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(54) Title: NON-INVASIVE OPTOGENETIC STIMULATION METHOD TO REGULATE GLUCOSE METABOLISM IN THE LIVER AND BROWN ADIPOSE TISSUE

(57) Abstract: Methods and devices are disclosed for non-invasive optogenetic stimulation of autonomic efferent fibers to regulate glucose metabolism in the liver and brown adipose tissue and to treat metabolic disorders.

NON-INVASIVE OPTOGENETIC STIMULATION METHOD TO REGULATE GLUCOSE METABOLISM IN THE LIVER AND BROWN ADIPOSE TISSUE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional patent application No. 62/487,599, filed on April 20, 2017, the contents of which is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Throughout this application various publications are referred to in parenthesis. Full citations for these references may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated by reference in their entireties into the subject application to more fully describe the art to which the subject application pertains.

[0003] The liver plays a major role in blood glucose homeostasis by maintaining a balance between the uptake and storage of glucose via glycogenesis and the release of glucose via glycogenolysis and gluconeogenesis. This process is highly regulated through neural connections between the liver and the autonomic nervous system. The liver is innervated by both sensory afferent and autonomic efferent nerves. Autonomic efferent innervation of the liver originates mainly in two areas including the intermediolateral cell column of the spinal cord and the dorsal motor nucleus of the vagus. Both of these regions contain preganglionic neurons that innervate liver. Adrenergic receptors for the sympathetic neurotransmitter epinephrine and norepinephrine are present in the liver and cholinergic receptors for the parasympathetic neurotransmitter acetylcholine are involved in control of liver metabolism. The liver consists of parenchymal cells (*i.e.*, hepatocytes) and non-parenchymal cells such as stellate and Kupffer cells. These cells express cholinergic and adrenergic receptors that contribute to diverse liver function. Early pharmacological studies demonstrate that cholinergic and adrenergic receptors on hepatocytes contribute to the regulation of hepatic glucose production *in vitro* and *in vivo*.

[0004] Interscapular brown adipose tissue (BAT) is the principal site of nonshivering thermogenesis and contains a high density of mitochondria with high amount of uncoupling protein-1 (UCP-1), a protein that allows for the uncoupling of fatty acid oxidation from ATP production to generate heat (1-3). In addition, BAT possesses a great capacity for glucose

uptake and metabolism (2, 4). For instance, cold exposure increases glucose uptake by BAT in rodents as well as humans (5, 6). Importantly, both BAT thermogenesis and glucose uptake are highly regulated by the autonomic nervous system (3). Although the function of the parasympathetic nervous system in BAT remains elusive, mice lacking the β-adrenergic receptor are cold intolerant (7) and activation of the β3-adnergic receptor in BAT stimulates glucose uptake (8-10). Therefore, the sympathetic nervous system (SNS) plays an essential role in regulating BAT thermogenesis and glucose utilization (4,11). BAT plays a critical role in regulating glucose hemostasis in rodents and humans (17-19).

[0005] Although the autonomic nervous system (ANS) innervates both liver and BAT and regulates glucose metabolism in these organs, studies to date have not examined the effects of "selective" stimulation of sympathetic innervation of BAT mainly due to lack of sufficiently precise experimental approaches. Most studies have examined the effects of systemic administration of adrenergic and cholinergic agonists that regulate not only liver and BAT but also other peripheral organs such as pancreas, heart, and muscle (12-16). As such, altered blood glucose levels following drug administration may well be due to alterations in insulin release from pancreas, altered insulin sensitivity in muscle and liver, hepatic glucose production, and increased BAT activity.

[0006] Recent advances in optogenetics have enabled precise control of specific cells or fibers at the millisecond timescale in freely moving animals (25). Optogenetics is the technology that uses visible light to trigger changes in proteins that modulate membrane potentials in neuronal cells through excitatory or inhibitory membrane currents. For instance, channelrhodopsin 2 (ChR2) is a transmembrane ion channel found in green algae that becomes permeable to cations in the presence of blue light. ChR2 can be used in neurons as ion channels are a main contributor in electrical signal transduction in the nervous system. This ability to modulate neuronal cells has proven instrumental in preclinical studies and holds enormous potential for the treatment of diseases such as Parkinson's disease, epilepsy, and depression.

[0007] Although the ability of optogenetics to modulate neuronal activity has proven helpful in preclinical studies and has the potential for the treatment of neurological disorders, the current techniques are too invasive for practical clinical application in humans. In current implementations, optical fibers are surgically implanted in organs to deliver light. Moreover, optical fibers need to be connected to an expensive and relatively big laser or a LED as the light source. These technical limitations make it difficult to develop clinical devices. The

present invention addresses the need for methods and devices for *non-invasive* optogenetic therapy.

SUMMARY OF THE INVENTION

[0008] The invention provides methods and devices for non-invasive optogenetic therapy.

[0009] Non-invasive optogenetic methods are provided for modulating the activity of metabolic organs, such as liver and BAT, in a subject, where the methods comprise applying light through the skin of the subject to activate a transmembrane ion channel channelrhodopsin virally-induced in autonomic efferent nerve fibers innervating the organ, where the light is applied in a wavelength, amount and duration effective to modulate activity of the organ in a subject.

[0010] The invention also provides devices for non-invasive optogenetic stimulation, where the devices comprise one or more surface-mounted-device light-emitting device (SMD-LED) modules, a Transistor-Transistor Logic (TTL) pulse generator, and a power supply, wherein the devices are configured to be mounted on the skin of a subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1. Conventional optogenetic stimulation of autonomic efferent fibers in the liver reduces blood glucose levels. An optogenetic fiber was placed directly in the liver parenchyma of ChAT-IRES-Cre::ChR2-YFP mice. Optogenetic stimulation of cholinergic fibers at 20 Hz for 1 hr strongly reduced blood glucose levels.

[0012] Fig. 2A-2E. Non-invasive optogenetic stimulation method. (A) A small (2 X 2 mm box) three watt SMD blue LED was connected to a TTL pulse generator controlled by a computer. (B) Images showing placement of SMD LEDs on abdominal skin of mice. (C) Illustration showing optogenetic stimulation protocol. ChAT-Cre::ChR2 (D) and Th-Cre::ChR2 (E) mice were generated to study the physiological functions of the autonomic nervous system. Non-invasive optogenetic stimulation of cholinergic efferent fibers reduced, whereas activation of catecholaminergic efferent fibers increased blood glucose levels, supporting the use of this non-invasive method as being as efficient as the invasive method (n = 6 and 5 mice, respectively). Efferent fibers were stimulated at 20 Hz for 1 hr. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

[0013] Fig. 3A-3G. Selective activation of sympathetic efferent fibers of BAT. (A) Expression of Th-positive fibers (arrow heads) in BAT of Th-Cre::tdTomato mice. 50 μm calibration bar. (B) Optogenetic stimulation of sympathetic efferents of BAT of Th-Cre::ChR2 mice increased BAT thermogenesis (n = 7 mice). Open circle, core temperature

and gray square, BAT temperature (C) Expression of ChR2 exclusively in sympathetic efferents innervating BAT via injection of retrograde viral vectors. Th-Cre mice injected with retrograde ChR2. Optogenetic stimulation increased BAT metabolism (n= 7 mice). (D) Injecting a β3 receptor antagonist blocked the effect (n= 7 mice). (E) Images showing the novel non-invasive optogenetic stimulation method. Hair on the neck and shoulders was removed and then two SMD LED modules per each side were placed directly on the skin. The skin was illuminated with blue light. (F) Increased plasma norepinephrine (NE) levels during stimulation of sympathetic efferent fibers with the method (n= 6 mice). (G) Pooled data showing changes in body temperature and blood glucose levels before, during, and after non-invasive optogenetic stimulation of sympathetic efferent fibers (n = 6 mice). ***$p<0.001$ (unpaired t-test)*$p<0.05$, **$p<0.01$, ***$p<0.001$, unpaired t-test.

[0014] Fig. 4A-4B. Channelrhodopsin expression in sympathetic efferent nerve fibers using an AAV having a tyrosine hydroxylase (Th) promoter. An AAV vector carrying ChR2 expression cassette under control of the Th promoter was injected in BAT of wild-type mice. Graphs showing effects of optogenetic stimulation of ChR2-expressing fibers on body temperature (A) and blood glucose levels (B) in wild-type mice injected with AAV8-Th promoter-ChR2-eYFP (n = 2 mice).

DETAILED DESCRIPTION OF THE INVENTION

[0015] The invention provides a non-invasive optogenetic method for modulating the activity of a metabolic organ in a subject, the method comprising applying light through the skin of the subject to activate a transmembrane ion channel channelrhodopsin virally-induced in autonomic efferent nerve fibers innervating the organ, where the light is applied in a wavelength, amount and duration effective to modulate activity of the organ in a subject.

[0016] The organ can be, for example, liver or brown adipose tissue.

[0017] The channelrhodopsin can be, for example, channelrhodopsin2 (ChR2), and the light is blue light. The blue light can have a wavelength of ~450 to 475 nm. In another embodiment, the channelrhodopsin can be a red-shifted variant of channelrhodopsin, and the light is red light. The red light can have a wavelength of ~590 to 630 nm.

[0018] For the present invention, light-activated transmembrane ion channel proteins need to be expressed in target neurons. This can be accomplished, for example, using adeno-associated viruses (AAV) for expression of a transmembrane ion channel channelrhodopsin (*e.g.*, ChR-2 or a red-shifted variant of channelrhodopsin (24)).

[0019] Adeno-associated virus (AAV) is a non-enveloped, single-stranded, small DNA virus of the Parvovirus family. As infection with wild-type AAV is not associated with any known illness, AAV has been used for long-term, stable gene expression in neurons in mammals, with little or no toxicity. The naturally occurring AAV integrate its genome into the host chromosome, but the deletion of the rep genes (that are required for the AAV life cycle) from the vector form of AAV lead to the loss of this integration. In other words, most AAV genomes exist as non-integrated episomes as single viral genomes. This feature is particularly important because the random integration of the therapeutic vector genome within the host DNA can lead to malignancy. No AAV vector-induced malignancy has been reported in clinical gene transfer studies.

[0020] Gene transfer studies using AAV have shown significant progress at the level of animal models and clinical trials and have been noteworthy with respect to the safety of AAV vectors (*e.g.*, 23). There are a number of clinical trials using AAV vectors (as of 2011, there are more than 20 clinical trials). Among them, three groups undertook gene therapy trials for the retinal degenerative disorder Leber's congenital amaurosis (early-onset blindness) (NCT00643747, NCT00516477, and NCT00481546). They found that immune responses to AAV serotype 2 (AAV2) and transgene product were minimal and more than 25 subjects injected with the AAV vector showed some improvements in visual function that persisted for periods of over 3 years. Moreover, a recent clinical trial of RetroSence Therapeutics (NCT02556736; Phase I/II) uses the AAV2 that encodes channelrhodopsin-2 (ChR2) for retinitis pigmentosa.

[0021] Adeno-associated virus (AAV) is replication-defective and cannot cross synapses without replication. In general, AAV particles are taken up by axon terminals of the neurons projecting to the infected organ and then AAV is transported either anterogradely or retrogradely to neuronal cell bodies. Importantly, the infection and direction are determined mainly by the serotypes of AAV. AAV serotypes 1, 8, and 9 are anterogradely and retrogradely transported (20). AAV6 is also retrogradely transported to cell bodies.

[0022] Importantly, the natural promoters of AAV can be replaced with other promoters. This means that one can use AAV vectors that have the choline acetyltransferase (ChAT) promoter for the parasympathetic cholinergic efferents and the tyrosine hydroxylase (Th) promoter for the sympathetic adrenergic efferents. For example, an AAV vector carrying ChR2 expression cassette under control of the Th promoter (21, 22) can be used. The presence of the Th promoter will drive expression of ChR2 exclusively in catecholaminergic neurons (see Fig. 4).

[0023] In one embodiment, the channelrhodopsin is induced in parasympathetic cholinergic efferent nerve fibers using an adeno-associated virus (AAV) having a choline acetyltransferase (ChAT) promoter. In another embodiment, the channelrhodopsin is induced in sympathetic adrenergic efferent nerve fibers using an adeno-associated virus (AAV) having a tyrosine hydroxylase (Th) promoter.

[0024] A similar approach has been used in clinical trials of AAV delivery to liver (*e.g.* AAV vector with a liver-specific promoter consisting of the human α1-antitrypsin promoter with the apolipoprotein enhancer and elements of the hepatic control region; NCT00076557, NCT00515710, and NCT00979238). Interestingly, AAV vector was administered through intramuscular, hepatic, and intravenous routes in these clinical trials. In other words, direct injection of AAV to target organs is not absolutely necessary.

[0025] Viral injections can be made directly into peripheral target organs, such as liver and brown adipose tissue.

[0026] The activation of human brown adipose tissue (BAT) represents an opportunity to increase energy expenditure and weight loss alongside improved lipid and glucose homeostasis (11). Accumulated evidence has suggested that sympathetic innervation contributes to the regulation of BAT activity (4,11). To selectively stimulate sympathetic efferent fibers of BAT, ChR2 can be expressed in sympathetic efferent fibers of BAT by directly injecting a retrograde AAV encoding the ChR2 transgene into BAT.

[0027] The penetration depth of light into tissue depends on several factors including light wavelength, wattage, spot size, etc. In general, red light penetrates the skin to a depth of about 8 to 10 mm. Recently, a red-shifted variant of channelrhodopsin that is activated with orange to red light (~590 to 630 nm) has been reported (24). They show that red light can penetrate the mouse skin and even the skull and then activate this newly-developed red-shifted channelrhodopsin in rodents.

[0028] Non-invasive optogenetic stimulation of autonomic efferent nerve fibers to the liver can be used to regulate hepatic glucose metabolism. Activation of cholinergic parasympathetic efferent nerve fibers to the liver can be used to reduce blood glucose levels in a subject. Activation of catecholaminergic sympathetic efferent nerve fibers to the liver can be used to increase blood glucose levels in the subject. The subject can have type 2 diabetes or pre-diabetes or hyperglycemia.

[0029] Non-invasive optogenetic stimulation of autonomic efferent nerve fibers to brown adipose tissue (BAT) can be used to regulate one or more of BAT thermogenesis, core body temperature and blood glucose levels. Activation of catecholaminergic sympathetic efferent

nerve fibers to BAT can be used to do one or more of increasing BAT thermogenesis and core body temperature, and decreasing blood glucose levels in a subject. The subject can have type 2 diabetes, pre-diabetes or hyperglycemia.

[0030] In the non-invasive optogenetic stimulation method, the light can be applied to the subject using a device mounted on the skin of the subject over the organ of interest, where the device comprises one or more surface-mounted-device light-emitting device (SMD-LED) modules, a Transistor-Transistor Logic (TTL) pulse generator, and a power supply.

[0031] The light applied to the subject can have an intensity of, for example, 180 lumens. The light can be presented to the subject, for example, in 20 msec pulses at a frequency of 20 Hz. Pulses of light can be applied to the subject in, for example, on/off cycles of 1-3 sec duration. The light can be applied to the subject for a duration, for example, of 30 minutes to three hours.

[0032] The invention also provides a device for non-invasive optogenetic stimulation, where the device comprises:

- one or more surface-mounted-device light-emitting device (SMD-LED) modules,
- a Transistor-Transistor Logic (TTL) pulse generator, and
- a power supply,

where the device is configured to be mounted on the skin of a subject.

[0033] The light-emitting device can comprise one or more SMD-light-emitting diodes. Each module of the device can be, for example, about 2 mm by 2 mm square. The TTL pulse generator can, for example, be incorporated in a pulse generator circuit board. The TTL pulses can, for example, use 5 volt TTL logic levels. Preferably, the device uses no more than about three watts of power.

[0034] The SMD-LED device can, for example, emit blue light or red light. The light can have an intensity of, for example, about 180 lumens. The light can be generated, for example, in 20 msec pulses at a frequency of 20 Hz. Pulses of light can be generated in, for example, on/off cycles of 1-3 sec duration.

[0035] All combinations of the various elements described herein are within the scope of the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0036] This invention will be better understood from the Experimental Details, which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims that follow thereafter.

EXPERIMENTAL DETAILS

Introduction and Overview

[0037] A non-invasive optogenetic stimulation method was developed to regulate glucose metabolism in the liver and brown adipose tissue (BAT) in freely moving animals. It is known that blue light (453 nm) readily penetrates human skin. We took advantage of this property to develop a non-invasive optogenetic stimulation method that permits direct stimulation of channelrhodopsin-expressing autonomic efferent fibers to the liver and BAT. Surface-mounted-device light-emitting device (SMD-LED) modules were used as a light source. The SMD-LED can be mounted onto and soldered onto a tiny circuit board and is quite small. Importantly, the SMD-LED gives off almost no heat and has low voltage and current requirements. This physical feature of the SMD-LED allows it to be applied directly to the abdominal skin since the skin is not irritated by heat during light stimulation. Individual modules are controlled by 5 V TTL logic levels. This innovative method can be easily applied to humans since it does not require surgery to implant optogenetic devices into target organs.

[0038] In our studies of mice expressing light-activated stimulatory proteins in cholinergic and catecholaminergic preganglionic neurons, 3 W SMD-LED modules controlled by TTL logic levels were applied directly to abdominal skin below the ribcage of mice. This non-invasive optogenetic stimulation method significantly altered plasma blood glucose levels in mice. Specifically, non-invasive optogenetic activation of sympathetic adrenergic fibers in the liver increased, whereas stimulation of parasympathetic cholinergic fibers decreased blood glucose levels. Activation of catecholaminergic sympathetic efferent fibers to BAT increased BAT thermogenesis and core temperature, and decreased blood glucose levels. Therefore, the present non-invasive optogenetic stimulation method will open up new therapeutic strategies to control blood glucose levels in people with diabetes in particular.

Liver Parenchyma Contains Autonomic Efferent Fibers

[0039] The distribution of autonomic nerve fibers was examined in the liver parenchyma. To this end, two strains of mice were generated: the tyrosine hydroxylase (Th)-Cre:: tdTomato strain to identify sympathetic catecholaminergic fibers, and the choline acetyltransferase (ChAT)-IRES-Cre::tdTomato strain to examine the distribution of hepatic parasympathetic cholinergic efferent fibers. Both parasympathetic and sympathetic efferent fibers were clearly observed throughout the liver parenchyma. Most Th- and ChAT-positive fibers appear to be adjacent to the sinusoids and possibly hepatocytes. Therefore, both

animal models are ideal for studying the functional roles of autonomic neural circuits of the liver.

Conventional Invasive Optogenetic Stimulation of Autonomic Efferent Fibers in the Liver

[0040] The liver expresses adrenergic and cholinergic receptors that are activated by neurotransmitters released from autonomic sympathetic and parasympathetic efferent fibers. To examine the functions of the parasympathetic cholinergic fibers, the ChAT-IRES-Cre::ChR2-YFP mice were generated. Under isoflurane anesthesia, parasympathetic efferent fibers were illuminated by directly placing an optogenetic fiber coupled with a laser or an LED in the liver parenchyma. Bursts of light pulses were applied for 1 second followed by a 1 second break that repeated continuously for 1 hour (Fig. 1). This optogenetic stimulation readily reduced blood glucose levels, consistent with parasympathetic activation. Although these findings are very promising, surgical implantation of an optic fiber into the liver was needed to selectively stimulate autonomic efferent fibers under isoflurane anesthesia. Moreover, one cannot monitor blood glucose levels in freely moving animals with this invasive optogenetic stimulation method.

Development of a Non-Invasive Optogenetic Stimulation Method

[0041] To overcome existing limitations, a non-invasive optogenetic stimulation method was developed. Small (2 mm x 2 mm) 3 W SMD-LED (180 lumen of blue light) modules were directly applied to the abdominal skin just above the liver (Fig. 2). Individual modules were controlled by 5 V TTL logic levels that were generated by a TTL pulse generator (Doric Lenses). The pulse train parameters were controlled via an interface of open source OTPG4 controller software (Doric lenses) (Fig. 2A). Under these experimental conditions, the abdominal skin of ChAT-Cre::ChR2 and Th-Cre::ChR2 animals overlying the liver parenchyma was illuminated without surgical implantation of optic fibers into the liver (Fig. 2B). Bursts of light pulses at 20 Hz were applied for 1 s followed by a 1 s break that repeated continuously for 1 hr (Fig. 2C). This non-invasive optogenetic stimulation of parasympathetic efferent fibers effectively decreased blood glucose levels (Fig. 2D). Additionally, optical stimulation of sympathetic efferent fibers elevated blood glucose levels. Hence, results from these studies support the fact that blue light not only penetrates the skin but also is sufficient to stimulate channelrhodopsin-expressing autonomic efferent fibers to the liver.

Target Organ-Specific Expression Of Light-Activated Proteins

[0042] The method can use Cre-inducible retrograde AAV (*i.e.*, AAV6, 8, and 9) that encodes ChR2. Importantly, the natural promoters of AAV can be replaced with promoters from other viruses or host cells. This means that one can use AAV vectors that have the

choline acetyltransferase (ChAT) promoter for the parasympathetic cholinergic efferents and the tyrosine hydroxylase (Th) promoter for the sympathetic adrenergic efferents (e.g. Fig. 4). In this case, one does not need Cre-expressing neurons to induce expression of ChR2 in autonomic efferent fibers.

Direct Activation of Sympathetic Efferent Fibers of BAT Increases BAT Activity

[0043] Th-Cre::tdTomato mice were used to examine BAT sympathetic efferent fibers. Immunohistochemical staining showed tdTomato-positive fibers in BAT, indicating that BAT receives catecholaminergic innervation (Fig. 3A). Next Th-Cre::ChR2 mice were generated to optogenetically stimulate sympathetic efferent fibers of BAT. An optic fiber was surgically placed just underneath the BAT pad and sympathetic efferent fibers were optogenetically stimulated at 20 Hz for 1 hr. This optogenetic stimulation readily elevated body core temperature that was associated with reduced blood glucose levels (Fig. 3B, n = 7). These results support the interpretation that optogenetic stimulation of sympathetic efferent fibers of BAT enhances BAT metabolism.

[0044] To express ChR2 in sympathetic efferent fibers exclusively innervating BAT, replication-incompetent retrograde herpes simplex virus (HSV) encoding the Cre-inducible ChR2 transgene was bilaterally injected into BAT of Th-Cre mice. At four weeks post viral injection, sympathetic efferents of BAT were optogenetically stimulated, resulting in increased BAT thermogenesis (Fig. 3C). Increased BAT thermogenesis was due to activation of β3-adrenergic receptors in BAT as injection of the β3-adrenergic receptor antagonist (SR59230A) completely abolished the effect of optogenetic stimulation (Fig. 3D). These experiments provide direct evidence for retrograde expression of ChR2 in sympathetic efferent fibers that exclusively innervate BAT.

Non-invasive Optogenetic Stimulation of Sympathetic Innervation of BAT

[0045] It was then examined whether non-invasive optogenetic stimulation is also able to stimulate sympathetic efferent fibers, resulting in norepinephrine (NE) release. SMD-LED modules were directly placed on the skin of the neck and shoulders (Fig. 3E). This novel non-invasive method was as efficient as the invasive method in that non-invasive optogenetic stimulation significantly increased plasma NE levels, indicating that this non-invasive optogenetic stimulation activates sympathetic efferent fibers of BAT, leading to NE release.

[0046] ChR2-expressing sympathetic nerves of BAT were illuminated with bursts of light pulses for 1 hr. Likewise, this non-invasive optogenetic stimulation method was sufficient to raise body temperature and glucose uptake by BAT (Fig. 3G). Therefore, our results support the interpretation that both conventional and non-invasive optogenetic stimulation methods

have the capability to stimulate sympathetic nerves of BAT, resulting in nonshivering thermogenic responses and glucose uptake by BAT.

Conclusions

[0047] This invention has several advantages over current optogenetic methods. 1) Since the method is completely non-invasive, it does not need a surgery to implant optogenetic devices into target organs. 2) Since the SMD-LEDs are quite small and can be mounted directly on a circuit board, it is relatively easy to make a small device that can be applied directly to the skin. 3) Since the SMD-LEDs do not produce heat, they are safe to use in animals and humans.

[0048] This invention is designed to control blood glucose levels and other metabolic functions in freely moving animals without surgical implantation of optogenetic devices. The non-invasive optogenetic stimulation method will enable new therapeutic strategies to control blood glucose levels in people with diabetes.

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What is claimed is:

1. A non-invasive optogenetic method for modulating the activity of a metabolic organ in a subject, the method comprising applying light through the skin of the subject to activate a transmembrane ion channel channelrhodopsin virally-induced in autonomic efferent nerve fibers innervating the organ, where the light is applied in a wavelength, amount and duration effective to modulate activity of the organ in a subject.

2. The method of claim 1, wherein the organ is liver or brown adipose tissue.

3. The method of claim 1 or 2, wherein the channelrhodopsin is channelrhodopsin2 (ChR2) and the light is blue light.

4. The method of claim 3, wherein the blue light has a wavelength of ~450 to 475 nm.

5. The method of claim 1 or 2, wherein the channelrhodopsin is a red-shifted variant of channelrhodopsin and the light is red light.

6. The method of claim 5, wherein the red light has a wavelength of ~590 to 630 nm.

7. The method of any of claims 1-6, wherein the channelrhodopsin is induced in parasympathetic cholinergic efferent nerve fibers using an adeno-associated virus (AAV) having a choline acetyltransferase (ChAT) promoter.

8. The method of any of claims 1-6, wherein the channelrhodopsin is induced in sympathetic adrenergic efferent nerve fibers using an adeno-associated virus (AAV) having a tyrosine hydroxylase (Th) promoter.

9. The method of any of claims 1-8, wherein the light applied to the subject has an intensity of 180 lumens.

10. The method of any of claims 1-9, wherein the light is presented to the subject in 20 msec pulses at a frequency of 20 Hz.

11. The method of any of claims 1-10, wherein pulses of light are applied to the subject in on/off cycles of 1-3 sec duration.

12. The method of any of claims 1-11, wherein light is applied to the subject for a duration of 30 minutes to three hours.

13. The method of any of claims 1-12, wherein stimulation of autonomic efferent nerve fibers to the liver is used to regulate hepatic glucose metabolism.

14. The method of claim 13, wherein activation of cholinergic parasympathetic efferent nerve fibers to the liver reduces blood glucose levels in the subject.

15. The method of claim 13, wherein activation of catecholaminergic sympathetic efferent nerve fibers to the liver increases blood glucose levels in the subject.

16. The method of any of claims 13-15, wherein the subject has type 2 diabetes or pre-diabetes.

17. The method of claim 13 or 14, wherein the subject has hyperglycemia.

18. The method of any of claims 1-12, wherein stimulation of autonomic efferent nerve fibers to brown adipose tissue (BAT) is used to regulate one or more of BAT thermogenesis, core body temperature and blood glucose levels.

19. The method of claim 18, wherein activation of catecholaminergic sympathetic efferent nerve fibers to BAT does one or more of increasing BAT thermogenesis and core body temperature, and decreasing blood glucose levels in the subject.

20. The method of claim 18 or 19, wherein the subject has type 2 diabetes, pre-diabetes or hyperglycemia.

21. The method of any of claims 1-20, wherein the light is applied to the subject using a device mounted on the skin of the subject over the organ of interest, wherein the device

comprises one or more surface-mounted-device light-emitting device (SMD-LED) modules, a Transistor-Transistor Logic (TTL) pulse generator, and a power supply.

22. A device for non-invasive optogenetic stimulation, the device comprising
- one or more surface-mounted-device light-emitting device (SMD-LED) modules,
- a Transistor-Transistor Logic (TTL) pulse generator, and
- a power supply,

wherein the device is configured to be mounted on the skin of a subject.

23. The device of claim 22, wherein the SMD-LED device emits blue light or red light.

24. The device of claim 22 or 23, wherein the light has an intensity of 180 lumens.

25. The device of any of claims 22-24, wherein the light-emitting device comprises one or more light-emitting diodes.

26. The device of any of claims 22-25, wherein each module is about 2 mm by 2 mm square.

27. The device of any of claims 22-26, wherein the TTL pulse generator is incorporated in a pulse generator circuit board.

28. The device of any of claims 22-27, wherein the TTL pulses use 5 volt TTL logic levels.

29. The device of any of claims 22-28, wherein the light pulses are 20 msec in duration and generated at a frequency of 20 Hz.

30. The device of any of claims 22-29, wherein pulses of light are generated in on/off cycles of 1-3 sec duration.

31. The device of any of claims 22-30, wherein the device uses three watts of power.

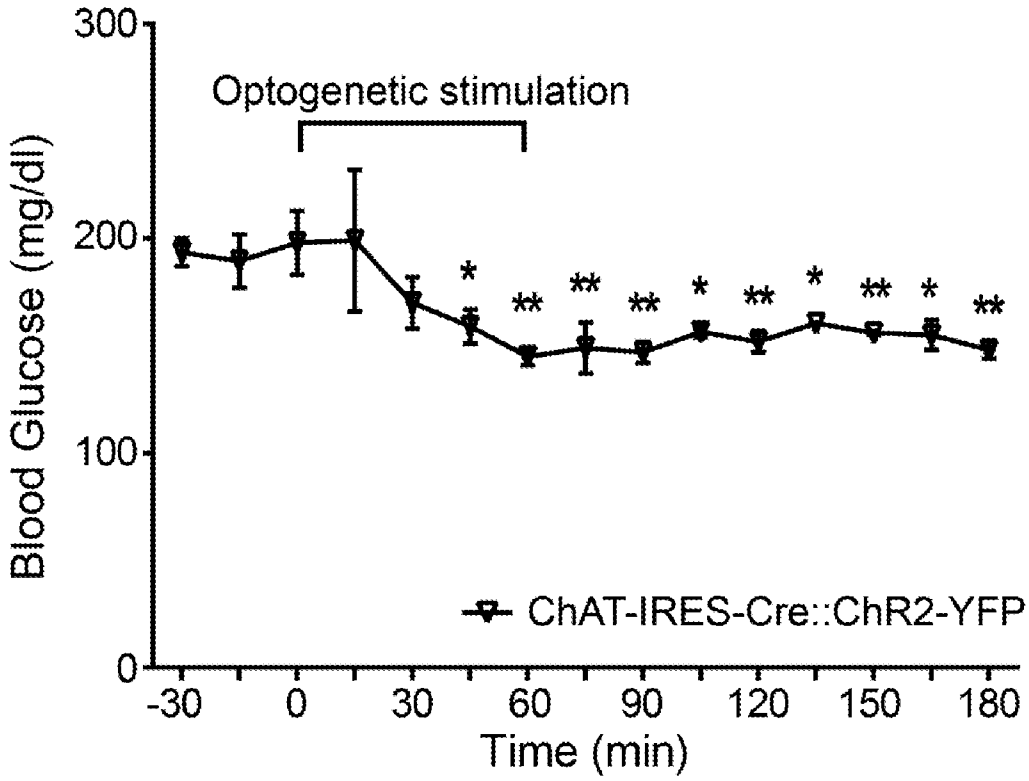
300
200
100
0
Blood Glucose (mg/dl)
Optogenetic stimulation
*
**
**
**
*
**
*
**
*
**
ChAT-IRES-Cre::ChR2-YFP
-30
0
30
60
90
120
150
180
Time (min)

FIG. 1

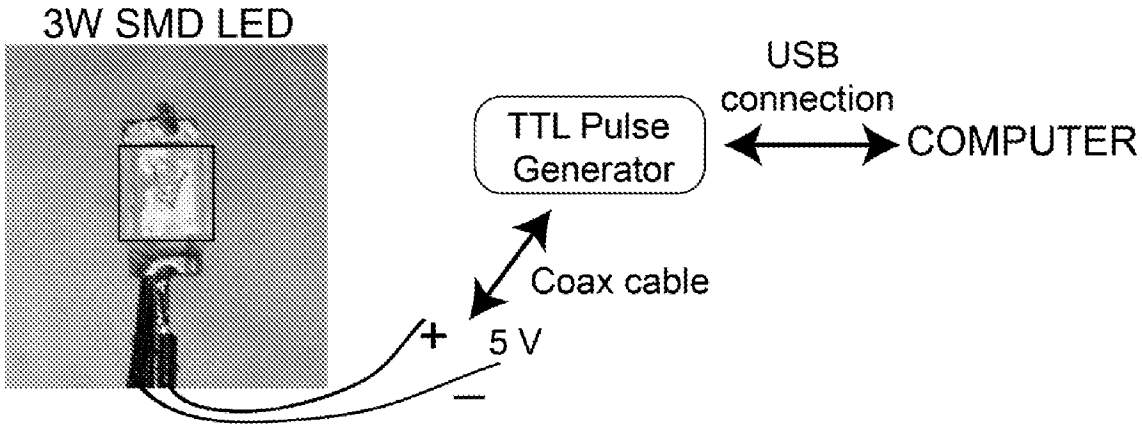

FIG. 2A

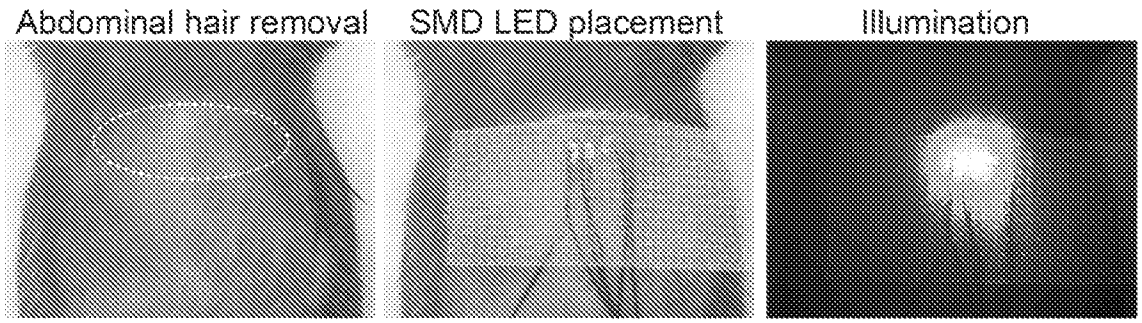

FIG. 2B

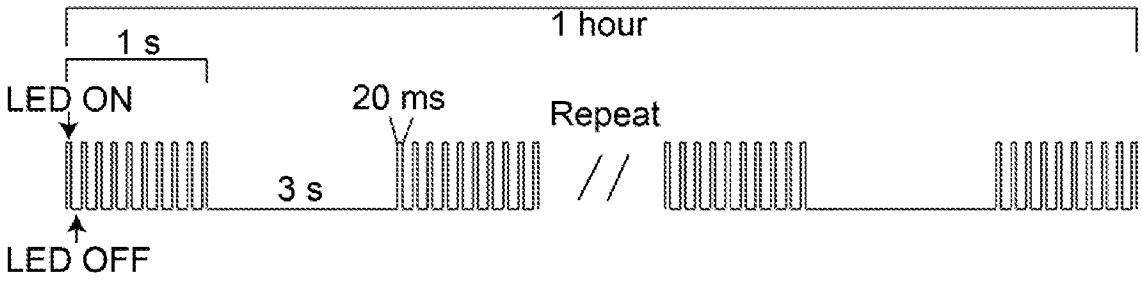

FIG. 2C

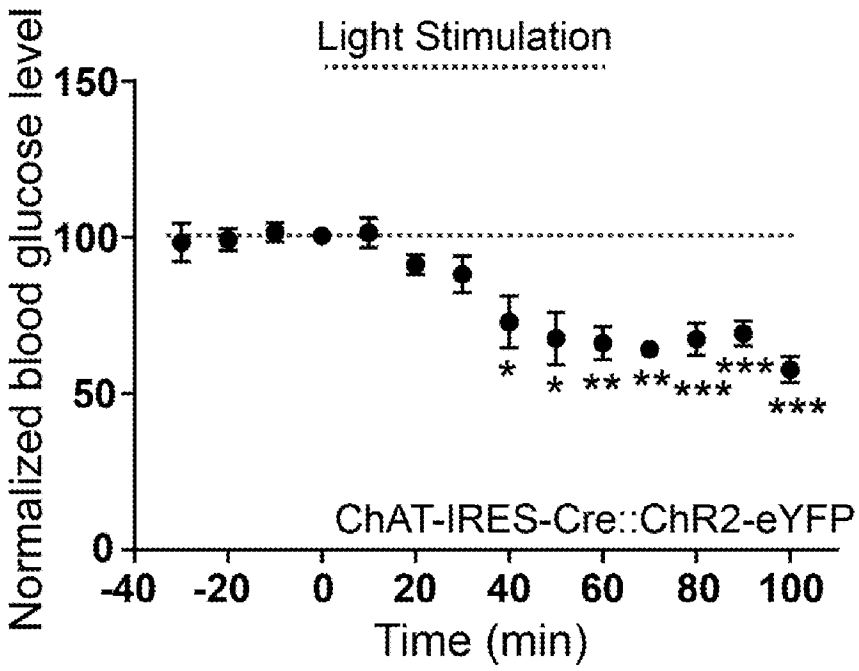
Light Stimulation
Normalized blood glucose level
150
100
50
0
-40
-20
0
20
40
60
80
100
Time (min)
ChAT-IRES-Cre::ChR2-eYFP

FIG. 2D

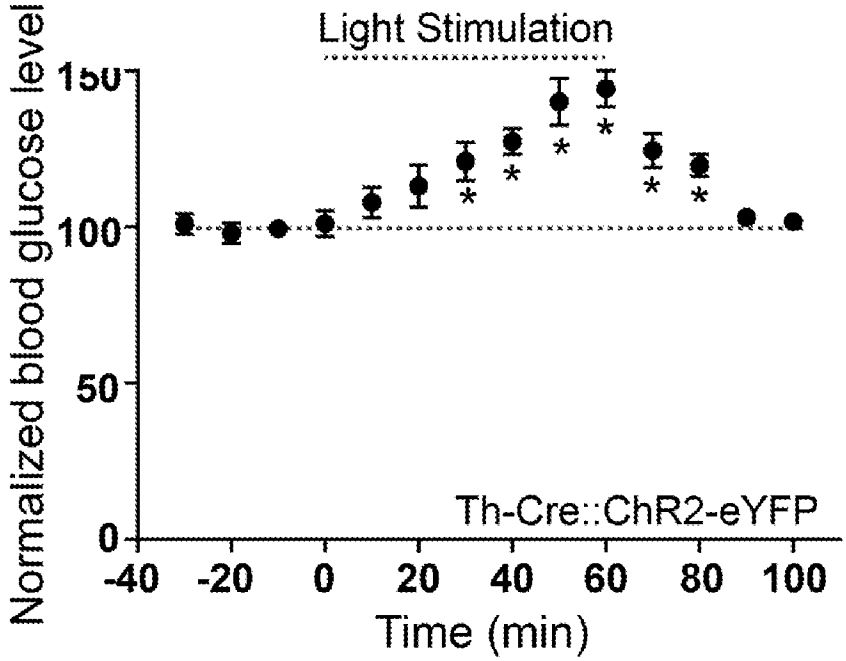
Light Stimulation
Normalized blood glucose level
150
100
50
0
-40
-20
0
20
40
60
80
100
Time (min)
Th-Cre::ChR2-eYFP

FIG. 2E

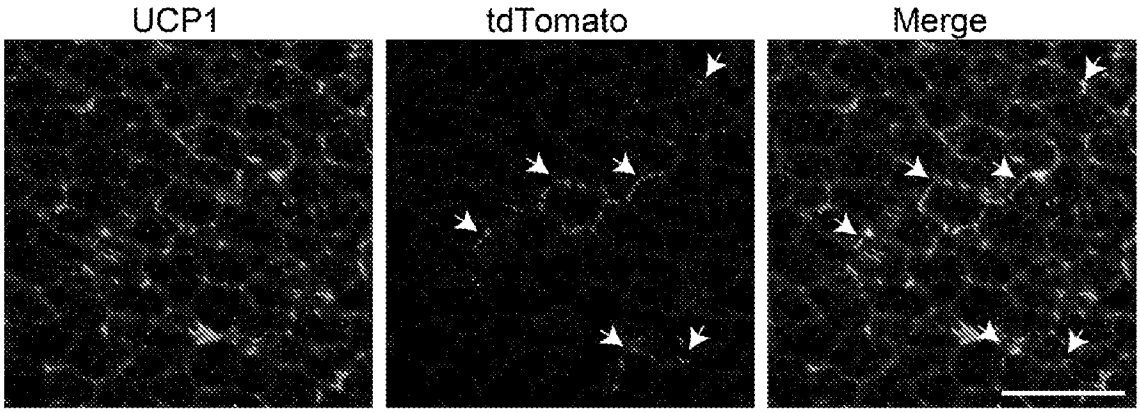

FIG. 3A

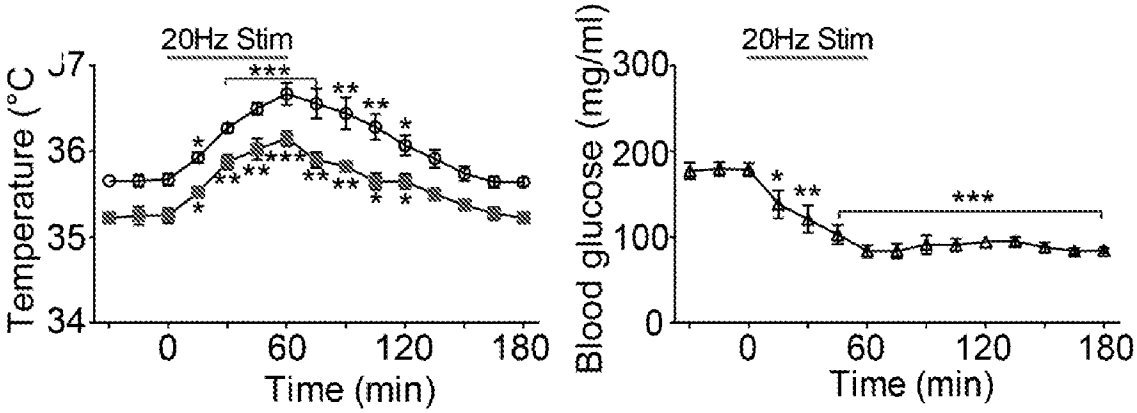

FIG. 3B

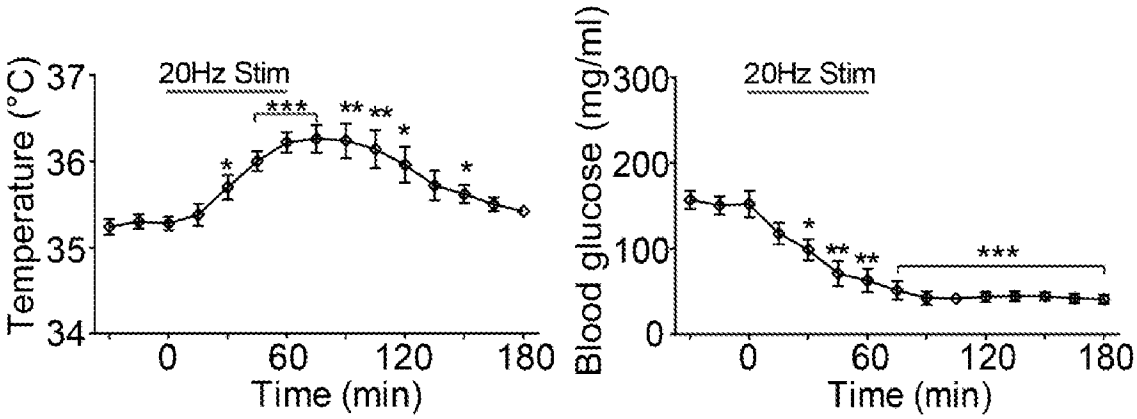

FIG. 3C

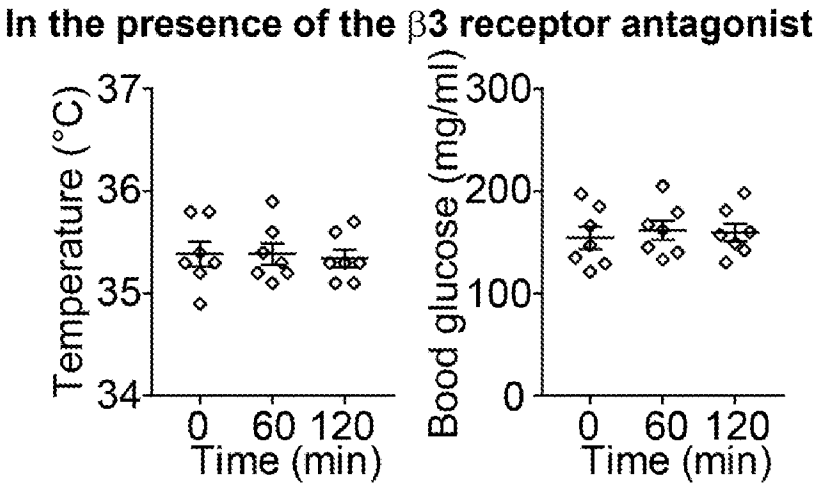
In the presence of the β3 receptor antagonist
Temperature (°C)
37
36
35
34
0 60 120
Time (min)
Bood glucose (mg/ml)
300
200
100
0
0 60 120
Time (min)

FIG. 3D

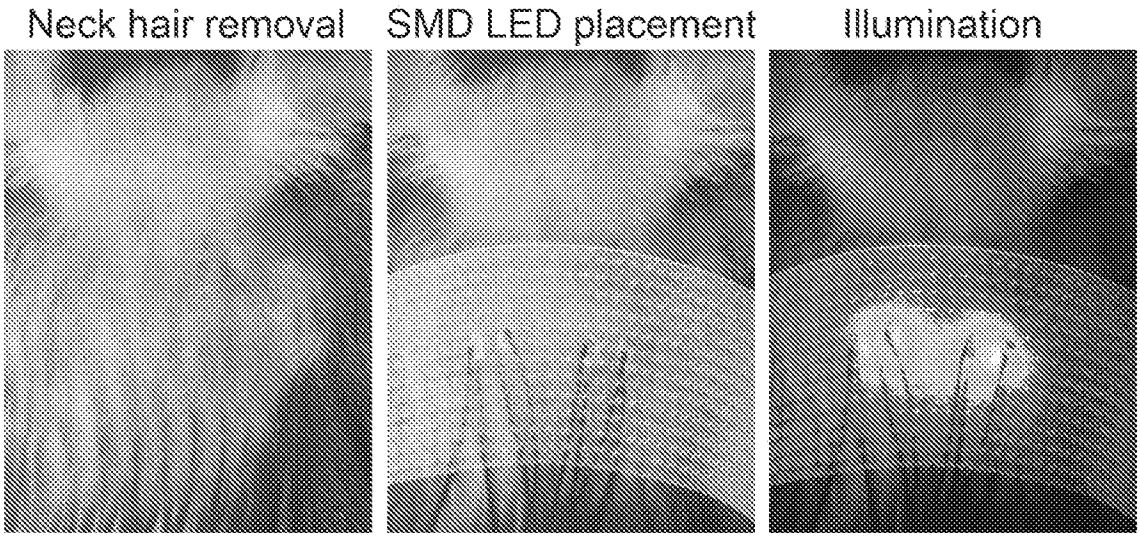
Neck hair removal
SMD LED placement
Illumination

FIG. 3E

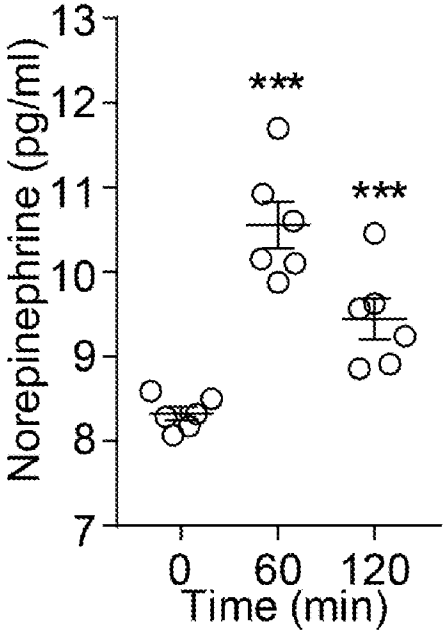
Norepinephrine (pg/ml)
13
12
11
10
9
8
7
***
***
0
60
120
Time (min)

FIG. 3F

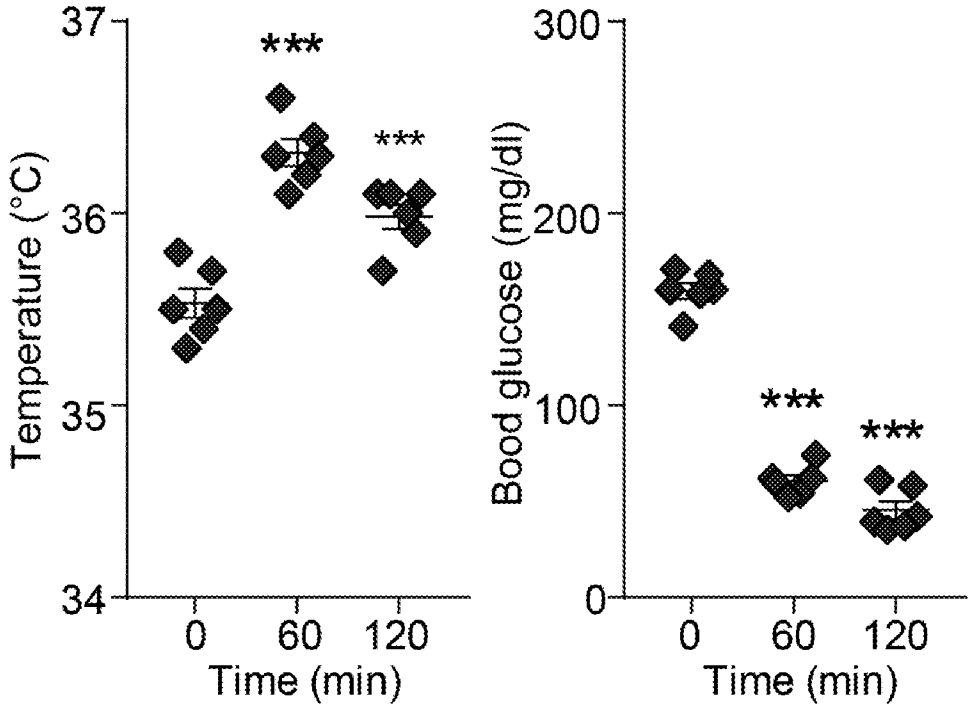
Temperature (°C)
37
36
35
34
***
***
0
60
120
Time (min)
Bood glucose (mg/dl)
300
200
100
0
***
***
0
60
120
Time (min)

FIG. 3G

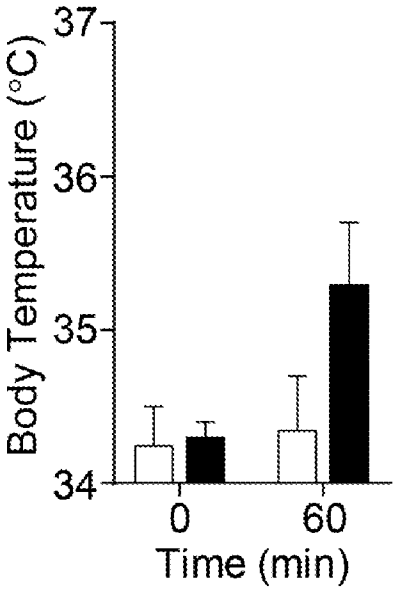
Body Temperature (°C)
37
36
35
34
0
60
Time (min)

FIG. 4A

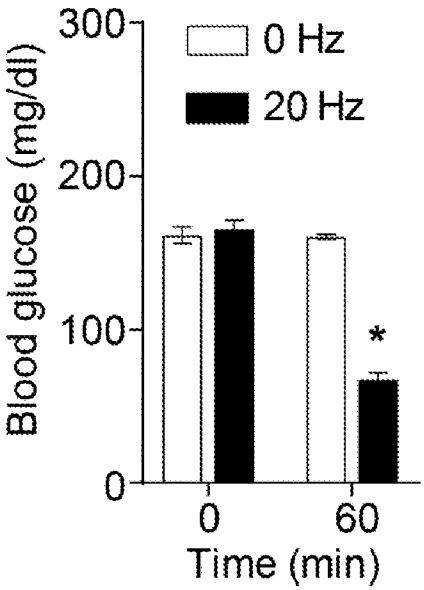
Blood glucose (mg/dl)
300
200
100
0
0 Hz
20 Hz
*
0
60
Time (min)

FIG. 4B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/25639

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 41/00, A61K 48/00 (2018.01)
CPC - A61N 5/0613, A61K 48/0083, A61N 5/062, A61N 2005/0663, A61N 2005/0662

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2016/0030765 A1 (CIRCUIT THERAPEUTICS, INC) 04 February 2016 (04.02.2016) abstract; FIG. 98; para [0018], [0055], [0065], [0092], [0178], [0180] [0182], [0243], [0246], [0251]-[0252], [0305], [0316], [0341].	22-24 --- 1-6
Y	WO 2016/070286 A1 (THE GOVERNORS OF THE UNIVERSITY OF ALBERTA) 12 May 2016 (12.05.2016) abstract; pg 3, ln 13-17; pg 7, ln 18-24, ln 30-32, ln 35 to pg 8, ln 1, ln 4-5; pg 13, ln 1, ln 14-16; pg 14, ln 32-37.	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 May 2018	28 JUN 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/25639

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-21, 25-31
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.