BRAIN, MIND & BEHAVIOUR

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INDEX

ABSTRACT 3

KEYWORDS 3

EXECUTIVE ABSTRACT 4

INTRODUCTION 5

CHALLENGES

DECODING THE EMERGENCE OF NEURAL CIRCUITS 12

FROM GENES & CIRCUITS TO BEHAVIOUR 31

COGNITION, COLLECTIVE BEHAVIOR & CONSCIOUSNESS 53

THE MOSAIC BRAIN: SEX/GENDER & NEUROSCIENCES 80

BODY-BRAIN-MICROBIOME INTERACTIONS 106

EXPOSING THE ROOTS OF MENTAL DISORDERS 123

AGING & BRAIN DEGENERATION 141

BRAIN & SPINAL CORD DAMAGE & REHABILITATION 161
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 main challenges described in this chapter.

Keywords
Neural circuits; neurological diseases; neurodegeneration; learning and memory; sex and gender; brain-body interactions; aging; cognition; collective behavior; mood disorders.
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 on 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 these changing connections 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 a 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 significant number of excellent groups with the potential to contribute significantly to the 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 the **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 as 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 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, that not only should be kept 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 dispersed 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 CI, the IN, IEGD (Instituto de Economía, Geografía y Demografía) or CAR (Centro de Automática y Robótica) 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 also of 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 postdocs devoted to favor their training in a cross-disciplinary manner. Second, 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

DECODING THE EMERGENCE OF NEURAL CIRCUITS

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**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.
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, squizofrenia, 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 XX century and the first years of this XXI, 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; PMID:16469913). 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 PJ 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; PMID:29689229). 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 (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.

**Key challenging points**

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.

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. 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 (Velasco et al., 2019; Chen et al., 2016; de León Reyes 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 (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.

**CSIC advantage position and multi/inter-disciplinarity**

There has been a long tradition of research in the field of both development and neuroscience in Spain and this has left a deep mark on the CSIC. At present, the CSIC concentrates a number of excellent scientists working in Developmental Neuroscience that are international leaders in their fields. This field accumulate a significant number of excellence CSIC groups that are or have been ERC-grant holders and are working on some of the key points highlighted in this document, including leading research at the international level carried at the Centro Andaluz de Biología del Desarrollo (CABD-CSIC), at the Instituto de Neurociencias (IN), at CBM Severo Ochoa, at the Instituto de Biomedicina de Valencia, etc. Most of these groups are currently associated to the Sociedad Española de Biología del Desarrollo (SEBD) and to the Sociedad Española de Neurociencia (SENC). This community has nested a generation of young scientists with excellent training, that now look to even further horizons than their mentors. They are
highly educated, more specialized, endowed with more interdisciplinary tools and best fitted to be competitive at the international levels.

The many CSIC institutes that work on neuroscience and development and their rooting bonds to the CSIC community makes them establishing their line of research’s in the CSIC and this sets a positive feedback loop in the CSIC that should contribute to increase inter-disciplinarity. However, although this is clearly a strength and the CSIC is a major node for neurodevelopment in Europe, its appeal to the new generation of scientists as compare to other European and Spanish institutions is dramatically decreasing. Technology evolves nowadays at an incredible fast speed and equipment, software’s and facilities rapidly get outdated while CSIC actions are slow and a clear disadvantage of being a CSIC researcher is the excessive bureaucracy we have to deal with when acquiring equipment or hiring personnel. The lack of technical permanent positions in the laboratories is also a hurdle that laid too many tasks onto the principal investigator. The retention and transfer of technical and scientific knowledge to new generations of researchers through permanent personnel is absolutely essential if we want to build a more efficient institution. The increasing differences in the offering of prospective opportunities in CSIC compare to other research centres, both Spanish and European, is clearly taking its toll in all biomedical sciences including developmental neuroscience.

Developmental neurobiology is itself a multidisciplinar field with many of the current remaining questions at the crossroad between physics and biology, bioinformatics and computational modelling, etc. CSIC counts with excellence researchers in all these fields but their connections must be favored. Unfortunately, the current strategy of the CSIC seems to go on the opposite direction preventing interdisciplinary by favoring small teams size and barely supporting in-house multidisciplinary initiatives. The CSIC requires a more rapid and efficient system that rewards multidisciplinary teams, in terms of grants, resources, and professional status. This may encourage CSIC developmental neuroscientists to get more involved in interdisciplinary projects.

Plan and resources
This section describes the actions and resources planned to achieve the key challenges listed above:
1. To develop integrative computational models that explain brain shape and growth. A systems biology approach will be required to uncover the hidden principles of brain self-organization. It is very likely that the research community sustained efforts, and the exponential growth of computing power (see Moore’s law), will materialize in improving computational modelling methods and applications within the next years (Sharpe 2017). It is essential that models for growth and morphogenesis operate at a multi-scale level integrating signaling events and gene regulatory networks, together with cell shape changes and mechanical forces. Based on what we already know about the extreme complexity of morphogenetic mechanisms involved in the formation of relatively simple structures such as the neural tube or the retina, this task seems particularly challenging (Martinez-Morales et al 2017). Even more demanding, and perhaps still impossible within the next decades, will be to concatenate morphogenetic models to follow the shape of the organ through entire developmental programs: i.e., from the specification of a given brain domain, over the proliferative phase of its precursors, and the differentiation of the different cell types and their final arrangement.

2. To generate new analytical tools at the imaging/biophysics/genetics interface. Despite recent advances in imaging and bioengineering, there are still significant gaps that are expected to be filled in the near future to get a more precise map of how the initial phases of brain and sensory organs development take place. As examples, reliable and direct methods to measure and interfere with morphogenetic tensions in vivo is still experimentally needed (Monguera et al 2018). In the short to medium term, the refinement of existing methodologies, such as optogenetics and magnetic beads/droplets, will certainly help to circumvent these limitations. In the long term, biological engineering, and particularly nanotechnology, should provide not only new sensors to follow the rheological properties of neural tissues, but also to manipulate and “correct” developmental programs in vivo. This is a fundamental step towards a “personalized medicine” approach directed to neurodevelopmental disorders.

3. To understand mechanical feedback loops and robustness operating in brain development. From a conceptual point of view, it is increasingly clear that mechanical feedback loops are essential to coordinate tissue patterning, shape changes, and even fate specification during development and homeostasis (Hannezo et al 2019). However, the true nature of mechano-transducing mechanisms is not so well understood. These
mechanisms need to be investigated at the molecular, cellular and supra-cellular level. Among other things they are important to understand basic properties of stem cell niches such as self-renewal and self-assembly. One of the most fascinating properties of development is its robustness. In the case of the brain and sensory organs this translates, for a given species, in precise neuronal morphologies, defined topology of sulci and gyri in the cortex, or constraint dimensions for the optic globe. However, we know very little about the developmental mechanisms that lead to this phenotypic stability within the species (Hiesinger and Hassan 2018). This phenomenon is highlighted when we compare the less constrained development of organoids vs. that of their organ counterparts. Understanding robustness is not a merely academic exercise, but it is a fundamental step to obtain faithful in vitro representations of the developing brain.

4. To understand the molecular mechanisms generating cell diversity in the developing brain. Gene regulation has been demonstrated to be much more complex in the neural lineage than in any other cell type. Therefore, it will be essential to identify the linear progression and gene regulatory networks hierarchies that lead to specific mature neuron-specific fates from progenitor commitment to mature neuron. It will be also essential to understand the role of stochasticity in the regulation of cell fate decisions and how different regulatory routines co-habit in a neuron (e.g, parallel regulation of neuron-type specific genes, pan-sensory genes, pan-neuronal genes, ubiquitous genes, etc). In this regard, the description of in vivo neuronal and glial specific transcriptomes and regulatory landscapes, including important information including isoforms or small non-coding RNAs will be critical to define cell fate and states.

Also, the interpretation of protein-protein interactions among different TFs, the importance of TF complexes for enhancer function, the role of genetic robustness and the importance of splicing will be all essential to understand how functional diversity is provided. Also, how TFs modulate chromatin states and how chromatin defines TF actions and the interplay between both in time and space need to be addressed as well as to have a more profound comprehension of the role of non-coding RNAs in transcriptional regulation of neuron-specific fates. The identification of homologue neuronal types among distant species and sister neuronal types in specific species should be tackled by implementing enhancer modelling and predictive approaches in order to identify rules underlying gene regulation during brain formation. Finally, the analysis of other mechanisms important in neuronal diversity, including post-transcriptional
modifications of TFs, regulation of basic translational machinery, RNA binding proteins, microRNAs, RNA granules, etc. need to be also deeply analysed. CSIC researchers working on gene regulation and evolution need to boost collaborations with neuroscientists to address these questions as well as to define the changes in gene regulatory networks that underlie the appearance of neuronal novelties through evolution.

5. To understand how circuits assembly and brain function emerge.

In this regard it will be essential to address how circuits progressively engage into one specialized function. Brain circuits, specially most complex and higher order circuits, do not fully segregate from other functional circuits until mature states (Sahel et al., 2019; Marín, 2016; de León Reyes et al., 2019; Antón-Bolaños et al., 2019). For example, the same cognitive process activates similar brain regions in adults, but shows an enormous variability in young individuals. These suggests that they are not unique functional trajectories for the same final outcome and potential diversity within young circuits. Brain functions might be multidimensional equations with multiple solutions. Understanding these issues, the potential diverse development, the influence of the outside world and the unfolding of multiple functional states before the segregation into mature circuits is key. Spontaneous neural activity and sensory-evoked activity are known to play important roles in intra- and inter-circuits assembly and therefore on the progressive engaging of the circuit into one specialized function.

A key question is to understand early patterns of activity and their modifiers such as regulators of excitatory and inhibitory balances, developmental expression of ion channel in neuronal subtypes, and neuromodulators, that are responsible on the elaboration of optimal circuits (Escalante et al., 2013; Benjumeda et al., 2013; Murcia-Belmonte et al., 2019; Hutson et la., 2019; Antón-Bolaños et al., 2019; Favuzzi et al., 2019). Within them, there is a particular need to understand which ones are the circuital nodes and neuronal hubs that emerge during circuit wiring and dictate central rules of assembling (de la Prida 2019; Favuzzi et al., 2019). Also, what kind of activity they orchestrate to instruct both the structural and functional topology of the future adult circuit. Neuronal hubs are mostly known for their role in epilepsy and they likely hold keys to other neurodevelopmental disorders and to normal development. The maturation of sensory organs and circuits clearly identifies the time of entry of experience in the game, but we still need to understand how this is done (de Leon Reyes et al., 2019; Antón-Bolaños et al., 2019). The progressive developmental organization also occurs for higher-
order circuits processing intellectual and cognitive computations, and modelling putative forms of secondary waves of activity that refine them is an important challenge that will be addressed.

To understand the dynamic molecular states of neuronal and glial differentiation that determine circuit connectivity is also necessary to advance our knowledge on how neural circuits emerge. Defining the dynamic changes and molecular states of differentiating neurons and glia at a single-cell level during the temporal windows of wiring is key to identifying molecular routes and cellular nodes determining functional networks in vertebrates and invertebrates.

6. To uncover the role of the immune system in the formation of developing circuits. In the last decade many interactions between the immune system and developing circuits at many levels have been proposed. Though immune processes within the brain are not identical to those occurring in the periphery, the brain has resident immune cells, namely microglia, which produce cytokines and other inflammatory molecules in response to disturbances in homeostasis in a manner similar to peripheral immune cells and they seem to affect brain development. However, it is still not clear whether or how the immune system impact brain development and these are important question that need to be addressed in the future again by using imaging and electrophysiology techniques together with computational analysis of gene regulation.

7. To develop reliable in vitro and in vivo approaches to model human neurodevelopmental disorders.
What makes the human brain different is a long-standing question that deserves much investigation. It will help to uncover the causes of many neurodevelopmental diseases and rare disorders considered as human specific disorders (Doan et al., 2016; Marin, 2016). Interestingly, bipolar disorders, schizophrenia or autism spectrum disorders, are now seen more like a continuum that reflect common underlying disruptions. Therefore, we do not only require to understand the degree of genetic and phenotypic variability of human functional circuits, but also the diversity within normo-typic individuals (Doan et al., 2016; Sahel et al., 2019). Very important to this task will be to define gender differences occurring during development. Solving these questions will require interdisciplinary studies joining electrophysiologist, research in behaviour, state-of the art-imaging and 3-D microscopy, and multi–omics approaches. Principal to this goal is
the development of proper animal’s models and appropriated comparisons with human systems, i.e. patients and brain organoids derived from them (Velasco et al., 2019). This task also requires stronger translational interactions with health professionals.

Collaborations with genetic neurologists and clinicians managing these patients incorporating latest tools of diagnosis and even applying them to animal models, such as diffusion tensor imaging, is indispensable (Arango et al., 2018). The ability to generate organoids from intrinsic or induced stem cells derived from human samples will expand our capacities to understand human characteristics (Velasco et al., 2019, Chen et al., 2016; Kupferman et al., 2016). While it is still a long way to define the limits of brain organoids as reproducing the complexity of neural circuits, it is clear that they are major allies to understand the development of mature circuits and what goes wrong in specific neurodevelopmental pathologies.

8. To capitalize the plasticity of development circuits to design brain repair strategies.

Understanding circuit wiring and plasticity at the levels of complexity described above will be rewarded with an increased ability to manipulate the adult brain. Developmental plasticity creates alternative circuits when there is malfunction of circuital systems during the protracted period of development (de León Reyes et al., 2019). In many of these cases, such as in children born blind or deaf, blocking the generation of these alternative circuits is crucial to make sure that when blindness or deafness are repair, the circuits are not dedicating to their tasks. They should be plastic and ready to build up optimal sensory systems (Sahel et al., 2019). Unblocking the developmental plasticity that is locked in the adult brain, is a current strategy to try to re-establish axonal connections after trauma. Also, the use of compensatory plasticity by engaging circuits that were processing other levels of information before disruptions or where secondary to these functions. Because even when the recoveries are partial, the impact on the patient’s life is enormous, which makes these strategies of great interest. Hence, the tasks are to identify drugs and manipulations that target the plasticity of any of the cell types of the brain and also to define the temporal windows of possible interventions known as critical periods (Marín, 2016; Sahel et al., 2019, Karow et al., 2018; Hutson et al., 2019).

All these specific actions are interconnected and need to be studied and globally integrated. These specific tasks will require the use of state of the-art tools addressing population analysis at the single cell level including techniques such as barcoding, the analysis of epigenetic landmarks, open chromatin, RNA-seq, exon usage for RNA
stabilization, RNA and DNA-chromatin immunoprecipitation, ATAC-seq, Hi-seq, etc. Comprehensive data processing, such as pseudo-timing analysis of the progression of differentiating states in neural populations is necessary to establish subclass specific intra-state and inter-state relations. It is known that activity modifies circuits both at the cellular and genetic levels and defining how these changes take place during development to shape the mature circuits requires a interdisciplinary exchange among neuroscientists, geneticists, developmental and evolutionary scientists that will need to use approaches traditionally used on the study of adult circuits, such as of state-of-the-art circuit imaging, electrophysiology and optogenetics couple them with genome-wide studies to analyze functional and structural connectivity that define the degree of plasticity in which nascent circuits are predetermined by molecularly unidirectional routes and to what extent these molecular programs are influenced by external actors such as experience (Velasco et al., 2019). This will be possible only by implementing and maintaining common facilities such as imaging, sequencing and biocomputing analysis platforms at the very edge of the state-of-the-art.

REFERENCES


**ONE SLIDE SUMMARY FOR EXPERTS**

**Decoding the emergence of neural circuits**

**Explaining how to make a Brain**

**Developing new tools**

**Explaining how the brain is constructed during development**

**Understanding and providing solutions to birth defects**

Microcephaly, Holoprosencephaly, Lisencephaly, Spinal Bifida, Cerebro and other neurodevelopmental pathologies related to late stages of brain development.

**ONE SLIDE SUMMARY FOR THE GENERAL PUBLIC**

**Decoding the emergence of neural circuits**

**What?**

Understanding the Basic Principles for Making the Brain and Sensory Organs

- Generating the shape and size
- Generating Cell Diversity
- Assembling Functional Circuits

**How?**

Gene editing, systems biology, in vivo imaging of animal models and 3D organoids

Atlas of emerging cell-types

Atlas of Brain Connectome

**What for?**

Making a Brain and Sensory Organs on a dish for:

1. Mathematical Modelling
2. Pharmacological Platforms
3. Developmental Malformations

**By whom?**

CSIC researchers from different disciplines

Leading international experts
Challenge 2
FROM GENES & CIRCUITS TO BEHAVIOR

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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.

Figure 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.

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 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 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 (Fig.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 and behavior by identifying elementary processes underlying basic behaviors such as motor control, freezing, place preference, navigation to then scale them bottom-up to the level of 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.
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.

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, 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.

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 improved diagnostic tools, the development of novel strategies to prevent disease and identification of new therapeutic strategies.

**Health impact**

A better knowledge of the normal brain may offer an opportunity 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 others 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. Behaviors such as stress and anxiety could result in depression, addiction or aggressive comportments. Research on these areas will increase our understanding of conditions that facilitate (or obstruct) the onset of child, adolescent and adult mental health as well as the adoption (or desistance) of harmful behaviors.

**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.

**Societal impact**
In the modern world, threatened by strong 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 a mandatory weapon to combat false beliefs and fake news.

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. These 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.

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 in 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.
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 cells and 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.

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). 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.

Importantly, brains are shaped across millions of years of evolution. For example, using single-cell transcriptomics to study turtle, lizard, mouse, and human brain samples the lineage of different cell types of the mammalian six-layered cortex could be disentangled (Tosches et al., 2018). While many basic behaviors such as fear or navigation may indeed share common circuits across species, their appropriate expression is strongly shaped by years of evolution. Thus, adopting an evolutionary perspective could confirm 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 (Cárdenas et al., 2018; Kohl et al., 2018).

To successfully accomplish this challenge we need to breakdown efforts in the following specific tasks, as described under Plans and Resources:

a1) Developing the atlas of brain cell-types

a2) Dissecting cell-to-cell communication

a3) Identifying cell-type specific circuit motifs

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 processes such as spike-timing dependent plasticity (STDP) have highlighted the importance of the precise timing of 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:

b1) To elucidate synaptic modifications during behavior
b2) Identifying and manipulating cell ensembles on demand

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 RNAs sequencing at population and single-cell level are shedding light on the molecular underpinnings (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. These elementary substrates are to be revealed at the optimal spatiotemporal resolution, and so we will need addressing two key tasks:

c1) Characterizing engrams of specific behaviors

c2) Developing novel neurotechnologies for imaging engram activity

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 require different synaptic interactions that in turn shape circuit dynamics. 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. Thus, adopting novel perspectives for deconstructing behavior requires merging computational methods and more sophisticated statistical tools:

d1) Developing new machine learning and computational tools
d2) Testing correlation and causation

CSIC advantage position and multi/inter-disciplinarity

The complexity of decoding brain function and behavior requires a multidisciplinary and multi-center approach. Over the past decade, catalyzed by transformative high-throughput technologies and the advent of artificial intelligence and machine learning,
the field has expanded massively, forcing interactions between different areas of knowledge.

Many CSIC teams and centers are widely recognized as international leaders in their particular fields. At the most basic levels, leading laboratories at the Instituto de Neurociencias de Alicante and the Instituto Cajal in Madrid are currently covering the study of brain circuits from elementary cell-type perspectives up to an integrated systems level. While these two dedicated centers are at the forefront of the CSIC strategy in neuroscience, some others (e.g. CNB and CBM in Madrid or the Basque Center for Biophysics in Euskadi) incorporate several individual teams with major focus in understanding brain function as well. This strong specialization represents a major advantage of the CSIC, but as we claim in the next section, our institutional position in the international arena requires reinvigorating attention. Linking genes with cells, circuits and behavior advocate the construction of unique shared capabilities such as more versatile animal facilities, dedicated genomics and imaging cores as well as large-scale computational platforms, which cannot be efficiently considered without such a level of institutional integration.

At a technological side, the field of material science and micro- and nanotechnologies is covered by a widespread network of research centers in Madrid (IMN), Barcelona (CNM), Euskadi (CFM), Sevilla (ICMS) and Zaragoza (ICMA), providing strong experience in new materials and advanced fabrication processes. If appropriately catalyzed, this unique expertise can help transforming the field in close collaboration with neuroscience centers and teams nationwide. Similarly, transformative research and concepts emerging at the interface with artificial intelligence and social sciences can further capitalize some of the existing CSIC resources at Barcelona (IIIA) and Madrid (CCHS). Therefore, institutional multidisciplinarity within and between the three CSIC global areas; Society, Life and Matter is another strength of CSIC that can be exploited.

Finally, the leading role and international visibility of some of our most outstanding researchers and strong interaction with industry and patent leadership suggest that there is ground to fertilize the very competitive research programs required to properly lead some of the key challenges described above. As can be seen in the next section we will propose some specific actions, resources and plans to be considered at the institutional level.
Plan and resources

Modern neuroscience is an alliance between disciplines. Only an interdisciplinary approach will allow us achieving the challenge of decoding brain function and behavior. This is reflected in several worldwide public initiatives such as the BRAIN initiative from the United States (https://braininitiative.nih.gov/) and the European Human Brain Project (https://www.humanbrainproject.eu/en/). These programs, together with other private projects such as the Allen Brain Map (https://portal.brain-map.org/), the International Brain lab (https://www.internationalbrainlab.com/) and the Chan Zuckerberg BioHub (https://www.czbiohub.org/) aim at boosting neuroscience research by mobilizing an unprecedented amount of funding and resources. Thus, at the topmost institutional level, the CSIC should consider leading similar initiatives in Spain.

In addition to institutional and national initiatives, novel strategies for data sharing and open science are transforming the research culture in all fields. Projects such as Open ephys (https://open-ephys.org/), the Miniscope (http://miniscope.org/), EASI Genomics (https://www.easi-genomics.eu/) or DeepLabCut (http://www.mousemotorlab.org/deeplabcut) all emerged from individual laboratories that team-up to overcome complex technological issues impeding major advances. Thus, the field urges for transformative policies at the institutional level. For instance, intersectional crossbreeding between transgenic lines together with other emergent methodologies will not only provide new tools, but it also increases the pressure over CSIC existing facilities. Novel solutions enabling to target deep brain circuits in vivo will require faster high-powered laser sources to deal with higher number of photons. Computational analysis of all these data will pose demand on the configuration of computers and operative systems. While many of these emerging solutions have been considered institutional so far, they should rather be at the reach of small teams and consortia. It is critical to facilitate cloud-computing and versatile cloud-storing resources and to provide CSIC researchers with easy access to secure hosts and other emerging resources. Some of the current existing institutional solutions such as SACO for instance work well for administration purpose but fail to adapt to the quickly evolving research landscape.

Implementing appropriate politics for human resources are also mandatory to provide the necessary flexibility. The inter-disciplinary character of the challenges outlined above requires attracting the most competitive students and postdocs from broad fields of knowledge, nationalities and minorities. The CSIC will need to consider reforming the current personal recruiting system to facilitate this endeavor. Last but not
least, to ensure success, it will be critical to reinforce international collaborations with leading labs and institutions to fill gaps in knowledge, supporting the education and training of scientific and technical personnel and promoting collaborative strategies worldwide.

1. **Atlas of brain cell-types:**

The large repertoire of cell activities supporting behavior stems from an equally diverse range of specialized cells. Traditionally, major brain cell categories have been viewed as largely homogenous populations. For instance, identification of region-specific neuronal and glial subtypes suggests the existence of highly specialized interaction with major role in health and disease (Batiuk et al., 2020; Hammond et al., 2019). Similarly, in the hippocampus more than 40 groups of genetic, anatomical and physiologically different types of inhibitory GABAergic neurons and different subsets of glutamatergic pyramidal cells are revealing critical for microcircuit operation (Klausberger and Somogyi, 2008; Valero et al., 2015). By exploiting molecular and genetic high-throughput approaches together with cutting-edge fast and efficient gene editing techniques, the role of heterogeneous cell types could be further evaluated, both phenotypically and functionally. Is there any particular role of cellular heterogeneity in the elementary processes underlying memory encoding and retrieval? Is the coordinated activity of ensembles of heterogeneous cell types critical for the operation of simple tasks and functions? Our current understanding of the functional impact of this diversity and its temporal evolution is still limited and represents a major challenge for the forthcoming years. The goal here is to develop a detailed atlas of brain cell types to better understand their functional diversity.

**Implementation:** Given the horizontal character of this task we propose building a new dedicated research center to implement action across the different sections of this chapter involving >100 researchers. Currently, there is no such integrated facility in the CSIC. Such a center should follow the spirit of large-scale resources such as the Allen Brain Institute to ease transcriptomic, epigenetic and proteomic analysis of the diversity of single brain cells. While progressing towards such a new institutional facility, we recommend the CSIC launching a virtual platform with 10-20 experts in bioinformatics, electrophysiology, molecular biology and data analysts working in close contact with experts specialized in the study of brain cell-types. The project requires the most
advanced equipment as well as specific computational resources. The expected budget for implementing this flag ship plan is significant and requires careful consideration.

2. **Dissecting cell-to-cell communication:**
Understanding the mechanisms by which neurons communicate requires a better knowledge on the key molecules determining circuit organization (adhesion kinases, neuroregulins, etc) and configuration (synaptic receptors, trafficking proteins, etc), as well as those others underlying their plasticity (activity-activated transcription factors, epigenetic mechanisms, etc). Communication between neurons and different cell types is equally important. For example, communication between neurons and astrocytes regulate synaptic transmission (Perea et al., 2009), while microglial surveillance may be tightly controlled by circuit activity (Liu et al., 2019). Revealing the composition of the synapses and their cell-type specificity require adopting a horizontal approach across genetic, molecular and functional levels. Remarkably, unbiased genome-wide analyses suggest that the repertoire of mRNAs localized in neuronal compartments is dynamically regulated over time in response to neuronal activity (Rangaraju et al., 2017; Vickovic et al., 2019). Thus, questions remain regarding the mechanisms of different forms of plasticity and their cell-type and circuit-specific dependency. Novel super resolution microscopy will enable the analysis of single molecules in specialized subcellular regions like dendritic spines while gaining scalability across cell types and regions. These techniques in combination with the next generation of voltage and calcium sensors and optogenetics will hopefully help to dissect cell-type-specific connections and their molecular dynamics with high temporal and spatial resolution.

**Implementation:** This is a large-scale program requiring long-term support of 10-20 experts in molecular and cellular neuroscience in combination with microscopy, electrophysiology, bioinformatics, biophysics and data science experts over a minimum of 10 years. An inter-center strategical research program is required to coordinate currently scattered groups at the CSIC institutes, facilitating the incorporation of not only major equipment (state-of-the art super-resolution microscopes; single-cell ohmics platforms, etc…) but also several smaller electrophysiology stations to test molecular manipulations on synaptic transmission and plasticity). Moreover, adequate computational resources for mass-storage and data analysis are mandatory. Such a program would require over 1 million euros/year to cover a startup package and running costs.
3 To identify cell-type specific circuit motifs

A major next step is to connect the cell atlas and cell-to-cell communication to brain circuits. Combination of retrograde viral tracing with single cell RNA sequencing will help to elucidate the projecting taxonomy of cells within (microcircuits) and between functionally distinct areas (networks). Furthermore, the combination of these techniques can be used to uncover the molecular identities of upstream neurons in a specific circuit and the signaling molecules they use to communicate. At the cellular level, the recent development of Patch-seq techniques will enable profiling of gene expression of cells that have been analyzed for their morphology and electrophysiological response pattern. Intersectional crossbreeding between transgenic lines together with other emergent methodologies will represent a novel tour de force to target cell types and circuits more specifically (Luo et al., 2018). The goal of this challenge is thus to identify cell-type specific motifs of circuits and networks that will be later associated to specific behaviors. **Implementation:** This can run as a medium scale collaborative task requiring concerted actions across different labs and centers with a flat budget of 2.5 million euros for 5 years. A team of about 10 live imaging experts, electrophysiologists, physics and bioengineers would be needed. To ensure addressing the challenge across different cell types, experts in the study of specific neuronal populations (e.g. interneurons, brain stem cells, and specialized projecting cells), microglia, astrocytes and other underrepresented cell types is mandatory. This challenge would require buying small equipment (e.g. several two-photon imaging set ups and ephys rigs, microscopes, neurotracking systems such as Neuropixels and novel optoelectrode systems, interface cards for acquisition and video streaming, high-quality cameras and high-speed next-generation computers or any other emerging equipment) which are currently not accessible to most individual laboratories. This package would update existing technical resources and prepare the ground for more elaborated programs.

4. To elucidate synaptic modifications taking place during behavior

Novel super resolution microscopy techniques will enable the analysis of cell-type specific single molecules in specialized subcellular compartments like dendritic spines. These techniques in combination with next generation of voltage and calcium sensors and optogenetics will allow for all-optical monitoring and manipulation of cell-type specific ensembles at a resolution never attained before. In addition, many neuromodulatory
nuclei buried deep in the brainstem modifies synaptic transmission in local circuits with
great precision. Genetic and molecular heterogeneity in these nuclei represent a major
obstacle to identify specific strategies to monitor and control these cells in vivo. As a
consequence, we know very little about their specific role in modulating basic behaviors.
Future research should capitalize novel transgenic lines based in intersectional strategies
(e.g. Cre/Flp, Cre/Dre double recombinase systems). These models in combination with
opto- and chemogenetic approaches will allow targeting specific neuromodulatory
systems simultaneously while providing a framework to study molecular modifications
taking place during behavior.

Implementation: This is a medium scale program running along 10 years by teams of 5-
10 experts in imaging, molecular biology, behavioral neuroscience and
electrophysiologists. Overcoming this challenge would require about 0.5 million
euros/year to cover running costs, small equipment which are currently not accessible for
individual teams (optical systems, behavioral chambers, emerging head-fixed
technologies such as Neurotar, etc). Additionally, the challenge may require a significant
start-up grant package to buy major equipment by strategical centers (e.g. all-optical
holographic microscopes by Brucker and Thorlabs, etc..), which can act as technological
hubs for the program.

5. Identifying and manipulating cell ensembles on demand
Neuronal ensembles are considered the minimal correlate of cognition. While available
data show strong relationship between sequences of neuronal firing and specific
behaviors, causal evidence is elusive. Thus, a key challenging question is to overcome
the correlational curse that links ensemble activity and behavior. For instance,
navigational ability appears to rely in spatial and temporal representations of the events
in the form of place and time cells in the hippocampus, a brain structure critical for
episodic memory. A dream experiment is to manipulate precisely cells and ensembles
representing specific contextual associations and modulate navigation accordingly. To do
so we will need exploiting novel all-optical approaches that combines simultaneous
monitoring and imaging and stimulation of hundreds of genetically defined specific cell
types (Zhang et al., 2018). Other recent high-throughput methods such as next-generation
Neuropixels and/or opto-electrodes will facilitate recording massively the activity from
thousands of neurons from different brain regions simultaneously, allowing to evaluate
circuit operation brain-wide (Stringer et al., 2019). The goal here is to identify candidate
circuits for specific behavioral traits. This will transform our understanding of how basic sensory modalities integrate into the ongoing brain dynamics.

**Implementation:** This is a large scale challenge requiring concerted action from some of the experts involved in the preliminary programs describes above (e.g. a3 and b2). The program should run over 10-20 years as consecutive overlapping cycles of 5 years allowing appropriate adjusting of major goals. Given the complexity of the tasks, each cycle should involve a team of the very best 5-10 experts identified from the different programs. This challenge would require about 2 million euros/year covering running costs. To launch the program start-up grant money is mandatory for acquiring major equipment (including but not limited to holographic two- and three- photon microscopes for in vivo imaging and electrophysiology rigs). A major budget should be allocated to expand institutional animal core facilities in order to accommodate several transgenic lines by intersectional approaches.

6. **Characterizing engrams of specific behaviors**

By combining transgenic model for activity-dependent engram-specific labeling and single-cell transcriptomics we should be able to uncover the transcriptional landscape underlying elementary processes of memory consolidation and recall during basic behaviors. This kind of approach can reveal how sustained activity and specific transcriptional alterations in diverse populations of cells could persist even weeks after the experience. We still ignore whether ensemble activity translates directly into engrams or whether early activity-dependent gene expression cascades differ within and across cell-types. What is the role of engrams in memory? How do they subserve recall? How are they modified by emotional and environmental factors? Using a combination of emergent tools, more elaborated processes such as memory editing and erasure, learning transfer and generalization can be addressed at an unprecedented level of details. Our main goal in this task is to interfere with memory as a mechanism to modulate behavior.

**Implementation:** This is a large-scale very specific task running over 10-20 years by >10 experts in bioinformatics, molecular biology, biotechnology, and engineering, etc... This program should look to accelerate translation of major discoveries, generating novel solutions, cutting edge-techniques and tools to be used transversally across disciplines. Overcoming this challenge would require investment of 1 million euros/year to cover running costs and small equipment. The program should consider nurturing a successful relationship with industry and private investors.
7. Neurotechnologies for imaging engram activity.

In recent years, emergent technologies with single-cell resolution have transformed neuroscience, from multi-cellular optical imaging to ultra-dense electrodes. Further developing of precision tools is 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 allow us to monitor cell activity at unprecedented resolution (Jayant et al., 2017). Imaging and manipulating the large repertoire of cells put strong demands for higher spatial and temporal imaging resolution (Stringer et al., 2019). Thus, improvement of optical switches that selectively shift signals across channels will allow for new generation of all-optical techniques to monitor and activate selected cell types on demand.

The field of device miniaturization will keep growing to provide solutions such as miniscopes to facilitate chronic imaging during behavior. Other approaches such as neural dust designed to create a mesh of distributed elements without the requirements of wired connections (Neely et al., 2018), will hopefully change the game by permitting parallel high-throughput imaging and electrophysiology. In parallel, new generation of genetically encoded sensors will be created for ions, active molecules such as glutamate and enzymes such as protease and kinases while improving the signal-to-noise ratio of existing solutions for monitoring voltage changes. This will foster demands for faster high-powered laser sources to deal with higher number of photons so that images can improve in resolution and depth reach. The main goal here is thus to develop a new set of high-throughput approaches for precise monitoring and intervention of engram activity.

Implementation: This is a large scale program that should run in parallel to the previous program along 10-20 years with a flat funding level of 1 million euros/year. In contrast to the previous one, this is more oriented towards neurotechnologies. Different teams of 3-5 experts in nano-technologies, electronic design, clean-room technology processes and modeling of electronic devices should interact with experimentalists to develop new cutting-edge solutions. The program will need covering small electronic instrumentation and equipment, with a dedicated budget to cover access to clean room facilities at CSIC centers, the Spanish ICTS Networks and other platforms abroad required to develop advanced and custom new prototypes. Similar to the previous program, this workpackage should have a strong technological appeal to be specifically oriented to the industry. This challenge is a spin-off of the previous one and should be linked together.
8. New machine learning and computational tools

Understanding how cell-type and region-specific ensemble activity is transformed into engrams will open doors to deconstruct behavior. However, the more information we acquire about all possible contributing factors, the more challenging will be to understand how they are integrated. Modeling the precise dynamics and evolution of these events is a first step in this endeavor. These models will have to account for the highly nonlinear and nonstationary dynamics of brain activity during movement, decision-making, memory retrieval, etc. Prompted by the recording technologies described so far, an exponential increase of available data will call for transformative infrastructures and computational resources to ease application of artificial intelligence to neuroscience. Thus, we envisage a strong demand for building models and theories beyond the current state of the art to bridge across description levels. Critically, models for emergent brain properties will have to be based on robust and falsifiable theories of the brain function as a whole.

Implementation: This is a large-scale project to be addressed over 10-20 years by a team of 10-20 experts in systems neuroscience, computational models, machine learning and data science. This task would require 2 million euros/year to cover running costs and small equipment (dedicated computers, data-storage, etc.), and to attract the most competitive postdoc, engineers and data scientists worldwide. At a large scale, access to supercomputer clusters should be better customized to meet specific needs and this program should include the generation of a brain-science oriented supercomputer hub. For instance, computational platforms providing access to popular neuroscience tools, pipelines, data processing software such as Neuron+Python environment are critical for efficient modeling of neuronal circuits; while some other emerging toolboxes for multicellular imaging or high-throughput neurophysiological recordings demands for specific requirements. Having thematically dedicated supercomputer clusters will significantly improve efficiency.

9. Testing correlation and causation

Understanding the elementary processes of basic behavior requires testing for correlation and causation. The science of causality is living a surge while evolving from their original epistemological and metaphysical perspective into a more modern mathematical conceptualization of counterfactual and manipulation theories (Pearl, 2000). Such
emergent approaches are quickly permeating neuroscience research and should crystalize in the conceptual shifts of our current experimental paradigms. Other concepts such as Bayesian inference in which observations are taken to inform and update the probability of hypotheses could impact the way experiments are designed. Here, better closed-loop technologies are imperative to couple recording, modeling and analysis with different actuators for more effective interventions in real time.

Implementation: This is a very important medium scale task should be addressed together with the previous one as a complementary program running over 10 years by a team of 5-10 experts in complex science, mathematics and statistics working in close collaboration with experimentalists. This challenge would require 0.5 million euros/year to cover running costs, small equipment (including super-computers and cloud-resources), and contracts very competitive mathematicians, engineers and data scientists.

REFERENCES


ONE SLIDE SUMMARY FOR EXPERTS

From genes and circuits to behavior

What

Basic individual behaviors
- Sensory perception
- Motor control
- Navigation
- Aggression & escape
- Decision-making
- Place preference
- Blending
- Singing
- Encoding
- Removal

Genes, cells and circuits
- Elementary processes: gene expression
- Cell signaling, synaptic transmission
- Protein, neurotransmitters, enzymes, oscillations, organelles, epigenetics

Within and between species behaviors
- Social hierarchies
- Maternal behavior
- Communication
- Learning
- Sleep
- locomotion

How

Genomics/proteomics

Neurotechnologies & Imaging

Atlas of cell-types

Artificial intelligence & modeling

Transformative Tools

By whom?

Multidisciplinary CSIC centers

Leading international experts

From different disciplines and techniques.

ONE SLIDE SUMMARY FOR THE GENERAL PUBLIC

From genes and circuits to behavior

Explaining brain function

Developing new tools

Explaining what makes us human.
Understanding and providing solutions to mental illnesses and other problems
(Schizophrenia, depression, Parkinson’s, Alzheimer, Autism)

National and international scientists, experts in different fields and techniques.
(Biologists, engineers, psychologists, physics, medical doctors, informatics, and others)
Challenge 3
COGNITION, COLLECTIVE BEHAVIORS & CONSCIOUSNESS

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Introduction and general description

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 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. We would say that our cognitive capacities make us humans.

Cognition exists throughout biological systems

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. No doubt, the Central Nervous System (CNS) supports these functions in many organisms. Throughout evolution, the CNS has developed specialized molecular and signaling mechanisms, cell types and neural circuits to support them. However, brains (or neural networks), have no monopoly of the signaling functions that implement many of these remarkable algorithms (Baluška and Levin 2016). Pagán (Pagán 2019) and Turner (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. Cognitive networks have evolved a broad range of solutions to the problem of gathering, storing and responding to information. Some of these networks are describable as structured sets of neurons linked through plastic and highly dynamic synaptic contacts forming a complex and adaptive web of connections. These are ‘solid’ networks, with a relatively stable physical architecture. Other systems are formed by sets of agents that exchange, store and process information but without persistent connections or move relative to each other in physical space, the so-called ‘liquid’ brains, 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 multilayer complex goal-directed behaviours also at their cellular and subcellular levels.

Therefore, one of the big challenges that we need to consider when thinking in 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).

FIGURE 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)

*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 behavior, social dynamics), society-ecosystem (e.g. economy, climate, epidemics).

Behavioral 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 instances, paintings, written records, songs or stories allow humans to ease memory burdens by storing information in the environment. Thus, just like 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 ends? 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 Kikuyo and 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 behavior 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 a third big challenge in the field, which involves the study of so-called “extended” cognitive capacities (see below).

**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 on starting 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 type 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, it 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 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 (AI)-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 principled knowledge about cognitive processes.

Impact in basic science panorama and potential applications
To put it briefly and directly, the path that cognitive science needs to take, and 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. This refoundation must necessarily be disruptive at all levels, e.g. academic, health, technological, social, economic, educational. Suffice it to say that the
perception 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**

- Refund 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 behaviors 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.
- 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.

**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:

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 el 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.

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), 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 (solid and liquid). 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**

As we have said, 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 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, the immune-brain axis, or the constraints that the musculoskeletal system impose on the perception of the environment, planning of our 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.


Human cognition and social interactions take place in an increasingly AI-dominated world. This research line would explore how artificial intelligence is changing human thinking and behaviour. In turn, this understanding will feedback into AI development and its multiple implementations in autonomic systems like robots and the improved integration and performance of human-machine interfaces in a cognitive continuum. 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. Their implementation in robots has provided them with increasingly sophisticated social skills. The long standing promise of incorporating these machines to improve our daily lives
seems closer than ever. However, there is still a big difference between our expectations and the reality of social robots (Yang et al. 2018). The study of the cognitive mechanisms that support the interaction between humans and robots is undoubtedly one of the next frontiers that will allow us to obtain critical knowledge to optimize social encounters between humans and robots. A new generation of robots that respond to and trigger human emotions not only allows for a more efficient collaboration, but can also stimulate human users to develop long-term social bonds with these 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.

AI algorithms will become part of the battery of tools available to cognitive sciences to probe the essential characteristics (actions, emotions, intentions) as well as the flexibility of social cognitive processing in the human brain. Here again, however, the data available indicate that this 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?

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 a discontinuous phenomena across species? For instance, are there cognitive capacities exclusive for humans? How stable is consciousness as an emerging state? We pursue the study of cognition as pluralistic and evolving scene with diverse opportunities for grounding future research.
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). The concept of reflex must not be taken out of context. 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). Let us also mention that cognition is arguably found in non-neural living organisms, such as plants (Calvo Garzón and Keijzer 2011). In fact, exploring the neural basis of cognition is necessary but not sufficient. Machine cognition, as the quest to engineer intelligent machines, is also key; especially when articulating the challenges of building machines that learn and think like people (Lake et al. 2017). Furthermore, cognition can and should also be studied beyond the brain itself and beyond being informed by behaviour, such as in the neurophenomenological approach championed by Varela (1996), whose aim is to include introspection as a source of scientific evidence. This leads into the upper limit.

At the upper limit of cognition, we find 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). Objectives ways to measure consciousness are 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 behavior 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), analyzed better in terms of its behavior 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 covid19 pandemic, have 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, even if diffuse, 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, but mainly, because of the impact that is expected from them in society.

**CSIC advantage position and multi/inter-disciplinarity**

The main strength of the CSIC lies in the quality of the research groups that cover many of the areas of knowledge that are necessary for this challenge. The main weakness lies in the currently reduced communication between them and the difficulties in establishing stable communication channels. Therefore, the strategy seems clear: to create a long-term structure whose cornerstone is trans-disciplinary collaboration. This will involve, as we will see in the next section, developing a common language in traditionally distant disciplines, defining common scientific questions with different time frames, having institutional funding to create and maintain transdisciplinary and multi-centre research groups, and new dynamic staff recruitment policies.

The CSIC has research groups of international prestige working on different aspects of cognition. Groups of neuroscientists are located in dedicated centres, such as the Institute of Neurosciences (IN, Alicante) and the Cajal Institute (IC, Madrid), as well as distributed in centres such as the National Centre for Biotechnology (CNB, Madrid), the Severo Ochoa Centre for Molecular Biology (CBM, Madrid) or the Basque Center for Biophysics in Euskadi. Researchers studying emergent cognitive capacities in
collective behavior can be found at the Institute of Interdisciplinary Physics and Complex Systems (IFISC, Palma de Mallorca), the Centre for Advanced Studies of Blanes (CEAB, Girona) or the Institute of Evolutionary Biology (IBE, Barcelona). The interface between cognition and artificial intelligence is of great interest to groups from the IBE and the Institute for Research in Artificial Intelligence (IIIA, Barcelona), and its application in bio-inspired robotics, autonomous systems and artificial perception is being studied in the Centre for Automation and Robotics (CAR, Madrid) and the Institute of Robotics and Industrial Computing (IRI, Barcelona).

CSIC's great capacity to address biological, psychological, computational and technological aspects of cognition is matched by the same capacity and interest in cognitive problems of its research groups in the field of social sciences and humanities. The importance of cognitive abilities such as memory in the development of language and the architecture of social spaces is of interest to research groups at the Institute of Language, Literature and Anthropology (ILLA, Madrid), the Institute of History (IH, Madrid), or the Institute of Advanced Social Studies (IESA, Córdoba). Groups interested in population dynamics and social identity or the effects of pluri-culturality can be found, for example, at the Mila and Fontanals Institution for Research in the Humanities (IMF, Barcelona). Archaeology research institutes, such as the Institute of Heritage Sciences (INCTEP, Santiago de Compostela), the Archaeological Institute of Merida (IAM, Badajoz) or the School of Arab Studies (EEA, Granada), study the cognitive role of archaeological artefacts, architecture and city design.

As a whole, we believe that the CSIC has the necessary basis to face the challenges we propose here. We are confident that a small number of actions on a well-defined plan, as we will propose below, can transform this excellent substrate into a CSIC flagship in the coming years.

Plan and resources
As a result of our assessment of the state of the field at the international level, we consider that any plan devoted to advance the understanding of cognition necessarily goes through the hybridization of knowledge disciplines beyond the brain boundaries. After decades of a successful reductionist research program, it’s time to revisit the work done with more holistic and synthetic views, being the idea of cognition a clear integrative hub in neurosciences. We will face many challenges, some of which have been delineated above, but the task could not wait any longer. The pillars of the Research Plan on Cognition are:
(1) to focus in organisms (not only organs) and collectives of organisms, (2) to take seriously the ecological dimension of cognitive behavior (the study conditions can be either controlled or natural, but always need to be meaningful) and (3) to consider an evolutionary, cultural, and historical perspective.

We have also reviewed the strategic position of the CSIC, and found that it has the necessary wicks to weave the basket. Research groups cover most of the aspects of knowledge that we have identified as necessary for a real progress, and additional first class national or international alliances could be easily implemented in the plan (below) to fill existing knowledge gaps. We have also identified the main weakness, the lack of effective communication between groups across disciplines, with some exceptions.

Therefore, the cornerstone of the action plan is undoubtedly to establish channels of communication between these disciplines, which is a task with a scientific component, but also a very important institutional one. The scientific component essentially involves (1) developing a common language so that communication between traditionally separated (almost isolated) disciplines is effective, and (2) defining research programs that bring together the interests of the different academic worlds. For the latter, the common language will also help formulate scientific questions in such a way that convergences are greater than divergences. The institutional component, without which all else will be in vain, involves (1) providing a management structure that supports transdisciplinary interaction, (2) stabilizing that structure with incentives for research, such as specific programs for recruitment of personnel and recognition of the transdisciplinary CV, and (3) support in the creation of innovative cognition-oriented scientific and technical infrastructures. Some of these points are elaborated further below.

**Development of a common language in cognitive sciences**

When moving towards complex cognition and real world problems, there is the danger of inheriting the strong constraints that carry the study of concepts that are in our daily vocabulary. The study of “big words” (ie. empathy, morality) is threatened by the imprecisions that imply folk psychology or intuitive understanding, which are hindering scientific advances. An operational definition of brain function and cognition must be created bottom up, from data to concepts and back again. As an example, psychologists and psychiatrists are moving away from general classification of syndromes (diagnostics based solely on DMS) towards a quantification of symptoms to obtain more useful information to guide treatments (Ghaemi, 2018). However, animal research on cognitive
disorders is far from this operationalization of behaviour. Furthermore, when consider
cognition and the need for transdisciplinarity, operational definitions are key.

We find an 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 broad
applicability of these tools discussed in dedicated cognition-focussed symposia with
groups from all relevant disciplines is a first valuable action in this direction. Previous
initiatives in this direction organized by our research groups were successful and already
demonstrated the validity of this strategy not only for sharing powerful tools, but mainly
for development of a common language.

Finally, a fundamental action to facilitate interaction between disciplines and a
common language is to introduce the holistic perspective of cognition into academic
training. Undergraduate courses and specialized masters should teach the diversity of
concepts from biological, human, psychological, physical and computational sciences
needed in the study of cognition. This action will naturally bring strategic alliances
between the CSIC and university institutions.

*Development of trans-disciplinary research programs: IVIT*

We propose to develop a new formula, compatible with the concept of research centre,
but virtual, delocalised. In this way, funds would be invested in research programmes,
rather than in bricks. These *Institutos Virtuales de Investigación Transdisciplinar del
CSIC* (IVIT) should allow a smooth and independent management of the resources
obtained by the research program, the free exchange of personnel and an annual budget
for coordination, support exchanges (short and mid-term stays), inviting international and
reference researchers in the field, and frequent meeting (some of which could occur
regularly in well-equipped video-conference rooms). These centers could be organized
around well define objectives (with an average life span of approximately 5 years) or
broader research themes (10 years or more), always with a fundamental transdisciplinary
character. The required expertise could be complemented with the participation of
researchers from other national or international institutions. IVITs would represent
dynamic scientific associations, in which their members could change according to the needs of the scientific question, and limited in time. Both the member groups and the IVIT as a whole would be subject to periodic evaluation by an external Scientific Advisory Board for funding continuation. With the intention of being concrete, we include some examples:

- **IVIT on Memory.** Aimed to explore the role of ‘memory’ *sensu latu* in cognition, including internal and external storage structures, from digital resources to ancient, tribal objects.
- **IVIT on Neuroethology:** aimed at looking for unifying principles in a range of cognitive networks, including both solid and liquid brains, with high-throughput experimental infrastructures.
- **IVIT on Collective Minds:** aimed at studying the underlying principles of collective/social cognitive processes in the real-world.
- **IVIT on Consciousness:** aimed at exploring the limits of Consciousness and free-will with a multidisciplinary and quantitative approach.

An analogue system to IVITs already implemented in the Spanish research portfolio is CIBERESP, one successful example in the CIBER networks (Centro de Investigación Biomédica en Red). Still, we would envision a different, likely more modern and agile approach, for the constitution of IVIT, learning from structural and organizational gaps in CIBERESP Groups.

Among the advantages that these structures would offer, besides fostering transdisciplinarity and reaching a critical mass for scientific discussion, they would increase the competitive weight of individual groups and of the CSIC as a whole, in the international scientific scene, which in turn would result in greater competitiveness in accessing international resources. IVITs, recognised as research institutes, could become Severo Ochoa Centers of Excellence. The cost associated with the creation of a thematic IVIT would be minimal compared to the construction of a classic research building. The saved resources could thus be invested in scientific and technical research infrastructures (see below) and in personnel recruitment programs. The CSIC should seriously consider having its own research funding program for such centers, as an important incentive.

CSIC’s current mechanisms for recruiting researchers need to be revised. This is not the place to discuss it, but suffice it to say, for this particular challenge, that profiles with a
truly trans-disciplinary background are *rara avis*, very costly to produce in terms of investment in training, and very difficult to maintain, as they are internationally prized. It would be appropriate to emphasize this important curricular characteristic in new personnel recruitments. More flexible hiring formulas in the medium and long term would also be desirable.

*Cognition-oriented infrastructures (capacities, not buildings)*

Studying behaviour under ecologically relevant conditions and scales, including the interaction between two or multiple organisms, the interaction between biological systems in embodied processes, and real-world experiments, requires specific and sophisticated experimental capabilities. In many cases, in fact, they require technological development and innovation. Analysing the global situation of the CSIC in comparison with other institutions, and the research needs, we propose a resources plan with two levels. First, at the individual institute level, in house fabrication labs with mechanical and electrical prototyping abilities are required. These allow researchers to be fast and flexible when designing and troubleshooting their novel experimental paradigms. We are recently living a surge of open source platforms for the creation of automatized high-throughput behavioural paradigms (e.g. Bonsai), brain activity recording systems (e.g. Open-Ephys, in which members of the CSIC collaborate) and behavioural analysis (e.g. DeepLabCut), to mention a few, which should be reinforced and harnessed by CSIC researchers. The institutional support at this level, enhancing technological capacities (workshops) at a local scale will be fundamental and synergize with the structure we propose for IVITs.

Second, at the supra-institute level, distributed technological research platforms (PTIs) dedicated to more sophisticated developments, like multi-organ physiological recording systems, virtual reality laboratories, new behavioral arenas in large environments equipped with telemetry, portable equipment for recording activity in real conditions, and any sophisticated solution not realizable at the local scale and requiring the advice and expertise of engineers, will be fundamental. Importantly, these platforms will represent an opportunity for technological development and transfer to the industry. Again, with the aim of being concrete, we indicate here some possible examples:

- Full-body physiological recordings devices for embodied cognitive research
- Wireless technologies for enriched cognitive experiments in the lab and real-life experiments with animals
- High-throughput, large-scale movement tracking platforms, to adjust to ecological scales in behavioral studies of model organisms.
- Adaptation and development large scale wearable technologies for real-life experiments with humans

Both levels, as a whole, will work as a fast and efficient resource for cognition-oriented technological development, where the solution found by individual groups will be immediately available to the scientific community, avoiding duplication of effort. Where the local capacity to produce prototypes speeds up and makes research more flexible, while the specific and timely support of high technical level (PTIs) makes possible more sophisticated developments, optimizes (e.g. miniaturization) the prototypes developed at the local level and approaches the transfer to the industry. Both levels would be coordinated in a joint (virtual) structure that would identify niches of opportunity, for development and transfer, and would maintain an updated catalogue of technological capacities for the different challenges.

Similarly, current and future cognition studies require big data collection and storage systems, including shared data privacy and ethics protocols, and automated processes to gather different sourced data (from experimental data to other sourced data (e.g. use of social networks to study cultural evolution or the impact of technologies in cognition). A research program where computational infrastructures, data collection protocols, and data processing software, are shareable and constantly under development through a research community from different research centers, requires some institutional organization and coordination.

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ONE SLIDE SUMMARY FOR EXPERTS

Cognition, collective behavior & consciousness

Cognition Throughout Biology

Common Language and Methods across systems and disciplines

A Unified Cognitive Science

Multidisciplinary Research Teams

ONE SLIDE SUMMARY FOR THE GENERAL PUBLIC

Cognition, collective behavior & consciousness

Understanding Cognition

Fusing Knowledge Disciplines

To understand what is to be a human and the humanity.
To improve education, society, well-being and medicine.
To build a sustainable existence.
Challenge 4

THE MOSAIC BRAIN: SEX/GENDER & THE NEUROSCIENCES

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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 sex of the newborn in human beings 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 and his anatomical and intellectual traits as well (Lopez-Beltran 2004). From this beginning, the meanings of nature were constructed, 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 both at current research projects in the neurosciences and at 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.

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.

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 and sex is a biological category, not necessarily dichotomous.

The biomedical complex –the research community of biology and biomedicine, its technologies and funding systems– has focused in a good part 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.

*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 animal. 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, biomedialized (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. Questions themselves and their answers should contribute to an inclusive research, one that takes into account the whole agents involved in a culture open to the recognition of the differences among individuals in addition to and beyond the sexes.

*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 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.

*Impact in basic science panorama and potential applications*

For many years, it was thought that women did not suffer cardiovascular disease or autism as a result of the biased approaches to women narratives of their own suffering and the lack of research on them in. This misconception came from 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). One of the best illustrations of the implications that our proposal could have is 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 the females are more variable 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; and 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 include in our funding system requirements of including 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.

**Key challenging points.**
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 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 similar 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 mechanisms 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.

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 (3.1.).

With the discovery that epigenetic modifications of the genome can be caused by specific environments and that sex can have an effect on as could be affected by these modifications (see below the discussion), we can envision that experience, nutrition, stress can impact on the brain function. 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 tells 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 (this implies no differences in neuroanatomy but rather molecular) between males and females, however it is not fully understood how molecularly defined are the neurons within these brain regions, how do they interact 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 operated 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, 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. 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).

**CSIC advantage position and multi/inter-disciplinarity.**

A very productive scientific community of expert scholars in the neurosciences and genetics is being very active in Spain for decades already. The role played in this by CSIC researchers is remarkable as it is shown by scientific production numbers at least since the 1990s (the works of Isabel Gómez Caridad and María Bordons so demonstrate, one among the most recent is found in reference (Bordons 2019)). This scientific community has obtained international recognition, extensive European funding and also national science policy support. Younger scholars have joined in when back from their postdoctoral periods, or their long stays in the US or other European countries’ research institutions. Although there is still a serious need for budgetary growth regarding the recovery of the so-called brain drain, it is the youngest researchers who are displaying the riskiest and innovative research projects nowadays.

CSIC does not have an institute that only treats sex and gender issues such many women health institutes across USA and Europe and institutes of women and gender studies in many Spanish universities. However, a few institutes already study the brain or brain disorders and their social and biomedical developments from many different perspectives: Instituto de Neurociencias de Alicante, Instituto Cajal, Centro Nacional de Biotecnologia, Centro de Biología Molecular Severo Ochoa, Centro Andaluz de Biología
del Desarrollo, as well as the Instituto de Filosofía, and Instituto de Historia. With the guidelines and challenges exposed here, the advance will be pronounced, but only if the suggestions proposed here are acknowledged and implemented. CSIC has an excellent position to achieve the leadership in sex and gender research because of its multidisciplinary nature. The creation of a virtual institute or a platform for instance through the website proposed will position the CSIC as a leading institution, implementing the guidelines and challenges proposed in this text but also developing a role of curating preexisting research.

**Plan and resources**

We propose an experimental scheme and research approaches in order to tackle the challenges exposed. In many cases, the proposed plans go beyond the study of the brain and should be applied to other fields of research. Importantly, we would like to emphasize the mandatory requirement of including women and female in research conducted on both human subjects and animals. The experimenter needs to acknowledge that as human beings we, scientists, are part of society and carry the baggage of culture. Then, we need to abandon stereotypes that can feed sex and gender inequalities in our research and finally communicate our results making use of an inclusive and accurate language.

1. **Guidelines for research**

This group would like to propose a set of guidelines for the research projects in the neuroscience, in biomedical research that involved sex, sex determination or that have sex as a variable. Such guidelines would aim to contribute to the awareness among the research community of the need to fit in the EU requirements in the designing and performance of research projects and to encourage a productive interaction with gender studies (Schiebinger and Klinge 2013, Gendered Innovations. European Commission 2013).

 Neuroscientists need improving expert communication and with the public by adopting a few changes in the way we report effects of, or correlation with, sex. There is a bidirectional flow between language and our convictions and perception. This has been reviewed elsewhere (Maney 2016):

1) We should use the term “sex effect” or “correlation” instead of “sex difference”.
2) When possible, data are best presented in a way that allows readers to see the degree of overlap.

3) When we see that our results have been misinterpreted or misrepresented, we should publicly take issue.

4) Sex needs to be included in experimental designs. This is especially relevant when not hypothesis driven data collections are produced.

We need to create surveys that make sure every researcher, and every member of an evaluation committee of any kind are aware of the language and the existence of CSIC’s available resources regarding gender biases in research and academic life (see the reports available at the website of the Comisión Mujeres y Ciencia del CSIC). Moreover, as article and grant reviewers, we should be proactive and demand an experimental design, explicit justification and the required language that follows these guidelines.

2.. Creation of a website or platform

This challenge on sex, gender and the brain is presented here by proposing a particular way of sharing: An open network of research projects and its gendered innovations/meanings/embodiments is proposed here as a space of interchanging and encounters. This would require an attractive and simple, user-friendly designed website to promote access for both researchers and a public audience – at a time when public science in being regarded as a new space for social thinking and scientific practice in everyday life for non-experts but committed citizens. This space would propose, include and publicize periodical activities to promote encounters between researchers from the diversity of disciplines in the CSIC areas and other academic institutions in Spain and abroad. Good references for this are NIH Office of Research on Women’s Health (ORWH) and EU European Institute for Gender Equality (EIGE) guides on gender and the website examples in setting up guidelines.

Moreover, the general CSIC website needs to become a reference platform where we can encounter a good description of the institutes, projects, etc. in general for CSIC but particularly to find validated resource for sex and gender research. Additionally, this would be a basic and powerful tool to establish collaborations between the high diversity of CSIC institutes.
3. How to study sex differences in the human brain

Rigorous studies in their conception, interpretation and in the way data are shared are necessary. The research being performed nowadays does not allow to reach clear, definitive conclusions for several reasons:

- Every research study is based on human interpretation as empirical evidence of pre-existing stereotypes, going backwards from a searched behavior and correlating it with a certain anatomical feature. We have to be cautious, critical and aware that we, as researchers, are immersed in an unbalanced society and therefore our data interpretation could be biased. The human brain is influenced by environment and experience (plasticity and permeability) that modulate a basal layer of a hardwired design. We are far from understanding to what extent the connections between anatomy and behavior work.

- Most studies are based almost exclusively on neuroanatomy that might not be the best characteristic and traditionally was measure with an imperfect methodology: Early reports were restricted to postmortem, usually of unpaired, old or unhealthy human samples. Nowadays, most of the studies are based on global imaging techniques that measure brain activity (fMRI) and connectivity (DTI) and we lack fine evidence coming from histological or molecular analysis (more prevalent in animal models).

- Imaging techniques are not intuitive or accessible and it is not easy to interpret so we rely on the investigator running the experiment and therefore requiring an unconditional confidence. Thus, a critical reading of those works is required. Sharing the data through open platforms and encourage the performance of meta-analyses.

- The controlled conditions of the experiments with living organisms in artificial environments are usually taken for granted, and details should be given so as to know the isolation/artefactual situation of the experiments and the animal system used related to sex, chromosomes and neurons. The transition from species to species animals to humans is usually taken for granted while it has to be taken with caution even if it is the best approximation we can take. There is extensive work on rodents and we should be careful and take into consideration that sex determination is a process highly specie-specific. But this can also be viewed as a strength, once we observe a common feature phylogenetically conserved, it is parsimonious to assume the same is occurring in humans, yet the diversity of variables impacting human brain development can never be fully modeled in a rodent.
4. Study of the effect of environment on the brain of males and females

We have identified several challenges (3.2.) but of course these are only a modest representation. Our aim is to emphasize the need of implementation of the role of sex and gender norms into epigenetic mechanisms that operate in the development of the brain. Researchers need to perform their studies by comparing male versus female subjects in different environmental conditions. The categorization applies to whole-genome analyses of epigenetic marks, ATAC-seq, ChIP-seq and similar techniques to eventually understand the impact that the environment has onto epigenetic marks, how the microbiota affects these epigenetic marks or to understand how the “epigenetic echo” operates (a delay in the effects of testosterone on the brain methylome and transcriptome in the male) (Ghahramani, Ngun et al. 2014).

5. Genderizing brain areas and social behaviors

With traditional approaches, it is not possible to identify or manipulate the relevant subpopulations within an area that controls sex-typical social behaviors. The use of genetics strategies to selectively manipulate defined neuronal subpopulations would be essential. We need to start by knowing whether there exist differences in gene expression in the whole brain among sexes or if any other variable, in addition to or instead of sex, may apply. It is necessary to obtain genome-wide expression profiles, in conjunction with spatial information like the recent technology MERFISH (a single-molecule imaging method that allows thousands of RNA species to be imaged in tissues) (Chen, Boettiger et al. 2015), comparing the two sexes. The use of Cre-lox system to express Cre in molecularly define neuron types has permitted projection-mapping as well as functional characterization of these neurons.

Interdisciplinary effort:

- Strategies to map anterograde and retrograde connectivity of molecularly defined subpopulations of neurons. Invest in connectomics to generate refined maps in conjunction with computational neuroscience that will allow us to generate different technologies to eventually observed the brain in action.

- Optogenetic manipulation of specific targets and recordings of activity in vivo during sex-typical social behaviors.
- Information processing: Imaging approaches defining activity patterns in molecularly defined populations (calcium sensors in conjunction with fiber optic cables or microscopy).
- Role of sexually dimorphic gene expression pattern in behavior with conditional knock-out transgenic animals that allow for spatial and temporal disruption.

6. Hormonal effects on neuronal circuits and interaction with sex chromosomes

In male rodents, testosterone is metabolized into estradiol by P450 aromatase. Therefore, in the male brain both estradiol, through estrogen receptors, and testosterone, through androgen receptors can exert its effects. Advances in understanding how steroid-mediated brain differentiation occurs within the sexes is still necessary, as well as the elucidation of the cellular mechanisms of steroid exert in their target cells, the pathways involved in the local production of steroids in the brain. Moreover, is necessary to get a deeper knowledge in the behaviors of genetically modified animals for the hormonal receptors (esr1, esr2, androgen receptor, including transgenic models carrying polymorphisms associated with hormonal receptors themselves and regulatory regions).

Furthermore, animal models are needed to investigate on the diverse interconnected mechanisms that could operate in the control the gene expression in the brain of male and females. The use of mouse models like the Four Core Genotype, where the gene SRY gene has been deleted from the Y chromosome and inserted in an autosome (De Vries, Rissman et al. 2002), are fundamental to approach this kind of intricate mechanisms where chromosomes, hormonal signals and epigenetic mechanisms might converge. These models are powerful tools to study the impact of sex chromosomes in sex differences and uncoupled them to the role of hormones and moreover, they can offer a possibility to unmask additional mechanisms acting in sex determination.

7. Stem cells have sex chromosomes

Sex should be analyzed at all levels, from cells to whole organisms. Cells from both sexes should be used and needs to be reported. To illustrate this, we will mention how the genome of embryonic stem cells with two X chromosomes is hypomethylated relative to those with one X chromosome, and they have reduced expression of the de novo DNA methyltransferases Dnmt3a and Dnmt3b (Zvetkova, Apedaile et al. 2005).
8. Model and non-model organisms and the human sex

In these days where translational science is absorbing funding resources, we should not forget and emphasize that major discoveries in biology have been discovered by studying model organisms like the invertebrates Drosophila or C. elegans. We take advantage of this opportunity to claim the need to continue funding the research of such models of study, for they are cheaper in their maintenance, less bio-ethical problems are involved and moreover, and pertinent to this challenge, they offer the precious opportunity to discover unanticipated molecular mechanisms of sex determination.

More recently, advances in transcriptome analyses and genome editing by CRISPR/Cas9 technology in virtually any specie have made nearly any system genetically accessible. Schemes using CRISPR to perform loss- and gain-of-function experiments will soon provide the genetic manipulations required to inquire the interplay between genetics, and the lack of its impact, and environment in many organisms and, by extension, yield insights into how many other bipotential progenitors manage the same problem during development.

9. Human resources

- Stable positions where a career can be initiated without interruptions.
- Interdisciplinary internships and fellowships for PhD students. Not only enrichment for the students and an opportunity given to them that will not be found in other institutions, but also a way to promote collaboration between different groups that would need to co-mentor the students.
- The CSIC website has to be a reference platform that takes care on the content and this needs constant and good IT support. For this particular sub-challenge, it will be necessary to have dedicated personnel to run, curate, coordinate, find the experts and the platform we propose (see section 2).

10. Big data analysis.

CSIC can emerge as a repository of all the data generated not only by CSIC researchers but in other studies. The power of having accessible data formatted in a common way through user-friendly graphical interphases will give the CSIC an advantage position. Of course, relevant for this challenge is that the data would have to be always available for the different sexes and conditions.
11. Specific funding initiatives for research that includes sex and gender.

- Intramural funding to facilitate the use of research platforms in neurobiology projects considering sex/gender as relevant variable (the “omics”)
- Transgenesis facilities. The generation of animal models
- Connectomics. This item requires hugh investment in technology to image, etc.

REFERENCES


ONE SLIDE SUMMARY FOR EXPERTS

The mosaic brain: Sex/Gender and the neurosciences

- Research on sex/gender and the brain
  - Female brain mechanisms
  - Sex-determinant factors
  - Sex/gender and epigenetics
  - Hormones and neural circuits
  - Model organisms

- Guidelines to generate & communicate science
  - Language matters
  - Experimental designs
  - Interpretation of data
  - Eradicate stereotypes
  - Surveys

- Website
  - Design for Researchers & general public
  - Reference platform to find institutes/projects
  - Collaborative encounters

Uncover Impact of sex and gender in brain disorders
Eradicate biases in society and scientific community

ONE SLIDE SUMMARY FOR THE GENERAL PUBLIC

The mosaic brain: Sex/Gender and the neurosciences

- Research on sex/gender and the brain

- Guidelines to generate & communicate science

- Website

Uncover Impact of sex and gender in brain disorders
Eradicate biases in society and scientific community
Challenge 5
BODY-BRAIN-MICROBIOME INTERACTION

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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). They develop symbiotic relationships that benefit both organisms. The human gut microbiome is mainly constituted by representatives of Bacteria, but also include 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.) contributing to human physiology and health maintenance (Sanz et al., 2018). Gut microbiota alterations may also 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 in the bidirectional crosstalk between gut and the brain, known as the gut-brain axis, constitutes a research field of growing interest (Dinan and Cryan, 2017). This axis is regulated through hormonal, immunological and neural signals, and represents a route through which the gut microbiome impacts neurodevelopmental processes and brain functions (Agustí et al., 2018). Emerging evidence reports causal effects of the gut microbiome on cognitive functions as well as on social, eating and emotional behaviours, like depression and anxiety-like behaviours (De Palma et al., 2015; Dinan and Cryan, 2017, Agustí et al., 2018). These effects are thought to be mediated through different mechanisms, including modifications in factors regulating synaptic plasticity and neural function (like the brain-derived neurotrophic factor and neurotransmitters) and through the regulation of the endocrine and inflammatory pathways. These are effects are driven by microbially produced dietary metabolites as well as by microbial stimuli (lipopolysaccharide, lipoteichoic acids, etc.) of non-dietary nature. In spite of this evidence, the mechanism and molecular mediators of these complex interactions are far from being fully understood.

Physical disorders contribute to the risk of developing mental conditions and vice versa, indicating that our mind and our body are deeply interconnected. This also accounts for the development of comorbidities, which complicates the diagnosis and treatment. Co-existence of different disorders poses a major social challenge, since clinical practice basically addresses individual disorders. Modifiable dietary and lifestyle factors (physical
activity, stress, drugs, social behaviour, etc.) are known to influence brain and body functions. Specially, unhealthy dietary habits have been identified as major risk factors for the development of physical and mental disorders (GBD, 2017). Therefore, the adoption of healthy dietary and behavioural habits could play an essential role in the promotion of health and reduction of disease vulnerability. The diet is also a major driver of gut microbiota composition and function (Portune et al., 2017). Consequently, dietary-health effects could theoretically be mediated and optimized as a function of the individual’s gut microbiome and its response to the diet (Sanz et al., 2018).

Elucidating the biological and molecular basis of the complex systemic communication between the brain, body and gut microbiome as well as the interactions with the diet and the lifestyle may open new diagnostic, preventive and therapeutic avenues for these 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. Deciphering the mechanisms underlying body-brain-microbiome interactions will help to identity new molecular targets and common therapeutic solutions for comorbid conditions.
2. Developing microbiome-based therapies and predictive tools might open new opportunities for improving treatment and management of psychiatric and neurologic disorders.
3. Personalizing lifestyle and nutritional strategies for effective disease prevention as a necessary step toward reduction of the societal and economic burden due to unhealthy dietary patterns and sedentary lifestyle.

**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 health and nutritional applications, and across sectors, from industry to health care and nutritional professionals and associations and the society. At the institutional level, this will promote interactions between the three CSIC global areas; Life, Material and Society.
**Scientific impact**

The integration of the gut microbiome and lifestyle factors in the investigation of the communication between the body and the brain will provide a new conceptual framework to understand biology and medicine. This broader multi-disciplinary approach and vision will enable us to deepen 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 a great potential to opening-up new directions for improving prediction (early diagnosis/prognosis) and diverse aspects of 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’s researchers have been central to this shift, this research field is strategic to project CSIC values and strengthening 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, based on the integration of the personal microbiome and dietary variables and applying holistic approaches that target the body, the brain and the lifestyle of the individual. These advances will boost innovative capacities, especially of the health, biotech and food industries by providing innovative solutions. The results of this research approach will broaden the focus in terms of diagnosis as well as in terms of drug discovery and development. Opportunities will also arise for further developments of personalized lifestyle and dietary strategies for disease prevention. In particular, the human microbiome market represents a great opportunity since 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 represent a major economic and societal burden, particularly when these conditions 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. This 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 reducing disease stigmatization and inequalities (job losses and reduced professional opportunities) and favouring social integration and cohesion, all essential pillars of the sustainable development agenda of the United Nations for 2030 and of the EU priority policies.

**Key challenging points**

1. **Deciphering the mechanisms underlying body-brain-microbiome interactions**

The understanding of the mechanisms governing the connection between the microbiome, the body and the brain is the basis to identify new molecular targets and common therapeutic solutions for comorbid conditions. This communication occurs through neural, endocrine and immune pathways through which our gut microbes and the diet 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 dense crosstalk between enteroendocrine cells and the enteric nervous system, and the multiple connections between the immune pathways and the enteric, autonomic and central nervous systems.

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 vague nerve) and sympathetic branch of the autonomic nervous system (ANS). The vagal nerve is identified as one of the most important neural pathways mediating the bidirectional communication between the gut and the brain (Forsythe et al., 2014), while the connection with the sympathetic nervous system remains largely unknown. 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).

- **Enteroendocrine pathway:** Enteroendocrine cells (EECs) are specialized cells of the gastrointestinal tract that produce neuroendocrine molecules with primary functions for example in energy metabolism (e.g. appetite, insulin signalling), which vary in response to the gut micro-diet interactions. Emerging evidence indicates EECs also modulate the
ENS activity and express innate immune receptors, suggesting additional roles in neural and immune signaling.

• **Immune and HPA pathway:** 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). On the other hand, functional adrenergic receptors and glucocorticoid receptors also expressed in immunocompetent cells, which suggest new types of interactions to be explored.

The development of new experimental model is also critical to discriminate interactions between the different organs and systems and to facilitate the identification of possible preventive/therapeutic targets. The advances in the development of in vitro models such as 2D and 3D cell cultures, based on established cellular 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 important to better mimic 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 permit to 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 are still very elementary. Furthermore, advanced approaches that enable us to monitor the real-time bidirectional communication between the body, the brain and the gut microbiome in vivo are 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 further therapeutic trials.

### 2. Developing microbiome-based therapies and predictive tools

Considering the limited efficacy of current therapies, medical or psychological, for psychiatric and neurologic disorders and, specially, the difficulties in managing mental and physical comorbid conditions, the discovery of new mediators and moderators of these disorders offers new opportunities for improving their management and reduce their high societal burden. Of these, the gut microbiome through connection to the brain and peripheral tissues represents a tractable personal 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 faecal transplants demonstrated that those strategies might ameliorate or intercept the disease
development in experimental study models. Further understanding of which are 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 efficacious microbiome-based therapies (Romani-Pérez et al., 2017).

When considering the potential of the microbiome to better inform therapeutics, it will be critical to gain a deeper understanding of the microbiome-drug interactions and their consequences. Scientific evidence suggests that a large number of non-antibiotic drugs (up to 24% of human drugs) might impact on keystone bacterial species of the intestinal microbiota, with possible downstream effects on human health (Maier et al., 2018). Gut microbiome, in turn, might be involved in the primary or secondary biotransformation of drugs using its enzymatic machinery or through host-micro co-metabolic processes, influencing the pharmacokinetics, efficacy and side effects of drugs (Turnbaugh, 2018). A special case seems to be antipsychotics, which showed important ability to inhibit commensal intestinal bacteria and this could 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 could 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 of the person’s microbiome as well as for drug repurposing.

The microbiome information could also serve together with other variables for early disease detection, prognosis and prediction of response to therapies. 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, so far, diseases have been investigated and clinically addressed as individual entities. The discovery of modifiable factors that help maintain the 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 of the individual 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.
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 key to reduce the societal and economic disease burden. Unhealthy dietary patterns and a sedentary lifestyle are major contributors to non-communicable diseases, varying from cardio-metabolic to psychiatric disorders. For example, the adherence to Mediterranean Diet and diets rich in fibres have shown promising results for both cardio-metabolic and mental disorders, like depression (Dinan et al., 2019). The role of specific essential nutrients (e.g. PUFAs, vitamins, 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 have not always shown definitive results, 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 amount 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, the understanding of the microbiome’s influence on dietary health effects is rather 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, improve cognition, improve mitochondrial function of neural cells, and even increase neurogenesis. However, an important challenge that remains is to understand why a percentage of exercise practitioners may not benefit and how exercise routine could be tailored to each person according to the hormetic characteristics. The 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 from which, potentially negative effects accumulate. There is no definitive evidence about 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 usually based on the net neurobiological evidence obtained from laboratory animals, lacking the subjective components obtained in human studies.
CSIC advantage position and multi/inter-disciplinarity

CSIC covers all research fields relevant to this topic, from the most fundamental to applied. The wide experience and expertise of the CSIC research groups ranges from neurobiology, animal behaviour, neurogenesis, molecular biology, microbiology, nutrition, food technology, biochemistry, chemistry and metabolism. The multidisciplinary character of our institution represents an important asset to address the above-described challenges. CSIC counts on excellent groups focusing on the human microbiome (IATA, CIAL), the central nervous system (IN, IC, CBM), the immune system (CBM, CNB), food science and technology (CIAL, IIM), and the interactions between external factors related to the lifestyle habits and individual’s health status (transversal aim). CSIC should favour integrated, multidisciplinary research within the framework of the Spanish State Research Agency (Agencia Estatal de Investigación, MICINN) and through new initiatives like the deployment of cross-cutting scientific programmes designed to foster national research consortia to reinforce research on the gut-brain axis. These programs should bring together experts in different areas of research to tackle the development of new models to study gut-brain axis and to elucidate how it regulates brain function and dysfunction as well as systemic effects. CSIC should develop and support structural initiatives to promote interactions and ensure effective response to these major societal and health care challenges through reactive research or research on emergent topics such as microbiomics that may offer some unique opportunities to science and medicine. These interactions should help building bridges of collaboration between CSIC and other national research institutions such as the network for the cooperative research in biomedicine of the Instituto de Salud Carlos III (ISCIII - CIBER). These collaborations would strength the connection between CSIC groups and research driven within the Spanish national hospital network. Promoting the collaborative work between CSIC research groups and clinical investigators would help translational research, which is at the heart of the Spanish Strategy for Science, Technology and Innovation.

CSIC should step up the development and dissemination of new scientific technologies to address research on emergent topics such as the gut-brain axis. Good research not only relies on state-of-the-art shared platforms/infrastructures but also helps to improve and develop them. Although some CSIC institutes maintain small sequencing facilities (e.g. IPBLN), the lack of adequate support and investment in next-generation
sequencing technologies and bioinformatics is greatly affecting competitiveness of CSIC researchers, who miss strategic opportunities. This aim should be developed in the form of a new institute or as a transversal program. It is of utmost importance for CSIC to prioritize large and sustained investments in research facilities and to foster the incorporation of innovative cutting-edge technologies to its research laboratories and groups. These efforts are instrumental to help secure CSIC’s position at the forefront of biomedical research in the coming decade.

**Plan and resources**

To maintain a strategic national and international position in this research field, the following needs should be covered:

**Human resources:**
- Stabilization of positions for young scientists (Ramon y Cajal, Juan de la Cierva and Marie Curie fellows). This could be facilitated by specific actions that help to prolong the short contracts of Marie Curie and Jun de la Cierva fellows who are still unable to get a Ramon y Cajal.
- Creation of new positions for technicians and “titulados superiores”, who are as critical as researchers, to maintain the activity of our labs and facilitate the uptake of innovative techniques.
- Direct support for preparation of project proposals, specially, European projects (for identifying partners, drafting proposals, etc.)
- Additional administrative support for project justification, travels and meeting arrangements, etc.

**Infrastructures**
- Germ-free facility to perform high level research related to microbiome-host interactions. This will be a unique distinctive infrastructure of CSIC in Spain.
- A more complete next generation sequencing service with competitive prices.
- Bioinformatics and biostatistics units and computing infrastructures for big-data analysis
- Repositories of curated big-data associated with epidemiological and clinical metadata
- Biobanks of biological material (stools, brain tissue, etc.) that will enable CSIC to have a competitive position in large projects, validation of new biomarkers, perform retrospective studies in the biosamples to test new hypothesis, to complete the follow-up of large cohort studies, etc.

Collaboration with clinicians/hospitals and stakeholders
- Creation of a platform that facilitates the collaboration between CSIC and ISCIII and patient organizations for sharing epidemiological data of existing cohort studies, samples, equipment, participation in common projects, etc. Standardization of protocols for sample collection and storage and rules for sampling sharing (e.g. with an emphasis on samples for microbiome analysis not systematically included in epidemiological studies so far).
- Creation of an environment (project calls, platforms, meetings, etc.) that facilitate the cooperation with the productive sectors (pharma and food industry) and the transference of knowledge to the society.

1. Deciphering the mechanisms underlying body-brain-microbiome interactions

a. Exploring the routes for microbiome-body-brain communication. This challenge is essential to develop new therapeutic strategies. This is a medium-large scale challenge due to its ambitious nature. This could be addressed in 10-15 years with great resources and in collaborations with a network of research groups.

  • Neural pathway: Key tasks are (i) to fully understand how gut bacteria and bacterial by-products activate the vagal afferences that transmit the signals from the gut to the brain and the neuro circuitry underlying the effects of vagal stimulation on brain, behaviour and body functions, (ii) to identify new interactions between the gut microbiota and the sympathetic nervous system and how they influence the gut to brain-body communication and functions, and (iii) understand whether the production of neuroactive molecules by the gut microbiota could influence the functioning of the ENS and the CNS and the biological consequences.

  • Enteroendocrine pathway: Key tasks are as follows: (i) understanding the mechanism whereby the stimulation of EECs (through the contact with bacterial by-products) impact the immune system and the downstream effects on brain and peripheral...
organ functions and (ii) the paracrine signalling between EECs and neurons of ENS, which may act a sensorial channel in the bidirectional communication between the gut and the CNS.

**Immune and HPA pathway:** Key research tasks are: (i) to understand how the gut microbe and the diet modulate immune signalling through interactions with the ENS and the sympathetic arm of the ANS and (ii) to understand the downstream effects of the HPA axis on brain and body functions. This understanding could help, for example, to identify 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 the HPA-immune crosstalk.

*b. Developing new experimental models.* This is a medium scale challenge. This could be addressed in 10-15 years with ambitious resources and a team of experts in neuroscience, microbiology, bioengineering and bioinformatics. It will be necessary to advance in the development of new *in vitro* models, such as (i) microfluidic gut-on-a-chip models that permit mimicking 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, and (ii) multi-organ/body-on-a-chip models to explore *in vitro* inter-organ interactions (Harjes, 2019), including those occurring through the gut-microbiome brain axis (Raimondi et al., 2019). All these will serve as discovery platforms that will enable us to perform large scale screenings of potential therapeutic molecules and bioactive agents (intestinal bacteria and products thereof) with higher predictive potential before using costlier *vivo* models.

In addition, development of *in vivo* models will also present specific challenges to be addressed such as (i) the design of microdialysis probes that enable a prolonged, automatized and more comprehensive monitoring process of the brain activity under other body site stimuli. The application of optogenetic techniques to *in vivo* models coupled to microbiome-related assessments would be very useful to progress in the understanding of the underpinning mechanism that govern the brain-microbiome communication and functions at cellular level.
c. Developing microbiome-based predictive tools and therapies. This is a medium-large scale challenge requiring funding over 10-15 years with a team of experts in different fields like microbiology, molecular biology, neuroscience, pharmacy, nutrition and computational modelling, among others.

To advance in the development of microbiome-informed predictive tools and biomarkers of the 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 the diet, lifestyle and psychosocial stress, as well as big data generated by advanced technologies, including brain imaging and multi-omics readout (metagenomics/transcriptomics, metabolomics, etc.), which would reflect the result of body-brain-microbiome interactions with the environment. This will allow attaining a more comprehensive understanding of the mediators and moderators that determine our health trajectory and leverage information from larger-scale but less phenotype epidemiological studies. These advances are key to identify robust drivers of the inter-individual variability and disease susceptibility, validate biomarkers for early detection of departure from “normality”, develop friendly use prototypes for the biomarker detection and computational models/algorithms that help to predict our individual 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.

To advance the development of microbiome-based therapies, key challenges include (i) prove and validate causality between specific bacteria/bacterial consortia and health outcomes in robust study models; (ii) leverage existing bioinformatics tools and combinatorial chemistry for the discovery of structurally new microbiome-produced metabolites/molecules and their targets as candidates for new therapeutics; (iii) replication and up-scaling of intestinal bacterial cultures for ensuring safe microbiota enrichments and replacements; (iv) develop miniaturized delivery systems of microbiome-based products targeting specific organs and functions.

Furthermore, the assessment of the therapeutic action in humans will also benefit from new technologies. Advances could also 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, in humans. This combined with other existing
strategies for monitoring gut microbiome activity and body functions could be of much help to obtain information about the gut microbiota-brain axis function in humans.

The future development of any new potential therapeutic drug will have to take into account 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: (1) integrate the microbiome as an additional biological variable in pharmacokinetic and pharmacodynamic studies for fine-tuning dose-response and side-effects assessments and identifying new pharmacological uses for existing drugs (“drug repurposing”); (2) Integrate microbiome data in pharmacological modelling to precisely predict the patient responses and new generation of therapeutics based on microbiome components and functions or targeting the person’s microbiome.

d. Personalizing lifestyle and nutritional strategies for effective disease prevention. This is a medium-large scale challenge requiring funding over 10-15 years with a team of experts in different fields like nutrition, food science, microbiology, molecular biology, neuroscience and computational modelling, among others.

Key challenges in this field are to progress towards (i) personalized nutrition and lifestyle strategies (physical exercise) through the integration of all biological variables into algorithms that predict the individual responses to dietary changes and to physical exercise with the support of more robust assessment tools; (ii) identify differences in sensitivity to dietary effects considering different developmental stages, age and the overlapping comorbidities; (iii) develop microbiome-directed foods tailored to the individual. All in all, this will increase the efficacy of dietary 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 important.

REFERENCES


ONE SLIDE SUMMARY FOR EXPERTS

Body-brain-microbiome interactions

**What**
1. Deciphering underlying mechanisms
2. Identifying therapeutic targets and predictive biomarkers
3. Lifestyle and nutrition

**How**
- Experimental models of gut-brain, microbiota and multi-organ interactions
- Multi-omics, imaging, computational biology, machine learning
- Personalizing nutrition/lifestyle

**What for?**
- Improving health, well-being, productivity and social integration
- Disease prevention
- Early disease detection & improved therapies

**Multidisciplinary CSIC centers + Leading international experts**
From different disciplines and techniques.

ONE SLIDE SUMMARY FOR GENERAL PUBLIC

Body-brain-microbiome interactions

**Explaining microbiome-body-brain interaction**

**Developing new predictive tools and therapies**

- Making the microbiome more human and meaningful for our health
- Understanding and providing microbiome-based solutions to mental and physical conditions (depression, cardiovascular disease, type-2 diabetes, etc.)

- National and international scientists, experts in different fields and techniques
  - Microbiologists, bioinformaticians, nutritionists, food scientists, pharmacists, psychologists, psychiatrists, and others
Challenge 6
EXPOSING THE ROOTS OF MENTAL DISORDERS

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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 1) (GBD, 2016).

Figure 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 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.

Mental disorders are likely to have multiple etiological causes, including genetic and
epigenetic, biological, psychological, social and environmental risk factors, i.e. stressful
eye 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 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.

Figure 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.

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, 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 3).

**Figure 3.** Translational readouts measured in animal models of mental disorders and in the evaluation of drugs and other therapies.

**Impact on 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.

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

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. The generation of suitable cellular and animal models is vital to achieve these goals.

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 integrate findings from animal 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 cognitive behaviour therapy (CBT) are attracting serious attention. 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. Therefore, development of diagnostic tools is urgent.

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.

CSIC advantage position and multi-interdisciplinary approaches

The CSIC counts with excellent research groups devoted to study mental disorders from different perspectives. These include basic brain function, pathological mechanisms, therapeutic strategies and social, cultural and historical aspects. These groups have multidisciplinary expertise that comprise, among other disciplines, systems, molecular and cell biology, genetics, biochemistry, behaviour, electrophysiology, neuroimaging and neuropharmacology.

They are spread in different research institutes all over Spain. Researchers at the Institute of Neuroscience (IN, Alicante), Severo Ochoa Centre for Molecular Biology (CBM, Madrid), Cajal Institute (Madrid), Institute of Biomedical Research (IIBB-IDIBAPS, Barcelona), Andalusian Centre of Molecular Biology and Regenerative Medicine (CABIMER, Seville), Institute of Parasitology and Biomedicine “López-Neyra” (Granada), among others, investigate the biological and pathological mechanisms of synaptic plasticity, neuronal development, brain circuits, glial physiology and brain genetics and epigenetics. Identifying new therapeutic targets, as well as applying stem cell therapy to mental disorders represent some of the main focuses of interest. Design and generation of new compounds fostering brain self-repairing mechanisms and neurogenesis are goals of research at the Institute of Medicinal Chemistry (Madrid).
Neuroimaging is used to determine brain structural and functional changes in mental disorders at the IN and at the Institute of Biomedicine and Biotechnology (Cantabria). At the IN they also have a platform for omic analysis in the brain including single cell level analyses. Researchers at the Institute of Microelectronic (IMSE-CNM, Seville) are involved in the development of microelectronic devices that can be implanted in the brain of patients and could serve to register alterations, predict crisis, and contribute to overcome them by local stimulation or drug delivery. Social, cultural and historical aspects of mental disorders are analysed at the Centro de Ciencias Humanas y Sociales (Madrid). The CSIC is in an excellent position to lead the generation and characterization of much needed mouse models for mental disorders and share them with the national and international scientific community. The Transgenesis Service of the CBM-CNBM allows rederivation of mouse embryos and cryogenic preservation and uses advanced technology, including TALENs, ZFNs and CRISPR/Cas-mediated gene editing, to generate mouse models. In turn, the IN has a dedicated space and state-of-the-art equipment for mouse neurological phenotyping, particularly oriented to assess endophenotypes associated with mental disorders.

To achieve their aims researchers at CSIC institutes join efforts with an extensive network of national and international collaborators. Among these cooperative efforts is the association with the stem cell network Iniciativa Andaluza en Terapias Avanzadas (Junta de Andalucía) and collaborations with the University of País Vasco (UPV/EHU), the Center for Autism Research and Treatment of the University of California Los Ángeles (US) or the Neuroscience and Mental Health Research Institute at Cardiff University (UK). The CBM-CNBM hosts the Spanish EMMA (European Mouse mutant archive) node for the generation and characterization of mouse models (INNOTECH) and takes part of the EU framework project INFRAFRONTIER towards enduring mouse resources and services advancing research into human health and disease. A special mention deserves the collaboration with clinicians and hospitals, many of whom belong to large Networks focused on mental disorders such as CIBERSAM (Salagre et al., 2019). Beyond sharing patient samples and tissues, several clinical trials are ongoing to assess safety and efficacy of compounds that had been pre-clinically tested in basic research CSIC laboratories (e.g. IIBB-IDIBAPS, Bellvitge Hospital, Barcelona). CSIC also holds a leading position in the study of social, cultural and historical aspects of mental disorders. Cooperation in this regard involve the interaction with the Instituto Interuniversitario
López Piñero de Valencia and the Universities of Málaga, Castilla-La Mancha and Rovira i Virgili. CSIC leads a European network “The Politics of the Mind. Connections and trajectories” and the Iberoamerican Network of History of Psychiatry, which integrates research groups at the Universities of Buenos Aires (Argentina), Chile, Antioquia (Colombia), Fiocruz (Brasil), Autonoma de México and Instituto Mora (México). Collaboration of research groups at the CSIC with patient associations is another key collaborative element that fosters research funding on one hand, and on the other, diffusion of results to social stakeholders.

Plan and Resources

Below we summarize a number of strategies, which might be taken to address the identified key challenging points in mental disorders, as well as the resources that would be necessary to accomplish this plan in the short and medium term future:

1. Understanding the biological origin of mental disorders:

- **To identify genetic alterations** predisposing to mental disorders that can then be tested by generating animal models or assaying induced pluripotent stem cells (IPSCs) from patients. Twin studies have revealed that major psychiatric disorders have a heritable component that rise to more than 75% in cases of schizophrenia, bipolar disorder or ASD. Large genome-wide association studies (GWAS) have identified thousands of SNPs spread across the entire genome as risk variants for psychiatric disorders. Conditions such as ASD, schizophrenia or bipolar disorder have been associated with copy number variations and splicing deficits on microexons.

- **To establish the patterns and roles of epigenetic modifications in mental disorders.** The studies investigating epigenetics in the human brain face the challenges of difficult access to the brain tissue and the extreme complexity of the nervous system, both in terms of cell diversity and number. It has been proposed that each neuron within the brain may have a unique epigenome that differs from the ones of neighbouring neurons depending on their activation history. New techniques for single-cell transcriptome and epigenome analyses may overcome some of these limitations both by addressing cellular diversity and reducing the amount of necessary tissue. The ongoing refinement and new development of genome-
wide techniques to explore the neuronal transcriptome and epigenome announces an era of discoveries that have the potential to radically change our understanding of brain function and dysfunction. In the near future, these techniques will allow the analysis of epigenetic and gene expression changes in restricted neuronal populations, for example, the cells responding to a given stimuli or specifically affected by a given pathology. Evidence for causality is another important challenge. Although changes in DNA methylation and HPTMs have been reported in many neurological disorders and may correlate with some environmental factors, it remains unclear whether these epigenetic alterations are cause or consequence of the pathology. Innovative technologies for precise manipulation of the neuronal epigenome may enable us to tackle this causality conundrum in the near future (Voigt et al., 2013).

- **To identify specific neurodevelopmental stages or processes related to the pathophysiology.** Many mental disorders have their origin in deviations from normal developmental trajectories, often occurring during “critical” or “sensitive” periods of brain development in childhood or adolescence. These periods are characterized by heightened neuroplasticity that directs the selection of synapses from initially exuberant connections. Mapping the effects of putative causative genes on specific developmental processes continues to be needed. In the case of addiction, it has been proposed that drugs of abuse hijack developmental mechanisms that are very potent at remodelling circuits. Targeting the mediator molecules or signalling might provide therapies with low incidence of secondary effects, as presumably their function is less prevalent or even dispensable in adult brains.

- **To identify and establish the role of alterations in synapse function.** Most mental disorders are characterized by defects in synapse numbers or structure that are caused by defective synapse maturation or pruning or by alterations in specific forms of synapse plasticity. To target these deficits it is critical to define the underlying signalling deficits, map the cell biological and molecular pathways disrupted and determine which circuits are dysfunctional.

- **To precisely determine the output range of particular brain structures** and how multiple regions/areas connect establishing functional circuits taking into account that most mental illnesses are considered as connectopathies, which present complex pathological
mechanisms at the level of connectivity of brain circuits and their encoded information. Optogenetic and pharmacogenetic techniques to selectively stimulate specific cell populations will help to uncover the alterations in connectivity underlying psychopathologies.

- **To define the involvement of non-neuronal cell types such as astrocytes and microglia.** The involvement of astrocytes in nutrient supply and tripartite synapses is crucial for proper neuronal function. The study of astrocyte metabolic properties, ionic and neurotransmitter control in physiological conditions and in the context of mental disorders is needed. Microglia has been linked to synapse pruning during critical periods, and might contribute to the effects of environmental risk factors (e.g., maternal infection or inflammation). Inflammatory mechanisms should be strongly considered as they might explain the periods of disease and relapse that are common in many mental disorders. The high heterogeneity of astrocyte and microglia populations has to be taken into account. Single cell sequencing (RNA-seq) and optogenetic and pharmacogenetic techniques will also help to uncover the individual contribution of glial cells to psychopathologies and their potential role as therapeutic targets.

- **To settle the influence of environmental factors that enhance/diminish predisposition to mental disorders.** Many of them are described by the recently coined term of “environmental diseases”. A broad variety of external inputs have been linked to mental disorders. These include maternal stress or infection, early-life stress, toxicants, diet, education, traumatic events, or socioeconomic problems.

2. **Bridging basic science progress to therapies**
   - **To generate suitable cellular and animal models.**
     
     - To obtain iPSCs from patients and differentiating them to neurons (frequently referred to as iNeurons) and other brain cell types it will help to open new avenues to analyse the cellular pathology in the human scenario and bring us closer to personalized medicine.

     - Modelling of human mental disorders in animals is extremely challenging given the subjective nature of many key symptoms and the lack of biomarkers and objective diagnostic tests. The knowledge acquired on pathological mechanisms will help to design new animal models. An invaluable tool to generate transgenic mice is the CRISP technique, which
greatly reduces time and costs. Optogenetics and designer receptors exclusively activated by
designer drugs (DREADD) techniques will allow the specific stimulation of brain cell
populations in vivo.

- To find effective therapies

- Pharmacology. A major option for many mental disorders is currently the
psychiatric medication. Antidepressants, anxiolytics, mood stabilizers, antipsychotics,
stimulants are commonly used and can help patients but there can be problems with adverse
effects and adherence. Design and synthesis of new small molecules, with ability to cross
the brain blood barrier, which avoid these effects and can specifically treat a given mental
disorder is an urgent need. A promising strategy is the search for compounds that improve
brain self-repairing mechanisms and promote neurogenesis, which has been found to be
decreased in certain mental disorders. Epigenetic drugs may also open up new avenues for
therapy.

- Cell therapy. The capacity of multipotent stem cells to self-renew and differentiate
into a desired type of cell can be exploited to treat mental disorders. More studies are needed
about the appropriate origin of these cells, the process of grafting, differentiation and
maintenance, and on the strategies to avoid neurosurgical procedures for delivery. Lessons
from the attempts of using cell therapy for neurodegenerative diseases can be useful in the
context of mental disorders.

- Oligonucleotide-based therapy. RNA interference (RNAi), antisense
oligonucleotides (ASO) and microRNA strategies provide experimental therapeutics agents
with great potential for cancer therapy and treatment of other diseases. Advances in our
understanding on RNAi and ASO mechanisms and in vivo studies which them indicate that
oligonucleotide-based therapies might soon provide a powerful new arsenal against
comorbid mental disorders/neurodegenerative diseases for which treatment options are
currently limited. Design and delivery strategies for RNAi/ASO effector molecules must be
carefully considered to address safety concerns and to ensure effective, successful treatment
of mental illnesses.

- Genome and epigenome editing. There is a great deal of interest in exploring the
possibility to directly correct genetic alterations using CRISPR/Cas9 technology. In
addition, the recently developed epi-editing methods (Hilton et al., 2015), based on the
CRISPR/dCas9 system or similar tools, also opens up new avenues for therapy. Although
the application of these incipient technologies confronts the common and prominent challenges associated with gene therapy in the brain (i.e., biosafety, cell specificity, accessibility to diseased tissue, etc.), it still represents an important area of development for the near future.

- **Transcranial stimulation.** Development of microelectronic devices with the ability not only to register, for instance epileptic crisis, but also to predict and avoid them by timely and localized drug delivery.

- **Psychotherapy**, CBT, behavioural activation and related techniques. There is a need to favour the development of inter-personal therapies increasing attention for the control, prevention and perhaps (at the least) **durable** treatment of mental disorders both alone and in combination with pharmacotherapy. Moreover, the relationship of pharmacotherapeutic approaches and both psychological and social support is an important part of any drug treatment program.

**To achieve accurate diagnosis.**

- Given the non-availability of brain biopsies, a main goal is to find biomarkers in blood or cerebrospinal fluid that can ideally be linked to acute or chronic disease stages and relapses. Immune-inflammatory markers, autoantibodies (for instance to NMDARs) could be explored in this regard as well as markers of synaptic function for the development of new therapeutic and diagnostic strategies.

- Define genetic mutations, polymorphisms, mis-splicing and epigenetic alterations as biomarkers that could contribute to the diagnosis of specific conditions

- Use neuroimaging to establish patterns of grey or white matter alterations.

All the above could be used not only for diagnosis but also to predict responses to treatment and guide therapeutic options in a step towards personalized medicine.

**Addressing the social impact of mental disorders**

- **Abolish stigma and discrimination.** Too often, the widespread stigma attached to mental health problems jeopardizes the development and implementation of mental health policy. Stigma is the main cause of discrimination and exclusion: it affects people’s self-esteem, helps to disrupt their family relationships, and limits their ability to socialize and get housing and jobs. It also contributes to the abuse of human rights in some large
institutions. Actions can be taken in order to empower people at risk or suffering from mental health problems and disabilities to participate fully and equally in society.

- **Mental health promotion.** It is fundamental to the quality of life, enabling people to experience life as meaningful and to be creative and active. Promote the mental well-being of the population as a whole by measures that aim at creating awareness and positive change for individuals with mental problems and families, communities and civil society, educational and working environments, and governments and national agencies.

- **Prevention.** Consider the potential impact of all public policies on mental health, with particular attention to vulnerable groups, demonstrating the centrality of mental health in building a healthy, inclusive and productive society. Offer targeted support and interventions sensitive to the life stages of people at risk, particularly the parenting and education of children and young people and the care of older people. Develop and implement measures to reduce the preventable risk factors of mental health problems, i.e. harmful stress, violence, alcohol consume, comorbidity and suicide.

- **Assistance.** Build up the capacity and ability of general practitioners and primary care services, networking with specialized medical and non-medical care, to offer effective access, identification and treatments to people with mental health problem. Offer people with severe mental health problems effective and comprehensive care and treatment in a range of settings and in a manner which respects their personal preferences and protects them from neglect and abuse. Design recruitment and education and training programmes to create a sufficient and competent multidisciplinary workforce. Assess the mental health status and needs of the population, specific groups and individuals in a manner that allows comparison nationally and internationally. Provide fair and adequate financial resources to deliver these aims; initiate research and support evaluation and dissemination of the above actions.

- **Empowerment.** Empowerment and advocacy are important mechanisms to address the mental health problems. Poor advocacy and a lack of financial support for service users’ and carers’ organizations hinder the design and implementation of policies and activities that are sensitive to their needs and wishes. The absence of the voices of users and carers from the
process reflects the stigma of mental health problems, and can reinforce negative attitudes. Therefore, it is necessary that all society recognize the importance and the urgency of facing the challenges on mental health and building solutions based on evidence.

To accomplish the above described plan CSIC needs to improve or implement resources like:

**Human resources:**
- In the last ten years the economic crisis has led to an important reduction of research personnel at the CSIC. The necessary renewal has been very limited and, as a consequence, the number and the age of researchers has significantly decreased and increased, respectively. Provision of new staff CSIC positions that allow the stabilization of valuable junior Ramón y Cajal researchers working on mental disorders is urgently needed.

- We also acknowledge the high geographical dispersion of researchers devoted to mental disorders. Different from other areas there is not a coordinated group/platform with focus on these disorders in the CSIC and research groups are spread across the country. Some initiatives to counteract the dispersion and facilitate interaction among researchers could be implemented such as:
  - To foster intramural coordinated projects and internal workshops on specific subjects related to mental disorders.
  - To develop technological platforms (PTI) on these issues.
  - To create a network at the CSIC similar to the CIBERSAM at the ISCIII. In fact, interaction between ISCIII and CSIC teams should be encouraged and the concurrence for same funding sources avoided.

**Common services and specific platforms essential to accomplish this challenge:**
- Generation of animal models, optogenetic DREADD or generation of viral vectors should be centralized.
- Characterization of animal models. Common services with suitable state-of the art equipment to assess mouse behaviour are especially relevant in the context of mental disorder research.
- Next generation sequencing.
- Design and screening small molecules, peptides or ASOS that modulate protein-protein interactions.
- Big data analysis.

**Collaboration with clinicians and hospitals**
- The bench-to-bed transition requires the smooth collaboration between basic researchers and clinicians treating patients with mental disorders. Clinical trials depend on such collaboration. The CSIC must do an effort to encourage and guide its researchers to actively participate in the design of clinical trials and in the search for clinicians and hospitals that can conduct them.
- Sample repositories of fluids, cells and tissues from patient suffering from mental disorders are difficult to find. CSIC could contribute to make them available.

**Contact with other stakeholders**
- Patient associations are key stakeholder in mental disorders. Communication of scientific and clinical advances to these communities by CSIC researchers and mutual feedback is important. Patient associations are often in the position of funding research projects. CSIC should have a flexible administration to manage this type of funding.
- Pharma companies are necessary to bring potential therapies to the market and make them available to patients. CSIC should facilitate contact of its researchers to these stakeholders and provide support for technology transfer activities.

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ONE SLIDE SUMMARY FOR EXPERTS

Exposing the roots of mental disorders

What

CHALLENGES IN MENTAL DISORDERS

Understand their origin
Bridging basic science progress to therapies
Addressing the social impact

How

Understanding:
- neuronal development
- brain circuits
- synaptic plasticity
- genetics and epigenetics
- glial physiology
- Psychosocial factors
- Environmental factors

Developing:
- animal models
- stem cell therapy
- Genome/wide editing
- pharmacology
- psychotherapy
- neuroimaging
- biomarkers
- oligonucleotide-based therapies
- transcranial stimulation

Implementing:
- mental health promotion
- welfare policies
- social awareness
- Patient empowerment

What for?

Understand, prevent, treat and accept MENTAL DISORDERS

Reduce burden of one of the most relevant public health problems

Understand how our brain works

By whom?

CSIC scientists and external collaborators devoted to study basic brain function, pathological mechanisms, therapeutic strategies and social, cultural and historical aspects.

Interacting with clinicians, patient associations and pharmaceutical industry.

ONE SLIDE SUMMARY FOR THE GENERAL PUBLIC

Exposing the roots of mental disorders

What

CHALLENGES

Disturb mind, thinking & behaviour affecting 600 million people worldwide.

Understanding their origin
Bridging basic science progress to therapies
Addressing the social impact

How

Cellular &
- animal models
- Biological &
- psychosocial causes

Diagnostic tools
- Therapies
- Avoid stigma
- Models for mental health care

What for?

Understand, prevent, treat and accept MENTAL DISORDERS

Reduce burden of one of the most relevant public health problems

Understand how our brain works

By whom?

CSIC scientists studying basic brain function, pathological mechanisms, therapeutic strategies and social, cultural and historical aspects.

In collaboration with national and international scientists, clinicians, patient associations and pharmaceutical industry.
Challenge 7
AGING & BRAIN DEGENERATION

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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 cause of disease and death (in the developed world) and the increase in the half-life 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 familial cost, emotional and financial, the total cost 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) (Martinez-Martinez 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.

One 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, frontotemporal 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.

**Impact on basic science 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, acting in concert, 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.

Key challenging points

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.

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; Francesci 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. 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 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 isolated. Evidences demonstrate that lonely older adults are more prone to frailty, mental illness, and are exposed to greater risk of all-cause deaths. 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 (transcranial 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. Finally, 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, as evidences verify.

CSIC advantage position and multi/inter-disciplinarity

CSIC supports and conducts genetic, biological, clinical, behavioral, social, and economic research to better understand the aging process, as well as diseases, conditions, and other problems or needs associated with growing older. One way or another, every CSIC institute with a focus on Neuroscience (i.e. Cajal Institute in Madrid, and Institute of Neuroscience in Alicante) or in cell and molecular biology (CBM, CIB, IIBM, CIB, ICTAN in Madrid and IIBB Barcelona, CABIMER Seville, and many others) perform
pioneer research in many of these lines of research, utilising state-of-the-art methodologies. Furthermore, the majority of scientists leading research in these institutions have a high reputation internationally, entitling them to take part in international networks of research. The combination of institutional support and excellent critical mass puts the CSIC in an exceptional position to attract scientists to lead the aging research field in Spain during the coming decades.

In addition to the hard-core genetics/biochemistry/cell biology research centers, social sciences institutes and teams, such as those in the IEGD, IESA and IPP institutes, have also proven research capacities and developed long-standing strategies to focus on the social components of the aging process. A particular way of delving into research is taking full advantage of social research tools (qualitative methods, interactions with civil society and political organisations, user involvement engagement, national and international, interdisciplinary research networks).

**Plan and resources**

In order to leave a mark in the scientific -international- arena and at the same time be able to generate economic revenues, we need to implement a serious plan that contemplates:

a) hiring the best scientists in the different fields, by creating new PI (Científico Titular) positions, a plan to stabilize the best Ramón y Cajal and Juan de la Cierva fellows whose future research projects fit within the lines here indicated, and a scheme to invite national and international scientists on aging. This strategy should be implemented rapidly to allow for proper training and continuity with the current investigators, who have been sustaining excellent research and are now approaching their own retirement age,

b) equip the potential receiving institutions with the most modern infrastructure: on equipment, to imperatively establish space properly prepared in the different institutions (acoustic isolation, filtered air) for (multiple) behavioural tests that can be used by an ever increasing need to validate cell/genetics/biochemical data with behavioural traits. It is also mandatory that selected centres are equipped with state-of-the-art microscopes for single molecule tracking and manipulation, namely confocal, two-photon microscopy, light sheet microscopy, swept field microscopy and super-resolution imaging.
c) ensure the availability of central or institution-based facilities and services for “omic” approaches, with the corresponding bioinformatics analysis. CSIC should also guarantee the institutional and administrative conditions which can facilitate the setting up of interdisciplinary teams and networks, not only with other CSIC’ ICUs, but also with national and international university research teams. The CSIC brand must be protected and fostered.

Specific research and intervention plans are described below.

1 Systems genetics approaches

There is a need to profile genetic and epigenetic patterns in single cells, cell populations, and circuits (e.g., engrams). These studies will capitalize on previous genome-wide association studies (GWASs), which have identified thousands of genetic loci that contribute to physiological and pathological forms of aging in humans. However, this information provides little mechanistic insight until the loci are translated into genes and pathways that can interact with each other and with environmental factors. Furthermore, it is also necessary to conduct new unbiased longitudinal studies in which the traditional groups (e.g., healthy vs. Alzheimer) are replaced by quantitative variables that can be intrinsic to multiple diseases (e.g., Amyloid/Tau/Neurodegeneration definition).

Epigenetics is an intrinsic aspect of this approach. Epigenetic marks provide a framework for long-lasting changes in gene expression. These changes can accumulate through the years and, if deleterious, undermine brain physiology. Epigenetic marks also operate at a genome-wide scale, and display regulated dynamics whose time-scale is still virtually unknown.

It is also necessary to visualize and modify epigenetic changes in real-time and in vivo (e.g., using opto-epigenetic tools). The causal role of epigenetic changes for disease is just starting to be addressed, for example by CRISPR-based epigenetic editing. In addition, these approaches will engage high-throughput technologies, as well as single-cell genetic and epigenetic manipulations. Accordingly, advanced statistical methods will be required to globally identify epigenetic marks and understand the time-scale of their dynamics.

2. Cell biology approaches
Cell biological approaches will require that selected centres are equipped with state-of-the-art microscopes for single molecule tracking and manipulation, namely confocal, two-photon microscopy, light sheet microscopy, swept field microscopy and super-resolution imaging. In addition, the cell biology of the future should also focus on synthetic biology approaches, to model pathways and circuits at the nanoscale level (in silico cell bio-engineering). Importantly, these approaches should reflect the complexity of the different cell types in the brain, as well as their intercellular communication. All these venues should increase our chances to identify biomarkers and establish treatments that reflect—and could perturb—the status of the brain at a broader level than our current, largely based on single cell approaches.

CSIC should guarantee all omics (peptides, lipids) and sequencing services with a company-like style (advice and delivery), perhaps centralised to a single institution, capable of providing the service to several projects at the same (roughly) time though giving priority, and at subsidised prices, to CSIC researchers. The omics and sequencing services ought to be supported by Bio-informatics/Systems Biology company-like for data analysis/grouping/target identification.

Finally, there is also an increasing need to validate genetics, biochemical, molecular and cellular data with behavioural assays. Dedicated infrastructure for comprehensive behavioural tests will also have to be available to test the functional significance of the identified factors for cognitive function.

3. **Addressing the influence of chronic inflammation and the microbiota in the aging brain**

Chronic inflammation is typically associated with neurodegenerative diseases, and is also characteristic of the aged brain. Since chronic inflammation is well known to be deleterious, anti-inflammatory medicines had been repeatedly tested to stop progression of reduced brain function. However, these attempts have met with failure. Nevertheless, this is not necessarily interpreted as faulty hypothesis, because it may simply reflect our incomplete knowledge on the brain inflammatory response. Therefore, another area to boost in the future should be that of age-related brain inflammation. This is a highly competitive area in which the CSIC must embark to avoid missing important discoveries for targeted intervention.
Inflammatory responses and the neuroendocrine system are also strongly modulated by the interaction with gut microbiota. Still, there are not yet good biomarkers that can be used to link differences in gut microbiota and predisposition to illness. It will be critical to introduce new longitudinal studies to determine human microbiota composition (individual microbiota genome composition, metabolomics) so to make predictions about functional implication (i.e. relation to morbid state).

These combined studies are likely to demand the development of artificial intelligence systems to simulate the interactions between the multiple elements involved and to understand the implications of the massive amount of data that will be generated (see Timmers et al., 2019; Martínez-Martínez et al., 2020; Tan et al., 2020; Livingston et al., 2017).

4. Improving brain function of the old through pharmacological interventions.
There is a wealth of small clinical tests based on approved compounds that show improvement of brain function in the old. One of these is the hypoglycemic drug metformin, which targets a multiplicity of pathways affected during aging, including inflammation, cellular senescence, oxidative stress, proteostasis, microbiome changes (Barzilai et al., 2016). This drug is currently under scrutiny as anti-aging in a clinical trial. Another family of drugs with possible beneficial effects are the cholesterol lowering statins, which also target pathways of inflammation and vascular defects associated to cholesterol deposits and as consequence reduce molecular damage by oxidative stress. The use of angiotensin converting enzyme (ACE) inhibitors and other anti-hypertensive drugs also showed general beneficial effects for the elderly. More recently, the mTOR inhibitor rapamycin or other rapalogs (affecting pathways of autophagy, immunomodulation, cerebrovascular function) showed clinical anti-aging effects (Mannick et al., 2018). A new generation of anti-inflammatory agents targeting the inflammasome is on the way, as well as senolytic agents. Anti-aging drugs generally improve brain function and may prevent or delay dementia, either crossing the blood-brain-barrier or acting through peripheral connections.

In line with the notion of the polygenic nature of neurodegenerative diseases, we need to carry out research to explore new medicines/compounds with capacity to interfere with the negative action of gene polymorphism-trait. This strategy implies the creation of a unit specialised in identifying molecular pathways affected in different gene-
environment combinations leading to disease, in order to then design and test compounds in these models, which would be later transfer to the human situation.

5. **Improving brain function of the old through natural approaches.**

In addition to pharmacological strategies, we must attempt to obtain better brain aging by natural approaches, as old people are, in general, under medication for the treatment of other conditions (hypertension, diabetes, gout, arthrosis, insomnia), which could result in counter-acting pharmacological effects. One of the “natural” strategies with high potential is caloric restriction, which has already shown positive effects on brain functions (such as pattern separation and recognition memory). But, it is not easy to impose caloric restriction to anyone, even less to old people who in general are reluctant to accept new limitations to their lifestyle. It is at this point when dietary supplements may be advantageous. The benefits of some of them have been demonstrated in epidemiological studies (Comhaire and Declerq, 2020; Féart, 2020). We need now to address more specific questions related to the identification of the optimal biomarkers of consumption of the different bioactive compounds in the diet, and the identification of which and how dietary supplements work on the pathological feature characteristics of neurodegeneration. We should also attempt to identify in different animal models the optimal consumption of bioactive compounds through the diet to obtain the desired effect in disease animal models. These goals will require the use of omic approaches for a better knowledge of the mechanisms and food components with neuroprotective activity. In addition, the effectiveness of these treatments should be based on epidemiological and intervention trials with humans grouped by clinical and genetics grounds exposed to different diets.

A third type of strategy to improve brain health refers to physical exercise. There is a substantial and ever-increasing body of evidence indicating that physical exercise interventions have enormous beneficial impact on cognitive performance. This is particularly relevant in modern society since a large proportion of middle-aged and older people are reluctant to practice physical activity, which has been associated with poorer health, increased risk of chronic diseases, lower functional capacity, cognitive decline and ultimately reduced health span and longevity (Kennedy *et al.*, 2008). These facts have moved the field to identify the mode of action of exercise, leading to the demonstration that physical activity increases neurogenesis and neuroplasticity, improves cardiovascular
function (with beneficial impact on the cerebrovascular system), enlarges hippocampal volume (which is accompanied by improved memory function), reduces stress and anxiety, decreases inflammation, and improves insulin sensitivity. Yet, the precise molecular mechanisms through which physical activity improves function remain to be addressed and this should be intensely searched for, to facilitate the identification of molecular mediators of improvement (keeping in mind that it is not always possible to make the elderly practice exercise with the intensity required to obtain beneficial effects).


The cure of hearing and vision deterioration with aging ought to be tackled from a multidisciplinary angle, putting together behind this goal basic researchers working in hearing and vision physiology and regeneration with medical and organic chemists for the design of new drugs and physicists and bioengineers for the design of hearing devices, ocular prosthesis and artificial retina implants. It should be noted that the CSIC houses the single Spanish service intended for non-invasive hearing evaluation (https://www.iib.uam.es/portal/en/web/enni). The essential collaboration with sociologists and clinicians should not be forgotten, the latter has improved in the last ten years with the creation of the CIBERER (https://www.ciberer.es/en/research-programmes/sensorineural-pathology-programme). In summary, CSIC houses the necessary experience, and should only recognize the magnitude of the problem, its implications, its potential and the required means to promote communication between disciplines and support collaboration to fight hearing loss.

7. Improving cognitive performance through social behaviours.

Older people live in social and residential environments that they have built throughout a long life-course, where social networks have been generated that act as a social support. When such social support and networks decrease or disappear, social isolation or loneliness tend to emerge, then affecting their physical and mental health, among other consequences (Rokach, 2019; NASEM, 2020; Kuiper et al., 2016).

The declining of social relationships is usually linked to brain activity, its most important mechanisms, processes and mental and psychological expressions. The plasticity of the brain and its adaptability to personal, social and environmental changes of the aging people is linked to neuronal connectivity and brain functions. This means
that there are some brain areas (amygdala, anterior insula, anterior cingulum, ventral striatum, ...) that respond to stimuli related to social relationships that pose threats (eg. loneliness) or are pleasant (eg. rewards). When cognitive impairment appears as one ages, the brain adapts to the aging process, mediated by various stimuli, being most of a social nature. Indeed, the deterioration of cognitive function has been related to the loss of social relationships that do not help stimulate certain regions of the brain. Mental illnesses also have a notable link with the maintenance of social networks or with their deterioration (loneliness) (Kuiper et al., 2016). The limited structure and density of social relationships (scarce frequency of contact with a reduce number of family members and friends), lack of social support, reduced participation in community activities, etc. all have been linked to depression, dementia, and Alzheimer's disease.

Intervention processes will need to be implemented to alleviate or improve cognitive decline when social behaviors are modified, through psychological or pet therapies, educational programs, shared social activities, leisure and volunteering, befriending or the use of technology.

8. Physical-Social Environment and Brain Aging: an Environmental Gerontology approach

An emerging line of research is indicating that environmental factors influence the underlying biological mechanisms that determine the risk of cognitive dysfunction and suffering from neurodegenerative diseases in older people. Certain environments favor maladaptive behaviors and higher levels of environmental stress among the old people, and are associated with an increased risk of brain degeneration, disease and death (Leon and Woo, 2018). Conversely, enriched living environments, sometimes called ‘therapeutic’ (Calkins, 2018), can stimulate brain plasticity in older (demented) subjects, which contributes to better cognitive and social interactions, sensory and functional skills, and benefits in learning and memory. However, today it is a question of deciphering how the physical-social environments influence the prevention of neurodegenerative diseases and the promotion of successful brain aging.

Environmental gerontology is aimed at understanding and modifying the interaction between the aged person and their physical-social environment to favor active, healthy and successful aging (Luciano et al., 2020). Most older people prefer to age in their usual environments (‘aging in place’), at different scales (housing, community and
neighborhood, community and support environment). In these settings, autonomy and independence are optimized and institutionalization is delayed. However, in many cases, these environments do not adjust to the changing needs of their inhabitants as they age and limit healthy lifestyles, which has adverse effects on their physical and cognitive health. Therefore, it will be necessary in the future to promote age-friendly cities and communities that favour healthy lifestyles and reduce chronic stress, as supported by the World Health Organization (Fernández-Mayoralas et al., 2020). At the same time, we will need to deepen the knowledge of the relationship between the physical-social environment and brain plasticity for a more successful aging among the older people.

9. Enhancing cognitive function by deploying communication technology devices and applications

Structural and functional brain imaging studies conducted in older adults have shown age-related gray and white matter shrinkage, with anterior brain regions exhibiting greater losses than posterior brain areas. The greatest shrinks occur in the lateral prefrontal cortex, the caudate nucleus, the cerebellum, and the medial temporal lobe-hippocampus complex (Ballesteros et al., 2015). Cognitive functions that depend on the prefrontal cortex and the medial temporal lobe-hippocampus complex, including processing speed, working memory and long-term episodic memory suffer the largest declines with age. The failure of these abilities predicts difficulties in the performance of daily living activities compromising independent living and the possibility to prolong a sustainable working life.

Based on the existence of neuroplasticity in the older human brain, different types of interventions have been implemented for boosting the cognitive functioning of healthy and cognitively impaired older adults, particularly the use of computer-based training programs and video games (Ballesteros et al., 2018). An important topic for future research will be the design and validation of ease to use ICT (Information Communication Technology) products and software applications to maintain and/or improve the declining cognitive functions like long-term episodic memory, attention, executive functions, and processing speed. For a future spreading and deployment of ICT device and software products, an interdisciplinary collaboration between different professionals, including technicians, is needed. The involvement of psychologists and technology developers will be required for conducting applied research to investigate the validity and effectivity of
the ICT products, as well as to train the older on how to use the new ICT products. Finally, it is vital to put the older users at the centre, not forgetting they always should play an important role in the development of new technologies (the user-driven approach).

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Aging and brain degeneration

**What?**
- Discovery: Genes and environment diseases behind normal and pathological aging.
- Development: Neurobiology, genetics, epigenetics, programming.
- Understanding: Cell, tissue, circuit, behavior, disease.
- Interventions... to reduce emotional, social and economic impact of pathological brain aging.

**How?**
- Intervention prevention: Genomics, proteomics, metabolomics, transcriptomics.
- Systems Biology: Understanding the whole brain system.
- Functional assays: Imaging, biomarkers, pharmacology, behavior.

**What for?**
- Better understanding... of how our brains lose capacities with age: cognitive, motor, metabolic, of sleep.
- Better knowledge... about why some develop dementia with age and others do not.
- Better strategies... to reduce the emotional, social and economic burden of brain aging-associated deficits.

**By whom?**
- CSIC must take ACTIONS: Foster research capacities and groups to face the challenges on brain neurodegeneration in order age, by using various perspectives.
- Promote the human, technical and organizational conditions, collaborative research networks and synergies with industrial, societal and political actors interested.

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Aging and brain degeneration

**What happens to our brain as we age?**
- How do we lose capabilities? Are there specific clusters of genes that make some of us more ‘normal’ and others not?
- How does the external environment contribute? And our peripheral senses? Our internal organs? And the society?

**What can we do?**
- Reduce the emotional, social, economic impact of diseases associated with brain aging.
- Improve life conditions of the old population.

**New human gene-environment sequencing data**
- New devices
- Cultural strategies
- New animal models

**CSIC is the Spanish leading research institution.**
- Able to put its research structures to meet the future challenges on brain neurodegeneration through various perspectives.
- Qualify to call up its human, technical and organizational conditions, collaborative research networks and synergies with industrial, societal and political actors interested.
Challenge 8
BRAIN & SPINAL CORD DAMAGE & REHABILITATION

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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 dysfunction due to the interruption of blood supply to a brain region that may occur after blockade of a cerebral artery (ischemia) that 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 immune depression that reduces immune competence of the patients and renders 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 an 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. In addition, 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 may be life threatening and requires urgent control (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), and 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 nanoprotesis (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.

**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 economical 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 from up to 30% in 2050. As a result, it is estimated that by 2023 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 & 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 of 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 1000/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 this 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 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 perspective. 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.

Key challenging points

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 neurotransmitter 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 critical 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 brain or spinal cord injury induce depression of the immune system that is mediated by complex humoral and neural pathways connecting the brain 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 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, IncRNA-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 and SPECT (single photon emission computed tomography) imaging provides molecular and functional information, such as inflammation, but it requires the development of novel radiotracers. 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 be able to obtain a better and greater number of brain registration points and better understand the functioning of the brain, but they will also allow better monitoring in brain surgeries and even be able to detect precocious epileptic episodes and be able to act early on these patients.

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, 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 & 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 or how cells "decide" between 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 in brain or spinal cord injury. However, much effort should be devoted at the basic level 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 (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. Further, 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 & 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 and 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 much more 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 a 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 also 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. 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 better diagnosis and better targeted treatments 4) Multimodal data recorded during the longitudinal study 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 BNCl as a major goal for rehabilitation of stroke. Such BNCl 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.

**CSIC advantage position and multi/inter-disciplinarity**

Interdisciplinarity is needed in this field to achieve the above-mentioned goals that need strong coordination between different research disciplines. Notably this field requires collaborations between neuroscientist and immunologists, engineers, chemists, biologists, physiologists, pharmacologists, and bioinformaticians. At the same time, this basic research needs to be developed in close collaboration with clinicians, in order to ask clinically relevant questions, to avoid conflicts with the strict regulatory conditions, and to facilitate translation of research findings to the patients. CSIC is in a good position to lead this scientific challenge because of its rich interdisciplinarity and expertise in different and complementary scientific disciplines. CSIC could take leadership by promoting interactions between the fields of neuroscience, molecular and cell biology, immunology and chemistry for drug development. CSIC should promote working networks to facilitate the transfer of these technologies to in vivo experimental models.
first, and eventually to the clinic. Furthermore, CSIC could reach agreements with health institutions that will improve the access to samples, patients, and their needs. Clinical/basic research needs active networks sharing samples and methodologies. Solid and well-based channels for interaction/collaboration with physicians and clinical experts, to warrant access to human samples and more effective transfer of results to the clinic. This will result in the design of more practical and ambitious projects with better opportunities for funding and capacity of translation from bench to bed.

CSIC has strong capabilities in Neurosciences subareas that are particularly relevant to address this challenge. For instance, there are groups working in neuronal cell death and viability, neuro-glial and vascular communication, cell therapy, experimental models of diseases, and neurorepair and neuroregeneration. CSIC teams working in related domains of research include teams in Neuroscience Institutes like the Instituto de Neurociences in Alicante and the Instituto Cajal in Madrid, and teams in CSIC Biomedical Institutes like the Instituto de Investigaciones Biomédicas ‘Alberto Sols’ in Madrid, Instituto de Investigaciones Biomédicas de Barcelona, Centro Andaluz de Biología Molecular y Medicina Regenerativa, Instituto de Biomedicina de Sevilla, just to mention a few. On the other hand, Neuroimmunology is an incipient field of research that may greatly benefit from the collaboration of CSIC researchers experts in immune cells and immune cell responses in different teams of biomedical Institutes like the Centro de Investigaciones Biológicas Margarita Salas, and the Instituto de Parasitología y Biomedicina López Neyra in Granada. CSIC is well positioned to build up interfaces between immunologists and neuroscientists, and there are groups with background in the field of immunology in acute brain damage.

CSIC has also strong Medical Chemistry teams with activities that are essential for new drug discovery and development of therapeutic targets. Drugs need to be tested experimentally first and pharmacologic studies are prerequisites for translation. Impressive novel developments in photopharmacology allow generation of drugs where the control of drug action on neuronal function is regulated by light. These and other novel developments may have future applicability for the treatment of acute CNS injury and are currently under investigation in teams of CSIC Institutes like the Instituto de Química Avanzada de Cataluña, Instituto de Química Médica in Madrid, Instituto de Química Física Rocasolano in Madrid, just to cite a few examples.
Nanotechnology developments have made notable advances in the last few years for future application in neurogerenerative strategies as well as monitoring biological parameters with interest in diagnostics and prognostics of patients with acute brain injury. Excellent CSIC nanotechnology Institutes developing applications in the field of acute brain damage include the Centro de Investigación en Nanociencia y Nanotecnología in Bellaterra. In addition, there are a number of teams working in nanomedicine in other CSIC Institutes, like the Instituto de Tecnología Química in Valencia.

Microelectronics is emerging as an attractive tool for recovery of neurological function. In the last 5 years, CSIC has invested numerous efforts to produce advanced neural interfaces. These efforts have focused on the research of new materials and the development of microfabrication processes in biocompatible flexible substrates, which makes CSIC the reference in the production of flexible neural interfaces based on graphene for future experimental use and transfer to the clinic. Several lines of research at CSIC combine regenerative medicine and bioengineering technology, including nanotechnology, for treating brain disorders. CSIC also counts with globally recognized experts in nanotechnology that may develop nanosized implanted devices/nanoprosthesis, nanoparticles/nanocarriers, nanospheres, liposomes, and mesoporous nanostructures for drug delivery with putative application to improve the outcome of acute brain injury. Several CSIC Institutes devoted to microelectronics, like the Instituto de Microelectrónica de Sevilla and the Instituto de Micorelectrónica de Barcelona have teams interested in neurorepair and neuroregeneration.

Importantly, CSIC has world-class research groups working on Robotics. The research program in this field heavily builds on the combined research experience in neural engineering, motor rehabilitation and brain plasticity, novel technologies and analysis methods that jointly study how the brain and the spinal cord control movement to then engineer neurotechnology with the goal of restoring motor function. There are teams with strong expertise in this biomedical field in the Instituto Cajal (Neural Rehabilitation Group), and Centro de Automática y Robótica (Group of Neural & Cognitive Engineering). There are reference groups in Europe that focus on similar goals, for instance, the Center for Neuroprosthetics and the Brain Mind Institute, EPFL (Switzerland) or the Bioengineering group of Imperial College London (UK). The main strength of the robotics research lines at CSIC is the focus on novel frameworks based on computational neurorehabilitation modelling as a rigorous methodology that incorporates
longitudinal data, neural correlates of locomotor function, modelling of the patient-robot interaction, and kinesthetic teaching methods for robot control, to boost recovery by promoting beneficial neuroplasticity. Such interdisciplinary mission could revolutionize the state-of-the-art in robot-mediated training and therapy and put CSIC in a leadership position, through identifying and applying a novel neurorehabilitation approach, and thereby unifying the study of computational neurorehabilitation, electrophysiology, robotics and neuroscience.

Plan and resources

Improving CNS protection and diagnostic strategies after CNS injury.

To develop therapeutic strategies to minimize CNS lesions after acute injury we need to extend current knowledge about the pathophysiological mechanisms involved in CNS damage and search for additional mechanisms possibly involved but yet unknown in the pathophysiology of these disorders. Edema can be a critical complication after acute brain injury. The contribution of BBB breakdown and its dynamics is very important but there are many open questions, such as the contribution of transcellular and paracellular traffic of molecules and cells across the BBB after CNS damage. Development of nanovehicles for drug transport across the BBB offer attractive possibilities to improve drug delivery to the injured brain tissue. Fluid dynamics in the brain are complex and there are current controversies about CSF traffic in the brain, dural lymphatics, and other anatomic connections for fluid drainage. Following stroke, impressive developments in the last few years have changed dramatically the management and treatment of ischemic stroke patients. This is due to the growing implementation of endovascular therapies for clot removal from major brain arteries that excel thrombolysis in promoting recanalization of the occluded vessels. Reperfusion offers a novel opportunity to administer drugs intended to prevent or decrease neuronal death or the causes of it (ie. excitotoxicity, edema, oxidative stress, neuroinflammation).

Many biological questions remain still unanswered, like whether and how stress-dependent humoral and neural pathways, circadian rhythms, sleep, the microbiome, and co-morbidities modulate CNS damage. The identification of novel targets and development of drugs for therapeutic intervention are required, as well as investigate repurposing old drugs. In addition, photopharmacology developing caged drugs and photoswitchable compounds where light controls drug activity are also interesting
strategies with application in the study of neuronal function after brain injury that should be explored. Future research should aim at designing and obtaining drugs, compounds, nanoparticles, and/or viral vectors to target specifically and selectively molecules/proteins or cellular/subcellular processes in a particular cell type and in a particular patient (molecule specific, cell specific, patient specific).

Unraveling the mechanisms underlying the crosstalk between the CNS and the immune system will require the identification of signals, e.g. alarmins and danger signals, that initiate inflammation after CNS injury and the subsequent response of glial cells. Injury triggers the infiltration of leukocytes to the brain tissue, but the underlying molecular cues and anatomic pathways for leukocyte recruitment to the CNS, need further investigation. Moreover, further knowledge is required on the dynamics, molecular signatures, phenotype and function of infiltrating leukocytes, as well as the putative long-term consequences of the acute inflammatory responses in the different scenarios of brain injury. The exciting possibility that innate immune responses can be educated through epigenetic modifications to modulate exaggerated inflammation upon CNS injury offers a putative strategy for therapeutic intervention. Targeting chronic neuroinflammation through modulation of immunometabolism or by treatment with stem cells, or extracellular vesicles such as exosomes carrying cargo, like non-coding RNAs, that seem to be able to enable communication between immune cells and neural cells are promising areas of research for the future development of treatments for acute brain injury. Given that post-CNS injury-associated infections can be very severe complications for the patients, we need to find out how to manage the immune response to CNS injury to prevent immune depression without aggravating brain damage.

Biomarkers may provide quick and accurate information with diagnostic and/or prognostic value, or monitor the response to treatment. Imaging systems and tracers to visualize molecules and their subcellular movements or function in vivo in animal models and humans are promising tools with translational applicability, e.g. advanced imaging to monitor neuroinflammation. Rapid high-throughput determinations (transcriptomic, proteomic, post-translational modifications) in biopsy, body fluids (particularly CSF) as biomarkers will be helpful at the bed side. Also, nanosensors for monitoring CNS lesion features, disease progression, and response to treatment are promising strategies to be used in the clinic. Drug delivery across the BBB based on nanotechnology may offer solutions to overcome this barrier.
Exploring novel approaches to promote neural repair and regeneration.

Cell therapies based on different types of stem cells are strategies under investigation that in certain countries are already in use to treat patients with acute brain injury, despite lack of demonstrated efficacy based on large clinical trials. Promising strategies for neuroregeneration include the use of lipid mediators (e.g. resolvins) that dampen neuroinflammation and promote resolution, neuroprotection, and functional neurological recovery after CNS injury (López-Vales & Samuel, 2019). Identifying the role of the immune system in lesion resolution and repair, and molecular signals enabling education and instruction of macrophages to carry out debris clearance, to increase healer activity, and to deactivate inflammation are fundamental steps prior to design immunomodulatory strategies that have a promising capacity to mediate injury repair and functional recovery.

Experimentally, drug- or cell-based neuroregenerative strategies can be used in combination with the development of optogenetic approaches or photoactivatable drugs to promote functional recovery. Studies on nanodevices and nanomaterials that can interact with biological systems are also promising novel tools with neuroregenerative capacity. Final applicability needs research and development in a collaborative effort between different scientific disciplines including nanoneurotoxicity, which is the main barrier before clinical translation. Current research supports the development of multifunctional nanostructures as carriers for drug delivery, or for diagnostic or treatment purposes, ‘theranostics’, with applicability in CNS injuries. Nanotechnology can also be applied to the study of neural cell communication and neuroregeneration. For instance, molecular deposition and lithographic patterning of neuronal-specific molecules with nanometre resolutions (micropatterning approaches) enables the study of cellular communication and signaling. Materials/devices to interact with neurons at the molecular level (such as methods to elicit an agonist response to cloned GABAA receptors are under investigation using techniques such as Atomic Force Microscopy (nanometre morphological responses to micro-electrode array stimulation of neuroblastoma cells). Silver nanoparticles allow studies of the interactions of neuron- and glia-like cells. Functionalized semiconductor quantum dot nanocrystals (quantum dots to elicit fluorescent optical properties of molecular and cellular processes) are also being developed, together with carbon-based structures (C-dots, grapheme, and nanodiamonds) to detect properties of neurons and glia.
Developing cutting-edge technologies for rehabilitation after CNS damage

There is need for determination of the suitable robot-assisted training dose and identification of what should be paired with robot-assisted training in future trials to enhance functional outcomes. Modern computational tools are required to facilitate the role of the clinical researcher to select and tune the available robotic devices for the level of severity of the patients in the rehabilitation hospitals. Developing such means for selecting and optimizing recovering after stroke or spinal cord injury is therefore of great importance. The challenges lie in recent clinical trials that compare robot-mediated vs. conventional therapy in motor impairments, which still show small absolute differences between therapies. We can hypothesize that the reason for this is that algorithmic computational models of learning behavior and of fine-level neural processes, are still to be developed and integrated into a context specific to robot-mediated rehabilitation. The breakthrough insight of computational neurorehabilitation modelling would be that locomotor control in clinical populations would involve an interplay of supraspinal circuits and spinal interneurons which, together with the suitable patterns of interaction with robot control structures, may constitute an interactive mechanism for functional recovery of the sensory motor system. Thus, most likely computational methods grounded in neurophysiological principles may actually improve existing robotic treatments, mapping neurophysiological changes during training with clinically acceptable techniques.

Resources

The current advance of science is strongly linked to novel technological developments. CSIC will need to invest in advanced infrastructures because state-of-the art technologies are mandatory to produce scientific breakthrough. In parallel, CSIC needs to incorporate trained personnel to run core facilities or nodes.

- Infrastructures.

This scientific challenge requires investment in core platforms that are fundamental for the technical advances critical in this field of research. Several CSIC Institutes have core facilities that should be reinforced and equipped with novel technology and should have
the capacity to provide service to CSIC groups of other Institutes by organizing thematic working networks.

1. Mass cytometry and high-throughput cell sorting (FACS, MACS). Cytomics technology is advancing very fast. New infrastructures are a new generation of equipment currently very scarce in Spain. A cytomics node would be a good opportunity for CSIC to lead this field of research in our country and it will represent an enormous advantage for this scientific challenge.

2. Advanced imaging technologies for animal research, including:
   a) Optical techniques: two-photon imaging of living mouse brain; light sheet microscopy for imaging and reconstructing 3D samples; spinning-disc microscopy for fast imaging of living cells/tissues; stereo fluorescent microscope for reconstruction of large fields of view: intracerebral mini-microscopes.
   b) MRI: multiparametric MRI-based imaging (e.g. Functional MRI, diffusor tensor imaging, tractography, spectroscopy).
   c) Software and technical support for image analysis and quantification

3. Omics (cellomics, proteomics, genomics, epigenomics, metabolomics, transcriptomics, single cell transcriptomics) are imperative in many fields of biology and our field is not an exception. The current situation is that laboratories search in the surroundings services that provide support in the different omics fields. This procedure is not very efficient and it is expensive. CSIC should be able to organize cores for research groups in the whole territory to provide high-quality results, including the bioinformatics supports needed for data analysis, at more efficient and competitive costs. Bioinformatics with experience in big-data analysis are critical. Deposit and analysis of big data and highly qualified scientist, bioinformatics, etc. to support multidisciplinary researchers.

4. Robotics: promoting and enhancing European-level efforts led by CSIC such as the H2020 EUROBENCH project* to consolidate a benchmarking framework for robotic rehabilitation systems in Europe. CSIC is a unique position to develop further the

* http://eurobench2020.eu/
infrastructures that are required to become a reference center for validation and testing of rehabilitation and assistive robotics for European stakeholders.

5. Global facilities to generate, phenotype and cryopreserve animal models. Advanced solutions for assessment of brain function using behavioral tests in rodents.

Human resources
Global CSIC facilities must be run by highly qualified researchers and technicians with training and experience in the use or design of these techniques and equipment. However, the current procedures to hire personnel at CSIC, at the different levels from Institutes to research projects is unacceptable because it is very hard to hire personnel according to their scientific expertise. It is not acceptable that scientific personnel has to be hired based on merits that may not be relevant to the specific job positions. Furthermore, foreign researchers, often from countries scientifically more advanced than ours, need to homologate their university titles and degrees to the Spanish system, which is not up to date with many international degrees (e.g. biomedicine).

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