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CLOSED-LOOP OPTOGENETIC STRATEGY IN EXPERIMENTAL EPILEPSY: HOW AFFORDABLE IS THE IMPLEMENTATION OF THIS EMERGENT TECHNIQUE?

ESTRATÉGIA OPTOGENÉTICA DE ALÇA FECHADA NA EPILEPSIA EXPERIMENTAL: QUÃO ACESSÍVEL É A IMPLEMENTAÇÃO DESTA TÉCNICA EMERGENTE?

ESTRATEGIA OPTOGENÉTICA DE BUCLE CERRADO EN LA EPILEPSIA EXPERIMENTAL: ¿CUÁN ASEQUIBLE ES LA APLICACIÓN DE ESTA TÉCNICA EMERGENTE?

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ABSTRACT

To explore complex mechanisms in the brain is an expensive task, which requires a combination of technological development and theoretical advances in neurobiology. In fact, it still is extremely challenging to diagnose accurately and treat some neurological diseases like drug-resistant epilepsy. In some cases, pharmacological interventions, electrical stimulation and surgery in epilepsy can be the specific cause of cognitive impairments and/or psychiatric comorbidities. Therefore, developing more selective strategies to control events produced by abnormal brain activity is mandatory. Our objective was to synthesize and organize information from the literature about the fundamental concepts that support the combination of optogenetics and closed-loop strategies in experimental epilepsy. We also sought to discuss how affordable would be the implementation of these emergent techniques. For this purpose, we first reviewed the literature on the closed-loop optogenetics and its applications for experimental epilepsy. Then, in order to evaluate the feasibility of this approach, we organized the information available in the literature on the materials necessary, and their respective costs. The combination of real-time detection and optogenetics has enormous potential to produce breakthroughs in neuroscience and its use for seizure control will certainly open new possibilities for more effective treatments of epilepsy. Overall, the costs of implementing a robust system with a high temporal precision and accuracy for detection and interference in seizures are relatively small. In addition, costs can be even lower if researchers choose open source hardware tools and software. Therefore, implementation of optogenetics with strategies of closed-loop in experimental epilepsy seems to demand more joint interdisciplinary efforts and innovative scientific questions than financial resources.

Keywords: Neurobiology; Epilepsy; Optogenetics; Neurosciences.

RESUMO

Investigar mecanismos complexos no cérebro é uma tarefa dispendiosa, que requer a combinação de desenvolvimento tecnológico e avanços teóricos em neurobiologia. De fato, realizar diagnósticos e tratar apropriadamente desordens neurológicas, como epilepsia resistente ao tratamento farmacológico, ainda é um grande desafio. Em alguns casos, as intervenções farmacológicas, a estimulação elétrica e a cirúrgica em epilepsia podem ser as próprias causadoras de prejuízos cognitivos e/ou comorbidades psiquiátricas. Portanto, é mandatório o desenvolvimento de estratégias mais seletivas para controlar eventos gerados por atividade anormal do encéfalo. Nosso objetivo foi sintetizar e organizar informações da literatura sobre os conceitos fundamentais que dão suporte à combinação de optogenética e estratégias de alça fechada em epilepsia experimental. Além disso, objetivamos discutir o quão financeiramente acessível seria a implementação dessas novas técnicas. Para isso, primeiramente revisamos a literatura sobre optogenética e estratégias de alça fechada e suas aplicações para epilepsia experimental. Em seguida, com o objetivo de avaliar quão acessível seria essa abordagem, organizamos a informação disponível na literatura sobre os materiais necessários e seus respectivos custos. A combinação de detecção em tempo real e optogenética tem um potencial enorme para produzir avanços

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em neurociências e seu uso para o controle de crises certamente abrirá novas possibilidades para tratamentos mais eficientes da epilepsia. Em geral, os custos para a implementação de um sistema robusto, com alta precisão temporal e acurácia para detecção e interferência em crises são relativamente pequenos. Além disso, eles podem ser ainda menores se os pesquisadores optarem por ferramentas de hardware e software de fonte aberta. Portanto, a implementação da optogenética com estratégia de alça fechada em epilepsia experimental parece demandar mais esforços interdisciplinares conjuntos e perguntas científicas inovadoras do que recursos financeiros.

Descritores: *Neurobiologia; Epilepsia; Optogenética; Neurociências.*

RESUMEN

Investigar los mecanismos complejos en el cerebro es una tarea costosa, que requiere una combinación de desarrollo tecnológico y los avances teóricos en la neurobiología. De hecho, todavía es um gran desafio diagnosticar con precisión y tratar apropriadamente trastornos neurológicos como la epilepsia resistente al tratamiento farmacológico. En algunos casos, las intervenciones farmacológicas, la estimulación eléctrica y la cirugía pueden ser por sí mismas la causa de los deterioros cognitivos y/o comorbilidades psiquiátricas. Por esta razon, es obligatorio el desarrollo de estrategias más selectivas para controlar los eventos producidos por la actividad cerebral anormal. Nuestro objetivo fue sintetizar y organizar la información de la literatura acerca de los conceptos fundamentales que soportan la combinación de la optogenética y estrategias de bucle cerrado en la epilepsia experimental. Además, tratamos de discutir cuán asequible sería la implementación de estas nuevas técnicas. Para ello, primero hemos revisado la literatura sobre la optogenética y las estrategias de bucle cerrado y sus aplicaciones en la epilepsia experimental. Luego, con el fin de evaluar cómo sería este enfoque económico, organizamos la información disponible en la literatura sobre los materiales requeridos y sus costos. La combinación de la detección en tiempo real y la optogenética tiene un enorme potencial para producir avances en la neurociencia y su uso para control de las crisis epilépticas sin duda abrirá nuevas posiblidades para tratamientos más eficaces de la epilepsia. Generalmente, los costos de implementación de un sistema robusto con una alta precisión temporal y la exactitud de detección y de interfencia en las convulsiones son relativamente pequeños. Además, los costos pueden ser incluso más bajos si los pesquisadores eligierenherramientas de hardware y software de código abierto y libre acceso. Por lo tanto, la aplicación de la optogenética con la estrategia de bucle cerrado en la epilepsia experimental parece exigir más esfuerzos interdisciplinarios conjuntos y preguntas científicas innovadoras que recursos financieros.

Descriptores: *Neurobiología; Epilepsia; Optogenética; Neurociencias.*

INTRODUCTION

Every cubic millimeter of our brain has hundreds of millions of neurons connected by trillions of synapses that work with the temporal precision of milliseconds. Of course, such complexity conveys to scientists an atmosphere of high motivation to unveil, at least partially, this intriguing and mysterious universe. On the other hand, this journey is quite demanding and requires a combination of technological development and theoretical advances in neurobiology. In the last 15 years, relevant tools were developed to investigate structural, molecular and functional aspects of intact circuits in the brain[1-5]. Despite this, to accurately diagnose and treat some neurological diseases, like drug-resistant epilepsy, is still extremely challenging. In fact, there is an enormous concern on how to overcome the lack of temporal and cell-type specificity of the current therapies in epilepsy. Pharmacological treatments, electrical deep brain stimulations or, in some patients with difficult-to-control seizures, surgical interventions can be causes of cognitive deficits and/or psychiatric comorbidities[6]. In this context, is not surprising that the epilepsy researchers have confirmed such enthusiasm regarding the development of more specific techniques for interventions in the central nervous system, such as Optogenetics[7]. Additionally, researchers have been implementing closed-loop strategies to perform electrical deep brain stimulation, transcranial magnetic stimulation or optogenetics, conditioned to the detection of a seizure or an abnormal oscillatory pattern. Closed-loop optogenetic strategy in experimental models of epilepsy has recently been validated, suggesting exciting future therapeutic avenues. In the present review, we sought to synthesize and organize information on the fundamental concepts that support the use of this strategy in experimental epilepsy and, discuss how affordable the implementation of this emergent technique would be.

Optogenetics

Optogenetics is defined by the integrated use of optics and genetics to control well-defined events within specified cells of living tissue[8]. This technique is based on the use of light-sensitive proteins called opsins that comprise inhibitory channels and pumps, excitatory channels and coupled receptors called G-protein. They can be expressed in selected cell types of selected cell areas, enabling the temporally precise control of genetically defined neuronal populations[8]. The excitatory channel opsins depolarize the cell, allowing cations to pass into the cell and generating an action potential when activated by light. For example, one of the most known opsins is the light-gated proton channel, channelrhodopsin-2 (ChR2). ChR2 opens upon blue light (473 nm) stimulation to produce a large permeability for monovalent and divalent cations[9]. On the other hand, the inhibitory opsins can hyperpolarize the cell. For instance, when exposed to yellow light (570 nm), halorhodopsin (HR)[10] pumps chloride ion into the cell, causing inhibition of neuronal activity. Another example is the Archaerhodopsin (Arch)[11], which can hyperpolarize the cell by pumping out protons (H+) upon green light stimulation (532 nm). Since the characterization of the expression and functionality of ChR2, HR, and variants, an impressive number of new opsins have been engineered. This effort was primarily focused on the improvement of its expression, temporal precision and phototransduction efficiency[12]. Besides, technology for light delivery in rodents in vivo alongside electrophysiological recordings has become very sophisticated and diverse. Therefore, optogenetics is now useful for the establishment of causal relationships between the activity of specific subpopulations of brain cells and mammalian behavior[13-15].

Optogenetic control of seizures

Once it is possible to manipulate specific populations of neurons, it becomes easier to inquiry the key networks and mechanisms involved in initiating, sustaining, propagating and terminating seizures[16,17]. Optogenetics can be used in epilepsy to activate or inhibit specific neuronal populations in a brain circuit of interest, allowing normalization of its excitatory/inhibitory balance[17]. For instance, artificially-induced epileptiform activity in the hippocampus *in vitro* can be strongly attenuated by optogenetic inhibition (yellow light activation of HR) of its excitatory and granule cells[16]. More recently, Sukhotinsky et al.[18] have demonstrated that optogenetic inhibition of hippocampal pyramidal cells is sufficient to delay electrographic and behavioral initiation of status epilepticus in the lithium-pilocarpine model of acute elicited seizures. Interestingly, optogenetic activation of interneurons has also been considered an attractive possibility of halting seizures[19]. For instance, Krook-Magnuson et al.[19] have reported that activation of parvalbumin positive neurons (PV+, a class of inhibitory interneuron) did interrupt seizures upon light application in a mouse model of temporal lobe epilepsy. Consistently, Ledri et al.[20] have reported a successful suppression of ongoing epileptiform activity in the hippocampus in vitro by a massive light-induced release of GABA from ChR2-expressing interneurons. Those are only a few examples of the enormous importance of the use of optogenetics in the experimental epilepsy research. However, determining when the interventions should start (e.g. a couple of minutes before, right before, during, just after the seizure) is still a challenge in epilepsy.

Closed-loop feedback in experimental epilepsy

Closed-loop systems are widely applied in engineering and easily found in everyday life. A simple thermostat used in air-conditioning devices is a good example of an on-off closed-loop system. It uses the information of an output signal - the measured temperature - and the input signal - the desired temperature - to guide the conditioning system to exert control over the temperature of the environment. In other words, these systems use the error signal between output and input to drive the control over the system accurately. In neuroscience, closed-loop systems are a lot useful because they allow one to control a neural system given target conditions. In an elegant work, Bérenyi et al.[21] have demonstrated that transcranial electrical stimulation triggered by spike-and-wave discharges in a rodent model of generalized epilepsy was very efficient in reducing its epileptic activity.

It is noteworthy to mention the main differences between a closed-loop and an open-loop control. Most of the works on electrical stimulation and optogenetics to date are based on an open-loop system. In this type of approach, the information used to control the input to the system is an off-line information that may be taken from the literature or previous neural recordings. The system works without feeding back the neural effect of the stimulation[22]. On the other hand, closed-loop control comprises: (1) the effector or actuator, that delivers the information, or that drives the feed-forward information to the system; (2) the system itself, that is the target neural circuit or cells; (3) the sensor to measure the output of the system; and (4) the controller to effectively compute the error signal between input and output and select the appropriate intervention to the system (Figure 1).

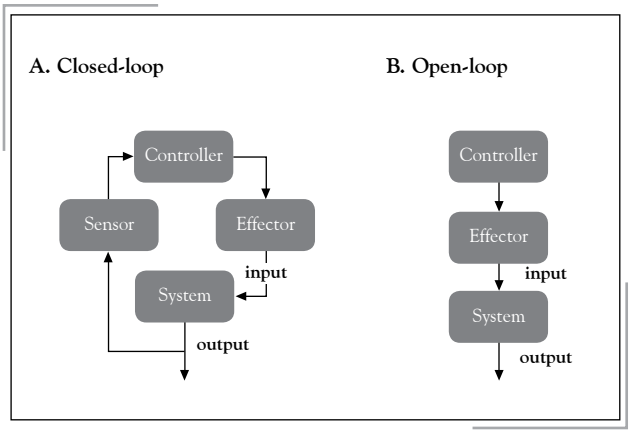

Figure 1. Comparison between closed-loop and open-loop systems. In closed-loop systems, the effector drives the feed-forward information to the system. The sensor sends online information regarding the output of the system to the controller to compute effectively the error signal between input and output. Then, the controller regulates the appropriate intervention to the system. In open-loop systems, the information used to control the input to the system is an off-line information that may be taken from the literature or previous neural recordings. This system works without feeding back the neural effect of the stimulation[22].

Closed-loop optogenetic strategy in experimental models of epilepsy has recently been described independently by two seminal studies from Huguenard and Soltezs laboratories[17,19].

Paz et al.[17] tested for the role of thalamus in post-stroke seizures. Also, they examined whether the thalamus could be targeted by optogenetic stimulation to interrupt seizures. They used a rodent model for induced photothrombosis that results in late epilepsy (>1 month) after stroke[23] in the right somatosensory cortex. The researchers then created a device with multiple electrodes and a chronic multisite optrode[12]. This method allows the selective illumination and registration of thalamocortical neurons while monitoring their firing during epileptic activity. To design the closed-loop system, the authors routed an EEG channel to a programmable real-time digital signal processor that calculated the EEG line length[24] and triggered laser stimulation upon crossing of a threshold. For each subject, the authors set the line length threshold for seizure detection manually at the beginning of the experiment. This approach was capable of detecting and silencing seizures within 1 s of initiation in rats with chronic implants. In summary, Paz et al.[17] have shown that selective optogenetic inhibition of thalamocortical neurons interrupted ongoing epileptic activities in thalamus and cortex, as well as the behavioral seizure.

Krook-Magnuson et al.[19] have developed a sophisticated closed-loop system to halt seizures with optogenetics. They generated a model of temporal lobe epilepsy by injecting kainate unilaterally into the dorsal hippocampus. Two weeks later, spontaneous and recurrent seizures have emerged. Then, the animals were implanted with electrodes and individual optical fibers in the hippocampus. Seizures were detected using custom software able to combine and evaluate signal power properties, spike features and frequency properties of the recorded EEG. The authors also individually tuned the system to the specific EEG signature of the animals. ChR2 or HR were expressed using Cre-lox strategy to target specifically pyramidal cells or PV+ interneurons in the hippocampus. The results indicated the closed

loop optogenetic system successfully halted more than 50% of the spontaneous and recurrent seizures by optogenetically inhibition of principal cells or by excitation of PV+ interneurons in the hippocampus upon seizure detection.

... and how affordable is it?

As discussed before, overall, closed-loop optogenetic technology requires: (1) light source (laser or light-emitting diode) plus optical fiber to work as the effector; (2) electrodes or optodes to probe the neural activity (e.g. seizure) in the system; (3) and an analog to digital system to transfer this information back to a controller. The computer (or controller), in turn, will run custom algorithms (usually written in MatLab, Labview, Phyton, C++) to detect specific events of interest and, with the lowest latency as possible, trigger the effector, closing the loop. Also, to make specific cell types responsible for light, it is required the use of virus vector to deliver the genes of the opsins and, in some cases, it is also necessary the use of Cre-expressing animals.

Table 1 synthesizes information on the main materials and its respective prices for an implementation of the closed loop optogenetic approach in experimental epilepsy. We also provide a more detailed list of supplies with costs estimation (Table 2); it is mainly based on Armstrong et al.[25], and the prices have been updated to 2015. Amplifier was listed twice in Table 2 mainly because A-M Systems amplifiers are cheaper and widely used, but the equipment use their own connector for input data, which can be inconvenient if the amplifier is used for a variety of experimental apparatus, while those from Neurophase (old known as Brownlee Precision) use Bayonet Neill-Concelman (BNC) connectors for it. Usually, the National Instruments (NI) digitizer board is an affordable and convenient option for closed-loop systems. The NI board digitizes the data while can simultaneously detect specific events, such as a seizure, that will activate the digital output to control the effector (e.g. laser). Besides, this board is compatible with LabVIEW, C++ programming and can be accessed by the MATLAB software through the Data Acquisition Toolbox. Among these options, LabVIEW is the user-friendliest option for investigators who are not familiar with programming. It is relatively straightforward use its graphical programming language to implement real-time event detections and to close the loop by controlling an effector. However, if the investigator wants to use a more sophisticated detection by using a high number

Table 1. Estimated cost for closed loop optogenetics.

Items	Price
Differential Amplifier	$2,230.00
Laser Source	$2,500.00
National Instruments A/D board	$2,129.00
Consumables for Optogenetics	$1,908.00
Total	$8,767.00

Table 2. Equipment and consumables required for implementation of closed-loop optogenetic approach in experimental epilepsy (modified from Armstrong C, Krook-Magnuson E, Oijala M, Soltesz I. Closed-loop optogenetic intervention in mice. Nature protocols. 2013;8:1475–93).

Company	Supply	Full name	Part Number	Cost in 2015	Per
Thorlabs	(Patch cable) optical fiber from laser/led device to commutator	Ø200 μm, 0.39 NA, FC/PC-FC/PC Fiber Patch Cable, 2 m	M72L02	$80.60	each
	Optical fiber	0.39 NA, Ø200 μm Core Multimode Optical Fiber, Low OH for 400 - 2200 nm, TECS Clad	FT200EMT	$1.50	per meter
	Fiber Stripping Tool	Fiber Stripping Tool, Typical Cladding/Coating: 285 μm / 500 μm	T14S21	$66.61	each
	Ruby Fiber Scribe	Ruby DualScribe™ Fiber Optic Scribe	S90R	$50.50	each
	Fiber Polishing Disc (Harder to use, but faster)	LC/PC Connector Polishing Disc	D50-LC	$84.10	each
	Fiber Polishing Disc (Easier to use, but slower	LC/PC Ferrule Polishing Disc	D50-L	$84.50	each
	Glass Polishing Plate	Glass Polishing Plate, 9.5" x 13.5"	CTG913	$36.00	each
	Polishing Sheets	13" x 9" Aluminum Oxide Lapping (Polishing) Sheet, 0.3 μm Grit (10 Sheets)	LFG03P	$15.50	pack of 10
		13" x 9" Aluminum Oxide Lapping (Polishing) Sheet, 1 μm Grit (10 Sheets)	LFG1P	$13.80	pack of 10
		13" x 9" Aluminum Oxide Lapping (Polishing) Sheet, 3 μm Grit (10 Sheets)	LFG3P	$13.80	pack of 10
		13" x 9" Silicon Carbide Lapping (Polishing) Sheet, 5 μm Grit (10 Sheets)	LFG5P	$13.80	pack of 10
	Syringes for epoxy	3 cc Empty Epoxy Syringe, Package of 10, Disposable	MS403-10	$10.20	pack of 10
	Epoxy	Epoxy for Fiber Optic Connectors, Long Pot Life, 10 Packets	F112	$106.00	pack of 10
	Light Power Meter	Compact Power Meter Console, Mechanical Analog & Graphics LC Display	PM100A	$906.00	each
	Photodiode Power Sensor	Standard Photodiode Power Sensor, Si, 200 - 1100 nm, 50 mW	S120VC	$407.00	each

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Company	Supply	Full name	Part Number	Cost in 2015	Per
Example: CNI Laser	Blue, Amber or Red Laser	Fiber-couple DPSS Blue (wavelength 473 nm), Amber (wavelength 589 nm) or Red (wavelength 635 nm), 50 mW TTL modulation (on/off)	-	$2500.00 **	each
NeuroPhase	Amplifier	Brownlee Precision 4-Channel Instrumentation Amplifier Model 410	Model 410	USA price / International Price $2550 / $3250	each
A-M Systems		Model 1700 Differential AC Amplifier, 110 V, 60 Hz	690000	$2,230	each
National Instruments	8 channel digitizer	NI USB-6221 M Series DAQ Device, BNC Term, U.S. (120 V)	780117-01	$2,129	each
	16 channel digitizer	NI USB-6229 M Series DAQ Device, BNC Term, U.S. (120 V)	780116-01	$2,661	each
Dell	Computer	Dell Inspiron Desktop, Intel i7 processor, 64-bit Windows 7 Professional, 16GB memory	Inspiron Desktop	$989.98	each
Tocris	kainic acid (available in 1, 10, or 50mg)	Kainic Acid, 10 mg	0222 10 mg	$149.00	10 mg vial
USA Scientific	5 mL pipette tips	5 ml pipet tip, type A, racks	1050-0700	$51.30	10 racks of 50
ACE Surgical Supply	Local Anaesthetic	Bupivacaine HCL - 0.5% 50ml	011663-01	$4.29	each
Valley Vet Supply	Analgesic + antibiotic	Neo-Predef	617RX	$17.99	each
	Analgesic	Banamine (Flunixin Meglumine) Injectable Solution Veterinary 50mg/ml 100ml	134RX	$27.95	each
Kent Scientific	Curver Iris Forceps	Iris Forceps, curved, 10cm long, 1 x 2 teeth, 0.8mm tips	INS650917	$35	each
	Pointed Forceps	Tweezer #5 Dumoxel, 11cm, 0.1 x 0.06mm Tips	INS600098	$55	each
A-M Systems	Mounted Alligator Clip	Helping Hands Soldering Stand	726200	$9.00	each
Doric Lenses	Patch cables (from commutator to animal)	Mono Fiberoptic Patchcord (zirconia ferrule connector) 30 cm long with flange	MFP_200/220/900-0.53_0.3_FC-ZF1.25(F)	$135*	each
		Branching Fiberoptic Patchcord (zirconia ferrule connector) 30 cm long with flange	BFP_200/240/900-0.22_0.30m_FC-2xZF1.25(F)	$195*	each
	Optical Commutator	1x1 Fiberoptic Rotatory Joint	FRJ_1x1_FC-FC	$595*	each
	Cannula holder for stereotax	Stereotaxic Cannula holder for 1.25 mm ferrule	SCH_1.25	$315*	each
Dremel	Hand Drill	Dremel 3000-1/24 1 Attachment/24 Accessories Rotary Tool	Model 3000-1/24	$62.06	each
	Flexible shaft	Dremel 225-01 Flex Shaft Attachment	225-01	$29.66	each
	Keyless chuck	Dremel 4486 MultiPro Keyless Chuck	4486	$11.78	each
Fine Science Tools	Fine tipped delicate forceps	Dumont micro-blunted, atraumatic tipped forceps #5/45	11253-25	$52.00	each
	Small surgery scissors	Iris scissors, delicate pattern 9cm	14060-09	$61.00	each
Fisher Scientific	Disposable scalpel	Fisherbrand Single-Use Scalpels	089275A	$90.00	pack of 20
	Sterile q tips	Fisherbrand Polyester-tipped applicators; Sterile, 2 per envelope	23400111	$24.00	pack of 200
	Gloves	Microflex Evolution One Powder-Free Latex Exam Gloves, medium	11-462-68C	$84.09	pack of 100
	Sterile gloves	50 pair sterile size 7.5 gloves	11-388-122E	$347.71	pack of 50
	Small petri dishes	BD Falcon Standard Disposable Petri Dishes, surface area 21.29 cm2	08-757-100B	$197.46	pack of 500
	Lint-free wipes	Kimwipes Delicate Task Wipers	34155EMD	$6.25	pack fo 280
	Weigh dishes for mixing dental cement	Fisherbrand Hexagonal Polystyrene Weighing Dishes Top I.D.: 1.4 in. (3.6cm); Base I.D.: 0.9 in. (2.4cm); Depth: 0.4 in. (0.95cm)	02-202-100	$95.00	pack of 500
Ikea	Lamp	NOT Floor uplight/reading lamp, white, white	301.451.29	$9.99	each
Intermatic	Light timer	Indoor plug-in timer	TN311	$18.16	each

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Company	Supply	Full name	Part Number	Cost in 2015	Per
Jackson Laboratory	PV Cre mice	B6;129P2-Pvalbtm1(cre)Arbr/J	008069	$464*	breeding pair
	CamKII Cre mice	B6.Cg-Tg(Camk2a-cre)T29-1Stl/J	005359	$464*	breeding pair
	Floxed ChR2 mice	B6;129S-Gt(ROSA)26Sortm32 (CAG-COP4*H134R/EYFP)Hze/J	012569	$296*	breeding pair
	Floxed eNpHR3.0 mice	B6;129S-Gt(ROSA)26Sortm39 (CAG-hop/EYFP)Hze/J	014539	$354*	breeding pair
Kientec	225um ID ferrules (ceramic)	Zirconia Ferrule, 225 μm inner diameter	FZI-LC-225	$3.25	each
Loctite	Gel super glue	Loctite Super Glues	LOC1364076	$12.19	each
Logitech	HD USB web cam	HD Webcam C270 - USB, 3MP, 1280 x 720	Logitech C270 960-000694	$39.99	each
McMaster-Carr	Screws for mice	1/8" length	91773A052	$8.20	pack of 100
	Screwdriver that fits these screws	#0 Philips blade miniature screwdriver	7026A18	$5.03	each
Office Max	Uninterrupted power supply	APC Back-UPS XS Series Batteryackup, BX1500G, 1500VA/865 Watt	21880582	$199.99	each
	External hard drives	WD My Book 6TB External USB 3.0 Hard Drive With Backup, Black	24828046	$249.99	each
Moore Medical	Suture	Coated VICRYL (polyglactin 910) Precision Point-Reverse Cutting Sutures Undyed Braided P-2 5-0	58723	$217.00	each
	Hydrogen Peroxide	Hydrogen Peroxide 3% 8oz	90153	$1.09	each
Heartland Veterinary Supply and Pharmacy	Antibiotic	Baytril Injectable 2.27% 100 ml	2900-RX	$52.95	each
Santa Cruz Animal Health	Inhaled Anaesthetic	Isoflurane, 250 ml bottle	sc-363629Rx	$34.00	each
Pearson Dental	Drill bit	S.S. White Carbide Bur HP #2 Pkg. of 10	W60-0234	$19.50	each
	Liquid for dental cement	Teets C.C. Liquid (16 oz.)	C73-0076	$25.50	each
	Dental cement powder	Teets C.C. Clear Powder (1lb.)	C73-0060	$44.95	each
Plastics One	Electrical commutators	2 channel electrical commutator with single brush	SL2C/SB	$120.24*	each
	Cables from commutator to amplifier	2 channel cable with mesh covering 305 to 2 banana plugs, 100cm length	305-491/2 W/ MESH	$36.73*	each
	Cables from animal to commutator	2 channel cable with mesh covering and 2 305 connections, 35cm length	305-305 W/ MESH	$42.11*	each
	Electrodes	Bipolar Electrode Unit for Small Animals; 2 channel electrode, untwisted length = 10mm	MS303/3-A/SP	$7.53*	each
Precision Fiber Products	Zirconia sleeve	PFP Ceramic Split Sleeve, 1.25mm ID	SM-CS125S	$0.95	each

*Cost in 2013; **Average price from different companies.

of parameters, LabVIEW maybe not be the best choice. For instance, Krook-Magnuson et al.[19] took advantage of a custom written MATLAB-script to combine different parameters of EEG signal to achieve a better accuracy of spontaneous seizures detection, although it will include the MATLAB license in the price, which would be with LabVIEW as well. Consequently, to achieve the lowest-cost would be a better option to use programming languages like C and Python (http://wiki.python.org.br/).

As a general rule, using open source tools for a closed loop approach will reduce costs, and increase flexibility and control of the desired application. In the last years, a considerable amount of free software programs and hardware tools were developed by research groups to use in neuroscience. For instance, we can cite Open Ephys (http://www.open-ephys.org) combined with Intan headstages (http://www.intantech.com/), to acquiring and real-time processing data, Pulse Pal (https://sites.google.com/site/pulsepalwiki/home) and Cyclops Driver (https://goo.gl/ItZlNO), to precisely drive light sources for optogenetic stimulation in closed-loop experiments using recordings and event detections with Open Ephys. Combined these hardware tools can significantly reduce the costs presented in Table 1.

So, how affordable is the implementation of this emergent technique? Despite the high temporal precision and accuracy in detecting and interfering with seizures, we can conclude the costs of the fundamental components for closed-loop system assembly are relatively small. For comparison, according the Table 1, the costs of the main materials needed for closed loop optogenetics makes approximately U$9,000 while commercially available multi-microelectrode electrophysiology systems can hardly be acquired for less than U$30,000. Regardless, costs can be even lower if the research group chooses freeware software programs and hardware tools. To facilitate the access to more detailed information on optogenetics, closed loop systems and open source tools for Brazilian researchers and students, we have made a website (openoptobrasil.wordpress.com).

FINAL CONSIDERATIONS

Closed loop optogenetic is an emergent technique with the astonishing potential to push the neuroscience field forward. Real-time seizure detection combined with optogenetic control is now a reality and will certainly open future therapeutic avenues. The fundamental principles behind this approach are (1) the use of light and genetics to control well-defined events within specified cells of living tissue, and (2) the combination of fast real-time processing to detect events of interest from a system and, then, precisely control effectors that will act on the same system, only if the event is detected, closing the loop. Therefore, implementation of closed-loop optogenetics in experimental epilepsy seems to demand more joint interdisciplinary efforts and innovative scientific questions than money resources.

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