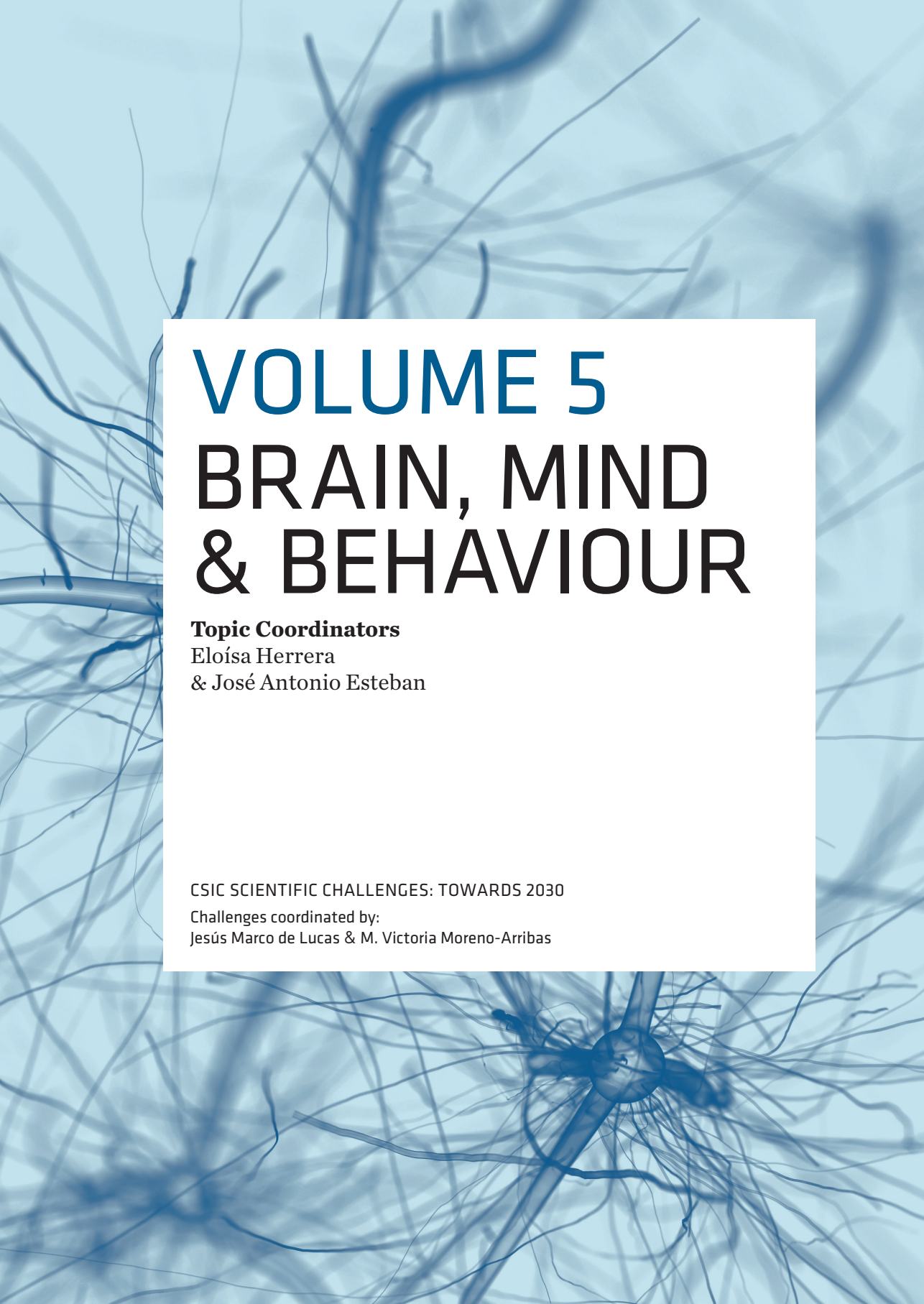

VOLUME 5
BRAIN, MIND & BEHAVIOUR

Topic Coordinators
Eloísa Herrera
& José Antonio Esteban

CSIC SCIENTIFIC CHALLENGES: TOWARDS 2030
Challenges coordinated by:
Jesús Marco de Lucas & M. Victoria Moreno-Arribas

VOLUME 5

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CSIC SCIENTIFIC CHALLENGES: TOWARDS 2030

What are the major scientific challenges of the first half of the 21st century? Can we establish the priorities for the future? How should the scientific community tackle them?

This book presents the reflections of the Spanish National Research Council (CSIC) on 14 strategic themes established on the basis of their scientific impact and social importance.

Fundamental questions are addressed, including the origin of life, the exploration of the universe, artificial intelligence, the development of clean, safe and efficient energy or the understanding of brain function. The document identifies complex challenges in areas such as health and social sciences and the selected strategic themes cover both basic issues and potential applications of knowledge. Nearly 1,100 researchers from more than 100 CSIC centres and other institutions (public research organisations, universities, etc.) have participated in this analysis. All agree on the need for a multidisciplinary approach and the promotion of collaborative research to enable the implementation of ambitious projects focused on specific topics.

These 14 "White Papers", designed to serve as a frame of reference for the development of the institution's scientific strategy, will provide an insight into the research currently being accomplished at the CSIC, and at the same time, build a global vision of what will be the key scientific challenges over the next decade.

VOLUMES THAT MAKE UP THE WORK

1 *New Foundations for a Sustainable Global Society*
2 *Origins, (Co)Evolution, Diversity and Synthesis of Life*
3 *Genome & Epigenetics*
4 *Challenges in Biomedicine and Health*
5 *Brain, Mind & Behaviour*
6 *Sustainable Primary Production*
7 *Global Change Impacts*
8 *Clean, Safe and Efficient Energy*
9 *Understanding the Basic Components of the Universe, its Structure and Evolution*
10 *Digital and Complex Information*
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12 *Our Future? Space, Colonization and Exploration*
13 *Ocean Science Challenges for 2030*
14 *Dynamic Earth: Probing the Past, Preparing for the Future*

CSIC scientific challenges: towards 2030
Challenges coordinated by:
Jesús Marco de Lucas & M. Victoria Moreno-Arribas

Volume 5
Brain, Mind & Behaviour

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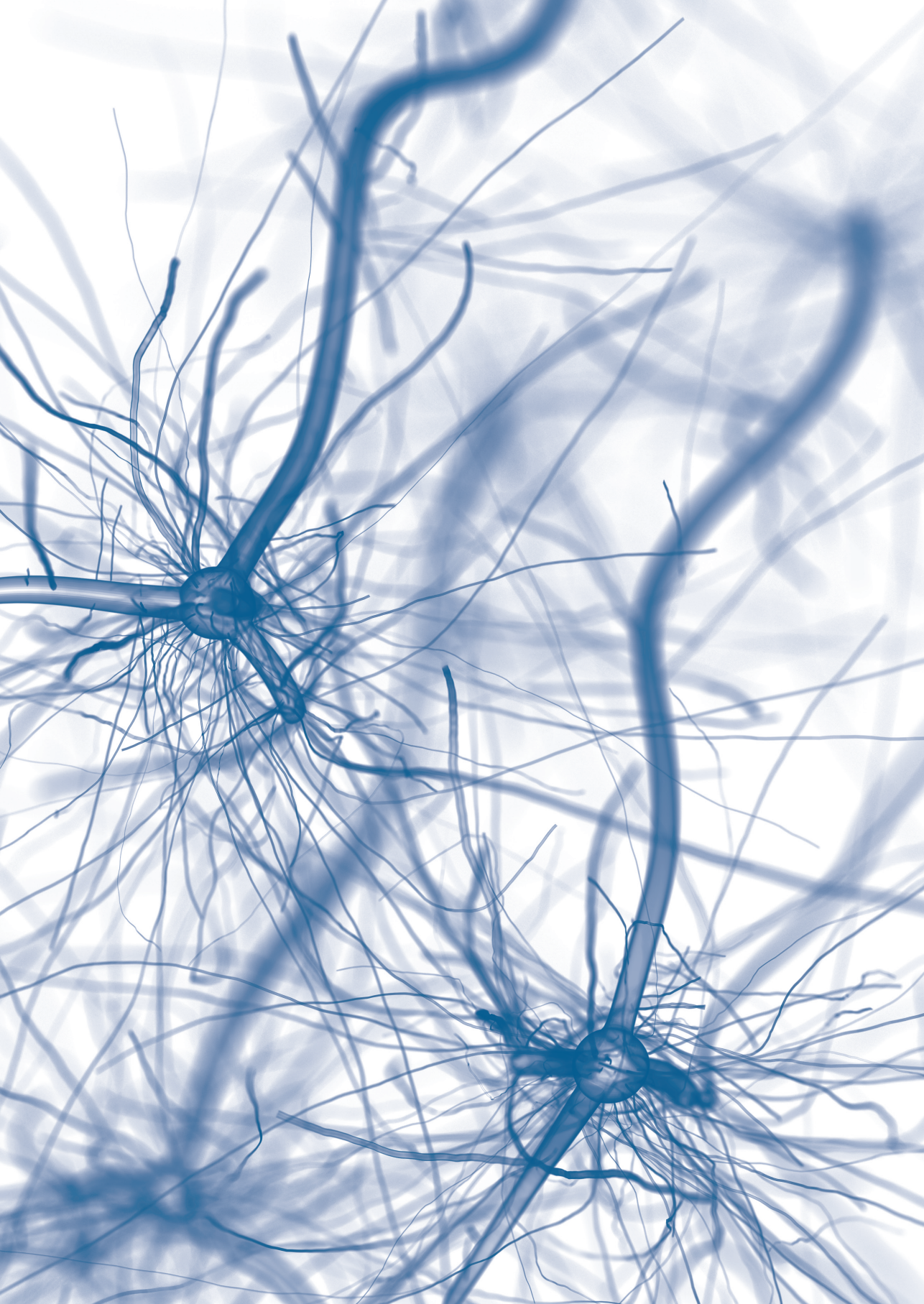

VOLUME 5 **SUMMARY**

ABSTRACT

The study of the brain will tell us what makes us humans and how our social behavior generates. Increasing our understanding of how the brain functions and interacts with the ecosystem to interpret the world will not only help to find effective means to treat and/or cure neurological and psychiatric disorders but will also change our vision on questions pertaining to philosophy and humanities and transform other fields such as economy and law. Neurosciences research at the CSIC is already valuable and should be intensified mainly focused on the eight major challenges described in this volume.

KEYWORDS

neural circuits | neurological diseases | neurodegeneration | learning and memory | sex and gender | brain-body interactions | aging | cognition | collective behavior | mood disorders

BRAIN, MIND & BEHAVIOUR

Topic Coordinators
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EXECUTIVE SUMMARY

The brain is arguably the most complex biological system in the known universe. The substrate of our thoughts, the way we built our societies through complex languages and the impressive cultural and technological advances at our disposal, have been all developed thanks to the activity of our brains. The next few decades are going to be strongly influenced by our capacity to integrate different levels of complexity to understand how neural circuits produce thoughts and behaviors. However, we are still far from achieving this goal. Understanding the development of the brain and its inner workings is already a formidable task. Even minor alterations of brain function may be responsible for mental disorders that have devastating impact for individuals and communities and are a leading cause of disability in developed countries. In addition, the nervous system is notoriously reluctant to repair itself after damage, which places a great burden on millions of people living with motor or sensory disabilities. Despite significant advances in recent years in the treatment of brain disorders, they are still considered a critical unmet health problem in Spain and Europe. This is due to the poor knowledge of their aetiology, the complexity and variability of symptoms, the demanding diagnosis, the limited therapies and public care as well as the social stigma they often pose. The incorporation of genetics, molecular and cellular biology to the study of the nervous system has greatly accelerated our understanding of some of these problems. However, we need to develop more sophisticated techniques of monitoring and

modifying brain function as well as better theoretical frameworks in which the valuable pieces of information that we collect are transformed in biological understanding. It is imperative that the CSIC participates on this revolution, as the risk of lagging behind may have long-term consequences. The CSIC counts with excellent biologists, chemists, mathematicians, physicists, engineers and outstanding experts in humanities and social sciences. Basic neuroscience researchers aim at understanding how the brain elaborates emotions, thoughts and behavior and the mechanisms by which these processes are altered in mental disorders. Translational researchers aim at using this knowledge to design and assess therapeutic approaches. Researchers in human and social sciences in this context aim at understanding the role of the various cognitive functions in the emergence of dynamic societies and civilizations. Approaching the study of brain function and mental disorders from different but complementary perspectives and research expertise is a critical strategy to achieve important breakthroughs in neurosciences in the upcoming years.

Introduction

The brain specifies and controls every aspect of our life, including rational thinking, emotions, heart-beat, breathing, food and liquid intake, sleep and sexual desire. Therefore, a high quality of life and well-being require that our brain stays healthy and properly operative. Disorders that are the consequence of brain dysfunction, such as depression, Alzheimer's disease, dementia, schizophrenia, migraine, sleep disorders, Parkinson's disease, pain syndromes, addiction, etc, have turned into a major health problem worldwide costing as much as cancer and heart diseases together. Brain disorders are currently estimated by health economists to account for 45% of Europe's annual health budget. In fact, the economic cost of brain disorders in Europe is estimated to be ca. € 800 billion per year and patients suffer a significant loss of quality of life during the course of the disease, which also impacts strongly on their families and their social network. With an increasingly aging population in Europe, the prevalence of the most common neurological and psychiatric disorders is expected to grow dramatically and it is imperative to find truly effective approaches that reduce this huge society problem, including the impact on care-givers and the resultant loss of productivity, employment and massive economic burden. Therefore, urgent solutions that prevent, diagnose, palliate or treat neurological diseases are needed.

The complexity of connectivity between neural cells in the brain is mind-boggling. The human brain contains eighty-six thousand million neurons and

many more glial cells. Each neuron can contact with thousands or even tens of thousands of others. Our brains form millions of new connections for every second of our lives. Furthermore, the pattern and strength of these connections is constantly changing and no two brains are the same. It is in this changing connectivity that memories are stored, habits learned and personalities shaped, reflected in reinforcing certain patterns of brain activity, and losing others. Therefore, finding out what is wrong in each particular brain disorder is extremely complicated and, as a consequence, diagnosing and treating brain diseases will require much more effort compared to other diseases. Brain research should continue at the most basic level to provide the bricks with which to build a comprehensive model of brain function and dysfunction. We believe that the best way to fight brain diseases is to solve the fundamental questions about brain development and function and to use these ideas to understand the mechanisms of brain dysfunction. We must intensify the scientific effort to understand normal and abnormal behavior emanating from impaired brain function and spanning molecular, cellular and network mechanisms to social and environmental determinants. Understanding the brain provides valuable knowledge (critical in a knowledge economy) that has the potential not only to treat disease, but also to innovate in the areas of artificial intelligence, brain-machine interface, robotics and new technology. The commitment of funding agencies to basic research in neuroscience has advanced our understanding of some of the mechanisms governing brain function in recent years, and recent methodological breakthroughs now offer a powerful opportunity to ease the societal burden of brain disorders and innovate at the frontiers of technology.

As the most important research institution in our country, the CSIC has the responsibility of contributing significantly to the knowledge of nervous system biology in both health and pathological conditions. Over the past decades, our institution has incorporated competitive lines of research on different neuroscience areas. We have identified eight specific challenges in brain research that are closely interconnected and to which the CSIC could contribute greatly because it has a significant number of excellent specialists capable of addressing them competently.

The first five challenges focus on fundamental mechanisms of how the nervous system develops and functions. The CSIC has a substantial number of excellent groups with the potential to contribute significantly to understanding **how neural networks emerge (challenge 1)** during embryonic stages and late

postnatal periods to establish a correct connectivity and how the different brain components integrate at different biological levels from **genes and circuits to orchestrate complex behaviours (challenge 2)**. The knowledge generated by basic scientists using simple model organisms combined with novel brain imaging techniques, large-scale computational analysis and machine-learning approaches will help to discover how the brain solves complex problems, such as managing emotional states, understanding languages, etc. Further diving into these issues will lead us towards higher emergent properties of the brain, such as **cognition, collective behaviour and consciousness (challenge 3)**. These investigations, in turn, should deliver innovative technologies that will impact on many areas of society, including ethics, philosophy or laws and legislation. For instance, in the search for a more egalitarian society, it will be essential to understand the influence of nurture vs nature in establishing stereotypic behaviors such as gender bias, both at the biological and social level. **The study of the neurobiology of sex and gender (challenge 4)** is as relevant as it is controversial, and one of the main challenges in this regard is to take into account the intrinsic biological diversities of females and males and, at the same time, not to feed the culture of gender dichotomy that is often articulated by society and its hierarchies through gender bias.

It has become increasingly evident that bidirectional communication between the nervous system and peripheral organs has an important effect on our mood and behaviors as well as on the pathogenesis of many brain disorders. This is approached by **challenge 5, body-brain microbiome interactions**. Therefore, it will be determinant in the next few decades to unveil the role of the immune system, metabolic processes, gut-brain axis and microbiome in regulating brain activity.

The following three challenges will make it possible to identify measures to help alleviate the burden of brain pathologies in our increasingly aging European society in order to maintain healthy individuals with functional cognitive abilities in old age. The challenges in this block should provide solutions **to diagnose and treat mental disorders as well as to advise on their social acceptance (challenge 6)**. Indeed, mental disorders have a devastating and growing impact on our societies and CSIC researchers should face the challenge of determining the biological and social causes and consequences of these disorders, and finding efficient therapies. It will also be essential to find ways to maintain the best possible cognitive performance as we age and to

guide society in caring for patients affected by **neurodegenerative diseases and other age-related brain conditions (challenge 7)**. Neurobiologists, mathematicians, informaticians, engineers, experts in robotics and nanosciences should then cooperate and capitalize on the new knowledge generated by basic researchers to devise methods to **improve brain regeneration and functional recovery after brain and spinal cord damage (challenge 8).** Injuries to the brain and spinal cord are amongst the leading causes of death and long-term disability in young people. Instruments of regenerative medicine such nanospheres, liposomes and mesoporous nanostructures or stem cell-based therapies, together with the stimulation of deep brain structures using nanotechnology strategies and new-generation activatable chemicals are emerging as future prospects for the treatment and diagnosis of acute brain damage and will be explored for further application. Finally, rehabilitation of patients with central nervous system (CNS) injuries driven by advances in novel robotics designs is now a powerful strategy for restoring disabilities, particularly in relation of motor functions, and will be also exploited.

Common actions to implement

To tackle these large-scale challenges and make breakthrough advances keeping our institution at the cutting-edge of nervous system research in Europe and the rest of the world, the CSIC should embrace ambitious steps towards implementing the following common strategic measures that are further detailed on the different challenges:

1. To increase the investment in centers of excellence and teams working on brain research. Spain is regarded as having a long tradition in neuroscience research, which not only should be maintained, but strengthened in order to improve the visibility of the country and the CSIC in the world. CSIC has two main centers that have played a fundamental role for development of neuroscience research in Spain: The Cajal Institute (IC) and the Neurosciences Institute (IN), the latter being a "Severo Ochoa" Center of Excellence for the last 6 years. In addition to these two medium-scale monographic institutes, neuroscience research in Spain is generally carried out by small teams distributed in university departments, hospitals and biomedical research institutes (e.g. CBM). Spanish neuroscience has acquired a privileged position nationally and internationally in recent decades. The CSIC should take advantage of this situation to expand and strengthen brain research in Spain, and should make strategic progress in organizing and promoting excellence neuroscience centers that should concentrate critical mass and infrastructure needed for

frontier investigations in this field. A pan-national network of virtual nodes would also help to enhance the international competitiveness of the CSIC-neuroscience. Research centers such as the IC, the IN, IEGD (Institute of Economics, Geography and Demography) or CAR (Centre for Automation and Robotics) have demonstrated that thematic institutes are an excellent way to nurture competitive science and therefore it would be crucial to maintain and reinforce this strategy. Nevertheless, it is important that other competitive brain research teams spread across CSIC's multidisciplinary centers are also supported and empowered as a complementary strategy to maintain diversity and exchange of ideas with researchers from other fields.

2. To foster interactions among different teams and centers. The inherent complexity of the nervous system has led us to realize that a higher level of integration of different biological areas and other disciplines is now essential to make significant progress in brain research. To obtain revolutionary and transformative results, as well as to maximize our translational impact, CSIC groups working on different aspects of neurobiology should move beyond their particular areas of expertise and embrace initiatives that reinforce and intensify contacts with clinicians, engineers, informaticians and social science investigators. Particularly, productive interactions with investigators in the health system will be critical to crystalize the translational potential of our investigations in brain disease. Actions to promote collaborative work among the CSIC groups and centers will certainly increase our productivity and raise the international competitiveness and visibility of our institution.

3. To launch technological infrastructures and platforms at the institutional scale. We are living a revolution in neuroscience thanks to the recent flourishing of technological developments that allow us to investigate questions that were unreachable only few years ago. However, technology keeps moving forward very rapidly and core facilities and platforms, such as imaging facilities or genomic platforms need to be continuously renewed with state-of-the-art equipment and be staffed with highly specialized personnel. Otherwise, the maintenance of competitiveness is unreachable. Many of the today's major challenges in relation to the development and dynamics of functional neural circuits arise as a natural consequence of the in-depth and detailed, but as yet unimplemented, knowledge provided by new technologies. Dispersion of platforms and common services for the generation of animal models, next generation sequencing, drug screening, big data analysis or neuroimaging should be avoided. Instead, common services working already in the different

CSIC centers should be strengthened, better funded and staffed with highly qualified technicians. Reinforcing and disseminating the already existing services would avoid redundancy in different CSIC centers and reduce costs.

4. To train and recruit researchers at the frontier of different disciplines. Understanding the brain will require not only to organize and share big-datasets in user-friendly repositories, but also to educate the younger generations into profiting of these data. We need investigators that can navigate comfortably between physics, biology and information theory. The elaboration of novel hypotheses that can comprehensively describe the complexity of the circuit development and functionality requires out-of-the-box thinking, the use of big-data languages to encode biological meaningful analysis, and also informed biological perspectives. This effort is going to require a true interdisciplinary approach between neurobiologists, physicists and biocomputational researchers. The CSIC should implement two main actions to accomplish this demanding challenge. First, by taking advantage of the large number of CSIC investigators working on distant disciplines, it should launch an intramural fellowship program for PhD students and young postdocts devoted to favor their training in a cross-disciplinary manner. Second, it should make an important effort in recruiting researchers with highly interdisciplinary profiles that serve as bridge between basic and clinical neuroscience or fill the existing gap among neurobiology, informatics, robotics and social sciences. The CSIC's recruiting policy should make a great effort to attract these type of exceptional professionals. This may require novel and more dynamic recruitment approaches to attract talent in a fast-paced and global environment, overcoming the constraints and rigidities of our 80-year old institution that often result in a loss of opportunities.

CHALLENGE 1

ABSTRACT

Santiago Ramón y Cajal quoted “Every man can, if he so desires, become the sculptor of his own brain” which means that we can refine the structure and function of our brains, the same way we can do so with the rest of our body muscles. Understanding brain physiology and associated pathologies requires a profound and detailed knowledge of how this complex organ is constructed. We need to decipher how the brain blueprint unfolds; from the general specification of its different regions and the astonishing diversity of its cellular components to the precise architecture of its connections. Finally, the challenge of a healthy aging also demands to grasp basic principles of brain repair and remodeling throughout the entire lifetime.

KEYWORDS

gene regulatory networks

neuroepithelial folding

neuronal connectivity

neuronal and glial cell fate

CHALLENGE 1

DECODING THE EMERGENCE OF NEURAL CIRCUITS

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1. INTRODUCTION AND GENERAL DESCRIPTION

The construction and maintenance of the brain and its associated sensory organs is an extremely complex multi-step process by which diversity is generated at all organizational levels. At the organ level, the relatively simple pseudostratified neuroepithelia need to bend, contract and grow to adapt their form to the functional requirements of the organ domains. At the cellular level, upstream genetic networks bifurcate into downstream sub-networks controlling the proliferative expansion of neural progenitor cells, their migratory behavior, their differentiation into a wide variety of neuronal and glial subtypes, and their specific connectivity patterns within functional circuits.

Generating and connecting such an impressive cell diversity requires regulatory mechanisms operating at all levels, from the ones controlling gene expression, cell signaling, or cell mechanical properties, to those directing the coordinated assembly of circuits in different brain regions. Throughout life, our daily thoughts and actions, learnings and meditation, cognitive therapies and more, are the "sculpting" tools that translate into adult neurogenesis, synaptogenesis and brain plasticity, refining the structure and function of our brain and mind.

The outbreak of genomics and technological developments including; (i) the irruption of deep sequencing and single cell technologies, that allowed the generation of detailed catalogues of neuronal and glial cell fates and states, (ii) the technological wave of advanced microscopy and quantitative imaging that allowed following cell and tissue behavior in vivo to an unprecedented level of detail, and (iii) the generation of cell-based in vitro 3D brain organoids (mini brains) to model the formation of the human brain and sensory organs and their developmental disorders, among others, provide unprecedented opportunities to accomplish a profound understanding of how the brain and its associated sensory organs develop in health and disease, including gender as a biological variable in brain development and regeneration.

Fundamental questions related to (i) the basic principles regulating the shape and growth of the brain and sensory organs, (ii) the mechanisms generating cell diversity in the developing brain and sensory organs, (iii) the mechanisms underlying the formation and assembly circuits, and (iv) the mechanisms regulating adult neurogenesis, are key to enhance our understanding of how the brain is constructed during development. This knowledge would provide a path, not only to understand how the brain is "sculpted" in a healthy life, but also a path to pave brain regeneration and healthy aging.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

Genetic and environmental injuries during development can cause two groups of neurological disorders; a) anatomical malformations of the brain, spinal cord and sensory organs such as, holoprosencephaly; lissencephaly; microcephaly, spina bifida, coloboma, among others and b) neuropsychiatric diseases of the mind such as autism, intellectual disability, schizophrenia, bipolar disorder, among others, in which an anatomical substrate is not always visible. Describing these mental illnesses as a malfunction of the developing brain will help minimize the social stigmatization associated with them.

Advances in developmental genetics during the last decades of the 20th century and the first years of this 21st, made possible the identification of core transcriptional regulators (TFs) and signaling molecules responsible for the specification of the different brain areas and sensory organs. This pioneering work uncovered that developmental blueprints were often conserved among

the different species of a particular phylum and sometimes even across phyla (Davidson and Erwin, 2006). Although this was a fundamental step to understand how developmental programs are triggered by upstream regulators it provided little information on how these programs unfold. Particularly, the precise morphogenetic mechanisms shaping each domain were not investigated until recently. Riding the technological wave of advanced microscopy, quantitative imaging in combination with model organisms' genetics allowed following cell and tissue behaviour in vivo to an unprecedented level of detail (Keller, 2013). Cytoskeletal and cell shape changes could be followed in numerous tissues and biophysics came into play to measure and perturb mechanical forces in neuroepithelia (Charras and Yap, 2018). Imaging advances were also instrumental to understand principles behind neuronal migration and precursors proliferation. Moreover, imaging data has been the starting material to build up the first computational models for relatively simple morphogenetic events (e.g. neurulation, retina folding, brain vesicles morphogenesis, cerebral surface folding) (Okuda et al., 2018). In parallel to these efforts, the fast development of next-generation sequencing technologies during the last decade (i.e. RNA-seq, ChIP-seq, ATAC-seq, etc) removed the barriers for the systematic investigation of gene regulatory networks (GRNs) (Martinez-Morales, 2016); those conferring identity to each neural domain and therefore determining their final morphology and size. Many candidate genes were identified through these approaches as key effectors and causative genes for neurodevelopmental diseases and their function further assessed using either classical genetics or CRISPR-based methods.

Finally, cell-based brain organoids (mini brains) have enabled the generation of powerful in vitro systems with reduced complexity and increased accessibility (Di Lullo and Kriegstein, 2017). These 3D cultures not only recapitulate many aspects of the different neural tissues ontogeny, but also can be used to model the formation of the human brain and sensory organs and their developmental pathologies.

Understanding the basic auto-organization principles of an organ as complex as the human brain is still a very demanding task. However, the technological advances above described are encouraging and set the ground for future challenges, as those described in the next section.

3. KEY CHALLENGING POINTS

3.1. Understanding the basic principles regulating the shape and growth of the brain and sensory organs

How neural tissues, including the different parts of the brain and sensory organs, acquire their exquisitely controlled shape and size is certainly a challenging question that demands a multidisciplinary approach. As the nervous system develops, even the relatively simple pseudostratified neuroepithelia need to bend, contract and proliferate to adapt their form to the functional requirements of the organ domains. The correct balance between precursors proliferation and neurogenesis acts afterwards as a main sculpting force. When neurons are born, additional mechanisms such as neuronal and glial migration, neuronal morphology, and axonal growth, came into play to determine the architecture of the different brain modules.

Over the years, the scientific community has accumulated a large amount of information from genetic screens, imaging studies, NGS-sequencing, and biophysical approaches. A first major challenge is merely organizational, how to integrate information derived from different model organisms, tissues, technologies, and developmental stages into cross-relational repositories equipped with user-friendly interfaces that allow navigating different datasets. This is a common challenge for many disciplines in biological sciences; however it is particularly pressing in the case of developmental neurobiology if we consider the volume of data already accumulated. But this is only the beginning; to integrate information on growth and shape into predictive computational models, to measure morphogenetic forces *in vivo* in an accurate manner, or to understand how mechano-regulatory feedback-loops confer robustness to self-organization, remain as tasks to be confronted in the near future.

3.2. Understanding the mechanisms generating cell diversity in the developing brain and sensory organs

The description of neuronal diversity per se is one of the first steps towards the understanding of the molecular mechanisms that guide neuron-type specification programs. The recent irruption of new technologies, especially deep sequencing and single cell technologies, has revolutionized the field of developmental neurobiology (Tasic, 2018). Cellular Atlases are being produced for several organisms, including humans, aimed to provide a detailed catalogue of cell types and their corresponding transcriptomes. These initiatives have

also increased our knowledge of neuronal diversity in the brain. More recently, single cell transcriptomics has gone a step further to include the study of the temporal dimension to try to reconstruct lineage progression or performing interspecies comparisons to identify homologous neuronal types (Arendt et al., 2019; Baron and van Oudenaarden, 2019; Konstantinides et al., 2018).

In addition to transcriptomes, other approaches provide a genome-wide view of the regulatory landscapes present in specific neuronal types (chromatin accessibility, description of epigenetic marks, transcription factor binding profiles, physical interactions between distant DNA sequences or massively parallel reporter assays to identify active enhancers) (Long et al., 2016). Application of these technologies at single cell level is still unfeasible (with the exception of single cell-ATACseq), this limitation, together with the cellular complexity of the nervous system, precludes the analysis of neuron-type specific regulatory landscapes in vivo. An alternative approach to circumvent this problem has been the use of alternative more simple animal models or the use of stem cells or iPSCs to generate and characterize specific neuronal types in vitro (Engle et al., 2018).

Finally, in vivo loss of function experiments, nowadays also revolutionized by CRISPR technology, is also providing some clues of the mechanistic processes that are behind neuron-type specific transcriptomes and regulatory landscape.

We are now starting to acquire a global, genome-wide understanding of many different neuronal types. This increase in knowledge is not only important for the basic science but has also important applications in biomedical research. Just to mention a few translational approaches, iPSCs from patients of different neurodevelopmental diseases are being used to produce specific neuronal types in vitro (Engle et al., 2018) and the identification of transcriptome or regulatory-landscape differences of these cells compared to controls can lead to a better understanding of the disease and to potential new therapeutic tools. These in vitro approaches can also be used to perform drug screens or genetic screens to identify relevant hits or pathways. Finally, massively parallel reporter assays are being used to profile thousands of different genomic SNPs to better characterize the biological relevance of these mutations (Kinney and McCandlish, 2019).

We are now in a very exciting moment for the field in which we have the tools to transcend from description to functional characterization and to move from

patchy descriptions focused on specific developmental times or focused on specific target genes to more global understanding of neuron-type specification processes.

In the next years we should be able to 1) find general principles behind neuron- and glial-type specification programs 2) learn how these principles are modulated in evolution to generate new neural cell types, 3) apply our knowledge to generate specific or even new synthetic neuronal types with therapeutic applications.

3.3. Understanding the mechanisms generating functional circuits

After the early descriptions of the cellular composition of the brain by Ramón y Cajal and Golgi, there have been giant steps in our path to understand the wiring of brain circuits. However, the finishing line seems far away and the road intricate. We are far from understanding normal wiring and much further from responding to the needs of patients bearing neurodevelopmental disorders. Moreover, we have just initiated the travel to explore possibilities to utilize developmental mechanisms to repair the brain, and just begun to mimicking bits of the complexity of our brain networks in artificial systems.

The difficulties we encounter when understanding the wiring of functional networks are in part due to the magnitude of elements involved and the complexity of their relations. After initial descriptions of diversity of progenitors, control of cell-type differentiation, chemo-attractive and repellent guidance cues, and others, we are now seeking integrative models accounting for much more comprehensive views. Luckily, this appears now more approachable thanks to the increasing power of experimental tools, mathematical analysis, and computational modelling (Escalante et al., 2013; Velasco et al., 2019; Chen et al., 2016; de León Reyes et al., 2019; Murcia-Belmonte et al., 2019), the latter being especially important because of the rising numbers of methods generating large data-sets.

Conceptually, one major focus of current investigations is set on electrical activity, and the plasticity it mediates, as fundamental to circuits assembly and functional wiring (Benjumeda et al., 2013; anton-Bolaños et al., 2019; Marín, 2019; de León Reyes et al., 2019). Activity triggers and governs a multitude of key developmental programs that we now start to perceive as highly interdependent. It is involved in very diverse processes, such as dendritic elaboration, synapses, neuron-glial dialogs, epigenetic remodelling, and the

regulation of the trajectory of subtype-specific molecular programs of differentiation (de la Prida et al., 2019; Hutson et al., 2019). It appears that the orchestrating action of activity over these different processes enables plasticity that guarantee the coordinated and robust wiring of local neuronal networks first, and then of brain territories. In mature circuits, this plasticity appears blocked, as if to ensure little alterations in these circuits since they were costly and required extraordinary encoding of information for their generation. However, most of the current knowledge, derived from the study of critical periods of sensory driven activity and spontaneous activity in sensory and motor pathways, open new avenues to manipulate or bring back plasticity to repair the brain (Sahel et al., 2019, Karow et al., 2018).

Another focus has been set in the dynamic roles of non-neuronal cells during wiring. These other cell types include not only supporting cells, glial and oligodendrocytes, but also, cells of the immune system, in particular resident microglial cells of hematopoietic origin. Many studies are turning their views on their roles during development.

Finally, the long-standing question of what makes the human brain human is still open (Velasco et al., 2019). It seems that several of the above mechanisms, such as the regulation of spine number and maturations, and possibly, plasticity, have evolved with the increasingly complex circuits of mammals, and more rapidly in humans, to generate new circuits and functions.

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CHALLENGE 2

ABSTRACT

Understanding the brain from genes and circuits to behavior is a major scientific challenge. The large repertoire of cell activities supporting behavior stems from an equally diverse range of specialized cell types, from neuron to glia. To untangle mechanisms underlying brain function, elementary processes should be dissected, from the complex machinery of signaling pathways at the level of single cells and synapses, to the intricate phenomena leading to orchestrated ensemble activity and the establishment of engrams driving memory-guided behaviors. In this chapter we identify the main key tasks required to address some of the open questions in the field, and discuss on the main issues and strategies.

KEYWORDS

omics | electrophysiology | cell type | ensembles | engram

CHALLENGE 2

FROM GENES & CIRCUITS TO BEHAVIOR

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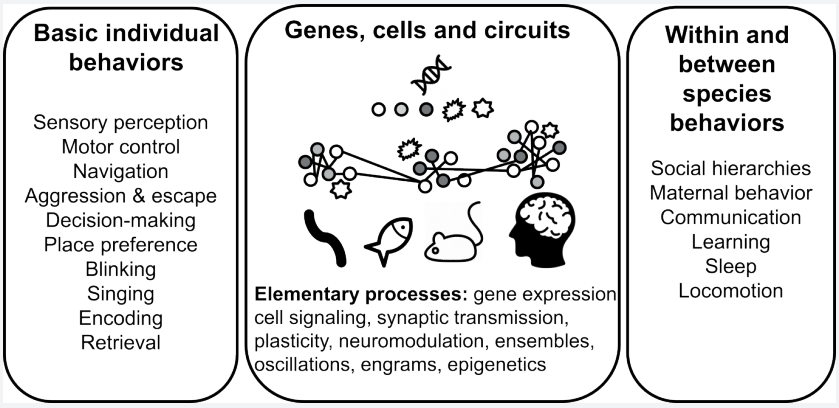
1. INTRODUCTION AND GENERAL DESCRIPTION

The hope to explain human behaviors is an old dream for the humankind. Can we identify specific genes or brain circuits responsible for our courage or resilience to cope with difficulties? How experience and emotions shape our brain in turn? Is there any inherit trait or region where fear and sensibility resides? Or is rather our ability to understand the world and to ask about our own nature something elusive even for the most qualified researchers and institutions? We believe this challenge is attainable in a long run, but in order to succeed we need to start from the basics.

There are some fundamental behaviors such as sensory processing, motor control, bird singing or rodent place preference, amongst others, that have been associated to the operation of specific circuits and regions in different species (Alstermark and Isa, 2012; Kim et al., 2017; Tovote et al., 2015). The ability to successfully deconstruct these behaviors is possibly associated to their evolutionary 'simplicity'. For instance, sensory processing and navigation are at the very bottom of the organizational principles of microcircuits from worms to mammals in order to survive. Similarly, basic neuronal solutions for decision-making are indeed conserved across species (Hanks and Summerfield, 2017). More elaborated abilities such as episodic memory formation and retrieval or the basic hierarchies of social interactions rather

FIGURE 2.1–Basic approach to understand basic behaviors in terms of genetic, cellular and circuit specificity. Basic individual behaviors such as those listed in the left box can be dissected from elementary circuits across different species and experimental models. Circuits are composed from specific cell types (e.g. neuron, microglial cells and astrocytes) which are determined by specific gene combinations and become assembled in a very specific manner (cell-to-cell communication). How function emerges from circuits is yet unclear, but it requires dynamic interactions between elementary processes (middle box). Adopting across-species approaches is critical to better understand the underlying mechanisms. Interactions within and between species are dominated by more complex behaviors summarized at the right box. The challenge to link genes and circuits with behavior will require assessing the different levels holistically with a combination of techniques.



suggest brain-wide network operation (Kimchi et al., 2007; Kitamura et al., 2017; Kohl et al., 2018). The way basic behaviors are related to our genetic heritage and whether they leave a durable footprint in our brain remains unclear. Specificity in terms of genes, cell types and circuits is critical to dissect this complexity (Figure 2.1).

In the last fifty years, neuroscience has grown spectacularly as a stronger interdisciplinary field at the interface between genetic and molecular engineering, neurophysiology, cell biology, psychology, and physics among many others. More recently, developments from materials and data sciences are rapidly permeating and transforming our view. Interactions between fields have prompted novel technological advances with remarkable development of high-throughput technologies and computational analyses and simulation never seen before. Today it is possible to screen the transcriptional landscape of single cells in situ and to simultaneously record from hundreds of them while acting with sufficient specificity to modify behavior. There is now a window of opportunity to address new challenges in the field and to foresee imaginative solutions to accelerate our understanding of brain function.

Here, after having evaluated the state of the field and the current strategical position of CSIC within the national and international scenario, we have identified the following main general goals to be fulfilled in the forthcoming years:

1. To dissect brain function by identifying elementary processes underlying basic behaviors such as motor control, freezing, place preference and navigation, to then scale them bottom-up to the level of more elaborated functions such as episodic memory, social interaction, communication, etc.
2. To foster interdisciplinary strategies that integrate genetic, molecular, cellular and microcircuit approaches together with next-generation theoretical and artificial intelligence tools to bridge data from genes and circuits to behavior.
3. To promote access of individual laboratories and teams to new high-throughput technologies aimed at monitoring and interrogating brain function in real time.
4. To enhance open science and transdisciplinary scientific collaborations at the interface across broad areas of knowledge.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

The scientific impact of addressing these general goals will apply horizontally and transversally along disciplines, from basic science and health care to humanities, and across sectors, from science, technology and education to impact on the society as a whole. At the institutional level, they aim to promote interactions between the three CSIC global areas: Society, Life and Materia.

2.1. Scientific impact

Deciphering the diversity of elementary processes underlying behavior requires a transformative vision. The classical separation between broad areas and fields will no longer hold, as molecular/cell biology, genomics, electrophysiology, bioinformatics, pharmacology and behavioral neuroscience will merge with other specialties such as virology, bioengineering, optics and materials science. For years, a reductionist approach was applied to distill basic mechanisms of brain function without wrapping that back with more holistic approaches. Today, we still lack a general theory of the brain and we are

puzzled with the pieces. Time is ripe to take another direction in brain research that, while deconstructing these pieces also aims for understanding the complexity of emergent levels. This approach will lean on new methodologies and push theoretical and analytical techniques beyond their limits. But also, knowing more about how the brain represents the world will change our vision on questions pertaining to philosophy and humanities and transform other fields that are currently considered unrelated, such as ethics, economy and law.

2.2. Technological impact

From an industrial standpoint, the techniques and approaches required to address these questions will accelerate the field of neurotechnology. Developing optical methods to monitor and analyze brain activity based on genetically-constrained functional sensors and high-throughput recordings will require new solutions for miniaturization and biocompatibility. Similarly, engineering chronic high-throughput probes that record activity of thousands of single neurons robustly over time requires unprecedented advances in materials science, electronics and nanophotonics. Novel materials and microfabrication processes, such as graphene or stem-cell coated probes, will surely be translated to a broad-range of industrial sectors. Moreover, brain-inspired solutions for self-navigation, pattern recognition and generalized learning will crystalize in more efficient algorithms for artificial intelligence and machine learning, transforming these pervasive fields. These investments would in turn impact on the generation of novel and better tools for diagnosis, prevention and treatment of brain disorders.

2.3. Health impact

A better knowledge of the normal brain is critical to understand disease and maladaptive behaviors. Some of the most prevalent neurological disorders, such as epilepsy, Alzheimer's and Parkinson's diseases, are calling for solutions hidden in the very same mechanisms that brain circuits use to operate. For many other mental disorders such as schizophrenia and bipolar disorder it is essential to understand how a diseased mind emerges from the brain itself. Other disease conditions result from early insults or traumatic experiences via processes that usurp the molecular machinery, originally evolved for plasticity and adaptability. Conditions such as stress and anxiety could result in depression, addiction or aggressive behaviors. Research on these areas would lead to the development of novel approaches to prevent disease and to identify new therapeutic strategies.

2.4. Educational impact

Neuroeducation is currently in its infancy as an emergent field at the interface between neuroscience and educational theory. Designing educational approaches that capitalize on the mechanisms used by the brain to learn will transform the way we teach at schools, and how we handle special cases such as dyslexia, attention deficits and reading delay. At the high education level, universities are now embarked in curricular development to train students more transversally in knowledge, attitudes, values and skills. The way these programs are drafted should be inspired by a better understanding of brain processes, where basic concepts such as iterative learning and memory consolidation are exploited. By addressing the challenge of incorporating the role of individual variability in learning, brain research will impact in drawing more advanced personalized educational tools, both for students with special needs and trainers.

2.5. Societal impact

In the modern world, threatened by unforeseen pandemics, ecological, environmental and demographic factors, science is more needed than ever. Communication with society will benefit from strong scientific inspiration without abandoning the humanistic perspective, by educating people in pursuing for truth and knowledge while accepting the limitations of our existence. This social dimension of education is essential to confront false beliefs and fake news.

2.6. Ethical impact

Advances in deconstructing behavior will also open major ethical questions. With the ability to decode brain activity in real time, novel brain-machine interfaces will change the way we interact with each other and with external devices. This will raise novel concerns and issues regarding mental privacy and cognitive autonomy. At another level, understanding basic mechanisms of behavior across species is likely to show us how similar (and different) we are from other animals and will certainly transform our perception of their rights. Finally, a better understanding of the neural mechanisms of behavior may open new questions regarding free will and responsibility, with impact in courts of law. Some of these concerns are already being put forward by the NeuroRights Initiative, from the Columbia University (https://nri.ntc.columbia.edu/), which is progressively gaining support worldwide

3. KEY CHALLENGING POINTS

Deconstructing the human brain is one of the great scientific challenges of the 21st century. To untangle mechanisms underlying brain function we need to reduce some basic behaviors to elementary processes at the level of cells and circuits. Elementary processes can be conceptualized from the complex machinery of signaling pathways within single cells, to the plasticity phenomena underlying synaptic transmission and communication between cell types. At the circuit level, neuronal activities of ensembles of cells during brain oscillations are considered elementary processes of brain computation during specific behavioral tasks. The way cell ensembles transform into memory engrams is at another level of complexity and from there to behavior will require embracing complexity at an emergent category. Below, we identify the four major key challenges that will transform understanding behavior across all these organizational levels.

3.1. Mapping genes and proteins to cell-types and circuits

Current efforts seek to elucidate cellular diversity of the nervous system and the connectome as a prerequisite to understand brain function. Traditional attempts to understand cellular diversity focusing on anatomical and physiological features have not resulted yet in a unified taxonomy of brain cell types. Development of massive deep sequencing has become essential for cost-effective profiling of single cell types.

Today, single-cell profiling methods are helping to define all cell types of the nervous system from laboratory animals (Tasic et al., 2016; Zeisel et al., 2018) to the human brain (Zhong et al., 2020). Moreover, recent evidences show successful combination of transcriptional profiling with chromatin accessibility or chromatin methylation to track epigenetic modifications at single-cell resolution. The concept of cellular identity is thus evolving to incorporate a temporal dimension of the molecular state of the cell (Lipinski et al., 2020).

These comprehensive categorization schemes are propelling the systematic study of physiological states, developmental trajectories, regulatory circuitry and interactions between cells, thus providing a novel framework for understanding cellular dysregulation upon environmental influences and along lifespan. For instance, different region-specific neuronal and glial subtypes play major roles in health and disease (Perea et al., 2009; Batiuk et al., 2020; Hammond et al., 2019). Similarly, in the hippocampus more than 40 groups of genetic, anatomical and physiologically subtypes of GABAergic and

glutamatergic cells are critical to understand microcircuit operation (Klausberger and Somogyi, 2008; Valero et al., 2015).

Importantly, brains are shaped across millions of years of evolution. While many basic behaviors such as fear or navigation may indeed share common neural circuits across species, their appropriate expression is strongly shaped by evolutive driving forces. Thus, adopting an evolutionary perspective could help identifying properties, mechanisms or behavioral outputs preserved across organisms. But also, the emergence of differences across species will provide new knowledge on the evolutionary solutions responding to the same adaptive demand, forcing for new hypotheses and paradigm shifts.

To successfully accomplish this challenge we need to breakdown efforts in the following specific tasks:

1. Developing the atlas of brain cell-types. By exploiting molecular and genetic high-throughput approaches together with cutting-edge gene editing techniques, the role of heterogeneous cell types can be disentangled.
2. Dissecting cell-to-cell communication. Novel super resolution microscopy will enable the analysis of single molecules in specialized subcellular regions (e.g. dendritic spines) while gaining scalability across cell types. These techniques in combination with next-generation functional sensors and optogenetics will permit dissecting synaptic dynamics at high temporal and spatial resolution.
3. Identifying cell-type specific circuit motifs. A major next step will be to link the cell atlas and connectome with brain circuits. Combination of cell-type specific circuit mapping and intersectional transgenic strategies with emergent recording approaches such as Patch-seq and high-density optoelectrodes will represent a novel *tour de force*.

3.2. Decoding brain function in real time

Brain circuits operation is not static and quickly adapts to behavioral contingencies under the influence of experience and environmental factors. Elementary processes such as synaptic plasticity, including long-term potentiation (LTP) and depression (LTD), are essential for neuronal adaptation in an ever-changing environment. In addition, other forms of plasticity, such as spike-timing dependent plasticity (STDP) and others operating at the behavioural time scale, have highlighted the importance of the precise timing of activity between neuronal ensembles. It is believed that memories are encoded

and stored in the brain by these sparse ensembles transformed into memory engrams (Josselyn and Tonegawa, 2020). However, several fundamental questions remain unsolved:

- Are there different plasticity rules operating in different cell types?
- How do transcriptional changes influence the function of cell-type specific microcircuits and the configuration of ensemble activity?
- Can we track these changes dynamically along elementary behaviors?

Importantly, tracking the activity at individual synapses, neurons and circuits in real time will offer the possibility to decode brain function on demand. This will foster unforeseen applications, from movement and speech production to remote device control, to give only few examples. To make substantive progress in this direction future neuroscience research needs to address the following critical tasks:

1. To elucidate synaptic modifications during behavior. Using novel technologies permitting 3D volumetric mapping in real time to allow for unprecedented analysis of microcircuits at multiple scales.
2. Identifying and manipulating cell ensembles on demand. By exploiting all-optical techniques for simultaneously imaging and stimulating hundreds of genetically-defined cell types during elementary behavior.

3.3. Mapping circuit activity to engrams

The brain has the ability to encode, store and retrieve information critical for adaptation and survival. A remarkable example is episodic memory that allows individuals to handle a stunningly large collection of experiences. The way in which activity across different circuits interacts brain-wide to make meaningful and enduring representations remains challenging. It is now clearly established that memory circuits are malleable and that activity-induced gene expression underlies structural and functional plasticity allowing us to cope with daily life contingencies. Pleasure and fear, acting as reinforcement, are two natural feelings with strong effects on the configuration of memory circuits. Thus, current efforts based on unbiased DNA and RNA sequencing at population and single-cell level are shedding light on the molecular underpinnings (Lopez-Atalaya et al., 2013; Fernandez-Albert et al., 2019; Jaeger et al., 2018). Sophisticated methods coupling mouse genetics and single-cell sequencing are helping to elucidate expression profiles of engrams associated to episodic memories (Kitamura et al., 2017). However, it is yet unclear what chain of elementary processes is required to consolidate/modulate memory

traces into engrams across brain regions. The associated neuronal substrates are to be revealed at the optimal spatiotemporal resolution.

Further developing of precision tools is thus essential to unravel ensemble dynamics and its relationship with behavior. For instance, novel graphene based solutions now allow for recording infra-slow brain activities (Masvidal-Codina et al., 2019), while nanotechnologies permit tracking single cell activity (Jayant et al., 2017; Neely et al., 2018). Imaging and manipulating the large repertoire of cells place additional technological challenges (Zhang et al., 2018; Stringer et al., 2019). To properly address these questions we will need to focus on the following key tasks:

1. Characterizing ensembles and engrams of specific behaviors: Using a combination of emergent tools, to address mechanisms of more elaborated processes such as memory editing and erasure, learning transfer and generalization.
2. Developing novel neurotechnologies for interrogating engram activity: Including, but not limited to, the exploitation of new materials and strategies to increase biocompatibility and multimodality of neural implants.

3.4. Deconstructing behavior

In order to understand how the brain operates, we need to embrace complexity at the emergent behavioral level. Decoding the complexity and dynamism of the natural behavior is at the limit of our current tools. Neuronal activity organizes differently across behavioral states such as sleep, wakefulness, attention, emotional alertness, etc., while the brain is coping with huge number of external inputs. Different cognitive or behavioral states involve distinct plasticity rules, which in turn shape circuit dynamics differently. Nevertheless, the number of activity states of a given neural circuit is limited by anatomical and functional constrains. Therefore, while we apply massive profiling approaches at the bottom level to dissect a large variety of cell types, high-throughput recordings of these cells during behavior suggests that the neural population dynamics may be indeed simpler. For example, the mechanistic complexity of cell types and processes underlying reaching and grasping can be operationally reduced to a low-dimensional mathematical object termed 'neural manifold' (Gallego et al., 2020). However, deconstructing the complexity of collecting items from a basket poses fundamental questions regarding how the elementary behaviors are integrated. It is not only a matter

of reaching and grasping, but understanding how the brain process and integrate the flow of information to make decision of what object to take. Here, a major problem is separating correlation from causation (Pearl, 2000). Thus, adopting novel perspectives for deconstructing behavior requires merging computational methods and more sophisticated statistical tools:

1. Developing new machine learning and computational tools. By leveraging in modern techniques for supervised and unsupervised learning, we aim at transforming neuroscientific data analysis and developing novel theoretical frameworks to understand brain activity.
2. Testing correlation and causation. By working at the interface between mathematics, statistics and complex systems, in combination with closed-loop real time engineering solutions, we aim at challenging current experimental paradigms.

Modern neuroscience is an alliance between disciplines. The complexity of decoding brain function and behavior requires a multidisciplinary and multi-center approach. Over the past decade, the field has expanded massively, forcing interactions between different areas of knowledge. Only an interdisciplinary approach will allow us achieving the challenge of decoding brain function and behavior.

CHALLENGE 2 REFERENCES

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CHALLENGE 3

ABSTRACT

Few things fascinate us as much as understanding human cognition. We owe it our ability to adapt to a complex environment in continuous change; it ensures that decisions are made on the basis of knowledge, previously acquired through experience or transmitted from generation to generation in human culture. It is also the cognitive capacities that allow us to imagine the future in order to anticipate it, being creative in problem-solving, but also identifying beauty in our perceptions and reproducing it in art pieces. Our cognitive capacities make us humans.

Looking for a comprehensive theory of cognition implies first recognizing that many of the key dynamics that enable information processing can, in fact, be implemented by different biological hardware, not only brains, and that this has been widely exploited by evolution. Therefore, future research plans need to study cognition as *the total set* of mechanisms and processes that underlie information acquisition, storage, processing, and use. This has to be done across organizational levels and biological systems connected to each other and with the environment. In short, the path that cognitive science needs to take, represents the refounding of a discipline of knowledge. We identify three pillars: (1) to focus in organisms (not only organs) and collectives of organisms, (2) to put forward the ecological dimension of cognitive behaviour and (3) to consider an evolutionary, cultural, and historical perspective. Cognitive science can no longer run on parallel paths of science, but must converge and define a new holistic direction that will bring about new understanding.

KEYWORDS

cognition | behavior | consciousness | embodiment | individuality | collectivity | society | context | extended mind | artificial intelligence | brain-AI | culture | evolution

CHALLENGE 3

COGNITION, COLLECTIVE BEHAVIORS & CONSCIOUSNESS

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1. INTRODUCTION AND GENERAL DESCRIPTION

Cognition (from the Latin *cognitio* 'examination,' 'learning,' or 'knowledge'), refers to the process of acquiring knowledge and understanding through thought, experience, and the senses. It underlies functions and processes such as attention, the formation of knowledge, memory, judgment and evaluation, reasoning, computation, and decision-making.

1.1. Cognition exists throughout biological systems

No doubt, the Central Nervous System (CNS) supports these functions in many organisms. Throughout evolution, the CNS has developed specialized molecular and signalling mechanisms, cell types and neural circuits to support them.

However, brains (or neural networks), have no monopoly on the signalling functions that implement many of these remarkable algorithms (Baluška and Levin, 2016). Pagán (2019) and Turner (2019), for example, extend the question of what is a brain to consider the wider picture, from the smallest brain to very different (solid and liquid) cognitive systems (Figure 3.1). Cognitive networks have evolved a broad range of solutions to the problem of gathering, storing and responding to information. Some of these networks are 'solid', i.e. with a relatively

FIGURE 3.1–Cognitive networks have evolved a broad range of solutions to the problem of gathering, storing and responding to information. Cognition is not a monopoly of the brain. Instead, cognitive processes occur across a broad range of biological systems with different organizational levels, expanding to the collectives and interfacing with the surrounding environment and all sorts of lifeless artefacts, which in turn bring back powerful transformation capacities to cognition. Common principles of cognitive networks can be found by using a common language and novel big behavioural data and quantitative tools across disciplines. The time is ripe to start an interdisciplinary research program to explore them. (Drawing by Alex Richter-Boix and Frederic Bartumeus).

stable physical architecture. Solid brains involve structured sets of neurons linked through persistent but highly dynamic synaptic contacts forming a complex and adaptive web of connections. Other systems are formed by sets of agents that exchange, store and process information but without persistent connections, including those that move relative to each other in physical space. These are the so called 'liquid' brains, or fluid neural networks, a category that includes ant and termite colonies, immune systems and some microbiomes and slime moulds (Solé, Moses and Forrest, 2019).

Looking for a comprehensive theory of cognition implies recognizing that many of the key dynamics that enable information processing can, in fact, be implemented by different biological hardware, and this has been widely exploited by organisms throughout the tree of life. Evolutionary pressure to optimize decision-making has led to the inevitable exploitation of past history (memory) and information processing (computation). Importantly, however, decisions are made at every level of biological organization

(Baluška and Levin, 2016). For example, multicellular organisms, such as animals and higher plants, exhibit goal-directed behaviours also at their cellular and subcellular levels.

Therefore, one of the big challenges that we need to consider when thinking of future research plans is the understanding of cognition as the total set of mechanisms and processes that underlie information acquisition, storage, processing, and use, at each level of organization (Lyon, 2015).

1.2. Cognition involves interactions across biological systems, the environment, and lifeless artefacts

The set of mechanisms and processes that build up cognitive functions involve different organizational levels across biological systems connected to each other and with the environment. Clear examples of such interfaces are the paired interactions cell-tissue (organs functioning, cancer), brain-body (e.g. brain-gut axis), individuals-groups (e.g. collective behaviour, social dynamics), society-ecosystem (e.g. economy, climate, epidemics).

Behavioural science and cognitive neuroscience have tended to treat the environment as a fixed set of constraints on action (Sterelny, 2004). However, recent developments in cognitive sciences have demonstrated the bidirectional relationship between living agents and their environments. So-called niche construction theory has shown that organisms do not merely passively respond to the challenges of their environments, but they actively modify them, thus transforming the action of the environment on themselves and on their descendants (Odling-Smee et al., 2003). This perspective builds on Dewey's ideas transforming the stimulus-response reflex-like view of the relationship between an organism and the environment, into a feedback loop in which sensory perception and motor actions keep a dynamic equilibrium through the interaction with a changing and transformable environment (Dewey, 1897). Humans are the most salient example of a permanent construction of our own niches, not only by means of material artefacts such as tools or clothes, but also through epistemic artefacts which extend our cognitive capacities (Clark and Chalmers, 1998). For instance, paintings, written records, songs or stories allow humans to ease memory burdens by storing information in the environment. Thus, just as environmental and cytoplasmic factors are considered together with genes as resources enabling development, extended cognition holds that external resources, such as notebooks used as memory stores and AI algorithms in our wearable smart devices, can play a fundamental

functional role within a cognitive process. This has led the advocates of the so-called "extended memory" to argue that memory (and generally cognition) exceeds the bounds of the individual brain, and should be regarded, not only as an embodied, but also as an extended capacity.

Even more, if we really want to be comprehensive in our definition of cognition, we cannot even restrict ourselves to the crosses between biological systems, but we also need to consider the crosses between biological and non-biological worlds. As posed by Maurice Merleau-Ponti (1963), consider a blind man with a stick navigating a city street, where does the blind man's self end? At his fingernails? At the handle of the stick? At its tip? It is not easy to answer this question because the representation of the limits of the self can be really dynamic, changing almost instantaneously depending on the context and the task. African women from the Kikuyoand Luo tribes can carry more weight on their heads than even the most capable army recruit (Heglund et al., 1995). They do so by an improved transfer of gravitational potential energy and kinetic energy during the inverted pendulum movement of each step cycle. Surprisingly, this improved motor behaviour cannot be applied when unloaded, as if the memory of the improved gait economy program was only available in that particular context, as if the load itself was considered part of the carrier's body. Thus, for our purposes, as we will see below, we might be better off by considering that our cognitive capacities, spread out beyond our bodies into culture and the material world. In this way, we define another big challenge in the field, which involves the study of so-called "extended" cognitive capacities.

1.3. Cognition requires an interdisciplinary approach with common concepts and tools

Examples of cognitive networks go from the nervous system to things like social insects, ecosystems, economies, cities, and civilizations. "Beyond the specific functional roles they play, and the forms they can take (e.g., small/large, distributed/centralized, modular/hierarchical, or alive/artificial), all cognitive systems are composed of multiple components that exchange and react to both environmental and internal signals to gather, store and process information" (Solé, Moses and Forrest, 2019). Importantly, common organizational principles are to be expected and should be explored. We believe the first steps towards an integrated view of cognition stem from sharing cross-boundary concepts and quantitative tools across disciplines (e.g. neuroscience, physics, ethology, ecology, humanities).

Some of the potential commonalities were suggested nearly three decades ago (Farmer, 1990), including two key properties shared by most connectionist models: (1) the interactions between the variables at any given time are explicitly constrained to a finite list of connections, and (2) the connections can change, in that their strength and/or pattern of connectivity can change with time. For example, in the case of a nervous system, synaptic contacts dynamically modulate the connectome; in the case of colony ants, different types of ant interactions are at play, from antenna contacts to pheromone release, and the "rate or density" of interactions rather than interactions themselves modulate the connectome (Gordon et al., 2010). Other core concepts in the study of cognition are those related to information. In living systems, is crucial to capture the dual nature of information, both structural and computational (Mitchell 2009, O'Connor et al., 2019). Some forms of information stored in biological structures have energetic value and constrain future possible states of a system. Quantitative measures of structural (syntactic) information fail to capture the content of information (semiotic information) and how it is interpreted, processed or transformed. The latter processes are much more related to the concept of biological computation (Mitchell, 2009). Although multiple systems can develop a cognitive network, they might differ in their levels of complexity. In the study of cognition, it is still a big challenge to fill the gap between the characterization of cognitive mechanistic properties (syntactic or structural information) and the emergence of complex semiotic systems. This difficulty clearly explains the existence of different levels of complexity in cognitive networks.

Overall, it is clear that pushing cognitive science to the next level will require trans-disciplinary research in which, (1) different biological hardware are studied as cognitive agents, (2) neuroscience is not separated from biology but integrated with it to understand an organism, (3) the cognitive function is acknowledged to have an evolutionary and ecological dimension, (4) cognitive by-products act on (and transform) the environment in which they live, which in turn, transform the adaptive needs of cognitive systems, evolving new cognitive functions. The study of human societies through innovation and the production of cultural artefacts, or more recently, the impact of artificial intelligence-brain interactions, come into play as fundamental cross-disciplines for a comprehensive understanding of cognition. From the above points, it appears obvious that only a trans-disciplinary research program will produce synthetic and first principles knowledge about cognitive processes.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

To put it briefly and directly, the path that cognitive science needs to take, whose first steps undoubtedly begin now and will extend for at least the next 10 years, represents the refounding of a discipline of knowledge. Cognitive science can no longer run on parallel paths of science, but must converge, define a new holistic direction, and then bring about new understanding. We find a clear opportunity to develop a common language in cognition in the common tools, both experimental and computational, that the different disciplines use. There is a range of exploitable experimental infrastructures and analytical capacities with application in the transdisciplinary cognitive spectrum, from experimental to social sciences (e.g. eye tracking systems, high-throughput movement tracking systems, statistical physics tools like networks analysis, machine learning, etc).

The refoundation of the discipline must necessarily be disruptive at all levels, e.g. academic, health, technological, social, economic, educational. Suffice it to say that the perceptions we have of ourselves in the world and the universe form our framework of thought. Changes in this framework often came in the past associated to scientific revolutions and represented a leap in civilization. This could be just a promise, but a huge one!

Academy

- Refound the cognitive sciences by unifying traditionally separate knowledge disciplines. This will bring about shared operational definitions of cognitive functions and the complementation of study viewpoints and methods. Overall, the impact is to have a more scientific approach for humans and humanities; and a more human and humanistic approach to science and scientists.
- Dissecting neuronal networks will not bring the understanding of cognitive functions by itself; embodied and extended properties of cognition are required. The holistic perspective will finally make accessible answers to long-standing cognitive problems.
- New questions will be formulated and theories proposed on the grounds of the hybridization between disciplines, and the experimental possibilities offered by the breakdown of standard experimental lab constraints. Novel and ecologically-based lab conditions and the so called real-life experiments, outside of the traditional labs, will also validate or contradict current theoretical propositions.

- By taking seriously the collectivity, the individuality (in contraposition to the average) will also gain relevance and dimension for a personalized understanding of cognitive functions.

Health

- Understanding collective behaviours may have numerous impacts on health. From designing resilient structures at the social scale, let's call them "cognition-friendly" spaces, to understanding of cellular processes in disease (e.g. cancer), or microorganism dynamics.
- The disease, in any of its forms, whether it directly affects the CNS or not, physical or psychological, is reflected in some of our cognitive capacities, as cannot be otherwise. Therefore, a better understanding of cognition, its dimensions, and entry points for treatment, will have an enormous impact on health. An obvious example is illustrated by the impact of brain-body interactions in cognition, mental health and wellbeing.
- Understanding cognition in brain-body interaction will also impact human-machine-interface technology used in rehabilitation, limb prosthesis, exoskeletons or extended memory devices.
- Real-life experiments, wearable technologies and techniques of artificial perception (e.g. artificial vision), by breaking the translational barriers imposed by the constrained laboratory conditions, will impact diagnosis of pathology at early stages (e.g. children in the school) facilitating the implementation of early therapies.
- Understanding cognition at the edge between humans and AI algorithms will impact on the understanding and treatment of addictive behaviours.

Technology

- Development of new technologies for integrated measures (i.e. whole-body electrophysiology, microbiota status, movement, ongoing measures of immune system, cognitive testing).
- Improved Human-Machine-Interphases (HMI) by including cognitive properties into machine designs, feedback information from memory and proprioceptive perception. Self-perception of machine.
- Bioinspired algorithms, resilient information processing networks.
- Humanized domestic robots, and optimized interaction with digital tools.
- Autonomous systems implemented with cognitive capabilities (i.e. decision making).

Education

- Incorporating knowledge about cognitive mechanisms into education methodologies, e.g. 'smart' usage of digital technologies, optimization of group dynamics, information processing and decision-making in the classroom.
- Designing spaces for optimal learning.
- Educational programs on the brain-body interaction to understand the value of nutrition, physical activity and the understanding of physiological reactions in stressful contexts, and the value of art as the maximum expression of a cognitive system.
- Fight technological negative impact on attention, and 'fake' news acceptance.
- Including a transversal and evolutionary notion of cognition in the curricula from the school.
- Teaching the value of transdisciplinarity in higher-level education.

Society

- Better understanding of the properties and capacities that make us humans, and those that we share with other species, together with importance of the collective and the habitat, should have a positive impact on the building a fairer and more sustainable global society.
- Moral and empathy are fundamental processes that guide our decisions, perceptions, actions, feelings and emotions. Rigorous understanding of these processes would educate society on how we organize, and of course, help identify mechanisms when this is not happening (bullying, borderline and antisocial personality disorder, psychopathy).
- Understanding the human-ecology interactions and climate change as an example of effects between cognitive networks.
- Detection of patterns of collective behaviour that can instruct institutional protocols for guiding mass behaviour in extreme situations.
- Improved ability to plan for and respond to climate migration or to incorporate changes in human mobility patterns into epidemiological models by understanding how people perceive and understand the spaces they move through.
- Design of private and social spaces and smart cities under the concept of "cognitive-friendly" architectures.
- Response to societal concerns, and the associated ethical issues, over the impact of artificial intelligence, particularly in terms of social relations.

- Fighting fake news and information overload phenomena which amplifies social inequality and weakens modern democracies.

3. KEY CHALLENGING POINTS

Reflecting on the multiple dimensions of cognition introduced above, we have organized the challenges that cognitive science should confront in 4 categories:

3.1. Cognition in the real-world

We define cognitive systems as networks of multi-agent systems capable of processing, computing, and storing information to generate action and decision-making. Cognitive systems can show different organizational levels as well as different type of relationships with the environment surrounding them. To understand how cognitive systems integrate information from the environment, past experience and internal states to produce useful behaviours we need to assess: (i) the agents' degrees of freedom (i.e. mobility, behavioural variability), (ii) the 'emergent' patterns produced by them (social and collective dynamics), and (iii) the causal mechanisms resulting in cognitive responses at adequate ecological scales and environmental complexity levels (from syntactic to semiotic).

What is the minimal set of fundamental processes in cognition? What are the key differences between solid and liquid brains, particularly in their cognitive potential, ability to solve particular problems and adapt to environments, and information-processing strategies? How did active exploration of the environment, the production of gadgets to relate with and understand the world, and other evolutionary innovations impact cognitive systems? The search for common organizational principles in cognitive systems will require from statistical physics, network theory (Barabasi, 2014), and a complex systems perspective (Mitchell, 2009) and make sure it copes with different sources of variability and uncertainty at different organizational levels (e.g. personality, labour division, internal states). From an experimental perspective, the challenge is to identify inherent variability in large-scale behavioural arenas (Bartumeus et al., 2016), represent better environmental complexity including the creation of virtual reality laboratories, and extract meaningful data from experiments in 'digital' behavioural arenas as provided by social networks (Twitter, Facebook).

Mobility: solid and liquid brains

Mobility at the agent level (e.g. molecules, cells, organisms), can strongly modulate the responses of cognitive systems. In particular, the collective dynamics exhibited by large populations of agents interacting nonlinearly depends critically on whether or not the basic network components are mobile. Indeed, density and movement of the agents account for most of the distributed intelligence in liquid brain models (e.g. Piñeiro and Solé, 2019).

Currently, systems biology (Krummel, Bartumeus and Gérard, 2016), ecology (Nathan et al., 2008) and social sciences (Palmer et al. 2012, Zagheni et al., 2014) are being revolutionized by the massive access to high-throughput mobility and spatial real-world data (e.g. video-recording techniques and massive data analysis, GPS and biologging devices, cell phone apps, geolocated twitter, GIS open data sources). This technological revolution will clearly improve our understanding of cognitive systems. Both fields can now start answering relevant (but still unsolved) questions about how cells, organisms, and humans perceive and use space, and how they chose where to go or to spend time. These questions are focused at multiple spatial and temporal scales, and gain much interest in the context of volatile and uncertain social and physical environments. How do animals/people search for things in geographic space under varying levels of information or no information at all (e.g. search strategies)? How are daily activity spaces and space uses determined and understood? How do migrants (cells/animals/people) choose their routes and destinations? How do individual spatial decisions aggregate and interact in collectives? What are the effects of different changes in social and physical environment on these questions? (e.g. wars, climatic emergencies, pollution, disease outbreaks).

Behavioural variability and biases

In addition to mobility, agents' behavioural rules of interaction and intrinsic variability can also strongly modulate the responses of cognitive systems. Behavioural variability (personalities, stereotypes) is an important dimension of ecologically and evolutionary variation within living systems (Wolf and Weissing, 2012). In this context, the new era of 'Big Behavioural Data' (Gomez-Marin et al., 2014) together with machine learning and artificial intelligence approaches (e.g. Berman et al., 2014), promises a much synthetic and comprehensive view of behaviour, unifying traditionally separate fields like neurosciences, ethology, and behavioural ecology (Gomez-Marin et al., 2014).

Key to behavioural variability analysis is the identification of cognitive evolutionary constraints, and more specifically, biases and errors. Considered in Prospect Theory (Kahneman, 2011), these types of biases are common across cognitive systems (Friggeri et al., 2014; Wendt et al., 2019; Oro, 2019). In long-lived social species, culturally biased information, rumours, and cheating not only modify information processing but they are often amplified, so that decision-making, e.g. staying or leaving a patch, is spread faster across a population, producing non-linear dynamics and critical transition phenomena (Oro, 2019). Among humans, cognitive biases are massively exploited and amplified in advertising, but also in other communication contexts in the form of 'fake news' or 'information overload'. Clearly, cognitive biases and their impact in collectives need to be part of cognitive research programs as it has relevant consequences for our societies and democracies.

Culture and technological innovation

It is only recently that we have started to seriously consider the impact of technologies in cognitive processes, and develop a quantitative approach to cultural evolution within cognitive frameworks (Heyes, 2018). Are cultural products (i.e. art, technology) the result of collective cognitive mechanisms ('cognitive gadgets' sensu Heyes, 2018) or are they neuro-genetically encoded? How does 'cultural cognition' evolve with the appearance of innovative artefacts both in ancient (fire, wheel, art) and modern (technological innovations) times? Developing these novel conceptual frameworks has practical implications, for example, in rehabilitation and education, but also fundamental ones, such as extending evolutionary theory to accommodate also the evolution of cultural artefacts and the notion of cultural cognitive mechanisms.

3.2. Individual cognition in complex contexts: neuronal networks embodied in organisms

From the concepts previously introduced, it follows that we will only be able to explain brain cognition if we study the neural circuits as they work to solve the tasks for which they evolved.

Neuronal circuits supporting/enabling cognitive capacities

There is an urgent need of a more comprehensive understanding of the neuronal circuitry underlying complex cognitive function. We are still lacking relevant knowledge on how the healthy brain works on sophisticated cognition, such as decision-making, the perception of time, creativity... and as transversal theme, how internal state (emotions) modulate all these. The lack of a model of the mind

explaining the interactions between cognitive processes explains why we are still assessing cognition in simple and highly controlled environments. Complex cognitive situations are virtually absent in the current experimental panorama, and this is the obvious next step that cognitive neuroscience should take.

Building upon of the superlative technical developments in neuroscience that allow us to monitor and manipulate specific neural circuits with increasing precision, specificity and resolution (Boyden, 2015) (see Challenge 5b), we should comparatively devote greater efforts to provide qualitatively improved behavioural tasks supported on the conceptualization of the cognitive processes under study, and enriched with strong quantitative approaches. We need to design ecologically-valid behavioural paradigms, constructed from the perceptual perspective of the organism in use, to study ethologically-relevant processes for which the brain has been shaped through evolution, but without renouncing to the tremendous advantage of laboratory well-controlled settings that will enable the fine dissection of the neural circuits of sophisticated cognitive processes. The use of virtual reality in human experiments and behavioural arenas for animals reproducing natural contexts will be strategic for achieving this goal in the next years.

The brain implementation of cognitive functions requires the interaction between specialized neuronal networks distributed in the brain-broad anatomy (Álvarez-Salvado et al., 2014; Canals et al., 2009), however, how these interactions develop a cognitive system with semantic properties is not known. Since humans, as many other social animals, rely on social information to learn the contingencies of the world and guide their motivated actions (Olsson et al., 2020; Márquez et al., 2015), the inclusion of a "second person" perspective in the study of the individual brain networks will be very relevant. Our current understanding of the brain is mostly based on single subjects. Put it in other words, understanding the function at any level of complexity involves a network of networks. Bringing the multi-agent perspective to the study of individual brain networks is a neuroscience challenge that cannot wait any longer.

Embodied cognition: brain-body interaction

Neuronal circuits do not have a monopoly on the communication/signalling functions implemented by the algorithms of cognition. We should not forget that neurons specialized cell signalling mechanisms (Figure 3.1) that existed long before the appearance of the CNS in evolution, to orchestrate physiology, embryonic development, behaviour. The fundamental features

of brain networks to process and store information, such as cellular excitability and activity-dependent plasticity, are present in different cell types in many tissues. Even in the nervous tissue, non-neuronal cells as glial cells are increasingly recognized as important elements contributing to the computational capacity and cognitive functions of the CNS. Therefore, it should not come as a surprise that information processing algorithms can be implemented on multiple biological hardware (Marr, 1984).

Embracing this simple and biologically funded idea opens up a fascinating world of possibilities to the concept of cognition. There are ways of communication that go beyond the boundaries of the CNS and that contribute to implement the algorithms of cognition. Thus, cognition permeates the complete organism. It becomes immediately clear the importance of understanding the bidirectional influence between the CNS and the body: the impact on the cognitive functions of the viscera-brain axis and the immune-brain axis, or the constraints that the musculoskeletal system impose on the perception of the environment, the planning of motor actions and other cognitive processes (Foglia and Wilson, 2013). For example, current initiatives try to unveil the role of the interaction between the brain and the body (heart, breathing, gut and microbiota) underlying emotional regulation, memory, and its deficits due to neurologic and psychiatric disorders like Alzheimer's or major depression.

3.3. Hybrid-cognition: human-algorithm, human-machine interactions

Human cognition and social interactions take place in an increasingly AI-dominated world. A key research line in the future should investigate how artificial intelligence changes human thinking and behaviour. This understanding will feedback into AI development and its multiple implementations in autonomic systems like robots and the improved integration of human-machine interfaces. At the same time, we highlight the emerging opportunities offered by last generation AI algorithms as tools to investigate cognition (Dabney et al., 2020). By making the differences and similarities between AI research and neuroscience progressively explicit, we move forward in the understanding of how brains work in the light of how differently machines work.

Advances in artificial intelligence have made it possible to develop algorithms with the capacity to emulate human cognitive functions. However, they still rely on syntactic operations lacking for a semantic thinking, which preclude AI systems to understand and develop complex cognitive and emotional behaviours.

There is still a big difference between our expectations and the reality of social robots (Yang et al., 2018). A new generation of robots that respond to and trigger human emotions not only would allow for a more efficient collaboration, but can also stimulate long-term social bonds between humans and these artificial agents. How do humans perceive these artificial agents in relation to other humans, pets and other animals, tools and objects? Answering these kinds of questions is not only fundamental to achieving a new generation of robots with which we can live, but will also help us to understand and support the resulting social changes in the fields of education, ethics and law.

Future AI research must be carried out under conditions of real-life experiments, collecting data during real-time interactions (Henschel et al., 2020). The effort will be worth it, as it will bring us answers to questions such as: what are the implications of turning over a growing array of cognitive activity and decision-making to machines? How are social interactions changing as they are increasingly mediated by algorithms? What are the implications for society?

3.4. Cognition at its edges: behaviour and consciousness

Another path for the next-decade refunding of the concept of cognition will involve exploring its boundaries. Over the past decades, neuroscience has been attacking the problem of cognition with increasing vigour. Yet, what exactly is cognition, beyond a general signifier of anything seemingly complex the brain does (Cromwell et al., 2011)? What is the minimum set of features defining "cognition"? Is it a continuous or discontinuous phenomenon across species? For instance, are there cognitive capacities exclusive for humans? How stable is consciousness as an emerging state?

At the lower limit of cognition, we find simple behavioural action-perception cycles. Noteworthy, the boundaries are fuzzy. Based upon the notion of 'intrinsic reflexivity' (Glasgow, 2017), one can articulate minimal forms of selfhood and cognition if they manifest self-maintenance, self-reproduction and self-containment. But, what is the real difference between cognition and a (complex) reflex? Integrating the distinction between motor and cognitive skills, it has been recently argued that all complex tasks (at least in humans), at any level of expertise, are a combination of intelligent reflexes (Krakauer, 2019). So, memory could be seen as the concatenation of multiple reflexes: stim-pain association in context A (encoding), context A-evoked motor action to avoid pain (retrieval), "understanding" of the environment based on experience (knowledge). If we take the concept of reflex literally, i.e. as the arc-reflex in the spinal cord,

where a nociceptive stimulus (note the difference between nociception and pain) triggers an involuntary (and uncontrollable) motor action, could we build cognition concatenating those arc-reflexes? For instance, is there "decision making" in a larvae foraging for food following a concentration gradient that samples by head-casting (flipping the head left and right)? Actually, based on the spontaneous actions and decision-making observed in invertebrates, free will has been proposed as a biological trait (Brembs, 2011).

Understanding the lower limit of cognition entails that exploring the neural basis of cognition is necessary but not sufficient, as cognition is arguably found in non-neural living organisms, such as plants (Calvo Garzón and Keijzer, 2011) or machines (Lake et al., 2017).

At the upper limit of cognition, we find introspection (Varela, 1996), consciousness and self-awareness. Since Crick's claim for a materialist view and scientific study of consciousness in The Astonishing Hypothesis (Crick, 1995), more evidence has been compiled that consciousness is an "emergent" and "homeostatic" state of a complex system, and that is not a monopoly of humans (Tononi and Koch, 2015). Objective ways to measure consciousness are currently looked for (e.g. IIT, Integrated Information Theory) and will progress in the future. IIT predicts that consciousness is graded, is common among biological organisms and can occur in some very simple systems. Conversely, it predicts that feed-forward networks, even complex ones, are not conscious, nor are aggregates such as groups of individuals or heaps of sand. Also, in sharp contrast to widespread functionalist beliefs, IIT implies that digital computers, even if their behaviour were to be functionally equivalent to ours, and even if they were to run faithful simulations of the human brain, would experience next to nothing. Intelligence is orthogonal to consciousness.

In the next decade, consciousness will be further explored deeper through meditation (Davidson et al., 2003), analysed better in terms of its behaviour under strong perturbations (e.g. facing degenerative processes) or the likelihood to be expanded as a collective (e.g. facing the "tragedy of the commons"). Without going any further, the present COVID-19 pandemic has taught us the importance of a collective consciousness and its dynamics, which can be understood, metaphorically or perhaps literally, as the cognition of the meta-organism that makes up the collective of individuals. Defining the limits of cognition, helps in identifying the areas that require greater attention in the neuroscience in the coming years. Both because they are still in their initial stages of development, and because their potential impact for society. from them in society.

CHALLENGE 3 REFERENCES

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CHALLENGE 4

ABSTRACT

The study of sex and gender in neurobiology is being as relevant as controversial. The main challenge identified by carefully looking at guidelines, research projects in the neurosciences and gender studies is how to design and perform experiments by taking into account the diversities found in females and males and at the same time not participating in feeding the cultures of gender dichotomy around which the society, its hierarchies and biases are articulated.

The CSIC has the opportunity to lead the path to a better understanding of diversity, and of the consequences, barriers, misconceptions, and loss of opportunities that gender biases impose in the advancing of knowledge in all fields and our society. In this chapter we expose the challenges we face in order to understand how sex and gender impact in the brain and mental disorders.

KEYWORDS

sex/gender differences

sex/gender bias in mental disorders

research guidelines

CHALLENGE 4

THE MOSAIC BRAIN: SEX/ GENDER & THE NEUROSCIENCES

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1. INTRODUCTION AND GENERAL DESCRIPTION

The study of sex and gender in neurobiology is being as relevant as controversial. It is inscribed in the long history of the biological and biomedical research regarding sex determination. Since old times the newborn's sex was regarded as important precisely because a man would be the heir and a woman would not. The whole concept of life was constructed on the idea of heredity-inheriting the father's estate, as well as his anatomical and intellectual traits (Lopez-Beltran, 2004). From this beginning, the meanings of nature were constructed and articulated around gendered powers and meanings. It is to handle sex as an agent, a variable among many others, what led to further knowledge and better practices in the treatment of human beings, no matter their gender.

The main challenge identified, by carefully looking at both current research projects in the neurosciences and at gender studies, is to design and perform experiments by taking into account the diversities found in females and males and at the same time not participating in feeding the cultures of gender dichotomy around which the society, its hierarchies and biases are articulated.

Research of sex differentiation, sex dimorphism and the social meaning of these, has conveyed old debates, frequent contradictions and few fine information about biological and human diversity. There is still controversy in the

fundamental question of whether there are sex differences in the human brain. This is a research topic in which many neuroscientists are focused, which still needs to be carefully addressed and therefore justifies the need for increase effort in research. Rigorous studies in their conception, interpretation and data sharing are necessary.

The CSIC has the opportunity to lead the path to a better understanding of sex/gender diversity and of the consequences, barriers, misconceptions, and loss of opportunities that gender biases impose in the advancing of knowledge in all fields and our society.

1.1. Background landscape of cultures and practices

Human culture and its skills as an educated community aimed at researching the biology of all kinds of living beings, from microbes and plants to human beings. The transference of the social order has often been an agent in the biological knowledge regarding sex, race/ethnicity and class. Local practices, global politics and social environments participate in the concepts of sex as a biological event while it is in society and culture where gender and biology have a diversity of expressions, observed by the naked eye. From Margaret Mead to contemporary studies on biology and gender, gender is understood as an analytical concept that questions biological determinism, while sex is a biological category, not necessarily dichotomous.

The biomedical complex –the research community of biology and biomedicine, its technologies and funding systems– has mainly focused on genetics and the processes involved in what molecules do by following the genetic code, from the cell nucleus and chromosomes, through membranes and all kind of proteins and molecules, to tissues and organs. Heredity and congenital traits, disorders and diseases investigated too often from two almost opposite positions, considering humans as one homogenous population of individuals in which sex had little influenced, or as a fully dichotomized binary population of individual of two sexes.

Biological relations, including those among sex and the brain, are interpreted by a fully gendered human culture, including the scientific community, that separated for centuries the society in two –women and men– (in addition to socioeconomic categories of class and race/ethnicity). The connection between the nervous system and the development of the gonads in embryos and living animals has become an attractive research subject. The brain, as its biology, remains the very unknown organ for contemporary biomedicine. Much

effort in research, research policy and clinical work has been done and many insightful results have been published. We propose an introspection on both gender and the brain, and on their connections.

1.2. The brain and the meaning of being human

The brain, as the core organ regarding the meaning of humankind and human skills, is being studied by experimenting with animals. The research agenda of the neurosciences embodies the whole and old gendered culture of differences between the sexes and genders (Fine, 2010; for further references, see the Neurogenderings network website). This means that what we already know and the ongoing projects in this area are fed, and contribute to feeding a gendered society that assumes differences to justify inequalities. The overcoming of this feedback loop has been the work of many scholars already. We propose here to include this aim among the main challenges to be faced by CSIC. The breakdown of such a feedback loop includes a careful use of the terms so as not to naturalize, biomedicalized (Clarke, 2003) the diversity of living beings.

The relationship between chromosomal sex and neurons, behavior and mental disorders should be carefully formulated, not only because terms do matter in a comparative way, but because the formulation of research questions and experimental designs do as well. Not to deny differences among the sexes, many of them visible for the naked eye, but to call for an awareness of the bias embodied when posing, and indeed trying to answer research questions on the relation on sex/gender and the brain. The questions themselves and their answers should contribute to an inclusive research that takes into account every agent involved in a culture open to the recognition of the differences among individuals in addition to and beyond the sexes.

1.3. Facing the challenge: sex/gender and the human brain

We face today the challenge of an integrative initiative from CSIC researchers in the biological and biomedical sciences and in gender studies so as to incorporate the knowledge and awareness of diversities in biology, society and culture. This challenge has been faced by feminist neuroscientists (in Spain, Barral Morán, 1999). It is at some websites where many debates are circulating and publications are shared. At the crossroad between neuroscience, gender studies and social policies, many scholars and research projects have met.

The proposals that follow aim at promoting these encounters between the neurosciences and gender studies by creating a space of interchange, by setting up

guidelines and supporting research on sex in its diverse expressions in the neurosciences while at the same time not contributing to gendered biases, social hierarchies and the naturalization of differences by sex and gender.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

For many years, it was thought that women did not suffer as much as men in cardiovascular or autism diseases. This misconception came from biased interpretations of women narratives of their own suffering, the lack of knowledge and research in women's health, and the bias of medical practices, mostly provided by the accumulated knowledge on diagnosis and treatment in men's bodies (Garcia, Mulvagh et al., 2016). This situation illustrates perfectly the implications that our proposal could have in preventing such misconceptions in medical diagnosis and therapy.

Research has traditionally focused more on males and too often excluded females (this point goes beyond the brain area). Therefore, there has been an exclusion of female subjects in preclinical and clinical studies (Zucker and Beery, 2010) that have hampered the effect of sex on basic neuroscience research (or in many other areas for that matter). The claim that this bias is partly due to concerns that females are more variable subjects due to cyclical reproductive hormones has been disproven in meta-analyses (Mogil and Chanda, 2005). Moreover, when studying human diseases in which symptoms are influenced by, for example, ovarian steroids or sex chromosomes, it is mandatory to include female animals in the sample under study. Based on European guidelines (Schiebinger and Klinge, Gendered Innovations. European Commission, 2013), it has been highlighted that the lack of inclusion of females in pre-clinical and clinical studies leads to:

1. less knowledge about disease processes in females which has a negative impact on the health of women;
2. inability to use sex as a variable in studies of basic biology;
3. missed opportunities to examine female-specific phenomena that often interact with disease progression.

It is, therefore, a MUST to follow the guidelines recommended by the European Union and require in our funding system the mandatory inclusion of women and female in research conducted on both human subjects and animals. This requirement should come accompanied by a primer written by

experts detailing how to proceed to incorporate sex as a variable in research studies.

However, it is the *biomedicalization* of sex in the society at large and its cultures that are at stake. Focusing on gonad cells, its chromosomes and its relation to the brain participated in a practice of classifications that take for granted differences instead of diversity, in which many traits in addition to sex could be taken into account as variables in the design of experiments. At the same time, the clinical significance of sex differences guides a fully justified interest, and fascination, that gonad cells and sex chromosomes play in medical practice, in diagnosis and therapy of disorders and diseases, while participating in keeping the borders between the sexes, the normal and the pathological. Such borders, however, are moving lines whose shifts are closely connected to the time, place and cultures that draw them. It is precisely the strong frontier among the taken-for-granted two human sexes –the gender dichotomy– what has sustained and justified many times experiments and research questions and programs.

3. KEY CHALLENGING POINTS

3.1. Investigating sex/gender and the brain

Based on experiments carried on in the forties by Alfred Jost and later on by Phoenix et al. (Phoenix, Goy et al., 1959), gonadal hormones, and in particular testosterone, have been traditionally considered as the solely responsible agent for masculinizing the genitalia and the rest of the body including the brain and other tissues (reviewed in McCarthy, Pickett et al., 2015). Fortunately, although some misconceptions about the biological basis of sex differences still prevail in the scientific community, in the last 20 years there has been sufficient evidence to offer a more nuanced and comprehensive view of sexual differentiation of the brain. If the gonadocentric theory does not explain all the effects that sex can have in the brain, what are the other factors that generate those actions?

We should now consider a wider view that includes both intrinsic or external influences produced by interactions within and outside the nervous system, or even outside the body (cultural or gender norms). In fact, it is currently assumed that genetic, epigenetic and environmental factors are incorporated along with the effects of hormones to cause or eliminate sex differences in the brain and other tissues (McCarthy and Arnold, 2011).

Identification of primary sex-development mechanisms

In animals with heteromorphic sex chromosomes, like in the case of rodents and humans, all sex differences stem from the inherent biological differences of the sex chromosomes (X and Y in mammals), which, so far, are the only factors known to differ consistently in male and female zygotes. However, we should be observant of additional sex-determination mechanisms that operate across phylogeny and allow ourselves to think of emergent interactions between genetic and environmental pathways.

A unique characteristic of the reproductive system (as opposed to other organ systems) is that its anatomical components arise from bipotential primordia. This is true for species as distant as *Drosophila melanogaster* and *Mus musculus*. This means that each embryo arises with the full potential to differentiate as either sex. Compared with our understanding of how the sex organs develop, we know much less about how sex determination acts –or to what extent, of even if at all– in the brain to establish morphological and molecular differences.

The direct role of sex chromosomes on sex differences in the brain are not yet fully understood. Primary sex-determinant factors define a phenotypic difference between males and females. The discovery of SRY in 1990 (Berta, Hawkins et al., 1990) triggered a revolution in our understanding of vertebrate sex determination as we began to define downstream pathways and gain a molecular landscape of the relatively well-studied system in invertebrates, mammals, birds, reptiles and fish.

Primary sex-determinant factors could fall in several classes (Arnold, 2012):

- Class I, Y-expressing genes. Expressed only in males. Sry is an example but it cannot be the only one since mice possessing SRY, but lacking other Y genes, cannot make sperm. There are approximately 27 genes in the Y chromosome that are transcribed to protein (Skaletsky, Kuroda-Kawaguchi et al., 2003).
- Class II, x genes that are expressed higher in females than males as a consequence of the 2:1 sex ratio in number of x chromosomes. Even after x inactivation has occurred, however, some x genes, "inactivation escapees", continue to be expressed from both x chromosomes. Some of these escapees are reliably expressed at higher levels in females than in males (Xu, Taya et al., 2005). For instance, it has been shown that 23% of X-chromosomal genes escape inactivation resulting in sex bias in gene

expression (Tukiainen, Villani et al., 2017). The use of mouse models varying the number of x chromosomes, so that XXY can be compared to XY, thereby revealing the impact of the number or x chromosomes, are also very useful (Arnold, 2014; Tukiainen, Villani et al., 2017).

- Class III, x genes that receive a parental imprint. The effect of a paternal imprint on x genes will occur in about half of female cells but never in a male cell.
- Class IV, factors that exert an effect on the epigenetic status of the rest of the genome. For instance, the heterochromatic inactive x chromosome is proposed to sequester factors regulating the epigenetic status throughout the genome in a sex-specific manner (Wijchers and Festenstein, 2011).
- Sex differences in gene expression localized to autosomes. In the past decade, it has become clear that although the upstream sex-determining signals are diverse and can be fast changing, they often act through more ancient downstream regulatory hierarchies that involve the autosomal genes of a specific family of transcriptional regulators, the DMRTs, that act as "effector genes" of sex differences in the brain (Knoedler and Shah, 2018). Moreover, sex differences might need to be maintained through the life of the organism and these factors have been shown to play a maintenance role in the gonad of mammals (Matson and Zarkower, 2012) and in the nervous system of nematodes (Serrano-Saiz, Oren-Suissa et al., 2017). Nevertheless, it is still unknown whether in the mammalian nervous system, DMRTs and other autosomal genes exert a similar role in the control of sex differences.

Not a default female brain anymore

For many years, it has prevailed the idea of a default female brain (as opposed to an active mechanism of masculinization in the male brain), paralleled by the misconception and lack of research on the regulation of ovary development (Jost, 1947), as if in the absence of the testis-determining factor gene SRY in XX individuals, the bipotential gonads would automatically differentiate into ovaries. However, the existence of cases of 46, XX DSD (Disorder of Sex Development) without SRY (McElreavey, Vilain et al., 1993) has cast doubts on this dogma and the "Z model" has been proposed. Under this model a Z factor would be produced by the XX gonad to actively promote ovary development. According to this model, Sry or another male specific primary factor functions to suppress ovarian development by repressing the Z factor. However, no Z factor has yet been identified, but several studies provide strong

evidence that male and female pathways actively suppress each other (Matson and Zarkower 2012; Minkina, Matson et al., 2014). There are known factors to play a role in ovarian development like Wnt, Rspo1, b-catenin, Foxl2 (Eggers, Ohnesorg et al., 2014). The regulatory networks implicated in the specification and maintenance of ovarian identities are not very well know and they must be investigated. Beyond the gonad, for instance, the potential role of Foxl2 could act as a primary factor outside the ovary, but this specific question has not been focused to the female brain yet (Egashira, Takekoshi et al., 2011).

Lastly, since DNA methylation is usually associated with transcriptional repression, this suggests that normal female brain morphology and behavior involve the active repression of masculinization, an interesting twist on the traditional view that female development is the 'passive,' or default, mode (Uhlenhaut, Jakob et al., 2009; Matson and Zarkower, 2012).

Model and non-model organisms and the human sex

Sexual reproduction is universal among animals but despite the universality of sex, the molecular mechanisms of sex determination are almost as diverse as the number of species there are. They have traditionally been classified as genetic sex determination (GSD) or environmental sex determination (ESD) like temperature, visual cues or social context (Capel, 2017). However, many vertebrate species have been identified in which both GSD and ESD mechanisms operate simultaneously in response to a continuum of heritable and environmental factors. Nevertheless, we have closed the door to think that the environment could have an impact on human brain sex determination. Detailed studies of sex determination and sexual differentiation have been carried out in model organisms, nematodes, insects and vertebrates and have revealed some general regulatory rules. For example, in the past decade, it has become clear that although the upstream regulators are highly diverse, there are ancient common effectors, like the family of Doublesex, mab-3 transcription factors (DMRTs) (Kopp, 2012; Matson and Zarkower 2012; Knoedler and Shah, 2018). Similarly, some unanticipated pervasive sex-determinant mechanisms in unrelated species could be operating in humans.

3.2. Brain plasticity, epigenetics and gender biases

We want to highlight several important concepts that are fundamental to progress in the development of an integrative effort in the behavioral neuroscience field. First, the concept of a mosaic brain may apply to any individual and not only establish a duality between males and females. The notion that there is a "female brain" or a "male brain", configured by the early role of the gonads and chromosomes in the brain shall be challenged and investigated from open perspectives as reality is likely more complex. Rather, most brains are unique "mosaics" of features, some more common in men and others in females. This mosaicism can be influenced by the environment (as we discussed later) and, as the animal studies demonstrate, some of them may be sex-dependent (Joel and Fausto-Sterling, 2016). And second, traditionally, the study of sex and social behaviors has been, and might be at present, conditioned by stereotyped modes of behavior that fit in the gendered hierarchies the researchers are socially embedded in.

The biological sex of a child immediately influences its social and physical environment, even before birth. Our gendered place in society strongly conditions our project design, life history, concept of self, and reaction to social and nonsocial events. The different environments for boys and girls contribute to strong sex differences in choice, social roles, stress and disease. Gender norms can actually impact on the developing brain, although no clear neuroanatomical substrate upon which socialization pressure would act has been found and the mechanisms are still largely unknown.

In mammals, sex determination relies on genetic mechanics. However, in many species, like lizards, that come in two sexes, and even have sex chromosomes, this genetic component is overruled by environmental signals like temperature (Capel, 2017). Similar mechanisms could be operating in mammalian brains and therefore the study of such species could be very interesting as discussed before (4.3.1.).

With the discovery that epigenetic modifications of the genome can be caused by specific environments –experience, nutrition, stress can impact on the brain function– similarly, sex can have an effect on these epigenetic modifications (see below). However, much research remains to be done regarding the interaction between sex and environment for whose design biomedical techniques, tools and approaches could only partially contribute. This is so because environment as an agent cannot be approached only on genetic terms but on cultural, social and historical terms.

The two best-studied types of epigenetic modifications are DNA cytosine methylation and the covalent modifications of histone tails, both of which have been linked to sexual differentiation of the brain (Forger, 2016). It is still controversial and not perfectly well understood how histone modifications and DNA methylation control gene targets and moreover, the answers will be complex and probably region specific.

In every cell of females, and in no cells of males, one x chromosome is inactivated. This occurs via countless epigenetic modifications of the silenced chromosome that must be continually maintained. If any of the epigenetic machinery involved in x chromosome inactivation is rate-limiting, this creates an uneven playing field for regulating the expression of autosomal genes. The majority of differences between the sexes genome-wide may actually serve a compensatory role (Tukiainen, Villani et al., 2017).

Based on Waddington's concept, canalization is a biological process where variability is constrained within a certain domain or a phenotypic trait is tightly constrained with little variability or perturbation. Pertinent to sex differences in the brain, hormones action endpoints are not partial and at the same time they are under a tight control, which suggests that there are mechanisms that act as a ceiling to maintain them within the appropriate range of action. Something acts to both prevent the steroids' actions in the female brain and similarly, higher doses of steroids in males do not trigger a greater masculinization. However, these mechanisms are not very well known. As important is the masculinization of the brain as the prevention of it, therefore debunking the argument of a passive, "default" female brain. Even though this concept was proposed in the 1980s by Döhler and Groski (Dohler, Hancke et al., 1984), the mechanisms participating in what the authors call the ¨feminization¨ of the brain are still missing. It is, therefore, necessary to know what are those mechanisms in both sexes. A few have been proposed like heat shock proteins (Beato and Klug, 2000), micro RNAs (Posadas and Carthew, 2014) and epigenetic modifications (Nugent, Wright et al., 2015) but more research is necessary.

How much does neuroanatomy tell us?

Anatomy is the basis upon which behavior is tethered but is subject to buffering and plasticity by its socio-cultural belonging. The challenge is to understand both parameters (connection or disconnection between brain and behavior) and whether and how they are interconnected. In mammals, most of the data of the regions implicated in, what has been termed, sex-typical social

behaviors come from rodents, mostly mouse models. The description as sex-typical behaviors emerged mainly from rodent studies, where they are qualitative and quantitative in nature and highly reproducible and stereotyped: in males, mating, territorial aggression or marking; in females, receptivity, pup retrieval or maternal aggression (Xu, Coats et al., 2012). Several regions have been implicated in these behaviors (Bayless and Shah, 2016). The brain circuits implicated in sexually dimorphic social behaviors are shared between males and females (this implies no differences in neuroanatomy); however it is not fully understood how the neurons within these brain regions are molecularly defined, how they interact within the circuit or even the configuration of the interconnected pathways. The neural pathways underlying sex-typical social behaviors are embedded within an assortment of neural circuits involved in many unrelated behaviors. For example, one region composed of a mixture of neurons can be controlling several behaviors. Reinforcing this idea of a mosaic brain, findings from Shah's group indicate that sex-typical social behaviors are modular in that specific components of behavior (such as male-typical aggressive attacks and male-typical marking behavior) are controlled by distinct sets of genes. Components of individual dimorphic behaviors are controlled in a modular manner by genetically separable pathways comprising sexually dimorphic molecularly defined neuronal subpopulations (Xu, Coats et al., 2012).

Nevertheless, compensation mechanisms might apply to sex behaviors, which means that the same physiological problem would be solved differently in each sex executed by different regions in the brain of females and males resulting however in similar systems; that is, different biological processes of brain development may lead to brains that may well be indistinguishable by sex. Several years ago Geert de Vries proposed that the functional relevance of sex differences in the brain may serve two functions: either to generate differences in overt functions (explicit organismal level) and behavior, or to prevent sex differences in overt functions by compensating for sex differences in physiology (De Vries, 2004). Yet, the challenge is still to demonstrate this dual-function hypothesis in the brain –if the differential region function is blocked, the difference in function or behavior will disappear; the second part predicts that blocking differential regions will create sex differences in other overt functions where they did not exist before–. The need for compensation gets to a bigger dimension when one considers that sex chromosomal genes may directly affect brain development. Every cell in the brain may express sex chromosomal genes in a sexually dimorphic manner to induce or prevent sexual

differentiation of the brain. This has been addressed in other domains of biology. For example, the X-inactivation evolved presumably to prevent deleterious effect of sex differences in the dosage of X-specific gene expression.

Hormonal effects on neuronal circuits

Even though we should displace hormones as the only factors responsible for sex determination, gonadal hormones have an impact on neuronal circuits, but again, we should be aware of the many misconceptions that still prevail in the neuroscience community about sex hormones and gender (Arnold, Rissman et al., 2003; Arnold, 2012; McCarthy, Pickett et al., 2015; Joel and Fausto-Sterling 2016).

Extensive studies in rodents show that testosterone is metabolized into estradiol by P450 aromatase (Naftolin, Ryan et al., 1971). Therefore, in the male brain both estradiol, through estrogen receptors, and testosterone, through androgen receptors can exert its effect. Moreover, the old idea that estrogens have no role in the female brain differentiation is been contradicted and genetic experiments in female mice have now shown that there is a requirement for estradiol in normal brain development (Dohler, Hancke et al., 1984; Bakker, Honda et al., 2003). Therefore, estradiol is unlikely to masculinize males and feminize females acting in the same site and at the same time (notably, the role for differential neurogenesis and cell death in brain regions outside those directly involved in reproduction, and multiple molecular pathways might respond to estrogen and androgens in males and females). Such a plethora of mechanisms suggest the operation of intrinsic, distinct molecular pathways involved in specific targeted cell types and further emphasizes the need to study whether there is a relationship between gonadal hormones and sex-dependent differences in neural circuits.

The idea of sex-specific circuits stems, in part, in the existence of anatomical sex differences in brain regions critical for sex behaviors but even in the most extreme cases like the sexually dimorphic nucleus of the preoptic area (SDN), both circuits exist in males and females. Even though macroscopically, shared circuits might look the same, intrinsically they might have differences. Instead of two distinct neuronal circuits, it is equally likely that there is only one but differentially weighted toward sex-specific responses (McCarthy and Arnold, 2011). Diverse mechanisms, including gonadal hormones, can impact on this weigh, from the number of neurons that form part of a circuit, synaptic number and density, and neurotransmitter load; however the exact mechanisms

are still elusive. Interestingly, even though original works from the Baulieu group that go as far as the eighties, showed that steroid hormones can be synthesized locally in the neurons themselves (Robel and Baulieu, 1985; Lanthier and Patwardhan, 1986), which leads to the elaboration of a new concept of "neurosteroids" as direct neuromodulators acting directly at the synapse (Remage-Healey, Saldanha et al., 2011). The mechanisms that regulate the production, metabolism and mode of action of these potential neuromodulators is definitely a challenge that needs further research.

3.3. Sex/gender in nervous system disorders

There are marked sex differences in the age of onset, prevalence and symptomatology for nearly every neuropsychiatric disorder, neurological and neurodegenerative diseases (Zagni, Simoni et al., 2016). Insults to the brain can occur prenatally, after birth or as a consequence of prematurity. Sex effects on neurological pathologies could have an unanticipated origin, such as the microbiome (there are sex differences in neuroendocrine and neuro-immune systems that eventually also impact on the gut microbiome), sleep dysregulation, as well as notable differences between microglia, astrocytes and neuro-immune signaling in the male and female brain throughout the life span (Lenz and McCarthy, 2015).

The clinical authority has given less attention to women's disorders and to what women described as their health problems, and this created one early difference. Hence, it is mandatory that we take into consideration sex and experimenter's gendered approach when we study mental disorders not only to elucidate effective treatments but also because taking this perspective can give light to the cause of some of the diseases for which we still don't know the origin. Lastly but equally important, as discussed previously in this text, the majority of neuroscience research is conducted in males and when both sexes are included, sex is rarely analyzed as a variable. Study of sex will not only generate knowledge in the protective and vulnerable factors but also will give light to general mechanisms involved in the etiology of mental disorders. For review see (Zagni, Simoni et al., 2016).

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CHALLENGE 5

ABSTRACT

Brain function is influenced by internal inputs from many parts of the body, including chemicals in the blood and bacteria in the gut. The gut microbiota is a fundamental component of the body that can be transferred across generations and contribute to the unique features of the human phenotype influencing both health and disease. Deciphering the controlling mechanisms of microbiome-body-brain interactions may help in identifying new molecular targets to prevent and/or treat a range of psychiatric and neurologic disorders as well as their physical comorbidities. Here we provide an update on the functioning of the gut microbiome-body-brain axis and outline open scientific challenges and future research directions.

KEYWORDS

microbiome | microbiota-gut-brain axis | lifestyle | dietary patterns | brain disease | comorbidities

CHALLENGE 5

BODY-BRAIN-MICROBIOME INTERACTION

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1. INTRODUCTION AND GENERAL DESCRIPTION

Multicellular organisms have co-evolved with complex communities of microorganisms (microbiota) and their genomes (metagenome), collectively referred to as microbiomes (Marchesi and Ravel, 2015). Host-microbe symbiotic relationships benefit both organisms. The human gut microbiome is constituted mainly by representatives of Bacteria, but also includes Archaea, lower and higher Eukarya and viruses. The gut microbiome orchestrates an array of bodily and brain functions (metabolic, immune, endocrine, neural, etc.) through interactions with the host and the environment (diets, antibiotics, stress, etc.) with profound impact on human physiology and health maintenance (Sanz et al., 2018). Alterations of the gut microbiome (dysbiosis) can contribute to disease susceptibility and pathogenesis as reported, initially, for physical disorders and, more recently, for neurological and psychiatric conditions including autism, depression, Alzheimer's disease and Parkinson's disease (Cenit et al., 2017; Dinan et al., 2019).

The influence of the gut microbiome on the bidirectional crosstalk between the gut and the brain, the so-called "gut-brain axis" is a relatively new research field with multiple applications in health and disease (Dinan and Cryan, 2017). This axis is regulated through hormonal, immunological and neural pathways, and represents a route through which the gut microbiome influences neurodevelopmental processes and brain function (Agustí et al., 2018). Emerging evidence supports causal effects of the gut microbiome on

cognitive functions as well as on social, eating and emotional behaviors, including depression and anxiety-like behavior (De Palma et al., 2015; Dinan and Cryan, 2017, Agustí et al., 2018).

These effects are believed to be mediated through distinct mechanisms, including modifications in factors regulating synaptic plasticity and neural function (e.g., brain-derived neurotrophic factor and neurotransmitters) and through the regulation of endocrine and inflammatory pathways. These effects driven by microbially-produced dietary metabolites as well as by microbial stimuli (lipopolysaccharide, lipoteichoic acids, etc.) of non-dietary nature. Despite the mounting evidence on the significance of these microbial products, the mechanisms and molecular mediators of the complex interactions are far from being fully understood.

Physical disorders contribute to the risk of developing mental conditions and viceversa, indicating that our mind and our body are deeply interconnected. This interconnection accounts for the development of comorbidities, which complicates diagnosis and management. Indeed, the co-existence of different disorders poses a major social challenge, as clinical practice fundamentally addresses individual disorders. Modifiable dietary and lifestyle factors (physical activity, stress, drugs, social behavior, etc.) are known to influence brain and body functions. Specifically, unhealthy dietary habits have been identified as major risk factors for the development of physical and mental disorders (GBD, 2017). Accordingly the adoption of healthy dietary and behavioral habits has the potential to play an essential role in health promotion and in mitigating the drivers of disease vulnerability. The diet is also a major determinant of gut microbiota composition and function (Portune et al., 2017). Consequently, dietary health-effects could theoretically be mediated and optimized as a function of an individual's gut microbiome and its response to the diet (Sanz et al., 2018).

Elucidating the biological and molecular bases of the complex systemic communication between the brain, body and gut microbiome as well as their interactions with diet and lifestyle might open new diagnostic, preventive and therapeutic horizons for highly prevalent physical and mental conditions that are often comorbid.

Here we summarize the main challenges in the field to be addressed in the upcoming years:

1. Disentangling the mechanisms underlying body-brain-microbiome interactions and their consequences on health and disease.
2. Developing microbiome-based therapies and predictive tools for improving treatment and management of psychiatric and neurologic disorders and associated comorbidities.
3. Personalizing lifestyle and nutritional strategies for effective disease prevention as a necessary step towards reducing the societal and economic burden of of non-communicable mental and physical disorders.

2. IMPACT ON BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

The impact of addressing these ultimate goals will apply horizontally and transversally across disciplines, from basic science and to health and nutritional applications, and across sectors, from industry to health care and nutritional professionals to associations and society. At the institutional level, this will promote interactions between the three global areas of the CSIC: Life, Matter and Society.

Scientific impact

The integration of the gut microbiome and lifestyle factors for investigating the communication between the body and the brain will provide a new conceptual framework to understand biology and medicine. This broad, multi-disciplinary approach and vision will enable us to extend our knowledge into the roots and pathophysiological mechanisms underlying both mental and physical disorders and identify shared risk factors and molecular pathways which, in turn, could translate into common solutions. This research field has great potential to provide new directions for improving prediction (early diagnosis/prognosis) and disease management (treatment and prevention) applied to mental and physical co-morbidities.

Furthermore, the gut microbiome-brain axis is considered as a paradigm shift in neuroscience and mental health. Considering that CSIC researchers have been central to this shift, this research field is strategic to project CSIC values and strengthen its already highly competitive international position in this area.

Economic impact

The knowledge generated will lead to a number of applications from more accurate predictive and diagnostic tools (algorithms and biomarkers) for early

disease detection to more effective preventive and therapeutic strategies, all based on the integration of the individual microbiome and dietary variables and applying holistic approaches that target the body, the brain and the lifestyle of the subject. These advances will boost innovative capacities, especially of the health, biotech and food industries by providing unique solutions. The results of this research approach will broaden the focus in terms of diagnosis and also in terms of drug discovery and development. Opportunities will likewise emerge for further development of personalized lifestyle and dietary strategies for disease prevention. Indeed, the human microbiome market represents a great opportunity as it is steadily growing and is expected to reach USD 899.1 million by 2025 from USD 506.5 million in 2022, with an annual growth rate of 21.1% during the period 2022–2025.

Societal impact

Mental and physical disorders are a major economic and societal burden, particularly when they are comorbid, which complicates diagnosis and treatment. This research line will have a positive impact on clinical diagnosis and therapy and also in self-management of health through dietary and lifestyle strategies. Our approach will contribute to reducing the socio-economic disease burden and ensure the sustainability of the health care system. It can also have other social consequences by reducing disease stigmatization and inequalities (job losses and limited professional opportunities) and favoring social integration and cohesion, all essential pillars of the sustainable development agenda of the United Nations for 2030 and of the EU priority policies.

3. KEY CHALLENGING POINTS

3.1. Disentangling the mechanisms underlying body-brain-microbiome interactions

Understanding the mechanisms governing the connection between the microbiome, the body and the brain is essential in identifying new molecular targets and common therapeutic solutions for mental and physical comorbidities. This interaction occurs through neural, endocrine and immune pathways through which gut microbes and dietary factors influence the brain and different body functions (Agustí et al., 2018; Dinan and Cryan, 2017). Dissecting the contribution of these different routes is particularly challenging, considering the local connections between intestinal immune and enteroendocrine cells and the enteric nervous system, and the links between the enteric, autonomic and central nervous systems and systemic immunity.

The different routes of microbiome-body-brain communication that need to be further explored include the following:

- Neural pathway: The gut is innervated by the enteric nervous system (ENS), which communicates with the central nervous system (CNS) through the parasympathetic (e.g., via the vagal nerve) and sympathetic branch of the autonomic nervous system (ANS). The vagal nerve is one of the most important neural pathways mediating the bidirectional communication between gut and brain (Forsythe et al., 2014), while the connection with the sympathetic nervous system remains largely unknown. More research is needed to elucidate how gut bacteria and bacterially-produced factors activate the vagal afferents that transmit the signals from the gut to the brain and the neural circuitry underlying the effects of vagal stimulation on brain, behavior and body functions. These new means of interaction between the gut microbiota and the sympathetic nervous system and how they influence the gut-brain-body communication and function remain to be explored. Gut bacteria are also known to influence the host production of neurotransmitters and contribute directly to the synthesis of neuroactive molecules (Dinan and Cryan., 2017). Additional efforts are required to fully determine whether the production of neuroactive molecules by the gut microbiota influences the functioning of the ENS and the CNS, and the biological consequences of this.
- Enteroendocrine pathway: Enteroendocrine cells (EECs) are specialized cells of the gastrointestinal tract that produce neuroendocrine molecules with numerous primary functions for example in energy metabolism (appetite, insulin signaling), and that vary in response to gut microbiota-diet interactions. Emerging evidence indicates that EECs also modulate ENS activity and express innate immune receptors, pointing to additional roles in neural and immune signaling. Understanding the mechanisms whereby the stimulation of EECs (through contact with bacterially-produced factors) impact the immune system and the downstream effects on brain and peripheral organ functions represent a major challenge in the field. Also, we need to unravel the paracrine signalling between EECs and neurons of ENS, which may act as a sensorial channel in the bidirectional communication between the gut and the CNS.
- Immune and hypothalamic pituitary adrenal pathways: The immune system interacts directly with the ENS, the ANS and the hypothalamic

pituitary adrenal (HPA) axis and plays a key regulatory role in the gut-brain axis (Foster et al., 2017). Interestingly, functional adrenergic receptors and glucocorticoid receptors are expressed in immunocompetent cells, which suggest new types of interactions for exploration. Further research is needed to gain better understanding into how the gut microbiota and the diet modulate immune signaling through interactions with the ENS and the sympathetic arm of the ANS, and to uncover the downstream effects of the HPA axis on brain and body functions. This understanding could help, for instance, in identifying strategies intended to modulate the gut microbiota to increase our resilience to chronic stress, a robust risk factor for the onset of both cardiometabolic and psychiatric conditions, via the regulation of HPA-immune crosstalk.

The establishment of new experimental models is also critical to classify interactions between the different organs and systems and to facilitate the identification of possible preventive/therapeutic targets. Advances in the development of in vitro models such as 2D and 3D cell cultures, based on established cell lines, as well as in ex vivo organoid-like structures (e.g. the 3D Brain Model), derived from animal and human tissue samples, have been instrumental in reproducing the complexity of the interactions between different intestinal or brain cell types. Nonetheless, these systems still have a number of limitations; for example, they do not fully recapitulate the intestinal oxygen gradient required for co-cultivation of microbes and for maintaining their stability and dynamics (Jalili-Firoozinezhad et al., 2019). Models to investigate multi-organ interactions remain very elementary. A multidisciplinary approach is needed to further develop this area including experts in neuroscience, microbiology, bioengineering and bioinformatics. This could be the case for the development of new *in vitro* models, such a microfluidic gut-on-a chip models can faithfully mimic the intestinal environment, including the complexity and diversity of microbial populations and of different epithelial and immune cell types, and their maintenance to explore long-term dynamic host-microbiome interactions. Also, new multi-organ/body-on-a-chip models will help to explore inter-organ interactions *in vitro* (Harjes, 2019), including those occurring through the gut-microbiome brain axis (Raimondi et al., 2019). These models will serve as discovery platforms enabling large-scale screening of potential therapeutic molecules and bioactive agents (intestinal bacteria and products thereof) with higher predictive potential before moving on to costlier *in vivo* models.

Advanced approaches that enable monitoring of real-time bidirectional communication between the body, the brain and the gut microbiome *in vivo* will also be critical to fully understand the mode of action of biological and environmental variables affecting our health status, and to validate effector molecules/bioactive agents as lead candidates for therapeutic trials. The development of *in vivo* models will also present specific challenges, such as the design of microdialysis probes that enable the prolonged, automatized and more comprehensive monitoring of brain activity under other body site stimuli. The application of optogenetic techniques to *in vivo* models coupled with microbiome-related assessments will be extremely useful to progress in the understanding of the underpinning mechanisms that govern the brain-microbiome communication and functions at a cellular level.

3.2. Developing microbiome-based therapies and predictive tools

Considering the limited efficacy of current therapies—medical or psychological—for psychiatric and neurologic disorders and, in particular, the difficulties in managing mental and physical comorbid conditions, the identification of novel mediators and moderators of these disorders will provide new opportunities for improving their management and reducing their high societal burden. Of these, the gut microbiome—through its connection to the brain and peripheral tissues—represents a tractable target to manage disease (Kashyap et al., 2017). The use of classical probiotics (bifidobacteria, lactobacilli, etc.) and other strategies directed to the gut ecosystem (e.g. prebiotic fibres, etc.) as well as fecal transplants demonstrated the potential of these strategies to ameliorate or intercept the disease development in experimental models. A better understanding of the specific bacterial consortia (beyond those classically used as probiotics) offering health benefits as well as derived metabolites/molecular mediators of such effects is critical for the development of rational and more efficacious microbiome-based therapies (Romaní-Pérez et al., 2017).

To advance the development of microbiome-based therapies, key challenges include (i) proving and validating causality between specific bacteria/bacterial consortia and health outcomes in robust models; (ii) leveraging existing bioinformatic tools and combinatorial chemistry for the discovery of structurally new microbiome-produced metabolites/molecules and their targets as candidates for new therapeutics; (iii) replicating and scaling-up of intestinal bacterial cultures to ensure safe microbiota enrichment and replacement; and (iv) developing miniaturized delivery systems of microbiome-based

products targeting specific organs and functions. The assessment of the therapeutic action of microbiome-based strategies in humans will also benefit from new technologies —for example, advances could be foreseen from the use of brain imaging technologies and wearable sensors that detect brain activity, to facilitate the assessment of real-time feelings like mood and emotion. This, combined with *omics*-based technologies for monitoring gut microbiome activity and body functions, could be of much help to obtain information about the gut microbiota-brain axis function and effects on interventions in mental and physical health.

When considering the potential of the microbiome to better inform therapeutics, it will be critical to gain a deeper understanding of microbiome-drug interactions and their consequences. Evidence suggests that a large number of non-antibiotic drugs (up to 24% of human drugs) might have an impact on key bacterial species of the intestinal microbiota, with possible downstream effects on human health (Maier et al., 2018). In turn, the gut microbiome might be involved in the primary or secondary biotransformation of drugs through its enzymatic machinery or through host-micro co-metabolic processes, influencing the pharmacokinetics, efficacy and side effects of drugs (Turnbaugh, 2018). This seems to be the case for antipsychotics, which have a significant ability to inhibit commensal intestinal bacteria and this might be part of the side effects or the mechanism of action (Maier et al., 2018). Human studies show that —for example, the medication (levodopa) for Parkinson's disease can be metabolized by gut microbiota, potentially reducing drug availability and causing side effects (Maini Rekdal et al., 2019). This evidence might be critical to predict efficacy and side effects as a function an individual's microbiome as well as for drug repurposing. The future development of any new potential therapeutic drug will have to consider the complex metabolic interactions between the host and the microbiome. In the light of the current evidence, there is a need to address the following aspects: (i) integrate the microbiome as an additional biological variable in pharmacokinetic and pharmacodynamic studies for fine-tuning dose-response and side-effect assessments and (ii) identifying new pharmacological uses for existing drugs (drug repurposing) or therapeutic effects mediated by the gut microbiome.

Taken together with other variables, information from the microbiome could also serve for early disease detection, prognosis and prediction of response to therapy. Specially the development of more accurate predictive tools is essential to move from reactive care to disease prevention and positive medicine.

This is especially needed for the management of comorbid conditions since, until now, diseases have been investigated and clinically addressed as individual entities. The discovery of modifiable factors that help maintain body-brain homeostasis and contribute to health promotion and resilience against disease (understood as an active process) is also an essential aspect to progress towards disease prevention as addressed by this challenge. Of these factors, the gut microbiome is considered as one of the missing pieces that could help explain our resilience or vulnerability to mental and physical conditions and, at the same time, represents a preventive target.

To advance the development of microbiome-informed predictive tools and biomarkers of health status and early disease detection, we need to progress towards the so called "human phenomic science" (FitzGerald et al., 2018) based on longitudinally deeply phenotyped subjects. This implies the integration of not only clinical endpoints but also environmental modifiable factors, like diet, lifestyle and psychosocial stress, as well as big data generated by advanced technologies, including brain imaging and multi-omics readouts (metagenomics/transcriptomics, metabolomics, etc.), which would reflect the outcomes of body-brain-microbiome interactions with the environment. This would help to attain a more comprehensive understanding of the mediators and moderators that determine our health trajectory and leverage information from larger-scale but less phenotyped epidemiological studies. These advances are key to (i) identify robust drivers of the inter-individual variability and disease susceptibility, (ii) validate biomarkers for early detection of departure from "normality", (iii) develop friendly use prototypes for biomarker detection and computational models/algorithms that help to predict an individual's health trajectory. Furthermore, this basic information on modifiable disease risk factors is essential for the design of personalized cost-effective preventive measures based on changes in diet and lifestyle, as described in the next section.

3.3. Personalizing lifestyle and nutritional strategies for effective disease prevention

Considering that suboptimal diets are responsible for more deaths than any other risks globally, including tobacco, and that ~7 million deaths and 255 million disability-adjusted life-years were attributable to unhealthy diets in 2017 (GBD, 2017), dietary changes are clearly key to reduce societal and economic disease burdens. Unhealthy dietary patterns and a sedentary lifestyle are major contributors to non-communicable diseases, varying from

cardio-metabolic to psychiatric disorders. Indeed, adherence to the Mediterranean Diet and diets rich in fiber have shown promising benefits in both cardio-metabolic and mental disorders, like depression (Dinan et al., 2019). The role of specific essential nutrients (e.g. polyunsaturated fatty acids, vitamins and minerals) in mental health is also well-established. Nonetheless, the effectiveness of dietary and lifestyle changes for ameliorating or reducing the risk of these disorders remains, in some cases, unclear, partly due to the large variability of the individual response. In turn, diet is instrumental for modulating the structure and function of the human gut microbiota, as well as for altering the type and abundance of bacterial metabolites and bacterial-host co-metabolic products, with a potential impact on metabolic and mental health (e.g., short chain fatty acids, neuroactive compounds, etc.). Yet, an understanding of the influence of the microbiome on dietary health effects remains limited to precisely inform dietary recommendations (Sanz et al., 2018).

Physical exercise is also a key lifestyle intervention with preventive and therapeutic potential. Its benefits and the mechanisms through which it exerts its effects are well documented. Focused on the brain and mental health, exercise can be antidepressant, anxiolytic and can improve cognition, improve mitochondrial function of neural cells, and even increase neurogenesis (Llorens-Martin et al., 2006; Lopez-Atalaya et al., 2011; Llorens-Martin et al., 2011). Nonetheless, an important challenge that remains is to understand why some exercise practitioners do not benefit and how exercise routines could be individually tailored according to a person's hormetic characteristics. Hormesis consists of the presentation of beneficial effects for the brain by the practice of a certain amount of physical exercise (mainly duration and intensity) up to a limit after which, potentially negative effects accumulate. There is no definitive evidence on how the amount of physical exercise affects each person in their cognitive ability or mood, due to the limited available tests and their intrinsic difficulties. Current knowledge is generally based on the net neurobiological evidence obtained from laboratory models, which lack the subjective components obtained in human studies.

We need to progress towards personalized nutrition and lifestyle strategies (physical exercise) through the integration of all biological variables into algorithms that predict the individual responses to dietary change and to physical exercise with the support of more robust assessment tools. We also need to better understand differences in sensitivity to dietary effects, considering different developmental stages, age and the overlapping comorbidities, which

are of key relevance. New efforts are needed to develop microbiome-directed foods tailored to the individual. Overall, this will increase the efficacy of dietary and lifestyle measures, empower citizens to take control of their own health, and contribute to disease prevention in the long-term. The development of new tools and devices to achieve this goal is thus very important.

Overall, much evidence on the role of the microbiome in the regulation of the gut-brain axis and body functions has emerged in the past few years. A truly multidisciplinary approach is required to address current and upcoming challenges in the field such as how the gut microbiota can be modulated by lifestyle and dietary habits to influence physical and mental health, and to elucidate the microbiome-mediated mechanisms influencing brain development and impacting both brain and body functions. This research may uncover new opportunities for improved diagnostic, preventive and therapeutic approaches for a number of mental disorders (e.g., anxiety, depression) and their physical comorbidities.

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CHALLENGE 6

ABSTRACT

Mental disorders have devastating and increasing impact in our societies. CSIC researchers face the challenge of determining the biological and social causes and consequences of these disorders, and of finding efficient therapies. To these aims, the collaborative effort of neuroscientists, neurologists, psychiatrists, psychologists and human and social scientists, the use and development of state-of-the-art technologies and the contact with patient associations and pharma industry are required.

KEYWORDS

mental disorders | behaviour | pharmacotherapy | brain stimulation | brain circuits | neuroimaging | mental health care | psychosocial causes | social stigma

CHALLENGE 6

UNDERSTANDING MENTAL DISORDERS

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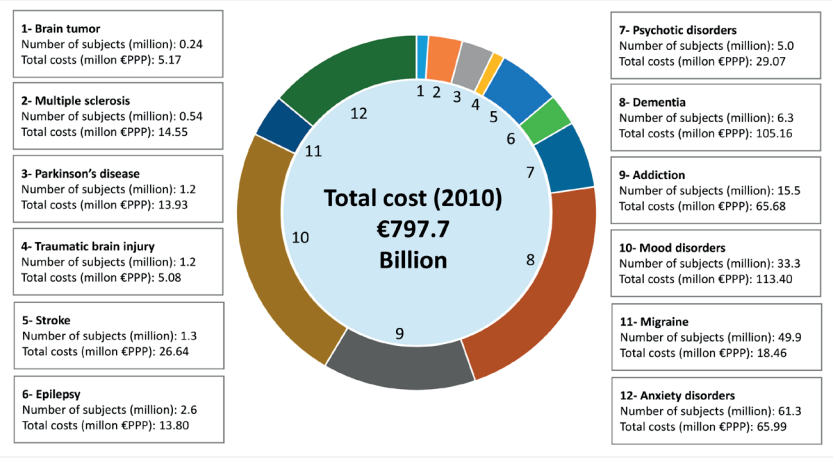
1. INTRODUCTION AND GENERAL DESCRIPTION

Brain diseases (mental and neurological disorders) represent a considerable medical, social and economic burden in Europe. With yearly costs of about 800 billion euros and an estimated 179 million people afflicted in 2010, brain diseases are an unquestionable emergency and a grand challenge for neuroscientists, according to the European Brain Council and the European College of Neuropsychopharmacology (Gustavsson et al., 2011; Diluca and Olesen, 2014). Brain research is at the forefront of science, but extensive work is still needed to understand brain functioning at molecular, cellular, and system levels as well as to unravel the pathogenesis of complex brain diseases. Brain research and brain diseases are relatively new terms.

Brain diseases were included in the global burden of disease study by the WHO (World Health Organization) (Murray et al., 1997; Olesen et al., 2003). They are responsible for 35% of Europe's total disease burden with one-third of all European citizens suffering from at least one brain disorder in the lifetime. These data were calculated in terms of so-called DALYs, or disability-adjusted life years. Several comprehensive studies have been carried out to date, which show that mood disorders and dementia represent the most costly brain diseases for European society (see Figure 6.1) (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators).

FIGURE 6.1–Cost of brain diseases in Europe 2010



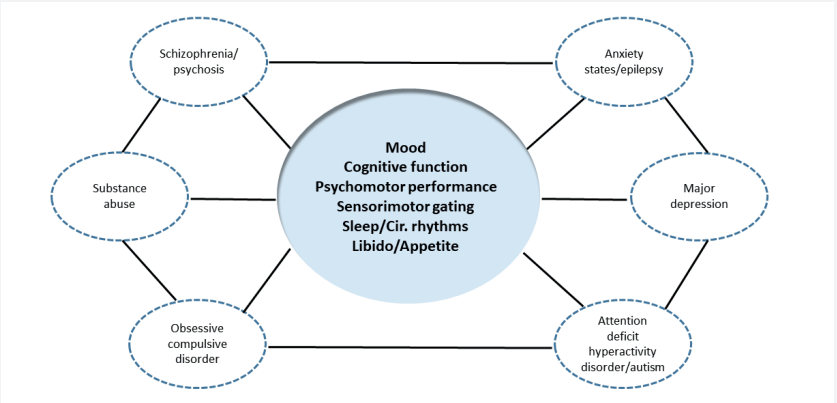
Mental disorders are defined as syndromes characterized by clinically significant disturbance that affect mood, thinking and behaviour (APA, 2013). Almost 300 different conditions have been listed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). They show highly variable symptoms that may be persistent, relapsing and remitting, or even occur as a single episode.

The incidence of mental disorders is steadily growing and has a strong impact on quality of life. They are associated with considerable comorbidity and mortality. Among mental disorders the most frequent include depression, bipolar disorder, dementia, schizophrenia and epilepsy, affecting about 500 million people worldwide (WHO, 2000). Addictive behaviour is another mental disorder with growing incidence that has recently led to public health crisis such as the opioid epidemic in US with 70,000 drug overdose deaths reported in 2018 (ASPA, 2018).

With the improvement of diagnostic methods, the incidence of previously minority mental disorders has increased dramatically in recent years. This is the case of autism spectrum disorders (ASD) with a prevalence estimate in Europe of about 1-2%. Still, diagnosing mental illness is a more subjective endeavour than diagnosing other diseases. No blood test exists for depression; no X-ray can identify a child at risk of developing bipolar disorder. At least not yet.

The high rate of comorbidity adds complexity to the diagnosis of mental disorders. A large number of studies have revealed that patients with mental disorders have higher rates of physical illness (Leucht et al., 2007; Walker et

FIGURE 6.2–Major classes of mental disorder and their main symptoms. Mental illnesses display a high degree of comorbidity, in particular depression, and share many common symptoms, which are not restricted to perturbed mood.

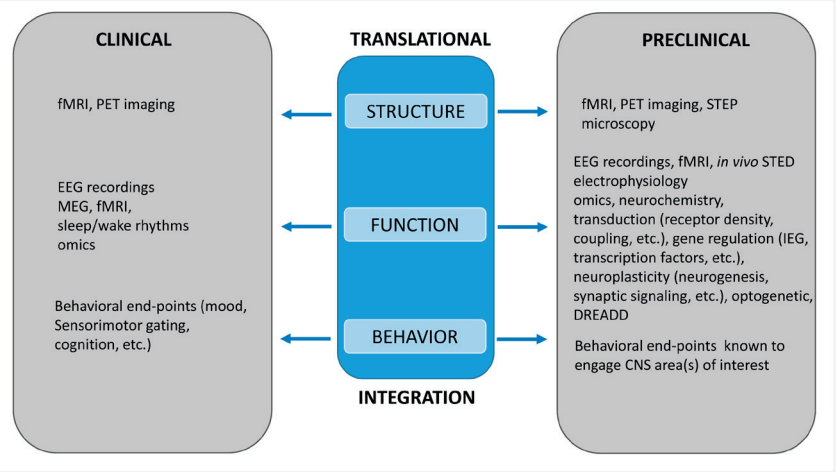


al., 2015; Weisser et al., 2009) and suicide (Turecki et al., 2019) than the general population. Comorbidity –the presence of two or more diseases– within mental disorders is pervasive, and the risk persists over time.

Psychiatric disorders are frequently reported in neurodegenerative diseases. Anxiety, depression, dementia, cognitive impairment, and psychosis are highly correlated in Parkinson's disease and other synucleinopathies and are associated with a range of early non-motor symptoms. Similarly, highly prevalent (i.e. Alzheimer's disease, fronto temporal dementia) and rare (i.e. genetic diseases such as neuropathic lysosomal storage disorders) neurodegenerative diseases show symptoms that mimic those seen in mental disorders confounding the diagnostic efforts (Figure 6.2).

Mental disorders are likely to have multiple etiological causes, including genetic and epigenetic, biological, psychological, social and environmental risk factors, i.e. stressful early life events, all may contribute to the development or progression of mental disorders (Arango et al., 2018). Different risk factors may be present at different ages, with risk occurring as early as during prenatal period (WHO, 2012). Age of disease debut varies, with ASD and epilepsy normally doing it in childhood, psychotic symptomatology and schizophrenia in adolescence period and depression or bipolar disorder in adulthood. We are far from understanding the interplay among these risk factors in mental disorders. Thanks to new tools in genetic and neuroimaging, scientists are

FIGURE 6.3–Readouts measured in animal models of mental disorders and in the evaluation of drugs and other therapies.



making progress toward deciphering details of the underlying neurobiology of mental disorders. Genes related to disease and abnormal brain growth and connectivity among brain regions have been reported.

Describing mental illness as a malfunction of the brain will help minimize the social stigmatization associated with them. Still, it is not possible to describe all mental illness in purely biological terms. Social and environmental factors are undoubtedly important. Mental representations, meaning and conditioning imply a whole level of processing that has to do with psychological abilities.

Available medications are effective in treating specific symptoms for subsets of individuals affected by mental disorders. However, these treatments do not improve quality of life in a significant proportion of patients, including children and adolescents, and may show serious side effects. Pharmacotherapy is not the only option adopted for the prevention and control of mental illnesses. Maintaining psychological equilibrium fulfils important roles in the lives of many patients. Indeed, approaches other than pharmacotherapy are often preferred for the alleviation of low mood, anxiety and heightened stress-sensitivity. Similarly, inter-personal therapy, cognitive behaviour therapy (CBT), behavioural activation and related techniques are attracting increasing attention for the control, prevention and treatment of mental disorders both alone and likely most effective in combination with pharmacotherapy (Figure 6.3).

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

Deciphering the biological bases of brain functioning and their association to emotion, thoughts and perception is essential for placing newly identified brain changes associated with mental disorders and treatment targets within a functional context. This requires the work of basic neuroscientists from different disciplines including cell, molecular and developmental biology as well as electrophysiology, brain circuits and behaviour.

It is necessary to move away from single-disease frameworks. Neurodegenerative diseases emerge as promising model systems for studying brain-behaviour relationships and the neural circuitry associated with psychopathology. Therefore, advance in the field would profit from the collaboration between basic scientists on neurodegenerative and mental disorders. The brain-only focus traditionally taken in research for these disorders should be extended to the study of brain-body connections. Basic research on the microbiome-gut-brain axis and on the brain effects of the immune response in peripheral tissues is particularly interesting.

Cross-interdisciplinary collaboration is essential for potential applications to come true. Basic researchers and clinicians (including neurologists, psychiatrists and psychologists) should join efforts. Beyond knowing that an intervention is efficacious, research initiatives are needed that clarify the mechanisms through which interventions work.

Together with neuroscientists and clinicians the contribution of researchers in human and social sciences is especially relevant in the context of mental disorders. There has been a historical confrontation between essentialist and non-essentialist perspectives to explain mental disorders. The first one stresses the biological nature while the second considers the cultural circumstance of the individual. Integrative approaches should overcome this debate.

3. KEY CHALLENGING POINTS

The complexity of mental disorders poses numerous defies at the scientific, clinical and social levels. We identify three major challenging points:

3.1. Understanding the biological origin of mental disorders

Although remarkable advances have been made in the past few decades, we are still far from understanding how emotion, perception, cognition, executive function,

motivation/reward and impulse control arise. This basic work is much needed to define which aspects of normal development, brain circuit structure or function are linked to the pathophysiology or to the emergence of behaviours that depart from a "normal" range. Among the urgent tasks to address the biological origin of mental disorders are: identifying genetic alterations; establishing the patterns and roles of epigenetic modifications; determining alterations in synapse function and brain circuit connections; defining the involvement of non-neuronal cell types such as astrocytes and microglia; and settling the influence of environmental factors that enhance or diminish predisposition to suffer mental disorders.

3.2. Bridging basic science progress to therapies

The progress in basic science discoveries has not yielded in parallel advances in the treatment of mental disorders. Indeed, most of the classes of drugs currently used to treat mental disorders were identified well before much of our current knowledge of brain biology was established (Spedding et al., 2005). In general, treatment planning in psychiatry depends on trial and error strategies. In view of the huge global burden of mental disorders and the inadequacy of current treatment, intensive efforts are needed to improve their management and prevention. Pharmacotherapy is likely to remain of central importance. In the research for better drugs, it is essential to clarify the mode of action of currently available drugs, and identify novel treatment targets and concepts. It is crucial to generate suitable animal models and to integrate the findings from these models, which are necessary to determine the therapeutic potential of novel pharmacotherapy, with human observations. Alternative therapies to pharmacology must be also developed. Examples as diverse as deep brain stimulation (DBS) and CBT are attracting serious attention, as well as strategies based on cell and oligonucleotide therapies and genome and epigenome editing. However, an efficient therapy may be harmful if a diagnostic is wrong. The difficult diagnosis for mental disorders prevents application of the right treatments and increases the risk of dangerous over-prescription. Diagnostic tools should be developed such as biomarkers for immune-inflammatory, synaptic and genetic and epigenetic alterations, together with improved neuroimaging techniques.

3.3. Addressing the social impact of mental disorders

Mental disorders are often associated with social stigma and discrimination together with poor public assistance. Psychosocial approaches that empower the individual suffering a mental disorder and promote community awareness and support are necessary. Analysis of welfare policies on mental health along history in different countries should illuminate the way ahead in this regard.

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CHALLENGE 7

ABSTRACT

Advanced age significantly increases the risk of developing chronic diseases such as cancer, diabetes, cardiovascular, immune and mental disease. Regarding the latter, advanced age is a necessary factor for the development of non-hereditary forms of neurodegenerative diseases such as Alzheimer's and Parkinson's. Despite years of intense research, we still do not know how these diseases occur, this being one of the main reasons for the lack of adequate interventions to prevent or cure these pathologies. To overcome the current limitations in the field, we plan to: 1) generate basic knowledge on the mechanisms responsible for cognitive, behavioral, motor, metabolic and sociability disorders that occur with age, 2) define the mechanisms that determine individual susceptibility to neurodegeneration, 3) design and develop strategies to improve brain aging, and 4) explore social and environmental conditions of the older population to know their influence in brain degeneration. Individual, social and policy interventions must be considered for future research.

KEYWORDS

genes | neurodegenerative diseases | Alzheimer's disease | diabetes | cardiovascular disease | immune system | microbiota | pharmacological interventions | nutrition | learning | memory | lifestyle | social support | environment | physical activity | public policies

CHALLENGE 7

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1. INTRODUCTION AND GENERAL DESCRIPTION

Spain, with one of highest life expectancies, is expected to become the world's second oldest country by 2050. Although this fact can be a matter of national pride, representative of both good standards of life and a good health system, it is also a matter of social and political concern. In fact, aging is the main risk factor for disease and death (in the developed world) and the rise in longevity of the population dramatically increases the number of individuals with chronic diseases, many of them disabling, such as cancer, cardiovascular diseases, type 2 diabetes, and dementias like Alzheimer's disease. It is estimated that there are more than 10 million new cases of dementia each year worldwide, that is, one new case every 3 seconds. And in addition to the personal and family, emotional and financial costs, the total burden of dementia represents more than 1% of GDP worldwide.

In the last two decades, thanks to genetics studies we have come to the conclusion that non-Mendelian age-related disorders are consequence of the interaction between multiple genes (i.e., polygenic) and environmental factors. In this scenario, neither of these two factors is sufficient in itself to produce

disease; this will occur in those individuals who have the most "complete" portfolio of both, i.e. predisposing genes and a harmful environment (internal or external) (Timmers et al., 2019). Additionally, biochemical and cell biology studies in cell and animal models have taught us about the effect of different genetic variants and age-associated systemic and local alterations on the function of brain cells. Unfortunately, even with the current state of knowledge, we do not have precise clues to explain causation of neurodegenerative conditions and therefore we are still far from being able to know how to prevent the development of neurodegenerative diseases. On top of these limitations, several studies have shown that the same genetic-environmental variants could be associated with multiple age-related disorders (i.e. pleiotropy) (Martínez-Martínez et al., 2020). As consequence, our current repertoire of interventions is also insufficient to treat the disease or to satisfactorily improve the quality of life of sick individuals. Therefore, there is a dire need for research aimed at improving the impact of aging in our society.

An important venue of future research will necessarily focus on the continuous elucidation of the mechanisms that determine susceptibility or resistance to pathological brain aging (e.g. Alzheimer's disease, vascular dementia, fronto-temporal dementia, dementia with Lewy bodies). Multiple disciplines will contribute to this endeavour, from human genetics and epigenetics to basic cell biology and biochemistry research (not only of neurons, but of all the other brain cell types). These studies should be linked to research on the aging of sensory and peripheral organs and how the environment and social behaviors influence our genes, cells and the whole organism. Naturally, translational strategies will also be needed to foster ties between researchers and medical and social agents, as well as care providers, to improve the general well-being of the old.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

Scientific impact

Research has demonstrated that in laboratory animals such as mice and worms a number of age-associated diseases, including those neurodegenerative, can be prevented (or at least ameliorated) by natural, pharmaceutical and biotechnological interventions like low-calorie diets, fasting, physical and mental exercise, food supplements, medicines, antibodies, peptides, nucleic acids. The question now is: do we know enough about aging so to start an intensive,

multi-year search and clinical trials for ways to delay brain aging in people? The answer is, as expected, no. We do not know enough about how different predisposing genes can lead to disease (or protect against it) nor how they interact with different environmental milieux so to determine, by acting all together, the type of brain aging we will have, either normal or pathological (demented). Without knowing more on gene-gene and gene-environment interactions we will not be able to establish appropriate animal models that resemble different human situations, allowing to better test the efficacy of therapeutic approaches. We must generate basic knowledge that is as close as possible to the tremendous individual, genetic-environment, differences that exist between humans, in whom the genetic and environmental background is so diverse.

Social and economic impact

A second question we need to address is how societal bodies and organisations are going to face the increased aging of our population, and what strategies should be promoted to keep older people independent for a daily living. The current trend is to channel many social and political initiatives, as well as the older individuals' voices, into public policies and intervention programs, which poses a significant challenge to our social care system. Indeed, a third question is also emerging: what will be the cost for our societies of having a longer-lived population? A simulation in the United States of reducing the development of age-associated diseases such as cancer and heart disease by 2 years predicts that the costs to the social security will raise substantially. However, having a population with higher possibilities of a healthier aging may largely compensate its financial costs and, if successful, give substantial financial return.

What our research organization should do?

If we accept the premise that only bio-medical and social research will allow us to have a less onerous aging, both emotional and economic, we, as an institution, must unconditionally support this type of research. The question is therefore: how? All starts by identifying the key questions that we need to address (see below, Key Challenging points). Next, we need to identify and hire, irrespective of geographical origin, the most suited scientist to address such questions. Thirdly, these scientists need to be funded at a competitive, international level. And fourth, to be surrounded by the best critical mass and infrastructure is an essential condition to succeed. Even though we may require some decades until having solid results that reach the public, we have to

establish a solid aging research programme as quickly as possible if we do not want to be simply consumers of goods and services produced by others. As a matter of fact, once we do improve aging the benefits to the society will not be restricted to the emotional aspects but also will produce financial revenues from the tools we have had discovered, in new medicines, supplements and new technological tools. We need to remember that aging is already a "business", and one that has grown extensively over the past decade, with more services and products that help older adults live a better life today than ever before. And research and development will only boost even more this industry, so much that market specialists expect this industry will give the highest revenue through the supply of health and financial benefits to keep the aged population thriving.

3. KEY CHALLENGING POINTS

3.1. To characterize brain aging at the genetic, molecular and cellular level from an integrating individual-environment perspective

It is now well established that the brain aging phenotype, whether normal or pathological (dementias), is the result of multiple genetic variations (polygenic traits) combined to environmental influences, both internal (e.g. hypertension, diabetes) and external, of early and/or adult life. Therefore, we will need a global view of the molecular architecture of aging as a complex trait, which integrates intermediate phenotypes, such as transcript, protein or metabolite levels, in different populations of interest and different environmental conditions. This system biology approach is needed for the identification of genes, pathways and networks that underlie brain aging in conditions that are closer to the human scenarios. These are more elaborated strategies than currently used and that have only recently started to be addressed.

While studies based on gene expression and quantitative cell biology have taught us about the consequences of age on all and every single metabolic, signalling and gene expression event of brain cells (Ballabio and Bonifacino, 2020), we still do not know how age-related dysfunctions of intracellular mechanisms affect circuit organization and communication. Thus, it becomes evident that in the future cell biology approaches will need to be extended to the cell biology of circuits, to define how the changes in the cells' biochemistry affect the maintenance and function of circuits. These studies will naturally feed from the systems genetic approach, as genetic/epigenetic

peculiarities of susceptible/resistant-to-disease individuals will be translated to the cells' signalling pathways and from there to circuits. Additionally, and in accordance with the genetic discoveries that age-related pathologies are polygenic, we need to consider cell dysfunction during aging as a multi signalling/multi organelle problem. This will require a comprehensive approach to define relationships between interconnected signalling pathways in the compartmentalized intracellular milieu.

Importantly, these considerations do not only apply to neuronal cells. It is now well established that normal brain function involves complex interactions and rich signalling between neurons and different glial cell types, together with the vascular system (Araque et al., 2014). One important question that will require intense scrutiny in the future is how these interactions are altered by age. Several major research programs may be envisioned as necessary to get a better knowledge of cellular aging in the brain. First, we need a better characterization of the phenomenon of cellular aging across the different cell types in the brain. For example, how similar is the aging program in neurons and glial cells? Some differences are expected because of their distinct metabolic programs and characteristic cell activity responses. Nevertheless, some similarities may be revealed, and these would offer clues about fundamental mechanisms of cellular aging. Second, emerging evidence is revealing the existence of a large degree of heterogeneity among glial cell types and among subtypes within and in different brain areas. This heterogeneity is also reflected in the nature of neuron-glia communication, which is integrated at the level of neuronal circuits (Poskanzer and Yuste, 2016). Are glial cell subtypes differentially affected in aging? Are the alterations region-specific? These are fundamental questions that may contribute to understand the existence of brain regions with different vulnerability to neurodegeneration and aging. Importantly, some alterations may contribute to enhance the aging brain phenotype, while others may help protecting from it.

In addition to neurons and glial cells, it will be pertinent to better understand adult mother cell niches: their ability to generate new neurons and glial cells in an aged environment, the efficiency of the newly generated cells to provide trophic support and circuit integration, and also to define whether stem cell implantation strategies have therapeutic potential.

Finally, we need to establish the best strategies to make use of all the previous knowledge for a better understanding of typical diseases of the aging, especially the most devastating Alzheimer's disease (AD). On the one hand, this

will come from a better general understanding of brain aging: how different genetic backgrounds contribute to the type of aging we will develop, how this background is affected by different environmental conditions (and in turn affects our responses to the environment), and how these changes at the genetic/molecular level affect the different types of biochemical processes of our cells and these to the circuits involved in brain function. And on the other hand, progress will come through direct actions to answer specific disease questions, such as the relationships between the classical features of disease (e.g. intra and extracellular aggregates, synaptic dysfunction and neuronal loss) and systemic alterations (e.g. inflammation, metabolic disorders, and the aging of our senses). In this regard, strong emphasis should be put into the study of age-related hearing loss (presbycusis) which is now being recognised as an important factor in the type of brain deficits that we will have with age, so much so that it is estimated that the risk of Alzheimer's disease will decrease by a 9% by preventing mid-life hearing loss.

3.2. The influence of systemic aging in the brain: gut-brain axis, the cardiovascular system, the immune system

There is a growing knowledge of the fact that age-related systemic diseases affect the aging processes of the brain, although specific mechanisms are poorly understood. In addition to the need for healthy musculoskeletal system and metabolic organs, attention has recently been focused on the state of the gut microbiota, the immune system and the cardiovascular system (Cowan et al., 2018; Franceschi et al., 2018; Kalaria and Hase, 2019). In the near future we should be able of designing strategies to increase brain resilience to aging and neurodegeneration through improvement of peripheral signalling to the brain.

The interaction between gut microbiota and brain, known as the "microbiota-gut-brain-axis", is a well-established fact these days. Deleterious changes of diversity and composition of microbiota have been proposed to play key roles in age-related cognitive decline and neurodegenerative illnesses, mainly Alzheimer's and Parkinson's disease, but also in psychiatric illness frequent of the old age, such as anxiety and mood disorders.

The activity and composition of the peripheral immune system also actively participates in defining the way our brain ages. As a matter of fact, the peripheral immune system is remodelled at old age with thyme atrophy and increased senescent T cells, resulting in the reduced capacity of the aged immune system to cope with immune stressors and the concomitant progressive

increase of pro-inflammatory mediators resulting in a state known as "inflammaging". This state can be aggravated by a concomitant chronic inflammation caused by metabolic diseases, which defines a particular state known as "metflammation". This chronic inflammatory environment is likely to have a major impact on brain aging.

A third element of our internal milieu that exerts strong impact on our brain's well-being is the cardiovascular system. Heart and the main components of vessels, the vascular endothelium and media arterial wall, suffer structural and functional changes with aging, which together with increase of arterial stiffness and endothelial cells' senescence lead to hemodynamics dysfunction of the blood entering to cerebral vessels and consequently reduced oxygenation and provision of nutrients. On top, the reduced cardiovascular efficacy leads to increased arrival to the brain of pro-inflammatory factors and detrimental signalling molecules. Despite the abundant knowledge on this matter at the vascular level, we know very little about how the defects in cardiovascular system impact on brain function.

3.3. The influence of lifestyle and social environment on brain aging

The influence of lifestyle factors in relation to healthy brain aging are attracting great attention because they are amenable to modification and therefore feasible for implementation of effective gero-protective policies. Indeed, all preventive measures towards a healthy aging are nowadays based on lifestyle modifications such as physical exercise, diet and promotion of sociality. The latter deserves more careful attention. In social species such as humans, balanced relations, both familial and social, are critical for building social networks and social support, both contributing to proper brain development and brain health throughout the entire lifespan. Sadly, family and social support for the aged population is currently very much deteriorating in advanced countries, propitiating that old people become more and more socially isolated and living alone. Evidences demonstrate that lonely older adults are more prone to frailty, mental illness, and are exposed to greater risk of all-cause deaths, among other consequences (Rokach, 2019; NASEM, 2020; Kuiper et al., 2016). Therefore, we envision for the future to improve our understanding of the inner workings of the social brain, and how they impact on healthy brain aging (Fried, L. et al., 2020; Tan et al., 2020; Ong et al., 2016). This approach will include analyses of the genetic basis of loneliness in humans. In addition, circuit and behavioural studies on animal models will be required

to describe social areas in the brain and their molecular, structural and dynamic adaptations to aging. Finally, we should define targets for drug development, and microcircuitry mapping for non-invasive interventions, including current and new technological tools (transcraneal stimulation, artificial intelligence, virtual reality, etc) to improve their cognitive conditions through stimulating training. Moreover, social interventions are tested to be beneficial and supportive measures for general population, and specially for the older, to alleviate cognitive dysfunctions and to boost the cognitive functioning of healthy and cognitively impaired older adults. Particular attention should be paid to the use of computer-based training programs and video games (Ballesteros et al., 2018). Future research should be addressed to the design and validation of easing use of ICT products and software applications to maintain and/or improve the declining cognitive functions.

Finally, environmental factors influence the underlying biological mechanisms leading to the risk of cognitive dysfunction and of neurodegenerative diseases in older people. Environments could favor maladaptive behaviors and higher levels of environmental stress, linked to an increased risk of brain degeneration, disease and death (Leon and Woo, 2018), but also can stimulate brain plasticity in older (demented) subjects and enrich their lives. These environments, sometimes called 'therapeutic' (Calkins, 2018), can contribute to better cognitive and social interactions, sensory and functional skills, and benefits in learning and memory. Policies addressed for healthier environments can contribute to favour the older people' autonomy and motivation as essential drivers for a cognitive development as people age and to improve their quality of life, as evidences verify. Policies promoting age-friendly cities and communities will favour healthy lifestyles, as promoted by the World Health Organization (Fernández-Mayoralas et al., 2020).

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CHALLENGE 8

ABSTRACT

Stroke and traumatic injury in brain or spinal cord are often life-threating conditions and major causes of death or permanent disability with high impact in the health care system. There are several stages of intervention to improve the neurological outcome. Acutely, fast interventions aiming to reestablish cerebral blood flow in ischemic stroke, to stop bleeding after brain hemorrhage, and to reduce edema after contusions are amongst mandatory actions. Current studies aim to develop accompanying strategies for brain cell protection based on enhancing endogenous protective mechanism, blocking cell death pathways, or through immunomodulation. After the acute phase, interventions are intended to promote recovery of function using rehabilitation with state-of-the-art technologies enabled by robotics. Other advanced strategies include cell, gene, and immune therapies, and brain function modulation with the aid of smart nanotechnologies. There is great expectation in the fast evolving novel approaches for improvement of neurological deficits in these unpredictable and devastating conditions.

KEYWORDS

stroke | traumatic brain injury | biomarkers for CNS injury | neuroplasticity | neural repair | neural regeneration | neurorehabilitation technology | neuroinflammation | neuroprotection

CHALLENGE 8

BRAIN & SPINAL CORD DAMAGE & REHABILITATION

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1. INTRODUCTION AND GENERAL DESCRIPTION

Injuries to the brain and spinal cord have a sudden onset and chronic evolution. They are common causes of disability or death, and they are a major health problem in the EU and worldwide. The most frequent causes of sudden injury to the central nervous system (CNS), and main focus of this section, are trauma and stroke. However, some aspects of the inflammatory and neuroimmune responses and regenerative strategies may be relevant for other pathologies including brain and meningeal infection, autoimmune diseases, brain cancer and paraneoplastic neurological syndromes. Moreover, acute brain injury may cause long-term secondary complications, such as depression, seizures, or cognitive impairment and dementia that are more frequent in the elderly. The latter consequences transversally connect this challenge with other challenges related to brain aging and neurodegeneration, as well as mental health.

Injuries to the brain and spinal cord are amongst the leading causes of death and long-term disability in young people. Traumatic brain injury (TBI) often leads to diffuse axonal injury, microglial activation, and microhemorrhages. TBI has different consequences depending on the degree, i.e. mild, moderate,

or severe as defined according to clinical criteria (Blennow et al., 2016). Any kind of spinal cord disease (traumatic or nontraumatic) presents with a distinct pattern of neurological dysfunction that has prognostic value for the neurological outcome. Stroke causes sudden neurological deficits due to the interruption of blood supply to a brain region. It often occurs after blockade of a cerebral artery that will cause tissue ischemia and will lead to brain infarction. About 20% of the stroke cases are due to rupture of a blood vessel (bleeding), which will generate intraparenchymal or subarachnoid hemorrhage. Stroke is a leading cause of death and disability worldwide. In Spain, it is estimated that stroke is the third leading cause of death (Soriano et al., 2018), but this ranking position is higher for women. Although stroke is more common in men, women have a higher lifetime risk of stroke, and a greater risk of death after stroke than men. Moreover, women have more severe disabilities and worse outcomes than men, but the reasons are not entirely known (Cordonnier et al., 2017).

Discovery of novel druggable targets for new therapeutic drugs together with investigations in drug repurposing are necessary to design strategies aiming to minimize brain damage and to promote brain repair. There are different stages of putative intervention to improve the functional outcome following injury to the CNS:

Damage in the acute phase: Stroke and trauma cause acute neuronal cell death and generate strong inflammatory reactions that alert the immune system and may exacerbate the initial lesion. Moreover, severe damage to the brain or spinal cord may induce immunodepression that reduces immune competence of the patients and rends them more susceptible to acquire life-threatening infections. The first line of treatment in the acute phase of brain injury aims to reestablish the cerebral circulation, reduce edema, and minimize neural cell death. Advances in monitoring devices using nanotechnology and biomarkers with prognostic value will also improve patient care in the acute phase of CNS damage. Given that the onset of traumatic injuries or stroke cannot be predicted, a rapid intervention is crucial for the best prognosis. The most effective treatment in ischemic stroke is vessel recanalization, either with thrombolysis or mechanical thrombectomy, to reopen the occluded artery, restore anterograde perfusion, salvage ischemic tissue, and improve clinical outcome. Nonetheless, arterial recanalization is not always followed by clinical improvement. One of the reasons could be the lack of entire microcapillary reperfusion in spite of opening previously occluded large vessels.

Addressing this problem will require better knowledge of the cerebral microvascular circulation, mechanisms involved in clot formation and removal, and secondary thromboinflammation. Indeed, reperfusion may bear unwanted side effects known as reperfusion injury involving oxidative stress, inflammation, and hemorrhagic transformation. The physiopathology of intracerebral and subarachnoid hemorrhage is not well known. Complex genetic factors emerge as potential contributors. However, there are no current treatments available for brain hemorrhage. Therefore, some therapeutic option for this condition is urgently needed. Edema is a complication in neurocritical patients that requires urgent control since it may generate life-threatening situations (Jha, Kochanek and Simard 2019). Dysregulation of CNS fluid homeostasis and brain water content may have critical consequences after TBI and stroke. Tackling this problem will require further understanding about cerebrospinal fluid (CSF) dynamics, the contribution of meningeal lymphatics to CSF drainage, and resolving important controversies on the glymphatic hypothesis (Nedergaard 2013; Abbot et al., 2018). Dysfunction of the blood-brain barrier (BBB) is also amongst key processes involved in acute CNS injury (Sweeney et al., 2019).

Subacute and chronic stages: Lesions to the CNS evolve following the acute phase of injury. The underlying physiopathology is complex and involves dysfunction of neuronal networks, glial reactions, and active participation of the immune system. There is a temporal window to improve the neurological dysfunctions ranging from weeks to months, where synaptic plasticity and possibly neurogenesis can be stimulated, particularly in spinal cord injury (SCI) (Hutson and Di Giovanni, 2019). Novel developments in strategies for regenerative medicine and rehabilitation are providing great expectation for recovery of neurological functions after acute CNS damage. Regeneration based on stem cell therapy (Ouyang et al., 2019), interneuron transplantation (Zhu, Eom and Hunt 2019), or genetic cell reprogramming (Pereira, Birtele and Rylander Ottosson 2019), have shown the capacity to restore neuronal function in experimental studies, and translation to the clinic is on the way through clinical assays in patients with CNS injuries. In addition, the past three decades have seen a shift in the focus of neurorehabilitation from the use of compensatory approaches to enable function toward an emphasis on functional neurorecovery or promoting the restoration of function through use of the affected limbs. Current approaches to boost spontaneous recovery after stroke and TBI rely on rehabilitation therapies, but high-quality clinical trials are needed to demonstrate efficacy (Winters et al., 2018). Nonetheless,

rehabilitation is used to promote recovery after acute injury, either using conventional techniques or implemented with virtual reality solutions that could positively affect stroke patient cognitive outcomes by improving patient motivation and participation (Maggio et al., 2019). Strategies to further promote recovery include neuronal stimulation, including novel developments based on nanotechnology to promote recovery of the neurological function. The use of transcranial magnetic stimulation (TMS) (Dionisio et al., 2018) and electrical stimulation can initiate functional response in neurons by steering current to depolarize their cell membranes (Caldwell, Ojemann and Rao 2019). Brain stimulation remains as a promising tool particularly combined with rehabilitation, in spite that protocol harmonization and standardization is needed. Future applications of neuronal stimulation will benefit from improved basic knowledge on the involvement of neuronal networks and neuron-glia communications in recovery of function after CNS injury. Optogenetics (Boyden, 2015) and optically activatable drugs (López-Cano et al., 2019) have emerged as potent tools enabling fine modification of neuronal activity with very high spatial and temporal accuracy. For regenerative purposes, these light-based techniques are applied for stimulation of transplanted stem cells (Yu et al., 2019). Also, nanodevices can contribute to promote neuroregeneration using nanoprotheses (Mosbacher et al., 2020). Our understanding of the potential for neuroplasticity following a CNS injury has contributed to the development of intensive physical interventions that aim to promote neurorecovery through repetitive movement training. One of the most relevant new therapeutic technologies developed to facilitate the intensive training process is based on robotic rehabilitation devices, including exoskeletons.

2. IMPACT IN BASIC SCIENCE PANORAMA AND POTENTIAL APPLICATIONS

Stroke is a leading cause of morbidity and death, and the increasing number of stroke patients, including new strokes and stroke survivors, is currently taking huge proportions (Hankey, 2017). Globally, it is estimated that there are near 14 million new strokes each year in the entire world (www.safestroke.eu). Moreover, epidemiological studies estimate that 1 in 4 or 6 people over age 25 will suffer a stroke in their lifetime. According to the Spanish Society of Neurology (SEN), about 120,000 people will suffer a new stroke every year in Spain, and 50% of them will suffer permanent disability or will die. The cost of stroke in Spain is near 2,000 million euros per year. Reducing permanent neurological symptoms and disability would translate into significant

economic savings. The expected impact of these projections would increase even further if we account for an ageing population where the proportion of those aged 65 and above is expected to rise up to 30% in 2050. As a result, it is estimated that in the coming years the absolute number of patients experiencing a first stroke will increase. Further, validation of clinical benefits in women would increase even more the expected impact, as women are more vulnerable to the consequences of stroke, and incur on average 16% more costs than men (Cordonnier et al., 2017).

TBI affects 10 million people worldwide, mainly due to different types of accidents. TBI is estimated to be involved in about one-third of all injury-related deaths in the US (Faul and Coronado, 2015). Overall, the magnitude of number of people affected by brain or spinal cord injury (SCI) and subsequent long-lasting disabilities or death makes these diseases a problem of first order for the health care systems and the society as a whole. The incidence of SCI including traumatic and non-traumatic lesions is estimated to be between 40 and 83/million/year with an absolute estimated annual number of new cases worldwide around 250,000-500,000. According to a study, the annual incidence of new traumatic SCI rose significantly in persons 55 years and older. The proportion of tetraplegia and incomplete injury also increased. Additionally, traumatic SCI occurs mostly at a young age, below 30 years, whereas non-traumatic spinal cord disease affects people at a higher age, above 55 years. A report of the World Organization (WHO) shows that 15% of the world's population is affected by disability, 0.1% by SCI. Hence, the global prevalence of traumatic SCI is estimated to be 1,000/million people (Singh et al., 2014). The highest cost occurs during the first year after injury, whereas the total costs are determined by the life expectancy.

The ultimate goal of research is to improve the neurological function of patients that suffer acute injury in the CNS, by minimizing the severity of the damage and progression of the injury, promoting repair mechanisms, and helping with novel strategies for rehabilitation. Given the very large numbers of patients affected by stroke and traumatic brain and spinal cord injury in Spain and worldwide, the societal and economic impact of reducing permanent disabilities is expected to be very high. Research in this field will also increase the understanding of fundamental questions regarding the physiopathology of the CNS as a functional unit, including the diverse components from neuronal networks to blood supply, and the interaction with the immune system and the microbiome. The physiopathology underlying acute CNS

damage will contribute to understand the determinants of CNS damage from the genetic, molecular, cellular, tissue, and systems biology perspectives. Regenerative medicine tools, stimulation of brain function using nanotechnology strategies and new-generation activatable chemicals will impact recovery of the neurological function in patients with CNS injuries. Nanospheres, liposomes, and mesoporous nanostructures all emerge as future prospects for treatment and diagnosis of acute brain damage. Improving diagnostic and prognostic tools through the development of biosensors based on expertise in high-sensitive sensor platforms, and the design and development of strategies for biomarker discovery, including imaging biomarkers, will have an impact on the management of CNS injury. Finally, rehabilitation in patients with CNS injuries is a promising area of research driven by advances in novel robotic designs with impressive potential to restore disabilities, particularly relating the loss of motor functions.

3. KEY CHALLENGING POINTS

3.1. Improving CNS protection and diagnostic strategies after CNS injury

Current treatment of acute ischemic stroke aims at restoring blood supply by inducing reperfusion with intravenous thrombolysis and/or mechanical thrombectomy, both of which can only be provided in dedicated hospital stroke units. However, not all patients receiving reperfusion therapies achieve functional independence. Therefore, the current view is that combination of mechanical thrombectomy and/or thrombolysis with protectant drugs may open new avenues to reduce stroke brain damage (Savitz, Baron, and Fisher, 2019). Depletion of brain energy after stroke induces neuronal depolarization and excessive release of neurotransmitter glutamate that triggers excitotoxicity. Increases of intracellular calcium, oxidative and nitrosative stress, and other cellular metabolic and molecular alterations leading to different forms of neuronal cell death (López-Menéndez et al., 2019). Therefore, various steps of the ischemic cascade are possible targets for drug treatment to prevent neuronal death (Chamorro et al., 2016). Novel experimental advanced drug designs include light-controlled allosteric modulation of neutrotransmiter receptors, like glutamate receptors, for fine regulation of excitotoxic signaling (López-Cano et al., 2019). Stroke also induces damage to glial cells, such as oligodendrocytes, and affects the structure and function of the white matter. Moreover, it causes vascular damage that alters the integrity of the neurovascular unit, increases the permeability of the BBB and promotes the formation

of edema. Thus, therapeutic strategies need to provide not only neuroprotection but also glial and vascular protection to achieve integral protection of the entire brain tissue (Chamorro et al., 2016).

Injury to the CNS elicits a distinct inflammatory cascade that begins with cell death and progresses through multiple molecular and cellular phases. Necrotic cell death causes spread of intracellular contents to the environment and releases danger signals that alert local immune cells resident in the brain parenchyma, i.e. microglia, and attracts leukocytes towards the damaged tissue. Microglial cells are the myeloid cells resident in the brain parenchyma that are critical for homeostasis, repair and response to injury. It is crucial to resolve inflammation upon tissue repair. Otherwise, inflammation will become chronic, with detrimental effect for the CNS. Thus, the immune system needs to be firmly regulated to elicit its beneficial effects after injury while avoiding its potential destructive capabilities. The magnitude of the CNS inflammatory response may depend on systemic inflammation mediated by co-morbidities, such as inflammatory conditions (aging, obesity), and the critical molecular mediators should be identified to develop protective strategies (Chamorro et al., 2016).

Stroke and traumatic CNS injury induce depression of the immune system that is mediated by complex humoral and neural pathways connecting the CNS and the immune system, i.e. mainly the hypothalamic pituitary adrenal (HPA) axis, the vagus nerve, and the sympathetic nervous system (Chamorro, Urra, and Planas 2007; Prüss et al., 2017). The nature and role of immune responses to CNS damage, and the cellular and molecular mechanisms mediating the communication between the CNS and the immune system are still not completely understood. The adrenergic response to acute CNS damage is believed to alter the permeability of the gut epithelium facilitating the translocation of gut bacteria and infection (Stanley et al., 2016). The microbiota may change in response to brain damage and the status of the microbiota can affect the severity of brain damage after stroke. Understanding the interplay between the CNS and the immune system could be instrumental for the effective control of infection and improvement of survival rate and quality of life of patients with CNS injury.

Diagnostic and prognostic tools are important to find out the most appropriate treatments and to predict outcome. The identification of molecules, images, or other biomarkers of a disease condition or response to treatment or other interventions is critical. Systematic and exhaustive proteomics characterization will be required to identify/validate/verify panels of biomarkers in

plasma/serum/CSF by using high-sensitive technologies (ie. protein microarrays, mass spectrometry) for simultaneous analysis in high-throughput format of hundreds/thousands of proteins/peptides with minimal amount of sample, which may be a limitation. In addition, it is also critical to establish well-defined workflows for biomarker identification covering from discovery-validation-verification phase and all the required techniques/methodologies in all these stages. Proximal biological fluids, such as blood, serum or plasma have been extensively studied after acute brain injury because they are important sources for biomarkers.

Transcriptomic profiles in blood or brain samples may provide valuable disease-associated signatures that can identify candidate regulator genes and putative molecular targets. CSF has been historically considered as a rich source of biomarkers for diseases of nervous system. The CSF omics characterization could provide information about the mechanisms of CNS pathologies, and also as a panel of biomarkers candidates (Galicia et al., 2017). However, CSF cannot be obtained routinely in acute patients with the exception of patients that for clinical reasons need drainage or receive craniotomy. CSF biomarkers have been more often studied in TBI patients, where several molecules are useful to indicate the integrity of the BBB, the extent of the neuroinflammatory reaction, or axonal, neuronal or glial damage (Zetterberg, Smith and Blennow, 2013).

The study of genetic and epigenetic mechanisms involved in disease pathology is expected to contribute to discovery of new targets and identification of useful biomarkers. Genetic polymorphisms should be considered as possible predictors or covariates in studies that investigate neuroplasticity, motor learning, or motor recovery after stroke. Today genome-wide association studies (GWAS) carried out in large populations are able to identify specific genetic variations and associate them with particular disease conditions in the CNS that may predispose to vascular pathology or post-injury complications. Future predictive models of stroke recovery will likely include a combination of genetic factors and other traditional factors (e.g. age, lesion type, corticospinal tract integrity) to determine an individual's expected response to a specific rehabilitation intervention. Bioinformatics tools for analysis and construction of multiple network types, including protein-protein-interaction (PPI) network, miRNA-target network, lncRNA-associated competing endogenous RNA (ceRNA) network, and miRNA-transcription factor (TF)-target network are useful for this purpose (Luck et al., 2020).

Imaging biomarkers have strong translational capability due to the implementation of imaging technologies to clinical diagnostic and their current use in clinical trials. PET (positron emission tomography) and SPECT (single photon emission computed tomography) imaging provide molecular and functional information, such as inflammation, but novel radiotracers should be developed. Multiparametric MRI provides very valuable information on the status of brain tissue viability, vascular remodeling, structural connectivity of major white matter tracts, and functional connectivity. In addition, paramagnetic contrast agents can improve functional (cerebral blood flow, BBB integrity) and structural information (white matter tracts) and provide molecular information (e.g. inflammatory molecules), but it requires the development of novel contrast agents. Overall, non-invasive imaging techniques provide useful biomarkers and allow longitudinal monitoring and follow up. Image generation, reconstruction, analysis, quantification, and automation is mandatory but requires the cooperation of multidisciplinary teams.

Nanoscience used for diagnostic and therapeutic applications is termed 'theranostics', enabling diagnosis, drug delivery and monitoring of response to treatment. In the acute stages of CNS lesion, implantable microtechnologies, neural interfaces, with new materials and processes (such as graphene transducers) are providing new records of wide frequency range that allow detecting neuronal signals such as Cortical Spreading Depression (CSD) that may have prognostic value in neurocritical patients after CNS injuries due to stroke and trauma (Dreier et al., 2017). It is possible that detection of CSD and treatment could improve the evolution of these patients. Advances in neural interfaces based on new 2D materials could generate novel tools enabling to monitor these neurocritical patients after stroke, brain trauma or brain surgery. The use of graphene in a transistor configuration offers an alternative to metal electrodes for recording the low frequency neuronal signals that occur in these neurological pathologies (Masvidal-Codina et al., 2019). Likewise, new biosensors made with microtechnologies allow with minimal samples to identify neurological or metabolic markers of interest that may be the door to perform differential and evolutionary diagnoses of neurological lesions. The design of biosystems with minimum size and weight enable generation of Lab-on-a-Chip technology for new measurements based on high performance integrated circuits (García E, et al., 2019). This technology is also important for the development of organ-on-a-chip (OOC) technology consisting of 3-D microfluidic cell culture chips simulating complex cell-cell interactions in an organ-based manner. Finally, advances in neuronal interfaces are in the

spotlight to obtain a better and greater number of brain registration points and better understand the functioning of the brain. Moreover, they will improve monitoring in brain surgeries and will facilitate the detection of precocious epileptic episodes for earlier interventions on these patients.

3.2. Exploring novel approaches to promote neural repair and regeneration

The severity of clinical impairment after CNS damage correlates with functional disability and quality of life. Cell and gene therapies are promising approaches to improve functional recovery after CNS damage. These experimental techniques are based on the use of cellular material (cell therapy) or genetic material (gene therapy) to prevent or treat a disease. For instance, the use of bone marrow-derived stem cells, such as mesenchymal, hematopoietic, or cord blood, has already been transferred to clinical assays for the treatment of stroke, but efficacy has not been demonstrated so far. Mesenchymal stem cells exert anti-inflammatory actions that may contribute to the putative benefit of this cell therapy. Other promising approaches include transplantation of GABAergic neuronal precursors (Alvarez Dolado and Broccoli, 2011). Cell-based therapies, by themselves or in combination with biomaterials, engineered devices, or nanotechnology, are becoming a reality for the treatment of CNS injuries. However, CNS is an extremely complex tissue where all cells are exquisitely regulated and cell communication is essential for correct function. Understanding how glial-neuron communication works, how different cell types regulate their behavior and proliferative or differentiation fates is required before embarking on cell or gene therapy approaches. For instance, it is known that glial cells, e.g. astroglia and microglia, are able to proliferate after CNS damage. Nevertheless, glial cells may produce brain tumors, thus their proliferation must be carefully regulated (Portela et al., 2019). Strategies to enhance natural neurogenesis and angiogenesis are also regarded as promising therapeutics to promote neurorepair after acute brain injury.

Another promising field is the combination of biomaterials with stem cells to bridge the lesion gap after stroke or traumatic CNS injury. However, much effort should be devoted to understand the underlying biology, to learn how to handle biomaterials and differentiate stem cells, their response to different biomaterials and immunological properties, and how they could interact with electronic devices. Biomaterials, like hydrogels, can act as scaffolds that also generate a pro-regenerative environment favoring CNS repair after injury. There are encouraging experimental findings in the field of biomaterials and

regeneration for application after acute injury in the brain (Nih et al., 2018) and for axon regeneration after spinal cord injury (Anderson et al., 2018).

Immunomodulation is also regarded as a putative strategy to promote regeneration. Macrophages exhibit a huge functional plasticity because their transcriptional and functional programs during inflammation are shaped by environmental cues (tissue-specific factors, pathogen-derived factors, danger-associated factors) (Ginhoux and Jung, 2014), and possibly by the barriers the cells encounter during migration to the CNS. Importantly, innate immune cells are also critically influenced by their previous history of exposure to stimulatory agents, exhibiting what has been termed as "memory" (innate immune cell training or tolerance). The ability of innate cells to "remember" previous "encounters" has an epigenetic, transcriptional and metabolic basis (Netea et al., 2020), and should be considered when analyzing co-morbidities in the context of brain injury. Because of their central role during inflammation and functional versatility, "macrophage reprogramming" has been proposed as a therapeutic strategy for numerous inflammatory diseases (Schultze, 2016). However, the identification of macrophage specific markers to distinguish macrophages in their different functional states (de Las Casas-Engel & Corbí, 2014), as well as to distinguish newly recruited from tissue-resident macrophages in inflamed tissues, is a requisite for the development of macrophage-directed therapeutic interventions for human pathologies without altering host protection or inflammation resolution. Furthermore, the mechanisms underlying the acquisition of the anti-inflammatory/resolving profile are not completely defined in the case of human tissue-resident macrophages. Further understanding of the communication between the immune system and the injured CNS, mediated by cells, extracellular vesicles (Mittelbrunn, Vicente Manzanares and Sánchez-Madrid, 2015), humoral factors, and epigenetic regulation, will enable designing more effective therapies for CNS repair. Epigenetic modifications may be induced by rehabilitation strategies and are expected to impact on axon regeneration after SCI (Hutson and Di Giovanni, 2019). In addition, a variety of molecules, such as bioactive lipids, have emerged as putative drugs promoting functional neurological recovery after CNS injury because of their potent pro-resolution features (López-Vales & Samuel, 2019). Enhancement of endogenous protective signaling routes is complementary to strategies designed to prevent cell death and may favor repair in the damaged brain tissue. It is based on the capacity of several natural molecules, including certain lipids, growth factors, heat-shock proteins, amongst other molecules, to favor restoration of brain homeostasis after injury.

Nanotechnologies emerge as promising tools to promote regeneration in the CNS. Increasingly optimized solutions have been provided after the sequelae of the nervous system lesions. Implanted devices or neuroprosthesis have allowed the restoration of certain motor functions of both paralyzed limbs and sphincter control, among others. The challenge is to achieve devices that by size, shape, materials and energy expenditure involve a friendly biological-artificial interaction, both short or long time, and with greater benefits. The rapid development of nanotechnology in other areas of modern medicine has ignited a widespread interest in its potential for the field of stroke. An important feature of nanoparticles is the relative ease in which their structures and surface chemistries can be modified for specific and potentially multiple, simultaneous purposes. Nanoparticles can be synthesized to carry and deliver therapeutics to specific cellular or subcellular compartments; they can be engineered to provide enhanced contrast for imaging based on the detection of changes in the blood flow; or possess ligand-specific chemistries, which can facilitate diagnosis and monitor the treatment response. More specifically for stroke, nanoparticles can be engineered to release their payload in response to the distinct extracellular processes occurring around the clot and in the ischemic penumbra, as well as aid in the detection of pathological hallmarks present at various stages of stroke progression. Nanomaterials may enable control and/or local activation of the immune response, among drug delivery or diagnostic systems, as adjuvant agents in order to potentiate the role of innate/adaptive immunity in CNS to increase the recovery from the CNS injury. Engineering and artificial intelligence studies promoted the development of artificial synapses and neuronal networks (Saïghi et al., 2015), which in the future may enable neuroregenerative strategies. Implantable neuroelectronic interfaces offer the possibility of modulating neural function under the control of external computer devices. Next-generation neuroelectronic interfaces use biological materials for the interfaces. Biohybrid architectures are built with artificial devices coupled and interacting with biological brain, in such a way that the artificial counterpart activity is activated and modulated by biological behavior (Adewole et al., 2019). The additional implementation of artificial intelligence to control the interaction between the biological tissue and the artificial devices has brought the concept of intelligent biohybrid systems.

Novel strategies to favor recovery aim at improving current limitations of brain stimulation techniques, such as indiscriminate stimulation of cell components, large electrical artifacts, and poor spatial resolution due to

unpredictable and time-variant propagations within the neural tissue. Optogenetics provides several distinct advantages, such as cell-type specificity, millisecond temporal precision, and rapid reversibility by using optical stimulation to activate or inhibit genetically modified targeted neurons, which express light-sensitive ion channels and pumps (opsins) (Deisseroth, 2015). Also because of its much smaller electrical disturbance, this technique enables simultaneous neural electrical response monitoring near the stimulation sites. Methods combining regenerative strategies, such as cell therapies, with optogenetics may be instrumental to enhance and/or entrain the action of the regeneration promoters. Experimentally, it has been also demonstrated that seizures, which can be a complication secondary to acute brain injury, can be largely suppressed through the optogenetic activation of GABAergic neurotransmitters with Thy-1 or parvalbumin promoters. Additionally, the optogenetics-regenerative medicine binomial has demonstrated its efficacy in vision restoration or for reverting paralysis in models with induced motor neuron disease, among other application scenarios for regeneration after CNS injury. Moreover, photoactive drugs allow sophisticated fine-tuning of neuronal activity, offering great promise to boost recovery of neuronal function (López-Cano et al., 2019).

3.3. Developing cutting-edge technologies for rehabilitation after CNS damage

Neurorehabilitation technology is a rapidly expanding field in research and clinical applications. The use of robotic trainers for neurorehabilitation applications has increased in the last decades, both in childhood and later life, and in several motor diseases such as stroke, spinal cord injury, cerebral palsy, Parkinson disease. Because of its robustness, adaptability, and capacity to integrate multimodal information about the patient, robotics technology is in a privileged position to take advantage of this ability and lead to unprecedented levels of recovery (Bayon and Raya, 2016). This approach has interesting advantages compared to traditional therapy, because robotic therapy integrates functional tasks with accurate and assembled movements instead of repetitive movements without goal.

The promise of robotics as a powerful tool in the treatment of stroke and brain injury continues to excite stroke survivors, careers, researchers, developers, and funders (Bernhardt & Mehrholz, 2019). There are more than ten different devices available for robot-assisted arm therapy. Devices are needed that deliver substantially better functional outcomes than current care. Despite

that, the quality of the evidence is low, and there are variations between the trials in the intensity, duration, and amount of training, type of treatment and participant characteristics.

Some issues deserve special consideration in future research, including the need for better ways of determining the most effective dose of treatment, with dose considerations including length of each session, total number of sessions, and their schedule (sessions per day or week) as well as the intensity of training within a session.

Neurorehabilitation is currently undergoing dramatic technological changes as a result of significant advances in robotics (sensors and actuators), clinical diagnosis, biosignal recording techniques, and signal processing. In particular:

1. Emerging sensor and actuator technologies will allow to engineer the rehabilitation robots acceptable by patients.
2. Recent developments in artificial intelligence (AI) support the development of user-driven therapies, which is crucial to promote more natural motor development.
3. Current clinical knowledge permits s better diagnosis and better targeted treatments.
4. Multimodal data recorded during longitudinal studies will help to elucidate how the brain and the spinal cord coordinate their activity while learning/recovering movements. By enhancing our knowledge on how to optimize motor learning, the potential impact of successful adoption of neurorehabilitation could be enormous for both healthcare and society.

Robotics in community settings

One of the major benefits of the advanced robot technologies is that training can be conducted with less direct supervision, hence allowing parallel sessions and also increasing the amount of therapy sessions that can be provided to individual patients. This approach is already followed by world leading rehabilitation clinics, giving priority to the intensity and duration of training sessions (while maintaining suitable associated costs). This approach can be exploited with community-based approaches, in which robot trainings are conducted in a group setting. This constitutes an opportunity to investigate both from the methodological perspective -to facilitate delivery of treatment in such group setting, and clinical perspective with regard to the impact in terms of effectiveness and cost.

Future human-machine interfaces

The major goal of the stroke rehabilitation therapy aims to activate and reorganize the brain areas related to the planning and execution of the motor tasks. But very little is known regarding the cortical function during task execution in stroke patients, and in particular with respect to the cortical planning and cortical effort. The cognitive planning time and cognitive effort must be characterized for complex motor coordination tasks, such as walking. Therefore, a Top-Down approach has been proposed aiming ultimately at encouraging plasticity of the affected brain structures to improve motor function.

A better insight of the cortical function will enable the design of more accurately targeted and sophisticated interventions for stroke survivors. Such improvement in neurorehabilitation must consider means to correlate the brain activation patterns observed in electroencephalography, which are related to motor control associated with planning, with muscle force, electromyography and the executed movements. Such systems based on non-invasive methods for brain/neuronal-computer interaction (BNCI) have become very common in research. Examples of such robotics-based systems cover treatments for various motor disabilities, including tremor, stroke, traumatic brain injury, cerebral palsy, multiple sclerosis, Lou Gehrig's disease, and spinal injuries. For instance, the EU grants BETTER and TOBI developed BNCI as a major goal for rehabilitation of stroke. Such BNCI systems, which combine measurements of cortical activity (electroencephalography, EEG) with muscular activity (electromyography, EMG) and other sources. These systems have shown promising results to optimize some clinical outputs, such as improving spasticity (Tamburella et al., 2019), however are still facing crucial challenges for their practical use in routine rehabilitation. Thus, the main goal is to demonstrate technology based interventions that could be merged with routine rehabilitation approaches to develop usable and acceptable tools.

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The last decade of the 20th century, officially designated as the Decade of the Brain, brought forth significant advances in our understanding of the biological basis that underlie brain function. Despite this notable progress, neurological and psychiatric disorders currently affect almost a third of the population, a situation that derives from our still uncomplete knowledge of basic principles ruling brain development and function. Today, we are also facing a new era of technological advances that affect our lives in profound ways and we are bound to recast our relationship with our brains. In fact, there is the prevailing view that we are on the verge of new discoveries that will challenge our concepts for self-identity and free will, the privacy of our thoughts, the origins of social behavior or the inner workings of a diseased brain. To accelerate the pace of discoveries in Neurosciences able to prevent and treat mental affections and contribute to reshape the landscapes of other fields, from psychology to economics, education and the law, we need seamless flow of information between neurobiology and other areas of science that provide different but complementary perspectives and research expertise. Given the multidisciplinary wealth of the CSIC and the privileged position of Spanish neuroscience, we are in an optimal position to make a qualitative leap in understanding the mechanisms that control brain activity and be able to turn it into useful knowledge for building a healthier, more responsible society.

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