Development of GABAergic neuron connectivity in health and in disease

The biological processes associated with neurodevelopmental disorders and diseases of the brain are still in part to be ascertained. **Drs Graziella Di Cristo** and **Bidisha Chattopadhyaya**, two scientists working at the Sainte-Justine hospital research centre, affiliated to the Université de Montréal, have conducted extensive research exploring brain development. Their latest work investigates the role of GABAergic neurons in brain disorders, including intellectual disability, autism and epilepsy. Drs Di Cristo and Chattopadhyaya use cutting-edge high-resolution imaging and gene manipulation techniques to gain insight on how disturbances in GABAergic circuits could lead to disruptions in cognitive function.

The brain is one of the most complex organs in the body, made of millions of neurons that communicate to each other through connections called synapses. To facilitate studies on such a complex biological network, neurons have been classified based on the neurotransmitters they release. In the mammalian neocortex, excitatory neurons that release glutamate predominate, but they are precisely modulated by a heterogenously diverse population of neurons that release neurotransmitter GABA – the GABAergic neurons.

Drs Di Cristo and Chattopadhyaya's research aims to gain a better understanding of GABAergic neuron development, plasticity and functions. Their work also provides insight into the potential causes and biological dynamics of a number of disorders, including intellectual disability, epilepsy, autism and schizophrenia, which have been associated with dysfunctions or unusual development of the brain.

BRAIN PLASTICITY

Brain plasticity (from the Greek word 'plastos', which means 'moulded') can be defined as the ability of the brain to change and adapt throughout life. The human brain has been found to modify itself over time, adapting to changes within the body or in the external environment by forming new connections between brain cells. These connections are formed via synapses, structures that allow neurons to communicate, passing electrical and chemical signals to one another. For instance, the brain of someone who suffered from a stroke that caused paralysis in a given part of the body could later shift activity related to the paralysed area to a different location, making new connections to adapt to the change.

DISORDERS OF THE BRAIN: AUTISM AND EPILEPSY

Drs Di Cristo and Chattopadhyaya's recent research investigates brain plasticity and function in individuals suffering from autism and epilepsy. Autism is a highly variable neurodevelopmental disorder that first appears during infancy or childhood, and generally follows a steady course without remission. A lifelong disability, it affects how people perceive the world and interact with others. It is characterised by impaired social interaction, impaired verbal and non-verbal communication, and restricted and repetitive behaviour. Although it is understood that autism affects information processing in the brain by altering how nerve cells and their synapses connect and organise, how this occurs is not well understood.

Epilepsy is a medical condition that affects the brain, causing affected individuals to have repeated seizures, the severity of which can vary from case to case. During these seizures, neurons have been found to fire abnormal electrical impulses. In some cases, onset of epilepsy can be attributed to stroke or brain damage. However, in most cases the exact cause for the condition is hard to identify.

THE ROLE OF GABAERGIC NEURONS

Dr Di Cristo and Dr Chattopadhyaya investigate the role of GABAergic neurons in relation to brain disorders such as intellectual disabilities, autism and epilepsy. GABAergic neurons are cells that generate gamma aminobutyric acid (GABA), an inhibitory neurotransmitter within the central nervous system (CNS). GABA, a chemical used as a means of communication between neurons, helps to reduce excitation in the nervous system. GABAergic circuits have been found to control the function of cortical networks, regulating the development of the brain by moderating the proliferation and connectivity of neurons.

Irregular development of GABAergic circuits has been associated with a number of neurodevelopmental disorders, including schizophrenia, autism, and Tourette's syndrome. Due to the complexity of the brain and its circuits, understanding how GABAergic neurons form synapses has so far been very challenging.

THE ROLE OF GABA IN BRAIN-RELATED DISORDERS

Both autism and epilepsy appear to be associated with defects in the development of GABAergic neurons, particularly in the

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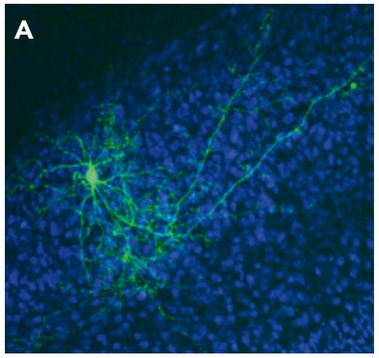

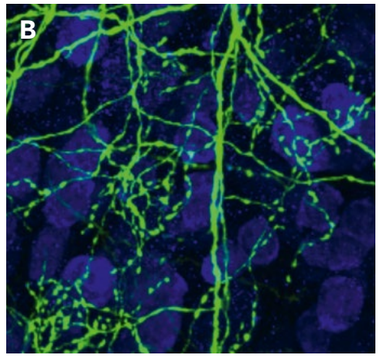

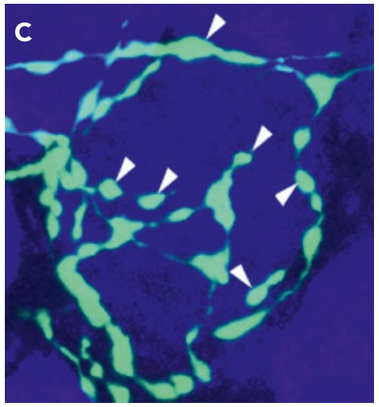

A. Parvalbumin (PV) expressing GABAergic cell in the mouse cortex. PV cell is green because it expresses Green Fluorescent Protein. Cell bodies of excitatory (or pyramidal) cells are labelled using immunofluorescence (blue)

B. High magnification image of the same PV cell. In green, we can see the axon. Please note how complex it is and how it seems to hug its target (neuronal cell bodies, in blue)

C. High resolution image showing the innervation made by the PV cell onto an excitatory (pyramidal) neuron soma. Axon is in green. The beads along the axon, indicated by arrowheads, are putative synapses between the PV cell and the cell body of the excitatory cell.

way in which these neurons connect and communicate with each other, via synapses. Drs Chattopadhyaya and Di Cristo applied an elegant combination of live imaging and gene manipulation techniques to try and shed light on the mechanisms regulating the formation of GABAergic synapses.

They conducted experiments on mice and found that manipulating neural activity by supressing or increasing the release of GABA neurotransmitter itself could affect the formation of GABAergic synapses. They also found that specific genes and molecular pathways regulated by those genes influence the formation of synapses.

In parallel studies together with Dr Carmant, Drs Chattopadhyaya and Di Cristo explored brain development following low-oxygen, or hypoxia-induced seizures (HIS), since such insults in the neonatal brain can lead to severe cognitive dysfunctions later in life. Drs Di Cristo and Chattopadhyaya found that HIS affects the maturation of different GABAergic neurons in the neo-cortex, which appears to be associated with an impaired working memory and altered social behaviour. Further investigation into molecular mechanisms involved could help to develop new drugs that could prevent the consequences of HIS.

More recently, Drs Di Cristo, Chattopadhyaya and Michaud further explored the neural dynamics behind intellectual disabilities and autism, with a particular focus on the role of GABAergic circuits. Previous research by Dr J. Michaud and other researchers has shown that SYNGAP1, a protein that in humans is encoded by the *Syngap1* gene, is critical for the development of cognition and formation of synapses, with mutations of this gene potentially causing intellectual disabilities, autism and epilepsy. Together with Dr Michaud, Dr Di Cristo and Dr Chattopadhyaya found that *Syngap1* gene mutations reduced the formation of connections between particular GABAergic cells. Such reduced inhibitory synaptic activity suggests that *Syngap1* mutations in GABAergic circuits could contribute to cognitive deficits associated with neurodevelopmental disorders.

Drs Di Cristo and Chattopadhyaya applied an elegant combination of live imaging and gene manipulation techniques to shed light on the mechanisms regulating the formation of GABAergic synapses

Q&A

When did you start researching brain development and plasticity?
Di Cristo: As an undergrad in Pisa, in 1995, I had the opportunity to work in the lab of Prof Lamberto Maffei, learning how to record visual cortical neuron activity to study mechanisms of brain plasticity, for example, what makes the brains of children more amenable to learning new things as compared to an adult, and this to me was such an exciting question to explore.

Chattopadhyaya: I was first exposed to the field of synapse development and plasticity when I started my PhD with Dr Josh Huang in Cold Spring Harbor in 2000, where we were developing new genetic tools to label and manipulate different types of GABAergic neurons, with the aim of understanding if and how they may alter plasticity in the developing brain. Since GABAergic neurons are so heterogenous in the brain, there were no established techniques to reproducibly identify them then in live tissue, and it was just amazing when we could first consistently label them and could actually visualise how they form synapses/connections.

What do you feel are some of the greatest challenges related to the study of brain function and development?
There are many critical aspects in brain development, such as, how different types of neurons are generated, how they establish their identity, how they connect to each other, and in doing so form neural circuits that make a functional brain that is responsive and adapts to its environment. In this astonishing complexity of neuronal types in the brain we chose to focus on the GABAergic interneurons, since they play such important roles in regulating brain activity and plasticity. Therefore, factors that affect their normal development and function may also underlie some of the deficits that we see in neurodevelopmental disorders. Within the field of GABAergic interneurons, we are fascinated by questions such as: How do neurons form synapses on another neuron, how many synapses does it decide to form, are there specific molecules that dictate this process and how is the process of connectivity regulated by experience? By exploring all these aspects of synapse formation in a normal developing brain we hope to understand the process in enough detail so as to understand when and if we should intervene, to ameliorate cognitive deficits caused by either genetic mutations (such as those that cause intellectual disabilities, autism) and/or early life insults (such as neonatal hypoxia).

What do you feel were some of your most interesting findings so far and why?
We are characterising different molecular mechanisms that regulate GABAergic synapse development and plasticity. One of the molecular pathways we discovered is that through modulating the activation of a protein called p75NTR (low affinity receptor for neurotrophin), we can alter the number of GABAergic synapses, both during development and in adulthood. For example, by activating this receptor in the adult brain, we can reduce the number of synapses formed by GABAergic cells and, in parallel, re-introduce juvenile-like levels of plasticity in the adult brain. Controlled activation of this receptor, coupled with rehabilitation, may help increase plasticity and aid function recovery after stroke, for example. This is the hypothesis that we're currently testing.

What are your upcoming plans for future research and investigation?
We will continue to look at the mechanisms controlling/modulating GABAergic synapse connectivity and plasticity during development, by using a combination of high resolution imaging (*in vitro* and *in vivo*), genetic manipulation and cognitive testing in rodents. We hope that such knowledge may indicate subcellular substrates potentially affected in specific neurodevelopmental disorders. Furthermore, it may help to develop novel tools to facilitate and foster brain plasticity in the adult. Further along the road, coupling cognitive rehabilitation with pharmacological treatments that facilitate brain plasticity could correct neuronal network connectivity and function and reduce cognitive deficits.

In addition to autism and epilepsy, what other neurological conditions may involve GABAergic synapse connectivity?
Multiple lines of evidence suggest that GABAergic synapse function is affected in schizophrenia (even if it is not the only deficit). The expression levels of proteins important for GABAergic synapses and neurotransmission are reduced in the brain of persons affected by this disease.

BREAKING THE BRAIN CODE
The dynamics of the human brain are incredibly complex, which makes the attribution of specific roles and functions to its different circuits a challenging task. Drs Di Cristo and Chattopadhyaya's research has substantially contributed to the study of brain plasticity and development, with their most recent work suggesting that dysfunction in the developing GABAergic circuits is related to the cognitive deficits seen in individuals with disorders, such as intellectual disabilities, autism and epilepsy. In future, this could help to develop effective pharmacological treatments for these often debilitating conditions, which would counteract the chemical imbalances in the brain of those affected.

Detail

RESEARCH OBJECTIVES
Drs Di Cristo and Bidisha Chattopadhyaya's research focuses on understanding brain development, plasticity and function. They aim to better understand the mechanisms of the many diseases affecting the brain.

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COLLABORATORS
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BIO

Associate Professor, Dept. of Neuroscience, Université de Montréal, Dr Graziella Di Cristo runs her lab at Centre Hospitalier Universitaire (CHU) Sainte-Justine Research Centre. Completing her PhD in Pisa, Italy, and postdoctoral training at CSHL (NY, USA), she was awarded the Canada Research Chair (Tier2) in Neural Circuit development in 2006, and NARSAD Young Investigator award in 2007.

Dr Bidisha Chattopadhyaya is Associate Research Scientist at the (CHU) Sainte-Justine Research Centre. She received her PhD from Stony Brook University (NY, USA) and postdoctoral training in Basel, Switzerland. She received the Steriade Award with the Savoy Foundation postdoctoral fellowship in 2008 and in 2009 was awarded the CHU Sainte-Justine Foundation Postdoctoral Fellowship for Excellence.

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