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Insights into the mechanism of adult neurogenesis - an interview with Arturo Álvarez-Buylla

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ABSTRACT Neurogenesis is the process by which new neurons are formed from progenitor cells. The adult nervous system was long considered unable to generate new neurons, especially in mammals. It was not until the 1960s that Joseph Altman and Gopal Das, using H^3-thymidine autoradiography to trace newly formed cells, that the first suggestions of new neurons added to the olfactory bulb and the dentate gyrus of the rat hippocampus came about. These observations remained controversial for many years as they went against the dogmatic view that the structure of the adult brain precluded processes of neurogenesis. It was not until two decades later that work in songbirds and then in mammals, not only confirmed that new neurons could be produced in the adult brain, but revealed basic processes of how young neurons are produced, how they could migrate long distances and become incorporated into adult brain circuits. Arturo Álvarez-Buylla has made important contributions to the understanding of the mechanism of adult neurogenesis, including the identification of adult neural stem cells. Here we summarize a discussion with him related to the field of adult neurogenesis, the root of his interest in neural development and the ramifications of some of his laboratory findings.

KEY WORDS: *adult neurogenesis, neural stem cell, neuron replacement*

Introduction

In the adult mammalian brain, neurogenesis is mainly restricted to two niches, the ventricular-subventricular zone (V-SVZ) of the lateral ventricles and the dentate gyrus' sub granular zone (SGZ) of the hippocampus. In both regions, the newly generated neurons contribute to circuit function and plasticity; neuroblasts generated in the V-SVZ migrate through the rostral migratory stream to reach the olfactory bulb where they integrate and differentiate into several types of local circuit neurons (interneurons), while the NSC of the SGZ gives rise to the glutamatergic granule cells of the dentate gyrus. Neurogenesis in the hippocampus has been associated with the process of learning and memory, although how it contributes to these processes is not completely understood. The decline of adult neurogenesis with aging or perturbations to this process, have also been associated with neurological or psychiatric disorders. Yet it remains highly debatable whether neurogenesis continues in the adult human brain. The Álvarez-Buylla laboratory is intimately involved in some of these debates. However, independently of whether this process continues in the adult human brain or not, the field of adult neurogenesis has grown rapidly and has provided some of the most basic insights into how new neurons are made. Some of these findings could help develop new therapeutic approaches to the treatment of neurodegenerative disorders and brain injuries.

Arturo Álvarez-Buylla's laboratory has studied for over 30 years the mechanisms of adult neurogenesis and neuronal replacement and has made outstanding contributions in those fields: the identification of adult neural stem cells and their regional organization in the V-SVZ niche (Doetsch *et al.,* 1999; Merkle *et al.,* 2004, 2007); establishing the embryonic origin of adult neural stem cells (Fuentealba *et al.,* 2015); a very detailed histological and ultrastructural characterization of the architecture of both adult neurogenic niches (Doetsch *et al.,* 1997; Seri *et al.,* 2001); the discovery of a new

Abbreviations used in this paper: BBR, Basic Biomedical Research; CNS, central nervous system; MGE, medial ganglionic eminence; NSC, neural stem cell; PGC, primordial germ cell; SGZ, sub granular zone; V-SVZ, ventricular-subventricular zone.

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Fig. 1. XII Congress of the Mexican Society of Developmental Biology in Lagos de Moreno, Jalisco. Mexico, March 2016. *From left to right: Marcos Nahmad, Susana Castro-Obregón, Enrique Salas-Vidal, Arturo Álvarez-Buylla, Fernando López-Casillas, Diana Escalante-Alcalde, Hilda Lomelí, Mario Zurita and Martín García-Castro.*

mechanism of neuroblast migration in the adult brain (Lois *et al.,* 1996); the discovery of novel migratory paths for young neurons in humans (Sanai *et al.,* 2011; Paredes *et al.,* 2016), the identification of embryonic brain progenitors that can migrate and integrate in the adult (Witchterle *et al.,* 1999); the characteristics and the kinetics of adult NSC division (Ponti *et al.,* 2013; Obernier *et al.,* 2018); identification of new interneuron types in the olfactory bulb (Merkle *et al.,* 2014), among others. The versatile and elegant use of a wide variety of technical approaches, both morphological and molecular, is evident in his works (https://pubmed.ncbi.nlm.nih.gov/?term=Alvarez-Buylla+A&sort=pubdate). Those of us who are fortunate enough to know him, would describe him as an excellent scientist, extremely creative, humble, with a brilliant mind, a broad and comprehensive vision of the scientific career and, with the unique characteristic that he thinks outside the box.

Son of immigrant Spanish scientists, Arturo Álvarez-Buylla Roces was born in Mexico City, obtained a bachelor's degree in Basic Biomedical Research at the Institute of Biomedical Research at the National Autonomous University of Mexico (UNAM) and completed his Ph.D. in Neurobiology and postdoctoral training at the Rockefeller University, USA. Álvarez-Buylla has received numerous accolades during his career, including the Prince of Asturias Award for Technical and Scientific Research in 2011, a prize that he shared with Joseph Altman for their contributions to the adult neurogenesis field. He started his laboratory at Rockefeller University, New York, and currently is Professor in the Department of Neurological Surgery at the University of California, San Francisco, and Principal Investigator in the Brain Tumor Research Center. He is also co-founder and scientific advisor of Neurona Therapeutics. His ties with science and education in Mexico, and Ibero-America in general (Fig. 1), are remarkably close and he enjoys conversing with graduate and undergraduate students at every opportunity (Fig. 2).

In this enjoyable interview, Arturo Álvarez-Buylla shares with us the early days of his interest in understanding how the brain is assembled and works, what his academic influences were, his vision in the field of adult neurogenesis in vertebrates, including the human being, and what he believes the future holds (Fig. 3).

You come from a family of scientists who came to Mexico as immigrants. How has it influenced you or influenced your decision to pursue a scientific career?

Very much, my sisters and I grew in an environment where discussion, curiosity, tinkering and scientific thinking were constantly promoted. We learned to love nature, to admire the working of the human organism, to be critical and creative. It was a stimulating environment. My father was a science enthusiast, extremely original in his research, an idealist who stayed away from politics to focus on education and research. He believed that science was the most humane of professions and where knowledge has the potential to contribute to the common good. My mother too, devoted her life to us, to education and science. I was extremely lucky to be surrounded by this very stimulating environment.

Did you ever think about following a non-scientific career?

Yes, I always liked using my hands and to see constructive things emerge from this. This is more tangible than writing grants, sitting all day in front of a computer, correcting text, or fighting reviewers. I think I would have enjoyed just being a mechanic, a construction worker or perhaps an electrician or plumber. I was always attracted to the equipment in my parent's laboratories, the machines with those fantastic analytical powers or that could automate complex tasks. I would say that deep down I am an engineer, I like taking things apart, trying to figure out how they work and, if I can, putting them back together. At some point I thought about engineering or physics, I spent a year at Queen's University in Canada studying physics, when my father was there as visiting professor. This was a year before signing up to the Basic Biomedical Research (BBR) program at the UNAM here in Mexico. The BBR program offered an opportunity to begin working in the laboratory and playing with incredible tools from the outset of the degree. At the time, it seemed like a unique and fantastic opportunity; and I think it was. However, I must say I probably spent as much time in the work-shop learning from carpenters, electricians and mechanics as I did in the classrooms and laboratories learning from the many great professors.

Why did you study the bachelor's degree in Basic Biomedical Research (BBR) and not Biology or Medicine?

My father insisted that I had to study Medicine, he thought it was a career with better prospects for research. My father was passionate about studying physiology to find medical applications. He was a medical doctor and saw the organism as a machine, so he thought it was much more important to study medicine than to study a novel career as BBR. While we were in Canada, I heard about the Basic Biomedical Research program from Alejandro Garciarrubio, a good friend of mine. Upon my return, the two of us signed up for this new program. As I mentioned above, being in a laboratory from the initial stages of our university training, appeared hugely attractive at the time and I do not regret the decision. My classmates and I had a great curriculum and enthusiastic teachers that gave us a world-class education. I then went on to Rockefeller University in New York to do my Ph.D. There too, I spent as many hours in the machine shop as I did in the laboratory. Through both, I was already becoming well trained for doing experimental work and for tinkering with machines. I realized I had some gaps, especially in structural biology, but the training from the BBR program as an experimenter and in thinking critically were extremely useful.

Your father was a neurophysiologist and your mother still does research in neuroendocrinology. Did this influence your decision to dedicate yourself to neurosciences in some way?

When I joined the BBR program, my original interest was and still is now, Developmental Biology. I am extremely curious about how organisms are assembled and organized. I think I can trace this interest back to chats with my father. He was a functionalist: he trained in Russia in the physiology school, in part initiated by Pavlov, where there was a heavy emphasis on the importance of the function in the organization of systems, including neural circuits. However, studies during the last 50 years have taught us that a remarkable amount of initial organization comes from the genes that pattern the early organism, including the nervous system. This part, my father did not like. He thought it was a reductionist perspective that could not explain the wonderful adaptation that you observe for all functions. Instead, he believed that the "function makes the organ". As with many things in science, there is an element of truth to this, but the initial shaping and working of all organs is orchestrated by phenomenally interesting genetic and epigenetic programs that we all are still trying to understand. How wonderful it would be to discuss some of this new knowledge, today, with my father. I am sure I will not be able to change his way of thinking, but it would be so much fun to have more ammunition for the discussion. But yes, the family influence must have had an impact on my interest in the brain. For example, in the rainy season in Mexico City, after collecting some tadpoles and witnessing their metamorphosis, I was incredibly intrigued by the change of shape and function. He challenged me to think how the newly formed legs emerged, a process that even to this day I cannot easily explain. To him, since the simple crushing of the nerve can bring to a halt to this fantastic morphogenetic event, the nervous system was the orchestrator of the entire process. He used this to reinforce his view that function makes the organ. The development of organs began to draw my attention, but the organ that interested me most was the brain, the nervous system in general, how it is assembled starting from a simple epithelium.

What other influences did you have?

Since I was interested in Developmental Biology and the Nervous System, during my BBR studies, I chose to join laboratories that worked in both areas. One was studying GABA neurotransmitter release in Ricardo Tapia's laboratory at the Institute of Cellular Physiology, UNAM. Later, I did my bachelor's thesis project with Horacio Merchant-Larios at the Institute of Biomedical Research, also at the UNAM. We described how primordial germ cells (PGC) use fibronectin for their migration (Alvarez-Buylla and Merchant-Larios, 1986) and characterized the alkaline phosphatase PGC express in mice (Merchant-Larios *et al.,* 1985). I was impressed by the long migrations that cells make to form organs, in this case, the gonads. These journeys of cells, still to this day, fascinate me: How do cells find their way, adjust their numbers or why do they

Fig. 2. Meeting with students of the Basic Biomedical Research and Neuroscience bachelor programs. *Institute of Cellular Physiology, UNAM. Mexico City, April 2019.*

have to come from such distant locations, if all cells have the same genetic code? Not only in the gonads, but in the nervous system and in most organs, cells make long journeys from their sites of birth (and specification) to the final locations where they become incorporated. The mechanism of these processes is not only intriguing but is fundamental to understanding how the organism is constructed and how tissue repair occurs. The most important lesson of my time in Horacio Merchant's laboratory was to realize the importance of studies at the cellular level: how understanding cellular behaviors would greatly help explain the way in which tissues become organized. The study of the gonad in that sense is fascinating, with the advantage that its organization is quite simple compared to the complexity of the nervous system's development. I was fascinated by the ability of cells to organize, migrate, to connect and to send each other information to produce structures. Back then, when the problem was beginning to be understood from a cellular point of view, it seemed to me it was going to be a matter of a few years and we would know exactly how organogenesis occurs. Now I realize it is much more complex, and further complexity keeps emerging.

However, several fundamental concepts have emerged over the years. For example, incredible progress has been made to understand how cells communicate with each other on the molecular and biochemical levels, or with the identification of transcription factors that control morphogenesis. Equally important, since I was in the BBR program, major corrections to fundamental concepts have been made. We were wrong about the nature of many glial cells and the identity of neural stem cells. We were also wrong about the origin of many cells in the brain, or the assumption that the adult brain parenchyma could not support long-range neuronal migration. Yet, if there is one organ where many more discoveries and corrections await it is in the brain.

Now that you touch on the subject, the beginning of your time as an undergraduate student coincided with the rise of the molecular age in Developmental Biology. How much did that influence your thinking? How much did you resist or get carried away in that sense?

Of course, the molecular revolution has been fundamental to all biological disciplines. In the BBR program, we were exposed to some of what was to come next. For example, we had courses on the most recent molecular cloning techniques with Francisco Bolivar, on the mechanism of genetic regulation with Jaime Mora and Rafael Palacios and a fantastic course in genetics with Fernando Bastarrachea. Mario Castañeda of the Institute of Biomedical Research at the UNAM organized an outstanding course in Developmental Biology and how genetic and molecular modeling helped us to understand development and the link to evolution, what has become so fashionable these days: *EvoDevo*. We also discussed in this course molecular clocks and the role of time in the control of development. I was fascinated to learn how heterochrony can lead to changes in the shape of organs and organisms.

At that time, however - and perhaps it was a mistake - I thought it was more relevant to try to explain the phenomena at the cellular level, rather than dig deeper into the molecular one. It is undeniable that the tools of molecular biology gave us, and continue to give us, tremendous experimental tools which have been essential to those with interests at any level of complexity. However, at that time and frequently to this day, to dig into the molecular level, you had to choose one gene, protein or molecular pathway to try to explain entire developmental processes. This is entirely absurd. However, the molecular tools have become very powerful to address developmental processes. For example, the problem of lineage in Developmental Biology, which cell gives rise to which other cells, or where do cells come from? These cellular questions have been partly solved by molecular tools (e.g. retroviral labeling and genetic barcoding) that enable us to tag individual progenitor cells in selective ways. It is the interactions among cells and the behavior of cells that ultimately result in the formation of tissues. In the case of the nervous system, how neurons find each other to form circuits is a cell behavior problem guided by specific molecules. Early physiological activity, once connections are made, is also key to the survival of both, the synapse and entire neurons. All levels of complexity are important.

Fig. 3. Arturo Álvarez-Buylla during the 5th International Meeting on Stem Cells and Regenerative Medicine, Faculty of Medicine, UNAM. *Mexico City, October 2019.*

Did you have other important influences on your scientific career after your undergraduate studies?

Absolutely, Fernando Nottebohm was my Ph.D. mentor at Rockefeller University and a huge influence on my training. Before I became a student at Rockefeller, I worked for one year in this great institution and attended one of his lectures where he was linking basic new findings on brain organization to behavior. I saw an opportunity to get training in Neurobiology linking molecular and cellular processes to behavior, the way animals manifest their brain functioning. Fernando's laboratory had identified a set of discrete, sexually dimorphic, brain nuclei and circuits that

control song, a learned behavior, in canaries. I was struck by how Fernando's lab was able to link brain plasticity to the growth and contraction of specific brain nuclei. Moreover, his laboratory had just discovered that in one of these nuclei, the high vocal control nucleus HVC, new neurons continue to be added in adulthood (Goldman and Nottebohm, 1983; Paton and Nottebohm, 1984). It was the perfect opportunity to study how processes though unique to embryonic development continue to be present in the adult, linking basic development with brain function. This is, I believe, one of the most basic and interesting problems in Developmental Biology: How, from basic developmental rules, neural circuits emerge that control the amazing animal behaviors we observe in nature. Fernando's laboratory was a place where discussions about behavioral problems were linked with developmental, cellular and molecular events. It was at his laboratory that I started working with birds, and my long-term interest in the mechanism of adult neurogenesis emerged. We wanted to better understand where the new neurons came from and how they migrated from their sites of birth to their final destinations (Alvarez-Buylla and Nottebohm, 1988; Alvarez-Buylla *et al.,* 1988; Alvarez-Buylla *et al.,* 1990). We described incredible long journeys of young neurons guided, in part, by the long processes of radial glia. It was not only surprising to find that radial glia persisted in an adult vertebrate brain, but that these "glial" cells divide and serve as the progenitors for the neurons. This new concept was suggested in songbirds almost a decade before it was realized that in mammals, radial glia are also the neural stem cells in development. The time I spent in Fernando's lab was truly inspirational, not only on how to think and approach science, but also on how to present (in writing or talks) and discuss scientific evidence.

I also trained for a few months in the laboratory of Nicole Le Dourain in Paris, who did the classic experiments for lineage-tracing neural crest cells. My love for Developmental Biology was further strengthened in her laboratory. I believe the transplantation techniques her laboratory pioneered continue to be an extraordinary tool to ask basic questions, such as to distinguish the cell-autonomous effects of cells carrying genetic mutations, and also to study the function of genes that when removed in the entire organism are lethal during embryonic development or shortly after birth. Using this approach we can bypass embryonic lethality, a side exit to study developmental defects. Although I was in her lab for a short period, I learned many things and I think this prepared us for some of the discoveries we were going to make years later using transplantation. Nicole is an extraordinary scientist with truly admirable strength and dedication for research.

That brings us to your field, neurogenesis, and in some measure neuro-regeneration, two incredibly important fields in Developmental Biology. How can we bring together both processes from a three-dimensional point of view - or even in the fourth dimension that is time - to understand if we can regenerate the brain?

This is a hot topic as people have now realized how development is key to understanding neurodegeneration and possibly to come up with new ideas on how to repair the central nervous system. We know that certain vertebrate species can make new neurons and that these cells can migrate through the dense and complicated terrain of the adult brain to become incorporated into neural circuits. This process has been best studied in birds, as indicated above, and also in rodents where new neurons are observed in the olfactory bulb and in the hippocampus. In mice and rats we, and others, have studied how new neurons are generated, migrate and become incorporated in the olfactory bulb. We have also identified the neural stem cells in the adult hippocampus dentate gyrus. This region of the brain is key to learning and other groups have made key discoveries on how adult born neurons contribute to plasticity. This simply tells us that basic steps for the therapeutic replacement of neurons may be possible one day but equating this with brain repair is premature. Firstly, only certain types of neurons (a minute subset from the enormous diversity of neuronal types present in the brain) are produced in the adult brain. During neurodegeneration, or after trauma, a broad variety of neurons generally die. In cases, where a specific neuronal type is primarily affected, e.g. motoneurons, these neurons are not produced by adult neural stem cells. Secondly, the nervous system has a developmental history, during which the sequential recruitment of different types of neurons, is key to circuit assembly and subsequent emergence of proper neural function. This history is not easy to replicate in adults. Thirdly, it remains unclear if, in humans, adult neurogenesis occurs and whether this phenomenon is significant to plasticity and possibly to repair. Our own group finds that in both the olfactory bulb and hippocampus, neurogenesis greatly decreases in young children and it is rare or not present in the adult human brain (Sanai *et al.,* 2011; Paredes *et al.,* 2018; Sorrells *et al.,* 2018). However, other groups believe that neurogenesis is a robust phenomenon in adult humans.

Glial cells may also offer an opportunity for repair. For example, myelinating oligodendrocytes die in multiple sclerosis with a range of debilitating neurological symptoms. There is evidence from the laboratory of Steve Goldman (another of Fernando's disciples), that these cells can be transplanted and that they may be able to re-myelinate adult CNS (Windrem *et al.,* 2020). However, there are still many events that we do not understand. For instance, recently, a group at Harvard has shown that axons are selectively myelinated depending on the kind of neurons. Intriguingly, in neurons that are myelinated, oligodendrocytes leave selective regions of the axons stripped of myelin (Zonouzi *et al.,* 2019). This extraordinary selectivity must have a functional meaning, but the logic remains obscure. Other glial cells that may become potentially useful for cell therapy are astrocytes as they are key in supporting neuronal function (Alvarez-Buylla *et al.,* 2001). Among the astrocytes, there are multiple subtypes and we still do not know exactly what each of them does (Batiuk *et al.,* 2020). One discovery we made in collaboration with David Rowitch's group was that astrocytes have a regional organization that is established early during development (Tsai *et al.,* 2012; Bayrakar *et al.,* 2014). We have speculated that the astrocytes may retain information of location according to the early epithelial map that gives rise to the brain, and this information may be key to neuronal allocation and circuit stability and development. If this is the case, astrocytes may need to be programmed to recover this region allocation for repair purposes. Therefore, the geographical relationship of origin that we described for the parcellation of early neurogenesis and for adult neurogenesis (Merkle *et al.,* 2007) is also crucial for the allocation of astrocytes. As a result, the manipulation and transplantation of astrocytes could help in regeneration or prevention of degeneration (Su *et al.,* 2014; Qian *et al.,* 2020), but if astrocytes' regional information is important for the function of specific neural circuits, the use of these glial cells

for therapeutic purposes may not be that simple.

My main interest is to understand the mechanism by which new neurons are made and incorporated into neural circuits in the adult. This has led us to a fundamental new understanding on each of the steps required to make new neurons (Lim *et al.,* 2007). Such knowledge may be useful to induce neuron replacement or increase plasticity. The fact that neuron birth, migration and replacement can occur in at least two regions of the nervous system in rodents does offer hope that some of these processes will one day have therapeutic benefits.

That brings us to the debate of adult neurogenesis in humans. Can you share your thoughts? What questions are still there?

I believe that one of the things that I learned clearly in Fernando's laboratory is the importance of objectivity: to always be conscious of possible bias because of how much we love our hypothesis, to be aware of the value and limitations of experimental data and to maintain a clear separation of inferences and hard facts. In other words, often our hypotheses and the interest of being right leads us to "wishful thinking", to make our ideas real. We have been working on the mechanism of adult neurogenesis for decades and it would be indeed satisfying if similar processes were to be demonstrated in the adult human brain. Evidence from my laboratory, in both the ventricular-subventricular zone and the hippocampus suggest that neurogenesis and neuron recruitment does continue postnatally but decrease rapidly in infants. However, in our recent study in the hippocampus (Sorrells *et al.,* 2018; Paredes *et al.,* 2018) or our earlier work in the V-SVZ (Sanai *et al.,* 2011; Paredes *et al.,* 2016), we are clear to point out the limitations of our techniques. For example, the use of human tissue presents many challenges, such as *post mortem* interval, fixation, tissue processing, among others. I do agree that technical issues may affect our observations on the possible absence of neurogenesis (see discussion in Paredes *et al.,* 2018). Yet, based on the many samples analyzed and the observations of other laboratories, our view is that if neurogenesis occurs in the adult human brain hippocampus, it is a rare phenomenon. We have studied more than 100 cases, some are intraoperative temporal lobe recessions, tissues that are very fresh and well fixed. We also have cases from our own collections or from collaborators, with an extremely short *post mortem* interval and in a particularly good state of preservation. In none of those tissues we do have clear evidence that neurogenesis is active or at least not as we observe in mice. It should be noted that in mice, hippocampus and olfactory bulb neurogenesis also decays with age and what we are proposing is that a similar decrease occurs in humans during childhood.

The controversy has heated up as other groups have published data that according to their interpretation supports that neurogenesis continues in humans into adulthood (Bhardwaj *et al.,* 2006; Boldrini *et al.,* 2018; Moreno-Jiménez *et al.,* 2019). However, these studies have important limitations. For example, the use of extremely low doses of bromodeoxyuridine (BrdU), that incorporates into DNA during cell division, to label newly formed neurons, is poised with possible alternative explanations (DNA repair or cell death in addition to the *post mortem* human brain tissue artifacts discussed above). Another method uses atmospheric ^{14}C from atomic bomb tests, to infer cell birth, but ^{14}C can be incorporated into nuclei by non-cell division associated processes (e.g., by methylation or DNA repair). So, we do not know if these techniques are actually giving us a reading of new cells. Additionally, for the ^{14}C studies, nuclei of neurons need to be isolated, a process that always carries some contaminating glial cells that may have divided and incorporated the ^{14}C. But even if this method gave a reliable read-out for cell division, if you read the ^{14}C studies in the hippocampus carefully, many of the patients fall in the area of zero neurogenesis. It would be good to continue to discuss the technical limitations of all approaches. Particularly important are initiatives to try to collect better-preserved human tissue and the development of new methods to interrogate the age of individual cells within organs. For example, it would be very useful to come-up with biomarkers that tell us how old a brain cell is in a human brain. Controversies are positive and usually drive forward scientific discovery.

In your work you mention the importance of adult neurogenesis as a process of neuroplasticity. How do you define plasticity?

Plasticity refers to the flexibility of the nervous system to modify its connections and functionality based on normal physiological or behavioral demands or following injury. However, the term plasticity is frequently abused just to explain any change in the nervous system. By many it is also considered unlimited, because of our enormous ability to learn and modify our behavior based on how the environment influences us. My impression is that it has limits and that it changes with age. Clearly, from the point of view of repair and healing of the nervous system, plasticity could be important for the recovery of lost functions. It is also essential for later developmental steps. For example, it allows sensory systems to become structured according to environmental influences, very much along the lines discussed above, regarding "the function making the organ". Song learning in canaries or language learning in humans are examples of such plasticity. One fascinating problem is how developmental timing appears to be key to inductions of periods of enhanced plasticity. These periods are called critical periods of plasticity. Recent work in collaboration with my colleague Michael Stryker at UCSF, a neurophysiologist who is a world expert in critical periods, indicates that the age of a neuron is tightly linked to these periods of enhanced plasticity. In work we have done together with his group, we have been able to induce new critical periods of plasticity by transplanting specific subtypes of young cortical interneurons (Southwell *et al.,* 2010; Southwell *et al.,* 2014; Tang *et al.,* 2014). Adult neurogenesis, by supplying young neurons continually, possibly allows these subregions of the brain to have open plasticity for life.

Do you think your work can influence regenerative medicine in the short, medium or long term?

Our work is not focused on repairing the nervous system, but more on the mechanisms that drive adult neurogenesis. Which are the stem cells? Where do they come from? How do they interact with other cells? What do the new cells do? How do they migrate? From all the answers we have come across over the years, certain opportunities have arisen that suggest strategies for brain repair. I have already given you the example of induction of plasticity by neuronal transplantations (Southwell *et al.,* 2014). We have decided instead to focus on the basic neuroscience question. There are already many groups doing translational work. There has been a remarkable advance in Developmental Neurosciences during the

past 30 years, but I think that most of the basic understanding is not there to have a good chance at CNS repair - it's like trying to fix a machine without understanding first how it works. This is where Developmental Biology is fundamental. When I was a student, Developmental Biology was considered a complementary discipline to the subjects that were thought to be more important, such as physiology and anatomy. That is absurd because it is the other way around; anatomy and physiology are the consequences of development. So, if we really understand how the organism develops, we will have one of the best tools to be able to repair it.

Your work helped founding a company, would you share with us how this story started?

I am co-founder and scientific advisor of Neurona Therapeutics, with three colleagues from the University of California: John Rubenstein, Arnold Kriegstein and Cory Nicholas. John identified the origin of cortical inhibitory interneurons in the medial ganglionic eminence (MGE) while Arnold identified the embryo's neural stem cells. Cory, working with Arnold and in collaboration with our lab, developed a method to turn embryonic stem cells into inhibitory interneurons. For many years we have been deeply intrigued by the possibility of modifying the activity of neural circuits for therapeutic purposes. Pretty much as it naturally happens in circuits that receive new neurons throughout life. With graduate student Hynek Wichterle, we discovered that transplanted young neurons from the embryonic MGE, dispersed and differentiated into neurons in multiple adult brain regions (brain cortex, striatum, even the spinal cord). We were fascinated by the observation that these cells, much like adult born neurons, had the capacity to migrate and integrate into existing circuits (Wichterle *et al.,* 1999). We had a tool for introducing new inhibitory interneurons into existing circuits of the adult nervous system. Following this discovery, several opportunities and collaborations have emerged. For example, the fact that these cells, once integrated, can modify the excitatory activity of local cells prompted a collaboration in which we suggested that these cells may be useful for suppressing seizures in epilepsy (Baraban *et al.,* 2007; Hunt *et al.,* 2013). Another collaboration also emerged with a group at UCSF that studies pain. They knew for years that GABAergic interneurons were essential for pain modulation and that, when lost to cell death, chronic pain occurs. We taught them how to do the transplants, and surprisingly they found that grafting inhibitory cells in the dorsal horns of the spinal cord reduce injury-related neuropathic pain (Bráz *et al.,* 2012). I also already mentioned the ongoing collaboration with Michael Stryker that resulted in the induction of a new period of plasticity that mimics the endogenous critical period. All this suggested that transplantation of cortical inhibitory interneuron cells could be helpful for developing therapeutic interventions.

We began realizing how important cortex inhibitor cells were going to be in new therapeutic approaches and began selling our ideas to venture groups. With the speed at which science advances, we now know the genetic signature of these cells and it is clear that it is not a single type, but several types that are key to normal brain function. This is how Neurona Therapeutics was born; with the ultimate goal to use cell therapy to modulate neural circuits. For me it has been a great educational process on how the entrepreneurial environment in the Bay area can take a dream derived from basic research and scale it up to provide possible new therapies. I have learned how intricate, complicated and expensive the process of transforming basic new ideas and knowledge from science into applications. For this to occur, in addition to the scientists, the transformative vision of investors and true entrepreneurs like Cory is required. I am learning a lot, and I hope it works.

All your work and your career led you to receive an important recognition, the "Prince of Asturias Award." What has it meant to you?

I was moved and a bit overwhelmed by the social aspects, but when you are within science, awards are not what people think; actually, very frequently there are very minor differences between those scientists who have or haven't received recognition. Receiving a prize, I do not think should be taught as the motivation to do research. It is discovery that is the real prize. That was very well said by Günter Blobel who received the Nobel Prize in 1999 and taught us Cell Biology at Rockefeller University. There is a big difference between the satisfaction of receiving a prize and the satisfaction of making a discovery. Those rare moments in science when you say: "Aha! That's how it works!". These moments of Eureka, frequently just partial Eurekas, are much more satisfying than receiving an award. Also, the part that displeases me about prizes is the social one, people start to think of scientists as extraordinary intellects, when we are normal people that have been given tools to play with our curiosity. Awards do raise the interest in science and make people realize the importance of science.

If you could turn back time and have the opportunity to interview Santiago Ramón y Cajal, what would you ask him? Or what would you like to discuss with him?

This is an interesting question. I have suggested to people in the Cajal Institute and the Cajal Museum, to bring Cajal back to life, in our times, in a movie. Cajal was actually the founder of modern neurosciences, by defining the neuron as the fundamental element of the nervous system and by revealing many of the circuits these neurons make. I just came back from Chicago, where we met at the Cajal Club, which is one of the oldest neuroscience foundations in America. This Club, in addition to being interested in developmental problems, cerebral cortex, function, anatomy, also recognizes the importance that Ramón y Cajal has had for neurosciences. In Fernando Nottebohm's laboratory, the importance of objectivity was constantly stressed, of separating in your work and writing interpretation from observation. This is where Cajal was extraordinarily accurate, the drawings he made of what he saw under the microscope were as accurate and detailed as techniques permitted. Yet, he was conscious that they were incomplete forcing him to frequently make inferences. However, he was particularly careful in the collection of the data to be as accurate as possible, but then explaining -"this is what I see, and these are my interpretations". The intuition of what he did not see (the inferences) was extraordinary. For example, from these deductions he correctly inferred the direction of information flow within neurons, neurotransmission in neurons and the synapse. I would love to ask him: How did he train his intuition?

I do not think intuition is magical, I think it has to do with the ability to make objective and careful observations and to think in a structured manner about them. The initial descriptive step in science, which is frequently downplayed and criticized, is essential! The inferences of mechanisms that emerge are a consequence

of a careful description. Science is at a different stage now since data production frequently exceeds the human capacity to absorb or integrate it. In bringing Cajal back to life, I would love to see his reaction, not only to the new understanding of the neuron, the synapse, and the connectivity of the nervous system, but his reaction to the tools (e.g. confocal, electron microscopes and computers), and to the vast amount of data these new tools generate. Could Cajal integrate all these new data and come up with a new set of inferences for the next 100 years?

Finally, you keep close ties with Ibero-American science and education. How do you see the advancement of Developmental Biology in Ibero-America and in Mexico in particular?

Unfortunately, I have only visited and participated in scientific meetings in a few countries in Latin America, so I don't have a full picture of Developmental Biology in Ibero-America. I have found interest and creative ideas, researchers and students interested in Developmental Biology in Chile, Argentina, Brazil and Mexico. I was particularly impressed by students I met at those meetings, full of enthusiasm, asking great questions. In Mexico, the field has expanded from when I was a student at the UNAM but it remains small compared to other research areas. The lack of a more directed focus to study Development is hard to understand given the potential transformative information the field is beginning to yield. I think, in Latin America, science in general and more specifically Developmental Biology, is considered secondary and unlinked to the economy or national progress. Science is simply confused with technology. What politicians I think don't realize is the power of unraveling the rules that guide the assembly of the brain and other organs or body structures not only in humans, but in many organisms. The rules that guide development, will not only help us understand the developmental origin and predisposition for disease, but will help in many fields including regenerative medicine, evolution, ecology, farming.

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