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Advanced approaches for selective investigation of neuronal function and circuitry: the future of developing novel therapeutic strategies in neuropharmacology?

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Abstract

Recent advances in neuroscience techniques and methods ushered in a new era in the research of neuronal function with unprecedented selectivity and temporal resolution. One of the main characteristics of these technical advances is the ability to selectively target and/or modulate specific neuronal subpopulations and circuits in both healthy and diseased brains. Although initially designed as tools to help researchers better understand the mechanisms underlying neuronal activity and complex behaviors, these novel approaches may also accelerate the process of drug discovery in many areas of neuroscience, and some may even potentially serve as novel therapeutic strategies. The application of different electrophysiological techniques is still considered essential in studying ion channel function and pharmacology, as well as network-level changes in brain activity. The cutting-edge methods for investigation of brain function include opto- and chemogenetics in freely behaving animals; both approaches enable highly selective control of neuronal activity using either a light stimulation (optogenetics) or a chemical ligand (chemogenetics) in both loss- and gain-of-function experiments. In this review paper, we aim to summarize recent scientific evidence on the state-of-the-art and provide information on these advances, taking into account both academic and pharmaceutical industry points of view.

Key words: neuroscience, drug discovery, electrophysiology, optogenetics, chemogenetics

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Introduction

The unprecedent rate of implementing new technologies in neuroscience research is advancing the field of neuropharmacology (1-3). From examining the activity of a single neuron to recording and/or imaging hundreds of neurons in freely behaving animals (4, 5), these approaches allowed for a better understanding of brain function, and helped illuminate the most intriguing questions in neuroscience, such as those related to neurophysiological basis of consciousness and memory. Equally important is their potential application in the pursuit of novel drug targets (6, 7), as our knowledge about how certain cellular and molecular processes modulate brain activity in healthy and pathological conditions is still rather limited. Almost in parallel, developing novel genetic tools, including gene-editing strategies, allowed for highly specific targeting of neuronal subpopulations and circuitry, as well as the creation of more suitable animal models to study and better understand the brain functions (8). Together, these technical advances stimulated renewed interest in research methodologies for the evaluation of novel drug targets and the effects of drugs on neuronal function.

Since molecular mechanisms underlying the activity of individual neurons should be viewed as integral parts of neuronal circuitry, it appears that an alternate approach to a conventional one focused on receptors and/or downstream signaling pathways may be to put more emphasis on understanding circuit functions through manipulation of specific neuronal pathways that control particular behavioral responses (9-11). Hence, local neuronal circuitry and projections between different brain regions may represent promising targets for innovative treatment strategies. Although these novel approaches show great potential in animal models, their potential application as a therapeutic option in humans remains to be determined. In this review article, we will discuss the most relevant advances in neuroscience techniques and methods from both academic and pharmaceutical industry perspectives. We will use a bottom-to-top approach, and first focus on the methods studying single neuron physiology, then proceed to those aiming to assess large neuronal populations or modulate the function of an entire neuronal circuit, and hence alter animal behavior. Finally, we will speculate about the use of circuit-specific strategies as potential therapeutic modalities.

In vitro and *ex vivo* electrophysiology

Although patch-clamp electrophysiology was developed almost 50 years ago (12), it remains a gold standard for studying ion channel physiology and pharmacology. To record current flowing through the patch of a neuronal membrane, one must first electrically isolate this patch of membrane from the external solution by pressing a fire-polished glass pipette, which has been prefilled with a suitable electrolyte solution, against the surface of a cell and applying light suction. Under such conditions, it is possible to electrically control the activity of parts or even an entire cell membrane. Since ion channels represent some of the most essential regulators of neuronal function, it is not surprising that this method is considered very valuable among scientists in both academic and industry settings. For example, voltage gated calcium channels are involved in many

physiological processes, such as synaptic transmission or action potential generation, and their malfunction underlies numerous disease states, making them important pharmacological targets. Therefore, recording their biophysical properties in isolated cells in the presence of agonists or antagonists may provide valuable information while testing the drug candidate.

Further advancement of this method to include electrophysiological recordings of neuronal activity in *ex vivo* acute brain slices allowed for a deeper insight into the analysis of local circuit function (13), as the fundamental circuit architecture in such preparation remained mostly intact. Due to these improvements, it became possible to readily record synaptic events, such as $GABA_A$-mediated inhibitory postsynaptic current, as well as to study in detail the firing properties of specific neuronal subpopulations and the contribution of different ionic currents to these processes. Testing the effects of acute pharmacological manipulations on these essential cellular and molecular processes also became a routine procedure for many laboratories. Furthermore, by combining patch-clamp *ex vivo* recordings with calcium imaging and/or molecular biology (e.g. single-cell PCR), scientists were able to make this approach even more powerful.

Using conventional patch-clamp electrophysiology, one may obtain extremely valuable information about neuronal function, but it requires highly trained personnel, and often only one experiment can be performed at a time. About two decades ago, the field of ion-channel research was revolutionized by the development of the automated patch-clamp instrumentation (reviewed in 14). The advent of higher throughput patch-clamp electrophysiology systems has begun to change the face of ion channel drug discovery. Systems such as the QPatch or the Qube 384 (Sophion Inc) should allow electrophysiology to be a frontline gene expression analysis tool and drug screening workhorse. A different automated system designed by Axon Instruments (now part of Molecular Devices Corporation), The PatchXpress, enables up to 16 parallel recordings to be made on a single ‘seal chip’. All these systems retain most of the functionality of the conventional patch-clamp, thus successfully bridging the gap between high throughput and high fidelity. However, while incorporating relatively simple cell lines (e.g. HEK, Human Embryonic Kidney cells) into the workflow of the automated system was relatively straightforward, working with the acute brain slices proved to be a difficult obstacle. Hence, *ex vivo* patch-clamp electrophysiology remains a cornerstone of fundamental neuroscience research, as well as certain phases of early drug target discovery.

Single cell recordings are usually time-consuming and low-throughput; therefore, significant effort has been invested in developing novel technologies that would enable faster assessment of novel therapeutic targets in cellular biology. One of these assays is the multi-electrode array (MEA) system that allows capturing the field potential or activity across an entire cellular population and detecting activity patterns that would otherwise elude traditional assays, such as single-cell patch-clamp electrophysiology. MEA assay is a plate-based assay with a grid of tightly spaced microscopic electrodes embedded in the bottom of each well, enabling continuous undisturbed recordings from

the cells cultured on top of these electrodes in wells. The system can record spontaneous as well chemically stimulated neuronal responses originating from either rodent or human cultured neurons.

Performing traditional electrophysiology assays in acutely prepared human brain slices or dorsal root ganglia (DRG) dissociated sensory neurons is very challenging due to the difficulties in obtaining the tissue. However, using neuronal cell cultures derived from human induced pluripotent stem cells (hIPSC) or cultured human DRG neurons in MEA assay has allowed researchers another opportunity in translating the results from their rodent or *in vitro* studies in human tissue as well. The work by Namer et al. (2019) is an excellent example of successful patient specific precision medicine using iPSC technology, MEA assay and individualized therapeutic treatment based on patient-derived sensory neurons (15). In order to identify potential treatment options for the patient, the authors reprogrammed the patient‘s fibroblasts into iPSC and differentiated these into peripheral sensory neurons using a small molecule approach. In the MEA assay, patient’s derived iPSC sensory neurons exhibited an increase in excitability manifested as increase in spikes and the number of active electrodes, which was significantly reversed with *in vitro* treatment of these neurons with lacosamide, a sodium channel inhibitor recently evaluated in clinical trials for small fiber neuropathies (16).

In vivo electrophysiology

Recording ionic currents or neuronal activity in *ex vivo* conditions is often only the first stage towards understanding the physiological processes and potential effects of drug candidates on neuronal function. The next important step would be to record neuronal network activity in CNS or periphery using *in vivo* electrophysiology by measuring single units and/or local field potentials (LFPs) generated by a large number of neurons. This type of study represents a direct way of measuring neuronal function, detecting brain circuit abnormalities, and/or investigating changes in electrical signals in response to therapeutics or modeling brain disorders, such as epilepsy or schizophrenia. In the past five decades, technologies used to record the spiking activity of individual neurons in concert with local field potentials have become extremely sophisticated, with the number of recording sites per electrode reaching 1000 (17). Silicon neural probes, for example, allow superior signal-to-noise, multi-channel recording across multiple brain regions in freely-behaving rodents (18, 19), pigs (20), and even non-human primates (21). This recording technique, however, is mostly restricted to academic laboratories, as it requires extensive training and analyzing large data sets is often challenging and time-consuming, which makes it difficult to incorporate into a routine use of a high-throughput laboratory in an industry setting.

On the other hand, simplifying the experimental procedure and lowering the number of recording channels may help address these issues and provide better translational value. Indeed, the scalp electroencephalography (EEG), often accompanied by LFPs recorded using depth electrodes targeting specific brain regions, is still an effective tool for analyzing neuronal oscillations and elucidating circuit-specific

mechanisms underlying therapeutic effects of different centrally acting drugs (22-24). One of the most utilized approaches in the context of preclinical studies is to evaluate how certain (patho)physiological conditions and drug candidates affect the spiking activity and/or spectral power of different neuronal oscillations in specific brain regions, which often yields a particular pattern of activity. In psychopharmacology, it is well established that antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), acutely reduce neuronal firing in the dorsal raphe nucleus (25, 26), the main source of serotonergic forebrain projection neurons, whereas antipsychotics alter high frequency (gamma) oscillations in the hippocampus (27); both of these effects may be related to their therapeutic properties. The use of *in vivo* electrophysiological recordings in studying epilepsy cannot be stressed enough, in both preclinical and clinical settings, as this type of recordings represents a mainstay of seizure detection and epilepsy management (28, 29). In the anesthesia field, an increase in cortical low frequency (delta) power is associated with deep states of hypnosis or anesthesia in both rats (30, 31) and humans (32). Finally, combining *in vivo* electrophysiology with simultaneous blood sampling or local drug administration can be used to correlate the actual effects of drug candidates on neuronal activity with its pharmacokinetics.

In vivo electrophysiology studies intended to investigate the conduction properties of peripheral nerves are of utmost importance for understanding the physiology of peripheral sensory pathways involved in pain transduction and transmission (33). The peripheral nerves innervate peripheral organs and tissues and provide valuable information on proprioception, sensation and motor coordination. Recording the electrical activity of peripheral nerves is clinically relevant and is commonly used to support diagnosis of various diseases that affect the integrity of the nerve (e.g. Guillain Barre, carpal tunnel syndrome and various neuropathies). Extracellular peripheral nerve recordings assay on saphenous nerve-skin preparation in rodents is commonly used for studying peripheral nerve activity and their properties in various animal disease models (34). Moreover, the development of novel pharmacological inhibitors or selective knockdown or knockout of receptors or ion channels involved in neuronal transduction and/or transmission further facilitates the exploration of peripheral sensory pathways.

Nerve conduction velocity and action potential amplitude are used as biomarkers of neurological disorder, stemming from either myelin or axonal damage in neuropathic conditions, such as diabetic neuropathy. Specifically, nerve conduction velocity depends on the integrity of myelin sheathing and changes are indicative of myelin damage, while axonal damage is usually manifested in changes in the action potential amplitude, which commonly reflects the number of recruited axons/neuronal fibers with electrical stimulation (35, 36).

In a clinical setting, small fiber neuropathies can be diagnosed with microneurography. First established in 1977 by Hagbarth and Burke as an electrophysiological technique to study the properties of peripheral nerves in awake humans (37), this technique enables researchers to collect the data from specific subsets of peripheral nerve fibers, such as C-fibers. These fibers are thin, unmyelinated and

encode sensory sensations, especially pain, itch, and temperature, or serving as efferent nerve fibers for the autonomous nervous system (38). By percutaneously inserting thin recording microelectrodes inside nerve fascicles of peripheral nerves, such as sural, tibial or peroneal, extracellular nerve recordings are collected, and subpopulations of specific fibers of interest (e.g. C-fibers) can be detected by using the so called “marking” technique (15, 39). This technique is based on the feature of C-fibers that manifests as pronounced activity-dependent hyperpolarization (causing slowing of conduction velocity) and enables the identification of specific C-fiber units. This assay can be performed in the same way in anesthetized rodents, and represents a powerful tool to study the neural signaling of pain and itch in healthy conditions, as well as in different pathologies of the peripheral neural system. Taken together, these findings testify that *in vivo* electrophysiology remains a powerful approach to elucidate abnormalities in neuronal circuitries and information processing, as well as to evaluate whether drug candidates can restore normal brain function.

Optogenetics

Many important brain functions rely on the finely tuned activity of neuronal circuits encompassing several brain regions, rather than individual neuronal subpopulations. Therefore, as an alternative to more traditional strategies targeting receptors, enzymes and/or their downstream effects, a more promising approach may be to focus on integrated circuit mechanisms. Indeed, activating or inhibiting specific brain circuits may have a profound effect on different behavioral outcomes. Optogenetics, or light-controlled neuronal modulation (40, 41), is one of the most innovative neural circuit technologies that allow us to control animal behaviors, such as learning and memory (42) or anxiety-related behavior (9). Pioneered by the Deisseroth lab at the Stanford University in California, this method opened new research avenues towards understanding the contribution of specific neuronal subpopulations and their projections to downstream targets in regulating brain functions. Although its use is by no means limited to *in vivo* conditions, here we will focus mostly on this approach, as it has clear advantages in terms of translational value.

In the first step, viral vectors such as adeno-associated virus (AAV) are used to selectively express light-sensitive transmembrane proteins (opsins) in neuronal populations of interest, followed by implantation of an optical fiber. These opsins represent ion channels, ion pumps, and enzymes that allow light-induced manipulation of electrical and biochemical activity with high temporal and cell-type resolution. The channelrhodopsins (ChR), light-gated nonspecific cation channels, are the most popular among the opsins which can be used to excite neurons (40, 43), whereas anion-conducting halorhodopsin is often used for neuronal inhibition (44). When light of a particular wavelength is delivered to these neurons through the optical fiber, it will cause depolarization or hyperpolarization of the cell membrane, thereby resulting in cellular excitation or silencing with a temporal precision in the millisecond range (45).

In cognitive neuroscience research, *in vivo* optogenetics has been utilized to dissect the role of hippocampal circuitry in memory formation and retrieval (46, 47), to manipulate memory engram cells (42), and to reversibly deactivate and reactivate new (48) and even remote memory traces (49). As pioneers in this emerging field, researchers from the Tonegawa lab at the Massachusetts Institute of Technology were able to implant a false memory in a mouse using the contextual fear conditioning behavioral paradigm (50). Although limited to animal models, these captivating findings shed light on the mechanisms underlying memory processing and opened new perspectives for the study of cognitive function in both healthy and diseased brains.

Using a mouse model of early Alzheimer's disease, the same group showed that optogenetic reactivation of hippocampal memory engrams may rescue long-term memory deficits in these animals (7). In epilepsy research as well, optogenetics was applied not only as a tool, but as a potential therapeutic strategy. Specifically, Krook-Magnuson et al. (2013) reported that a real-time, closed-loop, response system and *in vivo* optogenetics can stop spontaneous seizures in a mouse model of temporal lobe epilepsy (TLE) by selectively inhibiting hippocampal principal neurons or activating a rather small subpopulation of GABAergic cells (51). More recently, using a similar approach, Lu et al. (2016) showed that optogenetic manipulation can potently inhibit the spread of ictal seizures and rescue behavioral deficits in mice (52). In addition, studying seizure generation and propagation can be made more efficient by inducing seizure activity selectively and "on-demand", targeting only certain neuronal subpopulations or brain regions (53). Regarding anesthesia-related research, the use of optogenetic tools yielded a fascinating discovery that selectively stimulating dopamine neurons in a rather small brain region of the brainstem (ventral tegmental area) induced a powerful arousal response in mice that restored conscious behaviors during general anesthesia (54). It is important to note that the brain may not be the only focus of optogenetic manipulation (reviewed in 55); a selective expression of channelrhodopsins in mouse sensory neurons allowed modulation of pain pathways both in the periphery and at the level of the spinal cord in mice (6, 56).

These advances in basic neuroscience research made possible by the use of optogenetics provided a new momentum in drug discovery, as it seems that targeting neurotransmitter systems in their entirety is unlikely to lead to refined pharmacotherapeutic approaches, at least for complex brain disorders (1). Indeed, optogenetics is beginning to translate and transit into drug discovery by providing a circuit- and pathway-centric strategy suitable for drug target identification. For example, Prigge et al. (2010) were able to express channelrhodospin in a cell line that also expresses $Ca_V3.2$ T-type calcium channels, which allowed activation of these low voltage-gated calcium channels by short pulses of blue light, thereby creating a platform for an all-optical testing of calcium channel antagonists (57). A similar principle, more suitable for high-throughput screening, was used to study other ion channels, such as $Ca_V1.3$ calcium channel (58). It remains uncertain, however, in what capacity these approaches will be able to replace the protocols used routinely during the drug discovery process. In terms

of clinical applications, despite obvious advantages over other comparative therapeutic modalities, such as deep brain stimulation, optogenetics faces many translational obstacles that impede its further development (59).

There has been an increased interest in utilizing optogenetic approach in studying peripheral nervous system pathways that will enable better understanding of the role of specific subpopulations of sensory neurons in pain processing. It has been shown that ChR2-mediated stimulation of $Na_V1.8$-positive (6), TRPV1-positive (60) or C-fiber sensory afferents (61) resulted in nociceptive-like behaviors. It is interesting to note that acute sensitization with optical stimulation of TRPV1-ChR2 neurons leads to an increase of ubiquitin-specific peptidase 5 (USP5) expression, resulting in increased $Ca_V3.2$ T-type activity *in vitro*, and increased mechanical hypersensitivity *in vivo* (62), suggesting that optogenetic stimulation can lead to long-lasting behavioral sensitivity even after the stimulation discontinuation. There is also a possibility of using optogenetic stimulation to mimic the conditions of neurogenic inflammation; however, additional experiments are warranted to confirm such a notion.

On the other hand, inhibitory opsins can decrease responses to noxious stimuli in naïve animals (63), as well as in animals that have undergone chronic constriction injury (64). It is also possible to specifically express opsins in certain subpopulations of sensory fibers, such as A-delta fibers expressing Arch, the inhibitory proton pump archaerhodopsin, which enabled exclusive reduction in the activity of high threshold mechanoreceptors, but not in C-fibers or low threshold mechanoreceptors in rats with partial sciatic nerve ligation (65). Finally, *in vitro* studies in human dorsal root ganglion neurons have shown that opsins can reduce firing of human sensory neurons (66), implicating optogenetic approach as a potential clinically relevant avenue for the development of novel non-pharmacological strategies for pain alleviation in patients.

Chemogenetics

Another innovative approach developed relatively recently with a similar overarching strategy for specific targeting of neuronal circuits that attracted considerable scientific attention is chemogenetics. Instead of using opsins, this method involves the viral-mediated genetic expression of designer receptors activated exclusively by designer drugs (DREADDs) that remain “dormant” until activated by a biologically inert ligand (67, 68). The most often used DREADDs represent modified human muscarinic receptors (M3 and M4), named hM3Dq and hM4Di. The first DREADD was designed to increase neuronal excitability via the phospholipase C stimulatory Gq-coupled signaling pathway (67); this modified muscarinic receptor is rather insensitive to acetylcholine, but has high affinity for the synthetic ligand, such as clozapine-N-oxide (CNO). If neuronal inhibition is required, the binding of systemically applied CNO to the Gi-coupled hM4Di DREADD will activate a downstream signaling cascade leading to targeted silencing of neuronal activity (67, 69).

This strategy may produce prolonged neuronal excitation or inhibition, which is a great advantage in comparison to optogenetics. Furthermore, the use of DREADDs shows

more flexibility as it can be applied in a selective manner across multiple sites in the brain, and thereby regulate several different circuits at the same time. Chemogenetics also offers a unique opportunity to study signaling pathways relying on G protein-coupled receptors. It should be mentioned here that one of the main caveats of using optogenetics *in vivo*, unlike chemogenetics, is that it requires the optic fiber implantation in addition to a viral delivery, thereby causing unnecessary tissue damage.

As was the case previously with optogenetics, the potential of chemogenetics for *in vivo* applications was quickly recognized. This neuron- and/or circuit-specific strategy improved our knowledge about a wide range of fundamental processes and behaviors: sleep-wake cycle (70), feeding behavior (71), insulin release (72), learning and memory (73), etc. Furthermore, chemogenetics has significantly advanced our understanding of basic circuit mechanisms underlying different brain dysfunctions. For example, it allowed the dissection of neuronal circuits that contribute to the cognitive symptoms in psychiatric disorders (10, 11), and it helped identify neurons that mediate the addictive properties of cocaine (74), or those involved in anxiety and fear responses (75, 76).

In epilepsy research, chemogenetics has been used bidirectionally, to increase or decrease seizure activity (reviewed in 77), as well as to improve cognitive deficits in chronically epileptic mice (78). One of the most recent studies showed that DREADD administration effectively suppressed spontaneous seizures in the intrahippocampal kainic acid mouse model of TLE (79), an effect superior to that of levetiracetam, a commonly used antiepileptic drug (80). Regarding cognitive deficits related to TLE, chemogenetic inhibition of epilepsy-induced hyperexcitability in the dentate gyrus of the hippocampus significantly improved the spatial memory impairment in pilocarpine-treated mice (78). A similar approach was used to specifically target CA1 hippocampal neurons in a mouse model of 22q11.2 deletion syndrome (81), one of the highest known risk factors for schizophrenia (82).

In terms of translational value, it is noteworthy that chemogenetics has been successfully used in non-human primate studies. These studies reported changes in reward-related behaviors following repeated DREADD-induced inactivation of several brain regions including the orbitofrontal cortex and rostromedial caudate (83, 84). More recently, Magnus et al. (2019) developed an improved set of chemogenetic tools by mutating the ligand-binding domain of the α7 nicotinic acetylcholine receptor that is highly sensitive to a smoking cessation drug varenicline in rodents and primates (85). Although the use of chemogenetics in human patients is hindered by many limitations (e.g. expressing an exogenous receptor using gene therapy), there is an intriguing possibility to apply DREADDs in certain neurologic disorders, such as Parkinson's disease or epilepsy, at the same time when deep brain stimulation is being delivered (86). Another important avenue to pursue is the neuropathic pain treatment (87), as changes in neuronal excitability are one of the hallmarks of this pain syndrome (88). In summary, a growing body of evidence collected over the past decade shows that the chemogenetic manipulation of neuronal activity became not only an essential part of the neuroscience toolbox, but also a promising therapeutic approach for multiple brain disorders.

Lastly, it is important to mention that non-neuronal cells in CNS, such as astrocytes, may play an important role in the pathogenesis of different brain disorders, and thereby represent a viable target for potential interventions (89). For example, Habib et al. (2020) described a population of Alzheimer's disease-associated astrocytes, which was similar to that found in aged wild-type mice and in the aging human brain (90). In the field of psychiatry, an increase in the expression profiles of cortical astrocytes was found in patients with bipolar disorder and schizophrenia (91), and certain antipsychotics are known to regulate astrocytic functions (reviewed in 92). Although astrocytes comprise a significant portion of the cells in the brain, their role beyond providing support for neuronal function has only recently been recognized; it appears that astrocytes can modulate synaptic neurotransmission and plasticity, thus actively participating in learning and memory (93). Indeed, astrocytic activation using either optogenetics or chemogenetics can enhance spatial and contextual memory processing (94). Therefore, an extensive investigation of their functions using cutting-edge methods is warranted if we are to fully understand brain disorders and explore novel therapeutic avenues.

Conclusions

Modern neuroscience research and drug discovery are inconceivable without the state-of-the-art technological advancements. Along with their crucial role in dissecting neuronal physiology and brain functions, some of the techniques reviewed here (i.e. opto-/chemogenetics) show promise in developing new strategies to treat brain disorders. The therapeutic advantages of selective circuit manipulation might be transformative for many nervous system pathologies, particularly those associated with neuronal hyperexcitability, such as epilepsy or pain, or the conditions that already require some level of tissue ablation or resection. However, their potential application is still largely limited to preclinical animal models, and several substantial hurdles remain to be resolved to ultimately translate these technological advances to human patients. Moreover, it is not clear whether these novel approaches can selectively modulate neuronal circuitry in humans and thereby serve as a potential therapeutic strategy. A relative success of gene therapy in humans has potentially resolved the issue of safely delivering an engineered target into the central nervous system, thus increasing the translational value of these approaches. With its use of modified human receptors and relatively cheap and safe ligands suitable for chronic administration, it appears that chemogenetics has the greatest potential for bench to bedside transition.

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Savremene metode za selektivno ispitivanje funkcije neurona i neuronskih kola: budućnost razvoja novih terapijskih pristupa u neurofarmakologiji?

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Kratak sadržaj

Napredak u savremenim tehnikama i metodama u oblasti neuronauka poslednjih godina doveo je do nove ere u istraživanjima neuronske funkcije sa do sada nezabeleženom selektivnošću i visokom vremenskom rezolucijom. Jedna od najvažnijih karakteristika ovih tehničkih dostignuća jeste ciljana manipulacija grupa neurona i/ili neuronskih kola u mozgu zdravih i obolelih jedinki. Iako su inicijalno ovi pristupi bili dizajnirani kao alati koji bi trebalo da pomognu istraživačima da bolje razumeju mehanizme koji leže u osnovi neuronske aktivnosti i složenog ponašanja, oni bi mogli ubrzati put ka farmakološkim otkrićima u mnogim neuronaučnim oblastima, dok neki pristupi mogu potencijalno služiti i kao nove terapijske strategije. Primena različitih elektrofizioloških tehnika se i dalje smatra krucijalnom u istraživanjima funkcije i farmakologije jonskih kanala, kao i u ispitivanjima promena moždane aktivnosti na nivou neuronskih kola. Najsavremenije metode za ispitivanje funkcije mozga uključuju opto- i hemogenetiku kod životinja koje nesmetano ispoljavaju svoje ponašanje. Oba pristupa omogućavaju kontrolisanje neuronske aktivnosti sa visokom selektivnošću primenom svetlosne stimulacije (optogenetika) ili korišćenjem hemijskog liganda (hemogenetika), kako u eksperimentima sa gubitkom, tako i u onim sa pojačanjem funkcije. Ovaj pregledni rad ima za cilj da sumira najnovije naučne dokaze i pruži relevantne informacije o navedenim savremenim pristupima uzimajući u obzir ne samo ugao sagledavanja akademskih ustanova, već i farmaceutske industrije.

Ključne reči: neuronauka, istraživanje, elektrofiziologija, optogenetika, hemogenetika