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Innovative Light Therapy: 5. Anti-stress Effects of Polarized Polychromatic and Monochromatic Light from Halogen and LED Sources

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Abstract: In experiments on laboratory animals (n = 100) and in examinations of people (n = 42), the anti-stress effect of low-intensity polychromatic or monochromatic halogen light of the Bioptron device (PILER/PL: 480-3,400 nm, 40 mW/cm^2) and LED (light-emitting diode) light of the Medolight-polychrome device (401+467.5+527.5+640.5+940 nm) has been demonstrated objectively. We found out that adult rodents (white mice) showed that 30 min of immobilization stress increased grooming duration by 330%, and after a PL (polarized light) session to acupuncture point (AP) E-36—only by 170%-230% compared to the norm. PL's anti-stress effect was determined by its wavelength. Researchers found that red light (the long-wavelength part of the visible light spectrum) had a significantly greater anti-stress effect than green light (medium-wavelength). Red PL application reduced stress-induced grooming by 49.2% and other behavioural responses (sleeping, eating, and physical activity) were partially normalized, while green PL reduced grooming by 31% without affecting other behavioural responses. The short-term immobilization stress weakened the somatic pain response (formalin test) by 28.5% and the visceral one (acetate test) by 26.3%. Red PL has a less pronounced analgesic effect on animals under stress than on animals not under stress. In normal conditions, red PL suppressed somatic pain by 54.4%, visceral pain by 64%, and under stress by 31% and 46.1%, respectively. Under the action of low-intensity LED-light on AP, we have obtained experimental evidence of stress reduction in humans. The latent period for falling asleep in the subjects increased from 393.6 ± 47.1 to 749.3 ± 44.4 s under stress. Applying the Medolight-polychrome device to auricular AP weakened post-stress sleep disturbances: the duration of falling asleep was reduced to 512.5 ± 38.6 s. In persons with daytime stress, the frequency of dreams was 49% ± 5.7%, and after a PL session it was 14.79% ± 5.2%. The results of these studies can be used to develop recommendations for reducing stress in humans.

Key words: PILER-light, polarized light, Bioptron, LED-light, monochromatic light, polychromatic light, grooming, immobilization stress, analgesia, formalin test, acetate test, acupuncture points, sleep, stress auriculotherapy.

1. Introduction

Stress is a special state of the human and mammal bodies caused by a strong external stimulus. Stress was first introduced by Georg Selye, who used it for the first time in biology in 1936 [1]. Stress is defined by him as any external stimulus (stressor) strong enough to cause a state of internal protective tension within a number of physiological systems. Stress manifests itself in the development of a certain nonspecific (i.e., independent of the type of stressor) response in mammals. A general adaptation syndrome, depending on the severity of the stressor, can have both positive and negative effects on the functioning of the body (up to complete disorganization).

Stress factors have been identified experimentally and clinically as follows:

(1) Physical factors (ionizing radiation, high or low temperature, high or low atmospheric pressure, immobilization).

(2) Chemical (irritating and toxic substances).

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(3) Biological (intense muscular work, infection with microbes and viruses, trauma, burns).

(4) Mental (strong positive and negative emotions, including combinations of these emotions).

The general adaptation syndrome involves three phases, depending on the severity of the stressor.

They are functional indicators of the adrenal cortex cells:

(1) A phase of anxiety (such as shock), when the body's defenses are activated (the number of granules in the adrenal cortex that contain hormones such as corticosteroids sharply decreases)

(2) A phase of stability (granule numbers increase significantly from the initial phase).

(3) A phase of exhaustion occurs when an impact is too strong or too long, as well as when the body is unable to adjust enough to it. During the exhaustion stage, the number of granules declines again, and the stress reaction takes on a painful, pathological quality.

Chronic and frequent stress affects a person's health not just on a neuro-psychological level but also physically. These are the primary "risk factors" that contribute to the development and exacerbation of cardiovascular and gastrointestinal diseases.

Stress has become a common occurrence for most active working-age adults, especially those living in big cities. Pharmacological methods of combating stress are not always acceptable. Most of the drugs used for these purposes have side effects such as lethargy, drowsiness, weakness of attention, addiction, etc. These factors negatively affect the ability to perform professional duties, which in turn reduces the quality of life.

In our experiments with low-intensity electromagnetic radiation in the optical range for the treatment of pain syndromes, we demonstrated that polarized light (PL) also has a calming effect on laboratory animals [2], which suggests that it also has an anti-stress effect. The same pattern was also found for individual monochromatic ranges, although it manifested differently and depended on wavelengths [3, 4]. These data testified to the prospects of non-contact technologies achieved by using PL on reflex-therapy zones (acupuncture points, AP).

Originating in China, acupuncture is 5,000 years old. It was initially used only on humans. The first treatment schemes appeared later (1727, 1789), along with guidelines for physicians [5], which described physiological mechanisms and responses. There is evidence that acupuncture is effective in veterinary medicine; animals, like humans, possess meridians and AP linked to specific organs [6, 7]. The AP E-36 (Tzu-San-Lee) is very popular and has a multifunctional effect.

In this study, we investigated the effects of polychromatic (white) and monochromatic (red, green) PL on stress-modified behavioural responses in animals and humans.

The specific objectives of this study were: (1) to determine whether low-intensity PL, when applied to an analgesic AP, can attenuate stress-induced behavioural changes; (2) to test whether the length of the light wave (colour) affects the effectiveness of the analgesic; (3) to use models of formalin and acetate to test how low-intensity PL affects animal behaviour under stress; (4) to examine whether the light application has an anti-stress effect on a person.

2. Methods

2.1 Immobilization Stress Model

Literature describes a variety of experimentally induced stress models. The most common of these is probably movement restriction—immobilization (more than 2,000 publications). Single, short-term, intermittent or chronic immobilization is regarded as a serious stress factor and reliably causes all the known allostatic consequences [8].

We induced stress in animals by immobilizing them for 30 min. To evaluate these results, we developed quantitative methods to account for stress effectiveness, which made it possible to compare data from different experimental groups and increase the

reliability of the results. In stressed animals, we examined the effect of low-intensity PL on somatic (formalin test) and visceral (acetate test) pain.

The study was performed on 100 adult white male mice weighing 28-31 g. Experiments were conducted in accordance with the ethical guidelines of the International Association for the Study of Pain and with the permission of the A. A. Bogomolets Institute of Physiology NAS of Ukraine (Kyiv). The animals were grown up and kept in the Institute's certified vivarium under controlled temperature (18-20 °C) and 12-hour daylight hours. All animals had free access to water and food (special granular feed). Animals were randomly divided into groups. In each experimental group there were 10 individuals, and in the control group there were 20. The animals had been caged individually two days before the experiment. On the day before the start of the study, these cells were moved to the laboratory and placed near the computer to help the animals adjust to the experimental conditions. The experiment always started at 10 am. Fig. 1 shows the cyclogram of the experiments. Computer registration of the behaviour of the animal was first performed using a specially developed program. The following reactions were registered: grooming, eating, sleeping, and running for 60 min. A 30-min immobilization stress was then applied to the animal. In order to accomplish this, the mouse was placed in a round plastic chamber with air holes. During the last 10 min of immobilization, the left hind limb of the animal was taken out of the chamber through a special hole and held gently by the experimenter's fingers. During these 10 min, PL was applied to AP E-36 (Tzu-San-Lee) or an imitation of PL exposure for animals of the control group. At the end of the immobilization period, the mouse was transferred to its cage, and the above behavioural responses were recorded again for 60 min.

In a series of experiments examining the effects of PL on pain reactions caused by somatic or visceral pain, indicated by an arrow in the scheme, the animals received an injection of formalin or acetic acid (see below for more details).

2.2 Formalin Test: Somatic Pain

The formalin test is a classical model of hemogenic tonic pain [9-11]. Formalin-induced pain was created by subcutaneous injection of 25 μL of 5% formalin solution (in 0.9% NaCl) into the dorsum of the foot of the left hind limb. There are two phases of the pain

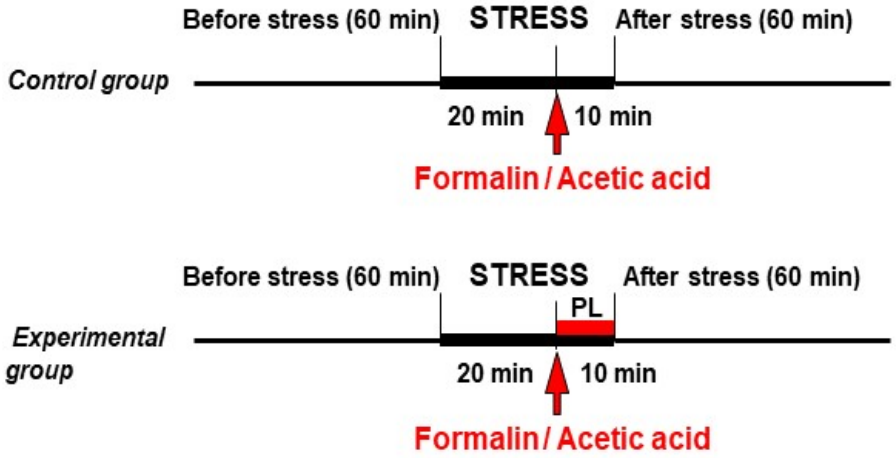


Fig. 1 The following sequence of events occurred during experiments to examine the effect of 30-min immobilization stress on animal behavior without polarized light application (control group) and after 10-min application of PL to AP E-36 (experimental group):

An arrow indicates the moment of injection of formalin solution into the hind leg (creating a locus of somatic pain) or acetic acid into the abdomen (modeling visceral pain).

response to a subcutaneous injection of formalin [9, 12]. The early phase lasts 5-10 min and is mainly associated with direct activation of the C-fibres. Late phase begins 10-15 min after formalin injection and lasts for more than 1 h (depending on the concentration of formalin). Late phase is the result of the development of an inflammatory process in peripheral tissues and changes in the function of neurons in the dorsal horn [13]. As PL was applied to AP for the first 10 min of the experiment, we only assessed the intensity and dynamics of the second phase, which, according to the literature, is the tonic component of the pain reaction [9, 12, 13].

As described above, experiments with 30-min immobilization of animals were conducted to study the effects of stress on tonic somatic pain. The experimental mice were exposed to PL on AP E-36 during the last 10 min of immobilization, while the control mice were exposed to PL session imitation. Formalin injection was performed immediately before the application of light or its imitation (Fig. 1). We observed mouse behaviour after the light or imitation session ended and observed the dynamics of pain responses (licking the affected limb for 60 min) as well as non-painful behavioural responses (grooming, sleeping, running, eating) were also recorded.

2.3 Acetate Test: Visceral Pain

To study visceral (in the abdomen) pain dynamics, we used the acetate test (the writhing test). A widely used animal model of visceral pain is the writhing test, which involves injecting an irritant intraperitoneally to induce a syndrome of writhing. It includes contractions of the abdomen, twisting and turning of the trunk, and extending the hind limbs [14].

For this purpose, each animal was intraperitoneally injected with a 2% solution of acetic acid (0.1 mL per 10 g of body weight). Painful forced postures (writhing) and licking of the abdomen are considered the most informative signs of visceral pain (acute peritonitis of chemical origin).

Unlike the mice that were injected with acetic acid, mice injected with 0.9% NaCl in the same volume did not display any writhing. Non-pain behavioural responses, including sleep and feeding duration, also changed significantly in animals with a locus of visceral pain.

Four groups of animals were divided according to their locus of visceral pain (which was induced by hydrochloric acid injection into the abdominal cavity). The first group did not undergo immobilization (without stress); the second group underwent a 30-min immobilization stress; group 3 received a 10-min session of red PL on AP E-36 (without stress); group 4 received both stress exposure (immobilization) and a red PL session.

The scheme of experiments to study the effect of PL on visceral pain in animals under stress was as follows. The animal was immobilized for 30 min. To do this, the mouse was placed in a narrow round plastic chamber with air holes. After 20 min, the animal was removed from the chamber for injection into the abdominal cavity (creating a locus of visceral pain). Immediately after the injection, the animal was placed in the chamber for another 10 min. In the 10 min, PL was applied to AP E-36 as described above (Fig. 1). The control group received imitation of the light session. The subsequent actions were similar to those described in Section 2.2 above.

2.4 Applications of Polarized Light on AP

The PL source in animal experiments was a Bioptron-compact device (Zepter/Bioptron Companies, Switzerland), which emits low-intensity (power density 40 mW/cm^2) halogen light (polarization 95%) in the wave range 480-3,400 nm (visible & infrared spectra). Light filters made it possible to obtain polychromatic light (480-3,400 nm) or monochromatic light (640+ nm red, 515 nm green). The distance between the light filter and the skin was 5 cm, and the exposure time was 10 min. A black diaphragm provided a light spot with a diameter of 5 mm. The beam of light was directed to AP E-36.

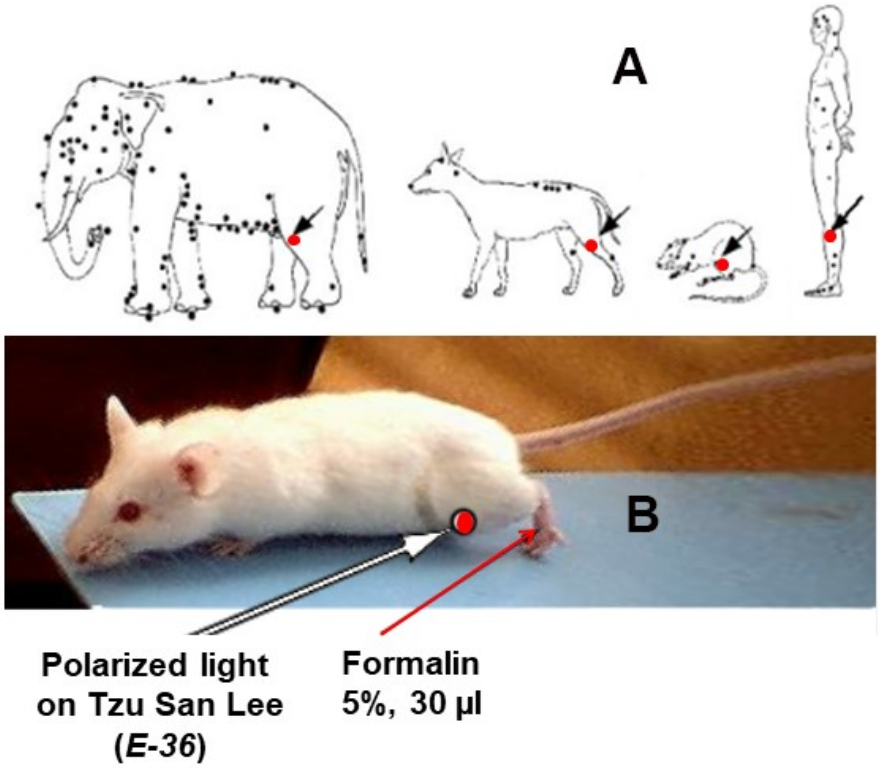


Fig. 2 Verification of acupuncture points in various animals and humans (A) and localization of the area of light applications during the formalin test in mice (B).
AP E-36 is highlighted in red.

The existence of meridians and acupuncture points in animals, like in humans, has now been proven (Fig. 2). The most well-known point in Chinese medicine is AP E-36 (Tzu-San-Lee). It is called the point "from 100 diseases". This point controls the functioning of the lower body organs. It regulates the normal functioning of the gastrointestinal tract, the genitals, and the kidneys through the spinal cord. A stimulation of this point induces an anti-stress and analgesic effect. Both humans and mammals possess this point.

2.5 Processing of Experimental Material

Using a specially developed computer program, we recorded the beginning and end of each episode of the animal's behavioural reactions. The total duration of reactions was calculated for successive 10-min time intervals, for the first 30 min, for the second 30 min, and for the entire observation period. We determined the mean value and squared error of the mean for a group of 10 (experimental) or 20 (control) animals. Student's t test was used to determine differences between groups. The difference was considered significant at $p < 0.05$.

2.6 The Study of Human Sleep-In Patterns

Auricular exposure was used to study the effectiveness of low-intensity polychromatic LED-light on humans under stress (volunteers). As part of the study, the rate of falling asleep and the presence of dreams were recorded in subjects in the norm, after exposure to stress factors without the use of light (placebo) and after exposure to light on the ear. Forty-two (42) healthy adult office workers (women) participated in the survey. They did not complain of stress. They were in a normal state or experiencing the effects of daytime production stress (emotional work with dismissed employees, operational meetings to overcome the crisis, management of reconstructive

Fig. 3 LED-light is composed of monochromatic components, which, when mixed, produce polychromatic light.
Original photos of the Medolight switched matrix;
Infrared LEDs are permanently switched in each combination.

office work, frequent car trips in heavy urban traffic, etc.). The Medolight-polychrome device provided low-intensity LED-light. It has a group of monochromatic LEDs (Fig. 3): violet 401 (397-405) nm + blue 467.5 (460-475) nm + green 527.5 (520-535) nm + red 640.5 (619-662) nm + infrared 940 nm = white. Their simultaneous luminescence provided poly-chromaticity like halogen polychromatic light in spectral components. The power density of the mixed LED-light was 45-55 mW/cm^2.

A light was directed to the ear, and the exposure time was 15 min. The examinee held the device very close to the ear with his hand. The sleep duration was measured in seconds. The fact of falling asleep was determined by the occurrence of a weakening of the tone of the muscles of the hand that was fixing the device near the auricle. The examinees (healthy women aged 45-55 years) were divided into three homogeneous groups of 14 each: First, intact (normal/placebo)—patients without stress and without exposure to light; Second group (stressed)—people who were stressed during their workday but received no light applications; Third group (stressed + Light)—individuals who were stressed during their workday and exposed to Medolight-polychrome LED-light for 15 min before sleep. The first two groups also held the Medolight device near their ears, but did not turn on the light (imitation a light session).

Based on the VAS (Visual Analogy Scale) and subjective data on the presence of dreams, we assessed the influence of light on the frequency of dreams in patients exposed to stress during the day. We examined people from the 2nd and 3rd stressed groups (n = 14 each) both before and after exposure to light.

The results of the observations were analysed statistically. We calculated the mean value and squared error of the mean for each group. We determined the difference between the groups using Student's t test. The difference was considered significant at $p < 0.05$.

3. Results

3.1 The Effect of Immobilization Stress on Mice's Behavior

Experimentally, we found that mice that were subjected to short-term immobilization became more excitable and their behaviour completely changed.

One of the most vivid indicators of stress is grooming. In rodents, grooming is a form of skin and hairline care behaviour, which is involved in thermoregulation, chemical distribution, etc. Grooming begins with licking the front paws and rubbing the nose, and then washing the entire muzzle, turning into washing the head and body, including hind limbs, and in the last stage—the genitals. Stress activates grooming, which is considered a behavioural marker.

Fig. 4 shows that in normal mice (without any influence on them), grooming duration remained the same for 1 h (about 50 s for consecutive 10 min of observation). After 30 min of immobilization, the duration of grooming increased sharply, exceeding 300 s in the first 10 min of observation. For 60 min of observation (Table 1), grooming, in intact mice, lasted on average 297.5 ± 59 s, but in animals stressed by immobilization, the duration was 986.2 ± 98.2 s, i.e., it increased 3.3 times.

Table 1 shows that immobilization stress affected the behaviour of animals in other ways. The differences are not significant (p < 0.5) when comparing indicators over 60 min of observation. The analysis of behavior immediately following a stressful event and the subsequent period of observation are more informative.

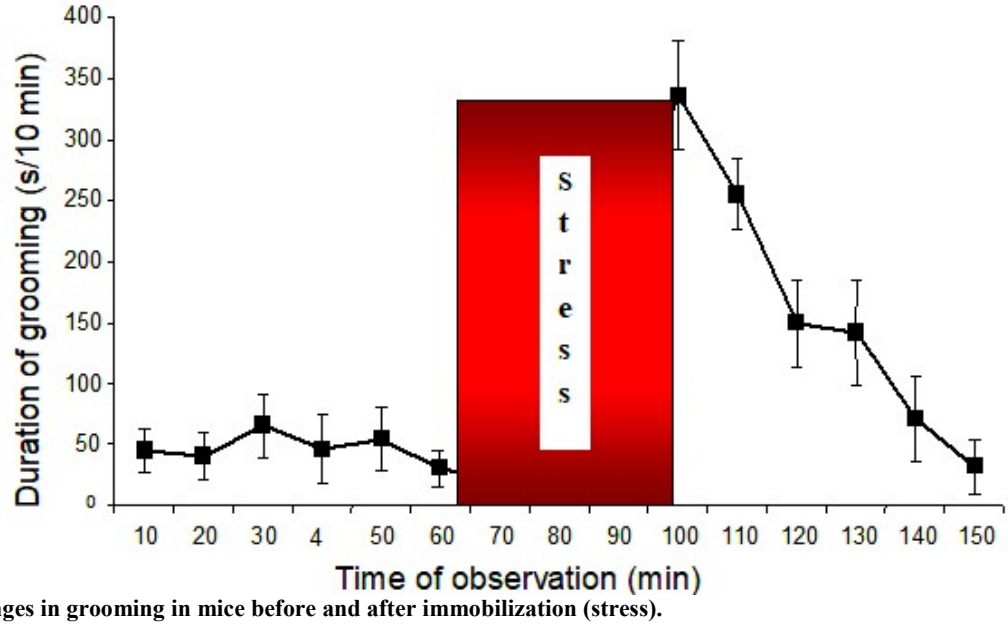


Fig. 4 Changes in grooming in mice before and after immobilization (stress).
The y-axis shows the duration of grooming (s) for successive 10-min intervals; the abscissa shows the duration of observation (min); The coloured rectangle denotes the period of immobilization of the animal.

Table 1 Behavioural reactions in the control group (without stress) and the group in which the animals were immobilized for 30 min.

Behavioural responses	Group 1 Without stress	Group 2 After stress
Grooming	297.5 ± 59 100%	986.2 ± 98.2*** 331.6%
Sleeping	407.7 ± 236.1 100%	702.9 ± 168.2* 172.4%
Running	336.1 ± 90.3 100%	169.2 ± 39.8* 50.3%
Eating	649.5 ± 178.2 100%	280.6 ± 242.1* 43.2%
State of rest	1,909.2 ± 280 100%	1,461.1 ± 126.7* 76.5%

Top line: duration of grooming in seconds, bottom line: percentage of the control group that received a simulated PL session. Significance of difference from control: *** p < 0.01, * p < 0.5.

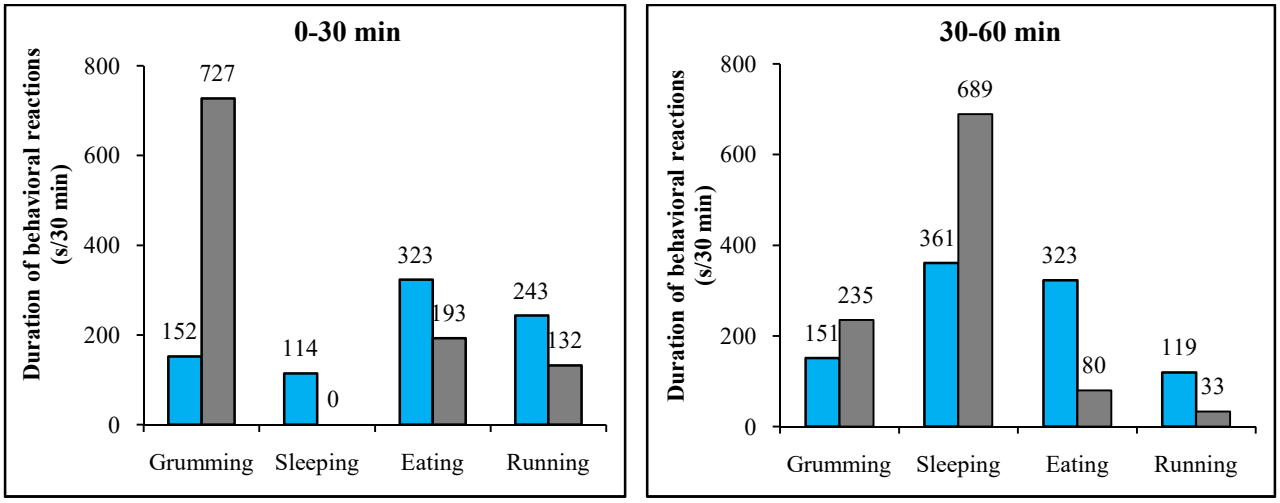


Fig. 5 Effect of immobilization stress on the duration of four behavioural responses in mice during the first 30 min and subsequent 30 min of observation:
Blue bars: reaction before stress; grey: after 30 min of immobilization stress. The numbers above the columns are the group average duration of the corresponding reaction in seconds.

Fig. 5 shows that changes in animal behaviour were most pronounced during the first half hour following stress. As a result, grooming in the first half hour of observation increased by 420% (727 s) compared to the control (152 s), while in the second half hour it increased only by 62% (235 s versus 151 s). Sleep was absent in the first 30 min after stress, although mice had slept an average of 114 s before stress. During the second 30 min, sleep duration increased by 139% compared to the control (689 and 361 s). The animals spent much less time eating and running after stress, as could be seen throughout the entire observation period (Fig. 5). Thus, mice exposed to 30-minute immobilization stress developed significant behavioural abnormalities.

3.2 The Effects of Polarized Light on Mice's Stress-Induced Behaviour. A Wavelength-Dependent Effect

We examined how behavioral responses in stressed mice change after exposure to low-intensity PL of the Bioptron device on AP E-36. Our previous studies on animals with an experimentally induced locus of tonic pain showed that the intensity of the analgesic effect of PL depends on the wavelength [3, 15]. Red PL was found to be the most effective, whereas cold colours suppressed pain less. We hypothesized that light with different wavelengths would also affect stress-induced behaviour differently. We applied polychromatic (white) and monochromatic (red and green) PL to AP E-36 to test this assumption. The three tested colours significantly suppressed the stress response (Fig. 6).

In animals that at the end of the immobilization period were exposed to a 10-min action of polychromatic, red or green PL on AP E-36, the duration of grooming for 60 min of observation was 54.2%, 50.8% and 69%, respectively (reaction in the group taken as 100%, receiving a placebo PL session). Differences from control are statistically significant (Table 2).

The red PL had the strongest effect. Fig. 7 shows a comparison of the dynamics of stress-induced grooming in animals exposed to red PL on AP E-36 and mice that received a simulated PL session. As can be seen, grooming was weaker throughout the entire observation period in animals that received PL. The intensity of stress-induced grooming decreased by 49.2% after the red PL session, as well as other behavioural responses (approaching norm) such as sleeping, eating, and physical activity. Green PL was less effective, reducing grooming time by 31% and having little impact on other behavioural responses.

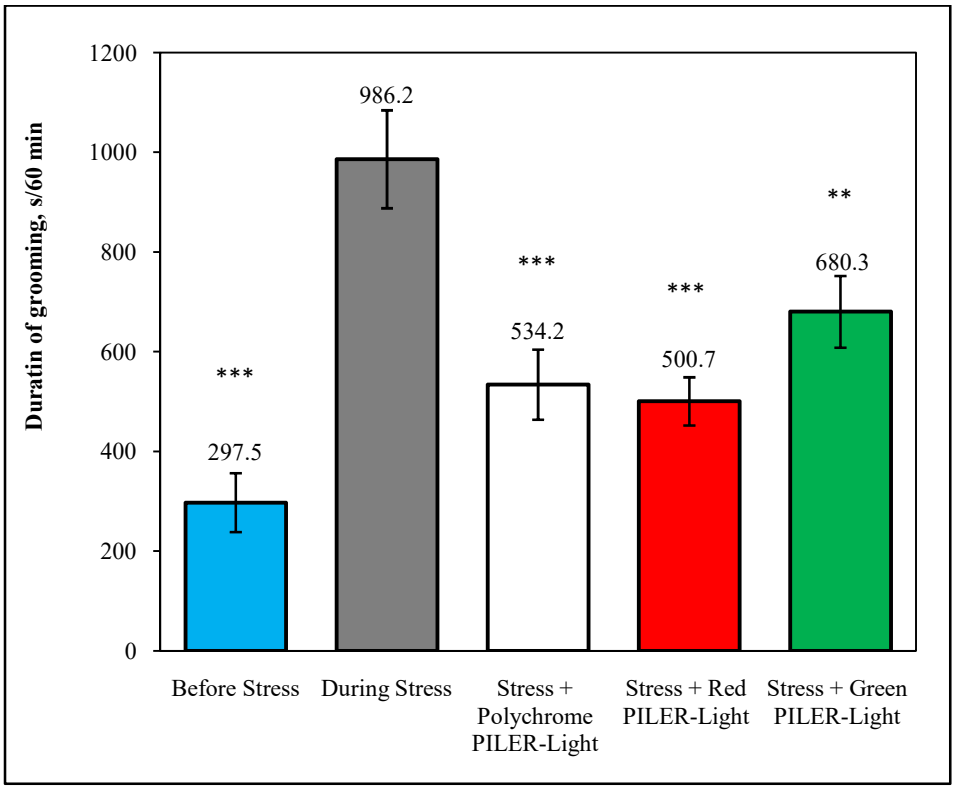


Fig. 6 Influence of poly- (white) and monochromatic (red, green) polarized light on the intensity of stress-induced grooming in mice.

The numbers above the columns indicate the duration (s) of grooming in each of the 5 groups. Significant difference from the group "During Stress": *** $p < 0.01$, ** $p < 0.05$.

Table 2 The duration of stress-induced grooming in mice after 60 min of observation without light and after a 10-min session of polychromatic and monochromatic PL on AP E-36.

Without PL	After the action of PL on AP E-36		
Stress (Placebo)	Stress + Polychromatic PL	Stress + Red PL	Stress + Green PL
986.2 ± 98.2	534.2 ± 70.4***	500.7 ± 48.2***	680.3 ± 72**
100 %	54.2%	50.8%	69%

The top line is the duration of grooming in s; the bottom line is the percentage of the control group that received a simulated PL session. Significance of difference with control: *** $p < 0.01$, ** $P < 0.05$.

This is the first time we have observed the stress response weakening under the action of low-intensity PL. Different wavelengths of light had different anti-stress effects. As with analgesia, red light was the most effective. The obtained experimental data suggest that low-intensity PL, especially its red spectrum, can increase stress resistance.

3.3 Red Polarized Light Effect on Somatic Pain in Mice Subjected to Immobilization Stress

In stress-induced animals, we have studied how the most effective red PL affects tonic somatic pain. As described above, stress was induced by immobilizing the animals for 30 min. The last 10 min of immobilization (after injection of formalin) we applied red PL to AP E-36, and in the control group, PL sessions were simulated.

Observation of behavioural responses during the formalin test revealed that licking of the affected limb was the most significant manifestation of pain (Fig. 8). The duration of licking the pain locus for 60 min of observation in control animals was 495.3 ± 82.8 s, in

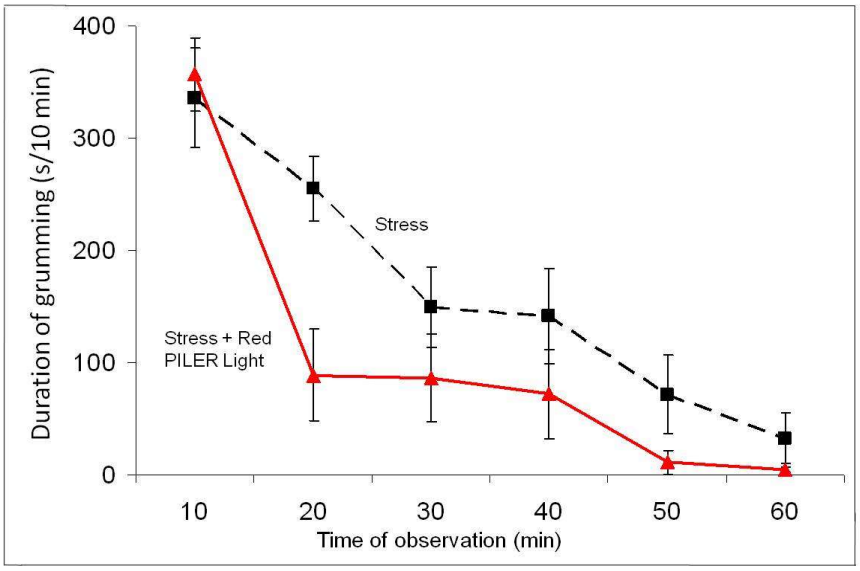


Fig. 7 A comparison of grooming dynamics in mice with immobilization stress, applied with or without 10 min red PL on AP E-36.

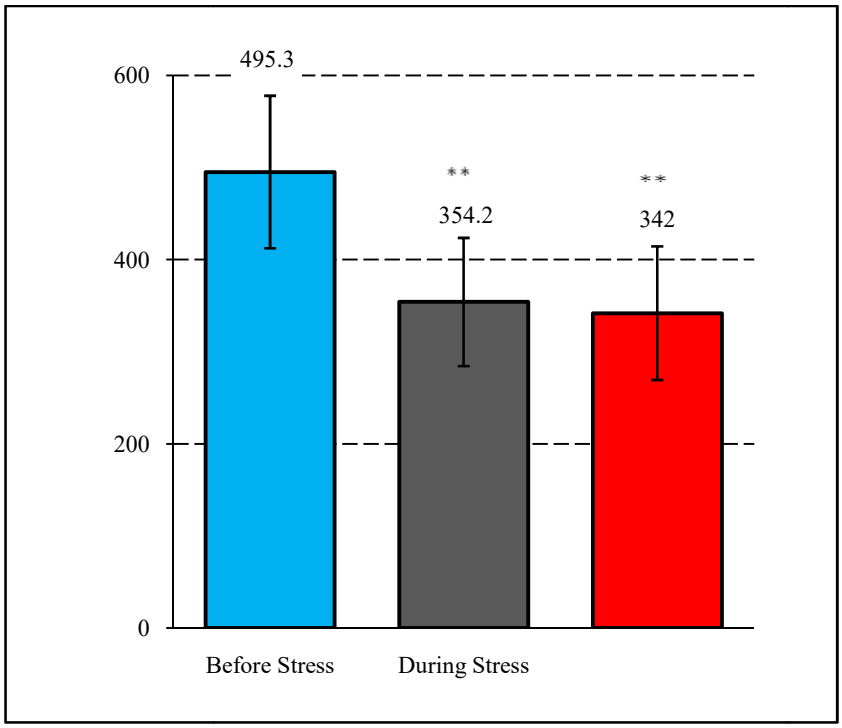


Fig. 8 Duration of the pain reaction caused by formalin (licking the pain locus) in three groups of animals for 60 min.
Difference from the control group (before stress): ** $p < 0.05$.

the stressed group—354.2 ± 69.6 s, and in the case of a 10-min session of red PL on AP E-36, the pain lasted 342 ± 72.5 s. The difference with control is statistically significant. In terms of non-pain response variables, there was no statistically significant difference between the three groups of animals.

A 30-min immobilization stress reduced somatic pain behavioural responses by 28.5%, and red PL, when exposed to AP E-36 in stressed animals, reduced pain by 31%. In our previous studies [15, 16], we found that red PL had a 54.4% analgesic effect on AP E-36 in animals not subjected to stress. Stress

therefore reduced somatic pain. Under stress, red PL was 31% analgesic. Evidently, this is because stress reduces the manifestations of pain.

3.4 Effect of Red Polarized Light on Visceral Pain in Mice Subjected to Immobilization Stress

In order to obtain additional evidence of red PL's anti-stress role, the visceral pain model was applied. To do this, we used an acetate test with a similar experimental method. When a 2% solution of acetic acid was injected into the peritoneal cavity, the animals exhibited behaviours typical of visceral pain, such as writhing and licking their abdomens.

The intensity of visceral pain was found to decrease when animals with a locus of visceral pain were subjected to immobilization stress. These animals writhed for 303.8 s over 60 min as opposed to 412.1 s in the control group, which indicates a 26.3% lower pain reaction (Table 3). The second recorded pain reaction (licking of the abdomen) was not significantly different from the control.

We demonstrated previously [17, 18] that low-intensity red PL reduced visceral pain. Using red PL as an analgesic, we assessed the effects of stress on its analgesic effects. Figs. 9A and 10A show a comparison of the dynamics of pain reaction development in different experimental groups. The control group (1) and the stressed group (2) experienced the most pain during the entire observation period. The pain reaction was weaker in the other two groups (3 and 4), in which red PL was applied.

Under the action of red PL, the duration of writhing in animals without immobilization stress was reduced by 64% over 60 min of observation. In stressed animals, red PL reduced visceral pain by 46.1%. This suggests that stress weakened the analgesic effects of red PL. In stressful conditions, the red PL significantly suppressed visceral pain. In both the first and second 30 min of observation, the PL effect is clearly visible. During 60 min of observation, writhing lasted 411.6 s. The red PL weakened writhing by 1.4 times (its duration decreased to 222.1 s). On the other hand, PL had a positive effect on sleep. The duration of sleep for 60 min of observation in animals under stress was 411.6 s (without PL application) and 641.7 s (after application of red PL to AP E-36). In other words, sleep duration increases by 1.6 times, which also indicates a decrease in pain. The responses of grooming and running were not different from those of controls. A source of pain in the peritoneal cavity resulted in mice completely losing interest in food.

Table 3 The effect of stress and red PL on pain and non-pain behavioural responses in animals with an experimental locus of visceral pain.

Behavioural responses	Group 1 Normal (without stress)	Group 2 After stress	Group 3 After red PL	Group 4 After stress + red PL
Writhing	412.1 ± 51 100%	303.8 ± 51.5* 73.7%	148.2 ± 25.3*** 36%	222.1 ± 30.7*** 53.9%
Licking belly	51.7 ± 12 100%	60.6 ± 25 117.2%	23 ± 12.1* 44.5%	20,9 ± 3.2*** 40.4%
Sleeping	266.7 ± 93,8 100%	411.6 ± 123.6* 154.3%	522.8 ± 152.5* 196%	641.7 ± 129.1** 240.6%
Grooming	26.4 ± 8 100%	34.7 ± 12.6 131.4%	45.9 ± 37.3 173.9%	12 ± 3.6* 45.5%
Running	19.5 ± 4.2 100%	36.7 ± 9* 188.2%	16.7 ± 3.4 85.6%	18.3 ± 4.5 93.8%

All four groups of animals received an injection of a 2% solution of acetic acid into the abdominal cavity: the 1st group was not subjected to any other influences; the 2nd group was in a state of immobilization (stress) for 30 min; the 3rd received a red PL session on AP E-36; and the 4th group received both stress exposure and red PL.

Top line: duration of reaction for 60 min of observation in s, bottom line: as a percentage of a similar reaction in the 1st group. Significant differences from control (without immobilization): *** $p < 0.01$, ** $p < 0.05$, * $p < 0.5$.

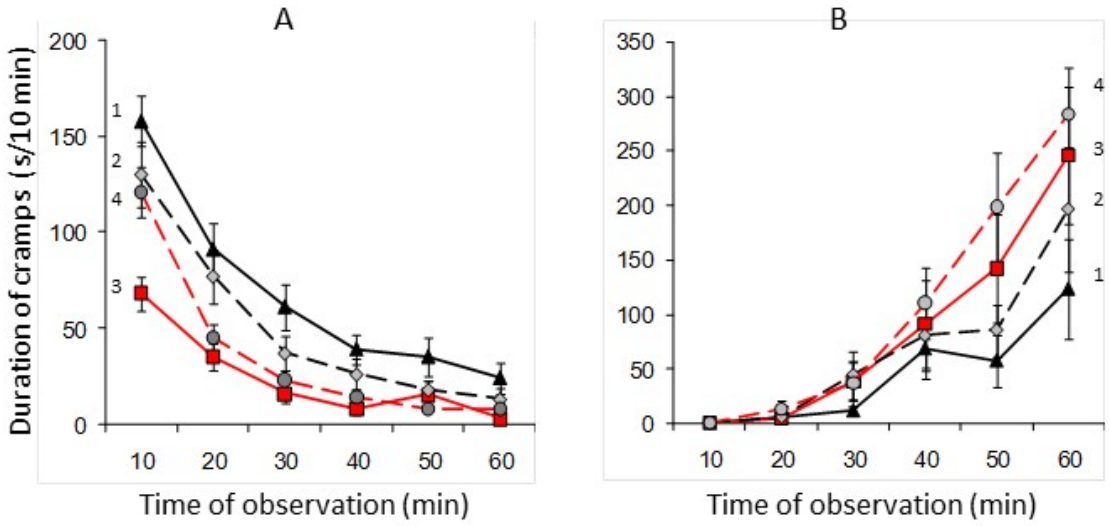


Fig. 9 Writhing (A) and sleep (B) in animals with a locus of visceral pain subjected to 30-min immobilization stress before and after application of red PL to AP E-36.

2% acetic acid was injected intra-abdominally into all four groups. First group: no stress; Second group: after stress; Third group: without stress but with PL application; Fourth group: after stress and PL application.

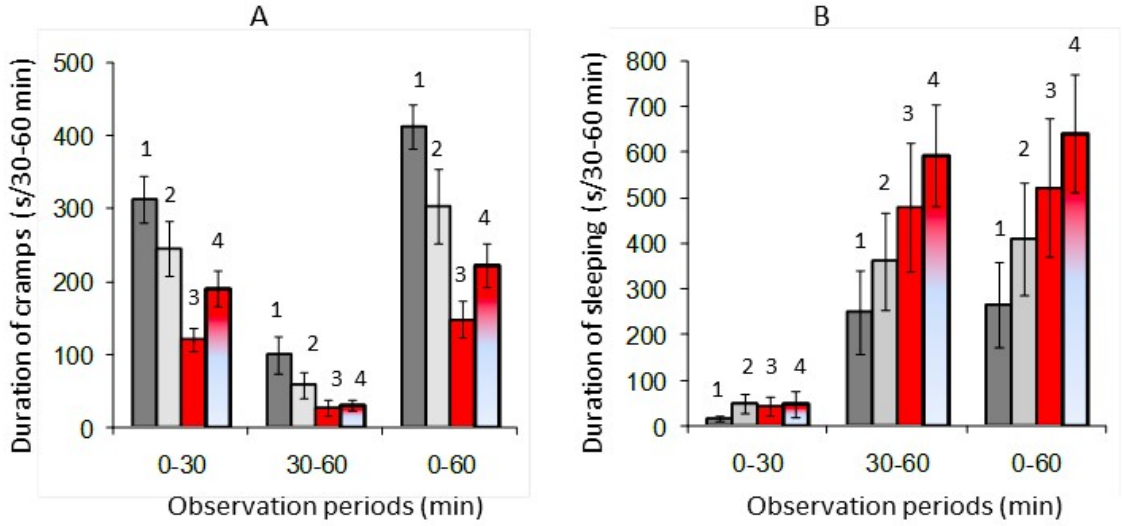


Fig. 10 Duration of writhing (A) and sleep (B) in mice of four groups with loci of visceral pain for the first 30 min, for the second 30 min, and for an hour of observation.

All four groups received an intra-abdominal injection of 2% acetic acid. 1st group: no stress; 2nd group: after stress (30-min immobilization); 3rd group: without stress, but with PL application on AP E-36; 4th group: after stress and PL application on AP E-36.

Eating behaviour was not restored either after stress or under the action of red PL against the background of stress.

3.5 Effect of Medolight-Polychrome Light on the Quality of Sleep in a Stressed Individual

Studies on animals have shown that light applications can level stress regardless of the wave range, making it logical to test the effects of such a technology on a person with stress disorders. In order to determine the effect of the low intensity polychromatic LED light of the Medolight device on falling asleep and dreaming in a person under stress, we examined the effect of low intensity polychromatic LED light of the Medolight device. Table 4 and Fig. 11 present the results.

Table 4 Effect of Medolight-polychrome light on sleep in stressed patients.

Number	Duration of falling asleep (s)			Having dreams (%)	
	Normal	Stress	Stress + Light	Stress	Stress + Light
1	375	750	500	40	10
2	370	760	525	50	18
3	375	680	490	35	22
4	315	830	580	40	16
5	460	700	610	58	24
6	390	620	550	52	5
7	430	710	440	49	8
8	465	790	485	55	21
9	350	735	460	42	18
10	250	790	490	54	8
11	400	780	550	49	11
12	430	770	465	60	21
13	490	740	500	54	12
14	410	835	530	48	13
M ± m	393.6 ± 47.1	749.3 ± 44.4	512.5 ± 38.6	49 ± 5.7	14.79 ± 5.2

Normal: a group without stress and without light application; Stressed: a group in a state of stress, but without light application; Stress + Light: the stressed group received a 15-min application of light on the auricle.

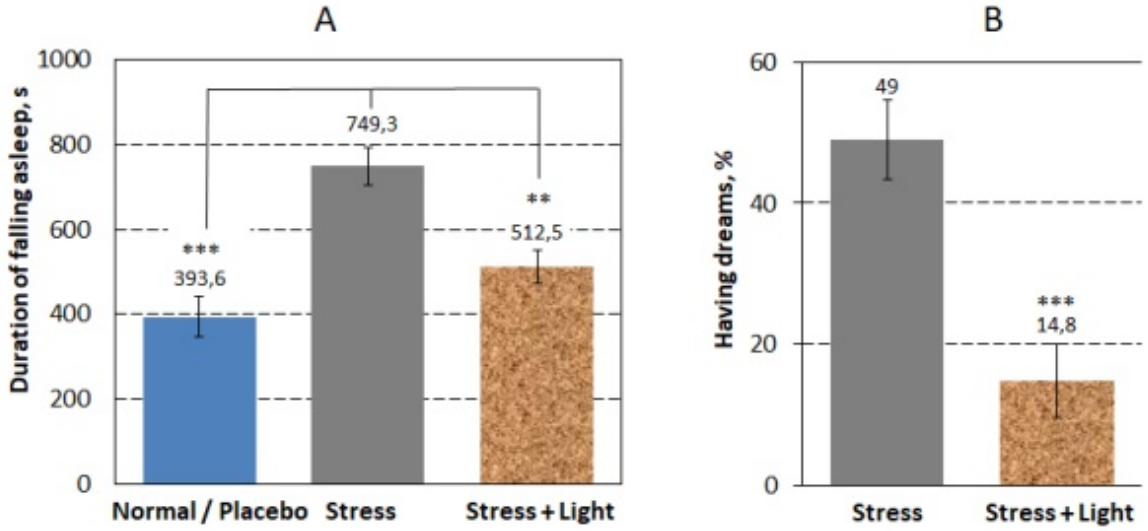


Fig. 11 The latent period of sleep (duration of falling asleep) (A) and the number of dreams (B) in the examined persons before and after applying the LED-light from the Medolight-polychrome device to the ear.

Normal: a group without stress and without light application (placebo); Stressed: a group in the state of stress, but without light application; Stress + Light: the stressed group received a 15-min application of light on the auricle.

The numbers above the columns: on "A"—the average duration of falling asleep for the group; on "B"—the average frequency of dreams for the group. Significant difference from the group "Stress": $^{***}p < 0.01$, $^{**}p < 0.05$.

Stressed subjects took an average of 749.3 ± 44.4 s to fall asleep, compared to 393.6 ± 47.1 s in the control group. The effect of light on these reflex zones of the auricle significantly reduced the time to fall asleep to 512.5 ± 38.6 s. The frequency of dreams in persons exposed to stress during the day was 49% ± 5.7%, and after a session of light therapy it was 14.79% ± 5.2%.

This difference is statistically significant.

Thus, the application of the light of the Medolight-polychrome device to the auricle weakened

the negative effect of stress on individual sleep indicators. The duration of falling asleep under the influence of light was shortened and sleep became more restful, as can be seen from the decrease in the number of dreams.

4. Discussion

We have demonstrated for the first time that low-intensity PL has an attenuating effect on behavioural manifestations of stress, suppressing somatic (based on the results of the formalin test) and visceral (based on the results of the acetate test) pain in mice under stress. The red PL had the most powerful effect.

The stimulation of E-36 has been known to produce analgesic and antistress effects, as well as other effects. This study confirmed those findings. We have achieved anti-stress and analgesic effects not by inserting a needle into the AP or stimulating it with an electric current, but by applying low intensity polychromatic and monochromatic PL.

Mice that are immobilized for 30 min show significant behavioural disorders. Grooming is one of the most prominent indicators of stress, which increased 3.3 times. In contrast, the time for eating was reduced and sleeping was disrupted (in the first 30 min after stress, the mice did not sleep at all), which is consistent with literature data. Stress-induced changes in sleep architecture [19] and eating behaviour [20] of animals are comprehensively described and used to monitor stress in a variety of experimental models.

In our study, low-intensity PL directed to the analgesic AP E-36 attenuated behavioural manifestations of stress [21]. In animals that at the end of the immobilization period received a 10-min session of low-intensity white, red or green PL on AP E-36, the duration of grooming for 60 min of observation was 54.2%, 50.8% and 69% of the similar reaction in the control group. The strongest anti-stress effect gave the red PL, and the smallest by green PL, that is, the wavelength of light had a significant influence on the anti-stress effect. Earlier, we observed a similar dependence on the analgesic effects of coloured PL [3, 15, 22-24]. There are cases described in the literature of AP influencing stress mitigation. Electroacupuncture has been shown to attenuate stress-induced c-fos expression in brain areas that are usually involved in stress responses [25]. Unlike electroacupuncture or classical acupuncture, in which special needles are inserted into an AP, the low-intensity PL treatment method is painless, non-traumatic, has no side effects, and is easy to use at home.

We observed a weakening of both somatic and visceral pain responses under stress. Stress reduces visceral pain by 26.3% and somatic pain by 28.5%. There is evidence in the literature that immobilization of animals can lead to significant changes in pain sensitivity [26]. Based on our results, we agree with those of other authors, who observed a decrease in the pain response induced by formalin in mice subjected to immobilization stress [27]. Our immobilization period was twice as short (30 min vs. 60 min) as the cited work. However, it is impossible to completely exclude the mechanism of pain relief caused by the redistribution of the area of pathology. The acetate test reproduces the symptoms of a perforated gastric ulcer and a subsequent acute peritonitis, which rapidly turns from local to diffuse. As a result of a decrease in the mobility of the intra-abdominal fluid, there was less irritation of the peritoneum during immobilization. The inhibition of somatic and visceral pain by immobilization is of clinical importance. Another experimental study has confirmed the expediency of immobilization in post-traumatic pain syndromes.

Analgesic effects of PL were weaker in animals that were subjected to immobilization stress than in controls. Under normal conditions, red PL reduced pain by 54.4% (somatic) and 64.0% (visceral), and in stressed animals by 31.0% and 46.1%, respectively. In

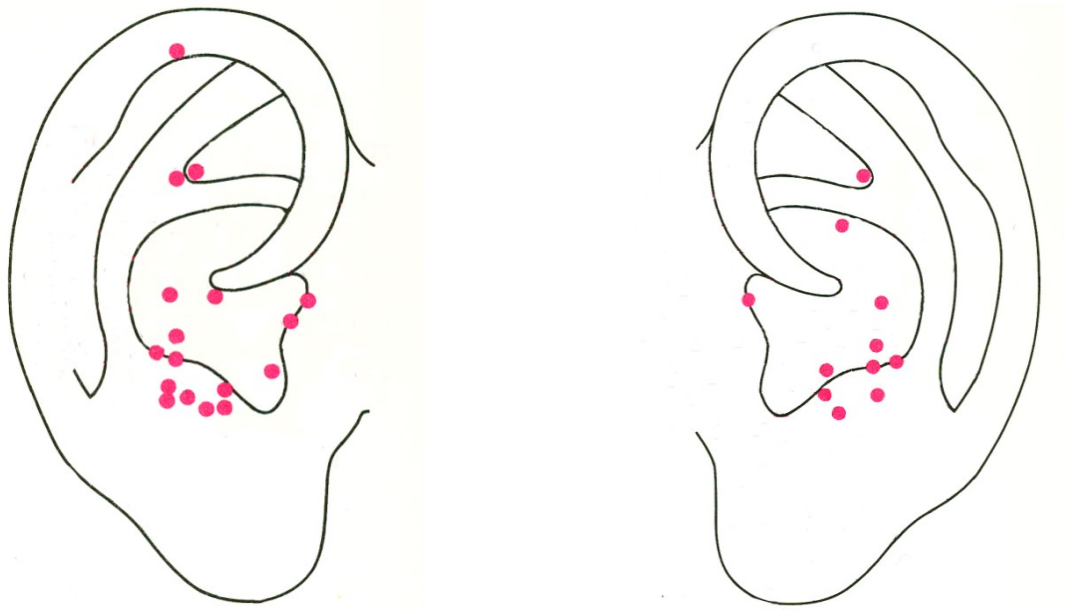
this case, it is possible that there is a conflict between the flow of pain impulses caused by immobilization and the flow of pain impulses from the source of chemical pain. Because of this, antinociceptive functions are depleted faster and the final analgesia is lower than what was observed with a single pain effect [2]. Further research is needed to determine the reasons for this difference and its mechanisms.

In human studies, low-intensity light is also found to reduce stress. Sleep disorders are one of the stress manifestations. About 45% of people worldwide suffer from insomnia. An increase in anxiety and stress caused by the Covid-19 pandemic has contributed to an increase in sleep disorders. Insomnia symptoms include difficulty falling asleep, increased sleep latency, and poor sleep quality.

We observed a significant increase in latent period of falling asleep in the examined individuals from 393.6 ± 47.1 to 749.3 ± 44.4 s. Application of the multi-component light of the Medolight-polychrome device to the auricle weakened the effects of stress. The duration of the falling asleep period was reduced to 512.5 ± 38.6 s. In addition to reducing the duration of the falling asleep period, light therapy also improved the quality of sleep by decreasing the number of dreams. In subjects exposed to stress during the day, dream frequency was 49% ± 5.7%, but after a session of light therapy, it was 14.79% ± 5.2%. A possible mechanism to improve sleep is light activation of specific acupuncture points (Fig. 12). About 200 different functional acupuncture points were found on the auricle. The stimulation of some of these points reduces the symptoms of stress and treats sleep disorders [28].

According to available literature on acupuncture's effectiveness for treating insomnia in humans, our data support these claims [29]. Acupuncture reduces the latency of sleep onset significantly [30]. The acupuncture applied to the ear is called auricular acupuncture. Sleep medicine is increasingly using this technique.

We conclude that the results of this study are not only theoretical, but also have practical significance. Stress-induced behavior was significantly reduced when PL of non-thermal intensity was applied to AP E-36. Stress in humans can be reduced through a non-traumatic method of PL influencing AP of the ear. By obtaining evidence-based data on the possibility of



(a) (b)

Fig. 12 Location of acupuncture points on the auricle that induce a sedative effect (a), facilitate sleep and improve sleep quality (b) [28].

increasing the body's resistance to stress, it will be possible to recommend PL for people who are frequently exposed to stress (entrepreneurs, civil servants, students, military personnel, industrial facility operators, etc.). The action of low-intensity PL on the reflexogenic zones of the human body may give it a high resistance to stress, relieve general fatigue, accelerate the recovery of working capacity, and strengthen it in general.

5. Conclusions

Experimental data have been obtained for the first time that statistically reliably demonstrate the presence of an anti-stress effect of low-intensity PL when it is applied to AP E-36. The duration of the main indicator of stress in rodents—grooming increased up to 330% after 30 min of immobilization stress, but only to 170%-230% after PL sessions.

The anti-stress properties of PL depend on its wavelength. Red light (the long-wavelength part of the visible light spectrum) had a significantly greater anti-stress effect than medium-wavelength green light. Red PL reduced stress-induced grooming by 49.2%, and other behavioural responses (sleeping, eating, motor activity) were partially normalized, whereas green PL reduced grooming by 31% without affecting any other behavioural responses.

A short-term immobilization stress reduced the somatic pain response (formalin test) by 28.5%, and the visceral one (acetate test) by 26.3%. These findings confirm that immobilization can relieve post-traumatic pain.

Red PL has a less pronounced analgesic effect on stressed animals than on non-stressed animals. Under normal conditions, red PL suppressed somatic pain by 54.4%, visceral pain by 64%, and under stress by 31 and 46.1%, respectively.

An experimental study demonstrated that PL could effectively reduce stress in humans. Stress increased the latent period of falling asleep in the subjects from 393.6 ± 47.1 to 749.3 ± 44.4 s. When the Medolight-Polychrome device was applied to the auricular AP, post-stress sleep disturbances weakened: the duration of falling asleep shortened to 512.5 ± 38.6 s. The frequency of dreams in individuals with daytime stress was 49.0% ± 5.7%, and after a PL session it was 14.8% ± 5.2%. The findings may serve as a basis for recommendations on how to level the effects of moderate stress in humans.

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