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NEUROSCIENCE AND NEUROTECHNOLOGY

Principal Investigator
Professor Owen Ko

Team Members
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Research Progress Summary

Over the period of 2019 January 1 – 2020 December 31, the research team led by Professor Owen Ko focused on three closely related research themes in neuroscience, namely, (i) glial and vascular dysfunction in neurological diseases, (ii) neuroimaging tools development, and (iii) neural circuits mediating sensorimotor behaviour in health and disease. Their major research accomplishments over the past two years are summarised below.

Glial and Vascular Dysfunction in Neurological Diseases

The fundamental mechanism for maintaining central nervous system homeostasis is accomplished by the cohesive functioning of different cells, including vascular cells (e.g. endothelial cell, smooth muscle cell and pericyte) and glial cells (e.g. astrocyte, microglia and oligodendrocyte). Each of the brain cell types serves its specific yet intertwined physiological roles. Over the past decades, accumulating evidence points to the causal roles of vascular and glial cell dysfunction in various neurodegenerative diseases. The team adopts a multimodal approach combining molecular assays, *in vivo* functional and structural imaging, as well as single-cell transcriptomic profiling to uncover how ageing and neurodegenerative disease-related genetic mutations culminate in neurovascular and glial dysfunction. Highlights of core output under this theme are as follows:

a. Uncovering arteriovenous axis zonation-dependent endothelial cell transcriptomic changes in the aged brain and demonstrating its reversibility (*Zhao et al., Nature Communications, 2020 Sep 4;11(1):4413*). In this work, they revealed that in the aged brain, the transcriptomic changes in endothelial cells depend on their subtypes, implying that the endothelium of different segments of the brain vasculature age differently. The expression changes are especially prominent in the capillary bed, and encompass important functional pathways impacting (i) blood-brain barrier (BBB) regulation, (ii) immune/cytokine signalling, (iii) energy metabolism and (iv) Alzheimer's disease (AD) pathogenesis. Importantly, they showed that some key expression changes with significant functional implications at the BBB in the aged mouse brain also generalise to human AD patients. They went on to demonstrate a practical pharmacological approach for reversing the ageing-associated vascular changes, whereby treatment with exenatide, a glucagon-like peptide 1 receptor (GLP-1R) agonist (GLP-1RA), can partially reverse the age-related differential expressions and strongly reduce age-related BBB leakage in the mouse brain endothelium. Their study thus offers novel insights into neurovascular ageing, neurodegenerative diseases and therapeutics development. Apart from the peer-reviewed publication, they have presented this work at the Society for Neuroscience (SfN) Annual Meeting 2019. They have also been invited to share the findings at the Diabetes Preventing the Preventables (DPP) forum 2020 and the 2nd Annual Conference of the Society of Cerebral Small Vessel Diseases, Chinese Stroke Association and Tiantan International Summit Symposium Cerebral Small Vessel Diseases 2020.

b. Demonstrating genome-wide reversal of glial and neurovascular cell transcriptomic ageing signatures by GLP-1R agonism (*Ko et al., US Provisional Patent Application, 2020 Dec 16;US63/126,122; Li Z et al., manuscript under review; preprint: bioRxiv, 2020 Dec 22;doi:10.1101/2020.12.21.423879*). Pharmacological reversal of brain ageing is a long-sought yet challenging strategy for the prevention and treatment of age-related neurodegeneration, due to the diverse cell types and complex cellular pathways impacted by the ageing process. As a follow-up work inspired by their previous finding that the age-related differential expressions in the brain endothelium can be partially reversed by GLP-1RA, they extended the pharmaco-transcriptomics approach to other neurovascular and glial cells whose age-related expression changes also play crucial roles in brain ageing and degeneration. Remarkably, they found that GLP-1RA treatment in fact results in genome-wide reversal of transcriptomic ageing signatures in multiple major brain cell types, including glial and mural cells. The age-related expression changes reversed by GLP-1RA are especially prominent in some glial cell types, such as astrocytes and microglia, and encompass both shared and cell type-specific functional pathways that are implicated in ageing and neurodegeneration. Concomitantly, AD-associated transcriptomic signature in microglia that arises from ageing is reduced.

c. Knowledge transfer and further developments: With the ever-increasing interest in the development of GLP-1RAs for neurological diseases, their studies have the following important practical implications: (i) they revealed novel mechanistic insights into the neurological benefits of GLP-1R agonism in diabetic patients that are beyond improving glycemic control, and (ii) they showed that GLP-1R agonism may be a

generally applicable therapeutics for patients at risk of age-related neurodegeneration (diabetic or not). To further translate these findings to applications, apart from (1) the provisional patent application filed by The Chinese University of Hong Kong (CUHK), they are also (2) initiating a clinical trial testing the efficacies of GLP-1RA in the treatment of age-related vasculopathy and associated cognitive impairment (*clinical trial ethics approval obtained*), (3) planning further mechanistic studies on the cellular target(s) of GLP-1RAs in the ageing brain mediating the treatment effects, and (4) exploring whether other anti-ageing pharmacotherapies may be possible by targeting the brain GLP-1R neuroendocrine signalling axis.

Under the theme, apart from these core publications and output, Prof. Owen Ko and his team members have also contributed to numerous other publications as contributing authors over the period. They are also honoured that based on this research theme, they have won funding support from the Croucher

Foundation, via a Croucher Innovation Award (2020) to Prof. Owen Ko.

Neuroimaging Tools Development

Studying the brain requires high-throughput molecular profiling tools, ideally in the three-dimensional context whereby the native spatial information of the molecules of interest is well-preserved. Over the past decade, they have seen rapid developments in tissue clearing techniques, which however are still limited in their applications due to a couple of factors, including (1) limited attainable depth of immunostaining, and (2) a lack of a clearing method that is simultaneously simple (immersion-only), fast (rapid clearing of whole-brain scale samples in hours) and offers high compatibility with other imaging modalities (e.g. RNA fluorescent in situ hybridisation, electron microscopy). They have therefore developed two new techniques, which they term thermo-immunochemistry with optimised kinetics (ThICK) and optical properties-adjusting tissue-clearing agent 2 (OPTIClear2) respectively, which are introduced below.
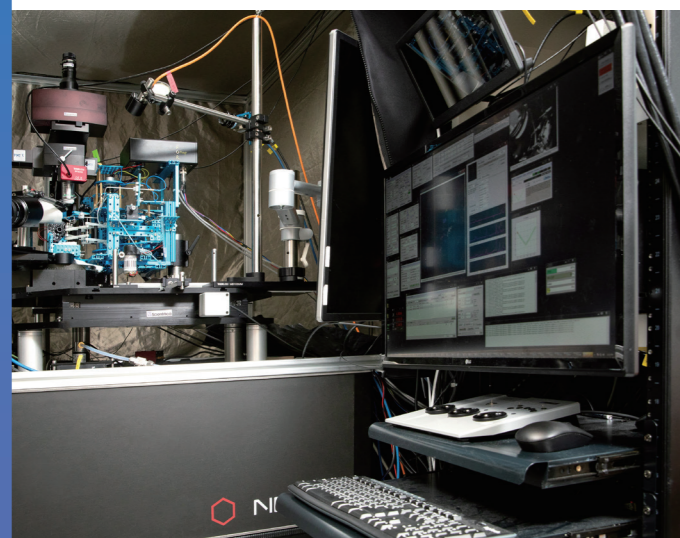
A general method for the thermostabilisation of antibodies and its applications in achieving fast, deep immunostaining by ThICK staining (*Lai & Ko, US Provisional Patent Application, 2020 May 21;US 63/028,022; Lai et al., manuscript in preparation*). They developed a new heat-facilitated method ThICK for achieving deep and fast immunostaining in thick biological tissue samples. ThICK is based on the principle that under a higher temperature, (i) there is faster antibody diffusions into deep tissue, and (ii) antibody-antigen binding is not favoured, thereby reducing antibody depletion when diffusing into a tissue. However, antibodies undergo denaturation and lose their antigen-binding property at high temperatures, therefore a heat-facilitated deep staining strategy is only viable when the antibodies can be thermostabilised. They demonstrated that by crosslinking with a multi-functional polymer and using

the Fab fragment of secondary antibodies as a conformation-stabilising chaperone, a variety of off-the-shelf primary antibodies can be conferred a high degree of heat resistance while retaining their antigen-binding capabilities. They demonstrated the applications of these processed antibodies, which they term synergistically protected polyepoxide-crosslinked Fab-complexed antibody reagents (SPEARs), for ThICK-based deep immunostaining with 15 commercially available antibodies. These include multiple neuronal subtypes, synaptic and neuromodulator markers. During this project, they have established collaboration with the Sainsbury Wellcome Centre for Neural Circuits and Behaviour of University College London for the application of SPEARs in high-throughput brain ThICK imaging. The preprint deposited will also be submitted for full publication soon.

Development of a new chemical cocktail for ultrafast clearing of biological tissues (*Lai & Ko, US Provisional Patent Application, 2020 Jun 3;US 63/029,582; Lai et al., manuscript in preparation*). They developed a new immersion-only chemical cocktail OPTIClear2 that is capable of rapid intact tissue clearing. OPTIClear2 has substantial improvements over its precedent OPTIClear and other state-of-the-art methods, including (i) faster tissue clearing time than even the fastest reported techniques (e.g. FDISCO, FOCM), (ii)
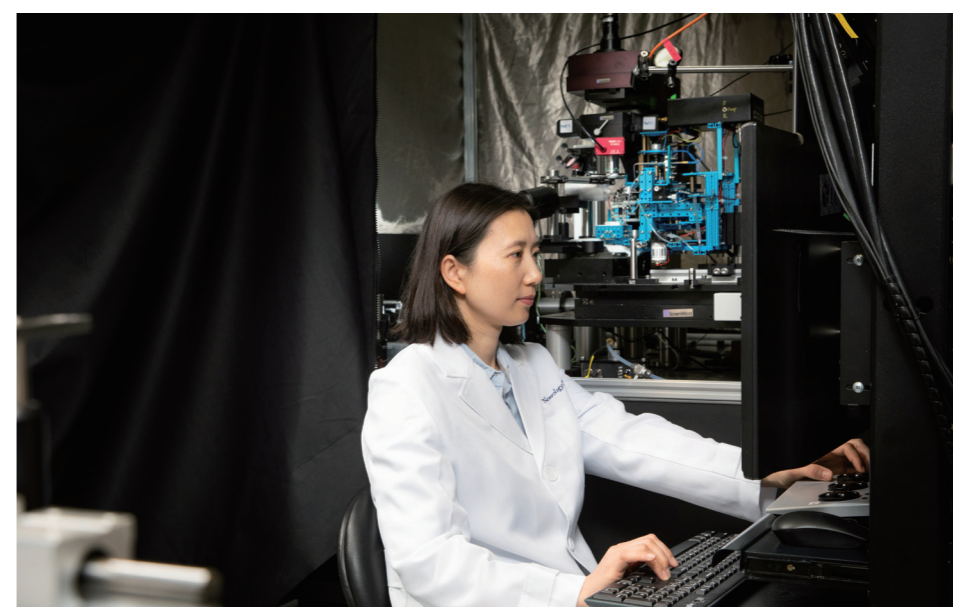
no distortion of tissue architecture, (iii) high endogenous molecules (i.e. proteins, mRNA) preservation, (iv) compatibility with multiple-round staining and imaging, (v) unique compatibility with lipophilic tracing, as well as (vi) permitting subsequent histological and pathological examination by other imaging methods (e.g. electron microscopy). They have tested OPTIClear2 on various tissues, including mouse nervous system, human archived / formalin-fixed brain samples, and fresh fixed squamous cell carcinoma biopsy samples. OPTIClear2 is also fully compatible with ThICK and SPEARs, thereby empowering their pipeline for investigations of the brain in both normal and diseased conditions. They are currently obtaining more data to substantiate the full patent application, and a manuscript is also under preparation.

Knowledge transfer and further developments: Based on this series of works, apart from the filed patent applications, they have won a Midstream Research Programme grant (HKD 4.7M, starting 2021 Mar 1) for further translational development. In the upcoming year, Dr. Hei-ming Lai, who led this line of research in the team, will establish his new independent laboratory at the Li Ka Shing Institute of Health Sciences. Prof. Owen Ko will maintain a close collaboration with Dr. Lai on the further development of the technologies for neurodegeneration, neural circuitry and human neuropathology applications.

Neural Circuits Mediating Sensorimotor Behaviour

They are currently investigating neural circuits mediating sensorimotor behaviour in two model systems:

Danio rerio (Zebrafish) larvae (*Sy SKH et al., manuscript in preparation*). Several days post-fertilisation, larval zebrafish can already perform an incredible repertoire of innate behaviours including chemosensory behaviour, alongside phototaxis, oculomotor and optokinetic responses, prey capture and various escape responses. As a model organism for neuroscience, larval zebrafish offers the distinct advantages of relatively high optical transparency and more tractable number of neurons in the brain (in the order of 10^5), permitting the interrogation of brainwide activities mediating sensorimotor transformation with cellular resolution. They are interested in fundamental questions of how neural circuits in the brain process survival-relevant sensory cues to drive motor response. To this end, they have thus constructed an integrated microfluidics-behavioural and brainwide imaging system, to precisely manipulate the sensory environment of zebrafish larvae while performing volumetric whole-brain imaging of neuronal activities.

In both vertebrates and invertebrates, sensory organs often come in pairs with lateral anatomical locations. Despite well-known chemosensory avoidance behaviours in various species, how bilateral olfactory inputs are integrated to guide these behaviours remains unknown. Utilising the system, in their recent work, they uncovered that the navigational strategies that larval zebrafish employs to efficiently escape noxious chemical zones require bilateral olfactory input. By brainwide neuronal activity imaging, they found that a distributed neural representation characterised by a dichotomy of on/off responses, continua of left/right input selectivity and summation linearity emerge in the early olfactory pathway and diverse forebrain regions. These results revealed the neural basis of paired sensory organ input integration that drives sensorimotor transformation for avoidance navigation. They have presented their findings at the SfN Annual Meeting 2019 and they will submit a manuscript for peer review soon.

Mus musculus (Mouse): To survive in the wild, animals must be able to associate sensory cues with the need to perform corresponding motor responses. These processes involve coordinated neural activity and signal flow across primary sensory, association and motor areas. Often, sensorimotor tasks also necessitate the acquisition of novel motor sequences from an existing motor repertoire. To understand sensorimotor learning, they must uncover how neuronal populations across different brain regions encode various aspects of task-relevant information, such as sensory features and predicted outcomes, and instruct the generation of complex sequences of motor commands.

In their laboratory, they have developed fully automated behavioural and stimulation platforms on which mice learn to execute complex forelimb motor and general locomotor tasks in response to visual cues, while neuronal activities are recorded from different cortical areas by multiphoton microscopy. Combined with circuit tracing techniques, they are currently studying the rules governing the encoding of distinct task-related information along pathways connecting higher visual areas to association and motor areas. Although this line of works was severely impacted by the pandemic, as a key piece of their key equipment (a Ti:sapphire femtosecond laser) was stuck for repair in California for seven months (from 2020 Jan to July), they have now fully resumed experimentation and making progress again.

Other Collaborations

They actively collaborate with both local and overseas academic researchers in other leading institutes, including (i) Professor Francis Szele and Hagan Bayley at the University of Oxford (with a joint publication *Zhou et al., Advanced Materials, 2020 Jun 14;e2002183*). (ii) Professor Yamei Tang and Wei-jye Lin at Sun Yat-Sen University (who contributed to *the Nature Communications* publication from their group this year), and (iii) Professor Nancy Ip at the Hong Kong University of Science and Technology, with whom they are working on a collaborative project on elucidating the neuronal and astrocytic calcium signalling dynamics in Alzheimer's disease, under a Collaborative Research Fund grant and an Area of Excellence Scheme grant from the Research Grants Council for which Prof. Owen Ko is a Co-PI for both grants.

Research and Scholarship

Research Awards and Recognitions

Member's Name	Details	
	Award	Organisation
Owen Ko	Croucher Innovation Award	The Croucher Foundation

Fellowships

Member's Name	Details	
	Fellowship	Organisation
Owen Ko	Member	The Nexus of Rare Neurodegenerative Diseases (NRND)
		The Society for Neuroscience (SfN), US
		The Hong Kong Epigenomics Project (EpiHK)

Academic Editorship

Member's Name	Details	
	Role	Journal
Owen Ko	Review Editor	Frontiers in Neuroscience
		Frontiers in Neurology
		Frontiers in Psychiatry

Reviewer of Journal / Conference

Member's Name	Details	
	Role	Journal / Conference
Owen Ko	Grant Reviewer	Medical Research Council, UK
	Manuscript Reviewer	Nature Communications
		Stem Cells
		Molecular Neurobiology
		Neural Regeneration Research
		Laboratory Investigation
		Neuroscience Letters
		BMC Genomics
		Biology Open

Grants and Consultancy

Name	Project Title	Funding Source	Start Date (dd/mm/yyyy)	End Date (dd/mm/yyyy)	Amount (HK$)
Owen Ko	Investigating Neurovascular and Astrocyte Dysfunction in Neurodegenerative Diseases – Matching Fund	Research Grants Council – Research Matching Grant Scheme	01/06/2020	31/05/2021	500,000
	Investigating Neurovascular and Astrocyte Dysfunction in Neurodegenerative Diseases	The Croucher Foundation	01/06/2020	31/05/2025	5,000,000
	The Role of IL33 in Synaptic Dysfunctions and Pathogenesis of Alzheimer's Disease	Research Grants Council – Collaborative Research Fund	01/04/2020	31/03/2023	5,586,951
	A Pilot Test in Search for Novel Plasma Biomarkers in REM Sleep Behavior Disorder, a Prodromal Stage of Alpha-Synucleinopathy Neurodegeneration, in a Prospective Family Cohort	CUHK Research Committee – Direct Grant	30/06/2020	29/06/2021	149,200
	Novel Carbobicyclic Nucleosides and Repurposed Drugs for the Treatment of SARS-CoV-2 Infection	The Chinese University of Hong Kong – Faculty of Medicine	12/03/2020	11/03/2021	1,050,000
	Elucidating the Cellular Functions of the Spinocerebellar Ataxia-Causing Gene CCDC88C and Its Pathogenic Roles in Neurological Conditions	CUHK Research Committee – Funding for Research Sustainability of Major Research Grants Council Funding Schemes	27/05/2019	30/06/2021	500,000
Owen Ko Hei-ming Lai	Establishing a Subcellular Spatial Transcriptomics Platform for Neuropsychiatric and Biomedical Research Applications	CUHK Research Committee – Academic Equipment Grant	27/02/2020	30/08/2020	625,000

Publications

A. Journal Papers

1. Li Z, Chen X, Vong JSL, Zhao L, Huang J, Yan LYC, Ip B, Wing YK, Lai HM, Mok VCT, Ko H. Genome-wide reversal of glial and neurovascular cell transcriptomic aging signatures by GLP-1R agonism. *bioRxiv*. 2020. doi:10.1101/2020.12.21.423879. (In Press)

2. Au CKF, Abrigo J, Liu C, Liu W, Lee J, Au LCW, Chan Q, Chen S, Leung EYL, Ho CL, Ko H, Mok VCT, Chen W. Quantitative susceptibility mapping of the hippocampal fimbria in Alzheimer's disease. *Journal of Magnetic Resonance Imaging*. 2020. doi:10.1002/jmri.27464. (Epub ahead of print)

3. Zhao L, Li Z, Vong JSL, Chen X, Lai HM, Yan LYC, Huang J, Sy SKH, Tian X, Huang Y, Chan HYE, So HC, Ng WL, Tang Y, Lin WJ, Mok VCT, Ko H. Pharmacologically reversible zonation-dependent endothelial cell transcriptomic changes with neurodegenerative disease associations in the aged brain. *Nature Communications*. 2020;11(1):4413. doi:10.1038/s41467-020-18249-3.

4. Mok VCT, Pendlebury S, Wong A, Alladi S, Au L, Bath P, GJ, Chen C, Cordonnier C, Dichgans M, Dominguez J, Gorelick PB, Kim SY, Kwok T, Greenberg SM, Jia J, Kalaria R, Kivipelto M, Naegandran K, Lam LCW, Lam BYK, Lee ATC, Markus HS, O'Brien J, Pai MC, Pantoni L, Sachdev P, Skoog I, Smith EE, Srikanth V, Suh GH, Wardlaw J, Ko H, Black SE, Scheltens P. Tackling challenges in care of Alzheimer's disease and other dementias amid the COVID-19 pandemic, now and in the future. *Alzheimer's & Dementia*. 2020;16(11):1571-1581. doi:10.1002/alz.12143. (Review)

5. Zhou X, Chen Y, Ip FCF, Lai NCH, Li YYT, Jiang Y, Zhong H, Chen Y, Zhang Y, Ma S, Lo RMN, Cheung K, Tong EPS, Ko H, Shoai M, Mok KY, Hardy J, Mok VCT, Kwok TCY, Fu AKY, Ip NY. Genetic and polygenic risk score analysis for Alzheimer's disease in the Chinese population. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*. 2020; 12(1):e12074. doi:10.1002/dad2.12074.

6. Zhou L, Wolfes AC, Li Y, Chan DCW, Ko H, Szele FG, Bayley H. Lipid-bilayer-supported 3D printing of human cerebral cortex cells reveals developmental interactions. *Advanced Materials*. 2020;32(31):2002183. doi:10.1002/adma.202002183. (Editorial)

B. Book Chapter

1. Ko H, Lam BYK, Mok VCT. Pathophysiology of Vascular Cognitive Impairment (II): Amyloid Contribution in Vascular Cognitive Impairment. In: *Springer*, Singapore; 2020:87-97. doi:10.1007/978-981-10-1433-8_8.

C. Poster Presentation

1. Liu W, Au LWC, Abrigo J, Luo Y, Wong A, Kwan P, Ma AHW, Ng AYT, Chen S, Leung EYL, Ho CL, Chu WCW, Ko H, Shi L, Mok VCT. MRI-based automated volumetric segmentation tool in the detection of early Alzheimer's disease. *Alzheimer's & Dementia*. 2020;16(S5). doi:10.1002/alz.042340.
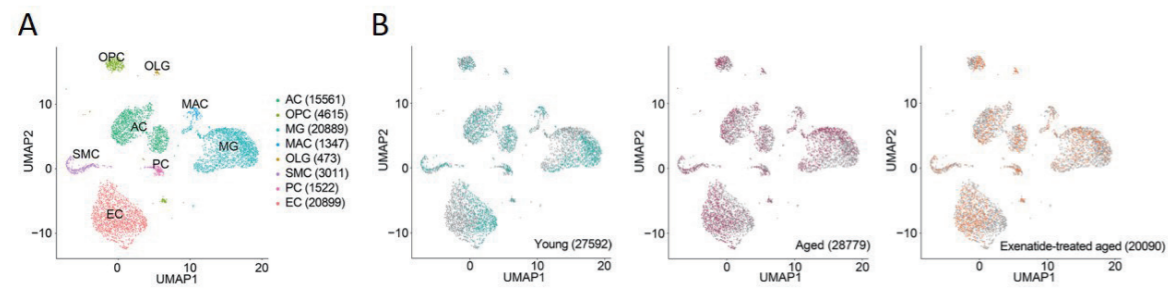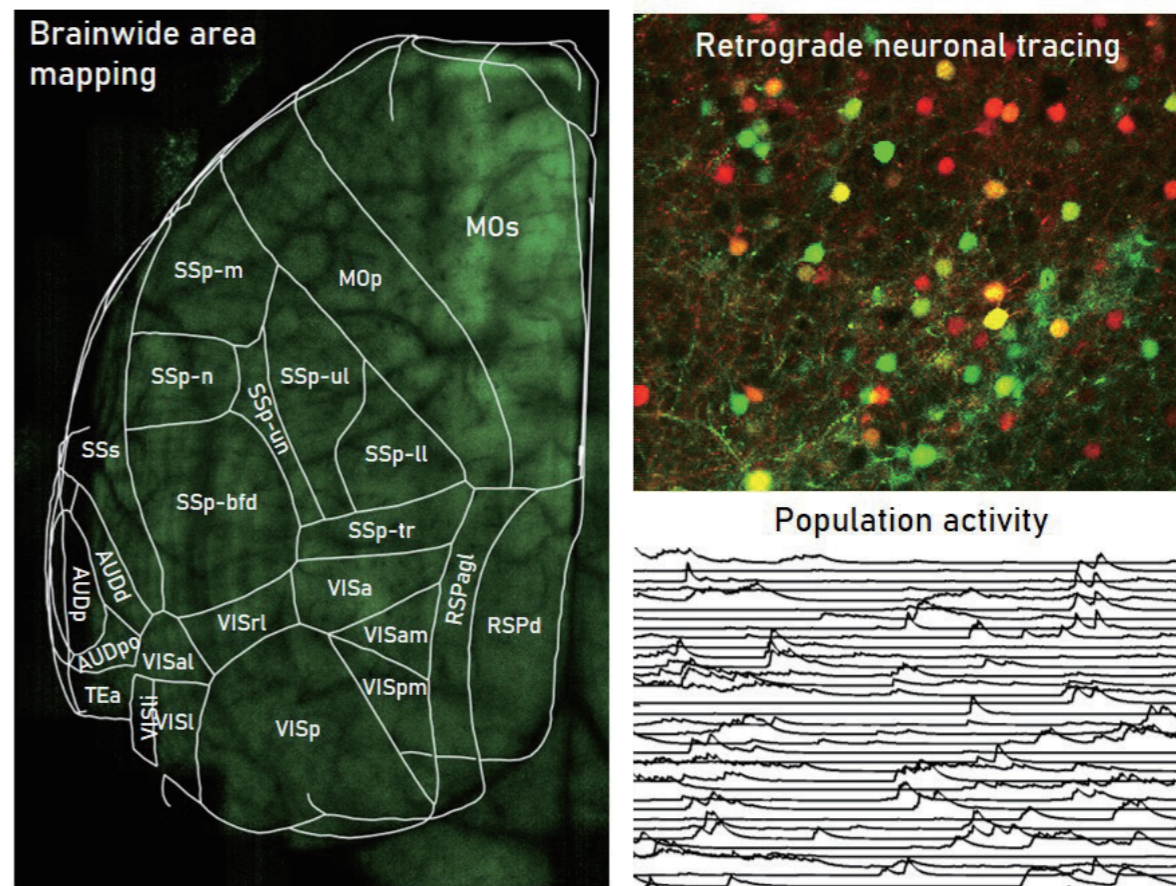
D. Patents

1. Ko H, Huang J, Jin YL, Li Z, Mok VCT. Methods for the treatment of Niemann-Pick disease type C. 2020 December 24; US63/130,213.

2. Ko H, Li Z, Chen X, Vong JSL, Zhao L, Huang J, Yan LYC, Lai HM, Mok VCT. A method for reversing aging brain functional decline. 2020 December 16; US63/126,122.

3. Lai HM, Ko H. Efficient antibody DNA-barcoding reagents for multiplexed molecular imaging. 2020 June 18; US 63/040,557.

4. Lai HM, Ko H. Efficient and effective tissue clearing agents and their compositions. 2020 June 3; US 63/029,582.

5. Lai HM, Ko H. Thermodynamically stabilized antibodies for deep immunolabeling and tissue imaging. 2020 May 21; US 63/028,022.

Single-cell transcriptomic profiling for assaying drug effects

(A) UMAP visualisation of the single-cell transcriptomes of eight major brain cell types from young adult and aged mouse brains. Numbers in brackets: cell numbers for the respective cell types. Abbreviations: AC: astrocyte; OPC: oligodendrocyte precursor cell; MG: microglia; MAC: perivascular macrophage; OLG: oligodendrocyte; SMC: smooth muscle cell; PC: pericyte; EC: endothelial cell. (B) UMAP visualisation of the single-cell transcriptomes from young adult (left panel), aged (middle panel), and GLP-1RA-treated aged (right panel) mouse brains. For each plot, colored dots highlight cells from the respective labelled group, while grey dots are cells from the other groups. Numbers in brackets: cell numbers for the respective groups (n = 3 animals for each group). For clarity, 6000 cells were subsampled for visualisation in each plot in (A) and (B).
Image credit: Dr. Richard Li
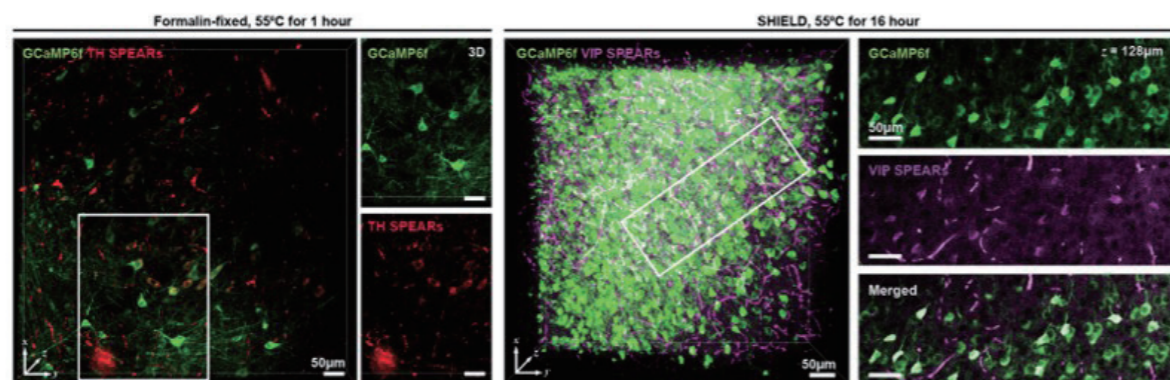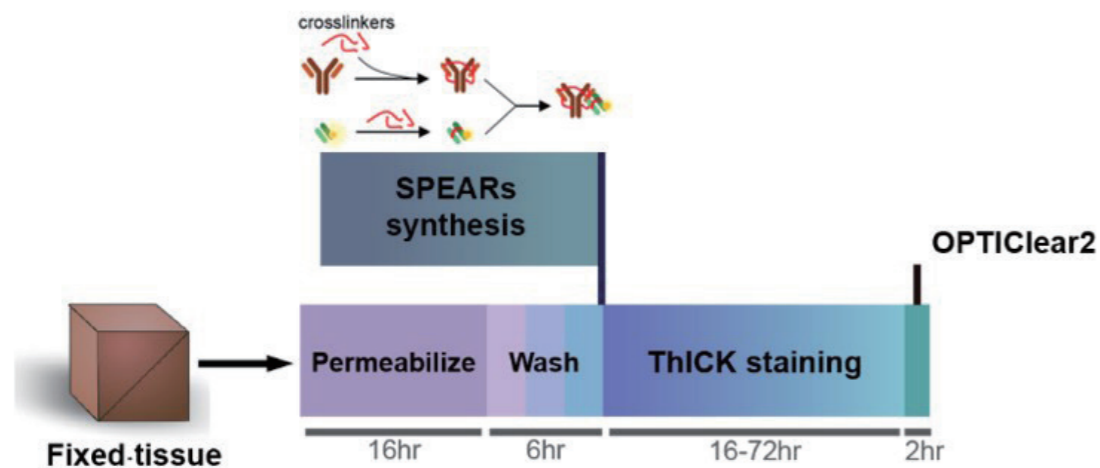
Source: Professor Owen Ko's laboratory

ThICK-staining using SPEARs

(A) The workflow of ThICK staining. SPEARs are made by complexation with a conformation chaperone and crosslinking with a polymer. They can then be used as typical antibodies while permitting high-temperature thermal cycling-accelerated deep immunostaining. (B) Examples of fixed tissues stained and imaged by ThICK-staining using SPEARs. Left panel: tyrosine hydroxylase (TH) staining (red) showing dopaminergic neurons (co-expressing GCaMP6, green). Right panel: vasoactive intestinal peptide (VIP) staining (red) showing VIP-expressing interneurons (co-expressing GCaMP6, green).
Image credit: Dr. Hei-Ming Lai

Source: Professor Owen Ko's laboratory

Techniques for studying neural circuits

(A) Identification of cortical areas by automated *ex vivo* imaging of mouse brain, image registration and anatomical reconstruction. (B) Cortical neurons imaged *in vivo* under two-photon microscopy labeled by fluorescent proteins via rAAV2 transfection, showing neurons with single- (red or green) or dual-target area projections (yellow). (C) Activities of a neuronal population during a sensorimotor behavioural task extracted from *in vivo* two-photon calcium imaging.
Image credit: Dr. Danny Chan

Source: Professor Owen Ko's laboratory