Table 1: **Gaps in knowledge and requirements to advance knowledge**

A summary of outcomes of discussion groups at the workshop are outlined. Several key mechanisms, the gaps in knowledge and requirements for early advances in knowledge are highlighted. (Abbreviations: AD, Alzheimer's disease; BBB, blood brain barrier; CBF, cerebral blood flow; CSF, cerebrospinal fluid; DTI, Diffusion Tensor Imaging; EEG, electroencephalogram; ISF, interstitial fluid; MRI, Magnetic Resonance Imaging; MMP, matrix metalloproteinase; NVU, neurovascular unit; SVD, small vessel disease; TIMPs, tissue inhibitor of metalloproteinase; VCI, Vascular Cognitive Impairment; VaD, Vascular Dementia)

Mechanism	Gaps in knowledge	Requirements for early advances in knowledge
Common to all mechanisms	• The most relevant model for human SVD is unclear • Regional variation in CBF, in vulnerability to low flow, hypoxia or inflammation, and in flow-metabolism coupling, in grey and white matter (periventricular and deep), in normal tissue and in SVD, is not well established. • Influence of regional differences (e.g. in arteriolar morphology between lenticulostriate and cortical perforating arterioles) in microvessel and perivascular structures on lesion development is poorly understood.	• Use different models to recapitulate components of SVD appropriate to the specific research question • Better in vitro and in vivo models of BBB function and dysfunction, including under conditions of shear stress, in cell co-cultures (3D >2D) and including the glycocalyx • A reliable 'neuro-glio-vascular unit on a chip' would help accelerate understanding of pathophysiological mechanisms, identify targets and allow high throughput testing of potential interventions; • Better molecular imaging probes to study different NVU cell types, determine molecular tissue changes and probe inflammatory processes; • Use larger mammalian models, eg well characterised porcine models, since rodents have low white matter:grey matter ratio.
BBB	• A dynamic complex structure whose dysfunction is a common feature of neurological diseases including cerebral SVD and related dementia syndromes (e.g. AD and VCI/VaD). • Lack of understanding of: a) how cellular morphology relates to function b) the temporal changes and sequence of damage c) how BBB dysfunction leads to brain parenchymal lesions.	• Core set of reference standard techniques for preclinical, neuropathological and clinical studies; • *In vivo* and *in vitro* methods to model small and transient BBB changes; • More sensitive neuropathological methods to identify WMH as seen on MRI; • New molecular imaging probes for pericytes and endothelial cell function status; • More use of the retina routinely in rodent models (as in human SVDs) could help visualise arteriolar/venular, retinopathic and nerve fibre layer changes that could advance understanding of brain changes in rodent models (as suggested in human SVDs); • Alternatives to gadolinium for human in vivo BBB imaging.
Hypoperfusion and ischaemia	• Often poor characterisation in models • Poor understanding of the influence of structural differences in perforating arterioles in different brain regions and arterial territories. • The primary trigger, whether inflammation,	• Longitudinal investigations in models • Better characterisation of tissue metabolic changes (eg pO2, glucose, metabolites) (particularly white matter); • More standard use of terminology for SVD in in vivo models and neuropathology, and for studying components of NVU in vitro would help, as

	altered flow, other, and their interactions. • Is flow-metabolism coupling altered in SVD?	has been implemented in clinical studies [3]
Inflammation	• How do microglia, pericytes, perivascular macrophages, fibroblasts, oligodendrocytes all interact to maintain homeostasis? • Are soluble inflammatory mediators (complement, chemokines, cytokines) involved in perivascular inflammation in SVD? • What is the role of fibrosis in SVD? • How is the matrix environment perturbed to influence cell function and signalling?	• Determine the contribution of inflammation in SVD and define whether there is a classic innate response and/or contribution of systemic inflammation or both • Undertake longitudinal studies across models to profile microglial proliferation and migration and the broader molecular/cellular neuroinflammatory environment including measures of circulating immune cells. • In relation to above a wider investigation of the 'matrisome'
White matter: axons, myelin sheaths	• The role of oligodendrocytes and myelinated axons in the pathogenesis of SVD is unclear as is their interaction with other cells, such as endothelial cells. • The extent of damage to myelinated axons in cortical grey matter in SVD is poorly defined • The relationship of demyelination and axonal loss to CBF changes in SVD is unclear	• Comparative studies of potential SVD mechanisms across models, species, and diseases of white matter that have potential similarities to SVD (eg multiple sclerosis) using the most innovative molecular, cellular and imaging approaches • Use MRI/DTI of white matter status as routinely as possible to link in vivo to tissue pathology more clearly; • Link white matter pathology stages and burden to vascular pathology load and CBF alterations more clearly • Investigate integrity of architecture of myelinated axons in cortical grey matter and white matter
Perivascular and interstitial fluid drainage	• To what extent are rodents relevant models of human drainage pathways and mechanisms? • What is the driving force for ISF clearance: a) vasomotion of cortical arteries?; b) perivascular innervation? c) does the smooth muscle action in veins have a functional role? • How does ISF clearance relate to SVD development or progression? • Does extracellular remodelling vary during the sleep-wake cycle?	• Determine how CSF and ISF and clearance pathways all relate to each other; • Determine if ISF and perivascular drainage vary with sleep-wake cycle; • Better methods to detect perivascular and ISF drainage in humans • Can CSF or ISF drainage pathways be targeted to inform treatment for SVD? • Does targeting of matrix proteins (~~TIMP3~~ TIMPs or MMPs) or vasomotion increase drainage?
Neuro-glia-Vascular mechanisms	• How do vascular changes drive glial and neuronal dysfunction and damage?	• Target white matter more in rodents; • Standardise anaesthetics and stimulation procedures; • Identify better ways to measure neuronal responses than blood oxygen dependent (BOLD) and related imaging methods (arterial spin labelling, blood volume measures, O2 metabolism, EEG and magnetoencephalography);

		• Expand cohort imaging similar to that achieved in the AD Neuroimaging Initiative; • Make more use of optogenetics and genetically encoded calcium indicators.