Unravelling microglial renewal and expansion during health and disease

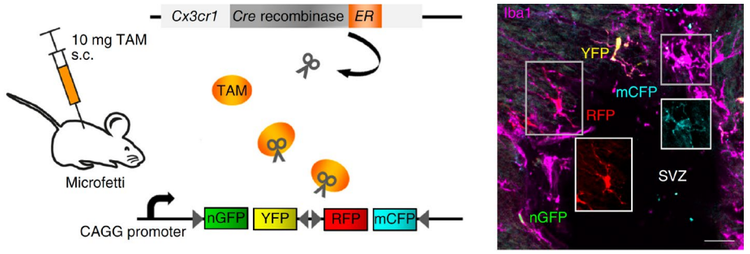
Due to their similarities with other types of white blood cells, microglia, a type of glia within the central nervous system (CNS), have been regarded simply as cells with immune functions by scientists for a very long time. However, recent technological advancements opened up new possibilities for the study of these cells, leading to a better understanding of their important roles in brain development and disorders. **Dr Tuan Leng Tay**, from the University of Freiburg, Germany, has been studying the role and dynamics of microglia within the context of health and disease.

Microglia are the major immune cells that permanently reside within the central nervous system (CNS), in addition to neurons and different types of glial cells. Over the past decade, research has made significant advances in understanding their housekeeping role and their functions in healthy individuals. More recent studies have also shed light on some of the patterns in microglial activity in the presence of diseases affecting the CNS.

THE ROLE OF MICROGLIAL CELLS WITHIN THE CNS

When thinking of cells populating the brain and central nervous system (CNS), neurons tend to be the most automatic association. However, a large part of the human brain is made up of other supportive cells, known as glia, which are involved in a variety of mental functions. Microglia are a particular type of specialised cells that comprise 5–12% of all glial cells in the CNS.

In contrast to neurons and other types of glial cells, mammalian microglia form very early, entering the brain cavity when the CNS is barely formed. A number of studies found that they reside in the brain throughout life, maintaining their numbers via a process of self-renewal. The presence of microglial cells in the CNS is evolutionarily conserved in vertebrates, all species of animals with a backbone or spinal column, which includes fish, birds, mammals, amphibians, and reptiles. Microglia have a number of important functions which combined make them central to the healthy functioning of the CNS. Firstly, being the chief permanent immune cells in the CNS, they are the main sentinels of infection, protecting the CNS from potential threats. They are now also known to help to maintain brain homeostasis (a state of relative stability between its different elements and mechanisms), as well as to control the formation of connections in the brain (synapses), a process that begins before birth and continues throughout life when learning, experiencing new things, and memorising information.



The Cx3cr1creER;R26R Confetti 'Microfetti' mouse model was developed to genetically track microglia in the intact CNS. 10mg of tamoxifen (TAM) was given in a single subcutaneous (s.c.) injection to adult Microfetti mice to induce Cre-mediated recombination that randomly produces one of four fluorescent reporter proteins (known as Confetti), in microglia. The scissors represent Cre recombinases that, upon binding of TAM, recognise and cut (or in addition flip) the reporter construct at loxP sites (triangles). This results in the expression of one Confetti colour from each pair comprising nuclear green fluorescent protein (nGFP, green) and cytoplasmic yellow fluorescent protein (YFP, yellow), or cytoplasmic red fluorescent protein (RFP, red) and membrane-tagged cyan fluorescent protein (mCFP, cyan). All microglia are positive for the Iba1 signal (magenta). However, the activity of single microglial cells labelled in each Confetti colour can be clearly distinguished. Subventricular zone, SVZ. Credit: Tay *et al.* Nat Neurosci. 2017.

STUDYING MICROGLIAL CELLS IN HEALTH AND DISEASE

For many years, the scientific community regarded microglia as mere immune cells of the CNS, due to their common features with other types of white blood cells. Yet, over the past decade or so, the development of more advanced technological tools revolutionised the study of these cells, allowing researchers to directly visualise their activity, expansion and movements in real-time.

Researchers have hence started more in-depth investigations of these movements, in the brain of individuals of different ages as well as in that of both healthy subjects and those affected by a dysfunction or injury of the CNS. These research studies significantly broadened the scientific community's understanding of the role of microglia within the CNS, which is now known to go beyond their immune functions.

MICROGLIA AND NEURODEGENERATIVE DISEASES

In the presence of neurodegenerative diseases such as Alzheimer's disease (AD), a condition that causes a progressive

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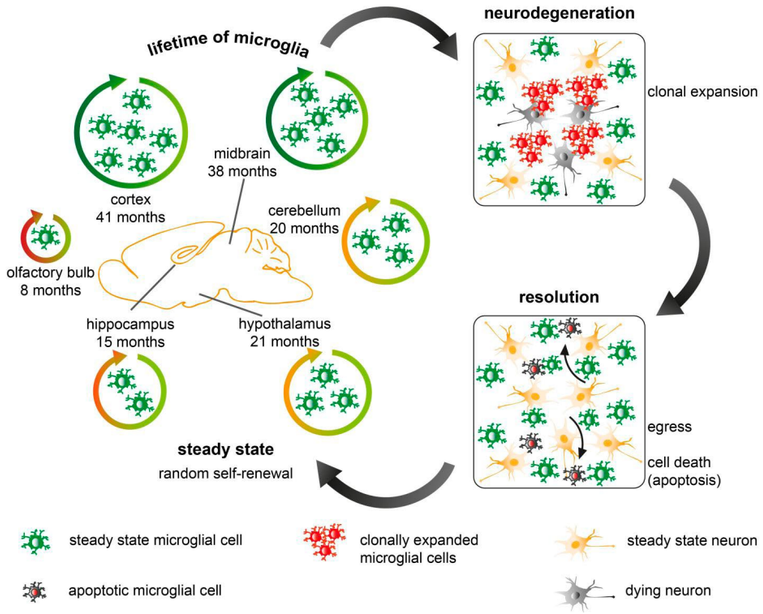
degeneration of the brain that leads to a severe decline in cognitive and memory functions, microglia were traditionally believed to act as causing agents or aggressors. This is due to research findings demonstrating that in patients affected by AD, the number of microglia increased and their forms altered, which suggested they were participating in worsening the state of the disease. However, this theory was often debated, as other studies provided substantial evidence that microglia release protective compounds in different disease conditions. For this reason and because of the characteristics that differentiate them from other brain cells, microglia might actually be an attractive target for pharmacological treatments targeting the brain.

After completing her doctoral studies at the Albert-Ludwigs University of Freiburg, Germany, Dr Tay has been investigating the functions and activity of microglia, particularly in the healthy, injured, or diseased brain. "We are especially interested in understanding the organisation of microglial cells in different brain regions and their longevity within different contexts of brain health," says Dr Tay. "Do our microglial cells accompany us from birth to death? What signals trigger them to alter their lattice-like network and expand in respond to injury or sickness? Subsequently, how do they regain their regular conformation when the brain has recovered?"

Dr Tay believes that providing evidence-based answers to these questions could help to develop effective ways to tackle defects and malfunctioning in the brain.



Context-dependent microglial renewal and clonal expansion.
Microglial renewal occurs randomly and steadily throughout the healthy CNS with regional differences in rate. The onset of neurodegeneration triggers rapid clonal expansion and activation of microglial cells. During recovery the altered microglial network gradually returns to homeostatic cell density and a non-reactive state. Excess microglia associated with disease are eliminated via cell egress and local apoptotic cell death to regain steady state microglial interfaces in the CNS. Credit: Tay *et al*. Nat Neurosci. 2017.

INITIAL RESEARCH STUDIES

Dr Tay and her colleagues investigated microglial activity over long periods of time, by carrying out genetic experiments in both healthy and diseased mice. By tagging microglial cells in different colours as if scattering confetti in the brain, they found that microglial renewal varies greatly between different brain regions. They also observed that microglial cells multiplied rapidly after an acute injury and that their network independently re-organised itself while the mice were recovering from illness.

Dr Tay says: "The rapid clonal expansion of microglial cells in response to an acute injury and the apparent self-reorganisation of the microglial network during recovery in an intact organism was fascinating. This was something that could not be examined previously because of the complexities of the brain and the lack of a clear cell-tracking method." Subsequently, Dr Tay and her team employed new generation sequencing techniques in order to uncover the genes involved in these observed expansion and resolution processes.

Dr Tay's research provides great insight into some of the expansion and renewal mechanisms of microglial cells, in healthy, diseased, and injured CNS

A PROMISING FIELD OF RESEARCH

Dr Tay's research has provided great insight into some of the expansion and renewal mechanisms of microglial cells, in healthy, diseased, and injured CNS. In future, her research and further investigations could help to develop innovative therapies to control microglial functions, which could improve the functioning of the brain in stressed and ageing individuals, as well as in those affected by psychiatric and neurological disorders. The next step in her research will be to identify and validate the genes involved in microglial expansion and re-organisation processes during recovery from injury or illness, by experimentally manipulating the instances in which microglia are exposed to these factors.

KEY PUBLICATIONS

Tay *et al*. Nat Neurosci. 2017; 20(6):793-803
Tay *et al*. J Physiol. 2017;595(6):1929-1945
Tay *et al*. Curr Opin Neurobiol. 2016;39:30-7
Prinz *et al*. Acta Neuropathol. 2014;128(3):319-31

Q&A

What is currently known about the functions and mechanisms of microglial cells and what specifics are yet to be unveiled?

Researching the microglia only became en vogue in the past decade and a half, even though these cells were described 200 years ago. We now know that they are similar to white blood cells, but they do not regenerate from the bone marrow. Rather, they enter the brain before birth and undergo self-renewal throughout life. Apart from immune functions, they maintain brain health and are important for synaptic function. It is important to carefully analyse microglial renewal over time; depending on how long they have been in their location since birth, they may be accumulating defects that promote disease later.

When and how did you start researching microglial cells in the context of health and disease?

I was studying neurons and neural networks for several years, not paying much attention to the other cell types in the brain. When I joined the Institute of Neuropathology in 2012, it was interesting that the main focus there was not neurons, but microglia! The enthusiasm of my colleagues for these tiny cells, which measure just a fraction of a neuronal body in size, got me on board. Soon, I learnt how important they are from their involvement in all known CNS pathologies, from autism, Rett syndrome, schizophrenia, and multiple sclerosis, to alcohol and substance abuse, depression, eating and sleeping disorders, and Parkinson's and Alzheimer's disease.

What do you feel are your most meaningful findings so far and why?

Since microglia are very sensitive cells that change their forms once exposed to stress or a different environment, we believe the most meaningful interpretations rely on having the cells in their original locations. With our genetic colour-coding method, we were able to take snapshots of the actual microglial dynamics happening in an intact brain. It was a breakthrough to be able to precisely track the movements of microglia during recovery from an acute brain injury. Previously we knew that the number of microglia went back to normal but did not know how this happened.

Our genetic colour-coding method can take snapshots of microglial dynamics in an intact brain

What are some of the most effective methods to study microglial activity that are currently in place?

In humans, the reactivity of microglia can be detected by PET scans. For rodents, a method to visualise microglial movement in a live animal in real-time has been established for over a decade. Scientists view the microglia through a glass cranial window embedded in the skull, similar to observing fish in their aquarium. Static high-resolution microscopy methods are also important, as they allow us to study the shape changes and interactions of microglia with other brain cells. Today, we are also able to distinguish various microglial activities by studying their genetic and protein profiles on a large scale.

What are your plans for future investigation?

We have been studying the dynamics of microglia renewal and expansion in healthy and sick adult animals so far. However, many CNS disorders that first present symptoms in young or aged adults have a very early onset in life. In particular, these diseases are often linked to dysfunctional microglia. Thus, I would like to understand the impact of early microglial dynamics on adult brain health and to find out what the mechanisms behind them are. It would be very pertinent to find out how to rescue the defects that arise during brain development, in adulthood.

Detail

RESEARCH OBJECTIVES

Dr Tuan Leng Tay's research interests lie in the role of the brain-specific innate immune system. In particular, her work focuses on microglia, the brain macrophages that act as the principle barrier to invading pathogens. Her work has applications for neurodevelopmental, neuropsychiatric and neurodegenerative diseases, such as autism, schizophrenia, multiple sclerosis, and Alzheimer's disease.

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COLLABORATORS

- Olaf Ronneberger, Thorsten Falk, Dominic Mai and Özgün Çiçek, Computer Vision Group, University of Freiburg https://lmb.informatik.uni-freiburg.de/
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BIO

Dr Tuan Leng Tay left Singapore to pursue her PhD in developmental neuroscience at the University of Freiburg. After studying the zebrafish model for 10 years, her postdoctoral studies at Columbia University focused on mouse dopaminergic systems. She joined the Prinz lab, University of Freiburg, in 2012 as an independent postdoc where her research focused on microglia. Dr Tay will commence her post-doctoral 'habilitation' thesis at the Faculty of Biology, University of Freiburg, in 2018.

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