DR JEROLD CHUN

On the **brain**

Dr Jerold Chun is listed among Thomson Reuter's most influential minds of 2014, and is known for his high-impact research in the fields of pharmacology and neuroscience. Here, he discusses his current work on congenital hydrocephalus and an underlying mechanism implicated in the condition

Could you discuss the objectives of your research into congenital hydrocephalus, more commonly known as water on the brain, and explain how your background has prepared you for this line of investigation?

We wish to understand how this disease arises and might be prevented and more effectively treated. This research came about through a confluence of scientific and medical experiences over the last three decades, which included basic science studies on the foetal brain, cellular and molecular biology, receptor pharmacology, knock-out technologies and drug discovery. All of these elements were used for the initial discovery of a new mechanism for hydrocephalus, and are being used in current research efforts.

How will your team overcome the treatment challenges posed by the condition?

As a receptor-mediated phenomenon, it is possible that hydrocephalus could be prevented, or at least attenuated, by drugs targeting the involved receptors. Such medical intervention has clear potential benefits over neurosurgical draining of the cerebrospinal fluid (CSF), which is itself subject to complications. Notably, G protein-coupled receptors are the target for nearly half of human medicines currently on the market, so the feasibility of producing such an agent is reasonable.

Can you elucidate the methodologies you are using to determine receptor selectivity of physiological and pathophysiological functions affecting the brain?

Lipids in general are messy and sticky, making their analyses problematic, especially relative to receptors. The literature is filled with erroneous conclusions on lysophospholipid (LP) receptor identification, functions and so forth, reflecting difficulties in handling lipids and, before receptor identification, interpretations on how these lipids in fact worked. The introduction of heterologous expression systems – cell lines that did not respond to a lipid unless made to express a specific receptor – allowed for the unambiguous identification of receptors. These systems worked in combination with mouse knock-out techniques and classical ligand binding strategies which, by themselves, are less than satisfying because the sticky, messy lipids produce lots of non-specific binding. Currently, new biophysical technologies are being pursued to solve the binding problem. In addition, receptor identification has allowed the production of receptor subtype agonists and antagonists, which have aided both *in vitro* and *in vivo* analyses.

What has been the impact of your discovery of a mechanism that causes hydrocephalus?

What was really striking about the identified mechanism was its effect on neural progenitor cells – those cells that later give rise to the neurons and glia of the brain – which moved our thinking about hydrocephalus from a purely 'plumbing' problem to a neurodevelopmental disorder. This insight places congenital or foetal hydrocephalus into a similar space as autism and schizophrenia, and indeed, some of the same risk factors are shared by all three – including brain haemorrhage, hypoxia and infection.

Ongoing research in your laboratory uses cutting-edge biophysical technologies to determine novel receptor-mediated physiological and pathophysiological functions affecting the brain. In what manner could this work eventually benefit patients?

One of the more exciting medical applications of basic science is in the detection of biomarkers for disease. Lipids such as lysophospholipids come in many 'flavours' that, when present as identified biomarkers, could sub-divide diseases into clinically relevant forms including those susceptible to specific treatments, as well as provide a prognosis.

To what extent have previous studies on hydrocephalus informed your current lab work?

Science progresses by building on the work of colleagues, and that is absolutely the case here. Defining the disease and its comorbid phenomenology – other changes to the brain, for example – have been instrumental in our thinking about how hydrocephalus arises.

Are there any particular partners or collaborators you would like to mention?

The work on hydrocephalus emerged from the PhD thesis project of Yun Yung, who is now carrying on hydrocephalus studies as a postdoctoral fellow. All the members of our lab interact extensively, and this exciting environment provides cross-fertilisation among projects that has aided our work. I am truly indebted to Paul Gross and the Hydrocephalus Association, as well as Jill Morris and Story Landis at the National Institute of Neurological Disorders and Stroke (NINDS); they have all encouraged our research. Early studies on basic brain development and LP receptor identification work were also supported by the National Institute of Mental Health (NIMH).

The secret life of lipids

A group of researchers based at **The Scripps Research Institute** in California, USA, is conducting vital work towards the development of new, effective treatments for neurological diseases based on receptors they discovered nearly 20 years ago

HYDROCEPHALUS IS A condition whereby cerebrospinal fluid accumulates inside the ventricles or cavities of the brain, causing pressure to build. Congenital hydrocephalus, a version of the illness that affects children before or around birth, is one of the most common neurological disorders in infants and young children. Affecting one in 1,000 newborns, the most recognisable characteristic of the disease is the rapid expansion it causes in the head of infant patients, whose skulls are still malleable. The cause of the disorder is usually genetic, but it can be acquired, and early warning signs include poor feeding and vomiting.

If it strikes within the first three years of life, before the cranial bones fuse, and is left untreated, hydrocephalus can seriously disrupt the development of the head – but even with treatment, there are other sequelae that cannot be alleviated. In current practice there is no cure for the condition, and even palliative treatments fail to counteract the psychiatric and neurological impact of the extreme pressure. As it stands, the front-line treatment for hydrocephalus is the neurosurgical removal of excess fluid – a process that, in itself, carries risks. In serious and ongoing cases, a cerebral shunt can be installed, again increasing patient vulnerability to complications.

TWEAKING TREATMENTS

Less invasive and more effective preventive measures for curtailing congenital hydrocephalus are desperately needed but, to date, researchers remain perplexed by this enigmatic condition. The causative molecular mechanisms behind presentation of the disease, as with many neurological disorders, are mysterious, and with so many molecular pathways and receptor networks already known to be active in the developing and adult brain, finding an appropriate target is like looking for a needle in a haystack. A newly discovered form of molecular signalling found to be prevalent in the brain, however, has spurred research into this disease.

The human brain, when water is discounted, is composed mostly of lipids. These molecules, which are sticky and difficult to work with, also make up the lipid bilayer that is fundamental to the walls of cells, and are in general an important building material for the body at a molecular level. For more than 50 years, evidence suggested that lipids also play a role in the production of extracellular effects, but it was not until 1996 that scientists identified the first lysophospholipid (LP) receptor, and demonstrated that LPs can act as receptor-mediated signalling molecules. Dr Jerold Chun, Professor of Molecular and Cellular Neuroscience at The Scripps Research Institute in California, led this initial demonstration, and today his research focuses on the ways in which LP biology could aid medicine, particularly helping patients who suffer from neurological disorders.

THE REVOLUTIONARY RECEPTOR

In the mid-1990s, Chun and his collaborators, then at the University of California, San Diego were looking for genes active during foetal brain development and found ventricular zone gene 1 (VZG-1), a G protein-coupled receptor of unknown function, activated by an unknown ligand. The researchers were working with neuron-like cells they had specifically produced from the brain at a time when most labs were expressing orphan receptors in more convenient cell types. Because of this, they found that VZG-1-bearing cells reacted strangely when placed in the culture medium. The medium

INTELLIGENCE

LYSOPHOSPHOLIPID MECHANISMS IN CONGENITAL HYDROCEPHALUS

OBJECTIVE

To understand how congenital hydrocephalus arises and can be prevented by investigating lysophospholipid mechanisms, in order to develop an effective treatment for the condition and other neurological disorders.

KEY COLLABORATORS

Dr Yun Yung, The Scripps Research Institute, USA

ACKNOWLEDGMENTS

Paul Gross, Hydrocephalus Association, USA

Dr Jill Morris; **Dr Story Landis**, National Institute of Neurological Disorders and Stroke, USA

Helen L Dorris, Dorris Neuroscience Center, USA

FUNDING

National Institutes of Health (NIH) • National Institute of Neurological Disorders and Stroke

CONTACT

Dr Jerold Chun
Professor

Molecular and Cellular Neuroscience
Dorris Neuroscience Center
The Scripps Research Institute
10550 North Torrey Pines Road
DNC-118
La Jolla, California, 492037
USA

T +1 858 784 8410
E jchun@scripps.edu

www.scripps.edu/chun/index.html

DR JEROLD CHUN is Professor in the Department of Molecular and Cellular Neuroscience at The Scripps Research Institute. He received MD and PhD degrees through the Medical Scientist Training Program at Stanford University, and pursued postdoctoral studies at the Whitehead Institute. The Chun Laboratory's overarching goal is to understand the brain – how it develops, carries out complex tasks, and is affected by diseases. This is being pursued through two scientific areas that have both basic science and therapeutic implications: lysophospholipid receptor signalling, involving small lipids that act as extracellular chemical signals; and genomic mosaicism among single brain cells – especially neurons – whereby cells from the same individual can vary at the genomic level. Within these areas, the group has placed a particular emphasis on translating basic science research into understanding and identifying potential treatments for brain diseases, with current efforts aimed at hydrocephalus, multiple sclerosis (MS), Alzheimer's disease, and other neurodegenerative diseases.

THE SCRIPPS RESEARCH INSTITUTE

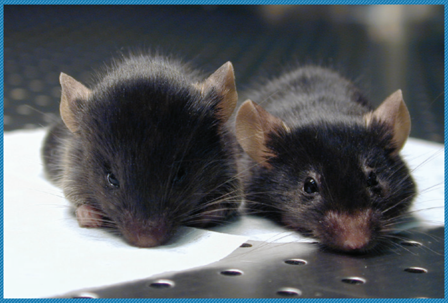
contained serum, and caused the neuron-like cells to round, just as they did naturally in the developing brain.

The group was intrigued by what they saw and began searching for the receptor's ligand in the culture medium. One by one, they ruled out all of the proteins present in the mixture and when they boiled the serum to destroy the proteins, found it still had the same effect. The ligand they were looking for was not a protein, but a lipid – a revelation that was new to the field at that time. After years of searching, the researchers discovered the culprit: lysophosphatidic acid (LPA). Though groundbreaking, the scientists' paper on the subject was initially rejected for publication, and even when it was published in 1996, was met with extreme scepticism.

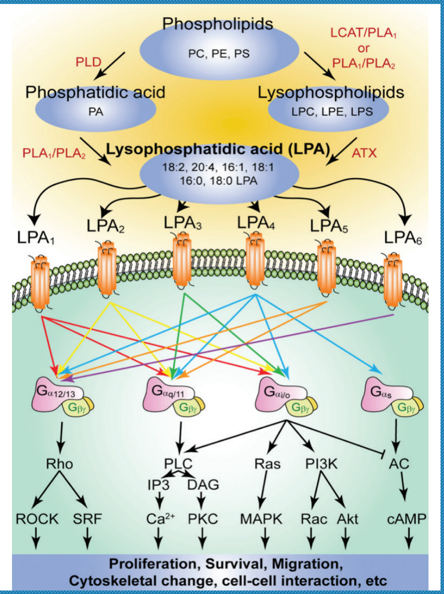
HOT TOPIC

Over the intervening years, interest in this field has grown exponentially. Today, Chun's discovery has led to the identification of around 15 receptors in the LP family; a number that is still growing. The contribution to basic biological science has been huge, but for the California researchers this was merely the beginning of their work. As they had initially suspected, this class of receptor has a marked impact on neural cells in the developing brain, and also plays a role in defining brain cell morphology and electrophysiology, as well as processes such as proliferation, myelination, organisation and even cell behaviour.

This diversity of function makes LP receptors an extremely promising target for novel drugs aimed at treating neurological disorders such as hydrocephalus and multiple sclerosis (MS). In 2010, fingolimod was approved by the US Food and Drug Administration (FDA) and became the first oral treatment for relapsing forms of MS. The drug is thought to work by trapping lymphocytes within the lymph node so that they are unable to contribute to inflammation and the destruction of myelin within the brain. The chemical itself looks very much like an LP in structure and achieves its protective effect by interacting with four species of LP receptor. Its mode of action is dissimilar to any previous treatment for the disease, and could therefore be revolutionary. Chun's lab was responsible for publishing an influential paper evaluating the mechanisms of fingolimod's action in 2011 that identified a new brain mechanism for the drug to treat MS.



LPA-induced hydrocephalic mouse (left) compared to a normal control (right).



LPA AND LPA RECEPTORS

TRIAL AND SUCCESS

In the same year, his lab released the results of a study into the causes of hydrocephalus. Using mouse models created for the purpose, the team injected either serum, plasma or red blood cells directly into the cerebral ventricles of the developing rodent. Because bleeding into the ventricles in the foetal brain appears to be one of the causative factors behind hydrocephalus, the researchers were keen to gauge what components of blood caused the problem. The mice were then observed at several time points following injection to investigate their progress.

Red blood cell injections had no impact on development – but the presence of either plasma or serum in the ventricles precipitated the accumulation of cerebrospinal fluid and the symptoms of hydrocephalus. Chun and his group hypothesised that LPA found in these blood fractions was the cause and, when they repeated the experiment with an injection of LPA in solution, the murine subjects developed severe symptoms. The final step of the experiment was to repeat the process a final time using murine models that did not express the necessary LP receptors, and the fact that they were unable to produce symptoms in these mice using the same methods suggests that this may be a possible medical route to treatment for hydrocephalus. Additionally, delivering an LPA receptor antagonist into the brain at the time of hydrocephalus appeared to also prevent its development, which provides a proof of concept for possible future therapy.

BETTERING THE BRAIN

"We would dearly like to identify tractable therapeutic mechanisms towards preventing disease – or at least reducing its severity," Chun remarks, and today it seems likely that this dream is finally within reach. The group has been responsible for elucidating a crucial class of receptor and, alongside Chun's more recent efforts towards clinical solutions, this achievement stands as a lasting legacy in the field.