Using fluorescent viruses to illuminate neuronal circuits

In recent decades, researchers have begun unravelling the complex web of connections that underpin all perceptual and cognitive processes in the brain. To map these details, researchers require methods to trace the individual connections between specific neuronal cells. **Dr David Lyon** and his team have developed a novel technique for specifically targeting inhibitory and excitatory neurones, with the capacity to label individual cells and their connections. Their work opens the door to detailed analysis of neural connectivity with unprecedented specificity, providing tools for understanding the intricacies of neuronal excitation and inhibition in the brain.

In the School of Medicine at the University of California, Irvine, Dr David C Lyon is an Associate Professor and Vice-Chair of the Department of Anatomy and Neurobiology. There he is leading research devising new methods for tracing neuronal circuits in the brain.

The human brain contains hundreds of billions of neurones which form an intricate array of neuronal circuits that facilitate brain function. They are involved in every sense we perceive, thought we have, and emotion we feel. How the complex circuits underlying these processes function is increasingly being revealed by researchers. However, progress has been limited by the tools available.

Employing cutting edge molecular biology techniques, Dr Lyon and his research group have been devising new methods to enable them to relate the structure of the mammalian visual cortex to its function. They are investigating the organisation of cortical areas of the brain, delving into the detail of the circuitry that underlies sensory capabilities at the level of individual neurones.

COMPLEX CORTICAL CIRCUITS

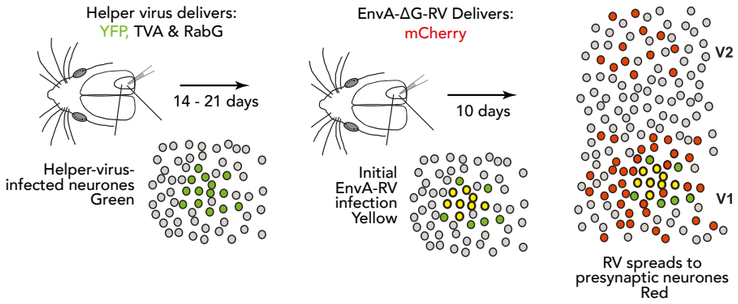
Neurones are specialised cells that are electrically excitable; they transmit sensory information through electrical and chemical signals throughout the brain. Series of interconnected neurones arranged in pathways form neuronal circuits, which can regulate their activity via feedback loops. Cortical inhibition and excitation work together through networks of inhibitory and excitatory neurones in these circuits to control and modulate complex cortical computations.

Neuroscience is progressing in building our understanding of these networks. However, there is still a vast amount of detail that remains unknown. Furthering our knowledge of the connections involved and how they change in the event of dysfunction is imperative if we are to better understand disorders of the brain that occur in the event of injury and disease. In doing so we will increasingly discover new avenues for the development of targeted therapeutics, meaning fundamental neuroscience is of great importance for medical progress.

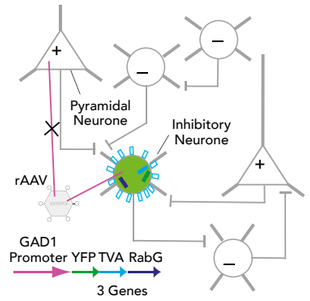
DIFFERENTIATING DETAILS

The details of how neuronal function is regulated by inhibitory and excitatory neurones have remained unclear, primarily due to technical limitations. As the two cell

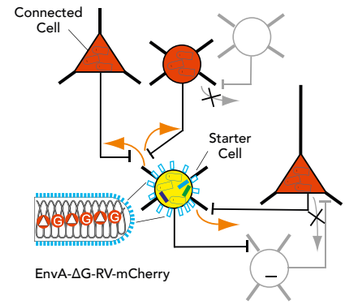
A. Targetting rabies virus infection with helper viruses



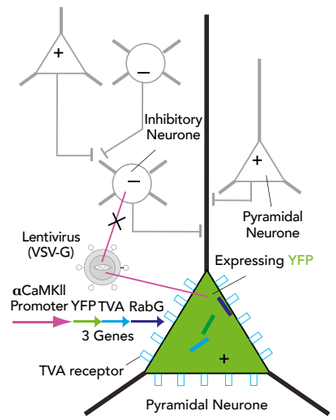
B. Cell-type specific AAV/GAD1 delivery of YFP, TVA & RabG



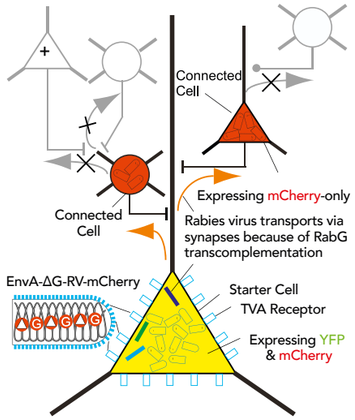
C. EnvA-ΔG-RV infection and spread



D. Cell-type specific LV/αCamKII delivery of YFP, TVA & RabG



E. EnvA-ΔG-RV infection and spread



Liu et al., 2013, Current Biology

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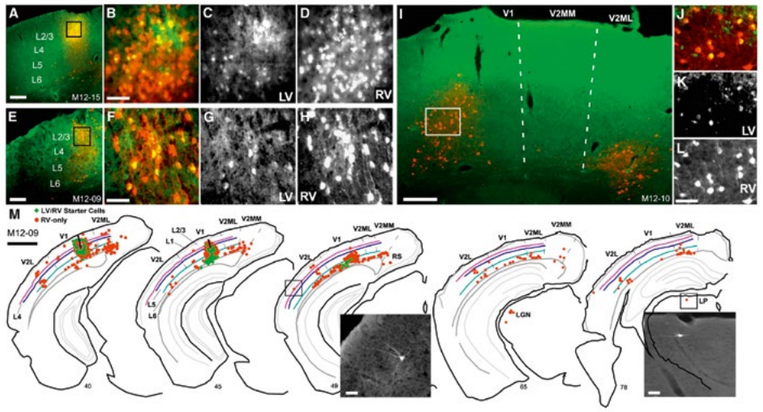
types are intermingled in the brain, study of each population in isolation has proved challenging, with existing neuronal tracers unable to easily differentiate between these cells. Therefore, despite several decades of research piecing together neuronal connections, it has been impossible to unveil the connectivity throughout the brain in cell-specific detail.

Using an innovative combination of genetic engineering and molecular techniques, Dr Lyon and his team have developed a method of differentiating between inhibitory and excitatory neurones. They use different colours of intracellular fluorescent protein labelling, allowing precise identification of the individual cells. Furthermore, their new technique allows routes of input to these neurones to be traced back upstream, towards the cells and brain regions they originated from.



Targeting EnvA-DG rabies virus infection to, and monosynaptic retrograde spread from, excitatory neurones in mouse V1 using the lenti-αCamKII-YTB helper virus
Liu et al., 2013, Current Biology

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HARNESSING THE POWER OF VIRUSES

Taking advantage of viruses that have the capacity to infect brain tissue, Dr Lyon and his team have devised an ingenious way of uncovering the complexities of neuronal circuits that have thus far eluded researchers.

Their first step in developing the technique involved creating a modified version of the rabies virus, which could specifically establish infection in inhibitory or excitatory neurones. The researchers genetically modified the virus so that it can only infect cells that present a specific protein on the cell surface. However, to differentiate between other cell types, they targeted the virus to a protein that is not usually present on cells in the mammalian brain. Therefore, they also needed to deliver this protein to the cells they wanted the rabies virus to infect.

To facilitate this, Lyon and his team utilised two other modified 'helper' viruses, one designed with specificity for excitatory and another for inhibitory neurones. Each of these viruses was genetically constructed so that they would introduce the gene coding for this crucial protein to the cells, which when expressed would allow the modified rabies virus entry.

DISTINCTIVE FLUORESCENT GLOW

In addition to requiring specificity of infection to the neuronal cells of interest, the researchers needed a way of identifying the individual cells and of differentiating between infection with the helper virus and modified rabies. Therefore, Dr Lyon and his team also engineered both viruses with genes encoding for fluorescent proteins that would be expressed once infection is established. They used a yellow fluorescent protein to identify helper virus infection and a red derivative, called mCherry, to highlight rabies-infected cells.

To test their new system, they introduced each helper virus to the brain tissue of mice in the region of the visual cortex. The helper viruses infected the target inhibitory or excitatory neuronal cells, expressing both the protein that renders the cells susceptible to infection by modified rabies virus and the yellow fluorescent protein. Next they introduced the rabies virus, resulting in targeted rabies infection of these cells. As a result, the rabies-infected starter cells were distinctively labelled with the presence of both mCherry and yellow protein fluorescence.

RETROGRADELY TRACING INPUTS

The system works with high specificity for labelling upstream presynaptic neurones from the starter cell due to another clever modification. The rabies virus the team used is also a deletion-mutant that is missing an essential gene coding for a protein known as rabies glycoprotein (RabG). RabG is required for the viral infection to spread to neighbouring cells. Prior to rabies infection, the helper viruses also delivered RabG to the starter cells. Therefore, these cells also contained this critical component for rabies to spread to connected presynaptic neurones, which once infected with the modified rabies were labelled red as the virus expressed the fluorescent mCherry protein.

REACHING HIGHER LEVELS OF COMPLEXITY

Dr Lyon's results showed that the technique is capable of fluorescently labelling thousands of presynaptically connected neurones in the visual cortex of any mammal. The data yielded wide-scale input patterns to each of the neurone types in this region, which allowed the researchers to conduct comparisons of these inputs that had never been possible before.

They further demonstrated the efficacy of the technique and its potential for research in the brains of different species by targeting inhibitory neurones in the cat brain visual cortex. This not only produced novel insights into the neuronal circuitry of a large and complex mammalian brain, but also proved that the method can be utilised in larger scale animal models. Therefore, this ground-breaking technique provides researchers with the first ever tool to explore the connections between specific cell types in the neocortex of higher-order mammals.

Q&A

What sparked your interest in neuroscience and led you to working in the field?

Seeing a psychologist as an adolescent initially piqued my interest in the great unknowns of the brain and drove me to be a Psychology major in college. At first I thought clinical psychology was my calling to better understand why people thought the way they did, then neuropsychology because it promised better insights into how different structures of the brain related to human behaviour. I finally settled on studying animal models because it offered more experimental control and because you could work more directly with the brain.

Why did you choose to use rabies virus as the viral vector for your novel methodology?

Rabies is amazing in that it has specifically evolved to spread across synaptic contacts between neurones in the brain, and saliva glands, so that animals become demented from eventual neurone death and then bite some other animal to pass the virus along. In some ways, rabies virus chose me – I happened to be at the right place at the right time in Ed Callaway's lab as a new postdoc where work by him and Ian Wickersham had just begun on genetically modifying rabies to control its infection of particular brain circuits. But, by that time I already had a keen interest and a lot of experience in tracing circuits of the brain; rabies offered a unique opportunity so I have taken advantage of it ever since.

Could this method be combined with other existing techniques to further enhance its potential for brain research?

Absolutely. Like many other viruses, rabies can be used to deliver genes that can express a wide range of proteins useful in manipulating and observing the anatomy and function of neural circuits: from fluorescent calcium indicators for measuring neural responses *in vivo* to opsins for optogenetic manipulation of neurone activity.

What are your plans for future research following on from this work?

Now we are building on our approach by incorporating opsins within the rabies virus to allow for optogenetic manipulation of cell-type specific circuits in the visual cortex. In addition, we are developing new helper viruses that allow us to target rabies virus infection of specific subtypes of inhibitory neurones. For example, at this year's Society for Neuroscience meeting we showed that in the cat primary visual cortex we could specifically target and optogenetically manipulate the parvalbumin-positive inhibitory neurones which make up around 40% of cortical inhibitory neurones and found that this had a significant effect on tuning properties of excitatory neurones.

What do you think is the greatest remaining challenge facing neuroscientists to constructing a complete map of the neuronal circuits in the brain?

Reconstructing the complete circuit in the mouse brain will take a lot of work, but seems entirely possible: doing so for a more complex brain such as in a non-human primate seems a far greater challenge, especially when trying to relate the circuits to complex functions.

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Detail

RESEARCH OBJECTIVES

Dr Lyon's research has led to the development of a novel method to track the presynaptic connections in neural pathways. Using genetically modified viruses, Dr Lyon and his team have traced pathways in the visual cortex of both the mouse and cat brain.

FUNDING

The Whitehall Foundation, the National Institute of Neurological Disorders and Stroke, the National Eye Institute

COLLABORATORS

- Ed Callaway, PhD, Professor, Salk Institute; key intellectual collaborator, helped with planning for cell type specific targeting using AAV and promoter fragments; Senior author on our 2007 Neuron paper showing EnvA-rabies targeted tracing technique
- Markus Ehrengruber, PhD, Visiting Scientist at UC Irvine, Lecturer, University of Zurich, Switzerland
- Ali Cetin, PhD, Allen Institute for Brain Science, Senior Manager, Viral technology
- Roberto Japelli, PhD, research scientist, Salk Institute

BIO

David Lyon is an Associate Professor and Vice-Chair of the Department of Anatomy and Neurobiology in the School of Medicine at the University of California, Irvine. He received his PhD from Vanderbilt University. He then received postdoctoral training at MIT followed by the Salk Institute for Biological Studies.

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