A chemical biology approach to explore lipid metabolism in neurological disorders

Vliet, D. van der

1

General introduction

Cells of the human brain

The brain enables all animals, including humans, to experience, explore and interact with the external world and our internal selves. The brain gives rise to our consciousness, perceptions, senses, actions, emotions and memories. How the brain is able to achieve all these high-level functions is still largely a mystery, but humanity is slowly learning more about itself through the science that focuses on the function of the brain, called neuroscience.

The human brain contains an estimated 86 billion neurons[1], cells that communicate with each other and send their signals to the rest of body. The neuron as the fundamental unit of the nervous system was established based on the works of Spanish neuroscientist Santiago Ramón y Cajal around 1891[2,3]. At the time, the dominating theory was that the nervous system functioned as a web of interconnected fibers without discrete units. However, detailed histological analysis by Cajal and his students showed that discrete cells could be identified, and the long fibers have discrete endings[4], from where they communicate to other neurons. This junction between two neurons is called the synapse and it is the location where two neurons interact through the release of neurotransmitters[5]. These neurotransmitters are small-molecule chemicals which are released from the presynaptic axon and then detected in the post-synaptic region of the receiving neuron, transferring information from one neuron to the next. Each neuron can have synapses with hundreds of other neurons, forming an intricate network from which complex brain function emerges.

While neurons have historically been credited the most for brain function, the brain contains as many cells that are not neurons: *glia*. Historically viewed as 'glue'[6] their contribution to the brain have long been under appreciated. However, research in the past decades has demonstrated that glial cells are indispensable for a healthy and functional brain. Astrocytes, *star-shaped cells*[7], were the first described glial cells and they are the main facilitators of all chemical processes in the brain, regulating the blood-brain barrier through contact with blood vessels[8], distributing lipids and other chemicals to neighboring brain cells and are even actively involved in synaptic transmission[9]. Oligodendrocytes, *cells with few branches*[10], facilitate fast and long-range communication between neurons through the myelination of neuronal axons[11]. Microglia, *small glia cells*[12], are the immune cells of the brain, a specialized community of macrophages[13] that survey the brain for molecular cues of defects[14]. These cues can induce a plethora of microglial activation states associated with functions that sculpt, protect and clean the brain[15]. For example, microglia prune synapses during development[16,17], remove apoptotic cells[18], sculpt myelin to enhance stability[19], and respond to damage and pathogens[20].

All these cells work together to shape the brain in an intricate anatomy (Figure 1). In human cerebral cortex, neuronal cell bodies reside in *grey matter*, where their dendrites receive inputs from nearby neurons and from neurons in other brain regions. Communication towards other brain regions happens through long-ranging axons, that travel through the *white matter* of the brain (Figure 1). In the white matter, axons are myelinated by oligodendrocytes to provide metabolic support[21], electrical insulation[11], physical protection and an energy reserve[22]. Myelin is a multilamellar membrane structure made up of many lipids and proteins, making the tissue appear whiter (Figure 3). It is organized into segments, produced by distinct oligodendrocytes, leaving small gaps called 'nodes of Ranvier'. These gaps allow the electrical signal to "jump" from node to node in a process called saltatory conduction[11], significantly increasing the speed of signal transmission[23].

Neurodegeneration

As the human body ages, brain function declines. The prevalence and severity of neurodegenerative disease is increasing worldwide. Dementia is the most prevalent neurodegenerative disease with 50-60 million patients currently worldwide, while this is estimated to rise to 130-180 million by the year 2050[24]. In the Netherlands, dementia cases are expected to rise to 0.5 million by 2050, almost a doubling from the current number of patients[24]. This is in part due through improved ability to prevent and treat many other diseases, including infectious diseases and cancer, resulting in increased life expectancy. As age is the primary risk factor for neurodegenerative disease[25], increasing life expectancy comes with an increased burden of neurological disorders for humans and society[26]. Thus, while diseases of the brain are currently already a societal challenge, this will become an even larger burden in the future. The development of new therapeutic strategies to prevent, manage and treat neurological disorders is of pivotal importance to sustain high quality of life and a prospering society.

The most common form of dementia is Alzheimer's disease, characterized by the accumulation of aggregates of proteins[27], most commonly amyloid-β and hyperphosphorylated Tau which spread around the brain, concomitant with loss of neurons[28,29] (Figure 2). Alzheimer's disease is also characterized by chronic neuroinflammation, in which microglia play an important role as the innate immune cells of the brain[30]. Despite considerable efforts of both academic researchers and the pharmaceutical industry, no effective treatments are currently available to stop the progression of neurodegeneration in Alzheimer's disease.

Neurodegeneration can also affect young people. The most prevalent neurodegenerative disease among young adults is multiple sclerosis (MS)[31]. With currently more than 2.8 million patients worldwide[32] and its lifelong chronic character, MS is a major burden for patients worldwide, for next of kin and for society as a whole. The dysfunction, damage and complete removal of myelin is the hallmark of MS[33] (Figure 2). When myelin is destroyed or damaged, in a process called demyelination, signal transduction through axons slows down or stops altogether, leading to a variety of symptoms such as muscle weakness, coordination problems, and sensory disturbances, but also cognitive problems. Myelin is not just vital for rapid signal transduction but also for protecting and maintaining the health of the underlying axons. Without myelin, axons are more vulnerable to further damage and permanent degeneration. Neuroinflammatory processes observed in Alzheimer's disease play also a key role in driving demyelination in MS[34].

Therapies addressing this inflammatory component, specifically by targeting adaptive immune cell infiltration, are effective in the early stages of MS[35]. However, for the progressive phase of the disease currently no effective therapies are available, which is a critical unmet clinical need[36].

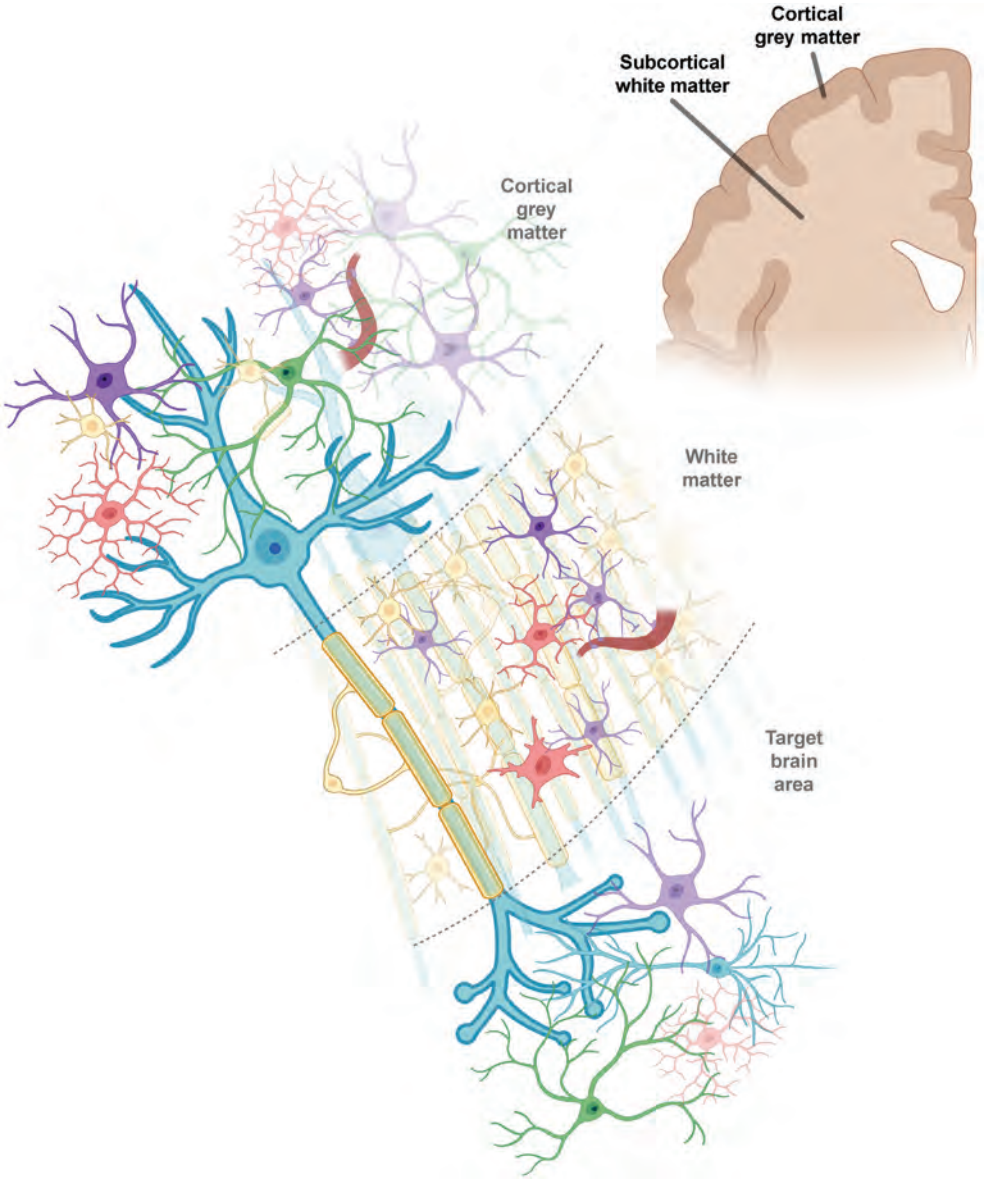

Figure 1. Cellular organization in the human cerebral cortex. Neuronal somas (blue) reside in the grey matter of the cerebral cortex, where they form synapses with nearby neurons (green) and incoming axons from other brain regions. Long-ranging axons travel to target brain regions with long myelinated axons through the white matter, where extensive myelination happens by oligodendrocytes (yellow). In both brain regions, astrocytes (purple) and microglia (red) maintain homeostasis and finetune myelination and synaptic transmission, leading to a balanced and fast working brain.

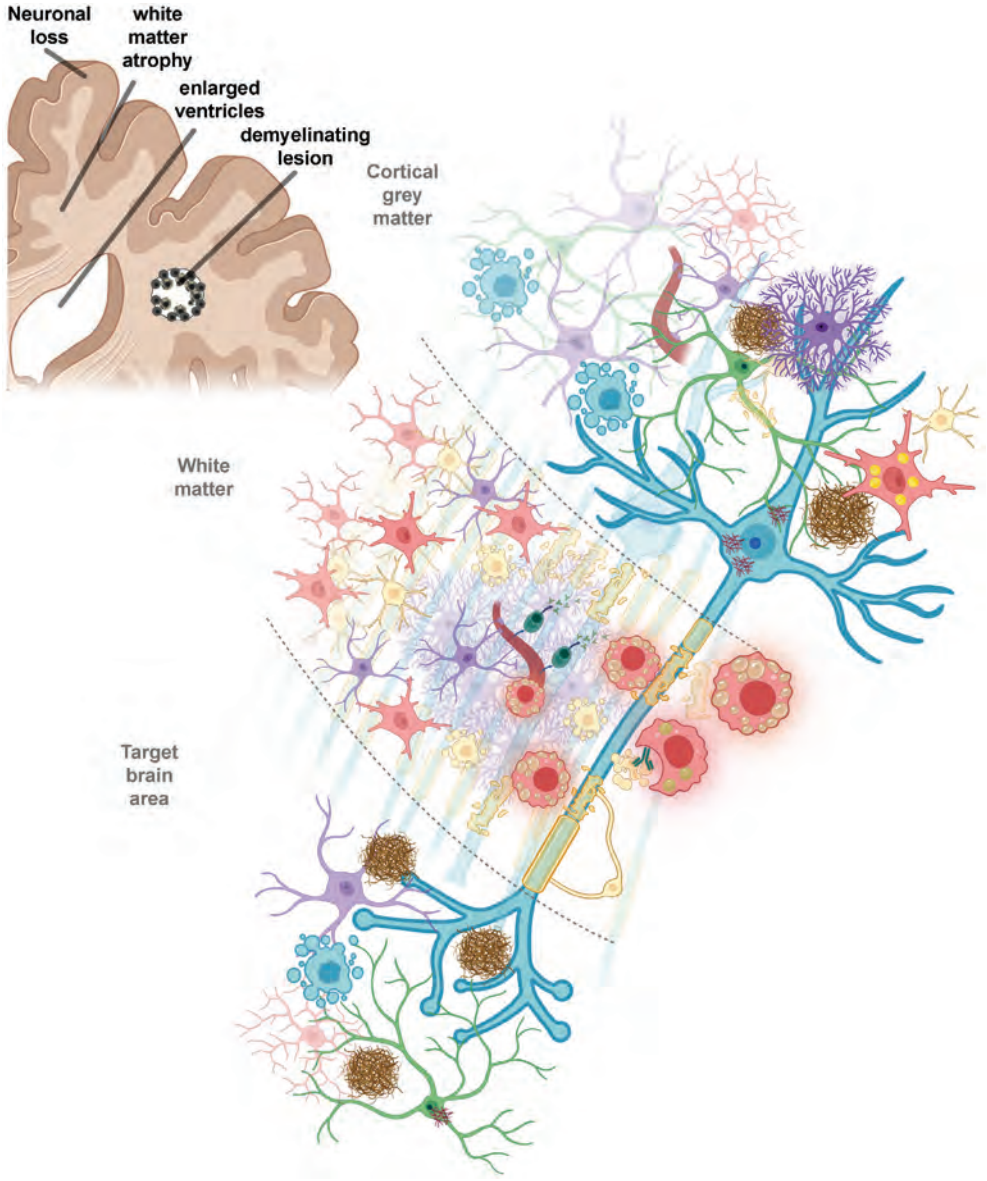

Figure 2. Neurodegeneration of the human cerebral cortex in Alzheimer's disease and multiple sclerosis. Neurodegeneration on the macro-scale is seen by cortical atrophy, enlarged ventricles and reduced white matter. In Alzheimer's disease, extracellular amyloid-β plaques accumulate and intracellular Tau-tangles emerge, concomitant with neuronal death, gliosis and microglial activation. In multiple sclerosis, demyelinating white matter lesions appear, impairing neuronal function. Infiltritating immune cells drive this process and immunoglobulins enter the brain. Phagocytosis of myelin by microglia leads to the formation of inflammatory foam cells and axonal damage, leading to gliotic scars with chronically impaired axonal function.

Drug discovery for brain diseases

While many effective treatments or cures have been developed for many diseases affecting peripheral organs, addressing brain diseases has proven to be notoriously challenging. This difficulty arises from four main factors:

1. The underlying causes (etiology) of many neurological disorders are currently still poorly understood.
2. The unique pharmacological challenges of the brain: Unlike peripheral organs, the brain is protected by the blood-brain barrier, which effectively blocks unfamiliar or potentially harmful chemicals.
3. The brain has a tremendous cellular and anatomical complexity (Figure 1), which makes it difficult to precisely target a single biochemical pathway. For example, a drug designed to act on microglia may have opposing effects on other cell types, such as neurons or astrocytes.
4. The vulnerability of brain cells, particularly neurons. Neurons have very limited capacity for spontaneous regeneration. Unlike most cells in the human body, neurons do not proliferate and thus loss of neurons in conditions like dementia is permanent. In addition, even if adult neurogenesis was possible, the ability of newborn neurons to contribute to the complex brain network is limited as neural networks are shaped over the course of many decades. As a result, many therapies focus on limiting further deterioration rather than reversing the damage already done and restoring brain function.

To improve the success of drug discovery for the central nervous system more research into the molecular mechanisms driving the progression of neurological disorders is imperative.

Lipids and brain function

Lipids play a central role in the biology of all brain cells. While lipids form the physical membranes of all cells throughout the body, the brain is uniquely lipid-rich compared to most other organs. In fact, lipids account for ~50% of the dry weight of the human brain[37,38]. A significant portion of these lipids are found in the fatty myelin sheath, which is even more lipid-dense[39] (Figure 3). Remarkably, the brain is largely self-sufficient in lipid synthesis and much less dependent on dietary intake of lipids compared to peripheral organs, especially for cholesterol, a major myelin lipid[40–42].

While lipids in the brain have traditionally been regarded as structural components of cellular membranes and the insulating myelin, there is growing recognition that lipid metabolism is a highly dynamic process that regulates many aspects of brain function[22,38,39,43–46]. For example, the turnover of phospholipids in the myelin sheath is only a few weeks, indicating dynamic remodeling and maintenance of the myelin sheath[47]. Myelin lipids can also be catabolized in times of glucose deprivation, indicating that myelin lipids dynamically regulate energy metabolism in the brain[22].

Beyond their roles in molecular structure and energy demands, lipids are increasingly understood to act as signaling molecules, facilitating molecular communication both within and between cells[45,48]. Exploring the links between lipid metabolism and neurodegenerative diseases holds the potential to discover new therapeutic targets for treating neurological disorders.

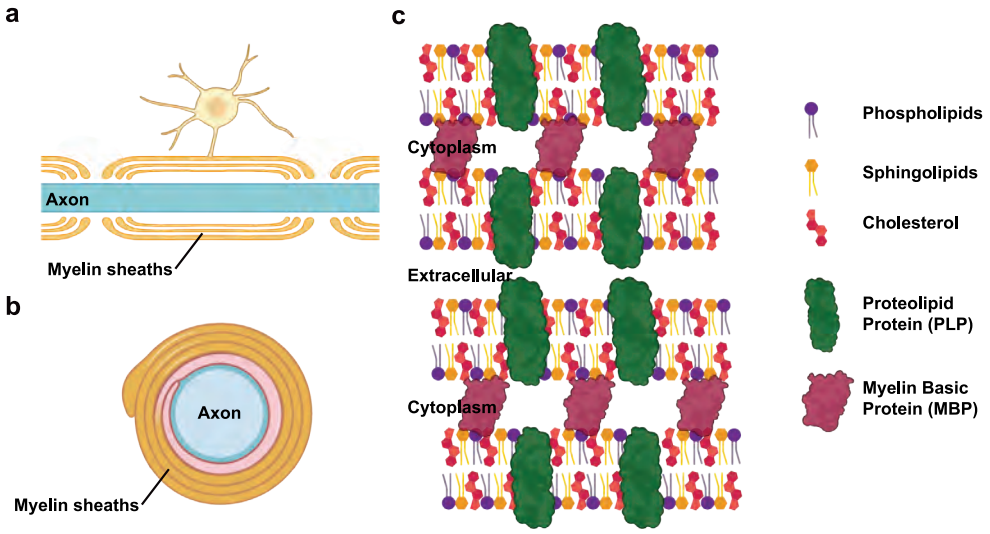

Figure 3. A simplified schematic of myelin structure and components. a-b, myelin wraps axons in a multilamellar structure. **c,** two lamellae of myelin are schematically depicted as a combination of myelin proteins such as proteolipid protein (PLP1) and myelin basic protein (MBP), in combination with the major lipids of the myelin sheath, cholesterol, sphingolipids (mainly glycolipids like gangliosides and galactosylceramides), and phospholipids (mainly phosphatidylethanolamines).

The endocannabinoid system: an endogenous lipid signaling system

The endocannabinoid system (ECS) is an example of such a lipid signaling pathway involved in brain homeostasis[49,50]. Two lipids are recognized as the main endocannabinoids: *N*-arachidonoyl-ethanolamine (AEA or anandamide) and 2-arachidonoyl glycerol (2-AG) (Figure 4)[51,52]. They activate the same G protein-coupled receptors, termed cannabinoid CB_1 receptor and cannabinoid CB_2 receptor, as the psychoactive component (Δ^9-tetrahydrocannabinol) in *Cannabis Sativa*, hence the name 'Endogenous cannabinoids' or endocannabinoids[50].

The inhibitory control of neurotransmitter release at synapses is the most studied function of endocannabinoid signaling (Figure 4). In this process, neuronal depolarization or metabotropic activation of receptors triggers the synthesis of 2-AG in the post-synaptic spine. 2-AG then travels retrogradely to activate the presynaptic cannabinoid CB_1 receptor[53], which leads to inhibition of neurotransmitter release by reducing presynaptic calcium release[54]. Through this mechanism, endocannabinoid signaling mediates phenomena like depolarization-induced suppression of excitation (DSE) at excitatory synapses[55], and depolarization-induced suppression of inhibition (DSI) at inhibitory terminals[56,57]. These effects were initially observed by patch-clamp recordings in acute slices[58], but have recently been demonstrated *in vivo* during spontaneous neuronal activity linked to behavior[59]. This system allows retrograde synaptic plasticity, which plays a crucial role in cognitive processes such as learning, memory formation and fear extinction[60,61]. Additionally, it provides neuroprotection by limiting glutamate release during excitotoxic insults and epileptic seizures[62–65].

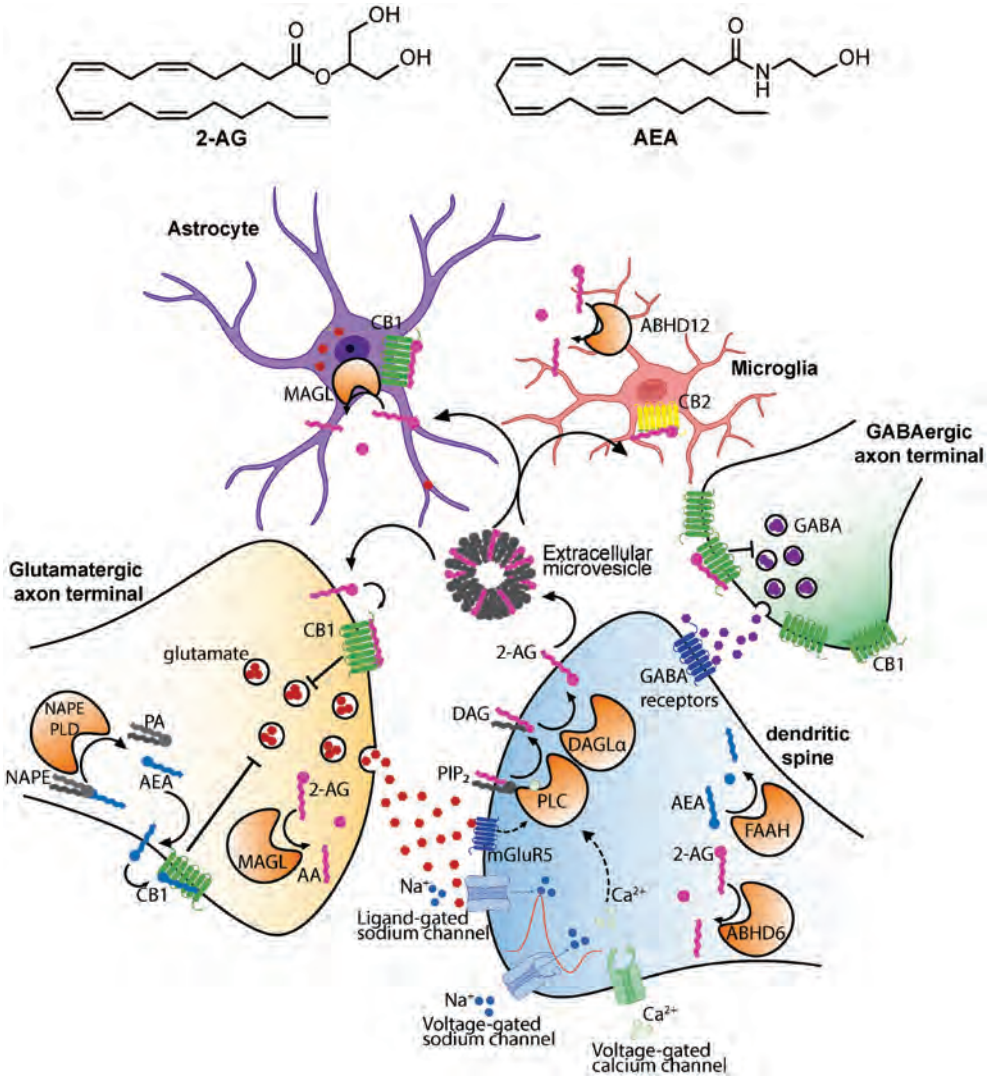

Figure 4. The endocannabinoid lipid signaling system. Schematic overview of components and signaling of the endocannabinoid system. Abbreviations: 2-AG, 2-arachidonoyl glycerol; AA, arachidonic acid; AEA, *N*-arachidonoyl ethanolamine; ABHD: α,β-hydrolase domain containing protein; CB, cannabinoid receptor; DAG, diacylglycerol; DAGLα, diacylglycerol lipase α; FAAH, fatty acid amide hydrolase; GABA, γ-amino butyric acid; MAGL, monoacylglycerol lipase; mGluR5, metabotropic glutamate receptor 5; NAPE, N-acyl phosphatidylethanolamine; NAPE-PLD, NAPE-specific phospholipase D; PA, phosphatidic acid; PLC, phospholipase C.

Diacylglycerol lipase α and β (DAGL-α and -β) are the main enzymes responsible for the biosynthesis of 2-AG through the hydrolysis of diacylglycerol (DAG)[66]. The hydrolysis of 2-AG to arachidonic acid (AA) and glycerol in the brain is predominantly mediated by monoacylglycerol lipase[67] (MAGL) and to a lesser extent α,β-hydrolase-domain containing proteins 6 and -12 (ABHD6 and ABHD12)[68,69]. In the brain, MAGL-driven hydrolysis of 2-AG serves as a major source of AA, which can subsequently be

oxidized to form prostaglandins, leukotrienes and other oxylipins controlling neuroinflammatory signaling[70,71].

AEA is primarily synthesized via the hydrolysis of *N*-acyl phosphatidyl ethanolamines (NAPEs) by NAPE-specific phospholipase D (NAPE-PLD)[72]. Its hydrolysis is predominantly mediated by fatty acid amide hydrolase (FAAH)[73], which also releases arachidonic acid. However, the brain-wide abundance of AEA is too low to significantly impact free arachidonic acid levels.

Beyond their role in mature neuronal signaling, endocannabinoids and their synthetic enzymes are also crucial for shaping neuronal circuits. For example, endocannabinoids regulate axonal pathfinding[74] and promote *de novo* synapse formation[75]. In addition, growing evidence suggests that endocannabinoids influence the phenotype of glial cells, influencing astrocytic signaling and metabolism[76,77], as well as oligodendrocyte differentiation[78,79]. The ECS also plays an important role in the immune system, mainly through the activation of CB_2 receptors. These receptors regulate polarization, translocation, and inflammatory response from various immune cells, including T-cells, B-cells, macrophages and microglia, both in the periphery and in the central nervous system[80–84].

Unlike classical neurotransmitters, which are stored in vesicles and *released on demand*, endocannabinoids are *synthesized and released on demand*[85] - produced only when needed at specific times and locations in the brain. This model highlights the critical role of the enzymes responsible for endocannabinoid synthesis and degradation in regulating this signaling system. Consequently, comprehensively mapping the activity of these enzymes is essential for understanding endocannabinoid biology.

A chemical biology approach to study lipid metabolism

Because lipid messengers are metabolized *on demand* and their metabolic pathways are tightly regulated by cellular mechanisms, traditional techniques such as gene expression analysis or immunohistochemistry are less effective to study the dynamics of lipid metabolism. Instead, chemical biology approaches have become one of the main driving forces to study and unravel the physiological role of lipid messengers in the brain. Among these, activity-based protein profiling (ABPP) has emerged as a key technique for mapping enzyme activity in complex samples, including brain tissue[86,87].

The metabolism of many lipids, including endocannabinoids, is regulated by enzymes belonging to a specific superfamily of enzymes: serine hydrolases. This superfamily comprises over 200 proteins that use a nucleophilic serine residue to hydrolyze ester-, amide-, or thioester bonds in small molecules, lipids and proteins via a covalent acyl-protein intermediate[87]. This enzymatic mechanism is the foundation of activity-based protein profiling (ABPP).

ABPP for serine hydrolases was pioneered by Cravatt and colleagues, who studied the reactivity of the toxic fluorophosphonates toward the AEA-degrading enzyme FAAH[88]. In ABPP, *a chemical probe*, typically consisting of a reactive *warhead* and a reporter tag, reacts with the catalytically active nucleophilic serine of an enzyme. The reporter tag can be a fluorophore enabling the visualization of the probe-protein adducts by gel-based electrophoresis[89,90] or microscopy[91–93], or a biotin group allowing protein enrichment from proteomes for identification by mass spectrometry[94]. ABPP is widely used in drug discovery to efficiently profile activity and selectivity of inhibitors across a protein family in native biological samples[95]. Additionally, it is also applied to compare various biological samples, mapping changes in enzyme activity across diverse biological systems[96,97].

Aim and outline of this thesis

The overarching aim of this thesis is to investigate lipid metabolism in both the healthy and diseased brain. To achieve this, new chemical probes have been developed, validated, and applied to study brain cells and tissues from both mice and humans. Furthermore, lipid metabolism in the diseased human brain is examined, with a particular focus on neuroinflammation as observed in multiple sclerosis and Alzheimer's disease. By integrating novel chemical tools with established classical techniques, this research provides a comprehensive overview of lipid signaling in the healthy and diseased brain.

Chapter 2 reviews how chemical probes support research into brain lipid signaling. It explores the design and applications of activity-based probes, chemical proteomics, fluorescent probes for imaging, and bio-orthogonal chemistry for visualizing and manipulating lipid signaling in living systems. **Chapter 3** describes the design, synthesis and characterization of a set of fluorescent chemical probes used to profile the activity of lipases that metabolize monoacylglycerol lipids. These probes demonstrate high selectivity and potency in engaging their target enzymes. Moreover, they are cell-permeable, enabling the study of enzyme activities in living systems, and revealing dynamic regulation in relevant biological processes. **Chapter 4** discusses the application of fluorescent chemical probes for imaging enzyme activities in the brain. It introduces a new protocol for imaging of MAGL activity in native mouse and human brain tissues. **Chapter 5** presents a study examining the molecular profile of demyelinating lesions in multiple sclerosis. Multi-omics analysis, including lipidomics and activity-based protein profiling identified dysregulated lipid metabolic pathways as potential new therapeutic targets. **Chapter 6** focuses on lipid metabolism in Alzheimer's disease, examining its relationship with the classical amyloid pathology observed in the brains of Alzheimer's patients. Finally, **chapter 7** summarizes the key findings of the research described in thesis and provides an outlook on future directions and emerging techniques for studying lipid metabolism in the human brain. It also highlights strategies for developing novel therapeutics for neurological disorders.

References

1. Azevedo, F. A. C. *et al.* Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *Journal of Comparative Neurology* **513**, 532–541 (2009).
2. Ramón y Cajal, S. *Textura Del Sistema Nervioso Del Hombre y Los Vertebrados*. (1904).
3. Jones, E. G. The neuron doctrine 1891. *Journal of the History of the Neurosciences* **3**, 3–20 (1994).
4. Ramón y Cajal, S. Estructura de los centros nerviosos de las aves. (1888).
5. Südhof, T. C. Neurotransmitter Release: The Last Millisecond in the Life of a Synaptic Vesicle. *Neuron* **80**, 675–690 (2013).
6. Virchow, Rudolf. Über das granulierte Ansehen der Wandungen der Gerhirnventrikel. *Allg Z Psychiatr* **3**, (1846).
7. von Lenhossék, M. *Der Feinere Bau Des Nervensystems Im Lichte Neuester Forschung*. (1893).
8. Hösli, L. *et al.* Direct vascular contact is a hallmark of cerebral astrocytes. *Cell Reports* **39**, 110599 (2022).
9. Hasel, P., Aisenberg, W. H., Bennett, F. C. & Liddelow, S. A. Molecular and metabolic heterogeneity of astrocytes and microglia. *Cell Metabolism* **35**, 555–570 (2023).
10. Río-Hortega, P. Son homologables la glía de escasas radiaciones y la célula de Schwann? *Bol. Soc. Esp. Biol.* (1922).
11. Cohen, C. C. H. *et al.* Saltatory Conduction along Myelinated Axons Involves a Periaxonal Nanocircuit. *Cell* **180**, 311-322.e15 (2020).
12. P Del Rio-Hortega. El "Tercer Elemento" de los Centros Nerviosos. I. La Microglía en Estado Normal. (1919).
13. Ginhoux, F. *et al.* Fate Mapping Analysis Reveals That Adult Microglia Derive from Primitive Macrophages. *Science* **330**, 841–845 (2010).
14. Nimmerjahn, A., Kirchhoff, F. & Helmchen, F. Resting Microglial Cells Are Highly Dynamic Surveillants of Brain Parenchyma in Vivo. *Science* **308**, 1314–1318 (2005).
15. Paolicelli, R. C. *et al.* Microglia states and nomenclature: A field at its crossroads. *Neuron* **110**, 3458–3483 (2022).
16. Schafer, D. P. *et al.* Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner. *Neuron* **74**, 691–705 (2012).
17. Pereira-Iglesias, M. *et al.* Microglia as hunters or gatherers of brain synapses. *Nat Neurosci* 1–9 (2024) doi:10.1038/s41593-024-01818-w.
18. Márquez-Ropero, M., Benito, E., Plaza-Zabala, A. & Sierra, A. Microglial Corpse Clearance: Lessons From Macrophages. *Front. Immunol.* **11**, (2020).
19. McNamara, N. B. *et al.* Microglia regulate central nervous system myelin growth and integrity. *Nature* 1–10 (2022) doi:10.1038/s41586-022-05534-y.
20. Li, Q. & Barres, B. A. Microglia and macrophages in brain homeostasis and disease. *Nat Rev Immunol* **18**, 225–242 (2018).
21. Chrast, R., Saher, G., Nave, K.-A. & Verheijen, M. H. G. Lipid metabolism in myelinating glial cells: lessons from human inherited disorders and mouse models. *Journal of Lipid Research* **52**, 419–434 (2011).
22. Asadollahi, E. *et al.* Oligodendroglial fatty acid metabolism as a central nervous system energy reserve. *Nat Neurosci* 1–11 (2024) doi:10.1038/s41593-024-01749-6.
23. Hartline, D. K. & Colman, D. R. Rapid Conduction and the Evolution of Giant Axons and Myelinated Fibers. *Current Biology* **17**, R29–R35 (2007).
24. Nichols, E. *et al.* Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health* **7**, e105–e125 (2022).
25. Hou, Y. *et al.* Ageing as a risk factor for neurodegenerative disease. *Nat Rev Neurol* **15**, 565–581 (2019).
26. Smith, E. E. *et al.* Systemic determinants of brain health in ageing. *Nat Rev Neurol* **20**, 647–659 (2024).
27. Ross, C. A. & Poirier, M. A. Protein aggregation and neurodegenerative disease. *Nat Med* **10**, S10–S17 (2004).
28. Braak, H. & Braak, E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* **82**, 239–259 (1991).
29. Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H. & Del Tredici, K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* **112**, 389–404 (2006).
30. Heneka, M. T. *et al.* Neuroinflammation in Alzheimer disease. *Nat Rev Immunol* 1–32 (2024) doi:10.1038/s41577-024-01104-7.
31. Filippi, M. *et al.* Multiple sclerosis. *Nat Rev Dis Primers* **4**, 43 (2018).
32. Jakimovski, D. *et al.* Multiple sclerosis. *The Lancet* **403**, 183–202 (2024).
33. Kuhlmann, T. *et al.* An updated histological classification system for multiple sclerosis lesions. *Acta Neuropathol* **133**, 13–24 (2017).
34. Klotz, L., Antel, J. & Kuhlmann, T. Inflammation in multiple sclerosis: consequences for remyelination and disease progression. *Nat Rev Neurol* **19**, 305–320 (2023).
35. Cross, A. & Riley, C. Treatment of Multiple Sclerosis. *CONTINUUM: Lifelong Learning in Neurology* **28**, 1025 (2022).
36. Kuhlmann, T. *et al.* Multiple sclerosis progression: time for a new mechanism-driven framework. *The Lancet Neurology* **22**, 78–88 (2023).
37. Piomelli, D., Astarita, G. & Rapaka, R. A neuroscientist's guide to lipidomics. *Nat Rev Neurosci* **8**, 743–754 (2007).

38. Vanherle, S., Loix, M., Miron, V. E., Hendriks, J. J. A. & Bogie, J. F. J. Lipid metabolism, remodelling and intercellular transfer in the CNS. *Nat. Rev. Neurosci.* 1–18 (2025) doi:10.1038/s41583-025-00908-3.
39. Schmitt, S., Cantuti Castelvetri, L. & Simons, M. Metabolism and functions of lipids in myelin. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1851**, 999–1005 (2015).
40. Berghoff, S. A., Spieth, L. & Saher, G. Local cholesterol metabolism orchestrates remyelination. *Trends in Neurosciences* **45**, 272–283 (2022).
41. Saher, G. *et al.* High cholesterol level is essential for myelin membrane growth. *Nat Neurosci* **8**, 468–475 (2005).
42. Camargo, N. *et al.* Oligodendroglial myelination requires astrocyte-derived lipids. *PLoS Biol* **15**, e1002605 (2017).
43. Berghoff, S. A. *et al.* Microglia facilitate repair of demyelinated lesions via post-squalene sterol synthesis. *Nat Neurosci* **24**, 47–60 (2021).
44. Bogie, J. F. J., Haidar, M., Kooij, G. & Hendriks, J. J. A. Fatty acid metabolism in the progression and resolution of CNS disorders. *Advanced Drug Delivery Reviews* **159**, 198–213 (2020).
45. Broos, J. Y. *et al.* Arachidonic acid-derived lipid mediators in multiple sclerosis pathogenesis: fueling or dampening disease progression? *Journal of Neuroinflammation* **21**, 21 (2024).
46. Grajchen, E., Hendriks, J. J. A. & Bogie, J. F. J. The physiology of foamy phagocytes in multiple sclerosis. *acta neuropathol commun* **6**, 124 (2018).
47. Ando, S., Tanaka, Y., Toyoda, Y. & Kon, K. Turnover of Myelin Lipids in Aging Brain. *Neurochem Res* **28**, 5–13 (2003).
48. Chiurchiù, V., Leuti, A. & Maccarrone, M. Bioactive Lipids and Chronic Inflammation: Managing the Fire Within. *Front. Immunol.* **9**, 38 (2018).
49. Cristino, L., Bisogno, T. & Di Marzo, V. Cannabinoids and the expanded endocannabinoid system in neurological disorders. *Nat Rev Neurol* **16**, 9–29 (2020).
50. Maccarrone, M. *et al.* Goods and bads of endocannabinoid system as a therapeutic target: Lessons learned after 30 years. *Pharmacol Rev* (2023) doi:10.1124/pharmrev.122.000600.
51. Devane, W. A. *et al.* Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor. *Science* **258**, 1946–1949 (1992).
52. Mechoulam, R. *et al.* Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochemical Pharmacology* **50**, 83–90 (1995).
53. Katona, I. *et al.* Presynaptically Located CB1 Cannabinoid Receptors Regulate GABA Release from Axon Terminals of Specific Hippocampal Interneurons. *J. Neurosci.* **19**, 4544–4558 (1999).
54. Kreitzer, A. C. & Regehr, W. G. Retrograde Inhibition of Presynaptic Calcium Influx by Endogenous Cannabinoids at Excitatory Synapses onto Purkinje Cells. *Neuron* **29**, 717–727 (2001).
55. Kreitzer, A. C. & Regehr, W. G. Cerebellar Depolarization-Induced Suppression of Inhibition Is Mediated by Endogenous Cannabinoids. *J. Neurosci.* **21**, RC174–RC174 (2001).
56. Wilson, R. I. & Nicoll, R. A. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410**, 588–592 (2001).
57. Ohno-Shosaku, T., Maejima, T. & Kano, M. Endogenous Cannabinoids Mediate Retrograde Signals from Depolarized Postsynaptic Neurons to Presynaptic Terminals. *Neuron* **29**, 729–738 (2001).
58. Pitler, T. A. & Alger, B. E. Postsynaptic spike firing reduces synaptic GABAA responses in hippocampal pyramidal cells. *J. Neurosci.* **12**, 4122–4132 (1992).
59. Dudok, B. *et al.* Retrograde endocannabinoid signaling at inhibitory synapses in vivo. *Science* **383**, 967–970 (2024).
60. Katona, I. & Freund, T. F. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. *Nat Med* **14**, 923–930 (2008).
61. Castillo, P. E., Younts, T. J., Chávez, A. E. & Hashimotodani, Y. Endocannabinoid Signaling and Synaptic Function. *Neuron* **76**, 70–81 (2012).
62. Marsicano, G. *et al.* CB1 Cannabinoid Receptors and On-Demand Defense Against Excitotoxicity. *Science* **302**, 84–88 (2003).
63. Monory, K. *et al.* The Endocannabinoid System Controls Key Epileptogenic Circuits in the Hippocampus. *Neuron* **51**, 455–466 (2006).
64. Soltesz, I. *et al.* Weeding out bad waves: towards selective cannabinoid circuit control in epilepsy. *Nat Rev Neurosci* **16**, 264–277 (2015).
65. Jensen, K. R., Berthoux, C., Nasrallah, K. & Castillo, P. E. Multiple cannabinoid signaling cascades powerfully suppress recurrent excitation in the hippocampus. *PNAS* **118**, (2021).
66. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *Journal of Cell Biology* **163**, 463–468 (2003).
67. Dinh, T. P. *et al.* Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proceedings of the National Academy of Sciences* **99**, 10819–10824 (2002).
68. Marrs, W. R. *et al.* The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* **13**, 951–957 (2010).

69. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A Comprehensive Profile of Brain Enzymes that Hydrolyze the Endocannabinoid 2-Arachidonoylglycerol. *Chemistry & Biology* **14**, 1347–1356 (2007).
70. Nomura, D. K. *et al.* Endocannabinoid Hydrolysis Generates Brain Prostaglandins That Promote Neuroinflammation. *Science* **334**, 809–813 (2011).
71. Piro, J. R. *et al.* A Dysregulated Endocannabinoid-Eicosanoid Network Supports Pathogenesis in a Mouse Model of Alzheimer's Disease. *Cell Reports* **1**, 617–623 (2012).
72. Tsuboi, K. *et al.* Enzymatic formation of N-acylethanolamines from N-acylethanolamine plasmalogen through N-acylphosphatidylethanolamine-hydrolyzing phospholipase D-dependent and -independent pathways. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1811**, 565–577 (2011).
73. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384**, 83–87 (1996).
74. Berghuis, P. *et al.* Hardwiring the Brain: Endocannabinoids Shape Neuronal Connectivity. *Science* **316**, 1212–1216 (2007).
75. Liang, J. *et al.* Axonal CB1 Receptors Mediate Inhibitory Bouton Formation via cAMP Increase and PKA. *J. Neurosci.* **41**, 8279–8296 (2021).
76. Jimenez-Blasco, D. *et al.* Glucose metabolism links astroglial mitochondria to cannabinoid effects. *Nature* **583**, 603–608 (2020).
77. Noriega-Prieto, J. A. *et al.* Distinct endocannabinoids specifically signal to astrocytes and neurons. 2023.06.13.544877 Preprint at https://doi.org/10.1101/2023.06.13.544877 (2023).
78. Bernal-Chico, A. *et al.* Endocannabinoid signaling in brain diseases: Emerging relevance of glial cells. *Glia* **n/a**,.
79. Sánchez-de la Torre, A. *et al.* Cannabinoid CB1 receptor gene inactivation in oligodendrocyte precursors disrupts oligodendrogenesis and myelination in mice. *Cell Death Dis* **13**, 1–13 (2022).
80. Chiurchiù, V., van der Stelt, M., Centonze, D. & Maccarrone, M. The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: Clues for other neuroinflammatory diseases. *Progress in Neurobiology* **160**, 82–100 (2018).
81. Maresz, K. *et al.* Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med* **13**, 492–497 (2007).
82. Maresz, K., Carrier, E. J., Ponomarev, E. D., Hillard, C. J. & Dittel, B. N. Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. *J Neurochem* **95**, 437–445 (2005).
83. Mecha, M. *et al.* Endocannabinoids drive the acquisition of an alternative phenotype in microglia. *Brain, Behavior, and Immunity* **49**, 233–245 (2015).
84. Mecha, M. *et al.* The endocannabinoid 2-AG enhances spontaneous remyelination by targeting microglia. *Brain, Behavior, and Immunity* **77**, 110–126 (2019).
85. Straub, V. M. *et al.* The endocannabinoid 2-arachidonoylglycerol is released and transported on demand via extracellular microvesicles. *Proceedings of the National Academy of Sciences* **122**, e2421717122 (2025).
86. Simon, G. M. & Cravatt, B. F. Activity-based Proteomics of Enzyme Superfamilies: Serine Hydrolases as a Case Study *. *Journal of Biological Chemistry* **285**, 11051–11055 (2010).
87. Bachovchin, D. A. & Cravatt, B. F. The pharmacological landscape and therapeutic potential of serine hydrolases. *Nat Rev Drug Discov* **11**, 52–68 (2012).
88. Liu, Y., Patricelli, M. P. & Cravatt, B. F. Activity-based protein profiling: The serine hydrolases. *Proceedings of the National Academy of Sciences* **96**, 14694–14699 (1999).
89. Patricelli, M. P., Giang, D. K., Stamp, L. M. & Burbaum, J. J. Direct visualization of serine hydrolase activities in complex proteomes using fluorescent active site-directed probes. *PROTEOMICS* **1**, 1067–1071 (2001).
90. Janssen, A. P. A. *et al.* Development of a Multiplexed Activity-Based Protein Profiling Assay to Evaluate Activity of Endocannabinoid Hydrolase Inhibitors. *ACS Chem. Biol.* **13**, 2406–2413 (2018).
91. Aaltonen, N. *et al.* High-Resolution Confocal Fluorescence Imaging of Serine Hydrolase Activity in Cryosections – Application to Glioma Brain Unveils Activity Hotspots Originating from Tumor-Associated Neutrophils. *Biol Proced Online* **22**, 6 (2020).
92. Withana, N. P. *et al.* Labeling of active proteases in fresh-frozen tissues by topical application of quenched activity-based probes. *Nat Protoc* **11**, 184–191 (2016).
93. Verdoes, M. *et al.* A Fluorescent Broad-Spectrum Proteasome Inhibitor for Labeling Proteasomes In Vitro and In Vivo. *Chemistry & Biology* **13**, 1217–1226 (2006).
94. van Rooden, E. J. *et al.* Mapping in vivo target interaction profiles of covalent inhibitors using chemical proteomics with label-free quantification. *Nat Protoc* **13**, 752–767 (2018).
95. Niphakis, M. J. & Cravatt, B. F. Enzyme Inhibitor Discovery by Activity-Based Protein Profiling. *Annu. Rev. Biochem.* **83**, 341–377 (2014).
96. Viader, A. *et al.* A chemical proteomic atlas of brain serine hydrolases identifies cell type-specific pathways regulating neuroinflammation. *eLife* **5**, e12345 (2016).
97. Baggelaar, M. P. *et al.* Chemical Proteomics Maps Brain Region Specific Activity of Endocannabinoid Hydrolases. *ACS Chem. Biol.* **12**, 852–861 (2017).