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

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

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

Using Human Pluripotent Stem Cell-Derived Neural Cultures to Assess Safety of New Drugs and Chemicals

Cassiano Carromeu

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.69434>

Abstract

The central nervous system (CNS) is a central pillar in safety pharmacology studies of new drugs. Characterization of serious adverse drug reactions to a new chemical entity involves extensive investigation using *in vitro* and *in vivo* models. However, primary culture of human neurons *in vitro* can be challenging, giving limited sample availability. Additionally, the inter-species differences between humans and current animal models impose a considerable obstacle to successfully predict the outcome of new drugs. New technologies also need to help address the 3Rs principles in animal research. Human pluripotent stem cells (hPSC) have the potential to change the current paradigm in pharmacological research. By using hPSCs and state-of-the-art differentiation protocols, researchers now have available an unlimited source of neural cells, able to mimic early and late stage of human CNS development. Moreover, hPSC-derived cells can be used at early stages of drug development, improving clinical predictability and reducing overall drug development costs. This chapter covers the advancements that resulted in hPSC-derived models intended to enable neurotoxicity assessment and drug screening. Finally, this chapter will also reveal the bottlenecks and the challenges to overcome of using hPSC as a predictive tool in research.

Keywords: human pluripotent stem cells (hPSC), induced pluripotent stem cells (iPSC), multielectrode array (MEA), Zika virus, neurotoxicity

1. Introduction

1.1. New therapeutic compounds and the nervous system

The human central nervous system (CNS) is a unique structure organized in an intricate network composed of different cell types [1, 2]. Its homeostasis is maintained by an orchestrated

signaling milieu composed of neurotransmitters, cell-cell interactions, and protein factors. Any compound acting upon one of the CNS components could potentially shift this delicate balance, resulting in untoward outcomes. Therefore, safety pharmacology profiling for compounds that crosses the blood-brain barrier represents a key step in the drug development process, particular prior to conducting studies in human subjects. Recently, the Biotechnology Innovation Organization (BIO) released the largest study of clinical drug development success rates to date [3]. In partnership with Amplion and Biomedtracker, BIO collected and analyzed a total of 9985 phase transitions in clinical trials between 2006 and 2015. Their data revealed a likelihood of approval being of only 9.6% for all developmental candidates. If segmented by diseases, candidates to neurology and psychiatry disorders fall under 9.6%, with 8.4 and 6.2% likelihood of approval, respectively. Moreover, adverse effects to the CNS account for a considerable proportion of all drug attrition cases. This demonstrates the poor predictability of current animal and *in vitro* models leveraged at the pre-clinical drug development stage.

Assessing the toxicological profile of new molecular entities requires extensive investigation using *in vitro* and *in vivo* models (**Figure 1**). This incremental accumulation of data helps to evaluate the toxicological profile and potential side effects of new compounds before moving to clinical trials. Studies to investigate the toxicity of drugs on the human central nervous system (CNS) relies mostly on animal (*in vivo*) and cellular (*in vitro*) models [4]. Although significant achievements have been accomplished using these models, there are many bottlenecks to overcome. For instance, efforts to fully recapitulate the human nervous system using animal models can be very challenging [5]. Rodents and human brains display major genetic, cellular, and anatomical differences [6]. Many compounds have failed in clinical trials even after being considered promising based on rigorous testing in animal models. Therapies to Alzheimer's disease (AD) for example have an attrition rate of 99.6% [7]. Many potential therapeutic compounds

Figure 1. *In vitro* and *in vivo* models available for toxicological screenings. As the complexity of the model increases, there is a substantial decrease in throughput. Common cellular models include immortalized cell lines, primary tissue culture and hPSC-derived cells. Organoids are 3D structures derived from hPSC differentiation toward neuroectoderm in suspension.

for AD displayed unacceptable toxicity in humans. Additionally, while animal models have unquestionable importance in toxicological studies, new technologies could uniquely help to address the 3R principles of refine, reduce, and replace their use in this research space [8].

There are two main *in vitro* cellular models available for toxicology studies: primary and established cell lines [9, 10]. Primary cell lines are isolated directly from tissues. Their main advantage is that they more closely remember the *in vivo* counterpart, displaying many features presented in the target tissue. However, primary cell lines need fresh tissue to establish the cell culture and have limited capability of expansion *in vitro*. This turns impractical Studies that require large numbers of cells, especially from difficult-to-obtain tissue such as the human central nervous system. Immortalized cell lines, on the other hand, can be kept in culture for extensive periods of time and expanded through passaging. The immortalized cells have the intrinsic ability to proliferate indefinitely in culture, usually acquired by multiple mutations or transformations in their genomes. Although the proliferative potentials for immortalized cells make them amenable to large-scale production, they may significantly differ from the tissue of origin. Given the limitations imposed by these types of cell culture, human pluripotent stem cells have gained credence as a new reliable source of human tissues, with many advantages over the traditional *in vitro* cellular models.

2. Human pluripotent stem cells (iPSC)

Human pluripotent stem cells (hPSC) have the ability to expand to large amounts and differentiate into any cellular tissue of the body [11]. Giving these extraordinary abilities, hPSC can potentially change the current paradigm in pharmacological research, offering unlimited access to a reliable source of neural tissue able to mimic early and late stages of human CNS development [2]. There are two types of hPSC: embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC). Although both types share the same core features that classified them as hPSC, such as the ability of differentiating to any adult tissue, there are major differences between them. The hESC are derived from inner cell mass of blastocyst stage embryos after 5 days from the fertilization of the oocyte [12]. As of January 2017, 378 hESC lines were eligible for NIH fund research [13]. Comparatively, this library is still small to fully explore the whole human genomic diversity landscape. Moreover, giving its origin, hESC carry many ethical issues [14]. In spite of that, hESC have been pivotal on advancing the human stem cells research, permitting unlimited access to any human tissue of interest for the first time.

In 2006, a scientific breakthrough introduced a technique able to generate pluripotent stem cells without the ethical controversies of embryonic stem cells [15]. The team used the technique of reprogramming to reverse an adult mouse cell (fibroblast) into a pluripotent stem stage: the induced pluripotent stem cells (iPSC). Soon after the same research group published the technique using human fibroblast to generate human-induced pluripotent stem cells (hiPSC) [16]. Once hiPSC have the same capacity as their hESC counterparts to generate human target cells *in vitro*, many scientists have shifted their focus into producing patient-specific iPSC to potentially validate disease phenotypes *in vitro* [17]. For the nervous system, many studies confirmed the great potential of hiPSC in recapitulating CNS diseases [18, 19].

Moreover, studies have revealed the potential of using hiPSC as a drug-screening platform to systematically evaluate spontaneous neurological disorders and drug-induced neurotoxicity [17]. The biggest challenge for this approach is to identify key phenotypes *in vitro* for reproducible outcomes. Neurodevelopmental disorders, for example, impose such a significant challenge. Recently, two studies that focused on different diseases (Rett Syndrome and MeCP2 Duplication Syndrome) successfully demonstrated the use of hiPSC-derived cells in identifying potential therapeutic candidates [20, 21]. Both neurodevelopmental disorders altered MeCP2 gene expression (loss of a functional copy in Rett Syndrome and overexpression in MeCP2 Duplication Syndrome). These studies identified core alterations in the synapses of neurons in both conditions. In the MeCP2 Duplication Syndrome study, researchers developed a simplified drug-screening platform able to quickly assess the synaptic phenotype. By using a library of epigenetic modifiers, they identified two compounds that able to reverse the synaptic phenotype *in vitro*. However, the study also displays an alarming finding: although both potential therapeutic compounds identified in the study induced rescue of the cellular synaptic phenotype *in vitro*, one of them demonstrated significant toxicity on the CNS function in selected electrophysiology assays. This study highlights the need for an extensive characterization of drug toxicity *in vitro* before further consideration in human studies.

2.1. The current state-of-the-art of using hPSC for CNS safety screening

The extraordinary ability of hPSC to differentiate to CNS components makes them an interesting platform to better understand the deleterious effects of compounds on neural tissue [11, 22, 23]. Paired with cellular, genetic, biochemical, and functional assays, hPSC-derived neural tissue can be used to generate a comprehensive toxicological profile of drugs on the CNS and help to address decisions of go/no-go during a drug development process. Moreover, the brain undergoes significant postnatal development and its structure and function differ significantly between infantile and adult stages. Many drugs can affect the CNS differently, depending on the maturity of the subject (i.e., embryonic, infantile, or adult). Taken together, researchers may leverage hPSC-derived neural cells in different stages of differentiation to explore the safety profile of drugs on mature and immature nervous systems.

Neural precursor cells (NPCs) are multipotent cells, with the potential to generate multiple mature CNS cells, such as neurons, astrocytes, and oligodendrocytes [2, 24]. They are able to self-renew and proliferate, being pivotal players in the developing human brain. Toxicity to these cells at young stages of development can predispose the CNS to the onset of neurodevelopmental disorders and neurological impairments [25]. NPCs can be expanded *in vitro*, which makes them amenable to incorporation into large-scale studies. Protocols to maintain and differentiate NPCs in their CNS derivatives are well established, with great consistency and reproducibility [2, 26]. Moreover, hiPSC from different individuals can be used to obtain a progenitor cell bank representative of genetic differences found on our population. Assessing toxicological profile in such a heterogeneous genomic population could improve predictability of safety profiles of drugs on different individuals.

Publications with human NPCs started to demonstrate their use in assessing toxicological profile of drugs [25, 27]. Using hESC-derived NPCs, a research group described a platform for detection of toxicity to neuronal induction in embryonic development [28]. Researchers

exposed differentiating cells to methylmercury (MeHg) and found that it could disrupt early stages of neural differentiation. In another approach, researchers described the use of hESC-derived NPCs in identifying compounds that were selectively toxic to progenitor, but innocuous to terminally differentiated cells (neurons and astrocytes) [29]. Although the work was primarily envisioned as a platform to identify compounds able to deplete proliferation cells from heterogeneous neural populations *in vitro*, with applications in purifying populations for regenerative medicine, similar approach could also be used to elucidate the safety profile of chemical compounds on the CNS.

Differentiated populations of neurons have also been used to assess the toxicological profile of compounds [11]. Phenotypic assays such as neurite outgrowth and neuronal morphology have been used to investigate the effect of chemical entities on these populations. In a recent study, a library of 80 compounds was screened for their ability to inhibit neurite outgrowth in iPSC-derived neurons using a high-content screening platform [30]. From the compounds tested, 16 selectively inhibited neurite outgrowth, confirming the usefulness of hiPSC-derived neurons in neurotoxicity screenings. Although this study represents a step forward in developing a relevant humanized safety screening platform, it still relies on dissociated neurons plated at low density *in vitro*, which does not represent well the developed brain. Additionally, more sophisticated platforms, able to capture functional phenotypes, such as electrical activity of the neural circuitry, and the interplay between different CNS cell types will greatly help to improve the predictability of safety screenings.

2.2. Neural culture *in vitro*: from traditional tissue culture to organized organoids

Given the easy accessibility to CNS cells that hPSC offer, we have witnessed in the recent years the rediscovery of three-dimensional (3D) cell culture technologies as a powerful tool to study the brain [31, 32]. Organoids are 3D agglomerates of tissue-specific cells self-organized in structures that more closely resemble the target organ. Once organoids exhibit key structural and functional properties of a target tissue, they hold great promise in advancing the studies of complex organs such as the brain, where the interaction of many different cells organized in a defined structure is pivotal for its functions (**Figure 2**). Moreover, giving the complex interplay between neuronal and non-neuronal cells in the CNS, the deleterious effect of drugs may rely on non-neuronal cells (and not directly on neurons) but still lead to a pronounced effect on the nervous system. In this scenario, brain organoids could better capture any deviation from the homeostatic balance of the interaction between different neural cells.

Two different types of cells can be used to obtain organoids: hPSC (using either ESC or iPSC) or multipotent adult stem cells [32]. Both approaches rely on the potential for expansion and self-organization of these precursors *in vitro*. Many recent studies have confirmed the used of brain organoids in the modeling of diseases by recapitulating *in vitro* the intricate and complex processes occurring during human brain development. Moreover, brain organoid constructs incorporating many different cell types (such as neurons, astrocytes, endothelial, and microglia) can be obtained and used in developmental neurotoxicity screenings [33]. By using the described model, a recent study assessed the neurotoxicity profile of a library of 60 compounds and correctly classified 9 of 10 chemicals. Although organoids containing different tissues would be a model more representative of the organ *in vivo*, the different tissue-specific

Advantages and Bottleneck of human PSC-derived models

Figure 2. *In vitro* models derived from hPSC. The main advantages and bottlenecks are listed. Brain organoids are 3D structures derived from hPSC differentiation toward neuroectoderm.

cells lack the structural organization found *in vivo* and may not fully recapitulate the organ function or multi-tissue interactions.

3. Using MEA technology to evaluate neuroactivity of new chemicals

Cell viability, gene expression and neurite outgrowth assays with neurons have been the standard methods *in vitro* to measure deleterious effects of compounds on the CNS. In spite of their importance, they have limited predictability, especially with drugs able to evoke a deleterious functional change but without noticeable biochemical or cellular changes. Electrophysiology techniques exploit ionic conductance of ion channels and transient modulation of the membrane potential of a neuron, being able to assess the functional status of the neural network *in vitro* [34]. There are many different techniques to record neuronal activity *in vitro*, with the most commonly used being the patch-clamp method. Although very sensitive, its low throughput and limitation of assessing only one or few neurons at a time precludes its use in investigating large neuronal circuitry dynamics [34]. Recently, new technologies of extracellular recordings have been developed. They have many advantages over traditional patch-clamp techniques, such as being noninvasive, capable of monitoring the culture for long periods of time, and

able to record multiple cells at once, allowing large-scale assessment of neuronal circuitry dynamics [35]. This allows their use to better understand neuronal communication, information encoding, propagation, processing, and computation of neuronal circuits *in vitro* [36].

One of the most promising technologies to record extracellular signaling is the microelectrode arrays (MEA). Uniquely, MEA platforms consist of hundreds to thousands of electrodes integrated in a cell culture dish and enable recordings of neural activity by sensing extracellular field potentials [37]. This technology has been used to investigate the neural network dynamics of hESC- and hiPSC-derived neuronal cultures, organotypic slice cultures and acute brain slices [34]. By combining multiple arrays, the MEA technology allows to investigate several conditions at the time in a high-throughput fashion. Moreover, because MEA is a noninvasive technology and the neurons are cultured directly onto the electrodes, this technology enables the repeated monitoring of intrinsic and inducible changes in neuronal network dynamics for several days which is extremely useful to investigate the relative effects of chronic drug exposure in a dish [35, 38].

Functional electrophysiology of neurons represents a powerful tool to investigate the safety pharmacology of drugs prior to first-in-human studies. It needs to be noted though that the human brain contains hundreds of different types of neurons, each with unique properties and pharmacological signal transduction pathways which may not be fully recapitulated *in vitro*. When mimicking the human brain *in vitro*, it is imperative to select the most appropriate cellular model to ensure unequivocally adequate and highly reproducible predictability [34]. Although mouse and rat primary neuronal cultures are the gold standard in MEA electrophysiology, interspecies differences in ion-channel expression profile and neuronal response can be significantly different; therefore, translation of data to the human brain is very challenging in many situations [39]. The potential of using human PSC as a source of neuronal circuitry mimicking the human brain just started to be explored [40]. While preliminary results are confirming the use of hiPSC-derived neural culture as a powerful tool to explore neurotoxicity of compounds on the human brain, more studies are warranted to address the variability and heterogeneity of such cell culture models.

4. Overcoming current bottlenecks of hiPSC-derived cultures

Giving the high degree of complexity of the CNS cellular components, full translation from *in vitro* studies of compound-induced neurotoxicity can be challenging [40]. In the recent years, the stem cell field has produced diverse protocols for obtaining hiPSC-derived neurons *in vitro*, making any attempt to standardize the field complicate once each laboratory uses its own protocol. Moreover, the field is also subjected to batch-to-batch variation and long period of time differentiation protocols, which introduce additional challenges in reproducibility and hampers its full adoption by screening companies. Recently, however, a number of hiPSC-derived neurons became commercially available. Homogenous populations of neurons with specific neurotransmitter profiles are an attractive alternative to study the human physiology. The reduced variability from batch-to-batch and controlled differentiation process make possible the reproducible use of these cells to investigate neurotoxicity on the CNS. One thing to be noted though

is the difference between hiPSC-derived products offered by different companies. Additionally, while most companies focus on highly pure populations of neurons, depleted of glia cells, this model may not be ideal to mimic the CNS complexity. The presence of astrocytes, for example, are important to modulate the response of neurons to neurotransmitters and can affect the vulnerability of neuronal cultures to toxic insults [41, 42]. Moreover, co-culture with astrocytes enhances synaptic maturation, with consequences on firing frequency and bursting behavior [43, 44]. Although still an emerging field with many questions to be answered, commercially available hiPSC-derived neurons and astrocytes will be pivotal in validating this model as a suitable solution to reduce (or even replace) animal experimentation in toxicology studies.

5. Conventional drug development process and Zika virus

In February of 2016 the World Health Organization (WHO) declared the Zika virus infection a Public Health Emergency of International Concern (PHEIC), which prompted scientist worldwide to an urgent and coordinated response to this new global threat. Zika virus was first identified in 1947, but only recently received public attention after being associated with microcephaly in newborns and Guillain-Barré syndrome in adults [45, 46]. Two recent studies screened libraries of FDA-approved drugs and identified potential therapeutics with novel activity against the Zika virus [47, 48]. Repurposing FDA-approved drugs can potentially accelerate the discovery of cures to diseases, reducing time, and costs. However, both studies lack extensive neurotoxicity characterization of the potential therapeutics. Although the library consisted of FDA-approved compounds, the active concentrations against the virus were relatively high and not necessarily safe to human use. Moreover, it is pivotal to assess the safety of these compounds in early stages of the CNS development before considering them to treat pregnant women or newborns. In a recent scientific communication, our group demonstrated that many of the compounds identified on the mentioned studies were in fact toxic at their effective concentrations against Zika virus [49]. We investigated the toxicological profile of 29 compounds described as potential therapeutic against Zika virus infection. By testing hiPSC-derived cells at different stages of the CNS development, we observed greater toxicity at early stages of the nervous system, with decreasing toxicity as the cells matured *in vitro*. Interestingly, Emricasan (a compound highlighted in a previous publication) demonstrated a safe toxicological profile in all stages of the CNS and did not interfere with the normal function of mature neural cultures, as assessed by calcium mobilization assays using a fluorescent imaging plate reader (FLIPR) platform and electrophysiology using MEA [48]. Nonetheless, this study emphasized the need for extensive early characterization of repurposed compounds before considering them to potentially alleviate new diseases.

6. Conclusion/remarks

There is an urgency to accelerate and streamline the process of the development of new drugs. From devastating neurodegenerative disorders, such as Alzheimer's, to global threats,

epidemics from known and unknown viruses, we need to be able to rapidly identify safe therapeutic compounds. The average time to translate a drug from the bench to the clinic is 10 years, with an approximate cost of \$2.6 billion dollars. One contributor for this is the fact that the current drug development process is very inefficient, with fewer than 10% of the drugs in development being approved for use [50]. Adverse drug reactions to CNS are responsible for a large amount of all drug attrition cases [51]. To change the current scenario, it is crucial to have available a toolbox able to quickly assess the toxicological profile in early steps of drug development. The incredible potential of hPSC to expand *in vitro* and differentiate toward any adult cell type makes them ideal tools to large-scale toxicology studies. Together with techniques able to assess functional phenotype in real time, such as MEA technology, terminally differentiated neurons derived from hPSC could help to improve clinical outcome predictability in early steps of the clinical trial, reducing overall costs and turnover of the drug development process.

In an attempt to streamline the discovery, development, and delivery of new cures, the House of Representatives of the United States of America recently passed the 21st Century Cures Act [52]. The bill will allocate funds to the National Institute of Health (NIH) and help to fast track the approval of new drugs by the Food and Drug Administration (FDA). The Cures Act will also provide funding for three innovative scientific initiatives: the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, the Precision Medicine Initiative (PMI) and the Beau Biden Cancer Moonshot Initiative [53]. Moreover, the Cures Act also simplifies the process of data sharing, allowing the quick use of data by the scientific community. The BRAIN initiative aims to elucidate how the neural network works in health brains and what is altered in neurological disorders. The building of knowledge on these brain states is pivotal to any drug development workflow. However, although initiatives as the BRAIN are required to advance our medical knowledge about the CNS, it is crucial to develop new platforms able to recapitulate these findings *in vitro*. To this end, platforms to assess adverse drug effects on the CNS using hiPSC are pointed as the most promising and currently being developed [17].

Human iPSC already started to revolutionize disease modeling *in vitro*, revealing disease mechanisms otherwise not seen using classical animal models. Moreover, once hiPSC can be derived from any individual, it enables their use in personalized medicine, including toxicological screening in individual-specific tissues to reveal the potential side effects of drugs before their use. One caveat though is the simplified representation of the nervous system tissue architecture that can be obtained *in vitro* using hiPSC-derived cell culture techniques. In an attempt to overcome this limitation, the field is seen as a re-emergence of 3D organoids. Recent studies with brain organoids have confirmed their potential in recapitulating steps of the human brain development and organization. The development of new 3D high-content screening technologies, such as Light Sheet Microscopy, and improved differentiation protocols will be critical to a broad adoption of this technology in drug development screenings. Moreover, they can be an attractive alternative in replacing animal use in certain applications as well in guiding conventional clinical trial studies for dose tolerance in humans. In principle, the use of brain organoids in screenings could help to provide a more fine-tuned and multipronged approach to understand the risks and benefits of new therapies [10].

In addition to hPSC, the repurposing of old drugs to new diseases have gained attention in the recent years and promise to revolutionize the drug discovery field [54]. Repurposing drugs could significantly decrease the time and costs to find new therapies. However, it is still crucial to re-evaluate their toxicological profile. When redirecting compounds to new diseases, their new efficacy dosage need to be extensively tested to assure safety on the clinic, once many redirected compounds present a higher effective concentration for 50% of the maximum response (IC_{50}) and may not be clinically relevant. The recent example of repurposing drugs to Zika virus found many hits with a higher IC_{50} than the safest dosage identified in a toxicological screening using hiPSC-derived neural progenitor cells, preventing their use in newborns and pregnant women [48, 49]. Drug combination therapy, using two or three compounds found in the repurposing screening, could potentially increase the success rate of such screening by synergistic effects of the combination [54]. Successfully synergistic combinations of drugs would enable the reduction of each drug dosage to nontoxic levels and allow to use a therapeutic concentration that is below or equal to their achievable human blood concentrations.

Finally, the Zika virus prompted the scientific community to react and collaborate in a fashion not seen before. Different fields joined forces sharing a common goal: discover new therapies and vaccines to an emerging global threat. It also highlighted the need to change the current drug development workflow. In face of such threats, new tools are needed allowing researchers to quickly identify new therapeutic compounds. Elements discussed on this chapter, such as hPSCs and their derivatives, combined with MEA electrophysiology will streamline this process and be the standard toxicological assays in the future.

Acknowledgements

This chapter was greatly improved with critical advisement from Ana Paula Diniz Mendes.

Author details

Cassiano Carromeu

Address all correspondence to: cassiano.carromeu@stemonix.com

StemoniX, San Diego, CA, USA

References

- [1] Fields RD, Woo DH, Basser PJ. Glial regulation of the neuronal connectome through local and long-distant communication. *Neuron*. 2015;**86**(2):374-386. DOI: 10.1016/j.neuron.2015.01.014

- [2] Carromeu C, Vessoni A, Diniz Mendes AP, Beltrão-Braga PCB. Differentiation of human pluripotent stem cells into cortical neurons. In: Ulrich H, Negraes PD, editors. *Working with Stem Cells*. 1st ed. Springer International, AG, Switzerland; 2016. pp. 163-180. DOI: 10.1007/978-3-319-30582-0
- [3] BIO. Clinical Development Success Rates 2006-2015 [Internet]. 2016. Available from: <https://www.bio.org/sites/default/files/Clinical%20Development%20Success%20Rates%202006-2015%20-%20BIO,%20Biomedtracker,%20Amplion%202016.pdf> [Accessed: January 29, 2017]
- [4] Dolmetsch R, Geschwind DH. The human brain in a dish: The promise of iPSC-derived neurons. *Cell*. 2011;**145**(6):831-834. DOI: 10.1016/j.cell.2011.05.034
- [5] Dragunow M. The adult human brain in preclinical drug development. *Nature Reviews Drug Discovery*. 2008;**7**(8):659-666. DOI: 10.1038/nrd2617
- [6] Clowry G, Molnár Z, Rakic P. Renewed focus on the developing human neocortex. *Journal of Anatomy*. 2010;**217**(4):276-288. DOI: 10.1111/j.1469-7580.2010.01281.x
- [7] Cummings JL, Morstorf T, Zhong K. Alzheimer's disease drug-development pipeline: Few candidates, frequent failures. *Alzheimer's Research & Therapy*. 2014;**6**(4):37. DOI: 10.1186/alzrt269
- [8] Thomas CE, Will Y. The impact of assay technology as applied to safety assessment in reducing compound attrition in drug discovery. *Expert Opinion on Drug Discovery*. 2012;**7**:109-122. DOI: 0.1517/17460441.2012.651122
- [9] Carney EW, Settivari R. Predictive toxicology: Biological assay platform. In: Faqi AS, editor. *A Comprehensive Guide to Toxicology in Preclinical Drug Development*. 1st ed. Academic Press, Cambridge, MA; 2012. pp. 777-799
- [10] Ko HC, Gelb BD. Concise review: Drug discovery in the age of the induced pluripotent stem cell. *Stem Cells Translational Medicine*. 2014;**3**(4):500-509. DOI: 10.5966/sctm.2013-0162
- [11] Kolaja K. Stem cells and stem cell-derived tissues and their use in safety assessment. *Journal of Biological Chemistry*. 2014;**289**(8):4555-4561. DOI: 10.1074/jbc.R113.481028
- [12] Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998;**282**(5391):1145-1147. DOI: 10.1126/science.282.5391.1145
- [13] NIH Human Embryonic Stem Cell Registry [Internet]. 2017. Available from: https://grants.nih.gov/stem_cells/registry/current.htm [Accessed: January 31, 2017]
- [14] de Wert G, Mummery C. Human embryonic stem cells: Research, ethics and policy. *Human Reproduction*. 2003;**18**(4):672-682. DOI: 10.1093/humrep/deg143
- [15] Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;**126**(4):663-676. DOI: 10.1016/j.cell.2006.07.024

- [16] Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;**131**(5):861-872. DOI: 10.1016/j.cell.2007.11.019
- [17] Shi Y, Inoue H, Wu JC, Yamanaka S. Induced pluripotent stem cell technology: A decade of progress. *Nature Reviews Drug Discovery*. 2016 [Epub ahead of print]. DOI: 10.1038/nrd.2016.245
- [18] Avior Y, Sagi I, Benvenisty N. Pluripotent stem cells in disease modelling and drug discovery. *Nature Reviews Molecular Cell Biology*. 2016;**17**(3):170-182. DOI: 10.1038/nrm.2015.27
- [19] Russo FB, Cugola FR, Fernandes IR, Pignatari GC, Beltrão-Braga PC. Induced pluripotent stem cells for modeling neurological disorders. *World Journal of Transplantation*. 2015;**5**(4):209-221. DOI: 10.5500/wjt.v5.i4.209
- [20] Marchetto MC, Carromeu C, Acab A, Yu D, Yeo GW, Mu Y, Chen G, Gage FH, Muotri AR. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell*. 2010;**143**(4):527-539. DOI: 10.1016/j.cell.2010.10.016
- [21] Nageshappa S, Carromeu C, Trujillo CA, Mesci P, Espuny-Camacho I, Pasciuto E, Vanderhaeghen P, Verfaillie CM, Raitano S, Kumar A, Carvalho CM, Bagni C, Ramocki MB, Araujo BH, Torres LB, Lupski JR, Van Esch H, Muotri AR. Altered neuronal network and rescue in a human MECP2 duplication model. *Molecular Psychiatry*. 2016;**21**(2):178-188. DOI: 10.1038/mp.2015.128
- [22] Ni M, Li X, Rocha JB, Farina M, Aschner M. Glia and methylmercury neurotoxicity. *Journal of Toxicology and Environmental Health, Part A*. 2012;**75**(16-17):1091-1101. DOI: 10.1080/15287394.2012.697840
- [23] Farina M, Aschner M, Rocha JB. Oxidative stress in MeHg-induced neurotoxicity. *Toxicology and Applied Pharmacology*. 2011;**256**(3):405-417. DOI: 10.1016/j.taap.2011.05.001
- [24] Seaberg RM, van der Kooy D. Stem and progenitor cells: The premature desertion of rigorous definitions. *Trends in Neurosciences*. 2003;**26**(3):125-131. DOI: 10.1016/S0166-2236(03)00031-6
- [25] Tofighi R, Moors M, Bose R, Ibrahim WN, Ceccatelli S. Neural stem cells for developmental neurotoxicity studies. *Methods in Molecular Biology*. 2011;**758**:67-80. DOI: 10.1007/978-1-61779-170-3_5
- [26] Muratore CR, Srikanth P, Callahan DG, Young-Pearse TL. Comparison and optimization of hiPSC forebrain cortical differentiation protocols. *PLoS One*. 2014;**9**(8):e105807. DOI: 10.1371/journal.pone.0105807
- [27] Yap MS, Nathan KR, Yeo Y, Lim LW, Poh CL, Richards M, Lim WL, Othman I, Heng BC. Neural differentiation of human pluripotent stem cells for nontherapeutic applications: Toxicology, pharmacology, and In vitro disease modeling. *Stem Cells International*. 2015;**2015**:105172. DOI: 10.1155/2015/105172

- [28] Stummann TC, Hareng L, Bremer S. Hazard assessment of methylmercury toxicity to neuronal induction in embryogenesis using human embryonic stem cells. *Toxicology*. 2009;**257**(3):117-126. DOI: 10.1016/j.tox.2008.12.018
- [29] Han Y, Miller A, Mangada J, Liu Y, Swistowski A, Zhan M, Rao MS, Zeng X. Identification by automated screening of a small molecule that selectively eliminates neural stem cells derived from hESCs but not dopamine neurons. *PLoS One*. 2009;**4**(9):e7155. DOI: 10.1371/journal.pone.0007155
- [30] Ryan KR, Sirenko O, Parham F, Hsieh JH, Cromwell EF, Tice RR, Behl M. Neurite outgrowth in human induced pluripotent stem cell-derived neurons as a high-throughput screen for developmental neurotoxicity or neurotoxicity. *Neurotoxicology*. 2016;**53**:271-281. DOI: 10.1016/j.neuro.2016.02.003
- [31] Bredenoord AL, Clevers H, Knoblich JA. Human tissues in a dish: The research and ethical implications of organoid technology. *Science*. 2017;**355**(6322):pii: eaaf9414. DOI: 10.1126/science.aaf9414
- [32] Lancaster MA, Knoblich JA. Organogenesis in a dish: Modeling development and disease using organoid technologies. *Science*. 2014;**345**(6194):1247125. DOI: 10.1126/science.1247125
- [33] Schwartz MP, Hou Z, Propson NE, Zhang J, Engstrom CJ, Santos Costa V, Jiang P, Nguyen BK, Bolin JM, Daly W, Wang Y, Stewart R, Page CD, Murphy WL, Thomson JA. Human pluripotent stem cell-derived neural constructs for predicting neural toxicity. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;**112**(40):12516-12521. DOI: 10.1073/pnas.1516645112
- [34] Accardi MV, Pugsley MK, Forster R, Troncy E, Huang H, Authier S. The emerging role of in vitro electrophysiological methods in CNS safety pharmacology. *Journal of Pharmacological and Toxicological Methods*. 2016;**81**:47-59. DOI: 10.1016/j.vascn.2016.03.008
- [35] Spira ME, Hai A. Multi-electrode array technologies for neuroscience and cardiology. *Nature Nanotechnology*. 2013;**8**(2):83-94. DOI: 10.1038/nnano.2012.265
- [36] Obien ME, Deligkaris K, Bullmann T, Bakkum DJ, Frey U. Revealing neuronal function through microelectrode array recordings. *Frontiers in Neuroscience*. 2015;**8**:423. DOI: 10.3389/fnins.2014.00423
- [37] Illes S, Fleischer W, Siebler M, Hartung HP, Dihné M. Development and pharmacological modulation of embryonic stem cell-derived neuronal network activity. *Experimental Neurology*. 2007;**207**(1):171-176. DOI: 10.1016/j.expneurol.2007.05.020
- [38] Johnstone AF, Gross GW, Weiss DG, Schroeder OH, Gramowski A, Shafer TJ. Micro-electrode arrays: A physiologically based neurotoxicity testing platform for the 21st century. *Neurotoxicology*. 2010;**31**(4):331-350. DOI: 10.1016/j.neuro.2010.04.001
- [39] Atack JR, Wafford KA, Street LJ, Dawson GR, Tye S, Van Laere K, Bormans G, Sanabria-Bohórquez SM, De Lepeleire I, de Hoon JN, Van Hecken A, Burns HD, McKernan

- RM, Murphy MG, Hargreaves RJ. MRK-409 (MK-0343), a GABAA receptor subtype-selective partial agonist, is a non-sedating anxiolytic in preclinical species but causes sedation in humans. *Journal of Psychopharmacology*. 2011;**25**(3):314-328. DOI: 10.1177/0269881109354927
- [40] Tukker AM, de Groot MW, Wijnolts FM, Kasteel EE, Hondebrink L, Westerink RH. Is the time right for in vitro neurotoxicity testing using human iPSC-derived neurons? *ALTEX*. 2016;**33**(3):261-271. DOI: 10.14573/altex.1510091
- [41] Beaman-Hall CM, Leahy JC, Benmansour S, Vallano ML. Glia modulate NMDA-mediated signaling in primary cultures of cerebellar granule cells. *Journal of Neurochemistry*. 1998;**71**(5):1993-2005. DOI: 10.1046/j.1471-4159.1998.71051993.x
- [42] Dugan LL, Bruno VM, Amagasu SM, Giffard RG. Glia modulate the response of murine cortical neurons to excitotoxicity: Glia exacerbate AMPA neurotoxicity. *The Journal of Neuroscience*. 1995;**15**(6):4545-4555
- [43] Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. *Nature Reviews Neuroscience*. 2013;**14**(5):311-321. DOI: 10.1038/nrn3484
- [44] Odawara A, Saitoh Y, Alhebshi AH, Gotoh M, Suzuki I. Long-term electrophysiological activity and pharmacological response of a human induced pluripotent stem cell-derived neuron and astrocyte co-culture. *Biochemical and Biophysical Research Communications*. 2014;**443**(4):1176-1181. DOI: 10.1016/j.bbrc.2013.12.142
- [45] Dick GW, Kitchen SF, Haddow AJ. Zika virus. I. Isolations and serological specificity. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 1952;**46**(5):509-520. DOI: 10.1016/0035-9203(52)90042-4
- [46] Pierson TC, Graham BS. Zika virus: Immunity and vaccine development. *Cell*. 2016;**167**(3):625-631. DOI: 10.1016/j.cell.2016.09.020
- [47] Barrows NJ, Campos RK, Powell ST, Prasanth KR, Schott-Lerner G, Soto-Acosta R, Galarza-Muñoz G, McGrath EL, Urrabaz-Garza R, Gao J, Wu P, Menon R, Saade G, Fernandez-Salas I, Rossi SL, Vasilakis N, Routh A, Bradrick SS, Garcia-Blanco MA. A screen of FDA-approved drugs for inhibitors of Zika virus infection. *Cell Host & Microbe*. 2016;**20**(2):259-270. DOI: 10.1016/j.chom.2016.07.004
- [48] Xu M, Lee EM, Wen Z, Cheng Y, Huang WK, Qian X, Tcw J, Kouznetsova J, Ogden SC, Hammack C, Jacob F, Nguyen HN, Itkin M, Hanna C, Shinn P, Allen C, Michael SG, Simeonov A, Huang W, Christian KM, Goate A, Brennand KJ, Huang R, Xia M, Ming GL, Zheng W, Song H, Tang H. Identification of small-molecule inhibitors of Zika virus infection and induced neural cell death via a drug repurposing screen. *Nature Medicine*. 2016;**22**(10):1101-1107. DOI: 10.1038/nm.4184
- [49] Society for Laboratory Automation and Screening (SLAS) Conference 2017 [Internet]. 2017. Available from: <https://www.eventscribe.com/2017/SLAS2017/ajaxcalls/postersinfo.asp?title=84821> [Accessed: February 11, 2017]

- [50] The Scientist [Internet]. 2017. Available from: <http://www.the-scientist.com/?articles.view/articleNo/48280/title/Opinion-Improving-FDA-Evaluations-Without-Jeopardizing-Safety-and-Efficacy/> [Accessed: February 3, 2017]
- [51] Arrowsmith J, Miller P. Trial watch: Phase II and phase III attrition rates 2011-2012. *Nature Reviews Drug Discovery*. 2013;**12**(8):569. DOI: 10.1038/nrd4090
- [52] The Scientist [Internet]. 2016. Available from: <http://www.the-scientist.com/?articles.view/articleNo/47639/title/Biomedical-Research-Bill-Approved-in-House/> [Accessed: February 1, 2017]
- [53] Hudson KL, Collins FS. The 21st century cures Act – A view from the NIH. *The New England Journal of Medicine*. 2017;**376**(2):111-113. DOI: 10.1056/NEJMp1615745
- [54] Sun W, Sanderson PE, Zheng W. Drug combination therapy increases successful drug repositioning. *Drug Discovery Today*. 2016;**21**(7):1189-1195. DOI: 10.1016/j.drudis.2016.05.015

IntechOpen