CHAPTER 17

# Deciphering the Systems Architecture of the Brain Using Molecular Can Openers

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## INTRODUCTION

With humankind's ability to land on the Moon, send rovers to Mars and study the vast expanses of space through propelled probes and far-reaching telescopes, the brain is viewed as the final frontier. Though investigating space was inevitable, given humankind's drive to explore, it was the ability to model and study the parameters of space flight that fundamentally enabled space travel. The accumulated knowledge of this industry now serves as the foundation for new space companies and the basis for an innovation S-curve in the domain of space travel. A similar epistemological revolution is required in neuroscience. Learning about the human 'three-pound gem' has been delayed despite progressive strides in other industries and even other facets of human biology. This discrepancy is due to the century's long difficulty in ethically attaining human central nervous system (CNS) tissue for scientific investigation. Thus, animal models have long been the only accessible biological source for scientists to study the brain. Though animal models have been valuable in drug development, there are obvious restrictions to the amount of clinical insight that animal models can yield, as the average rate of successful translation from animal models to clinical cancer trials is less than 8% (Mak et al., 2014). The human brain is more structurally complex than that of any model organism, and many aspects of human neuropathology are either absent or unobservable in lower order animals and organisms (Hofman, 1985, 1988). Therefore, with limited attainable knowledge from restricted models, the gap between brain studies and studies of other organs was always going to remain until it became possible to explore the human brain in an ethical and scientific manner. As a consequence, there has also been a delay in the

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development of marketable treatments for neurological disorders even though other fields such as immunology and oncology have seen a rise in effective immunotherapies (e.g., chimeric antigen receptor T cells) and advanced radiation therapies (e.g., proton beam therapy), respectively (Emami-Shahri et al., 2018; Evans et al., 2017). Despite this historical disparity that has produced contemporary gaps in knowledge and treatment options between clinical neurology and other medical specialties, relatively recent advances in the ability to model disease and different interventions are accelerating the fermentation of potential therapeutic products to treat neurological and psychiatric disorders.

The development of human induced pluripotent stem cells (hiPSCs) has expanded the reach of neuroscience (Takahashi et al., 2007). Since Yamanaka and colleagues discovered the first method for successfully converting human somatic cells into hiPSCs, countless discoveries have been made in neurology that were elusive due to technical difficulties (Tabansky et al., 2016). Researchers can now elucidate the complex biology of inaccessible tissues via the reprogramming of accessible biological sources such as skin and hair. As a result, almost an infinite number of neurological diseases, and a myriad of normal brain characteristics, can be modelled in vitro. Neurological diseases from Alzheimer's to Parkinson's, and many others, have since been studied with hiPSCs, which have even been chief players in the discovery of new and improved therapeutics by enabling drug screening and even by providing insight into disease pathophysiology to develop better therapies to attempt in the first place (Fig. 17.1).

Also critical to expanding the ability to understand normal and abnormal brain biology, as well as develop better treatment options, has been the capability to use functional agents in accurate in vitro and in vivo models. A functional agent is defined as the pharmaceutical compound or the active ingredient that exerts some biological influence whether or not the mechanism of action is fully understood, which is also an active ingredient that can aid in treating a condition, uncovering pathophysiology or studying normal physiology. The current, and longstanding, gold standard and most effective treatment for bipolar disorder (BPD) has been lithium salts. Therefore, lithium is a functional agent because despite having an incompletely understood mechanism of action, it is still used to treat and study BPD. When prescribed to BPD patients, lithium provides mood stabilisation for approximately 30%–40% of BPD individuals (Gershon et al., 2009). Lithium has also been deployed to study the brain and even reveal the pathophysiology and molecular mechanisms that may be central to BPD, which then facilitates the development of less toxic, more specific and more effective therapies. Therefore, a synergistic relationship between hiPSC-based faithful modelling and functional agent driven probing of normal or aberrant physiology enables these functional agents to serve as molecular can openers that pry open the black box of the brain to elucidate molecular and structural facets and empowers functional agents to support drug development.

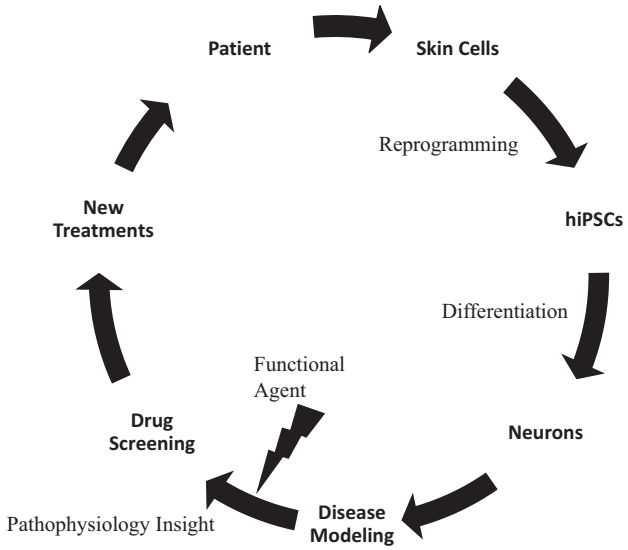


**Figure 17.1** Typical workflow for stem cell–based study of a genetic neurological condition (Wen et al., 2016). Human somatic skin cells (fibroblasts) are reprogrammed into human induced pluripotent stem cells (hiPSCs) with Yamanaka Transcription Factors and then differentiated into neurons to enable faithful disease modelling, relevant drug screening and ultimately the development of new treatments in a tractable in vitro system (Sirenko et al., 2014). Functional agents are used on hiPSC-derived differentiated cells to assess effects in a relevant model.

## USING LITHIUM TO GAIN INSIGHT INTO BIPOLAR DISORDER: A CASE STUDY

Recent work by Tobe and others demonstrates the synergistic power of hiPSCs, animal models and the functional agent lithium to collaboratively decipher the enigmatic neuropsychiatric condition of BPD (Tobe et al., 2017). By employing an hiPSC method to produce in vitro neuron cultures, using tractable in vivo animal models, and administering lithium, Tobe and colleagues were the first to reveal the molecular mechanism of BPD. BPD is a common psychiatric condition as 2.6% of adults in the Western world endure BPD. The putative behaviour associated with BPD is switching between depressive and manic moods or episodes (American Psychiatric Association, 2013). Other BPD symptoms include euphoria, grandiosity, delusions, impulsivity, hallucinations, social intrusiveness, anxiety, racing thoughts, inattentiveness, disorganisation, irritability, hostility and even suicide. Further proving a need for effective treatments, due to increased suicide rates, BPD is the most lethal psychiatric disorder such that a BPD diagnosis lowers life expectancy by 9 years. Additionally, the World Health Organization estimates that BPD is the world's seventh leading cause of disability and productivity loss (WHO, 1995). According to the American Psychiatric Association's Diagnostic and Statistics Manual, of the four varieties of BPD, which range in symptom presentation, BPD 1 is

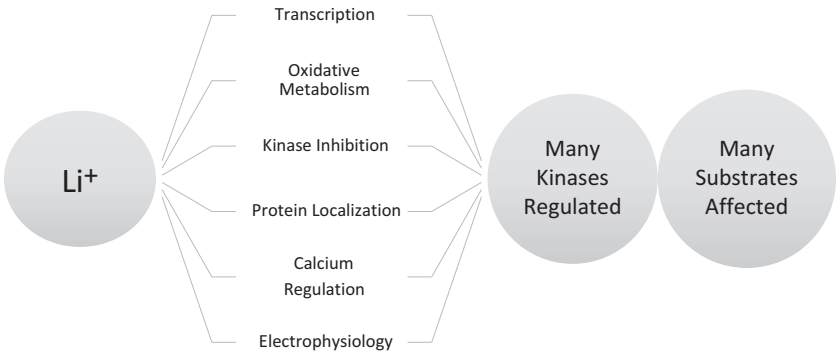


**Figure 17.2** Schematic of the many biological processes and downstream substrates that lithium influences (Brown and Tracy, 2013; Klein and Melton, 1996).

considered the most severe form of the disease, as affected individuals experience multiple manic episodes in their lifetimes (American Psychiatric Association, 2013). A very recently published review of available pharmacotherapies for BPD highlights current challenges in managing the depressive and stabilisation phases of BPD, and states that there is a dire need for novel treatments for BPD that are more specific and produce fewer side effects (Morsel et al., 2018). By studying the published breakthrough insights into BPD provided by Tobe et al., we demonstrate in this section that functional agents such as lithium can reveal the architecture of the brain and guide the development of better therapeutic options, which are so desperately needed by the many people in our society enduring BPD (Fig. 17.2).

## R&D: Studying Bipolar Disorder and Facilitating Drug Development Pipeline

A 'disease in a dish' approach using patient-derived induced pluripotent stem cells (iPSCs) and subsequently differentiated neurons in vitro is an appropriate way to study BPD due to its high heritability rate. BPD has a 70%–80% inheritance rate when evaluated in monozygotic discordant twin studies, which is unusually elevated for a polygenic disorder such as BPD (Althoff et al., 2005; Smoller and Finn, 2003). These findings prove the heritability of BPD and justify the in vitro modelling of BPD, as genetic fidelity and disease recapitulation is maintained. In the Tobe study, easily accessible skin fibroblasts or leukocytes were extracted from unaffected and BPD individuals and were then reprogrammed with episomes containing the Yamanaka factors (Oct4, Sox2, Klf4, c-Myc) to convert the somatic cells into hiPSCs (Tobe et al., 2017). The hiPSCs were then differentiated into mature cortical interneuron cultures. To validate the accuracy of the neuronal cell cultures, the generated neurons were found to be MAP2, CUX1 and vGLUT positive when immunocytochemistry was performed to confirm their neuronal fate (Tobe et al., 2017). With consistent interneuron cultures, the established

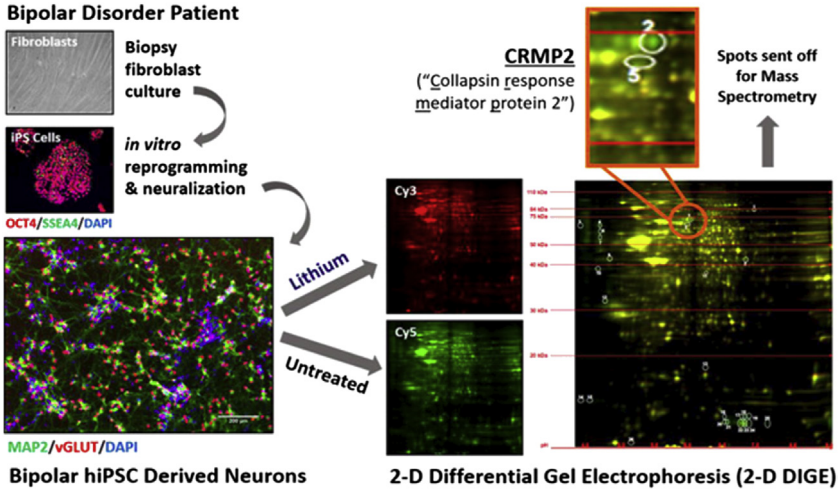


**Figure 17.3** Research workflow from patient cells to novel proteomic insight (Tobe et al., 2017). *(Courtesy of Brian Tobe.)*

pharmacotherapy for BPD, lithium, was administered to treatment groups and withheld from control groups. A two-dimensional differential gel electrophoresis was then performed to compare protein abundance and posttranslational modification status (i.e., phosphorylation) between lithium-treated BPD patient neuronal cell cultures (Fig. 17.3). All areas on the gel that differed were then extracted and identified via mass spectrometry. A subsequent unbiased and inclusive bioinformatics analysis was performed, which implicated collapsin response mediator protein-2 (CRMP2), as the keystone of the lithium response pathway in BPD neurons, showing for the first time what lithium actually does in BPD patients and newly identifying a principal protein altered by lithium (Tobe et al., 2017).

It was consequently discovered that lithium exerts its therapeutic action by reducing the ratio of phosphorylated CRMP2 at its Threonine-514 site relative to total CRMP2, a ratio which is abnormally elevated exclusively in BPD patients that respond to lithium treatment (Tobe et al., 2017). Fig. 17.4 highlights molecular phenomena that follow lithium administration.

In addition to these in vitro assays to determine the effect of lithium treatment on BPD neurons derived from patients via hiPSC-based reprogramming, animal models can also be employed to provide supplemental insight into genetic and pharmaceutical influences on behavioural outputs related to BPD. As it would be unethical to perform such experiments on humans, animal models are vital to providing corroboratory or causational observations into how certain genetic or pharmaceutical interventions alter behaviour and other in vivo characteristics. Moreover, animal models are often used in the drug development pipeline in the preclinical stages of target discovery, target

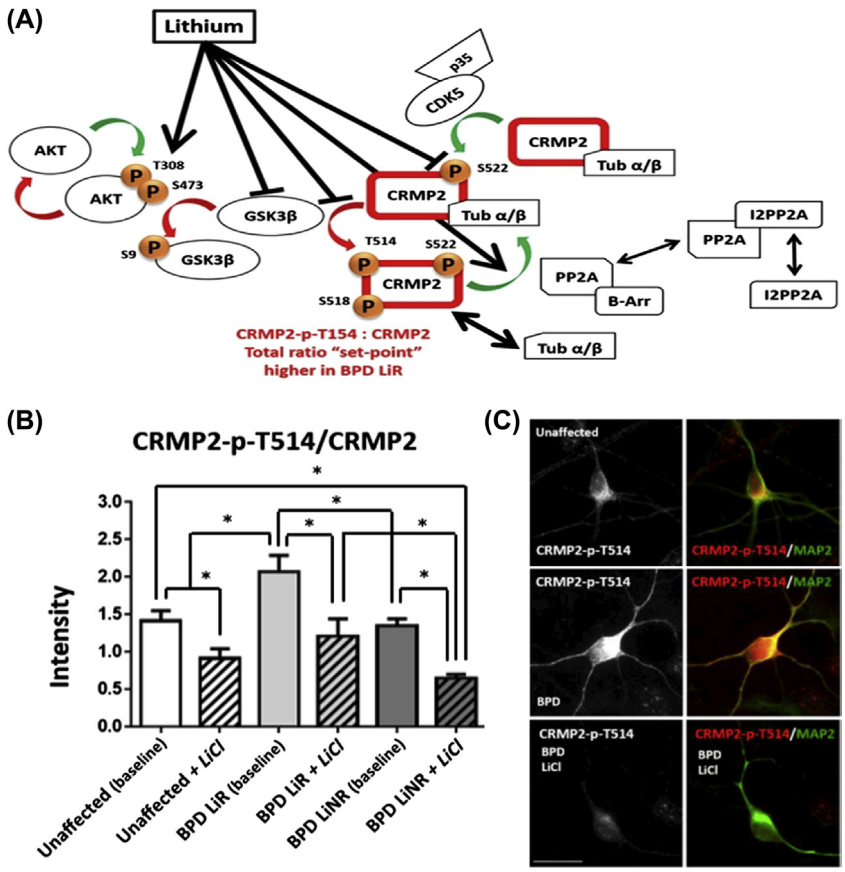


**Figure 17.4** (A) The proposed mechanism of action of lithium to treat lithium-responsive bipolar disorder (BPD) by preventing the phosphorylation of Collapsin Response Mediator Protein-2 (CRMP2). (B) Graph of fluorescent intensity via western blots across cell lines and treatment groups showing that lithium treatment lowers the ratio of phosphorylated to total CRMP2. (C) Photomicrographs showing reduction in phospho-CRMP2 (red) following $Li^+$ treatment in BPD patient neurons (Tobe et al., 2017). *(Schematics courtesy of Brian Tobe, photomicrographs courtesy of Stephen Haggarty.)*

validation, lead generation and refinement, safety, toxicity, bioavailability and efficacy studies, which makes these lower-order systems valuable to study to aid in the translation from the bench to the bedside (Taconic Biosciences, 2018; Fig. 17.5). For the study by Tobe and colleagues into the pathophysiology of BPD, as the functional agent lithium revealed that CRMP2 is the keystone protein in the lithium treatment response of BPD neurons in the patient neurons that are actually responsive to lithium, animal models with the *Crmp2* gene knocked-out (no expression of CRMP2) or knocked-in (constitutive expression of CRMP2) were generated to further explore the neurobiological and behavioural products of altered CRMP2 expression in the context of


**Figure 17.5** Typical drug discovery and drug development pipeline timeline schematic. Disease modelling via human induced pluripotent stem cells, animal models and functional agents can aid in all preclinical stages.

pharmaceutical treatment. CRMP2 had only been correlated with BPD and lithium's mechanism of action, so to determine if there is a causative relationship between CRMP2 and lithium-mediated behavioural changes in an accepted BPD animal model, the methamphetamine-induced manic hyperlocomotion model in mice was used. Though methamphetamine administration would normally make mice enter a manic state, lithium pretreatment actually prevents methamphetamine-induced manic hyperlocomotion and manic exploration of the periphery of a box, which makes this model the gold standard in the field as it accurately recapitulates the mood stabilisation afforded by lithium treatment. Yoshio Goshima provided a previously generated transgenic mouse line incapable of having its CRMP2 inactivated via phosphorylation (*Crmp2*-Knockin (CRMP2-KI)), which allowed for the determination if CRMP2 activation mimicked lithium treatment in an accepted BPD behavioural assay (Yamashita et al., 2012). Amazingly, the CRMP2-KI mice resisted methamphetamine-induced mania, just as if these mice had received lithium. This demonstrates that CRMP2's activity is causally linked to BPD behaviour. In the same way as lithium decreases pCRMP2 levels, it appears the CRMP2-KI mice mimic individuals who are receiving lithium, as lithium treatment leads to increased activation of CRMP2 (Fig. 17.6). This is the first ever genetic intervention to rescue BPD-associated behaviour. However, there are some potential negative effects with this mutation related to neuronal structure, as CRMP2-KI mice had some loss in their neuronal polarity, and an increase in the number of primary dendrites, which may alter neuronal and network signalling profiles (Tobe et al., 2017). Therefore, while the functional agent lithium has served as a 'molecular can opener' to reveal the key players and pathways in the pathophysiology of BPD, with the help of accurate disease models, lithium has also uncovered novel therapeutic target opportunities centred around CRMP2 and its phosphorylation status.

While the in vitro results in Fig. 17.4 highlighting lithium's ability to lower the ratio of phosphorylated to total CRMP2 in patient-derived neurons, and the in vivo data presented in Fig. 17.6 demonstrating that having constitutive expression of CRMP2 is able to prevent methamphetamine-induced manic peripheral exploration in the same way that lithium pretreatment prevents methamphetamine-induced manic hyperlocomotion are both scientifically noteworthy, as they further corroborate that CRMP2 is a key protein that mediates the lithium response pathway in BPD systems, these findings also critically support the development of therapies better than lithium. These established in vitro

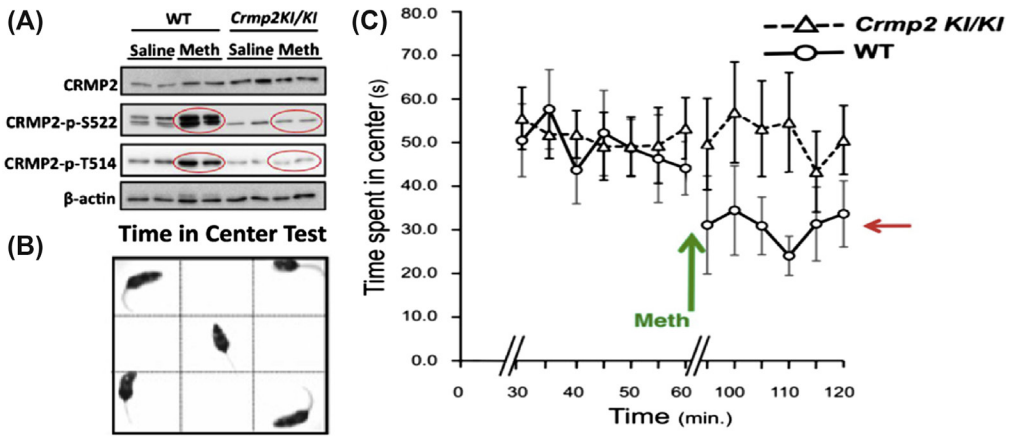


**Figure 17.6** (A) Western blot confirming that methamphetamine treatment increases phosphocollapsin response mediator protein-2 (CRMP2) in wild type (WT) (mimicking bipolar disorder (BPD) mania) but not *Crmp2*-Knockin (CRMP2-KI) mouse brains (mimicking lithium-treated BPD). (B) Open-field test for quantifying nonmanic behaviour (time spent in unprotected centre) versus manic behaviour (little time in centre, more time 'manically' circling the periphery). (C) Meth-treated CRMP2-KI mice spend no less time in the centre compared with baseline, whereas WT mice spend less time in the centre following meth treatment. This confirms that preventing CRMP2 phosphorylation (via CRMP2-KI) lessens BPD-like behaviours (Tobe et al., 2017). *(Western blot data courtesy of Brian Tobe, mouse behavioural data courtesy of Yoshio Goshima.)*

and in vivo disease models can now be used to study the efficacy of any newly proposed and developed therapeutics to assess whether or not the new drug also decreases the ratio of p-CRMP2:CRMP2 in BPD patient–derived neurons in vitro and also staves off methamphetamine-induced manic peripheral exploration in the gold standard animal model of BPD. This way a drug that is as effective, if not more effective than lithium, can be developed while also trying to make the drug safer than lithium. Even though the efficacy of lithium makes it the current standard of care for patients with BPD, lithium is not considered to be the safest of therapeutics. Lithium must be prescribed long term and at high doses, which often causes BPD patients to endure many deleterious side effects, such as renal failure. Therefore, there is a current considerable need for a therapy that is both safe for lifelong use and successful at stabilising manic behaviour. With these established in vitro and in vivo disease models that enable an evaluation of whether or not a particular intervention is normalising the posited pathologically relevant ratio of phosphorylated CRMP2 to total CRMP2 and preventing methamphetamine-induced hyperlocomotion, research and development of new and improved therapies will be easier. Now, these molecular and behavioural readouts can be employed to perform drug screening and assess efficacy, which will reduce time spent in the era of ferment, improve performance and expedite access to the take-off phase of the next innovation S-curve in the development of transformational pharmaceutical products for neurological disorders.

## Lithium Reveals Bipolar Disorder Brain Architectural Abnormalities

In addition to facilitating the acquisition of insight into the pathophysiology of disease, hiPSCs, when coupled with probing functional agents, can also dialectically uncover the systems architecture of the human brain. Nicknamed the three-pound gem, the human brain is also a three-pound computer. This analogy arises from the similarities in construction and function between the two systems. Axons and dendrites serve as the internal wiring that enables communication between close and distant parts of the nervous system. Neurons operate as computer chip components that integrate positive and negative electrochemical inputs to produce a neuron-specific output. Clusters or circuits of neurons, such as motherboards, serve as microprocessors of calculated input–output generators that then communicate through wiring to form pathways that ultimately govern cognition and behaviour. As previously described, studying the human brain and its comprising components has long been difficult due to the barriers to acquiring CNS tissue and cells. With the advent of hiPSCs through reprogramming, tractable assays to characterise neurons and even neural organoids such as 'minibrains', have been deployed to gain knowledge about the chips and microcircuits of the brain, especially for the study of brain development and architecture, in a healthy or diseased context (Kelava and Lancaster, 2016). These in vitro models can be effective ways to investigate diseases ranging from neurodegenerative conditions such as Alzheimer's disease (AD) to infectious diseases such as Zika virus–enabled microcephaly. Moreover, in vitro neuronal models can serve as platforms for drug screening for these and other conditions. With this said, animal models can also provide valuable in vivo insight to facilitate increased understanding of disease pathophysiology and multifaceted drug discovery (Hu et al., 2018; Qian et al., 2016).

Though the animal model employed by Tobe and colleagues to elucidate brain architectural aberrations was not a direct example of the disease modelling and probing powers of hiPSCs and functional agents, respectively, as the animal model was a product of investigations enabled by hiPSCs and the functional agent lithium that discovered which gene to model in the in vivo mouse system, the resulting observations prove that hiPSCs and functional agents are also valuable for informing downstream experiments. As stated before, Tobe et al. used a *Crmp2*-Knockout (CRMP2-KO) mouse line and a CRMP2-KI mouse line to verify CRMP2 as a key player in the lithium response pathway of bipolar disease neurons that respond to lithium treatment. While this mouse model provided useful proteomic and behavioural insights into lithium's mechanism of action (Fig. 17.6), the CRMP2-KO mouse in particular provided interesting observations about differences in neural construction between the CRMP2-KO mouse brain and a normal mouse brain. That is, knocking-out CRMP2 changed the characteristics of neuronal structure and thus the wiring of the cranial computer. As the phosphorylation status of CRMP2 regulates its activity such that it is inactive when phosphorylated, but active when not phosphorylated, these different phospho-forms were interrogated. It was

consequentially observed that only the active nonphosphorylated form of CRMP2 was found in spine neurite structures. Spines are formations along projections from neuron cell bodies (i.e., dendrites and axons), and spines are the critical structures enabling synapses between neurons. To see if CRMP2 regulates spine formation, spine density was juxtaposed between neurons with their CRMP2 gene missing (CRMP2-KO), and controls, and it was found that the neurons lacking CRMP2 had decreased spine density (Fig. 17.7).

To see if the human brain exhibits similar molecular and structural changes, human BPD brains were studied ex vivo, and it was valuably found that they have increased pCRMP2 levels, matching the discussed in vitro model of BPD (Konopaske et al., 2014). Moreover, using primary human postmortem samples, it was observed that BPD individuals had decreased dendritic spine density in their cortical neurons, and that lithium-treated BPD patients interestingly had no significant difference in spine density compared with unaffected controls (Fig. 17.7; Tobe et al., 2017). Overall, when all of these in vitro, in vivo and ex vivo observations are combined, new neuroanatomical insight into the pathology that BPD individuals endure results. Synapses are vital for signalling between neurons. As spines serve as the key players that construct a synapse, decreased dendritic spine density across all or some of brain could alter overall network signalling. Therefore, the etiological pathophysiology of BPD and other neurological conditions may not be consequences of cell loss, but rather in the loss or impairment of proper interneuronal networking, functionality and circuitry. As all of this new knowledge was the product of initial explorations into hiPSC-derived neurons from BPD patients using the functional agent lithium to identify a newly implicated keystone protein (CRMP2) in the lithium response pathway of BPD neurons, there is clear epistemic and practical value to using faithful cellular models of disease and compounds such as lithium salts to study human disease. It is the hope that these insights will further facilitate the development of novel therapies that are less toxic and more effective than lithium and that more BPD patients will successfully respond to, such that the newly discovered cytostructural aberrations of BPD can be reversed and that normal interneuronal and circuit-based communication can be restored. This challenge represents a significant drug development opportunity for BPD and likely other disorders.

## Connecting the Dots: Knowing that Lithium Influences CRMP2 Phosphorylation Reveals Therapeutic Opportunities for Alzheimer's Disease

Another common side effect of clinically relevant research that employs a functional agent in an accurate disease model is the opportunity for derived findings to inform additional drug discovery efforts for other diseases. Because AD is the most common neurodegenerative disease with devastating and terminal consequences, there is a

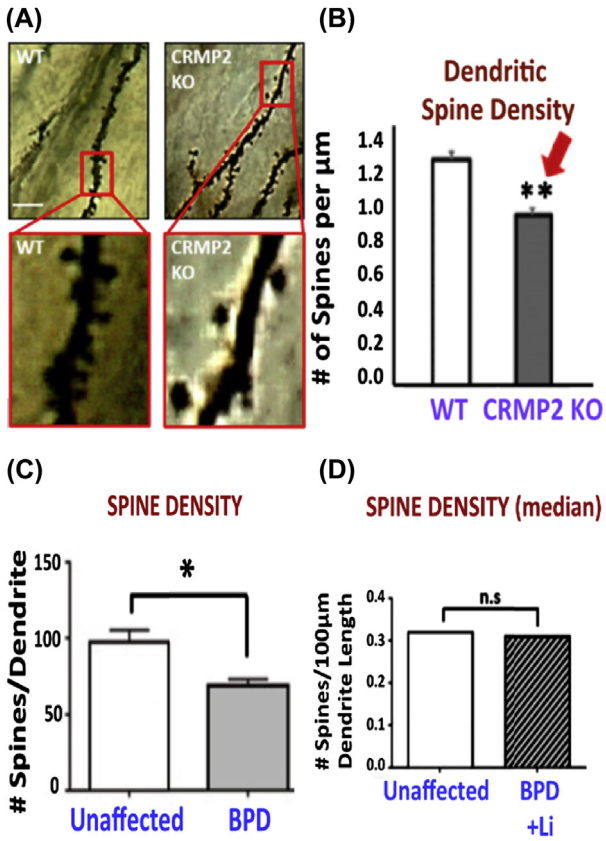


**Figure 17.7** (A) Silver stained mouse neurons demonstrating that *Crmp2*-Knockout (CRMP2-KO) neurons have fewer spines compared with wild type (WT). (B) Quantification of dendritic spine density showing that CRMP2-KO neurons have significantly decreased spine density compared with control neurons. (C) Quantified spine density in human postmortem neuronal samples showing that bipolar disorder (BPD) individuals have significantly fewer spines per dendrite compared with unaffected individuals. (D) Postmortem numerical spine density of neurons from unaffected human controls compared with the spine density of neurons from human BPD patients who were treated with lithium, showing that lithium treatment recovers the spine density decrease observed in BPD individuals untreated with lithium, as there was no significant difference in neuronal spine density between unaffected individuals and BPD patients treated with lithium (Tobe et al., 2017). *(Mouse dendritic spine data courtesy of Yoshio Goshima, postmortem human dendritic spine data courtesy of Glenn Konopaske.)*

significant need to develop effective therapies. The global financial burden of AD for 2018 is approximated to be $1 trillion US Dollars, and it is estimated that about 50 million people around the world are currently enduring AD and that 131.5 million people will be living with AD in 2050 (Prince et al., 2016). Plus, the cost to develop a disease-modifying therapy for AD is approximated to be $5.7 billion, with over 100 attempted products failing in preclinical and clinical trials (Doody et al., 2014; Scott et al., 2014).

Therefore, if the expensive and time-consuming drug discovery process can rely on existing knowledge and recent insights that decipher the pathobiology of the disease, the timeline and access to competitive, productive and profitable stages of the innovation S-curve can be accelerated. The relatively recent consideration of the potential of lithium to be used to treat AD and similar neurodegenerative diseases called tauopathies represents a new hope for patients facing these conditions. While the work by Tobe and colleagues demonstrated that lithium exerts its mood-stabilising effects by preventing the phosphorylation of CRMP2, CRMP2 has also been implicated in the pathogenesis of tauopathies and AD, and lithium has been explored as a potential therapeutic agent for AD (Tobe et al., 2017; Trujillo-Estrada et al., 2013; Yoshida et al., 1998). Thus, by using lithium as a functional agent or 'molecular can opener', new insight into lithium's mechanism of action and the crucial protein that lithium targets lends corroboratory evidence that lithium could be a potential therapeutic option for people with AD, and that lithium's possible ameliorative influence in AD could be mediated by its effect on CRMP2 just like in BPD.

The pathology of AD and other tauopathies is characterised by the presence of neurofibrillary tangles (NFTs) largely composed of aggregated hyperphosphorylated tau, which is a cytoskeletal protein, plus AD also has uniquely defining plaques of amyloid beta (Newell et al., 1999). Interestingly, lithium has been shown to prevent the hyperphosphorylation of tau by inhibiting cyclin-dependent kinase-5 and glycogen-synthase kinase 3-beta (GSK3β), which is one of the ways lithium is thought to prevent the hyperphosphorylation of CRMP2 (Lee et al., 2011; Noble et al., 2003; Phiel and Klein, 2001; Tobe et al., 2017). Beyond the common implication with GSK3β, tau and CRMP2 both regulate microtubule stability, among other functions, and fascinatingly, both tau and CRMP2 are prominently found in the NFTs that are a pathological hallmark of AD (Hensley and Kursula, 2016). Furthermore, it was phosphorylated CRMP2 that was found to be in AD neurons and in NFTs, and this hyperphosphorylated CRMP2 has been observed to accumulate in the cortex and hippocampus of AD transgenic mouse models that develop both amyloid beta plaques and NFTs with CRMP2 phosphorylation preceding plaque and NFT formation, which indicates CRMP2's potential pathogenic role in AD and the value of preventing the hyperphosphorylation of CRMP2 in the context of AD (Cole et al., 2007). While lithium could be therapeutic for tauopathies, as lithium treatment of an AD mouse model reduced the hyperphosphorylation of tau, lithium was unable to prevent the pathological accumulation of amyloid beta, failed to stave off neuronal loss and did not improve memory performance in the same AD mouse model (Sudduth et al., 2012). These connections between lithium, CRMP2, tau, GSK3β, BPD and AD highlight the considerable value of using established drugs to study diseases as these functional agents that operate as 'molecular can openers' may facilitate the discovery of new therapeutic options based on both old or new insights.

## REVISITING THE ESTABLISHED FUNCTIONAL AGENT LEVODOPA IN PARKINSON'S DISEASE

In James Parkinson's 1817 publication of 'An Essay on the Shaking Palsy', Dr. Parkinson defines the shaking palsy as 'involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace: the senses and intellects being uninjured' (Parkinson, 1817). The term shaking palsy would then be changed to Parkinson's disease (PD) to eponymously honour James Parkinson. PD is the second most common neurodegenerative condition, following AD, and it is characterised by the loss of normal dopamine signalling in the motor pathways of the brain, which produces its defining symptoms of bradykinesia, tremor, rigidity and postural instability with late-stage cognitive and autonomic impairment (Mastrangelo, 2017). Despite being first formally characterised in 1817, it took until 1960 to learn what brain system was most impacted in PD, as postmortem human studies at the University of Vienna discovered that dopamine levels were reduced in the striatum, and that the neurons responsible for providing the striatum with dopamine were located in the *substantia nigra* (Hornykiewicz, 2017). However, PD remains enigmatic as not all PD patients lose dopaminergic neurons, and some people who do lose dopaminergic neurons do not develop PD. With that said, the University of Vienna studies implicating dopamine deficiency in PD significantly facilitated progress in this field. With this important insight, a prodrug of dopamine, levodopa (L-dopa), was first tried in PD patients in 1961 and proved, in 1967, to be effective at managing the symptoms PD patients endure by increasing the dosage of L-dopa over time (Tolosa et al., 1998). It is now understood that unlike exogenous dopamine, L-dopa is able to cross the blood–brain barrier, after which L-dopa is taken up by neurons and then converted to dopamine by the enzymatic action of aromatic amino acid decarboxylase, thus replenishing dopamine levels, masking the symptoms of PD and making L-dopa the gold standard therapy for PD management (Poewe et al., 2010). However, after chronic L-dopa usage, with likely dosage increases along the way, many patients become refractory to L-dopa and actually develop L-dopa–induced dyskinesia or chorea-like movements, and patients can potentially even stop responding as well to L-dopa, which enables the symptoms of PD to resurface (Mehanna and Lai, 2013). Consequently, deep brain stimulation (DBS) has been developed to address the motor fluctuation side effects and therapeutic failures of L-dopa by implanting electrodes in the brain to stimulate specific motor areas with particular electrical properties encoded, to provide long-term management of PD, often with supporting pharmacotherapies such as L-dopa or dopamine agonists to yield the better management of PD compared with medical or DBS-based therapy alone (Fang and Tolleson, 2017). Therefore, while L-dopa has been essential to confirming dopamine's central role in PD, L-dopa has also served as a functional agent by managing the symptoms of PD, facilitating the acquisition of new knowledge about the parkinsonian brain and guiding the development of better

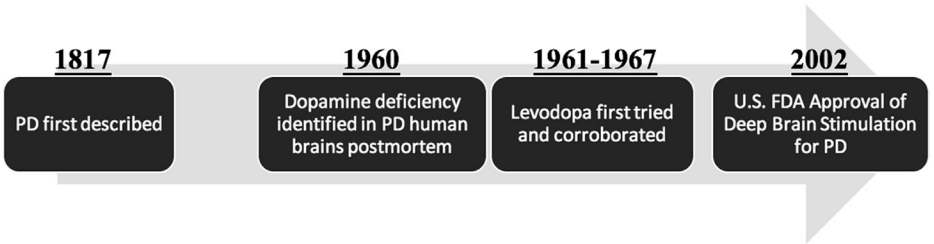


**Figure 17.8** Schematic timeline of epistemic and clinical breakthroughs in Parkinson's disease.

alternative and collaborative therapies such as DBS due to the insight, and clinical management, provided by L-dopa (Fig. 17.8).

## BRAIN STRUCTURE AND NETWORK CONNECTIVITY DISCOVERIES ENABLED BY LEVODOPA

While modern methods from functional magnetic resonance imaging (fMRI), positron emission tomography and transcranial magnetic stimulation have afforded considerable insight into healthy and diseased brains, combining these approaches with pharmaceutical or other interventions can amplify their investigatory potential (Annavarapu et al., 2018). Haslinger and colleagues demonstrated this enhanced study design by using fMRI to compare blood oxygen level dependent (BOLD) signal during voluntary limb movements in PD patients while they were either on or off of L-dopa (Haslinger et al., 2001). They found that 'levodopa led to a relative normalization of the impaired activation in the mesial premotor cortex and decreased signal levels in M1 (the main part of the motor cortex), lateral premotor and superior parietal cortex' (Haslinger et al., 2001). Similarly, Buhmann and colleagues compared fMRI BOLD responses during the performance of a motor task with and without L-dopa treatment in PD patients and healthy controls and found that 'signal changes in M1 and SMA (Supplementary Motor Area) were highly correlated with motor performance, which increased after L-dopa intake' (Buhmann et al., 2003). As the supplementary motor area is known to be vital to the planning and decision-making stages of intentional movement, its dysfunction due to decreased input from the basal ganglia via the nigrostriatal pathway as a consequence of the depletion of dopamine-producing neurons in the striatum and *substantia nigra pars compacta* is thought to produce one of the cardinal symptoms of PD, bradykinesia or slow movement (Gao and Wu, 2016). These studies depict the synergistic explanatory power of using functional agents (L-dopa) and modern brain activity measurement techniques (fMRI) to derive new information about dysfunctional brain areas, networks and circuits in the context of disease, namely PD in this case (Fig. 17.9).

Beyond providing further justification for the use of L-dopa to help PD patients manage their PD symptoms, the deciphering duo of functional agents and investigatory

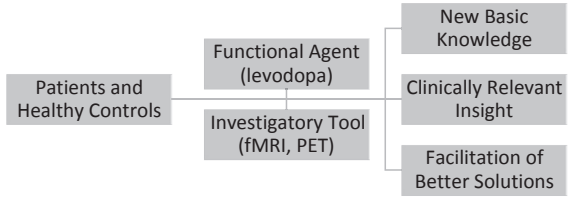


**Figure 17.9** General translational research pathway from human participants to theoretical and therapeutic advancements due to the enabling inclusion of functional agents and clinically accessible investigatory tools.

tools have also informed the development of improved ways to handle PD symptoms, such as through DBS. As of 2017, about 120,000 people have had DBS systems implanted in them (Hamani et al., 2017). One of the most well-established targets of DBS is the subthalamic nucleus (STN) (Anderson et al., 2017). The STN is a component of the basal ganglia motor circuit, and work by Gao and others found that L-dopa administration decreased bradykinesia through a L-dopa–mediated decrease in connectivity of the STN–thalamo–cortical motor pathway, which normalised the striato–thalamo–cortical motor and STN-cortical motor pathways and revealed a neural mechanism of L-dopa treatment in PD (Gao et al., 2016). These findings thus also importantly identify a neural mechanism of DBS treatment, as DBS often targets the STN, which is central to L-dopa's amelioration of PD symptoms.

In 2011, an international consortium of experts reviewed relevant literature, deliberated and then announced that 'patients with PD without significant active cognitive or psychiatric problems who have medically intractable motor fluctuations, intractable tremor, or intolerance of medication adverse effects are good candidates for DBS', and that 'deep brain stimulation improves levodopa-responsive symptoms, dyskinesia, and tremor; [with] benefits seem[ing] to be long-lasting in many motor domains' (Bronstein et al., 2011). While the consortium labelled the STN as 'an effective target [that] quickly became the most common site for DBS electrode placement', it was the work by researchers like Lin-Lin Gao that uncovered why the STN is an efficient and popular place to target through DBS, as the functional agent L-dopa revealed that normalisation of STN-involved motor pathways through L-dopa alleviates PD symptomology (Bronstein et al., 2011; Gao et al., 2016).

Though PD currently remains an unstoppable and incurable condition, as PD was first described in 1817, significant strides have been made in the ability to detect and address this considerable movement and neurodegenerative disorder. With use of L-dopa starting in 1961, 1 year after it was even discovered that dopamine was deficient in a movement centre, the striatum, of PD patient brains, the timeline of intellectual and clinical breakthroughs is a perfect and established rendition of how functional agents such as L-dopa can serve as 'molecular can openers'. Beyond aiding in the management

of the consequences of PD, L-dopa has elucidated the organisation of the motor system within the brain by confirming that the neurodegeneration of dopamine-producing neurons, and the consequential symptoms of PD, can be temporarily compensated for via L-dopa treatment. Therefore, dopamine is more positively understood to be a cornerstone neurotransmitter of the motor circuit. Moreover, the value of the connections of the multiple components of the motor system of the brain can be better appreciated as L-dopa's action on just one of those motor circuit parts, the STN, is able to normalise the entire circuit and mask the hallmark signs of PD. This insight has expanded the significance of L-dopa as a functional agent, as more effective control of PD symptoms and better quality of life for individuals enduring PD is now possible through DBS, which often targets the STN. Ironically, when L-dopa was first established as an often-efficacious medical management method in 1968, surgery approaches to treat PD effectively stopped despite the development of pallidotomy and thalamotomy surgical procedures to curb PD symptomology (Schwalb and Hamani, 2008). It is now clear that L-dopa would later encourage the deployment of surgical and medical device interventions, evidenced by DBS, due to the inability of L-dopa treatment to be a long-term PD management approach as L-dopa's efficacy reduces over time and because L-dopa can elicit unideal, involuntary, excessive movements. Thus, while L-dopa continues to be the gold standard early approach to managing the symptoms of PD, L-dopa has fundamentally facilitated the generation of theoretical and medical advancements in neurology.

## IMPORTANT CONSIDERATIONS IN THE TRANSLATION FROM THEORY TO PRACTICE: TECHNICAL, REGULATORY AND BUSINESS FACTORS INVOLVED IN hiPSC TECHNOLOGY

While the presented case studies of the theoretical insights and clinical progress enabled by lithium and L-dopa demonstrate the value of a research and development paradigm that deploys functional agents to probe relevant disease models, there are some important considerations that need to be kept in mind. From scientific and technical hurdles that could complicate the R&D pipeline to essential considerations such as cost, time and regulatory challenges, taking a step back reveals different factors that must be evaluated. The following tables summarise some of the challenges of hiPSC-derived disease modelling and drug screening, which can be overcome through significant financial and temporal investment.

These considerations posited by Tobe and others provide a sense of the logistical difficulties of developing a sufficiently robust disease model library and the resource-intensive processes of reprogramming and differentiating these cell models. Though seemingly specific to psychiatric diseases, these factors apply to all conditions that researchers seek to model through hiPSC-based systems, as these are criteria that characterise the achievement of construct, face and predictive validity in the context of disease modelling. These are vital scientific safeguards of translational research as they ensure that disease models are accurate and representative (Table 17.1).

**Table 17.1** Issues in applying human induced pluripotent stem cell (hiPSC) technology to psychiatric disease for drug discovery

- Establishing library of somatic and hiPS cells from adequately phenotyped patient populations
- Streaming laborious and costly hiPSC derivation and characterisation process
- Assessing relevance of genetic mutations inherently caused during reprogramming
- Developing more efficient differentiation strategies to generate the most relevant neural cell types and neural cells that are mature rather than young to more faithfully model age-associated neurological conditions
- Identifying discernible, meaningful cellular phenotypes, some of which may only arise in cyclic, stress-induced or relapsing-remitting manner
- Converting these phenotypes to high-throughput screening assays suitable for genome-wide mechanistic studies or large collection compound testing
- Controlling for variability in relation to disease specificity amidst low sample numbers
- hiPSC modelling only works well for genetic, infectious and triggerable diseases

Adapted from: Tobe, et al. Implications and limitations of cellular reprogramming for psychiatric drug development. Exp Mol Med 2013;45(11):e59. https://doi.org/10.1038/emm.2013.124.

While the above table continues to highlight the various technical challenges (i.e., episomal transfection) and various points of necessary quality control regulations (i.e., avoiding significantly mutated cell lines through screening for aneuploidy), these considerations help ensure that stem cell–based models are reproducible, representative and safe (Table 17.2). Additionally, with sustained focus on using stem cells to study disease, many of the limitations and doubt-inducing characteristics of this field have been addressed. There are companies that provide all of the necessary factors in a robust and standardised manner to facilitate access to these stem cell technologies. Despite this commercial homogenisation, scientists have developed different methods of reprogramming and differentiation that produce some disparities between models. This represents an opportunity for the cultivation of consensus amongst members of the scientific community and also regulatory intervention to standardise a best method for each specific disease or research paradigm. One such policy could be to ensure uniformity in the method of reprogramming. While the reprogramming methods used by Shinya Yamanaka in 2007 revolutionised science, resulting random insertion of transgenes into the genome was potentially problematic and so scientists had to develop mechanisms to remove these transgenes through additional genetic intervention via Cre/loxP and piggyBac transposon systems (Takahashi et al., 2007; Guo et al., 2017). Nowadays, there are newer ways to perform reprogramming of somatic cells, such as skin cells, into iPSCs through episomes or Sendai viruses, which do not integrate into the genome, to reduce genetic alterations in the first place due to integrating reprogramming methods such as through lentiviruses and via the Yamanaka protocol (Guo et al., 2017). What is more, doubts about comparative controls from healthy donors can be mitigated by using patient-isogenic cell lines with the mutation of interest removed via the CRISPR/Cas9 system (Tidball et al., 2017). Additionally, the Food and Drug Administration (FDA) rightly requires that stem

**Table 17.2** Cell type stages of reprogramming and associated variables in neuroscience

| Reprogrammed cell type | Technical factors | Potential variations |
|---|---|---|
| Somatic cells | Isolation and culture<br>Freeze/thawing | Somatic cell passage number<br>Age of biopsy<br>Karyotype |
| Human induced pluripotent stem cell | Retroviral transduction<br>Episomal transfection<br>Conditioned media<br>Feeder cell line layers | Insertional mutations<br>Copy number variants<br>Point mutations<br>Aneuploidy<br>Epigenetic reprogramming |
| Neural progenitors | Conditioned media<br>Feeder cell line layers<br>Extracellular matrix<br>Morphogens<br>Embryoid body, rosette steps<br>Chemical inhibitors<br>Purified protein activators | Patient and line variability<br>Purity from pluripotent stage<br>Clonality<br>Differentiation capability |
| Neurons | Specific media requirements<br>Purified protein<br>Molecular compounds | Selection of relevant cell type<br>Heterogeneity of cell types<br>Identification of cell subpopulations<br>Variable cell culture duration |

Adapted from: Tobe, et al. Implications and limitations of cellular reprogramming for psychiatric drug development. Exp Mol Medne 2013;45(11):e59. https://doi.org/10.1038/emm.2013.124.

cell–based products meet Good Manufacturing Practices, Quality Systems Regulations, Good Laboratory Practices, Good Clinical Practices and Good Tissue Practices, as these regulatory and technical hurdles serve as safeguards that ensure the reliability of hiPSC-based approaches (Mendicino and Weber, 2015). Despite these directives and scientific guidelines, market signals, significant interprofessional collaborations and positive regulatory actions provide confidence in the stem cell field. Moreover, the US FDA approval of human embryonic stem cell–based product clinical trials, the FDA's implementation of accelerated review processes that could help establish stem cell–involved products and the rapid innovation in this arena all evidence the potential in this field (Knoepfler, 2015). However, the European Union has banned the patenting of human embryonic stem cell–based products, which makes hiPSC-derived technologies more appealing. Despite the aforementioned logistical concerns to ensure that the used iPSC-derived models are accurate, relevant and ethically derived and manipulated, and that good practices are followed, the development of robust drug screening platforms centred around iPSC-based technology should further allay fears about the appropriateness of significant investment in iPSC-related paradigms.

High-throughput screening employing state-of-the-art automated robotic apparatuses to perform drug discovery takes place, for example, at the Prebys Center for Drug Discovery located at the Sanford Burnham Prebys Medical Discovery Institute in California (Sherman and Bang, 2018). This centre collaborates with many academic and pharmaceutical groups and facilitates target identification, target validation, lead optimisation, preclinical evaluation and drug discovery. As a result, hundreds of screens have been completed, dozens of published validated hits by chemical probes have been generated and new therapeutic approaches have been explored (Sanford Burnham Prebys Medical Discovery Institute, 2018). Furthermore, published work from this centre demonstrates the feasibility of hiPSC-based drug screening utilising high-throughput and automatic methods. Using hiPSC-derived neurons, researchers conducted high-throughput screening to determine which bioactive small molecules out of a group of 4421 different candidates influence neurite outgrowth, which is an important process for neural network formation and nerve regeneration (Sherman and Bang, 2018). Remarkably, this broad search in a relevant model identified 108 hit compounds, 37 of which are already US FDA approved drugs, some of which have been unambiguously associated with neurite outgrowth (Sherman and Bang, 2018). These recently acquired insights and the novel identification of putative compounds can now inform additional research and clinical progress. Moreover, the additional ability to use human cells, via differentiation of hiPSCs, to characterise the safety and toxicity of proposed therapeutics in a similarly high-throughput manner would also be valuable in preclinical trials (Ko and Gelb, 2014). These preclinical trials could also utilise improving microphysiological systems such as organ-on-a-chip apparatuses that enhance development and face validity of drug screens, as they have already been used to improve the detectability of drug-induced liver injury and cardiotoxicity to screen out deleterious drug candidates (Conant et al., 2017; Lin and Khetani, 2016; Low and Tagle, 2016). Thus, the development of adaptable and efficient drug screening technologies and facilities that use hiPSC-derived cells to test a myriad of functional agents should inspire additional confidence in these tools and the overall basic, translational and clinical approaches these tools enable.

It appears that high-throughput drug screening facilities would maximise the synergistic powers of iPSC technologies and functional agents such that drug development pipelines can be temporally shortened, thanks to the discovery of new or previously known drug candidates. Though this general iPSC-based drug discovery approach has been deployed by many major pharmaceutical companies, these efforts can be accelerated by using functional agents and 'Open Science' approaches to facilitate the 'bench to bedside' transition (Han et al., 2018). Moreover, the time and cost to perform safety and toxicity studies and New Drug Application work could also be reduced if drug candidates were selected from compounds already approved by the FDA for marketing in the United States or a similar regulatory group in another country such as the European Medicines Agency that authorises the marketing of medicines in the European Union.

This way a company with a specific active ingredient patent could apply it to a different disease indication, or if an active ingredient is off patent, the active ingredient could be repurposed to study disease pathophysiology, understand the mechanism of action of a drug and even treat another condition if the regulatory agency for the country the drug is aimed to be marketed in has approved the active ingredient. This represents another opportunity to leverage functional agents, which can be known approved drugs. However, what may be a greater opportunity would be to apply the understanding of the biological effects of each functional agent or active ingredient to guide the development of even better therapies rather than the often-attempted and frequently-failing, target-based approach for drug development (Peyvandipour et al., 2018). That is, knowing which drug elicits which desired biological effects that could help address conditions such as tauopathies whether they be increased autophagy to breakdown pathological proteins such as amyloid beta in AD or alpha-synuclein in PD, or some other cellular function, can facilitate translational success (Thellung et al., 2018). The previously referenced work by Tobe and colleagues on the mechanism of action of lithium in curbing BPD mania highlighted the potential therapeutic role of drugs that inhibit GSK3β in the same ameliorative way that lithium does in the context of BPD (Tobe et al., 2017). However, despite the synthesis of a very large number of GSK3β inhibitors, and the significant preclinical evaluation of GSK3β inhibitors, the clinical translation of GSK3β inhibitors has so far failed because the compounds tested so far elicit adverse off-target effects (Pandey and DeGrado, 2016). With the difficulty in repurposing existing medications, such as previously synthesised GSK3β inhibitors or the 37 already FDA-approved drugs implicated in neurite outgrowth in the aforementioned study, new drugs may have to be developed where side effects and efficacy would be dissociated. This presents another business hurdle that regulates whether or not the insights from iPSC-enabled drug discovery can be applied and monetised.

It is expensive and time consuming to develop a new drug. Some estimates claim that it requires about $1.2–1.7 billion and 12 years to develop and test a new drug (Sollano et al., 2008). While this financial and temporal cost encourages the repurposing of existing approved drugs, not every disease can be successfully managed through repurposing. New drugs need to be developed to address unmet medical needs. These efforts can be facilitated by in vitro hiPSC-derived cellular, organoid, tissue chip, organ-on-a-chip and 'human-on-a-chip' models that offer a more representative, cost-effective and high-throughput approach to safety, toxicity, preliminary efficacy and early-phase screening (Conant et al., 2017; Lin and Khetani, 2016; Low and Tagle, 2016; Mazzarella and Curigliano, 2018). However, despite these advancements it is very difficult to develop a new drug that can target a single phosphorylation site, a particular phosphatase or another specific kinase. This difficulty is evidenced by the disparity between the significant investment into phosphorylation-related and signal transduction–altering drug discovery and the paucity of FDA approved phosphorylated-related drugs. Even though

approximately 30% of drug discovery programmes by pharmaceutical companies target protein kinases, there are only 46 FDA approved kinase-modifying drugs (37 of which are only indicated to treat cancer) (Cohen, 2002; Xu et al., 2016). Commentators on this subset of pharmacology find hope in the increased abilities to study the structure of kinases and phosphatases, which can inform the structure of molecules and compounds used to target these phosphorylation regulators, and also in the enhanced capacity to perform small molecule discovery using novel high-throughput technologies (McConnell and Wadzinski, 2009). Thus, phosphorylation-related drug development can be guided by structure–activity relationship (SAR) data and models that reveal which active ingredients successfully target kinases and phosphatases through leveraging the nexuses between structural, chemical, physical, computational and statistical biology (Guha, 2013). This lead generation and lead optimisation analysis would achieve even greater translational significance if this process is applied to a disease-relevant system such as cell-based screens employing hiPSC-derived patient differentiated cells to validate and refine leads and even confirm efficacy in an accurate disease model, especially as many neurodegenerative diseases involve the malformation of likely target proteins, which could interfere with SAR-informed drug candidates (Bucciantini et al., 2002; Kayed et al., 2003).

Some of this optimism and enduring sense of the promise of hiPSC technologies derives from biotechnology success, translational progress and market buy-in. Many institutes and companies have capitalised on the automation of cell culture, organoid-development and bio-printing techniques (Fang and Eglen, 2017). In addition, many pharmaceutical powerhouses and corporations have significantly invested in iPSC-based drug studies for phenotypic and drug safety screening, displaying a strategic commitment to leverage iPSC technology for high-throughput R&D and preclinical evaluation. With many peer-reviewed publications demonstrating the translational value of hiPSC-derived drug screening, and with many institutions and companies investing in hiPSC-sourced drug discovery, this is a growing field with much potential to appropriately combine the powers of functional agents with hiPSC-based methods. This synergy will enhance drug development, boost profit margins by decreasing development costs and attrition rates, which will also benefit payers and patients through lowered drug prices, and ultimately help decrease the burden of diseases.

## PERSPECTIVES

iPSC-based methodologies have become sufficiently refined and established to enable diverse and high-throughput work. Therefore, the future of this modality will be defined by the inventive and variegated application of iPSC technology. This trend away from basic science and towards translation has begun. A literature review of articles referenced in PubMed and that were published between 2006 and 2016 and

contained the key phrase 'induced pluripotent stem cells' categorised the 3323 articles by theme (Negoro et al., 2017). These themes included reprogramming, differentiation, pathophysiological research of diseases, drug discovery and cell therapy. On counting the number of publications and the total impact factor score for each theme, it was interestingly found that publications on reprogramming and differentiation have declined after reaching their respective maxima in 2013 and 2014 (Negoro et al., 2017). However, research regarding the use of iPSCs in drug discovery and the study of disease pathophysiology have increased continuously despite initially lagging behind papers on the fundamental techniques of reprogramming and differentiation (Negoro et al., 2017). While this trend represents an intuitive and natural evolution, this shifting of gears also demonstrates the potential of iPSC-based methods to accelerate clinical progress as well as the technical maturity of iPSCs-derived in vitro assays. This transition also depicts an internal innovation S-curve within iPSC technology and its application. Reprogramming and differentiation were the techniques that, by definition, facilitated the take-off phase of the innovation S-curve. However, as the stem cell biology field became saturated with publications on reprogramming and differentiation, the maturity of the iPSC invention was realised. The intellectual and capitalistic drive to excel then brought about a discontinuity to the initial iPSC innovation S-curve based on the simple steps of reprogramming and differentiation and subsequently created a new innovation S-curve based on the varied application of established iPSC technology. It now seems as if this S-curve, that is characterising the myriad deployments of iPSC methods from investigating disease pathophysiology to performing drug screening, is maturing. Thus, the future of iPSC technologies will likely be found in incremental innovations that enhance the power of iPSCs rather than in progress in the iPSC arena itself. That is, the field of stem cell biology will be sustained and enhanced due to innovations in accompanying technological advancements that better facilitate the attainment of insight into disease pathophysiology and the development of novel therapeutic approaches.

However, before the next innovation S-curve takes over, the existing one is still relevant. New drugs will be successfully identified in drug screens, especially as ongoing innovation encourages the development of more cost-effective, more high-throughput and more precise drug discovery. Moreover, additional organoids will be cultured or printed to improve disease understanding and further refine drug screening. With this said, once these applications are exhausted, the consequential future of stem cell biology will be defined by the use of functional agents and novel technologies to unlock and amplify the power of iPSCs. The novel and creative application of functional agents such as lithium or L-dopa to study hiPSC-derived in vitro models of health and disease and the additional improvements in mass production of organs will govern the future of iPSC-based research and development. This application-centric transition has already

occurred, and if academia, industry, regulatory authorities and clinical medicine continue to collaborate, iPSCs and accompanying enhancement technologies can facilitate the achievement of a better future for everyone. Thus, institutional synergy would offer hope to patients facing intractable diseases in clinical areas that have vexed for many years the ingenuity and development power of big pharma, such as in neurology and particularly AD or PD.

Summing it all up, due to the advances in research tools achieved during the past decade, the brain is no longer an opaque black box. Neurological diseases are no longer indecipherable, as exemplified by the use of lithium as a 'molecular can opener' to gain access to a deeper understanding of the pathobiology of BPD and the mechanisms of action that can be leveraged to develop disease-modifying therapies. Furthermore, translational science is nowadays significantly more efficient than it was before the advent of iPSC technology. This progress is fundamentally due to the ability to faithfully recapitulate in vitro not only disease but also health. Thus, tractable models are constructed, thanks to the ability to reprogramme patient and donor somatic cells into iPSCs, which are then differentiated into the desired cell fate for investigation. In the context of the study by Tobe and colleagues, hiPSC-derived neurons from individuals with or without BPD were studied to assess lithium's mechanism of action (Tobe et al., 2017). Combined with corroboratory insights from an animal model, this hiPSC approach helped identify CRMP2 as the keystone protein in the lithium response pathway in BPD neurons (Tobe et al., 2017). This insight now facilitates the development of new drugs that are more specific to CRMP2 and more effective and safer than lithium. While the more recent BPD study demonstrates how hiPSCs and functional agents can accelerate the acquisition of disease insight, the ability of L-dopa to enable pathological and disease management progress in the context of PD shows that functional agents themselves hold significant explanatory power. While L-dopa offers temporary management of the symptoms of PD, L-dopa was also used to help identify brain regions to target with DBS, which is now an established method to provide the best control of the movement consequences of PD. Thus, while functional agents have helped decipher the systems architecture of the brain without the aid of iPSCs, combining these two tools mutually increases their abilities. As with most technologies, various technical, regulatory and business considerations must be kept in mind when employing hiPSCs and functional agents, but the positive trend in this arena demonstrates that these hurdles and checkpoints can be met. Moreover, the continued innovation in this field from enhanced high-throughput drug screening to improved organoid models indicates that the future is bright. Therefore, functional agents serve not only as 'molecular can openers' that elucidate the construction of the brain and reveal pathophysiology but also as 'molecular door openers' that invite the discovery of novel therapeutics that are capable of improving health outcomes for all.

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