Don't get overexcited! Restoring inhibition in neurological disorders

Dr Davies and **Professor Moss** from Tufts University have collaborated to successfully uncover a new way of treating one of the underlying causes of neurological disorders such as epilepsy and Fragile X Syndrome. Restoring the lost inhibitory action of $GABA_A$ receptors, their work combats the overexcitation of neurones associated with these conditions.

Q&A

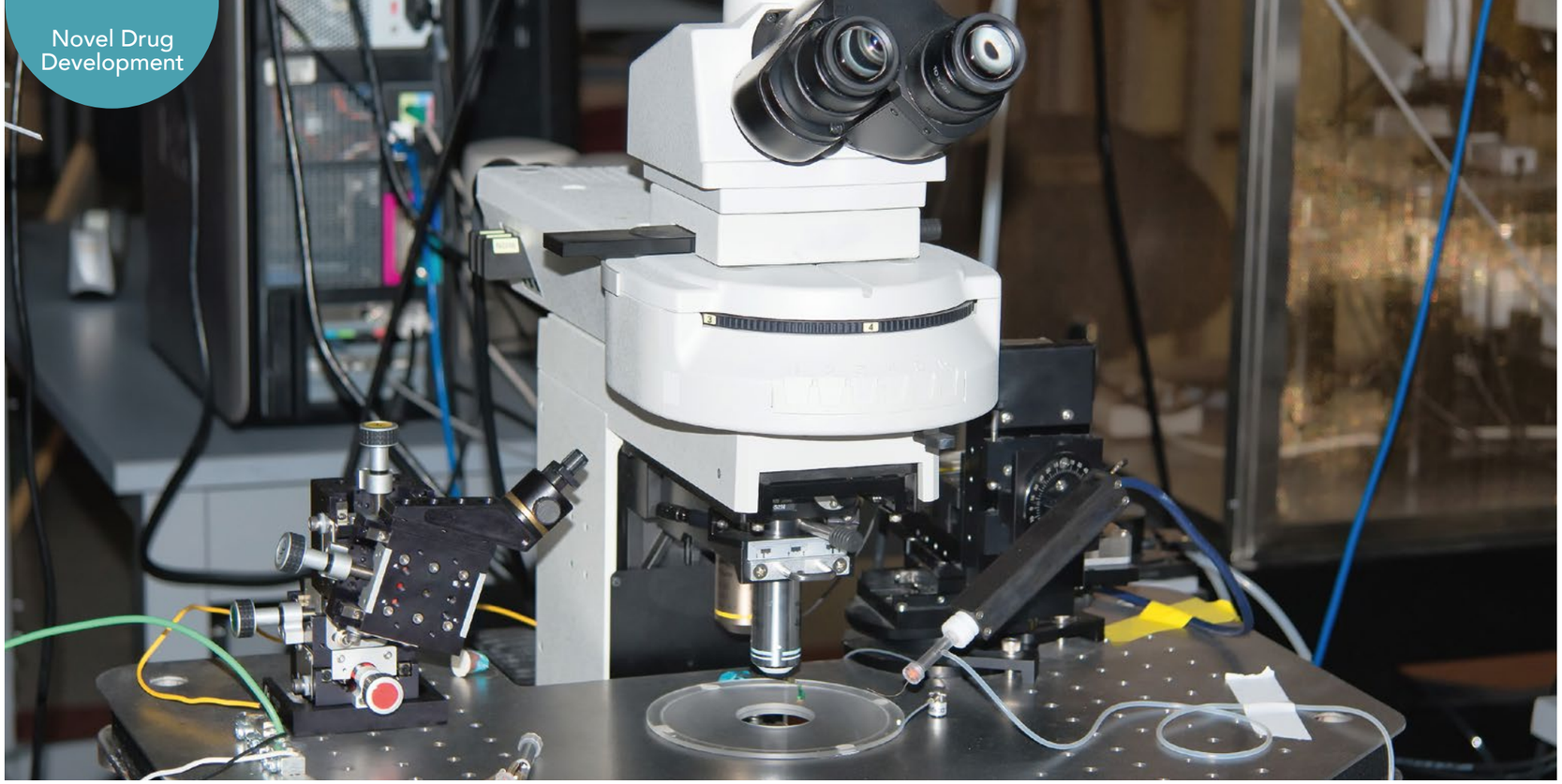
What first drew you to the investigation of neuronal disorders such as Fragile X Syndrome?
I have had a long standing interest in how neurosteroids modulate $GABA_A$Rs, especially extrasynaptic $GABA_A$Rs. When I started my collaboration with Steve, he was already examining how neurosteroids change the phosphorylated state of extrasynaptic $GABA_A$Rs and we saw a rapid change in the surface expression of functional channels leading to a rise in tonic inhibition. At the same time, there were reports on a reduced tonic inhibition in neuronal developmental disorders such as Fragile X and we thought that boosting the expression of $GABA_A$Rs would be a novel therapeutic approach. Traditionally, the aim would be to allosterically enhance the channels that are already there in the membrane but we are seeking to increase the number of receptors back to normal levels.

How has collaboration helped in moving the research forward?
We have distinct and overlapping skills: Steve is known for his pioneering work on $GABA_A$R phosphorylation and trafficking; I have experience on recording the currents that flow through $GABA_A$R ion channels and examining subunit-dependent changes in function and pharmacology. Together, we have the experience to examine the broader picture of how changes to protein phosphorylation affect neuronal excitability and how brain circuits are altered.

Why has it taken so long from the discovery of GABA to uncover these aspects of its regulation?
As with most experimental breakthroughs, it has taken so long because it is experimentally challenging to isolate and examine the individual steps involved. For our work we needed to identify the amino acid residues that are phosphorylated and then generate specific antibodies to identify changes in phosphorylation. Then, designing the experiments and experimental conditions to properly measure changes in receptor-mediated currents and neuronal excitability can take time to achieve through trial and error, luck, and insight. Because of the complex signalling mechanisms that regulate $GABA_A$R trafficking and function there is still much that is unknown.

How did SAGE Therapeutics become involved in the work?
SAGE had an interest in how their neuro-active steroids were modulating synaptic and extrasynaptic $GABA_A$Rs. When we identified a novel mechanism of neurosteroids changing the phosphorylated state of extrasynaptic $GABA_A$Rs to increase the trafficking of the receptor, we, together with SAGE, became interested in knowing whether synthetic neuro-active steroids could also work through this mechanism or if it was just naturally occurring neurosteroids that could phosphorylate $GABA_A$Rs. Discovering that certain synthetic compounds can enhance tonic inhibition through a trafficking mechanism differentiates these SAGE compounds from the typical allosteric modulator often used clinically to control excitation.

What is the next step in realising NAS as a therapeutic agent?
Firstly, we are awaiting results from clinical trials of some neuroactive steroids that are currently underway. However, we still do not understand the mechanistic pathway of how neuroactive steroids alter the phosphorylated state of extrasynaptic $GABA_A$Rs. Once we know more about the pathway then we could identify more selective compounds. In order for that to happen we are examining different pathways and different interacting proteins. Once the pathway(s) have been identified we could generate novel animal models which will demonstrate the specificity of the compounds generated before going forward into clinical trials.



The human brain is a complex organ and scientists have spent long careers attempting to elucidate the mechanisms underlying its activity. Central to this is the regulation of synaptic activity. A synapse is the point of communication between brain cells, called neurones, as well as their connection to other tissues such as muscle. Each of these synapses is bristling with receptors, proteins inserted into the cell membrane to sense the extracellular signalling molecules which are the messengers of the body. Gamma-aminobutyric acid (understandably shortened to GABA) was known of as long ago as the 1950s as one of these powerful neuronal transmitters, but its mechanism of action, and particularly the role of the receptors it stimulates, is still a matter of intense research.

GABA is almost exclusive to the brain, with only trace amounts found in other tissues. Couple this with the fact that almost all neurones are sensitive to it and it is clear that its function is vital to normal brain functioning. It has been estimated that about 30% of all the synapses in the central nervous system (CNS, consisting of the brain and spinal cord) utilise GABA as a transmitter; its role is to inhibit the effect of other transmitters which are exciting the neurones, providing an important regulatory mechanism in neuronal activity. Each transmitter has its own receptor – in the case of GABA there are two, designated $GABA_A$ and $GABA_B$. The first inhibits the reception of signals from other cells (post-synaptic), the second inhibits the release of signalling molecules to other cells (pre-synaptic). The $GABA_A$ receptor is a target for therapeutic drugs due to this ability to inhibit excitatory signals from other cells, the uncontrolled activity of which is found in disorders such as epilepsy. Anaesthetics and barbiturates have long been known to affect the intrinsic activity of the receptor, but less is known about how their physical presence at the synapse is regulated.

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THE BENEFITS OF COLLABORATION

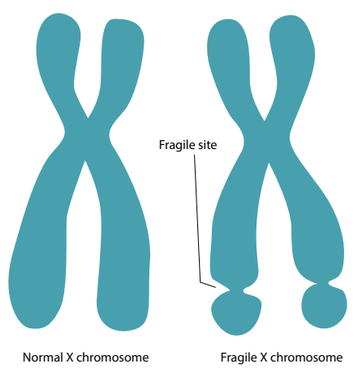
Professor Moss and Dr Davies have been collaborating since 2008, using their electrophysiological and molecular biochemistry skills to explore the role of $GABA_A$ receptors in neuronal inhibition.

A key focus of their research has been the movement of these receptors within the cell, known as trafficking. Receptors are proteins which are created from the template held in the cell's DNA. They are then often modified by cellular machinery before being moved into position on the cell membrane. Receptor populations are then managed through a process of continual recycling as new receptors are created and others degraded. Prof Moss and Dr Davies are currently examining how errors in this trafficking are implicated in disorders such as anxiety, neurodevelopment disorders, and epilepsy.

The researchers have recently discovered that a form of protein modification, known as phosphorylation, is critical for the correct localisation of the $GABA_A$ receptor at the synapse. In phosphorylation, one or more phosphate groups are added to the protein to achieve its final conformation (shape) and activity. Their experiments have elucidated the precise location of phosphate groups on the receptor, made up of multiple smaller proteins called sub-units. They believe that the phosphorylation of the alpha-4 and beta-3 sub-units is particularly critical, as it increases both receptor insertion into the membrane and its inhibitory activity once inserted.

KNOWLEDGE IS POWER

Taking this knowledge forward, the team identified neuro-active steroids (NAS) as potential therapeutics for the mis-trafficking of $GABA_A$ receptors within neurones. NAS have the potential to increase activity of the enzyme responsible for phosphorylation and are associated with improved membrane insertion of alpha-4 and beta-3 sub-units. Unfortunately, endogenous NAS, those naturally occurring in the brain, are relatively low. Administering additional supplies has been limited by problems around bioavailability (the proportion of the administered substance which enters circulation and is able to exert its effects) but Prof Moss and Dr Davies have been greatly assisted by the development of a novel NAS by SAGE Therapeutics. SAGE is a biopharmaceutical company which specifically develops novel therapeutics for CNS disorders. Their compound, SGE-516, has the equivalent efficacy as endogenous NAS but with radically improved bioavailability from oral administration. This opens up the possibility of new treatments for a multitude of neurological disorders, including epilepsy and anxiety.



THE FRAGILITY OF LIFE

Of particular interest to Prof Moss and Dr Davies' team is the role of mis-trafficking of the $GABA_A$ receptor in Fragile X Syndrome (FXS). FXS is an autism spectrum disorder, and the most common form of inherited intellectual disability. Deficits in neuronal inhibition by $GABA_A$ receptors are frequently implicated in FXS, along with a reduction in expression of a protein associated with the disorder, known as fragile X mental retardation protein (FMR1). A reduction in neuronal inhibition results in complex intellectual and behavioural issues in those with the genetic condition. An increased incidence of epilepsy is also a factor affecting individuals with the condition, and thought to be directly related to the deficits in neuronal inhibition.

Using a mouse model of Fragile X in which the FMR1 protein has been 'knocked out' through genetic modification, the researchers have shown that it is indeed the mis-trafficking of $GABA_A$ receptors which is responsible for the reduction in inhibitory activity at these neurones. It is the discovery of this novel mechanism by the team which has allowed for the investigation of high bioavailability NAS, with the aim of reversing the problem and restoring correct regulation of excitatory signals in the CNS. This is a breakthrough in the treatment of FXS, but the identification and successful modulation of this therapeutic target has created the possibility of addressing a much wider range of neurodevelopmental disorders.

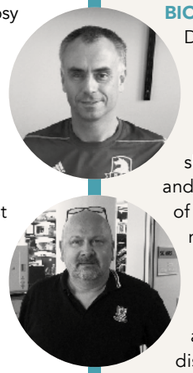
Detail

RESEARCH OBJECTIVES

Dr Davies and Professor Moss focus their work on the action of $GABA_A$ receptors in the brain. Their current research investigates how neuroactive steroids interact with the receptors and their potential use as a therapeutic in conditions where neuronal inhibition is deficient.

COLLABORATORS

- Professor Stephen Moss, Tufts University School of Medicine
- Drs Mike Ackley and James Doherty of Sage Therapeutics, Inc. http://www.sagerx.com/index.php



BIO

Dr Davies and Prof Moss have been collaborating since 2008. They have combined their electrophysiological and molecular biochemistry skills to explore how trafficking and pharmacological modulation of $GABA_A$ receptors impacts neuronal inhibition. They are currently examining how $GABA_A$ receptors are mis-trafficked in disorders such as anxiety, neurodevelopment disorders, and epilepsy.

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