Project Details	
Project Code	MRCNMH24Br Wilkinson
Title	Understanding neuronal dysfunction in Tuberous Sclerosis
Research Theme	Neuroscience & Mental Health
Summary	Mutations in the TSC1 gene lead to Tuberous Sclerosis (TS), a genetic disorder associated with severe neurological symptoms. However, how mutations in TSC1 affect the brain is not well understood. Using a range of cell culture and in vivo techniques, we will examine how deleting TSC1, or replacing it with mutant versions that cause TS, affect the health and function of neurons. The findings will help inform the future treatment of patients with TS.
Description	Tuberous Sclerosis (TS) is an autosomal dominant genetic disorder characterised by the formation of benign tumours and neurological symptoms, including epilepsy, cognitive disability and autism. TS is caused by mutations in TSC1 and TSC2, which encode regulatory proteins that control the activity of the metabolic master regulator mTORC1. Perturbation of TSC1/2 results in hyperactivity of mTORC1, leading to cellular over-proliferation and tumour formation. Currently very little is known about how loss of TSC1/2 function disrupts neuronal function to drive TS-associated neurological symptoms, and even less is known about how specific patient-derived mutations cause neuronal defects. Answering this question will help clinicians provide accurate prognoses and targeted therapies for patients who present with TS. Using TSC1fl/fl mice we will take a multi-disciplinary approach to understand how TSC1 mutations affect neuronal function, with a view to understanding how different TSC1 mutations underpin the diverse neurological symptoms of TS patients. Specifically, we will ask: 1. How does loss of TSC1 affect neuronal development and signalling? We will prepare primary cortical neurons from early postnatal TSC1fl/fl mice and transduce them with GFP-Cre, to knock out TSC1, or control lentiviruses. We will characterize how loss of TSC1 affects mTORC1 signalling and autophagic flux (BC, AT) and assay changes in neuronal development and survival, including analysis of: • dendritic complexity • excitatory and inhibitory synapse number • the shape and number of dendritic spines • cell viability We will also assess whether pharmacological interventions known to correct dysregulated mTORC1 signalling in TSC-null cell models can correct changes in neuronal function (AT), and perform surface proteome and kinase activity screens to provide a global profile of how TSC1 loss affects neuronal protein sorting and signalling. Alongside, we will collect conditioned media and carry out analysis of extracellular vesicles from control versus KO neurons (ED). These data will be correlated with severity of other cellular phenotypes as a potential avenue to identify biomarkers of TS. 2. How do TSC1 disease variants affect neuronal development and signalling? We will investigate how replacing endogenous TSC1 with disease-causing mutations affects the TSC1-dependent parameters we identify. To do this, we will delete endogenous TSC1 and reconstitute with either WT or well-characterised TSC1mutants using lentiviruses. Given the pleiotropic effects of different TSC1 mutations, we anticipate that these mutants will differentially affect the parameters identified above, providing

	insight into the cellular mechanisms underlying the diverse neurological phenotypes they cause. 3. What are the effects of TSC1 disease variants on neuronal function in vivo? Aims 1 & 2 will establish the neuronal impact of various TSC1 disease-causing mutations in vitro. We will then examine these effects in vivo using TSC1fl/fl mice. GFP-Cre AAVs will be injected stereotaxically for knock out studies, or co-injected with AAVs to re-express WT TSC1 or disease mutants in Cre-transduced cells. Experimental assays will be informed by Aims 1 & 2, but will include immunohistochemical analysis of neuronal morphology and protein localization, and electrophysiological measurements of neuronal excitability and synaptic connectivity (PA). The successful candidate will establish a pipeline to profile the effects of TSC1 loss in neurons and provide input to the experimental design across all aims, for example prioritising analysis of key signalling pathways, or cellular phenotypes. Alongside, interventions shown to restore signalling and/or neuronal function in vitro will be tested in vivo. Ultimately, this information will inform future therapeutic strategies designed to target the specific cellular defects caused by individual TSC1 mutations.
Supervisory Team	
Lead Supervisor	
Name	Dr Kevin Wilkinson
Affiliation	Bristol
College/Faculty	Life Sciences
Department/School	Physiology, Pharmacology and Neuroscience
Email Address	kevin.wilkinson@bristol.ac.uk
Co-Supervisor 1	
Name	Dr Paul Anastasiades
Affiliation	Bristol
College/Faculty	Health Sciences
Department/School	Translational Health Sciences, Bristol Medical School
Co-Supervisor 2	
Name	Dr Bernadette Carroll
Affiliation	Bristol
College/Faculty	Life Sciences
Department/School	Biochemistry
Co-Supervisor 3	
Name	Professor Andrew Tee
Affiliation	Cardiff
College/Faculty	Biomedical and Life Sciences
Department/School	Medicine