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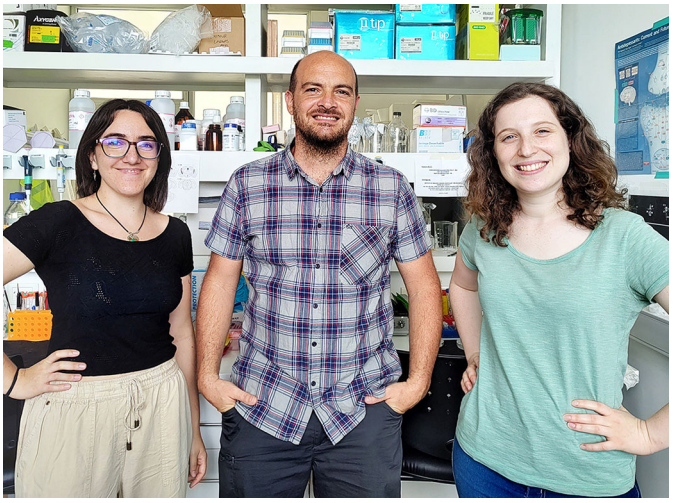
INTERVIEW

The people behind the papers – Carla Argañaraz, Tamara Adjimann and Mariano Soiza-Reilly

Serotonin neurons have been implicated in maladaptive neural mechanisms that could contribute to mental disorders such as depression and anxiety. In a new paper in Development, Mariano Soiza-Reilly and colleagues identify a key postnatal period when the synaptic inputs to serotonin neurons undergo profound refinement. We caught up with PhD students and co-first authors Carla Argañaraz and Tamara Adjimann, and corresponding author Mariano Soiza-Reilly, a group leader at the Instituto de Fisiología, Biología Molecular y Neurociencias in Buenos Aires, to find out more about their research.



Carla Argañaraz, Mariano Soiza-Reilly and Tamara Adjimann (L-R)

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

MS-R: I studied biology in Argentina at the University of Buenos Aires, where I also did my PhD in the field of neurobiology working in the developing striatum with antipsychotic drugs. Then, I moved to Boston for a postdoc with Dr Kathryn Commons at Boston Children's Hospital, who introduced me into the fascinating world of serotonin, its circuitry and its complicated architecture. During my postdoc, I adapted a novel technique at that time, called array tomography (Micheva and Smith, 2007), to investigate structural changes in synaptic circuits controlling the function of serotonin neurons that could be implicated in the pathophysiology of mood disorders. After that, I moved to Paris for a second postdoc in the lab of Dr Patricia Gaspar, a world-renowned expert in neuroanatomy of serotonin system. There, we studied a neurodevelopmental mechanism implicated in the early-life vulnerability to the effects of serotonin-related antidepressants, such as fluoxetine (Prozac). Our study revealed a novel neural mechanism by which fluoxetine can alter the synaptogenic capacity of certain prefrontal circuits implicated in stress responses and depressive-like and anxiety behaviours in the mouse. I am still intrigued by some of these questions. It is puzzling to me how neurodevelopmental mechanisms could lead to selective (and often undesired) effects of prescribed drugs during critical periods or why there are age windows of increased vulnerability for individuals to develop psychiatric disorders in adult life. These are our main questions in the lab, which I started in 2019 at the Instituto de Fisiología, Biología Molecular y Neurociencias (IFIBYNE-UBA-CONICET) after I moved back to Buenos Aires. Our team seeks to understand how maladaptive neurodevelopmental mechanisms engaged in the maturation of neural circuits could contribute to the early origins of psychiatric disorders, such as stress vulnerability, depression and anxiety.

Carla and Tamara, how did you come to work in Mariano's lab and what drives your research today?

CA: I was taking my final year courses of my degree in biology and was specialising in neuroscience. I really wanted to understand the mechanisms behind psychiatric diseases and was seeking a lab where I could do my final thesis and then hopefully continue on with a PhD project. I still thank my good luck, because Mariano was just returning to Argentina to start his own lab, and as soon as I saw the research focus I emailed him asking to be part of the team. This was 4 years ago; I have now got my degree and I am currently a PhD candidate in the lab, trying to uncover the complexities behind neuronal circuits development and function.

TA: During the last year of my biology degree at the University of Buenos Aires, I was looking for a lab where I could do research for my graduate thesis. I was struggling to find an option related to my interests in neuroscience and human health that felt right for me. I was starting to lose hope of finding the right match, when a friend shared a call for students from a new lab that was starting soon. The lab's main focus was the early origin of psychiatric disorders and had a very novel approach, with techniques that were fascinating to me. I answered the call and met Mariano for an interview; we understood each other very well and I joined the team. Carla and I were the first students in the lab and it has been very enriching to witness its assembly and growth. I believe that our topic of research is very important and to be able to contribute to the understanding of psychiatric disorders is what motivated me to stay in the lab after graduating, and to pursue a PhD in the same line of research.

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

MS-R, CA & TA: Our study reveals a postnatal period of synaptic refinement of certain axonal afferents that modulate the activity of serotonin neurons of the dorsal raphe nucleus. We found that only cortical glutamate afferents and subcortical GABAergic inputs undergo this ontogenetic refinement between the third and fourth postnatal weeks, whereas subcortical glutamate inputs were not reached by this process. Furthermore, other inhibitory mechanism controlling the activity of raphe serotonin neurons, such as 5-HT1A receptor-mediated inhibition, was not changed at these ages.

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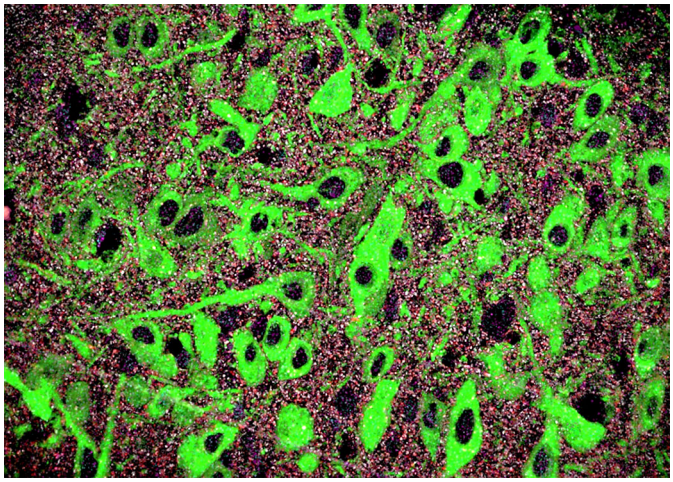

Synaptic neuropil of the developing mouse dorsal raphe nucleus visualised with the high-resolution immunofluorescence technique array tomography. A group of serotonergic neurons containing the serotonin synthesis enzyme tryptophan hydroxylase (green) is heavily innervated by both glutamate and GABAergic synaptic afferents containing the vesicular protein synapsin 1 (grey). Different populations of glutamate synaptic boutons are identified by the co-presence of the vesicular glutamate transporters 1 (VGLUT1, light blue) or 2 (VGLUT2, magenta), and GABAergic afferents by the co-expression of the GABA synthetic enzyme glutamate decarboxylase 2 (GAD2, red).

Your study uses array tomography – could you give us a short introduction to this technique?

MS-R, CA & TA: Kristina Micheva and Stephen Smith introduced array tomography to Mariano at Woods Hole at the Marine Biological Laboratory during the Neurobiology course, which Mariano attended as a student and then as Dr Commons' teaching assistant. This technique is a hybrid between electron and fluorescence microscopy. The idea is that you embed the tissue of interest in a plastic resin that, after curation, can be sectioned to give ultrathin serial sections of your sample. This gives you an extremely high resolution in the subsequent immunostaining, especially in the *z*-axis. The immunofluorescent detection of protein antigens is performed using primary antibodies and fluorophore-conjugated secondary antibodies, then serial images of immunostainings across the adjacent ultrathin sections are acquired, and finally these images are rendered in 3D. Using ImageJ, relationships between different antigens are quantitatively analysed in the whole tissue volume, giving a density of puncta when labelling synaptic proteins. The technique is very powerful, allowing us to investigate spatial associations of different molecular components of the synapses. But also, perhaps one of the most remarkable features of array tomography is that the antibody can be eluted and the sections re-labelled with a different set of antibodies, allowing many rounds of immunodetection and multiplexed analysis of many proteins in the same tissue volume.

Were you surprised to find that the synaptic refinement was not accompanied by changes in 5-HT1A receptor-mediated inhibition?

MS-R, CA & TA: We were interested in determining whether the decrease in GABAergic inhibitory inputs to serotonin neurons after the third postnatal week could be compensated for by changes in other inhibitory mechanisms controlling the activity of raphe serotonin neurons, such as the inhibitory feedback loop mediated by the 5-HT1A receptor. We knew from the literature that this inhibitory mechanism becomes active by the third postnatal week, and we hypothesised that this loop could be enhanced by the fourth week, in part compensating the decrease in GABAergic inhibitory inputs. However, the results surprised us and inhibition through 5-HT1A receptors was not changed between both ages studied. We also evaluated possible sex differences in the feedback loop, but we did not find any effects.

What implications will your study have on understanding or treating mental disorders?

MS-R, CA & TA: As we mentioned before, we are very interested in understanding how maladaptive neurodevelopmental mechanisms could contribute to the early origins of psychiatric disorders. We believe that having a more complete picture on the molecular and cellular actors engaged in brain mechanisms of circuit assembly and maturation will contribute to our understanding of the early pathophysiology of mental disorders, as well as identify novel therapeutic targets to prevent or treat mental conditions with developmental roots.

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

CA: I don't really recall having a particular eureka moment, it was more of a slow-developing feeling of excitement when we started to put all the pieces together and realised they began to fit in the bigger puzzle.

TA: To me, it was very striking to see how much a manuscript can grow after the reviewing process. Triggered by a comment from a reviewer, Mariano and Paula's knowledge and experience allowed us to do a more comprehensive analysis of part of the data that ended up in a new supplementary figure, which I believe contributes a lot to the work in general.

To me, it was very striking to see how much a manuscript can grow after the reviewing process

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

CA: We did have to face the COVID-19 pandemic during the time we were working on this paper, and that came with its own challenges. There was a lot of uncertainty about when we would be allowed to go back to work to get the lab going again.

TA: Well, those are always part of the process. Our work requires us to spend a lot of time watching a screen, taking and analysing images. It can be tiring sometimes, but it is all worth it in the end when you finally have the results.

Carla and Tamara, what is next for you after this paper?

CA: I will continue working on my PhD project, which involves trying to elucidate how early adversity influences the development of neuronal circuits involved in emotional behaviour, and may contribute to the aetiology of psychiatric disorders.

TA: This paper is the beginning of a series of studies that I am carrying out in the lab focused on understanding the neurodevelopmental maladaptive changes triggered by postnatal exposure to fluoxetine, and how we could design possible early interventions to prevent alterations in the circuits and adult behaviour.

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Where will this story take your lab next?

MS-R: We are studying how the synaptic modifications we described could interact with early-life environmental perturbations linked to the vulnerability of individuals to develop mental disorders later in life. Another aspect that we are exploring is the molecular/cellular mechanisms underlying the process of synaptic refinement. We hope to have some exciting results soon.

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

MS-R: I love swimming, playing football, cooking, playing some guitar, watching movies and sharing activities with my family and friends.

CA: For starters, I enjoy reading a good sci-fi or philosophy book, but since academia already requires a lot of reading, I sometimes need to do something completely different and preferably outdoors, like archery, climbing, cycling and yoga.

TA: I enjoy watching movies, reading books and spending time with friends and family.

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