Finding novel treatments for Angelman syndrome

Could Syn3 be the answer?

- Abnormalities in neuronal communication is associated with many neurodegenerative diseases, including depression and Angelman syndrome (AS).
- Dr John Marshall, professor at Brown University in the USA, is investigating the mechanism of synaptic dysfunction to develop novel therapies.
- His research focuses on the design of compounds that can activate and restore the strengthening of synaptic connections in AS.
- His team is designing novel compounds with significant antidepressant effects, paving the way for potential drug treatments for AS.

Angelman syndrome (AS) is a rare genetic disorder that affects the nervous system and causes severe physical and learning disabilities. People with AS have a normal life expectancy, but they present developmental delay and show minimal speech and movement difficulties, such as the inability to walk or balance well (known as ataxia).

Due to their condition, patients need support throughout their life, and while they receive medication for symptom management, there is a pressing need for a cure approved by the US Food and Drug Administration (FDA). Dr John Marshall, professor of molecular biology, cell biology, and biochemistry at Brown University in the USA, is a leading neuroscientist. His research on AS aims to contribute towards the development of novel treatments.

A problem in communication between neurons

AS is caused by deficiency of a gene called UBE3A, which is responsible for breaking down proteins during the clear-out processes taking place in cells. Abnormalities also stem from reduced brain-derived neurotrophic factor (BDNF) activity. BDNF plays a key role in nerve growth and survival, impacting the overall functioning of the nervous system. Studies by Marshall's group and several others have shown that, similar to the BDNF decrease, disruption in the function of UBE3A in the brain affects the efficiency of neuronal cells to communicate.

This is due to the abnormal breakdown of proteins essential for neuronal communication. The points of communication between neuronal cells in the brain are called synapses. Synaptic integrity is required for efficient brain function as well as for memory formation and cognition. The signals neuronal cells use are chemicals, called neurotransmitters. Once neurotransmitters are released and passed to the next neuron through the synapse, an electrical signal travels within this recipient cell leading, to its activation or 'firing up'.

Each neuron has between a few to hundreds of thousands of synaptic connections, altogether forming a network in the brain. The capacity for generating and removing synaptic connections is called 'synaptic plasticity'. Mouse studies show that in AS, the ability of neurons to amplify signals is compromised, affecting not only synapse formation but also long-lasting synaptic strength, or long-term potentiation (LTP).

Marshall and his team are working on finding ways to reverse this pathology for treatment. To manipulate the synapse, an understanding of synapse structure and function is necessary. Synapse formation involves the partnering of specific receptors that are found on neuron projections or dendrites.

The type of synapses impaired in AS involves the release of the neurotransmitter glutamate which binds to the respective glutamate receptor on the recipient cell. Glutamate receptors are made of a complex of different proteins, including postsynaptic density protein-95 (PSD-95), which is an essential molecule in synapse formation and important for synaptic strength.

Reactivating the synapse in AS

BDNF which is affected in AS can stimulate synapse activation. Additionally, downstream in the synapse activation pathway, PSD-95 needs to bind to another factor called tropomyosin kinase receptor B (TrkB). Marshall and fellow researchers have shown that this is a pivotal interaction as its disruption leads to synaptic dysfunction and is associated with different disorders of the central nervous system, including schizophrenia and depression, or diseases within the spectrum of autism, such as Rett syndrome, fragile-X syndrome, as well as AS.

A line of investigation towards treatment discovery would be to mimic the TrkB and PDS-95 coupling and trigger synapse activation. Taking advantage of the knowledge on the biochemistry of PSD-95 interactions, the team designed a compound, called CN2097, that can bind and activate PSD-95 to promote signalling. Marshall argues that targeting downstream the signalling pathway is a more efficient approach that can reduce off-target adverse effects.

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The team tested CN2097 in a mouse model of AS that was generated without the gene UBE3A in neurons to mimic the disease. These mice showed abnormalities in clearance of proteins participating in the synapse, which resulted in faulty synaptic function. They also presented clinical symptoms, such as difficulties in learning and movement.

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Administration of the compound inhibited the degradation of proteins that take part in the synapse and, therefore, restored synapse activation. Importantly, this effect was observed in a brain region – the hippocampus – that is responsible for learning and memory. The drug also successfully alleviated cognitive and motor dysfunction in these mice, thus showing great promise as a potential therapy.

Advancing compound formulation for treatment efficiency

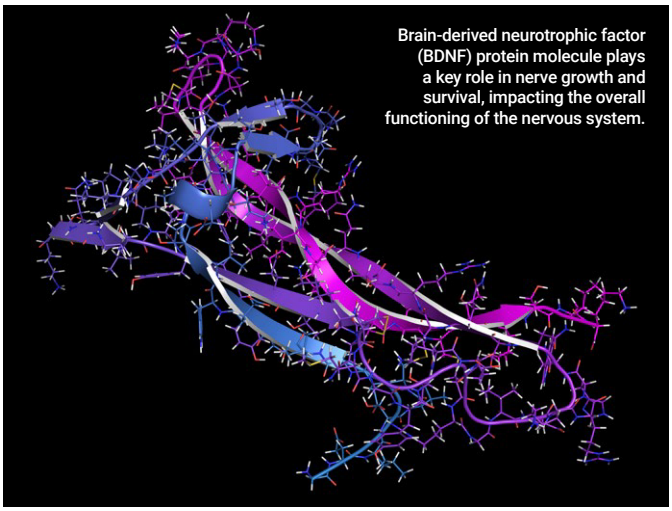
The team then attempted to check the efficiency of the compound in mouse models of anxiety and depression that show similar abnormalities in synaptic function. For stress/anxiety induction (CMS), mice were subjected for a total of five weeks to a number of mild and unpredictable stressors. In the more severe depression model (CORT), the mice were administered corticosterone daily for 21 days, which is the major stress hormone produced in the brain.

Treatment with Syn3 in mice with mild stress reversed abnormalities of synaptic function in the hippocampus, restoring the number of synapses as well as the levels of synaptic proteins.

Corticosterone delivery was enough to cause depressive behaviours in mice. Changes in behaviour in both models were examined in various tests, including swim tests to examine the extent of immobility due to anxiety or depression. The team also examined preference for sucrose as a measure of stress.

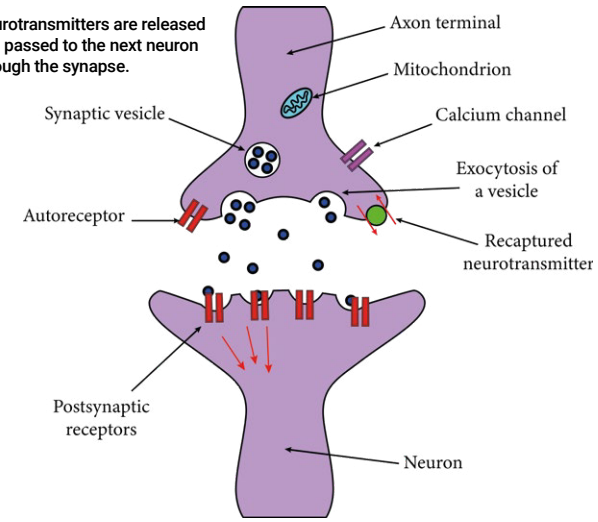
Considering previous findings that the PSD-95 pathway is implicated in depressive behaviour, Marshall and team evaluated the antidepressant effects of CN2097 in the mouse models of depression and anxiety. Treatment of mice with mild stress or depression had rapid antidepressant effects as less immobility and increased sucrose preference was observed. However, a high dose was required to achieve this effect, demonstrating the low affinity of the CN2097 for binding to PSD-95.

To tackle this, the researchers optimised the compound structure in order to develop novel drugs targeting PSD-95 with higher affinity. They designed an advanced compound, called Syn3, that demonstrated enhanced binding, including the ability to rapidly cross the blood-brain barrier and, therefore, achieve quick delivery to the brain.



Brain-derived neurotrophic factor (BDNF) protein molecule plays a key role in nerve growth and survival, impacting the overall functioning of the nervous system.

Neurotransmitters are released and passed to the next neuron through the synapse.



Treatment with Syn3 in mice with mild stress reversed abnormalities of synaptic function in the hippocampus, restoring the number of synapses as well as the levels of synaptic proteins within 12 hours. A similarly rapid antidepressant-like activity was also observed after administration of Syn3 in the CORT depression mice. The effect was so strong that a single dose rapidly reversed immobility or distaste for sucrose. Importantly, compared to CN2097, Syn3 showed anti-depression efficacy at a tenfold lower dose.

The way forward

Marshall and his team's work is aimed towards the development of novel drugs for synapse restoration. They formulated compounds that show great effects in synaptic restoration exhibiting antidepressant activity and ability to reverse clinical symptoms in AS. Their research is a step forward in opening avenues for novel treatment options for patients with AS.

Personal response

What inspired you to conduct this research?

It is impossible not to be inspired by the Angelman kids, or Angels as they are referred to. I have had the privilege of working with the FAST (Foundation for Angelman Syndrome Therapeutics) team, and have interacted with some of the most inspiring and courageous parents. Most people with Angelman syndrome have severe intellectual disabilities, seizures, and never learn to speak, but are incredibly social and never get tired of giving you hugs. There is no cure for Angelman syndrome, but FAST is on a quest to develop gene therapy approaches, such as gene editing. My hope is that the pharmacological drugs we are developing could also be beneficial as a therapy to improve learning in Angelman kids and for treating other autism-related conditions.

What would be the next steps in your research?

The next step is to translate this promising preclinical research into a human therapy. To get a drug into clinical trials, we will need to perform additional animal testing to prove that the drug is safe and does not cause harm. If we get to the stage of clinical trials, then the FDA approval process can be extremely challenging, often referred as 'the Valley of Death'. Moving a drug from mice to humans is difficult. Almost 95% of drugs fail in human clinical trials, mainly in phase III, due to a lack of efficacy in humans. We are 'cautiously optimistic' that the drug effects in mice can be replicated in human trials. It's a battle, but nothing like the battle of being the parent of a special needs child.

What are some findings in this research that have especially surprised you so far?

It always amazes me that our drugs can improve long-term memory in the mice. The beneficial effect of the treatment lasts for days, even after the drug is long gone from the body, indicating the drug quickly resets the brain to stabilise memories. A pleasant surprise in our Angelman study was that the drug demonstrated efficacy in adults, raising hope that treatment of this severely debilitating genetic condition can still be efficacious, even if it's not treated early on in its development.

We found that in both Angelman syndrome and depression, BDNF signalling was impaired; therefore, it is not surprising the drugs developed for Angelman syndrome can also elicit fast anti-depression-like responses. Depression affects approximately 1 in 6 people at some point in their lives. Major depressive disorder is often associated with cognitive problems which can severely impact young people. There is an urgent need for more effective treatments for depression as about 30% of people don't respond to current drugs. Therefore, developing a drug for Angelman syndrome, a rare disorder with an incidence of around 1 in 15,000 live births, may lead to new therapy for one of the most common mental disorders.

If Syn3 proves to be efficient for AS, what other avenues is this research likely to open in the treatment of AS and other neurodevelopmental disorders?

BDNF signalling is at the centre of a network of genes implicated in autism spectrum disorders, and drugs that enhance synaptic BDNF activity are likely to be useful in treating neurodevelopmental diseases that include Rett, fragile X syndrome, and cerebral palsy. The signalling pathways involved in learning and memory formation also protect neurons. Mounting evidence confirms the involvement of BDNF signalling in psychiatric disorders, such as major depression, schizophrenia, and post-traumatic stress disorder (PTSD). Dysregulated fear memories result in PTSD and Syn3 could provide an effective learning-based extinction of fear-related disorders.

Although BDNF treatment has been attempted for many neurodegenerative disorders, such as Amyotrophic Lateral Sclerosis (ALS), the trials have failed. Uniquely, Syn3 boosts BDNF signalling at localised synaptic sites different from the global stimulation previously attempted that causes adverse side effects and even neuronal death. Our studies show that Syn3 exhibits remarkable neuroprotective properties in several models of neurodegeneration, including glaucoma, neonatal hypoxic-ischemic encephalopathy (cerebral palsy) and traumatic brain injury (TBI). In cerebral palsy, which is the leading cause of childhood disabilities and shares symptoms with Angelman syndrome, our compounds have proven to reduce brain damage, partly through reducing microglial activation and neuroinflammation. The effects of Syn3 on neuroinflammation inhibition could be particularly beneficial in demyelinating (myelin-damaging) diseases, such as Multiple Sclerosis (MS), which is highly prevalent in Northern Ireland where I was born.

In some cases, cerebral palsy may be the result of a traumatic brain injury after birth. TBI-related disabilities, along with the adverse cognitive impairment, and repetitive concussions observed in activities like contact sports, can lead to progressive tauopathy and the later development of Parkinson's disease and dementia. Nearly 7 million Americans and 1 million people in the UK are living with Alzheimer's, figures which are likely to double by 2050. A reduction of BDNF signalling has been observed in AD and accumulating evidence demonstrates that drugs enhancing BDNF signalling provide protection against AD pathogenesis. While it is too early to say, drugs such as Syn3, that potently prevent neuronal death and improve cognition, represent a promising new therapeutic for neurological and psychiatric disorders.

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Competing interest statement

Dr Marshall has founded Engram Therapeutics – a Brown University spin-out biotech company working to develop new treatments for Angelman syndrome and other neurological disorders.

Bio

Dr John Marshall is a leading neuroscientist and a pioneer in the signalling and synaptic trafficking fields, and has made major contributions to understanding brain injury and neurodevelopmental disorders. He received his MSc from the University of Toronto and completed his PhD training in Neurobiology at the MRC at Cambridge University, England. He worked with Professor Len Kaczmarek at Yale University. Marshall assumed his position at Brown University in 1995 and has continued to produce cutting-edge research. His lab focus is on memory and behaviour in rodent models of Angelman syndrome.

Funding

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Collaborators

- Jill Lynn Silverman, PhD, UC Davis Health
- Cong Cao, Soochow University
- Dennis Goebel, Wayne State University
- Yu-Wen Alvin Huang, Xin Yang, and Mandar Naik, Brown University

Further reading

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