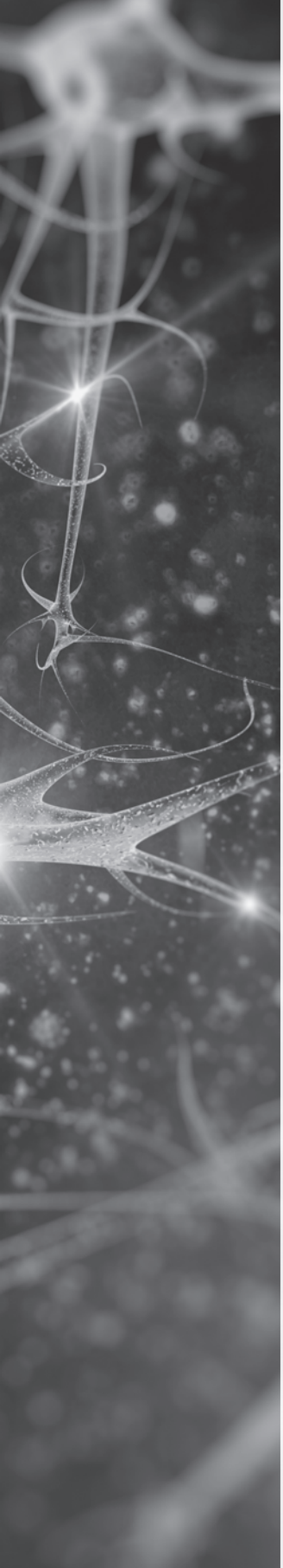
Neuronal identity and maturation



Valorization

Neurological and psychiatric disorders account for a substantial proportion of the world's total disease burden (i.e., 9% of global disability-adjusted life years (DALYs) in 2010) [1]. With the growth and changing age composition of the population, the societal and healthcare burden of mental health conditions is expected to become an even more significant problem in forthcoming years. For example, the World Health Organisation has estimated that depression will be the leading cause of lost DALYs by 2030 [2], and has calculated a 12% increase in DALYs from neurological disorders by that year from 2005 [3].

Mental disorders are often chronic and impact various (if not almost all) aspects of everyday living for patients and their families. Two major challenges that contribute to this personal burden can be identified as (i) a lack of compliance to treatments, and (ii) a huge knowledge gap between psychoeducation about the biological aspects of mental disorders as offered to patients in clinical setting, and current scientific knowledge as it is developing in the neurosciences. Overcoming this second challenge, for example by improving the information about neurobiological factors en mechanisms, may enable patients to formulate a better narrative and conceptual understanding regarding the development of their vulnerability. Knowledge development, as currently performed by studying living neurons from healthy subjects and patients "in a laboratory dish" (as presented in this thesis), may form—if interpreted with caution—a body of information on neurobiological factors and mechanisms involved in the onset and course of mental disorders, which may benefit patients and family members in better narrative and conceptual understanding on the development of their vulnerability, and may furthermore improve compliance to treatment.

Apart from the individual and societal burden that mental disorders represent for the patients and those who care for them, these brain-related disorders have a significant economic impact. The global cost of mental illnesses was estimated at US$ 2.5 trillion in 2010, and is predicted to escalate to US$ 6.0 trillion by 2030 [4]. It is important to point out that the true economic burden of mental health conditions may be considerably underestimated in these calculations due to the known comorbidity of these disorders with other chronic medical conditions, including cardiovascular disease [5, 6], diabetes [7], obesity [8, 9] and cancer [10], as well as with accidental death [11]. Moreover, the fact that only about one-third of total costs come from direct medical care, with the rest of expenses being incurred from lost employment or diminished work productivity due to the mortality and morbidity associated with these disorders [4], indicates that treatment options available to date are largely ineffective. Therefore, with a global surge in aging populations, the need for novel health discoveries through state-of-the-art scientific research has greatly exacerbated.

Although translational neurosciences research is relatively underfunded [12, 13], it has tremendous potential to dramatically impact the way we understand, prevent and

treat neurological and psychiatric disease. Preclinical trials with experimental animal models are essential and will continue to provide important insights into the biological mechanisms of mental health conditions; yet, pharmacological interventions found to be effective in *in vivo* animal studies almost invariably fail to translate to the human situation due to factors such as species differences, human brain complexity and patient/disease heterogeneity [14]. Most mental illnesses are complex disorders whose phenotypes can seldom be fully recapitulated by single-gene manipulation. The premise is therefore that preclinical trials with living human brain material are the most accurate and desired approach to study the precise physiological dysfunction of a neurological or psychiatric disorder, and avoid translational pitfalls. In this thesis, we describe a series of experiments that have resulted in the establishment of a biologically more relevant and accurate *in vitro* model of human neuronal cells derived from stem cells. The major implications of our findings and opportunities for valorization are detailed in this valorization addendum.

IMPROVING TRANSLATIONAL SUCCESS IN NEUROLOGY AND PSYCHIATRY: TOWARDS MORE EFFECTIVE PHARMACOLOGICAL TREATMENTS

Through recent advances in human cell reprogramming [15-18], it is now possible to generate virtually unlimited quantities of live human neuronal cells from readily accessible tissue sources, including skin cells. The ability to generate neuronal cell lines from patients afflicted with neurological and psychiatric disorders as well as from matched healthy control subjects has opened up new avenues for the modeling of both cellular and molecular pathophysiological features of brain disorders "in a laboratory dish" [19]. Research approaches using human patient-derived neuronal cultures represent a favorable intermediate between animal experiments and human clinical trials, and have vast potential to transform research and medicine by enabling drug discovery to be directly pursued on live human neuronal tissue [20-22].

Although *in vitro* human neuronal disease models derived using cell reprogramming technologies offer great promises to revolutionize medicine by facilitating the discovery of novel pharmacological interventions, it is imperative that such models are as realistic as possible to the human situation so as to optimize translational success to the clinic. Our results demonstrate that commonly used media for the differentiation and culturing of human neurons generally interfere with proper neuronal physiological function, and provide conditions that are much different from the living brain. More realistic neuronal models will be more likely to recapitulate the dysfunctional biology of neurological and psychiatric disorders, and improve the chances of discovery and translational success of novel pharmacological treatments. As detailed in this thesis (CHAPTER 3B), our design of a new neuronal medium that better resembles *in vivo* brain conditions, now

commercially available through STEMCELL Technologies Inc. as BrainPhys™, brings researchers around the world one step closer to this goal. The improvements made in this new medium, along with ongoing further developments in cell culture conditions and techniques, are expected to advance our understanding of mental health disorders and promote a more robust and accurate platform for preclinical drug development and testing.

Despite continuous advances in cell culturing methods, neuronal cultures remain inherently variable and are often characterized by a considerably heterogeneous proportion of functionally mature neurons [23, 24]. In comparing patient- and healthy control-derived neurons, we must strive to control for this source of variability by e.g. limiting cellular and molecular analyses to only mature functional neuronal cells. A major outcome of the research presented in this dissertation is the identification of a set of new live biomarkers associated with highly functional human neurons. As we demonstrate in CHAPTER 4A, these markers can be used to purify neurons of highly functional neuronal states from heterogeneously differentiating neuronal cultures. It may be expected that these and yet-to-be-discovered biomarkers of different neuronal types and states will be increasingly applied in future disease modeling experiments of brain disorders to reduce immanent phenotypic variability and streamline more accurate and robust investigations of drug development in high-throughput fashion. Without doubt, minimizing variation among neuronal populations and using more physiological neuronal models will importantly contribute to the successful translation of new discoveries into effective treatments.

PHARMACOGENOMICS APPROACHES TO PREDICTING DRUG RESPONSIVENESS: TOWARDS PERSONALIZED MEDICINE

Experiments like those described in this thesis may also offer future opportunities for personalizing drug therapy in patients. Although in this thesis we restricted our electrophysiological and molecular analyses to neurons derived from healthy donors, the research model we established based on cell reprogramming methodology can be applied to studying the molecular and neuronal dysfunction of any neurological or psychiatric disease *in vitro*. The electrophysiological and molecular properties of single patient-derived neurons can be correlated with patients' clinical and genomic profiles, an approach that may advance the discovery of disease mechanisms and potential new therapeutic targets. Additionally, the cellular effects of drugs prescribed to the patients can be studied in the patient-derived neuronal lines and correlated with observed behavioural symptoms, thus enabling a prediction of the clinical responsiveness of the patients to specific drugs to be made based on outcomes of *in vitro* cellular assays. Ultimately, such an approach that correlates cellular drug effects with clinical patient features will enable clinicians to

take a bold step toward personalized medicine by providing novel rationales for effective pharmacological treatment.

CONCLUSION

The work detailed in this thesis provides a promising framework for facilitating the discovery of novel biomarkers and treatments for various neurological and psychiatric disorders. Specifically, it is anticipated that creating more physiological *in vitro* culturing conditions and establishing more homogeneous neuronal cultures in disease modeling experiments will result in new mechanistic insights into the pathology of brain disorders and the identification of novel drug actions. The approach we outline in this thesis can be applied to model "in a laboratory dish" any brain disorder of public health importance in a more realistic and less variable manner, and improve rates of translational success. In a step towards personalized medicine, such improved *in vitro* disease models may be used in the future to study in detail the mechanisms of action of pharmaceuticals, and predict the clinical responsiveness of patients to prescribed drugs. Taken together, the framework provided by our studies offers manifold opportunities for medically relevant investigations aimed toward decreasing the personal, societal and economic burden of various neurological and psychiatric disorders. In this respect, productive collaboration of research scientists with clinical groups, biotechnology companies and industry is key to accelerating discovery and generating outcomes that benefit society.

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