A Synthetic Optogenetic Transcription Device Enhances Blood-Glucose Homeostasis in Mice

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Synthetic biology has advanced the design of genetic devices that can be used to reprogram metabolic activities in mammalian cells. By functionally linking the signal transduction of melanopsin to the control circuit of the nuclear factor of activated T cells, we have designed a synthetic signaling cascade enabling light-inducible transgene expression in different cell lines grown in culture or bioreactors or implanted into mice. In animals harboring intraperitoneal hollow-fiber or subcutaneous implants containing light-inducible transgenic cells, the serum levels of the human glycoprotein secreted alkaline phosphatase could be remote-controlled with fiber optics or transdermally regulated through direct illumination. Light-controlled expression of the glucagon-like peptide 1 was able to attenuate glycemic excursions in type II diabetic mice. Synthetic light-pulse–transcription converters may have applications in therapeutics and protein expression technology.

Light provides the energy to drive all metabolic processes and enables most multicellular life forms to see and adapt to their environment (*1*, *2*). The mammalian retina is a light-capturing multicellular assembly (*3*) that controls the environment-brain interface, facilitating vision (*4*–*6*) and a variety of non–image-forming activities, such as the entrainment of circadian rhythms (*7*, *8*). Image-forming and non–image-forming activities exhibit differential processing dynamics (*1*, *9*). Whereas pattern recognition processed by the rods and cones is optimized for rapid (microsecond range) and sensitive spatial and temporal contrast acuity (*10*), photoentrainment requires light stimuli of high irradiance (over 200 times brighter than cones) and long duration (over 30 s) in order to align the biological clock with the dawn/dusk cycle and remain unaffected by local fluctuations in light exposure as the animal moves through its environment (*3*, *11*).

Photoentrainment is processed by intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing the photopigment melanopsin, a member of the opsin subgroup of G protein–coupled receptors that is typically linked to a chromophore consisting of a specific form of vitamin A called 11-cis-retinal (or related compounds) (*12*, *13*). Although the exact mechanism of chromophore isoform processing, the specific types of G proteins involved, and the channels activated remain largely elusive, melanopsin was shown to trigger a phosphodiesterase-dependent cascade resulting in a calcium response in ipRGC somata that correlates with the action potential when illuminated by blue light (*1*, *3*, *14*). With its relatively high-brightness and long-exposure requirement, melanopsin seems to be an ideal light-input component for the design of a synthetic mammalian light-controlled transcription device (*15*). By rewiring the melanopsin-induced intracellular calcium increase to calcium-dependent activation of calcineurin and calcineurin-mediated mobilization of the transcription factor nuclear factor of activated T cells (NFAT), which initiates transcription from specific promoters (*16*), illumination of mammalian cells could be directly linked to transgene expression (Fig. 1). Simple cotransfection of several rodent and human cell lines with the constitutive melanopsin expression vector pHY42 (P_{hCMV}-melanopsin-pA_{SV40}) and the P_{NFAT}-driven luciferase reporter construct pGL4.30 (P_{NFAT}-luc2P-pA_{SV40}) showed that luciferase was exclusively induced whenever the cells were exposed for 24 hours to blue-light pulses (5 s ON and 10 s OFF, light power of 1.5×10^{18} photons s^{-1} m^{-2}) (Fig. 2A) (*17*). Differences in the availability of the promiscuous melanopsin-compatible G proteins and the efficiency of the intrinsic NFAT signaling pathway may in part explain the differences in basal expression and induction profiles among different cell lines. Despite capitalizing on the endogenous NFAT signaling pathway, basal transcription remains rather low in the absence of light, which indicates that pleiotropic input into this endogenous signaling cascade is insubstantial under standard culture conditions. Human embryonic kidney (HEK) 293 cells showed the best light-triggered transgene expression profile and were therefore used in all follow-up studies. A basic set of control experiments showed that (i) light was indeed increasing intracellular calcium levels (fig. S1, A and B); (ii) light-triggered transgene expression can be dose-dependently repressed by the addition of calcium-channel blockers, such as lanthanum chloride and ethylene glycol tetraacetic acid (fig. S1, C and D); (iii) the illumination regime had no negative impact on cell viability,

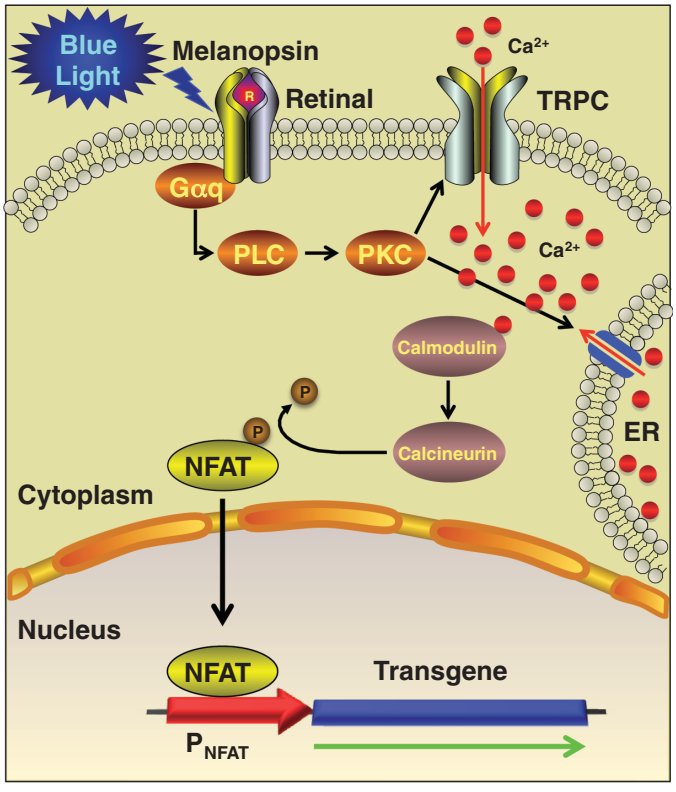

Fig. 1. Synthetic photoransduction cascade. Blue-light (~480 nm)–mediated photo-isomerization of the 11-cis retinal (R) chromophore changes the conformation of melanopsin, which sequentially activates the Gaq-type G protein (Gaq), phospholipase C (PLC), and phosphokinase C (PKC). This triggers the influx of Ca^{2+} by activation of transient receptor potential channels (TRPCs) and possibly also from intracellular storage organelles, such as the endoplasmic reticulum (ER). The blue-light–triggered intracellular Ca^{2+} surge is linked to the signaling pathway of NFAT via the calcium sensor protein calmodulin. Calmodulin activates the serine/threonine phosphatase calcineurin, which dephosphorylates the serine-rich region and serine-proline repeats in the amino terminus of NFAT. This activates a conformational change that exposes a nuclear localization signal, results in nuclear import, and enables binding of NFAT to specific promoters (P_{NFAT}) and cooperation with resident transcription factors to induce transgene expression.

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which is considered critical because exposure of cell culture medium to light could lead to the production of toxic radicals and destruction of vital additives (fig. S1E) (*18*); and (iv) supplementation of the culture medium with the chromophore retinal is essential for melanopsin-mediated light responsiveness (fig. S1F) (*12*, *13*, *17*).

When HEK-293 cells were cotransfected with pHY42 (P_{hCMV}-melanopsin-pA_{SV40}) and pHY30 (P_{NFAT}-SEAP-pA_{SV40}) encoding P_{NFAT}-driven expression of the human placental secreted alkaline phosphatase (SEAP), and exposed for 48 hours to 5-s ON/10-s OFF blue-light pulses, the SEAP levels had already reached near-maximum levels after 24 hours (Fig. 2B and fig. S2A). The maximum SEAP levels were comparable with those achieved by triggering the NFAT response, artificially using the ionophore ionomycin (Fig. 2B). This suggests that the light-induced melanopsin-triggered cascade was taking optimal advantage of the endogenous components of the NFAT pathway and may have been therefore operating at its maximum efficiency. Similar light-triggered expression kinetics could be visualized by means of fluorescence microscopy when using pHY41 (P_{NFAT}-EYFP-pA_{SV40}) instead of pHY30 (Fig. 2C). The SEAP levels of pHY30-/pHY42–cotransfected HEK-293 could be precisely fine-tuned by varying the irradiation times between 0 and 72 hours, which confirms that the synthetic control device is able to integrate the intensity of a pulsing light source over time and produce a sustained irradiance-adjustable transcription output (fig. S3). Exposure of engineered cells to a light-pulse of defined intensity and time period could also be used to precisely preprogram SEAP expression kinetics and predetermine the maximum SEAP levels reached after several days (Fig. 2D). Because light exposures of 3 to 24 hours lead to similar maximum SEAP levels, a standard 3-hour illumination is sufficient for optimal product gene expression (Fig. 2D). The 3-hour illumination provides sustained SEAP expression that rapidly declines after 24 hours and can be reversibly kicked on by 3-hour blue-light pulses at 24-hour intervals (Fig. 2E).

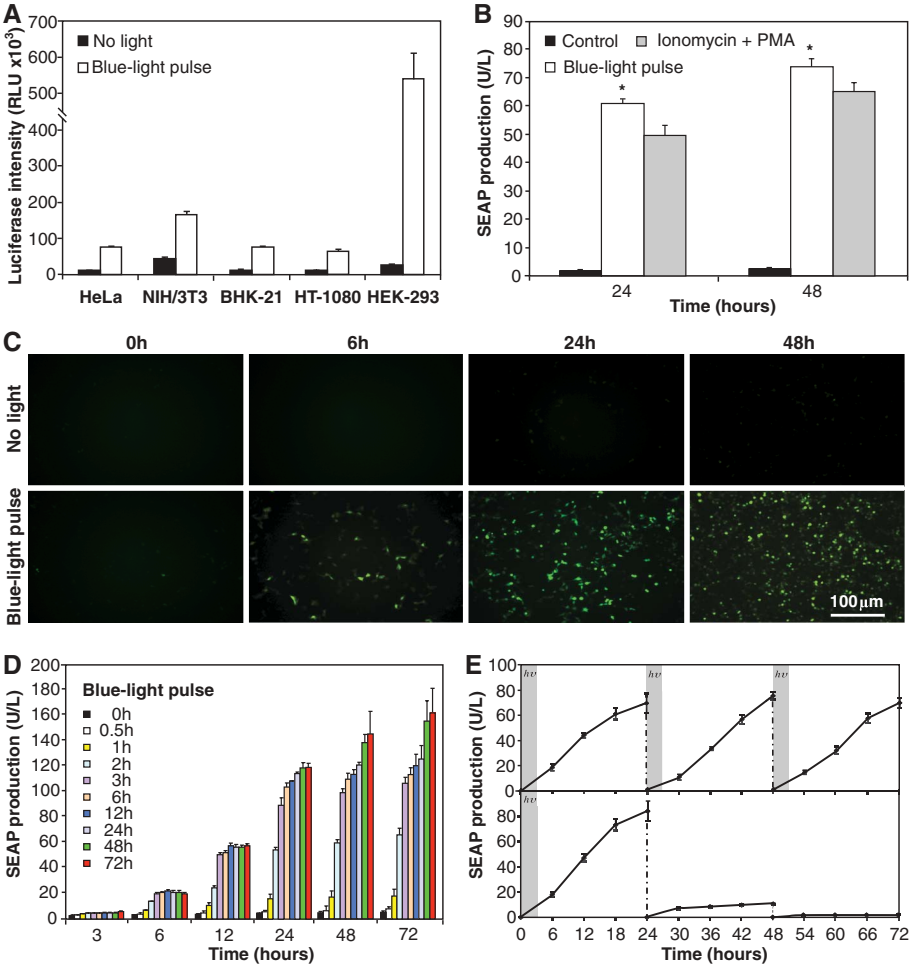

Fig. 2. Light-inducible transgene expression in mammalian cells. (**A**) Blue light–inducible intracellular luciferase expression levels in different mammalian cell lines 24 hours after cotransfection with pHY42 (P_{hCMV}-melanopsin-pA_{SV40}) and pGL4.30 (P_{NFAT}-luc2P-pA_{SV40}) and illumination for 24 hours with standard blue-light pulses. Data are mean ± SD; $n = 4$ independent experiments. (**B**) SEAP expression levels in the culture supernatant of HEK-293 cotransfected with pHY42 and pHY30 (P_{NFAT}-SEAP-pA_{SV40}) and either continuously illuminated with blue-light pulses or treated for the same time with 1 μmol ionomycin plus 10 ng/ml phorbol-myristate-acetate (PMA) to short-circuit Ca^{2+} signaling. Control cells were kept in the dark. Data are mean ± SD; $n = 4$ independent experiments. *$P < 0.05$. (**C**) Fluorescence micrographs profiling EYFP expression of HEK-293 cotransfected with pHY42 and pHY41 (P_{NFAT}-EYFP-pA_{SV40}) and illuminated with blue-light pulses for up to 48 hours. Control cells were kept in the dark. (**D**) SEAP expression profiles of pHY30-/pHY42–cotransfected HEK-293 illuminated with blue-light pulses for different periods of time. Data are mean ± SD; $n = 4$ independent experiments. (**E**) Reproducibility and reversibility of light-triggered transgene expression. pHY30-/pHY42–cotransfected HEK-293 were illuminated with blue-light pulses for 3 hours ($h\nu$, gray bar), and SEAP production was profiled for 72 hours. Every 24 hours, the culture medium was exchanged, the cell density was adjusted to 1×10^6, and the cells were illuminated with and without additional 3-hour blue-light pulses. Data are mean ± SD; $n = 4$ independent experiments.

The production of protein pharmaceuticals that impair the growth or viability of their production cell lines, such as cancer therapeutics, represents a major biopharmaceutical manufacturing challenge (*19*, *20*). Expression of these difficult-to-produce protein therapeutics requires precise titration of production levels into the viability and growth range of the production cell lines (*20*). Although many small-molecule–responsive transgene control systems are available to adjust product protein expression, the presence of trigger molecules in the production environment creates downstream-processing challenges and regulatory concerns (*19*, *21*). We therefore evaluated light-triggered product gene expression in a standard bioreactor setting (*17*). The illumination hardware used to irradiate the transgenic production cell lines was identical to that used for the basic cell culture experiments. Over a 10-day bioreactor run, the production kinetics of the human glycoprotein SEAP could be programmed in a noninvasive manner simply by using a blue-light source placed outside the bioreactor (Fig. 3 and fig. S2B). Thus, product gene expression can be externally con-

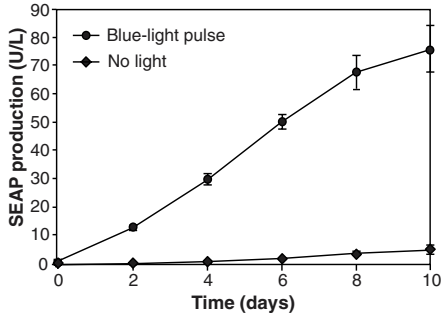

Fig. 3. Light-inducible product gene expression in bioreactors. 5×10^7 HEK-293 (200 ml, 2.5×10^5 cells/ml) transgenic for pHY42 (P_{hCMV}-melanopsin-pA_{SV40}) and pHY30 (P_{NFAT}-SEAP-pA_{SV40}) were seeded into roller bottles, and SEAP production was profiled for 10 days in the presence and absence of blue-light pulses. Data are mean ± SD; $n = 4$ independent experiments.

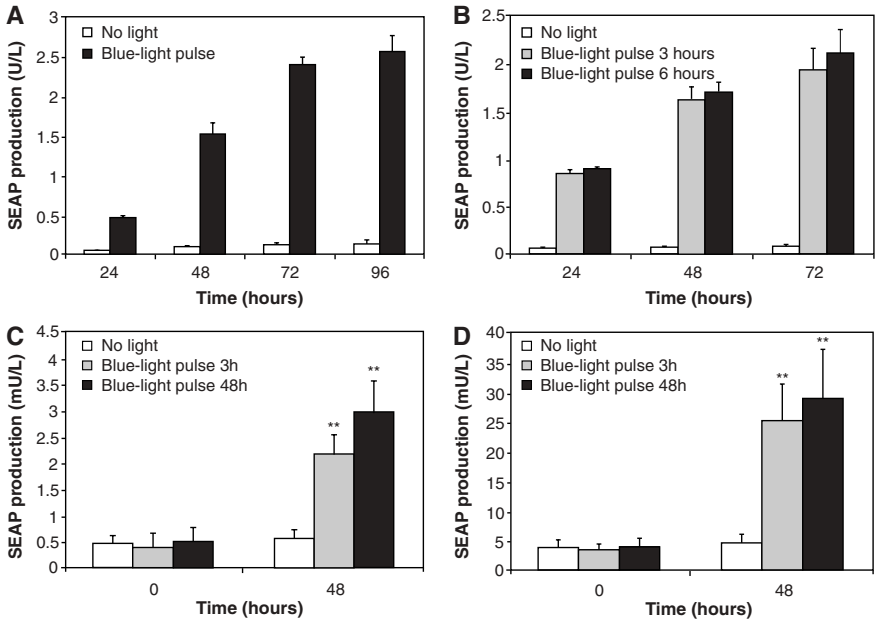

Fig. 4. Light-inducible transgene expression in mice. (**A**) SEAP production of hollow-fiber implants containing 1×10^5 pHY30-/pHY42–transgenic HEK-293 maintained for up to 96 hours in 2 ml of culture medium and continuously illuminated via the optical fiber with blue-light pulses. Nonilluminated implants were used as the control. Data are mean ± SD; $n = 4$ independent experiments. (**B**) SEAP production of hollow-fiber implants containing 1×10^5 pHY30-/pHY42–transgenic HEK-293 maintained for up to 72 hours in 2 ml of culture medium and illuminated via the optical fiber with blue-light pulses for 0, 3, or 6 hours. Data are mean ± SD; $n = 4$ independent experiments. (**C**) SEAP levels in the serum of mice containing optical-fiber–connected implants that were illuminated with blue-light pulses for 3 or 48 hours. Mice harbouring nonilluminated implants were used as the control. Data are mean ± SEM; statistics by two-tailed *t* test; $n = 8$ mice. $^{**}P < 0.005$. (**D**) SEAP levels in the serum of mice containing subcutaneous implants of 1×10^6 microencapsulated pHY30-/pHY42–transgenic cells that were illuminated with blue-light pulses for 3 or 48 hours. Data are mean ± SEM; statistics by two-tailed *t* test; $n = 8$ mice. $^{**}P < 0.005$.

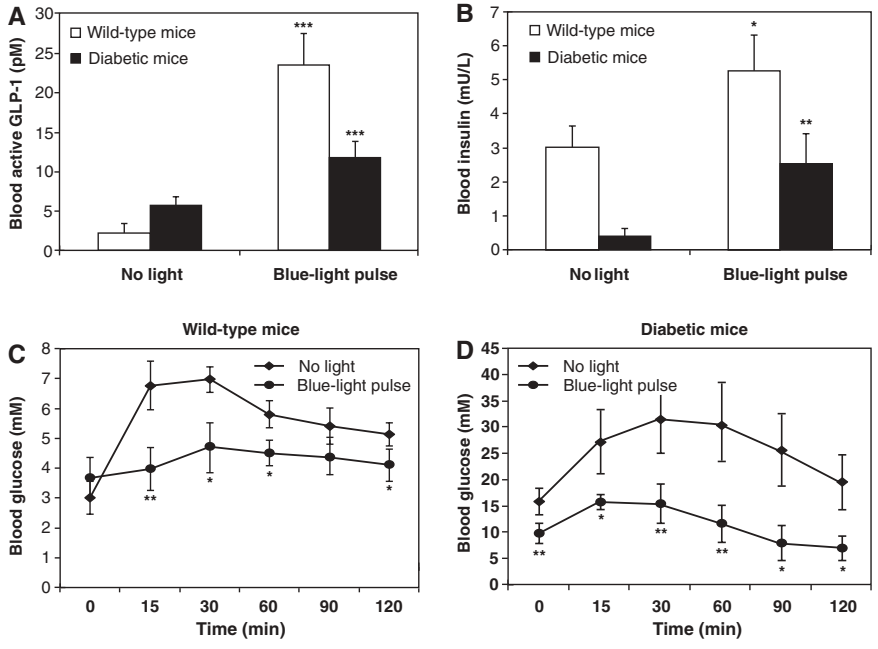

Fig. 5. Light-inducible expression of shGLP-1 in wild-type and diabetic db/db mice. (**A** to **C**) Wild-type and [(A), (B), and (**D**)] diabetic db/db mice subcutaneously implanted with 1×10^7 microencapsulated pHY42-/pHY57–transgenic HEK-293 cells were illuminated with blue-light pulses for 48 hours (control mice received no light) and then profiled for (A) blood active GLP-1 and (B) insulin levels as well as [(C) and (D)] for glucose tolerance. Data are mean ± SEM; statistics by two-tailed *t* test; $n = 8$ mice. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.0001$.

trolled, and production is triggered in cells as they move through a light beam. Also, the optogenetic transcription device shows improved product gene expression because it allows tight conditional expression of the highly toxic Rip death domain (fig. S4) (*22*, *23*).

A molecule-free electromagnetic trigger device to control transgene expression in vivo is particularly appealing for future therapeutic applications. Because fiber optics could deliver light of the desired wavelength and intensity to an intracorporeal site, we used optical fibers (200 μm in diameter) for lossless delivery of blue-light pulses to connected custom-designed intraperitoneal hollow-fiber implants containing 1×10^5 pHY30-/pHY42–transgenic HEK-293 engineered for light-triggered SEAP expression (fig. S2C) (*17*). Preliminary in vitro studies showed that the optical fiber could optimally trigger SEAP expression in connected hollow-fiber implants maintained in culture medium for several days when using a continuous light-pulse regime with a standard blue-light intensity validated in previous cell culture experiments (Fig. 4A). Illuminating the cells contained in hollow fiber implants via connected optical fibers for a limited period of 3 or 6 hours resulted in almost identical SEAP production profiles for 3 days, indicating that a 3-hour irradiation period is sufficient to program the cells to produce the desired heterologous protein for several days (Fig. 4B). To validate light-triggered product gene expression in vivo, HEK-293 variants engineered for light-controlled SEAP expression contained in sealed hollow-fiber packs and connected to optical fibers were intraperitoneally implanted into mice and subsequently irradiated for 3 or 48 hours. The implants in control mice were not exposed to any light. The resulting SEAP levels quantified after 48 hours in the serum of both treatment groups confirmed the light-triggered remote control of product gene expression in animals (Fig. 4C). We also tested whether external blue-light pulses could transdermally control transgene expression in subcutaneous implants (*17*). When mice subcutaneously implanted with pHY30-/pHY42–transgenic HEK-293 cells were illuminated for 3 or 48 hours with blue-light pulses, the plasma SEAP levels could be controlled accordingly (Fig. 4D and fig. S2D).

To validate light-triggered transcription control in a prototype therapeutic setting, we engineered HEK-293 cells for constitutive melanopsin production (pHY42) and P_{NFAT}-driven expression of a glucagon-like peptide-1 variant (shGLP-1; pHY57) (*17*) that has displayed potent glucose homeostasis-modulating characteristics and may have potential in treating type II diabetes (*24*, *25*). After control experiments confirmed the light-triggered expression of shGLP-1 and validated its capacity to induce insulin secretion in β-TC-6 cells (fig. S5), we implanted 1×10^7 microencapsulated pHY42-/pHY57–transgenic HEK-293 cells subcutaneously into wild-type and diabetic db/db mice and illuminated the treated animals for 48 hours

with standard blue-light pulses (the control group was not exposed to blue-light pulses). shGLP-1 (Fig. 5A) and the resulting insulin (Fig. 5B) levels were significantly increased in wild-type as well as in diabetic db/db mice after blue-light exposure, and the action of both proteins significantly reduced the glycemic excursion of treated animals after intraperitoneal administration of glucose (wild-type mice, Fig. 5C; diabetic db/db mice, Fig. 5D). On the basis of these glucose tolerance tests showing the improvement of glucose homeostasis, and recent reports suggesting that the glucose-dependence of shGLP-1 automatically shuts down insulinotropic actions upon reaching normal glucose levels and so prevents hypoglycemia (*25*), light-triggered expression of shGLP-1 may be considered for the treatment and prevention of glucose-related pathologies (*25*, *26*).

From plants to mammals, light-based energy and information is captured via receptors and processed via ion-based membrane potential (*2*, *3*, *27*, *28*). Some of these native light receptors, such as melanopsin (*3*) and channelrhodopsin (*29*), have been extensively used for heterologous intervention in native neuron-triggered activities in order to understand the photoentrainment of the circadian clock (*7*) or to restore visual function in retinal degeneration (*5*, *6*, *29*, *30*). Capitalizing on the principles of synthetic biology to assemble functional biologic devices from well-characterized components in a rational and predictable manner (*31*), it has recently become possible to engineer synthetic signaling cascades and control networks to program metabolic behavior (*32*–*35*), cell morphology (*36*), and therapeutic interventions (*37*) with high precision. By combining heterologous factors (melanopsin) and control modules (P_{NFAT}) with promiscuous complementary endogenous machineries (G proteins, NFAT pathway), we rewired melanopsin-mediated G protein–coupled receptor signaling to NFAT control, taking advantage of their common intracellular calcium-based, second-messenger–based signaling system as the interface (*16*). When engineered into mammalian cells grown in bioreactors or implanted into mice, this synthetic light-control device enabled conversion of a physiologically inert pulsed blue-light beam into a continuous transcription response, the level of which could be adjusted by the irradiation period. Remote control of transgene expression by means of electromagnetic waves may enable quantitative cell culture experiments, providing opportunities for economical manufacturing of difficult-to-express protein therapeutics and for low-risk dosing in gene- and cell-based therapies.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6037/1565/DC1
Materials and Methods
Figs. S1 to S5
References (*38*–*45*)

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Selective Attention from Voluntary Control of Neurons in Prefrontal Cortex

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Animals can learn to voluntarily control neuronal activity within various brain areas through operant conditioning, but the relevance of that control to cognitive functions is unknown. We found that rhesus monkeys can control the activity of neurons within the frontal eye field (FEF), an oculomotor area of the prefrontal cortex. However, operantly driven FEF activity was primarily associated with selective visual attention, and not oculomotor preparation. Attentional effects were untrained and were observed both behaviorally and neurophysiologically. Furthermore, selective attention correlated with voluntary, but not spontaneous, fluctuations in FEF activity. Our results reveal a specific association of voluntarily driven neuronal activity with "top-down" attention and suggest a basis for the use of neurofeedback training to treat disorders of attention.

Animal and human subjects can learn to alter their own brain activity when they are provided with feedback (*1*–*4*). Voluntary control of neuronal activity is likely associated with changes in behavior or cognitive functions, but that relationship is unclear. Operant control of motor cortical neurons is typically dissociated from movement production (*5*–*8*), and there are no clear behavioral consequences of operant control of neuronal spiking activity in other brain structures (*1*, *4*). Naturally, one might ask whether a chosen control strategy can elicit untrained behavioral or neurophysiological outcomes. To address this question, we examined the consequences of voluntary control of neurons in the frontal eye field (FEF), a visuomotor area within the prefrontal cortex with a known role in the programming of saccadic eye movements (*9*) and visual spatial attention (*10*), in rhesus monkeys (Fig. 1A).

We first asked whether the activity of FEF neurons can be controlled voluntarily by the monkey without explicit training on any task. We used an operant control paradigm (*2*) in which the monkey received juice rewards for alternately increasing and decreasing the firing rates (FRs) of FEF neurons during fixation (*11*) (Fig. 1, B and C). During trials, the monkey received auditory

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