We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

BOOK CITATION INDEX
CLARIVATE ANALYTICS
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Voltage-Gated Sodium Channel Drug Discovery Technologies and Challenges

Tianbo Li and Jun Chen

Additional information is available at the end of the chapter

Abstract

Voltage-gated sodium (Nav) channels represent an important class of drug target for pain and many other pathology conditions. Despite the recent advances in channelopathies and structure-function studies, the discovery of Nav channel therapeutics is still facing a major challenge from the limitation of assay technologies. This chapter will focus on advancement and challenge of Nav drug discovery technologies including nonelectrophysiological assays, extracellular electrophysiological assays, and the newly evolved high-throughput automated patch clamp (APC) technologies.

Keywords: voltage-gated sodium channel, drug discovery, uHTS, APC

1. Introduction

Nav channels are among the most well-characterized drug targets for pain, epilepsy, cardiac arrhythmias, and neuromuscular diseases. Current Nav drugs were developed empirically, in most cases without knowing their precise molecular targets. Even though new clinical indications have been found for these early-generation Nav modulators, their lack of sufficient isoform selectivity significantly limits the therapeutic outcomes. For example, when lidocaine is used to treat neuropathic pain, it causes cardiac toxicity due to inhibition of Nav1.5 and causes sedation, convulsion, and cardiac arrhythmias due to inhibition of CNS Nav channels.

Subtype-selective modulation of Nav channels is essential for target drug development but extremely difficult to achieve due to high sequence homology in the Nav family. The problem is further confounded by the current screening assays. Several assay platforms, such as radioligand binding, ion flux, and fluorescence membrane potential assays, have existed

for several decades. They are cost-effective and amenable to high-throughput screening. However these assays have significant limitations. For instance, the ligand binding assays could not provide functional effects (e.g., agonism vs. antagonism). Ion flux and membrane potential assays only measure channel function indirectly and are prone to artifacts such as autofluorescence, ionophore, and cellular toxicity. Electrophysiology is considered as the gold standard. However, traditional electrophysiology is significantly limited by its low throughput and labor intense. In the past decade, the emergence of automated electrophysiology has significantly increased the throughput, making it feasible to screen compounds using electrophysiology.

Same as any other drug targets, ion channel drug discovery refers to the process by which new candidate medications are discovered. This is a drug early development process lying between target validation biology research and drug clinic development. The process of ion channel drug discovery can be arbitrarily divided to three stages: primary screening, hit confirmation, and lead optimization (**Figure 1**). At the stage of primary screening, a large number (up to millions) of compounds are screened using various non- or indirect functional high-throughput assay, such as radioligand binding, ion flux, or membrane potential assays. Often, a small set of tool compounds are used to validate the assay. After obtaining correlation to electrophysiology, the assay is chosen for ultra high-throughput screening (uHTS) development and used for primary screening. Companion assays are also developed for counter screening to reduce assay artifacts or assess selectivity against other irrelevant targets. The uHTS assay performance is evaluated by assay stability, assay window, and hit rate, which is usually around 0.1% resulting in tens of thousands of potential hits. At the hit confirmation stage, often automated patch clamp (APC) platforms are used to test HTS hits. Hits are conformed, and their selectivity is confirmed by counter screening against other relevant isoforms. At the lead optimization stage, compounds are optimized on multiple fronts, including potency, selectivity, DMPK properties, efficacy, and toxicity.
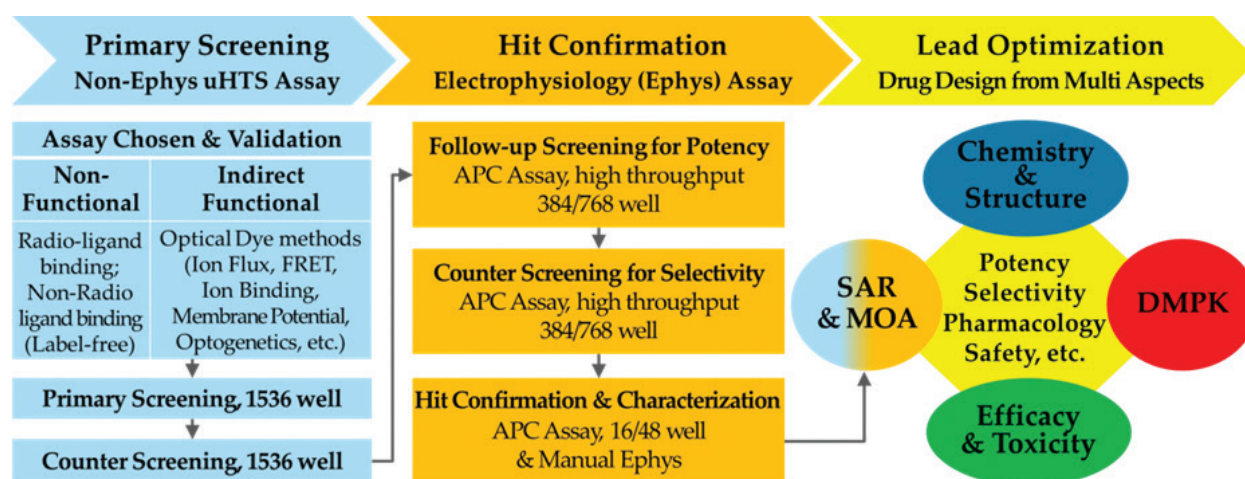

Figure 1. Ion channel drug discovery strategy. Strategies for the implementation of multiple assay platforms in Nav channel drug discovery in the three stages of drug early development: SAR, structure-action relation; MOA, mechanism of action; DMPK, drug metabolism and pharmacokinetics.

2. Nonelectrophysiological technologies

Nonelectrophysiological assays include nonfunctional assays and indirect functional assays. Under nonfunctional assay category, there are radioligand binding, fluorescence polarization (FP), fluorescence resonance energy transfer (FRET), and a series of fragment-based lead discovery assays. These assays can detect high-affinity binding, which makes them useful for kinetic studies and lead optimization. However, their major limitation is that the ligand binding sometimes does not translate into functional effects.

Indirect functional assays include ion flux assay and fluorescence membrane potential assays. These assays do not have precise voltage control and do not directly measure channel conductance. Hence, these assays are prone to false positives from autofluorescence, ionophore, off-target effect, and cellular toxicity and in many cases cannot detect state-dependent modulators, thus needing to be redesigned [1] and validated using tool compounds in early assay development stage. Overall, these indirect functional assays have been commonly used for Nav channel drug discovery primary screening, due to their functional correlation and uHTS amenable assay properties.

2.1. Radioligand binding assays

Radioligand binding assays have been extensively employed for various targets. These assays are cost-efficient, amenable for automation, and relatively easy to perform. Due to the lack of functional information, this assay format is usually not used for primary screening, but often employed during lead optimization stage to determine binding affinity, kinetics, and mechanism of action.

Ligand binding assays address the affinity between a ligand and its target: $K = 1/K_d$, which can be measured from kinetic experiments. In association experiments, multiple K_{obs} are measured at different ligand concentrations [C]. And K_{off} is measured in dissociation experiment at equilibrium condition. Then the association K_{on} and dissociation K_{off} rate constants can be calculated from Eq. 1, and K_d can be calculated from Eq. 2:

$$K_{ob} = K_{off} + K_{on} \times [C] \tag{1}$$

$$K_d = K_{off}/K_{on} \tag{2}$$

Binding experiments are relatively easy to perform and achieve high throughput. The assay development needs to avoid multiple issues, such as non-equilibrium dissociation and ligand depletions, which can be exacerbated by undetected impurities in the ligands studied.

Based on the nature of binding assays, the major limitation of this assay is that the studying subject compound needs to bind to the same or allosterically linked site of the ligand. This is particularly the case for Nav channels' target, due to Nav channels' high complexity and the high homology between intra-α subunit four domains and nine inter-Nav subtypes.

2.2. Nonradioligand binding assays

Traditional nonradioligand binding assays use fluorescence labeling to detect binding, including fluorescence polarization (FP) to measure the change in the rotational speed of a fluorescent-labeled ligand once it is bound; total internal reflection fluorescence (TIRF) labels target instead of ligand to measure fluorescence rotational change upon ligand binding; fluorescence resonance energy transfer (FRET) labels both ligand and target with a pair of donor and acceptor fluorescent molecules to measure fluorescent energy transfer. All these methods require that fluorescence labeling should not interfere with target-ligand interaction.

Another category of nonradioligand drug binding assays is fragment-based lead discovery (FBLD) technologies. Among them, nuclear magnetic resonance (NMR) spectroscopy is considered as the gold standard, as it measures chemical shift between free and bound targets in $^{15}N/^{1}H$, $^{13}C/^{1}H$, and/or other labeled atom's two-dimensional correlation spectra to provide molecular interaction information [2]. In the past decade, many label-free technologies emerged to provide new options (**Table 1**). Isothermal titration calorimetry (ITC) and microscale thermophoresis (MST) technologies measure thermodynamic change associated with ligand binding in solution. A quartz crystal microbalance (QCM) measures a mass variation per unit area via the change in frequency of a quartz crystal resonator. Surface plasmon resonance (SPR), second harmonic generation (SHG), and biolayer interferometry (BLI) measure optical change through a sensor, which usually requires immobilizing one binding reaction component on the sensor surface. Typical applications of these FBLD technologies include fragment-based screening, binding studies for target engagement, Kd measurements, and protein conformational changes. Many of these methods, such as surface plasmon resonance (SPR), can provide insights to enzymatic reactions by directly monitoring and quantifying the binding and depletion of the reaction components in real time. The advancement of increased sensitivity and implementation of automated systems have broadened the use of FBLD technologies in drug discovery, especially in lead optimization drug discovery stage mechanism-of-action studies.

2.3. Membrane potential assays

Membrane potential assays have been widely used in Nav channel drug discovery. Two types of membrane potential assays have been developed [3, 4]. One uses membrane potential dye and fluorescence imaging plate reader (FLIPR) (Molecular Devices, Sunnyvale, CA). Another measures fluorescence resonance energy transfer (FRET) between a voltage-sensing oxonol acceptor and a fluorescent membrane-bound coumarin dye. Both methods have been used to identify modulators for Nav1.6, Nav1.7, and Nav1.8.

The major drawback for these assays is their reliance on using nonphysiological relevant toxin openers (e.g., veratridine, deltamethrin, or batrachotoxin) to elicit channel response. These toxins remove inactivation and causes persistent channel opening through its binding to the intracellular pore domain (Site 2). The assay can successfully detect inhibition by TTX and tetracaine, but it fails to detect Nav1.7-selective aryl sulfonamides, such as PF-05089771 and GX-936. A new strategy of eliminating key LA binding site mutation N1742 K in Nav1.7,

	Nuclear magnetic resonance (NMR)	Isothermal titration calorimetry (ITC)	Microscale thermophoresis (MST)	Quartz crystal microbalance (QCM)	Surface plasmon resonance (SPR)	Second harmonic generation (SHG)	Biolayer interferometry (BLI)
Kinetics	No	No	Yes	Yes	Yes	Yes	Yes
Immobilization-free	Yes	Yes	Yes	No	No	No	No
Label-free	Optional	Yes	Optional	Yes	Yes	No	Yes
Throughput	Medium	Low	Medium	High	Medium	High	Yes
Sensitivity	High	Medium	Medium	High	High	High	Low
Mass dependent	No	Yes	Yes	Yes	Yes	No	Yes
Sample amount	High	High	Low	Low	Low	<1 ug	Low
Costs	High	Low	Medium	Medium	High	High	Medium
Vendor	Multi	NanoTemper, etc.		Q-Sense, etc.	Multi	Biodesy	ForteBio

Table 1. Fragment-based lead discovery (FBLD) technologies in drug discovery.

combined with using a new activator 1KαPMTX, was recently reported [1]. This new assay was able to detect aryl sulfonamides, with significantly reduced sensitivity to LAs, hence shifting the assay toward non–pore-binding mechanism. Such a mechanism-specific assay design can be applied to other ion channels to facilitate discovering subtype-selective drug.

2.4. Ion flux assays

Early ion flux assays were primarily developed on radionuclide ions such as $^{45}Ca^{2+}$ and $^{22}Na^+$. However, in the 1980s the development of ion-selective fluorescent dye-based indicators revolutionized the measurement of ion flux assay. This method provides an indirect functional detection for ion channel activity, especially for calcium and potassium channels due to the robust signals of calcium dye and thallium dye. Unfortunately, fluorescent Na^+ dyes have relatively weak signals and are not amenable to reporting Na^+ concentration. However, Na^+ concentration can be quantitated by using atomic absorption spectroscopy (AAS). Additionally, radioisotopic $^{22}Na^+$ still can be used as a tracer for Nav flux assay [5].

3. Extracellular electrophysiological technologies

Extracellular electrophysiological recording technologies employ direct or indirect electrical stimuli to a population of cell, in most cases a whole well confluent cell, and record target ion channel activity either from direct electrical signal or indirect fluorescent dye methods. These methods provide electrophysiological-involved functional assays at extracellular level, which usually represent a more physiological relevant environment, thus different from non-electrophysiological assays and typical single-cell and subcell-level electrophysiological patch clamp assays.

3.1. Optogenetics assay

The integration of optogenetics tools with membrane potential assays provides a powerful approach for Nav channel research and drug discovery [6]. Instead of using nonphysiological stimuli, optogenetics controls membrane potential by using light-activated channelrhodopsins (ChR2, ChR1, VChR1, and SFOs); therefore, channels can be populated to specific state, and state-dependent modulators can be identified. After optimizing the performance of channelrhodopsins and voltage reporters, this technique may contribute to Nav channel drug discovery. One potential drawback could be the introduction of false positives derived from interferences with channelrhodopsins and membrane potential dyes.

3.2. Electric field stimulation (EFS) assay

Recently, electric field stimulation (EFS) assay has been reported in studying Nav1.7 function in cultured rat sensory neurons. This method utilizes electrical field stimulation to evoke action potential and record action potential-driven calcium transients in the neurons through live cell imaging. This method provides a novel functional phenotypic assay platform to study voltage-gated ion channels in the network of excitable primary cell and induced pluripotent stem cell [7].

3.3. Multielectrode arrays (MEA)

Multielectrode arrays (MEAs) are a useful tool for monitoring the functional activity of several individual, electrically excitable cells within a larger population. MEA platforms enable noninvasive, longitudinal monitoring of cellular networks over weeks and months, so activity patterns during development and functional effects of acute and chronic treatment paradigms could be determined [8–10]. Recently, the availability of multiwell MEA plates has allowed for increased throughput, offering the ability to perform complete concentration-effect curves on cell populations.

4. Classical electrophysiological technologies

Single-cell-based electrophysiology remains the gold standard assay for Nav channel research, since it controls membrane voltage and measures ionic current directly, therefore assessing channel activities at distinct states. Despite the high information content, the utility of manual patch clamp electrophysiology is significantly limited by high demand for labor and skills. Therefore, automated patch clamp (APC) platforms have emerged to meet this challenge.

4.1. APC development and advancement

Many automated patch clamp (APC) technology platforms have been developed in the past decade [11, 12]. These include lipid bilayer recording (e.g., Orbit, from Nanion Technologies), *Xenopus* oocytes' two-electrode recording (e.g., OpusXpress, from Molecular Devices), glass pipette electrode recording (e.g., FlyScreen 8500, from Flyion), continuous microfluidic-based recording (e.g., IonFlux, Mercury, from Fluxion), and most notably chip- or plate-based planar recording technologies (**Table 2**, IonWorks HT, Quattro, PatchXpress 7000A, IonWorks Quattro, and Barracuda from Molecular Devices; QPatch16, QPatch HT, Qube from Sophion; Port-a-Patch, Patchliner SyncroPatch 96, 384PE, 768PE from Nanion). Among the planar platforms, the third-generation IonWorks Barracuda, Qube, and SyncroPatch 384PE/768PE have gained the most attention due to their high throughput, i.e., recording 384 or more cells in parallel.

IonWorks Barracuda was launched in 2010 and was applied for compound screening on hERG, CaV2.2, and Nav channels [13–15]. Barracuda uses perforated-patch configuration; the seal resistance was ~120 MΩ for single-hole mode and ~35 MΩ for population patch mode (64 holes). In 2014, Qube (Biolin Scientific, Sweden) and SyncroPatch (Nanion Technologies, German) were introduced with promised giga-seal data quality. Both platforms use 384 channel digital amplifier and 384 pipetting robot, borosilicate glass-based single- or multi-hole chips, and programmable negative pressure to achieve whole-cell configuration. However, they also differ in many regards. For example, Qube adopts an in-chip microfluid design to enable solution exchange, while SyncroPatch uses a liquid handler (e.g., BiomeK) so the system can be integrated for automation. SyncroPatch also can integrate two 384 modules into one robot platform, so 768 well parallel recording is feasible.

Feature	Manual	Dynaflow	Port-a-Patch	IonFlux/Mercury HT	IonWorks Barracuda	QPatch HT/HTX	Patchliner	Qube	SyncroPatch 768PE
Company		Cellectricon	Nanion	Fluxion Bioscience	MDC/MDS	Sophion	Nanion	Sophion	Nanion
Throughput/run	1	1	1	32	384	48	16	384	768
Substrate material	Glass	Silicone rubber	Glass	Silicone rubber	Polyimide	Silicon oxide	Glass	Glass	Glass
Seal resistance, GΩ	>1	>1	>1	~0.1	~0.2	>1	>1	>1	>1
Access resistance, MΩ	<10	<10	<10	>10	15	<10	<10	<10	<10
Parallel recordings	1	96	1	64	384	48	16	384	384*2
Unique recording	1	16	1	32	384	48	8	384	768
Amplifier channels	1	96	1	32	384	48	8	384	384/768
Number of pipettes	1 or 2	1	N/A	N/A	384	8	8	384	384
Solution switch time	<10 ms (100%)	30 ms (100%)	100 ms	50 ms (100%)	50 ms	80 ms	≤20 ms	50 ms	50 ms
Compound washout	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Compound additions	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Unlimited compound additions	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Internal perfusion	Yes	No	Yes	No	No	No	Yes	Yes	Yes
Rs Cslow compensation	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
In/out side out configuration	Yes	No	No	No	No	No	No	No	No
Single-cell recordings	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Population recordings	No	No	No	Yes 20/well	Yes 64/well	Yes 10/well	No	Yes 10/well	Yes 8/4/well
Implement date	1976		2004	2009		2009			2014

Table 2. Automated patch system comparison.

4.2. APC assay challenges

Even though the APC platform is superior to other nonfunctional or indirect functional assay methods by offering precise control of voltage-gated ion channels' physiological condition and direct measurement of channel activity, this technology faces many challenges including throughput, success rate, stability, integrating high-throughput data analysis, and lowering the equipment and consumable costs. The recently launched third-generation APC systems are intended to meet these challenges as a "primary screener" to perform both robust high-throughput screening and high-quality recording to support drug discovery and ion channel research. In order to bring out the best APC performance for each ion channel assay, there are many parameters that can be considered for optimization which will be discussed in the following section.

4.2.1. Cell line development and cell preparation

Since APC patches cell randomly from the cell suspension solution added into the system, the cell quality is critical for every APC assay. If it's possible, researcher should always pursue the best cell membrane electrophysiological property which includes homogeneous and high-target ion channel expression for good recording signal, high cell capacitance for easier cell catching, and good membrane property to facilitate achieving whole-cell configuration and stable recording. There are many methods that can be used to improve target expression such as choosing high expression host cell and expression system and test different medium and culture conditions. It is reported that using a gentle cell dissociation method followed by a thorough cell debris-removing protocol and adjusting cell solution density will improve APC success rate [16]. Also keeping the cell in serum-free medium and at lower temperature may improve current [17]. In cell line development and preparation optimization, it is vital to validate the target channel response by characterizing its electrophysiological and pharmacological properties by using reference compounds.

4.2.2. APC cell catching and forming whole-cell protocol

Two important parameters have to be optimized for APC recording, which are cell catching and breaking in pressure protocols and intracellular and extracellular solution compositions. Many APC systems allow programming advanced pressure protocol including holding, ramping, and rupturing steps to achieve best whole-cell configuration success rate. And some target and cell may be more sensitive to intracellular cesium and extracellular calcium ion concentrations, which usually help to achieve giga-seal. Since each target and cell line is different, one optimized pressure and solution protocol may not work well for other targets and cells.

4.2.3. Drug applications

Different APC systems utilize either vail (in lower-throughput platform) or plate (in higher-throughput platform) format source to deliver compound mostly by using automated liquid handler. Compound application usually is executed after recording signal reaching stable. Based on the APC system compound adding and washing capability and recording stability,

researchers chose to apply multiple concentrations of a compound to one recording, which usually decrease recording success rate but increase throughput comparing to single-dose application per recording. Also in order to avoid compound sticking to its container or pipette, the siliconizing reagent for glass and other surfaces can be included in the compound buffer after being validated using reference compounds.

4.2.4. Recording stability

In order to validate the pharmaceutical effect from adding compound, it's important to include positive and negative controls to monitor recording stability. In the ideal condition, the negative control recording should remain stable throughout the whole experiment, which requires cell membrane seal resistance, cell capacitance, and series resistance to remain stable. To achieve this, each APC assay usually needs to be optimized with various conditions, including intracellular and extracellular solution compositions, voltage protocol, pressure protocol, and experimental procedure design. Many times, the current decrease independent of any compound application can be observed, which is called "rundown." For Nav channel, rundown is usually caused by the holding membrane voltage (Vm), in which the closer Vm to half inactivation, the stronger slow inactivation-inducing effect will occur. So in order to exam inactive state-dependent modulator, the Nav assay voltage protocol has to be well designed and validated.

4.2.5. High-throughput data analysis

High-throughput data analysis is another major challenge in developing APC assay, especially in performing high-throughput screening. It is essential to build a robust high-throughput data analysis method in the assay development stage. To achieve this, two issues need to be overcome. One is data reduction strategy, which means extracting key parameters from each current sweep, such as peak current amplitude for signal change analysis, and three recording quality control (QC) parameters including seal resistance, cell capacitance, and series resistance. Another issue is to develop a robust and reliable data QC strategy to exclude poor-quality recordings, which can be achieved by using self-developed script program or commercial available software by setting proper QC criteria for the three QC parameters' value and stabilities at the desired time points of the experiment.

5. Concluding remarks

The advancement of technologies has made significant impact on Nav channel research and drug discovery in the past decade. As true for all drug screenings, no universal screening strategy can fit for all needs. Currently, primary uHTS is usually performed by using a validated non-electrophysiological functional assay and followed up by APC assay for potential hits' confirmation and characterization. Overall, the advancement of high-throughput APC coupled with rational assay designs has offered unprecedented capacity for Nav channel drug discovery.

Acknowledgements

Conflict of interest

The authors are all employees of Genentech Inc. (a Roche Group Company) and declare no financial and conflict of interest in this article.

Author details

Tianbo Li* and Jun Chen

*Address all correspondence to: li.tianbo@gene.com

Department of Biochemical and Cellular Pharmacology, Genentech (Roche Group),
South San Francisco, California, USA

References

[1] Chernov-Rogan T, Li T, Lu G, Verschoof H, Khakh K, et al. Mechanism-specific assay design facilitates the discovery of Nav1.7-selective inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 2018;**115**:E792-E801. DOI: 10.1073/pnas.1713701115

[2] Pellecchia M, Bertini I, Cowburn D, Dalvit C, Giralt E, et al. Perspectives on NMR in drug discovery: A technique comes of age. Nature Reviews Drug Discovery. 2008;**7**:738-745. DOI: 10.1038/nrd2606

[3] Finley M, Cassaday J, Kreamer T, Li X, Solly K, et al. Kinetic analysis of membrane potential dye response to Nav1.7 channel activation identifies antagonists with pharmacological selectivity against Nav1.5. Journal of Biomolecular Screening. 2016;**21**:480-489. DOI: 10.1177/1087057116629669

[4] Liu CJ, Priest BT, Bugianesi RM, Dulski PM, Felix JP, et al. A high-capacity membrane potential FRET-based assay for Nav1.8 channels. Assay and Drug Development Technologies. 2006;**4**:37-48. DOI: 10.1089/adt.2006.4.37

[5] Fitch RW, Daly JW. Phosphorimaging detection and quantitation for isotopic ion flux assays. Analytical Biochemistry. 2005;**342**:260-270. DOI: 10.1016/j.ab.2005.04.041

[6]   Zhang H, Cohen AE. Optogenetic approaches to drug discovery in neuroscience and beyond. Trends in Biotechnology. 2017;**35**:625-639. DOI: 10.1016/j.tibtech.2017.04.002

[7]   Yuste R, MacLean J, Vogelstein J, Paninski L. Imaging action potentials with calcium indicators. Cold Spring Harbor Protocols. 2011;**2011**:985-989. DOI: 10.1101/pdb.prot5650

[8]   McConnell ER, McClain MA, Ross J, LeFew WR, Shafer TJ. Evaluation of multi-well microelectrode arrays for neurotoxicity screening using a chemical training set. Neurotoxicology. 2012;**33**:1048-1057. DOI: 10.1016/j.neuro.2012.05.001

[9]   Newberry K, Wang S, Hoque N, Kiss L, Ahlijanian MK, Herrington J, Graef JD.Development of a spontaneously active dorsal root ganglia assay using multiwell multielectrode arrays. Journal of Neurophysiology. 2016;**115**:3217-3228. DOI: 10.1152/jn.01122.2015

[10] Massobrio P, Tessadori J, Chiappalone M, Ghirardi M. *In Vitro* studies of neuronal networks and synaptic plasticity in invertebrates and in mammals using multielectrode arrays. Neural Plasticity. 2015;**2015**:1-18. DOI: 10.1155/2015/196195

[11] Picones A, Loza-Huerta A, Segura-Chama P, Lara-Figueroa CO. Contribution of Auto mated Technologies to Ion Channel Drug Discovery. Advances in Protein Chemistry and Structural Biology. 2016;**104**:357-378. DOI: 10.1016/bs.apcsb.2016.01.002. Epub 2016 Mar 3

[12] Comley J. Automated patch clamping finally achieves high throughput! Drug Discovery World. 2014;**4**:45-56

[13] Mannikko R, Bridgland-Taylor MH, Pye H, Swallow S, Abi-Gerges N, et al. Pharmacological and electrophysiological characterization of AZSMO-23, an activator of the hERG K(+) channel. British Journal of Pharmacology. 2015. DOI: 10.1111/bph.13115

[14] Cerne R, Wakulchik M, Li B, Burris KD, Priest BT. Optimization of a high-throughput assay for calcium channel modulators on IonWorks Barracuda. Assay and Drug Development Technologies. 2016;**14**:75-83. DOI: 10.1089/adt.2015.678

[15] Cerne R, Wakulchik M, Krambis MJ, Burris KD, Priest BT. IonWorks Barracuda assay for assessment of state-dependent sodium channel modulators. Assay and Drug Development Technologies. 2016;**14**:84-92. DOI: 10.1089/adt.2015.677

[16] Li T, Lu G, Chiang EY, Chernov-Rogan T, Grogan JL, Chen J. High-throughput electrophysiological assays for voltage gated ion channels using SyncroPatch 768PE. PLoS One. 2017;**12**:e0180154. DOI: 10.1371/journal.pone.0180154

[17] Danker T, Möller C. Early identification of hERG liability in drug discovery programs by automated patch clamp. Frontiers in Pharmacology. 2014;**5**:203. DOI: 10.3389/fphar.2014.00203