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Wireless optogenetic modules for mice

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ABSTRACT

We present wireless optogenetic devices for mice freely moving in cages. The purpose of the wireless devices is to stimulate specific brain regions using light. The optoelectronic devices we have designed are similar to the NFC tags and consist of a resonant LC circuit, high-frequency current rectification circuit and 470 nm micro-LED chip. The device allows one to provide optogenetical stimulation in targeted neurons of mice brain with illumination intensities up to 40 mW/mm^2. We also discuss the results of wireless stimulation of arcuate nucleus neurons of living mice brain.

Keywords: optogenetic devices, wireless implantable optoelectronic module

1. INTRODUCTION

Optogenetics is a new powerful method in neuroscience rapidly developing in the last fifteen years[1,2,3,4]. Optogenetics relies on the light illumination for activating or inhibiting of genetically-modified neurons in the brain. Traditional approach to optogenetic research in brain requires an optical fiber to be attached to animal head to feed the light generated by an external source[5,6]. This approach provides significant restrictions on the movement of animals and changes their natural behavior. These mechanical limitations can be circumvented by optoelectronic technologies and wireless receivers, as recently demonstrated in optogenetics[7,8,9,10]. The idea is to use thin flexible implants equipped with wirelessly controlled micro-LEDs. An alternative approach relies on a very small battery-free wireless implant on the head of the animal and a special resonant cavity with 1.5 GHz signal generator[11], but devices for this radiofrequency band are very sensitive to signal absorption and reflection.

Yet another approach uses small flexible wireless devices with inductive coupling at frequency 13.56 MHz based on near-field communications (NFC) technology[12,13]. At this frequency range the coupling between the antenna and receiver less sensitive to physical obstructions effective operation is possible underwater or near metallic plates. Additionally, the production of the NFC tags from flexible circuit boards is well developed technology. Optogenetic systems based on NFC technology are available commercially[14]. This wireless optogenetic brain implant integrates ultrathin injectable needle with micro-LED, magnetic loop antenna and matching capacitor, rectifier and indicating LED.

In our previous report[15], we have described an mobile optoelectronic light emitting module for optogenetic research based on a standard cannula with a small light-emitting diode (LED) and module based on sapphire needle. In this work, we present a set for wireless optogenetic stimulation based on the Neurolux design[14] and results of behavioral testing.

2. METHODS

2.1 Implant fabrication

Battery-free wireless devices similar to the Neurolux implants[14] were designed and fabricated. The diameter of device is approx.10 mm and its thickness below 1 mm. The devices are fabricated from electro-deposited (Cu/Polyimide/Cu) material (Dupont, Pyralux) using a photolithographic technique. The circular coil consists of 8 copper turns (75 µm traces, 75 µm spaces) at the top and the bottom sides of polyimide substrate (see Fig.1). The top and bottom copper coils are connected through two connectors - laser-drilled holes filled with copper by electroplating. All copper conductors were gilded to prevent oxidation.

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Figure 1. Photography of the wireless device (indicating diode coated with orange phosphor ZIP 595I).

The injectable needle is connected with the ring coil through a flexible serpentine providing freedom of movement necessary in the implantation process in the brain. The needle width is 350 μm, maximal thickness 150 μm (see Fig.2). A micro-LED is soldered to the tip of needle. The needle is coated with a layer of UV-curing optical epoxy NOA 63 (Norland Products, Inc.) to increase the rigidity of needle and prevent mechanical detachment of the micro-LED. The remaining parts of the implant are coated with a transparent insulating lacquer PLASTIK 70 (CRC Industries Europe BVBA), which used to protect printed circuit boards.

Figure 2. The needle with stimulating micro-LED TR2227 (Cree Inc.) soldered to its tip.

The electronics scheme of the wireless device is quite simple (see Fig.3). The coil L1 was designed with an inductance 1.8 μH. The circuit resonates at 13.56 MHz with 22 pF matching capacitor C1. A Schottky diode (CDBZ0130R-HF, Comchip Technology) is used as rectifier.

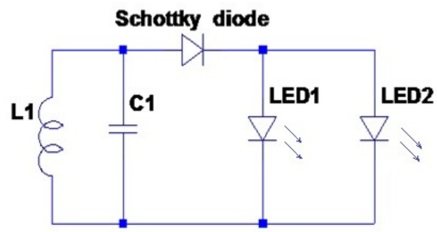

Figure 3. The circuit diagram of wireless optogenetic devices. LED1 and LED2 are stimulating and indicating LEDs respectively.

The indicating LED L2 (APT1608LVBC/D, Kingbright) is optional. Figure 1 shows an implant with the indicating LED coated with silicate LED phosphor ZIP595I (Beijing Yuji Science Technology Co., Ltd), but an ultrathin (0.4 mm thickness) device version with no indicating LED can be also used (see Fig.4.). The mass of this version is only 15 mg (20 mg for device with indicating LED).

Figure 4. Photography of the wireless device without indicating diode.

2.2 Wireless power supply system

The wireless power transfer system for optogenetic experiment was developed on the basis of standard HF consumer electronic devices. Wireless implants are connected inductively with a loop antenna in the ISM band 13.56 MHz. Control of the impulse mode of power supply system is performed by means of special software for Arduino Uno. The function generator is used in external gated burst mode as the signal generator, then the signal is fed into the broadband preamplifier followed by the power amplifier, a matching circuit and a loop antenna (see Fig.5).

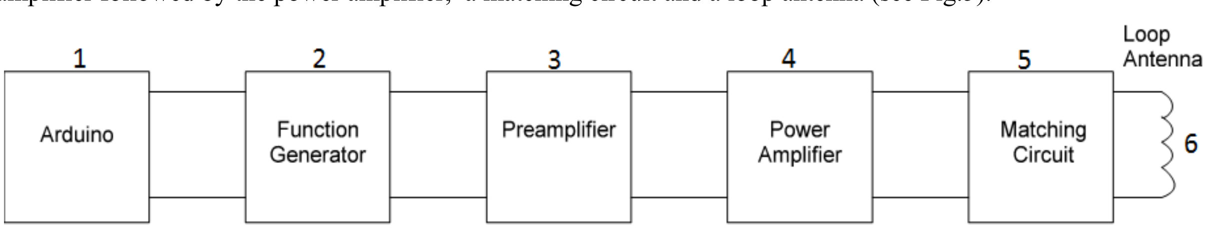

Figure 5. Wireless driving system block diagram.

The general view of the system is shown in the figure 6, where, as matching circuit is use high efficiency loop tuner for the antenna matching.

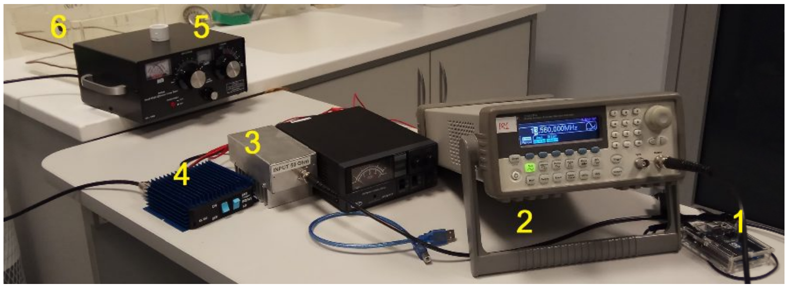

Figure 6. The wireless driving system: 1 - Arduino Uno, 2 - function generator, 3 – preamplifier, power amplifier, 5 – matching circuit, 6 – loop antenna.

We based our power amplifier on commercial model RM KL 203 modified to enable pulse operation regime.

3. RESULTS AND DISCUSSION

In our previous report[15], we have described two devices: an optoelectronic light emitting module for optogenetic research based on a standard cannula and a small light-emitting diode (LED) chip C470UT190 and an alternative design of the optoelectronic device made on the basis of sapphire needle with LED. The light-current characteristics shown in Fig.7 indicate that the LED chip C470TR2227 used in this work provides almost 3 times more light intensity for a given current than the chip C470UT190 of comparable dimensions used in the previous work.

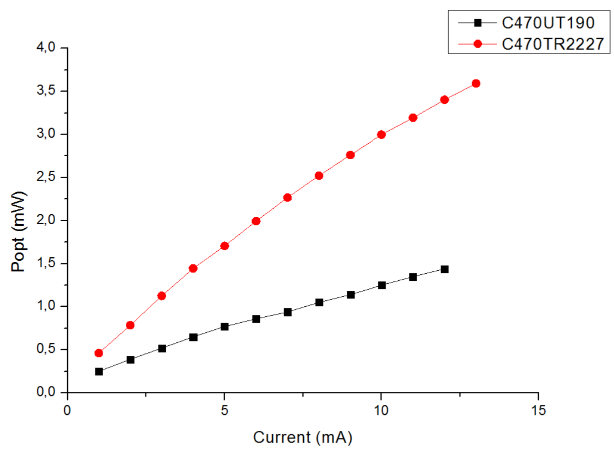

Figure 7. Optical power of LEDs CREE C470UT190 and C470TR2227 vs driving current.

The goal of this research was to construct a system suitable for relatively small cages for experiments with mice. An example of such a cage is shown in Fig.8 – a plastic container with the loop antenna wound around it. As the experiments planned concern freely moving animals one must ensure that the system will provide proper stimulus for every position of the animal in the cage. This has been verified in two ways. We have mapped light intensity emitted by a single implant placed in different positions inside the cage and found out that it varies by less than a factor of 2. We have also placed several devices inside a cage to illustrate the uniformity of the light emission (see Fig.8).

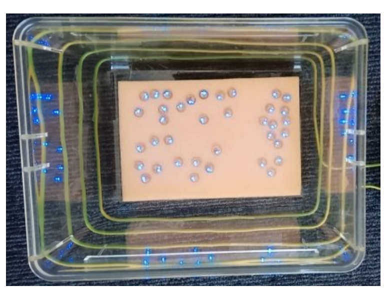

Figure 8. Loop antenna wound around a transparent cage (23 × 17 × 15 centimeters) with wireless optogenetic implants placed approx. 2.5 cm above the bottom.

We found out that the RF power emitted by the loop antenna disturbs the operation of our optical power meter when its head is placed inside the loop. So, in order to test our wireless devices we have attached an optical fiber to the stimulating LED and measured optical power at the end of the fiber away from the loop antenna. Such a procedure provides the lower bound on the light intensity of the wireless device. The results of the measurement of the light intensity versus the RF power fed into the antenna for a selected implant are shown in Figure 9. For the RF output power allowed by the government regulations (10W) light intensity of the stimulating LED is approx. 40 mW/mm^2 exceeding the intensity required for majority of possible applications. A further increase of the power in the loop antenna is technically possible, but would require RF safety coordination with proper government agencies.

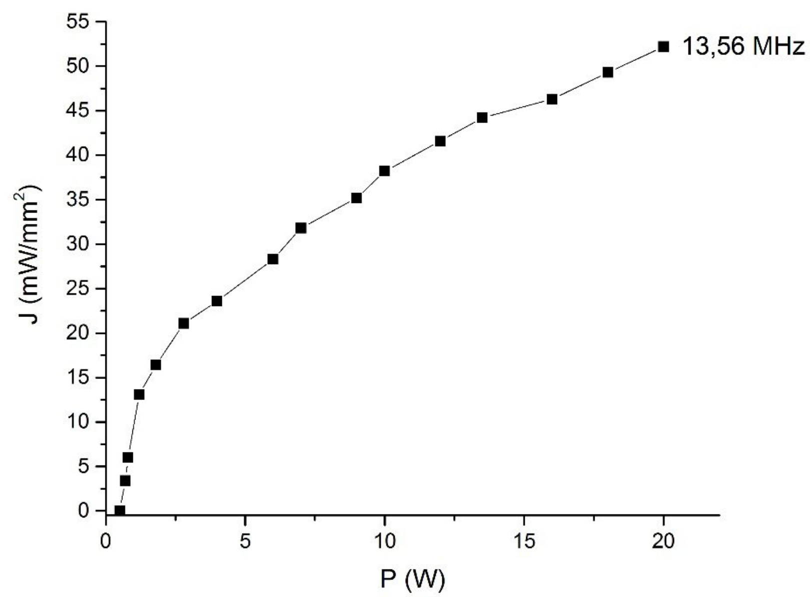

Figure 9. Experimental light intensity-antenna's RF power characteristic of a wireless implant.

Experimental data shows, that resonance curve of our wireless optogenetic implant is quite wide (FWHM ≈ 1MHz). It is not surprising as the resonant circuit is quite lossy due to LEDs. At the same time such a broad resonance guarantees that changes of the capacitance or inductance of components forming the resonance circuit due to temperature or other factors resulting in slight change of the resonance frequency will not influence the coupling between the loop antenna and the implant. The curve is also asymmetric for the reasons we do not know.

Preliminary in vivo experiments were performed with mice genetically modified with agouti-related protein neurons (AgPR) in arcuate nucleus(Arc), which regulate hunger. Four weeks after the surgery mice were tested for changes in behavior triggered by optogenetic activation. We observed an increase of the amount of food eaten during and after stimulation, but this preliminary data requires further studies and verification.

4. SUMMARY

We have developed wireless implantable optoelectronic devices with micro-LED for optogenetic stimulation of living mice brain tissue operating at13.56 MHz. We have built a near-field wireless power supply system for optogenetics with programming control. The devices have been tested in vivo on arcuate nucleus of the genetically modified mice with activated channelrhodopsin in AgPR neurons.

5. ACKNOWLEDGMENT

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