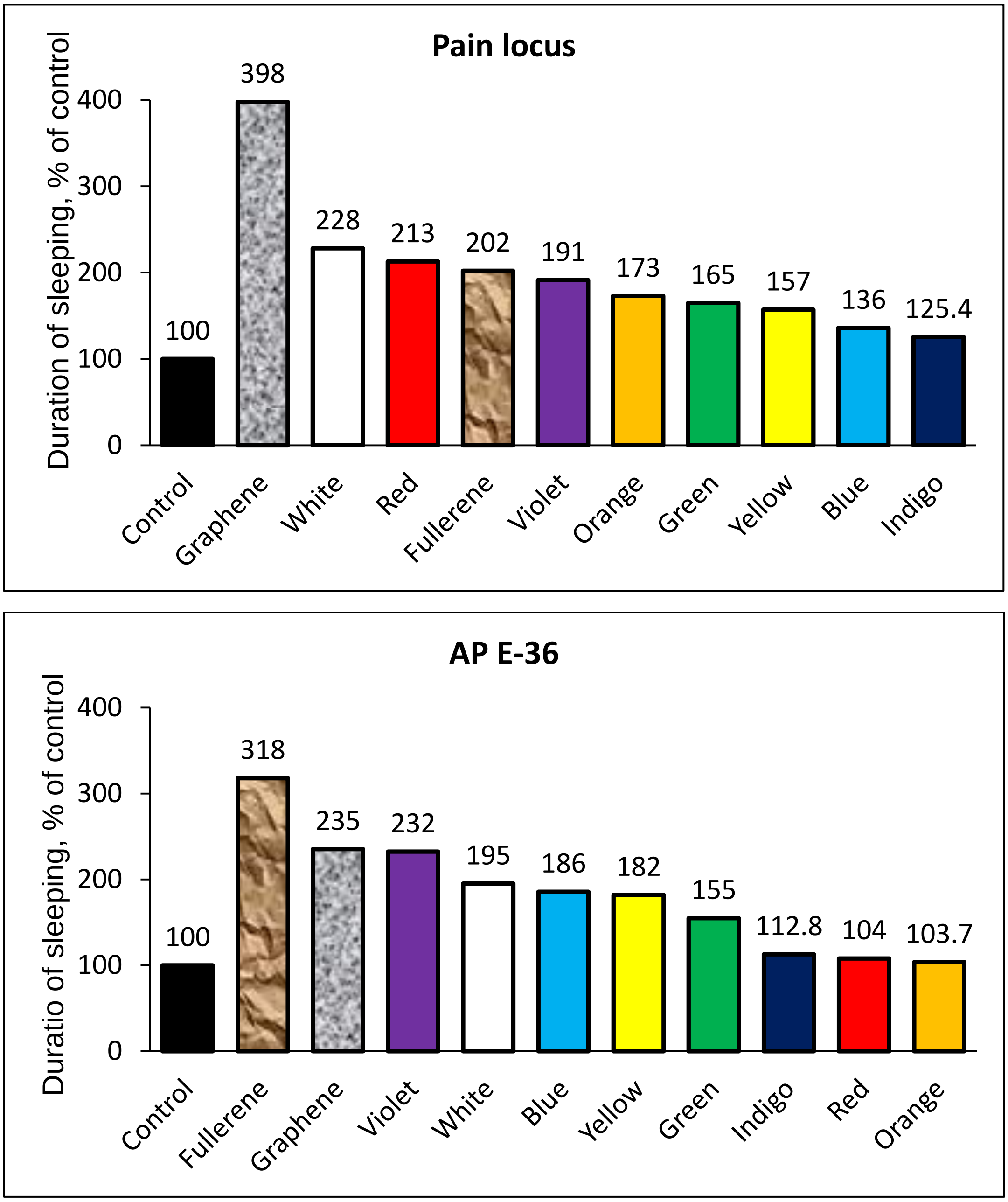


Fig. 11 Duration of sleep during 60 minutes of observation in mice with formalin-induced pain before and after the application of BIOPTRON device PL with different filters to AP E-36 or to the locus of pain: Sleep in the control group (without PL application) is taken as 100%. The figures above the bars (fractured to integers) are the average length of sleep in the corresponding group in % of the control group. The experimental groups are arranged in order from the maximum to the minimum duration of sleep.

minutes of application to the site of inflammation, the formalin-induced pain response (lysing of the affected limb) was shortened to 319.7 seconds from 566.2 seconds in the control. A significant reduction of pain was also found after the action to AP E-36. The duration of the pain response was reduced to 348 seconds. Analgesia made up 43.5% and 38.5%, respectively.

The biological efficacy of the PL, which passed through the nanophotonic fullerene filter, was manifested not only in the weakening of pain in animals with an experimentally evoked inflammatory locus (formalin test). The second indicator of the significant biological effect of such light is the sedative effect registered in our experiments. This is evidenced by the increase in duration of all non-painful behavioral reactions (sleeping, eating, running, washing). The most significant changes were found in sleeping and eating behavior. Duration of sleep compared with the control increased twice (application of light to the locus of inflammation) and 3.2 times (application to AP E-36).

The fact that the pain is weakened after the action of light of the BIOPTRON device with a nanophotonic fullerene filter is a new one, and it complements the list of physical factors that AP can react to. The results of the experiments described above also demonstrate a new opportunity for practical medicine to obtain additional opportunities for contactless analgesia.

In this paper, we also investigated the biological efficiency of polarized light passing through the graphene bilayer. This material is a two-dimensional sheet of carbon atoms located in a hexagonal lattice, making it the thinnest, the most electrically and thermally conductive material in the world, and also flexible, transparent and incredibly durable. It was discovered in 2004, and in 2010, the authors of this discovery Andre Geim and Konstantin Novoselov received the Nobel Prize in Physics [23]. This material is now being intensively studied in engineering. The medical capabilities of graphene are related to its unique properties (two-dimensional flat structure, large surface area, good biocompatibility and chemical stability).

In the present study on animals with a locus of inflammatory pain, we for the first time, showed that a low-intensity polychromatic PL, passing through a graphene filter, has an analgesic and sedative effects. Moreover, both effects took place when light was applied both to AP E-36 and to the locus of inflammation. Analgesia was 58.9% and 49.5%, respectively. Among non-painful behavioral reactions, the most significant changes have undergone sleeping and eating behavior. The duration of sleep compared with the control increased 4 times (application to the locus of inflammation) or 2.35 times (application to AP E-36). Consequently, the light that passed through the graphene filter, when applied to both AP E-36, and to the inflammation locus has a powerful biological effect, as a result of which there is a significant reduction of pain and sedative effect.

When explaining the effects of the action of the electromagnetic factor, in this case, the light effect, it may be suggested that the results obtained are related to the thermal effect. Therefore, we did appropriate measurements of the temperature on the surface of which light was transmitted through the carbon filters. It turned out that a 10-minute exposure to light using a standard red filter (BIOPTRON device) raises the temperature by 5.6°C. The light passing through the nanophotonic fullerene filter heated up weaker, by 4.6°C. The weakest heating was done by light passing through the graphene filter: the temperature rose by 3.3°C. These data convince us that the high biological efficiency of polarized light passing through the fullerene or graphene filters cannot be due to the thermal effect. This temperature level is insufficient to trigger thermal receptors. According to the published data [42], the average frequency of discharges of the population of thermal receptors begins to increase appreciably at temperatures above 35°C. The optimum temperature regime for thermal receptors is between 45°C and 47°C. Therefore, the effects of light transmitted through the fullerene and in particular the graphene filter are apparently not associated with tissue heating.

It is known that the tonic component of the formalin-induced pain (which was analyzed in the present work) is the result of an inflammatory process in peripheral tissues and changes in the function of the neurons of the dorsal horns of the spinal cord [43]. Perhaps the analgesic effect of the low-intensity polarized light passing through the fullerene or graphene filter is based on the same mechanisms as in the case of low-intensity polarized laser light. This may be an increase in the synthesis and release of endorphins, as well as a decrease in the release of bradykinin and serotonin in the area of inflammation [44, 45]. Another mechanism of easing pain is associated with a decrease in the rate of pain signals in sensory nerves [6, 47-51]. In addition, it has been shown that laser light (especially red) increases blood flow, which is accompanied by an improvement in oxygenation and metabolism, with simultaneous enhancement of lymphatic drainage and weakening of edema in the affected tissue, which also contributes to the relief of pain [7-10].

In the case of exposure to light not only of the zone of the inflammation (foot), but distantly, to the point of acupuncture E-36, located in the region of the knee joint, it can be thought that analgesia is not a consequence of the anti-inflammatory effect of light. It is more likely that polarized light through the acupuncture point triggers its own analgesic systems of the brain, which leads to suppression of the transmission of pain impulse at the neuronal level. Such a mechanism has now been proved in the case of an impact on AP by electric current or low-intensity electromagnetic radiation of the microwave range [12, 50, 51].

5. Conclusion

Having analyzed the effects of the light modified by molecular carbon, the question arises whether we are dealing with a unique phenomenon or we have expanded knowledge about the effects of different light variants, in this case, in regards to the pain syndrome. Using a unified experimental technology allowed us to compare the obtained results with the data for the main monochromatic light ranges. First, we understood that as to analgesic influence, lights modified in interaction vertically polarized light with fullerene and graphene are included in the group of the most effective ranges of visible light, which give a reliable analgesic effect. The second, and very significant, is the fact of sedation, which was not so pronounced in any of the studied monochromatic variants of light. It is important that these results confirm the possibility of both local and remote (through the point of acupuncture) effects obtained in contactless and non-pharmacological way. We take into account that the active factor is the polarized light that passes through the carbon layer. In this connection, it will not be incorrect to explain the described effects by increasing the volume of reacting biological structures due to the increase in the number of polarization planes. However, we also do not underestimate the proven value of the traditional components of light radiation: wave range and power density. All this indicates that we are dealing with a factor that can provide additional opportunities that are important for practical medicine.

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