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INTERVIEW

The people behind the papers – Roberto De Gregorio and Ji Sze

Serotonin uptake receptor (SERT) plays an important role in neural circuit development and reduced SERT function is associated with neurodevelopmental disorders. Now, a paper published in Development finds that SERT acts in a defined developmental time window in the hippocampus in a sex-biased fashion, and its absence from CA3 pyramid neurons leads to defects in neural circuit assembly, electrophysiology and behaviour. We caught up with first author Roberto De Gregorio and corresponding author Ji Sze, an Associate Professor at Albert Einstein College of Medicine, to find out more about their research.

Roberto De Gregorio (L) and Ji Sze (R)

Ji, can you give us your scientific biography and the questions your lab is trying to answer?

JS: Since the very start of my career, I have been interested in understanding the fundamental molecular mechanisms of plasticity in normal biological processes and in disorders associated with aberrant stress responses. I did my undergraduate research on limb regeneration in newt models, and my PhD dissertation on metabolic stress-induced gene expression changes in yeast models, at Purdue University. As a postdoc at Harvard Medical School, I utilized *C. elegans* as a genetic model to delineate transcriptional regulation of the biosynthesis of serotonin (5-HT), a well-established neuromodulator of cognitive function and stress responses. Unexpectedly, I found that three non-serotonergic neurons in the worm can take up 5-HT from the extracellular space. Moreover, feeding worms with antidepressant selective serotonin reuptake inhibitors (SSRIs) completely blocks 5-HT uptake by these neurons. Later, postdocs in my lab identified that these neurons express the serotonin uptake transporter (SERT/MOD-5). They found that SERT function in these non-serotonergic neurons is essential for a normal behavioural response to aversive environments, and SSRI exposure impairs the behaviour. My experience from studying multiple model organisms led me to hypothesize that the biological importance of SERT expression in non-serotonergic neurons is conserved across phyla. Currently, we focus on the role of SERT expression in CA3 pyramidal neurons and downstream molecular pathways of SERT in the establishment of neural circuits in the hippocampus in males versus females using mouse models. We are also very curious to understand whether the transient SERT expression in the non-serotonergic neurons in the CA3, cortex and thalamus, three core limbic regions, coordinates the circuit assembly in these regions to shape sensory perception, cognition and behaviour.

Roberto, how did you come to work in Ji's lab and what drives your research today?

RDG: In mid-2016, I was finishing up my PhD project studying the development of midbrain dopaminergic systems in Umberto di Porzio and Gian Carlo Bellenchi's lab at the IGB-CNR in Naples, Italy. Umberto passed away this year and I will always be grateful for his guidance during my very early years in science. At the time, both my partner – who is now my wife – and I were looking for postdoc positions. She found one at the Einstein, so I also searched at Einstein and Ji had a postdoc opening posted on Nature Jobs. I was actually hesitant about applying at first because I noticed that most of her previous work was on *C. elegans* serotonin signalling, and, I admit, I wasn't up to date on the *C. elegans* field. But when I read her work on SERT function in non-serotonergic neurons in *C. elegans* and mice, I got fascinated by it. During the Skype interview, Ji explained the project to me, and I started to truly appreciate its potential and importance. Now that this part of the project is published, there are still many important questions to be addressed. The most burning question for me is how to apply the knowledge we have gained from model systems to understanding the mechanistic origins of related human diseases and developing methods to facilitate accurate diagnosis and treatments of the diseases.

What was known about the role of SERT in both neural circuit development and neurodevelopmental disorders prior to your research?

JS & RDG: The developmental roles of SERT in neural circuits underlying cognitive function, emotion processing and stress responses have been studied for decades. Starting from the 1990s, a series of human genetic studies found associations between reduced SERT expression/function, resulting from a polymorphism in the transcriptional control region of the SERT gene *Slc6a4*, and abnormal anxiety, depression and neurodevelopmental disorders, including autism spectrum disorders (ASD) and attention-deficit/hyperactivity disorders (ADHD). In addition, the use of SSRIs during pregnancy has been found to increase risks for abnormal structural connectivity/activity of the corticolimbic circuits, a range of neuropsychiatric symptoms and ASD in the offspring. Reduced SERT function has been shown to impair cortical topographic map development, cause elevated anxiety and impair cognitive behaviours in rodents, through elegant studies from multiple labs using whole-body SERT knockout and timed early life exposures to SSRIs. However, the biological basis for SERT functions in

Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

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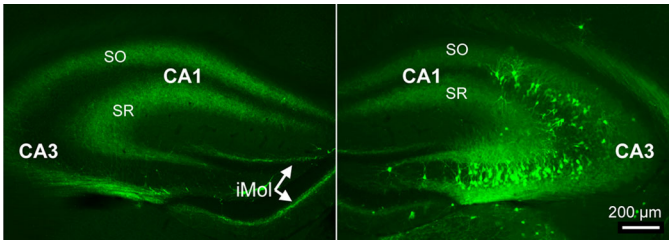

SERT-expressing CA3 pyramidal neuron axons innervate the stratum oriens (SO) and stratum radiatum (SR) of CA3 and CA1, and inner-molecular layer (iMol) of the dentate gyrus of the ipsilateral hippocampus, passing through the midline via the commissural pathway to the contralateral hippocampus.

brain development remains unclear. While our findings of SERT function in non-serotonergic neurons in *C. elegans* inspired us to investigate SERT function in non-serotonergic neurons in mammalian brain, a further stimulation came from Patricia Gaspar's work showing SERT expression in non-serotonergic neurons in specific brain regions and only during development in mice. Therefore, many prior studies with diverse organisms paved the way for this research.

Can you give us the key results of the paper in a paragraph?

JS & RDG: This collaborative work with Pat Stanton and Ian Maze revealed that the temporal-specific SERT function in non-serotonergic neurons is critical for normal neural circuit assembly in the male versus female hippocampus, an essential brain region for learning, memory and stress responses. Our electrophysiological and behavioural analyses suggest that disrupting this developmental SERT function may permanently alter the activity-dependent plasticity of hippocampal neural circuits and a range of cognitive behaviours in both males and females, but specific changes differ between the two sexes. This is noteworthy, as certain behavioural impairments, for example the fear-context memory deficits that we observed in the female mutant mice, may not manifest until later in life and only under particular environmental conditions. This raises the possibility that certain cognitive and behavioural deficits, particularly in females, may be missed using the current diagnostic protocols for neurodevelopmental disorders. Our RNA-seq analyses suggest that CA3 SERT function modulates gene expression in the hippocampus during the period of functional synaptic circuit assembly. This implies that there is a timed intrinsic genetic mechanism, where SERT is merely one component, which is dedicated to specifying neural circuit assembly and could explain the difficulty in 'reversing' aberrant neural circuits in the treatments of core phenotypes of certain neurodevelopmental disorders.

Are there any differences in timing or levels of expressions of SERT mRNA in CA3 pyramid neurons between male and female mice?

JS & RDG: Our data show a similar time window of SERT expression and comparable SERT mRNA levels in the CA3 pyramidal neurons in male and female mice. On the other hand, we found differences in mRNA levels of certain 5-HT receptor subtypes in male and female hippocampus during hippocampal circuit assembly. Therefore, SERT function may influence hippocampal circuit development in males versus females via, in part, inherent sex-biased expression of 5-HT receptor subtypes.

Astrocyte development is one of the GO terms that stands out from the male-specific differentially expressed genes. What role do astrocytes play in synaptic circuit assembly and how might serotonin affect their development?

JS & RDG: This observation is very intriguing. Astrocytes are specialized glial cells that relay signals between neurons and the vasculature of blood-brain barrier (BBB). A single astrocyte may envelope >100,000 synapses from multiple neuronal types, influencing synapse formation, maturation and synaptic function through precisely patterned expression/function of transporters, receptors, signalling molecules and metabolic enzymes. Astrocyte end-feet enwrap brain blood vessels, regulating the BBB properties and coupling energy source, peripheral physiological states and synaptic activity. Particularly intriguing to us is the establishment of neuron-astrocyte-vasculature coupling, which coincides with the period of functional neural circuit assembly, closely matching the time window of SERT expression in the non-serotonergic neurons. Currently, the functional relationship between disrupting CA3 SERT expression, 5-HT signalling, altered astrocyte gene expression and hippocampal circuit assembly is not known. One hypothesis is that excessive 5-HT perturbs the gene expression pattern in astrocytes in the hippocampus, which in turn alters the synaptic circuit assembly. An alternative hypothesis could be that CA3 SERT coordinately regulates 5-HT signalling in the astrocytes and the neurons, thereby coordinating the establishment of neuron-astrocyte-BBB functional coupling and neural circuits. Our mouse models may provide an entry point to address these questions in males versus females.

What impact will your research have on the debate surrounding the use of selective serotonin reuptake inhibitors during pregnancy?

JS & RDG: Our findings indicate the importance of SERT function in non-serotonergic neurons during a specific developmental time window. The database of recent human cortex single-cell RNA-seq shows SERT expression in non-serotonergic cortical neurons in mid-fetal gestation. This could be one explanation for the association between fetal SSRI exposure and a range of neuropsychiatric symptoms. It is important to define the brain regions and the time window of SERT expression in non-serotonergic neurons during human fetal brain development. This may help clarify the impact of reduced developmental SERT function on the mechanistic origins of certain neuropsychiatric symptoms and provide a basis for refining the time window of fetal brain development that is particularly susceptible to SSRI exposure. Together, this information will help physicians to provide better guidance for SSRI users and alternative medications for treating anxiety in pregnant women.

When doing the research, did you have any particular result or eureka moment that has stuck with you?

RDG: I think it was when Ji and I started to realize that there were sex-specific effects in our SERT mutant mice. It has been very exciting to confirm the idea through different approaches, examining the effects of perturbation of SERT in these non-serotonergic neurons on gene expression during hippocampal circuit development, hippocampal circuit plasticity and behaviours in the male and female mice. It was fun to speculate and plan the next experiments, and it was exciting when the results supported our hypothesis, especially those from our collaborators, who did not know our speculation. We still do not understand how disrupting this developmental SERT function produces different effects on

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males and females, and a lot more needs to be done. The conditional SERT knockout mice could be a model for addressing our outstanding questions.

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And what about the flipside, any moments of frustration or despair?

RDG: I did not really have any moments of despair related to the project, as I have been fascinated with my experimental results. There were, of course, many difficult moments. As SERT function in the non-serotonergic neurons during hippocampal circuit development has not been studied before, there are so many questions in my mind. Many times, I found myself struggling to balance doing experiments to address multiple interesting questions and prioritizing experiments for the first paper. Studying the sex-biased effects doubled the number of mice and assays we needed to analyse, and frequently the mice did not produce litters with mutant and control pups of both sexes for testing littermates. I have been fortunate to have confidence from Ji and support from collaborators, Ian Maze and Pat Stanton, and their postdoc and student. I am truly grateful to them.

What is next for you after this paper?

RDG: From the very beginning of my career, I have been interested in applying knowledge gained from basic research to help understand the mechanistic origins of human diseases and identify alternative paths for disease treatments. During the last phase of this research, I had the opportunity to learn the power of functional genomics through collaborations with Ian Maze's lab and Deyou Zheng's lab. I will focus the next phase of my career on applying cutting-edge genomic approaches to characterize human clinical samples, with the ultimate goal of contributing to the development of new diagnostic and prognostic strategies that may help to facilitate disease subgrouping and identify potential personalized treatments for common diseases.

Where will this story take your lab next?

JS: Clearly, many questions remain to be addressed about the fundamental mechanisms of neural circuit assembly in males versus females, and the role played by SERT and 5-HT signalling. My favourite project is utilizing the phenotypes we have observed in our mouse models as a tool to delineate SERT downstream pathways in astrocytes, in other glial cells and in neurons. We aim to characterize the impact of 5-HT signalling on functional maturation of the neuron-astrocyte-vasculature coupling and its relationship to normal regional neural circuit assembly, as well as cognitive and behavioural deficits associated with perturbations during development.

Finally, let's move outside the lab – what do you like to do in your spare time?

RDG: I like cycling, running, and hiking. I am originally from Massa Lubrense, a small town on the Amalfi Coast, which has amazing panoramic trails. I enjoy art exhibitions and music venues, and so I am lucky that I'm in New York! I love to spend time with my wife and our son, exploring surrounding small towns and meeting friends.

JS: Spare time is rare. My new hobby is speed walking in the woods around my home in the Westchester.

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