ENGLISH SUMMARY

the mind alone is the radiant jewel, from which all things borrow their temporal reality
- Anagarika Govinda

Undeniably the most complex structure in existence, equal to if not more than the universe itself, the brain is where everything begins and ends. All that makes us, us, be it humans or animals is because of the workings of our brain. Our cognition and behaviour are the end result of billions of neurons communicating through trillion connections in order to manifest reality. It is thus evident, that even minor disturbances in this exquisitely complex system, can have the most profound effects not only in our capacity to function but to even perceive the world we are in.

Such is no more apparent than in psychiatric and neurodevelopmental disorders (NDDs). Encompassing a range of syndromes including schizophrenia, attention deficit hyperactivity disorder, autism spectrum disorders (ASD), and intellectual disability (ID), NDDs can affect up to 15% of the general population. ID disorders comprise the most common form of NDDs, with patient IQ ranging from mild disability with $50 < \text{IQ} < 70$ and with considerable self-care skills, to severe $20 < \text{IQ} < 35$ or profound $\text{IQ} < 20$ and in need of constant attention, support, and care for their entire lives. In addition to deficits in the intellectual domain and reduced self-care capacity, ID is also characterized by behavioural, social, and communication problems, and patients are often co-diagnosed with ASDs. The research described in this thesis is focused on one such NDD, termed Fragile X Syndrome (FXS), caused by the silencing of a single gene. Intellectual disability in FXS ranges from mild in patients with borderline low IQ, to severe in patients with IQ below 40. Female patients are generally less affected, even in the presence of full gene silencing, exhibiting near normal to borderline low IQ ranges. Aside from deficits in the intellectual domain, FXS patients are frequently diagnosed with behavioural deficits in attention, hyperactivity, anxiety, impulsivity, and aggressive behaviour. Additionally, an increased incidence of seizures of up to one in five patients has been documented. Finally, FXS is often identified with social impairments, deficits and repetition in language, as well as stereotypic behaviours. Autism and ASD features are frequently observed in FXS, ranking FXS amongst the most common monogenic causes of ASD, accounting for ~5% of all autism cases.

As the most recently evolved brain structure, the prefrontal cortex (PFC) is hypothesized to orchestrate highly complex cognitive functions including attention, inhibitory control, working memory, goal oriented behaviour, sociability, and personality. Therefore, PFC dysfunction can cause severe impairments on an individual's ability to not only function in society but to one’s self-sufficiency and survival, and has been linked with NDD pathophysiology. The research described in chapters II and III of this thesis, thus focuses on the functionality of the PFC in FXS, either on the cellular level - chapter II, or at the level of behaviour that is mediated by the PFC - chapter III.

In chapter II the functionality of the PFC in the FXS mouse model was studied during two crucial postnatal periods of cognitive and behavioural development. Namely, prepuberty and adolescence. The research was focused on one system of neuronal communication, termed inhibitory since it is responsible for tuning and turning off the excitatory activity of the brain. Consider the example of volume control on a television set, where volume up would be excitatory making the signal travel louder and further, whereas volume down would be inhibitory making the signal softer. Through this trivial example one can imagine how if
volume up did not have a way to go down, significant problems would arise. Similarly, when the brain’s inhibitory system (volume down) is not working properly severe cognitive and behavioural deficits arise. During prepuberty, the rate of inhibitory communication was found increased while the strength of inhibition was augmented and less desensitised over time. We observed an increased in the number of cells generating inhibitory signals, and our data also indicate putative increases in the number of receptor that mediate such signalling, providing us with a possible explanation for some of our observations. During adolescence the number of cells generating inhibitory signalling were found reduced, concomitantly with a reduction in the rate of inhibition. Furthermore, the ability of inhibitory receptors to open and close properly was observed to be significantly slowed down, likely due to a change in their protein composition. Together, these data add to the existing body of literature pointing toward significant disturbances in inhibitory signalling in NDDs. Moreover, for the first time in FXS we demonstrate inhibitory dysregulation in the PFC, a region related to several cognitive and behavioural deficits observed in the syndrome. Finally, inhibitory dysregulation occurs during prepuberty and adolescence, periods of heightened PFC maturation, and could thus impair the proper formation and function of this vital brain region.

In chapter III we challenged the FXS mouse model with PFC mediated behavioural tasks. Sustained attention was found intact during early adulthood, mirroring findings in late adolescent FXS patients. However, FXS mice exhibited compulsive behaviour and hyperactivity akin to observations in patients. Notably, both of these phenotypes normalised with repeated exposure to the task or testing environment, while hyperactivity transiently remerged when task contingencies or testing environments were altered. The transient nature of these phenotypes could imply that underlying neuronal circuits are not necessarily impaired, but instead are frequently engaged while presented with novelty or unexpectedness. Normalisation of perseveration and hyperactivity upon familiarisation could highlight the value of cognitive behavioural therapies over the direct use of pharmaceuticals for some FXS patients.

The holy grail of translational research is the identification of dysregulated mechanisms, and subsequently the prevention or restoration of disease pathologies through mainly pharmacological or surgical means. The apparent difficulty is further amplified when considering neurological disorders, given the stupendous complexity, significant rigidity, and attenuated capacity for regeneration of the nervous system. To date there have been at least twenty-seven clinical trials for FXS. Considering the trials completed, although minor improvements have been reported with some compounds, none has met the criteria for primary outcomes. It is thus safe to say that all trials were unsuccessful in improving the quality of life for FXS patients, and currently no medication is approved specifically for the disorder. The disappointing yet astounding failure of FXS clinical trials, especially considering the overwhelmingly promising preclinical findings, underscores limitations often faced with translating work from rodents and projecting it to human pathophysiology.

In chapter IV we begun addressing such limitations through the utilisation of brain tissue that has been resected during surgery from patients suffering from intractable epilepsy. During such operations, a piece of generally healthy brain material is removed in order to access and subsequently remove epileptic brain tissue. We have utilised the healthy brain tissue collected to characterise electrical communication in the absence and presence of a pharmaceutical that was extensively tested in FXS patients. Our recordings were performed from cells that generate excitatory or inhibitory signals, thus providing a comprehensive description of how FXS related pharmacology affects human excitatory and inhibitory communication. Our data mirror
those found in rodents, while also highlighting putative cell-subtype specific effects of FXS pharmacology. Collectively, these results add to the existing literature on the effects of FXS pharmacology on neuronal signalling, now for the first time in human neurocircuitry. Furthermore, this work brings to focus a neglected aspect of the FXS pathology, namely the pharmacology’s effect on inhibitory interneurons.

Although surgery resected brain tissue is invaluable to furthering our understanding of the human brain in health and disease, even with this approach limitations still remain. Namely, the majority of surgically resected tissue is derived from patients with mesial temporal sclerosis. As such, studies with the resected tissue are constrained to either temporal lobe physiology or to epileptic hippocampal pathophysiology. To better understand the human brain physiology, tissue from other cortical and subcortical brain areas is imperative. Moreover, brain tissue from patients with neuropsychiatric, neurodevelopmental disorders, and from healthy controls would be required.

In chapter V the first step toward affording such possibilities has been committed, demonstrating feasibility for electrophysiological recordings from post-mortem tissue, and setting the post-mortem delay ceiling under which such an approach can be viable. Our results thus far have proved promising in enabling studies from all brain areas and from virtually all neurological disorders. The utility of this approach can in the future be further expanded, through acquisition of brain tissue from trauma or euthanasia cases. With further characterization of several vital electrophysiological measures, this approach can provide a great leap forward into our understanding of human brain physiology both in health and disease, by affording access to any brain region, neuronal disease, and importantly also to healthy controls.

**Conclusion**

The work described in this thesis provides the first evidence for inhibitory communication deficits in the prefrontal cortex of the mouse model for the Fragile X Syndrome. Furthermore, these changes occur during epochs of postnatal prefrontal cortex development, thereby jeopardizing typical neurocircuit maturation while also providing a window for therapeutic intervention that could have substantial beneficial impact. Assessment of executive functioning in the FXS mouse model did not reveal deficits in sustained attention during early adulthood. However, novelty induced hyperactivity and perseveration were observed that normalised upon familiarisation, indicative of possible benefits of cognitive behavioural therapy in FXS. The first data for the effect that FXS pharmacology has on human pyramidal and interneuron cells are described in this thesis. These data mirror observations in rodents, and aim to enhance our understanding of human physiology in health and disease. Finally, this is the first time that whole-cell recordings from human post-mortem tissue have been described. This opens the possibility of a tremendous leap forward in fundamental and translational research through electrophysiological studies from virtually any brain region and disease.