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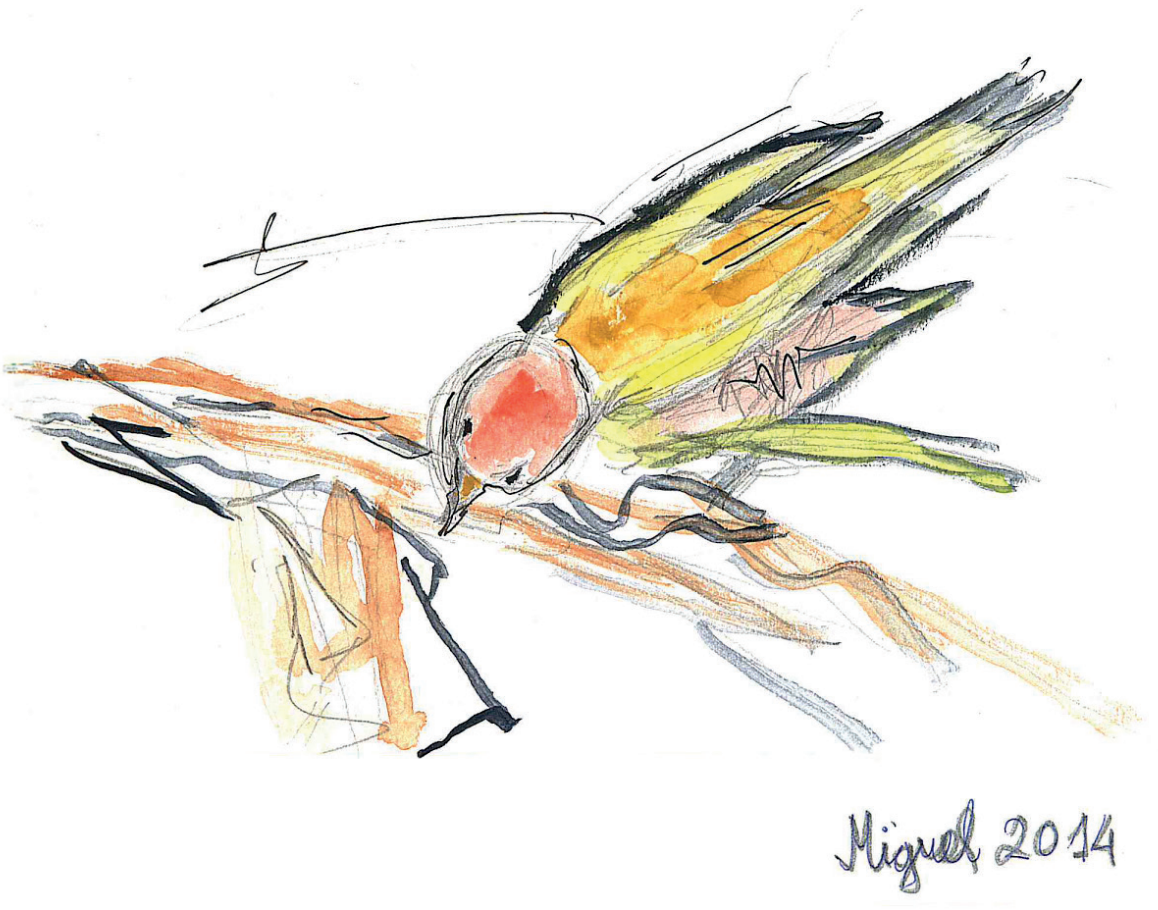
The challenges of Neurodiversity: A Translational Journey into Personalized Medicine in Autism Research

MIGUEL CASTELO-BRANCO



Miguel Castelo-Branco

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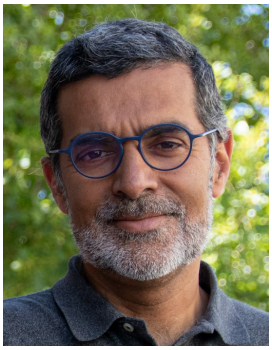

Miguel Castelo-Branco

MCB (MD PhD) obtained his PhD at the Max-Planck Institute for Brain research, Frankfurt, Germany and is now Full Professor at the University of Coimbra. He has held a Professorship in Psychology in 2000 at the University of Maastricht, the Netherlands, where he is now an affiliate Professor. Before, he was a Postdoctoral fellow at the Max-Planck-Institute for Brain Research, Germany where he had also performed his PhD work (1994-1999). He has been the Scientific Coordinator of IBILI, a leading Research Institute in Portugal and is the Scientific Coordinator of the National Functional Brain Imaging Scientific initiative. He is also the Director of CIBIT, a Translational Research Institute, which was evaluated as Excellent by an International panel, was the Director of ICNAS, the Medical Imaging Infrastructure at the University of Coimbra and has entrepreneurial activity.

We won major competitive scientific prizes (single winner in all fields of Research in Human Research, Pfizer and FLAD awards, Great Bial award 2008, Bial Clinical Medicine Award 2023, Best international paper of the year, Human Visual Science, Obstbaum Prize, World Molecular Imaging Society and MR Society) as well as several important European grants (STIPED, BRAINTRAIN, AIMS-2-Trials) and was recognized by the President of Portugal with the title of Great Officer of Order of Prince Henrique, the Navigator. He was until 2021 the Vice President of the European Association for Vision Research (EVER) and from 2014 the Chair of the Visual Function Section, He just finished a major competitive grant (2.5 million €), as PI for a "molecule to man" project of 5 major research

Institutes in Portugal, demonstrating the ability to coordinate scientific teams and complex projects. He has been able to secure funding for individual research projects over 20 million Euros, with top publications in cognitive and clinical neuroscience, invitations for keynote/invited talks in top institutions (MIT, Max-Planck, Karolinska, Univ. of Cambridge, Antwerp and Brussels) and was an invited contributor to the Encyclopedia for Brain Mapping. This also led to important international and national prizes. He is the President of the Brain Imaging Network and many of his previous students have professorship/research positions all over Portugal and several parts of Europe (Donders Centre for Cognitive Neuroimaging, Nijmegen; Univ. of Budapest, Hungary; Univ. of Surrey, Fac. of Psychology, UK, Univ. of Maastricht the Netherlands, and 4 Universities in Portugal).

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I dedicate this book to my son, Minô,
who illuminates the life of our family
every day

Acknowledgements

This work and this perspective on a personal research path would not have been possible without the work of many people from our Research Institutes (CIBIT, IBILI and iCBR) at the Faculty of Medicine, ICNAS and the University Hospital of Coimbra. All their names are mentioned in the articles that are cited throughout this book, but I would specially like to mention the year long fruitful collaboration with Guiomar Oliveira and Jorge Saraiva, from the Pediatric Hospital, and António Macedo and Nuno Madeira from the adult patient clinic.

I would like to give a special word to the Patient Associations, in particular of Viseu and Coimbra who opened their doors and knowledge to help us undergo this endeavour. Without the collaboration of Prazeres Domingues and Elsa Vieira this path would not have been doable.

Finally and most importantly, I would like to deeply thank my Family, Sandra, João and Minô, for all their love, patience, support, and the positivity that they bring into my life.

The challenges of Neurodiversity: A Translational Journey into Personalized Medicine in Autism Research

Introduction

This book represents the summary of about 15 years in research in research in Autism and related neurodevelopmental disorders. This research included genetic, molecular and imaging approaches investigating disease related biomarkers, but therapeutic approaches as well, including formal clinical trials addressing core symptoms in autism. This area has shown huge progress in the last decade, but in spite of all the gathered knowledge, the understanding of its underlying neurobiology still poses daunting challenges. However, we do believe that critical cues are emerging which are informing research and design of therapies. In spite of all the investment, it is now understood that genetic models of disease are still far from transforming psychiatry, and a paradigm shift taking into account the need to integrate multimodal and multilevel information is already taking place. Regardless of the progress made in neurology, where genotype-phenotype relationships are more transparent, we will need a more holistic approach, taking into account that in more complex conditions single factors make small contributions and we will need systems medicine approaches. Eric Kandel (2018) expressed the notion that if we understand autism, we will understand the brain. A key aspect in such comprehension is the concept of neurodiversity. We discuss in this book concepts and tools to address this issue.

This scenario provides a strong challenge to reductionist approaches to address fundamental questions about the neural basis of autism spectrum disorder. Is it looking at synapses sufficient? Based on our collected evidence, we will argue that this is not satisfactory, because autism maybe related to emergent circuit level phenomena, as we will see, and not just synaptic properties. It has been often claimed that autism is a "synaptopathy" (Grant, 2012) but we propose that it may well be a "circuitopathy".

This book is about a particular scientific path in autism research. One could question whether this is a biased trajectory, because in addition to being a medical scientist I am also a father of a young adult with autism spectrum disorder and a member of the board of a local patient association (APPDA-Coimbra) and of the national Federation.

I remember when one fellow senior researcher told me: "researchers in autism often have no clue of what autism really is. Only the ones that observe

them every day in the consultation really understand what it is about, and researchers often forget this". I feel this is to some extent indeed true, but other perspectives must also be considered. I contact with autism 24 hours a day, not just my son, because of the contact with other autistic people in the community, in everyday activities, irrespective of being a medical doctor. What does it mean to observe an autistic individual every year in a short consultation? Does this necessarily mean comprehensive knowledge? Fortunately, it is becoming increasingly understood that knowledge and its application comes from multiple, complementary perspectives. We hope that the focus in the future will be not just on diagnosis but also on intervention and rehabilitation even in public hospitals. Observing the system as a user makes a completely different perspective. Obviously, this influenced the way I perceive translational research and my perspective on how the efforts should be directed "from bench to bedside".

The neurodiversity debate in Autism

The theory of evolution has its foundations on diversity (Darwin, 1859). Variability of features drives evolution, because it increases the repertoire of available options for adaptation to new challenges and environments. Yet our society is driven by "normalization". Should one size fit all? This is particularly problematic when one considers neural and behavioural diversity.

We humans are driven by social norms, established by what "neurotypical" people define as acceptable. However, some non-neurotypical people, especially in the spectrum of autism, may have distinct patterns of social perception and interpretation. For them life maybe extremely challenging in a neurotypical world. Their literality might not be easily accepted, leading to often hidden ethical issues.

History of Medicine in particular has shown that a lot of barriers had to be overcome before homosexuality was neither legally punished nor considered an abnormal condition, and the debate was extended for example to transgender identity. The debate on neurodiversity in autism has increased with the growth of autistic self-advocacy movements, initiated in the late 90s (Singer, 1998). They had the merit to raise ethical and ideological debates

which will for sure influence future research and clinical practice.

There is however a circular conundrum to solve: neurodiversity implies that any advocacy group should be sufficiently diverse to represent all types of neurodiversity. Autism is characterized by extreme diversity of phenotypes and how can all be represented in self-advocacy groups? Some subsets of people with autism spectrum disorder do not have sufficient intellectual ability to engage in self-advocacy and somehow their needs deserve at least the same level of attention from society. Such heterogeneity is well recognized in systems medicine of autism and requires stratification of different profiles.

Autistic people with significant language or intellectual disability are not able to promote self-advocacy and may therefore be deprived of resources which they would definitely need. Can one advocate for those who are unable to do so themselves? This is a very challenging ethical issue. The big question is whether one can recognize that some neurological differences are disadvantageous and might deserve targeted intervention. Other differences not so much and just require a more tolerant social acceptance. The concept of "normalization" or "masking" of autism to resemble neurotypical individuals may place huge burden on mental health, and is not acceptable. Interventions should target improved adaptation to the environment from the social and emotional point of view.

This debate has tremendous impact not only in diagnostic practices in Autism but most importantly in the design of intervention strategies. In fact, this led us to frame in our own research the concept of effectiveness, by using measures of autistic prioritized outcomes. The whole of our design strategy was indeed to involve autistic people as partners of the process. Our intervention practice did increasingly align with the neurodiversity framework and this is directly related with the discussion of our research focus with known advocacy groups at the national and international level. Interestingly, our published clinical trials using neurorehabilitation approaches had significant impact on coping strategies, autonomy, and well-being (Direito et al, 2021; Amaral et al, 2018). These may be considered general effects, not necessarily related to specific neural mechanisms, but they are of extreme significance for autistic people.

The fact that autism phenotypes are highly diverse per se, while keeping some common patterns, led to very interesting insights into

neurodiversity. For example, why are some autistic people endowed with remarkable mathematical skills, while others are lost even with simple counting operations? How does the increase of the rate of diagnosis affect this debate?

The increase in Autism Spectrum Disorder (ASD) prevalence estimates over the last decades (Zeidan et al., 2022) raises the question on whether this increase reflects a new diagnostic trend reflecting an improved perspective on neurodiversity in autism spectrum disorder.

The individual results of the clinical trials we have run were related to emotion recognition and motivation to learn new skills. How relevant are these features to autistic people? We argue that sometimes the results might not be relevant for the researcher but extremely important to the individual. This is the case when results are not meaningful at the group level but are for that particular individual. We had one study participant who was not able to recognise disgust in faces but after the intervention he acquired this ability. Is this new skill relevant for an autistic person in terms of adaptive behaviour? I would say that undeniably yes, because this person can now detect signals in other people's faces that might be of personal value because this can guide simple actions. Learning if a given type of food should not be eaten is an undeniably useful skill.

In the interventional study that we ran using a virtual tool to teach people to use public transportation, we had significant results in terms of learning and anxiety reduction and two of the participants thereafter manifested will to start using public transportation. Here again we had positive group and individual results, but the latter are likely the most significant. Even if hypothetically only 2 individuals would benefit from the intervention in a clinical trial, the cost reduction that is achieved by the increase in autonomy cannot be underestimated.

Personalized Medicine and the Neurodiversity debate

A substantial part of our work has been devoted to the links between personalized medicine and the very heated debate on neurodiversity in autism spectrum disorder. Regarding the concerns of the neurodiversity movement, a central question is which variations in human neurological

development and functioning across humans are just a reflection of natural and likely valuable variation (from the point of view of evolution theory) and which ones might be considered truly pathological or likely maladaptive in any environment.

A major problem is that we cannot simply consider natural but instead social environments. In this case disability might emerge not as a simple a defect in the autistic individual, but rather from his/her unhelpful social environment. This features a social model of disability whereby the main issue is the mismatch between neurotypical and non-neurotypical individuals. We previously have shown in other clinical populations the high relevance of social and family factors in chronic diseases where habit control and impulsivity are extremely relevant (Jorge et al., 2021, 2022). These are also key aspects for the everyday life of autistic individuals.

Neurodiversity and gender bias

Gender effects are known to show strong interactions with neurodiversity in health and disease (Santos et al., 2022). Prior to understanding these effects, it is important to take into consideration temporal aspects underlying variation in phenotypic manifestations. Neurodevelopmental disorders are characterized by variable time of onset, which may be relatively late in the case of schizophrenia. Temporal variations may indeed occur within and across conditions. Variability of first conspicuous manifestations is quite striking in autism spectrum disorder (ASD). Its shows huge lifelong variations in social and communication impairments and cognitive deficits.

In recent years, the importance of biological sex as a pivotal factor in determining variability in behavioural and cognitive features of ASD has emerged under the spotlight. This has a direct impact on diagnosis, estimates of prevalence and therapeutic response. Several theories have been raised as an attempt to explain sex bias not only in autism but in neurodevelopmental disorders in general. These include the extreme male brain theory, female protective effect, maternal stress, and perinatal inflammation and have been claimed to be relevant into variable extents in ASD, attention deficit/hyperactivity disorder and schizophrenia (SCZ) (Santos et al., 2022). This scenario may explain differential disease vulnerability. The combination of

genetic predisposition and biological and environmental factors may explain diversity of time of onset, progression, and severity. This raises the notion whether autism has a fundamentally distinct expression in females, and what biological factors determine these differences remains a big question.

Some forms of autism with more simple and interpretable genetic association may provide some clues into this issue. These forms are known as syndromic autism, because of their more characteristic and stereotyped manifestations. They are less complex because of a single genetic aetiology, which may help disentangle some of questions raised by sex bias.

One can list a set of outstanding questions in this regard: 1. How does sex bias contribute to differences in social interaction, impaired communication, and repetitive behaviours? 2. Why is ASD diagnosed in a proportion of 3:1 ratio regarding diagnosed boys and girls? 3. Is the likelihood of diagnosis dependent on sexual dimorphic mechanisms underlying ASD symptoms, leading to a more masked condition in females? 4. Are animal models helpful in disentangling these questions?

Concerning the latter question, which is the one more directly addressable with experimental approaches, Tsc2+/- mice are an established genetic animal model for some of the manifestations of ASD. We performed an in vivo brain neurochemistry and social vocalization study in this model (Ferreira et al., 2022). In vivo proton magnetic resonance spectroscopy in juvenile male and female showed a distinct metabolite profile in the hippocampus and prefrontal cortex. Behaviour and ultrasonic vocalizations during social and repetitive tasks were analysed. Female mutant animals had differential patterns of social behaviour and presented an increase in repetitive behaviour. Mutant females displayed less complex communication during social tasks, while mutant males only exhibited similarly reduced vocal repertoire during repetitive tasks. These sexually dimorphic behaviours and communication observed in social and repetitive environments are quite intriguing and call for a more detailed consideration of sex bias when investigating autistic phenotypes.

Unfortunately, it still remains quite difficult to address the first three questions, because they require powerful data science approaches that are only now emerging, with the development of national biobank initiatives such as the UK Biobank.

Neurodiversity and brain structure

Uneven profiles of structural abnormalities are quite common in neurodevelopmental disorders. Morphometric techniques investigating cortical and subcortical anatomy have clearly disclosed this fact in autism spectrum disorders, and we were able to observe this scenario even in single gene disorders such as neurofibromatosis type 1 (Violante, et al., 2013). Even the way cortical structures are folded, defined as gyrification, seem to vary dramatically in these conditions (Violante, et al., 2013).

Given that Neurofibromatosis type 1 (NF1) is a monogenic disorder with links with autism spectrum disorder (Bernardino et al, 2021, Lubbers et al, 2022), one would think that it would be easier to understand its associated cognitive impairments and how mutations in the single NF1 gene impact on brain structure. But even in this condition contradictory findings maybe observed. When performing studies in children it is critical to match for age, gender, IQ and right/left-handedness, as we did in the above-mentioned studies. However, careful matching often leads to reduced sample sizes. This leads to a present-day conflict: should one invest in large scale studies, which may often suffer from quality and other methodological issues, or is careful inclusion, matching and more comprehensive evaluations in smaller sample sizes more advisable? One can argue this may depend on the stage of the study, like in the distinct phases of clinical trials, from proof of concept to final validation.

Methodological approaches also deserve careful and constant reappraisal. For example, interpretation issues emerge when one attempts to control for the increase in total intracranial volume, which is a feature in many developmental conditions. Total intracranial volume is taken as a confounding variable but conversely one should consider that it reflects genuine disease related changes.

We found overly larger thalami, right caudate and middle corpus callosum in patients with NF1 as compared to cortical structures (Violante et al, 2013). Remarkably, children with NF1 had significantly reduced gyrification indices than typically developing children in a larger array of regions, in particular frontotemporal ones. Neuroanatomic changes seemed to be quite specific. Our results raise the question that the lower gyrification

indices maybe a result of the fact that a large increase in brain size is not accompanied by a proportionate increase in folding in patients with NF1. Although the identified changes in brain organization and their location may explain patterns of cognitive deficits in the NF1 phenotype, they also point to inextricable levels of neurodiversity.

Brain asymmetries as a signature of diversity

Symmetry breaking is desirable

Diversity is a major trigger of evolution, but evolution itself creates diversity. This is the case of hemispheric asymmetries and this is very relevant to understand neural development (Duboc et al, 2015).

Studying asymmetries of low-level visual processing (Silva et al, 2008, 2010, 2018) is an approach that we have followed in relation with development (Silva et al, 2014). We investigated visual performance asymmetries in development and normal aging using very simple sensory contrast sensitivity behavioural tasks. The hypothesis was that the brain shows distinct and asymmetric response patterns in space and time even for low level stimuli. We probed distinct spatial and temporal channels. One type of stimulus had relatively larger spatial detail (higher spatial frequency) and was not changing in time. The other was less detailed in space (lower spatial frequency) but was changing quite fast in time (at a high temporal frequency - 25 Hz flicker). Different patterns of functional asymmetries were investigated along the life span (N = 258 participants; 8-65 years). We found a left visual hemifield/right hemisphere advantage (better performance) for the larger spatial frequency that was present early in life and remained stable throughout adulthood. In contrast, inferior/superior visual hemifield asymmetries, with a direct ecological meaning, because for mammals most interesting things happen below the line of the meridian, were found for stimuli of higher spatial frequency. This inferior visual hemifield advantage, evolutionarily advantageous for the type of animal we are, emerged early in life and persisted throughout aging. These findings show that both right hemispheric and up/down diversity patterns of dominance in low-level vision emerge early in childhood, and are maintained during aging.

These results suggest a sort of tension, leading to symmetry breaking. Once again, variability and neural diversity are desirable. How can we interpret these notions with the fact that autism spectrum disorder is associated with altered structural hemispheric asymmetries in a very broad range of brain areas? This occurs into a much larger extent than other neuropsychiatric conditions, as shown by large-scale neuroimaging studies (Mundorf et al, 2021). The functional impact of such differently organized hemispheric asymmetries in ASD remains a topic of interest for future research.

Diversity of social attention styles:

which ones are adaptive and how can they be disturbed during neurodevelopment?

The way we visually scan social scenes is widely variable, and patterns of scanning are even more variable in neurodevelopmental disorders. Social attention deficits represent a central feature in autism spectrum disorder (ASD), and implications of the nature of such patterns for adaptive behaviour remains controversial. If visual attention regarding social (faces) as compared to non-social stimuli (objects) is reduced this should in principle be a problem for daily life where demanding social interactions dominate. This distinct cognitive style may therefore lead to challenges in adaptive behaviour. We studied this pattern in an ecological diagnostic context, in children and adolescents with ASD and typical neurodevelopment. They were matched for chronological age and intellectual performance (Mouga et al, 2021).

One way to study visual attention is to use eye-tracking measures of visual scanning, while exploring and describing scenes. We used three known tasks from the Autism Diagnostic Observation Schedule: "Description of a Picture," "Cartoons," and "Telling a Story from a Book." A substantial novelty of this work is that we translated standard neuropsychological assessment into a computerized approach (Figure 1).

We found that diagnostic category (ASD or neurotypicals), task, and the social nature of the stimuli mattered. While the neurotypical participants attended first and longer to faces, ASD participants became similar to this

neurotypical pattern when they were asked to look at pictures while telling a story. In other words, social attention allocation is task dependent, and can change only if specific instructions are provided in ASD. If ASD people decide to be goal directed, their apparent spontaneous attention deficits disappear. When they adopt goal-directed action strategies there are no visible deficits. This raises important questions on the true nature of social attention deficits, and in fact shows that ASD can engage in adaptive behaviour if they are motivated to do so. The issue is how to drive this motivation internally instead of solely by external instructions. This raises important questions on the design of interventions to improve motivation to engage in social interactions. This conceptual point was used in all interventional approaches that are described in this book.

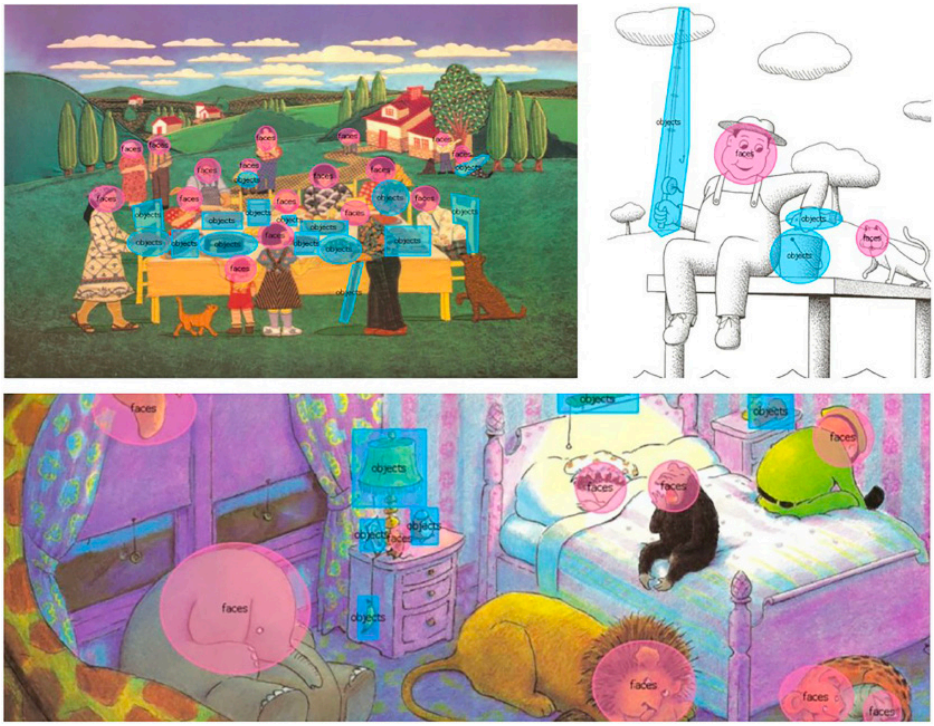


Figure 1. Example of the different areas of visual eye scanning interest (faces vs. objects) defined for our eye-tracking measures, to investigate differences in scene visual analysis. Mouga et al., 2021. Author owned Creative Commons Frontiers Copyright.

The impact of the neurodiversity debate on the development of new therapies

Interventional attempts should be carefully designed and not reduce or eliminate natural coping strategies, such as some patterns of repetitive behaviours that are actually self-regulatory. This is actually on the points that is often raised by self-advocacy groups. Regardless of the case-by-case dependency of the relevance of each type of concern, the voice of autistic people is very important to tailor intervention strategies and we have carefully followed this policy by constant dialogues with people with autism and patient associations. This was the case for the virtual travel game study (Simões et al., 2018) which did actually run at the headquarters of the patient association of Viseu, Portugal. Within the scope of the European project BrainTrain, we developed a job interview serious game with strong feedback from technicians and ASD people from the patient association of Coimbra, Portugal.

Concerning the travel game, we used VR technology to expose subjects to rich immersive exploratory virtual travelling environments (requiring changing buses in a virtual trip) while also recording biosignals over multiple sessions. We used both objective physiological and behavioural measures of exploratory immersive states. We used dynamic moving actors providing a rich and compelling naturalistic explorative environment while monitoring of subjects' visual experience. This interventional study is described in a separate section of this book.

VR environments can immerse users in a virtual world which is perceived as if it were real. Can we generate appropriate perceptual experiences in ASD participants, delivering the expected sensory input one expects when they move within an environment? We could show that this is case, but also realized that the fundamental nature of their experience is different (Simões et al., 2020). Virtual reality is used to train pilots in flight simulators, firefighters with real houses and masks in fire simulations, and to treat phobias or obsessive-compulsive disorders (van Loenen et al., 2022). Concerning neurodevelopmental disorders, it is important to first understand how real and virtual worlds match, and if was is learned in the virtual world can really be transferred into reality. Below we will present some studies on this type of validation.

Constant and critical re-evaluation of intervention targets will be very important in this context. It is a priority to identify what causes distress or performance issues to people with autistic people with an emphasis on quality of life and personalised requests for support. Promotion of autonomy is one of the main priorities of the families and individuals with autism and improving levels of independence is quite important.

The concept of effectiveness used in clinical trials has to be considered in respect to these ultimate goals of improving adaptive behaviour and autonomy, which are extremely variable along the autistic spectrum. I would argue that one should not simply design interventions may be solely effective at reducing autistic patterns, if they leave the child without coping mechanisms or at risk of mental health difficulties. They should be effective in improving their quality of life. We need to reframe effectiveness to concentrate on the outcomes that are most important to the long-term well-being and autonomy of the children and adults involved and the preferences and priorities of autistic people.

Neurodiversity in the representation of space

Can virtual world games really help people with autism?

How do people represent space? Cognitive neuroscience posits that we can navigate in the world using at least two types of distinct personal perspectives. One is labelled as egocentric and the one that is focused on world-based views, is called allocentric. This is another source of neurodiversity.

We initially addressed this issue in Williams syndrome (Bernardino et al., 2013), a neurodevelopmental disorder where some of the deficits claimed to be present in Autism are actually more exaggerated, such as visuospatial deficits. The impact of such features on spatial navigation and wayfinding remains a question. It is possible that it is desirable to be able to shift between egocentric (viewer-dependent) and allocentric (viewer-independent) representations of space to better perform. We studied egocentric and allocentric frames of reference in 75 participants (18 with this condition, 20 chronological age-matched participants, 20 non-verbal mental age-matched controls and finally 17 controls with intellectual disability. We

used computerized and 3D spatial judgment tasks. Participants with that condition were specifically impaired when performing both egocentric and allocentric spatial judgments and this was not even explained by intellectual disability. What leads to the impairments of spatial representations? Some have suggested that egocentric impairment is consistent with dorsal brain visual pathway changes while allocentric spatial judgments are more related to ventral visual functioning (Ring et al., 2018).

Autism spectrum disorder is characterized by impairment in relational binding, which is defined by the ability to form relations among items and context. This ability is required for spatial navigation. A recent study, using a computerised version of the Morris Water Maze spatial memory task, suggested that ASD participants have specific difficulties in performing allocentric when compared to egocentric navigation. Viewpoint independent route finding seems to be affected. Viewpoint dependent navigation is overall intact. Other studies have shown similar findings (Umesawa et al., 2020). For example, it has been found that autistic people show decreased utilization of allocentric coordinates during simple reaching movements. This suggests a bias in utilizing fewer visual landmarks during movement, but whether this strategy implies a true deficit remains an open issue.

It remains a debated question on how navigating an environment is really difficult for people with ASD. Neurodiversity may also play a role here, because flexibility in changing routes, the ability to memorize landmarks and allocating attention to them may all be relevant in this context. It is also unknown whether training at route learning might be helpful. Our evidence from the travel train game that we developed suggests that this is the case.

Egocentric bias?

Egocentric bias is defined as the propensity to over rely on the self-perspective.

Egocentric bias can be tested using false belief tasks. An example is the Sandbox Task in which objects are initially hidden at one location by a protagonist and then moved to a second location in the same are but with the protagonist's absence. Participants are asked to indicate either where the protagonist remembers the item to be, believes to be, or where he/she

will look for it. The distance away from the original one towards the new location can be taken measure of egocentric bias, which can be considered controversial (Samuel et al., 2018). What is the evidence that people with autism have excessive egocentric bias?

Previous studies suggested that individuals with autism have indeed the tendency to indicate locations closer to where they themselves know the object to be located, showing a specific difficulty with reasoning about false mental states. This interpretation as evidence of egocentric bias when they are requested to attribute mental states to others, has been challenged by a recent study which suggested a more general difficulty with reasoning about false representations (Samuel et al., 2018). In any case, the evidence for specific egocentric biases even in children with autism spectrum disorder is being increasingly challenged (Begeer et al., 2016).

Heterogeneity in ASD: lessons from a genetic syndromic model, Neurofibromatosis type 1

We have been studying disorders that share phenotypic characteristics in the autism spectrum, such as Neurofibromatosis type 1 (NF1). The advantage of studying this condition is that it is a monogenetic disorder, affecting the neurofibromin gene. In spite of being much simpler from the genetics point of view we found that NF1 still shows quite large heterogeneity in terms of its clinical and cognitive phenotype. Despite the challenge to establish Genotype-phenotype correlations in even in more simple conditions such as NF1 we were able to explain the heterogeneous disease expression observed in humans, by studying the impact of subtle variations of the type of mutation on inhibitory neurotransmission mediated by GABA (gamma-aminobutyric acid) (Violante, Ribeiro, Edden, et al., 2013, Bernardino et al., 2021, Figure 2).

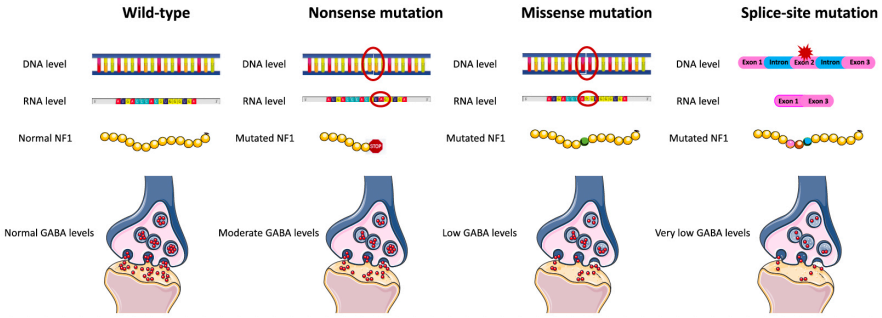


Figure 2. Genotype-phenotype correlation in NF1. Schematic representation of genotypic impact on GABA levels. Missense or splice-site mutations have significantly lower GABA levels than unaffected individuals, resulting in a more severe phenotype than nonsense mutations. DNA, deoxyribonucleic acid; GABA, g-aminobutyric acid; RNA, ribonucleic acid. From Bernardino, Gonçalves and Castelo-Branco, 2021. (Authors Figure with permission from the publisher under a Copyright Clearance Center's RightsLink®).

The excitation inhibition debate: new insights into variability using animal and human studies

To further understand heterogeneity , we also used an NF1 animal model to document variability of neurotransmission also at the within-subject level. We focused on excitation-inhibition imbalance (glutamatergic versus GABAergic neurotransmission), which is considered a hallmark of neurodevelopmental disorders. We were able to show for the first time that excitation/inhibition imbalance in NF1 is brain-region-specific with distinct pre- and postsynaptic mechanisms in prefrontal, striatal and hippocampal regions (Gonçalves et al., 2017, Bernardino et al., 2021, Figure 3). This suggests that no single pharmacological treatment will work because it cannot address the need for region-tailored therapies directed to different neurotransmission phenotypes of ASD. Adverse effects of medication are the price to pay, because some regions will benefit from treatment and others not, or even the opposite.

Excitatory/inhibitory imbalance has been suggested as a neurobiological substrate of the cognitive symptomatology in Autism Spectrum Disorder (ASD) (Bernardino et al., 2022). Studies using magnetic resonance spectroscopy (MRS) attempted to characterize GABA and Glutamate brain

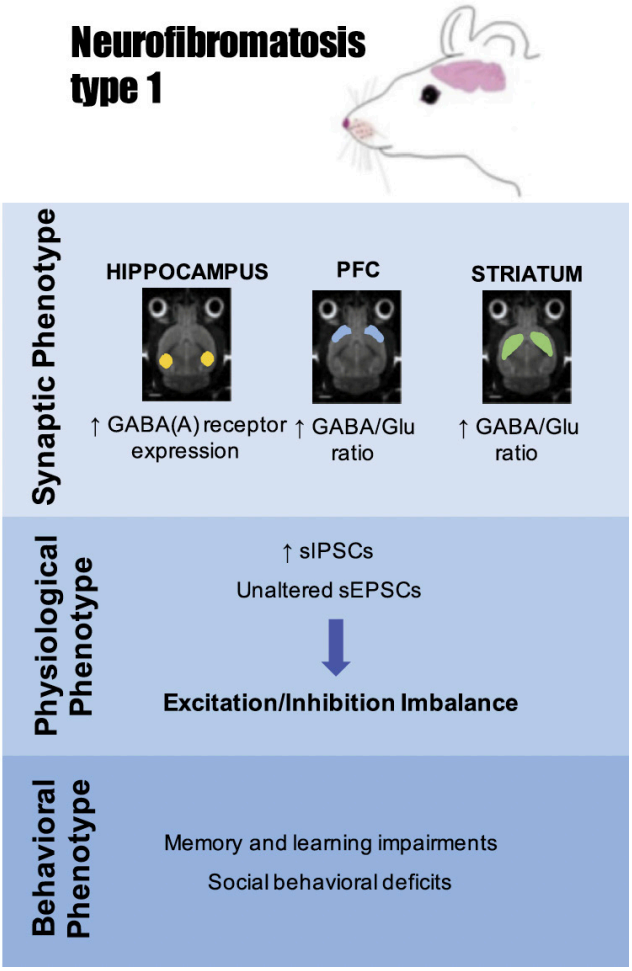


Figure 3. Variable brain regional phenotypes of excitation/inhibition imbalance in the Nf1+/- mouse model of NF1. GABAergic patterns vary across areas, showing within individual variability. The synaptic phenotype in hippocampus is characterized by GABAA receptor massive increases while in the prefrontal cortex and striatum it is the GABA/glutamate ratio that increases. In spite of the differences in synaptic phenotype in these brain regions, the physiological phenotype is similar with converging increases of sIPSCs (spontaneous inhibitory postsynaptic currents) relative to sEPSCs (spontaneous excitatory postsynaptic currents) resulting in increased inhibitory drive. This is associated with behavioural phenotypes such as memory and learning impairments and social behavioural deficits. GABA, g-aminobutyric acid; GLU, glutamate; PFC, prefrontal cortex. Adapted from Bernardino, Gonçalves and Castelo-Branco, 2021. (Authors Figure with permission from the publisher under a Copyright Clearance Center's RightsLink®).

levels in ASD (Carvalho Pereira et al, 2018). However mixed findings have been reported. The question then remains whether such mixed findings are explained by genuine biological variability, which would enforce group stratification. To achieve this biological understanding, it is important to combine different methods to investigate cortical excitability and inhibition. We recently characterized both neurochemical and physiological aspects of GABA system in ASD by implementing a more comprehensive approach to understand variability combining MRS and transcranial magnetic stimulation (TMS) (Bernardino et al, 2022). This latter technique measures cortical excitability directly using single or paired stimulation approaches. We employed the magnetic resonance spectroscopy technique to assess motor cortex GABA+ and Glutamate+Glutamine (Glx) levels using innovative acquisition protocol sequences. Additionally, a TMS experiment was implemented including paired-pulse stimulation pulses leading to cortical inhibition (short and long intervals – SICI and LICI, respectively) or excitation, respectively. We also measured input-output curves and the length of cortical silent periods after stimulation to probe cortical excitability. Our results showed a significantly increased Glx, with unchanged GABA+ levels in the ASD group compared with controls. Single TMS measures only suggested impaired inhibition in SICI for short stimulation intervals of 5ms, in ASD. These data are consistent with changes in excitation/inhibition balance but their subtlety challenges generalization, and suggest that group stratification of variability may indeed be necessary. Importantly, we observed a correlation between GABA levels and measures of the input-output TMS motor recruitment curve only in the control group, as demonstrated by direct between group comparisons. Increased Glx levels may contribute to ASD excitatory/inhibitory imbalance but larger-scale studies are needed, because it remains possible that only subsets of ASD patients will show such changes. In other words, current research is showing that population stratification is required to identify subgroups where these changes hold.

In sum excitation/inhibition (E/I) imbalance has been in the last years a major hypothesis in autism research, but our evidence suggests that it has diverse manifestations at within and between subject levels. If such an imbalance is indeed causal in some cognitive manifestations and comorbidities in the pathophysiology of autism, it could potentially define a therapeutic target for groups where such E/I balance is well defined.

The study of monogenic disorders related to autism, called syndromic autism, could be helpful in this regard. Although they are rare causes of autism, because they are simpler from the genetics point of view, they offer a unique opportunity to study excitation/inhibition imbalance. Neurofibromatosis type 1 (NF1), is one such example, which we studied from the point of view of animal models and the human disease, to isolate mechanisms underlying ASD-related impairments. The NF1 mouse model showed some surprising distinctions by showing increased gamma-aminobutyric acid (GABA) neurotransmission, in contrast with the GABA deficiency shown by the human disease. The latter showed overall reduced cortical GABA levels and GABA receptors. It is therefore important to clarify similar and distinct aspects of Excitation Vs Inhibition imbalance, for the GABA dysfunction hypothesis in NF1. We found that Excitation Vs Inhibition imbalance may depend on distinct pre- and postsynaptic push-pull mechanisms that are region-dependent. It was possible to achieve this goal, by assessing two critical components of Excitation Vs Inhibition regulation: neurotransmitter and GABA(A) receptor levels. We investigated brain regions such as the hippocampi, striatum, and prefrontal cortices using techniques such in vivo magnetic resonance spectroscopy (MRS) and molecular approaches in the Nf1+/- mouse model. We found indeed evidence for distinct regional phenotypes. GABA/glutamate neurotransmitter ratios were increased in cortical and striatal regions in contrast with the disproportionately small reduction of GABA levels found in the hippocampus (see Figure 3 above). In this region, at the postsynaptic level, very high receptor GABA A receptor expression was found. It therefore seems that distinct regional mechanisms lead to overall increases in GABAergic tone, either by receptor levels change or alterations in GABA/glutamate ratios. In other words, GABA neurotransmission seems therefore to be enhanced in all regions, but through distinct local mechanisms. Our data provide support for the Excitation Vs Inhibition hypothesis but show that different that pre- and postsynaptic mechanisms are regionally operating. Physiological evidence of changes in inhibitory tone do however show differences across regions and species. Such heterogeneity provides a challenge to therapeutic approaches aiming at restoring neurochemical imbalance in ASD. We propose a therapeutic focus on targets where convergent physiological mechanisms can be found.

The results observed in the animal model helps explain the variable nature of neurochemical changes in autism spectrum disorder (ASD), an issue that we have addressed by combining studies in idiopathic ASD and syndromic models. In the previous study by Carvalho Pereira et al. (2018) we compared medial prefrontal cortex neurochemistry of high-functioning children and adolescents with ASD without associated comorbidities with control participants. Under these conditions, we observed reduced total N-acetylaspartate (tNAA) and total creatine, suggesting that metabolic changes are at least as relevant as neurotransmitter changes. Accordingly, we found increased Glx/tNAA ratios but unchanged glutamate + glutamine (Glx) and unchanged absolute or relative gamma-aminobutyric acid (GABA+) in the ASD group. It is however important to take into account that even apparently unchanged neurotransmitter levels may have impact on clinical measures. This can be investigated using regression analysis. Accordingly, we found that both lower absolute and relative GABA+ levels were associated with worse communication skills and developmental delay scores assessed by the autism diagnostic interview-revised (ADI-R). This work raises questions on the relation between tNAA, a marker of neurometabolism and neural integrity, which is reduced in the medial prefrontal cortex in ASD and glutamatergic metabolism which may be altered because of unbalanced Glx/tNAA. The fact that GABA+ levels are related to autistic symptoms assessed by the diagnostic tool ADI-R, irrespective of its mean levels, will require additional studies. Our results from animal models suggest that voxel sizes typically used in human studies may be too large for some of the research questions by encompassing regions with distinct profiles of excitation/inhibition.

Understanding the relation between abnormal GABA levels and newly discovered patterns of neurophysiological changes and impulsive behaviour

Neurofibromatosis type 1 is characterized by a broad spectrum of cognitive deficits, and it is a very good example of the case that even a monogenic condition may lead to widely variable phenotypes. This may also lead to the consequence that some symptoms that are co-morbid

across neurodevelopmental conditions become overlooked. Impulsivity and inhibitory control are examples of such manifestations that have been very much disregarded and which we addressed in a previous study linking behaviour, neurochemistry and neural responses (Ribeiro et al, 2015). We addressed this question by studying inhibitory control in a visual go/no-go task in children and adolescents with NF1 and age- and gender-matched controls, from a multimodal perspective. We combined electroencephalography (EEG) with magnetic resonance spectroscopy (MRS) to measure the levels of GABA and glutamate + glutamine in the medial frontal cortex (Ribeiro et al, 2015). This brain region was selected because it that plays an important role in inhibitory control, in addition to a control visual region. We found a relationship between loss of inhibitory control leading to impulsivity (Figure 4), levels of neurotransmitters (Figure 5), and EEG markers of neural function (Figure 6).

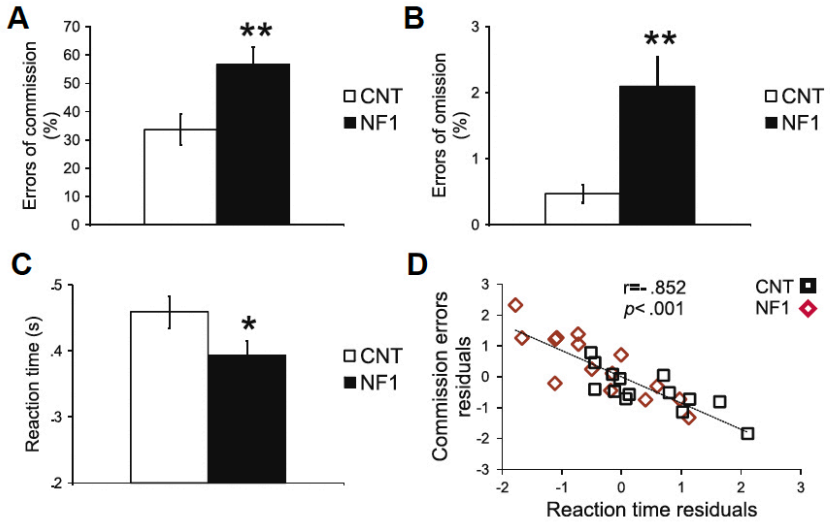
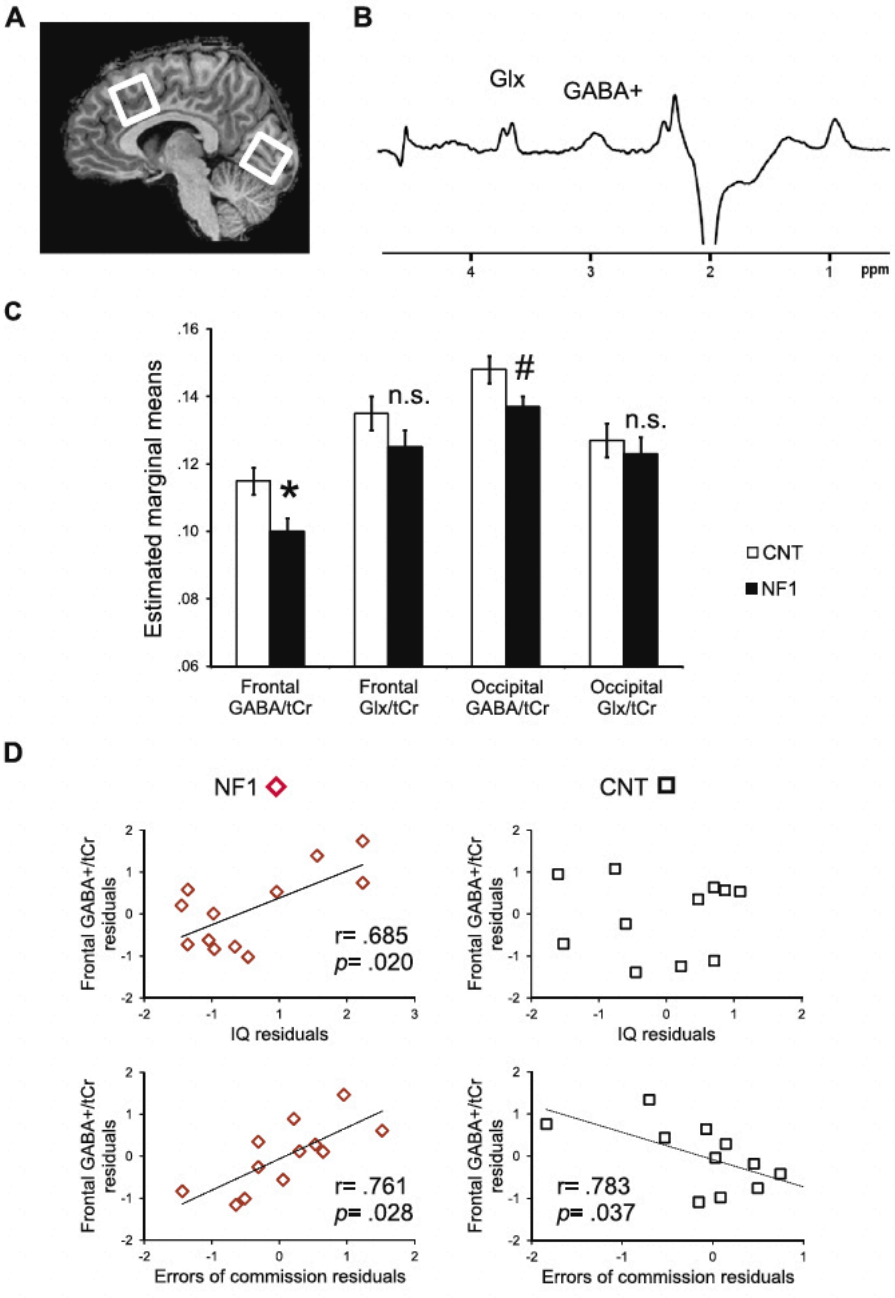


Figure 4. Individuals with NF1 (Neurofibromatosis type 1) show more omissions, and commit more errors with excessively fast responses, than neurotypicals. (A) Percentage of errors of commission (inability to inhibit the response to no-go stimuli). (B) Percentage of errors of omission inability to respond to go stimuli). (C) Reaction time of the correct responses to go stimuli, showing a speed vs accuracy trade-off. (A, B and C) Data are represented as mean ± 1 SE. Control group - white bars; NF1 group - black bars. *p < .05, **p < .01. (D) Partial correlation controlled for the effect of age, gender, and IQ between reaction time and number of errors of commission. Adapted from Ribeiro et al., 2015. Copyright Clearance Center's RightsLink® service, Elsevier.


A
B
Glx
GABA+
4
3
2
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C
Estimated marginal means
.16
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#
n.s.
CNT
NF1
Frontal GABA/tCr
Frontal Glx/tCr
Occipital GABA/tCr
Occipital Glx/tCr
D
NF1
CNT
Frontal GABA+/tCr residuals
IQ residuals
r= .685
p= .020
r= .783
p= .037
Errors of commission residuals
r= .761
p= .028

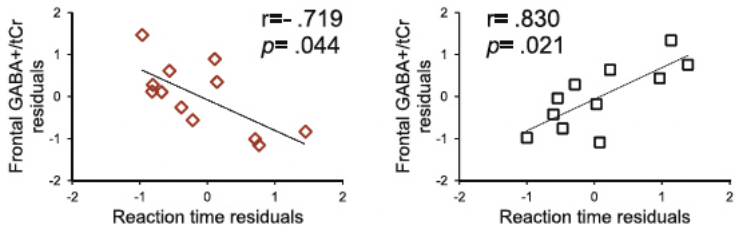


Figure 5. Relationships between GABA levels and neurobehavioral profiles. The Gaba/total creatine (tCr) levels of the medial frontal and occipital cortices of individuals with NF1 are significantly reduced. (A) Localization of the two MRS voxels (white squares) in the medial frontal and occipital cortices of a representative participant. (B) Edited MRS spectrum from a representative participant showing clearly resolved peaks for GABA+ and glutamine + glutamate (Glx). (C) GABA+/tCr and Glx/tCr levels adjusted for the percentage of grey matter in the spectroscopy voxels, for the control (white bars) and NF1 (black bars) groups. Data are represented as mean ± 1 SE. *p < .05 # p =0.057. (D) Upper graphs, partial correlations between levels of medial frontal Gaba/tCr and IQ (intellectual quotient), controlled for the effect of grey matter within the spectroscopy voxel. Middle and lower graphs, partial correlations between levels of medial frontal Gaba/tCr and number of errors of commission (middle) and between levels of medial frontal GABA+/tCr and reaction time (bottom), controlled for the effect of age, gender, IQ, and grey matter within the spectroscopy voxel. Graphs presenting NF1 data are shown on the left, control data are shown on the right. Adapted from Ribeiro et al., 2015. Copyright Clearance Center's RightsLink® service, Elsevier.

Patients revealed disrupted impulse control and abnormal early visual processing as well as impaired neural responses related to inhibitory control.

Magnetic resonance spectroscopy data revealed a reduction in medial frontal GABA+/tCr (total Creatine) levels in the NF1 group, which extended our prior dual replication of reduced occipital GABA levels. Excitatory species (glutamate + glutamine/tCr) levels were at normal levels, suggesting the existence of abnormal inhibition/excitation balance in this disorder. Medial frontal GABA levels showed to be specifically associated with inhibitory control, but in strikingly diverse ways. While higher GABA was associated with a faster and more impulsive response style in patients, in controls it was related to a more adaptive non impulsive strategy. Abnormal GABAergic physiology seems therefore to affect in NF1 and neurotypical participants in diverse ways. We also found altered neurophysiological responses, in particular of the inhibitory control related P3 component (Figure 6), which was associated with GABA levels. Correlation analyses revealed indeed a significant negative correlation between GABA+/tCr levels and no-go P3 amplitude, but only in the clinical NF1 group.

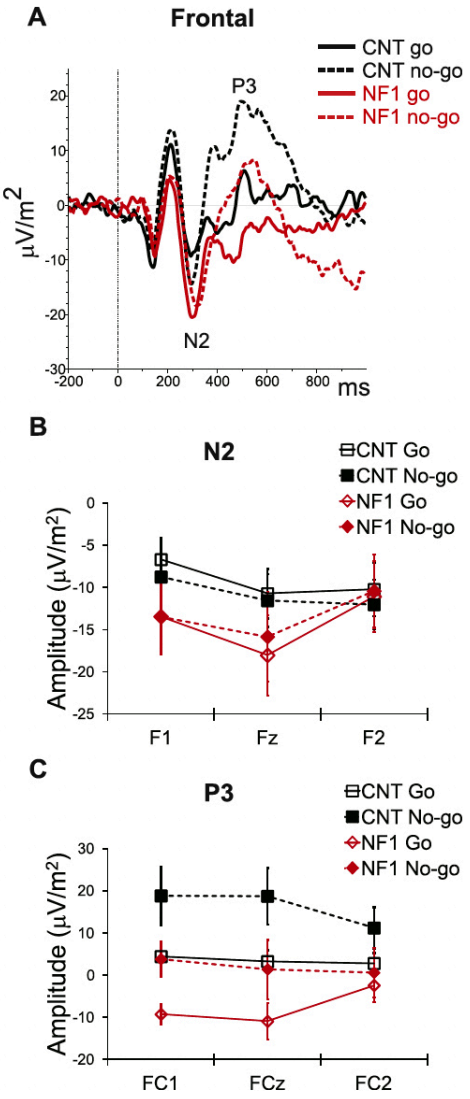


Figure 6. Decreases in inhibitory control related frontal cortical event-related neural signals in NF1 and relation with the GABA levels. Reference-free Current Source Density (CSD) (uV/m2) grand average traces detected over midline frontal electrodes, showing the N2/P3 frontal complex. Correlation analyses revealed a significant negative correlation between GABA/tCr levels and no-go P3 amplitude. Adapted from Ribeiro et al., 2015. Author owned Copyright Clearance Center's RightsLink® service, Elsevier.

Placing in context our ground-breaking work in studying GABA phenotypes for the first time in human neurodevelopmental disorders

Looking at both pre and postsynaptic levels

Our group was the first to go beyond animal models of NF1 and show for the first time a GABA phenotype in the human disease. This was quite an important discovery, because alterations in the balance between excitatory and inhibitory synaptic communication had been debated in a very broad range of neurodevelopmental disorders (Violante et al, 2013). Neurofibromatosis type 1 has been one the most highlighted early on because it is one of the most common monogenic disorders causing cognitive deficits, and a mouse model (Nfl(+/-)) had been shown to lead to increased gamma-aminobutyric acid-mediated inhibitory neurotransmission as the neural mechanism underlying these deficits (Costa et al., 2002). We were the first to show that a similar mechanism translates to the human disorder (Violante et al, 2013). We were able to implement a magnetic resonance spectroscopy technique to measure gamma-aminobutyric acid levels in the visual cortex of children and adolescents with neurofibromatosis type 1 and matched controls. We could confirm a GABA phenotype also in humans. However, patients with neurofibromatosis type 1 showed surprisingly lower gamma-aminobutyric acid levels than control subjects, in a genetic mutation type dependent manner (Figure 2). These results showed physiological significance because functional imaging of the visual cortex indicated a correlation between brain activity and gamma-aminobutyric acid levels (Violante et al, 2013). Our demonstration of a reduction in gamma-aminobutyric acid levels in human patients provided critical insights to the physiological profile of the disorder in human and animal models, which obviously has implications to the design of therapeutic approaches.

To further understand the role of gamma-aminobutyric acid (GABA) system in NF1 it is important to understand its nature at pre- and postsynaptic levels. In a subsequent cross-sectional study (Violante et al., 2016), we employed multimodal pre and postsynaptic imaging of the GABA system. Accordingly, we used measures to investigate GABA type A (GABAA) receptor binding, using a radiotracer that binds this receptor,

[(11)C]-flumazenil PET, and GABA concentration, using magnetic resonance spectroscopy (Figure 7).

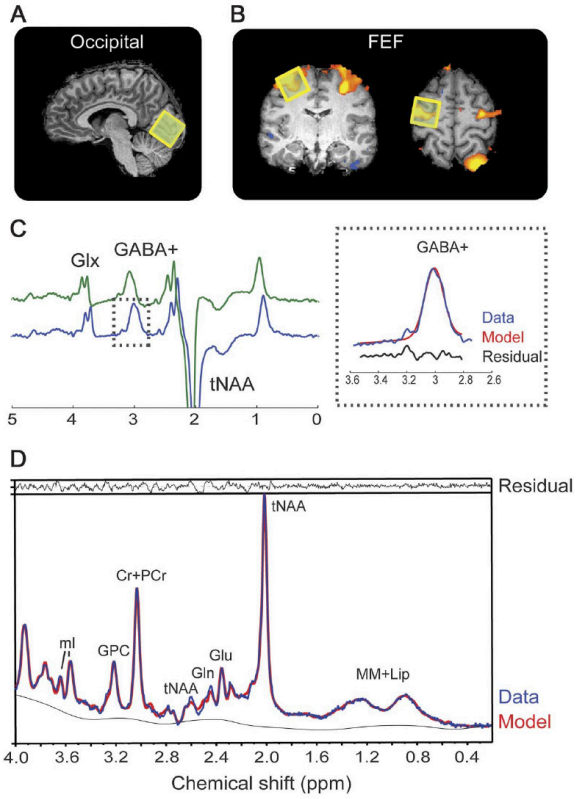


Figure 7. Definition of brain regions for measurement of neurotransmitters in health and NF1 based on functional criteria. Localization of the magnetic resonance spectroscopy voxel (yellow square) in the visual cortex (A) and FEF as localized with a functional brain imaging localizer (B). (C) Edited magnetic resonance spectroscopy spectrum from a representative participant showing clearly resolved peaks for GABA+ and glutamine + glutamate (Glx). The inset on the right shows the fit output for the GABA+ signal. MEGA-PRESS spectra were processed using the Gannet 2.0 toolkit, the green line shows the raw GABA data, the blue line the post-phase and frequency aligned GABA data, and the black line is the residual difference between the experimental data and the curve fit. (D) Single-voxel–localized PRESS spectrum (blue line) from a representative participant with spectral fits (red line) and the residual difference between the experimental data and the curve fit (black line) determined using LCModel. Cr=creatine; FEF =frontal eye field; GABA = g-aminobutyric acid; Gln = glutamine; Glu = glutamate; GPC = glycerophosphocholine; mI = myo-inositol; PCr = phosphocreatine; tNAA = total N-acetylaspartate (NAA [N-acetylaspartate]; NAAG [N acetylaspartylglutamine]). Adapted from Violante at al. © Creative Commons Licence.

We replicated for the third time the finding that in NF1 there is reduced concentration of GABA+ in the occipital cortex and confirmed this in another functionally localized brain regions, the frontal eye fields (Figure 8).

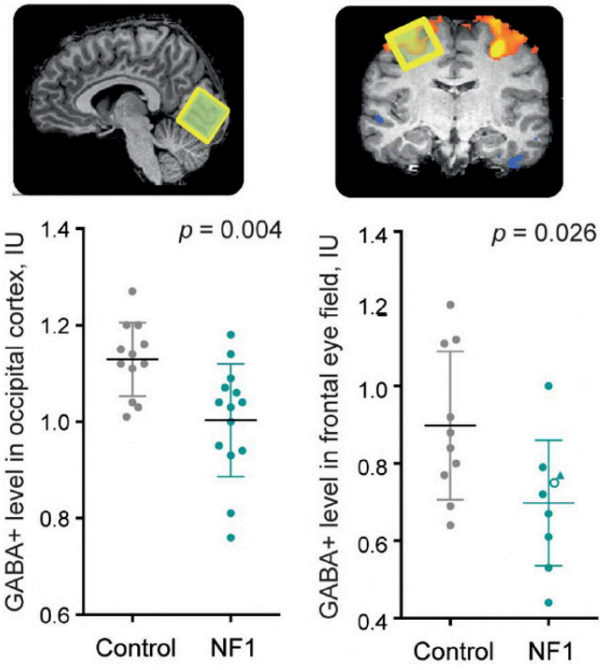


Figure 8. Metabolites showing differences between patients with NF1 (green) and controls (gray) in the occipital cortex and the FEF. FEF - frontal eye field. Adapted from Violante at al. 2017 © Creative Commons Licence.

We were surprised to find that PET results that binding of GABAA receptors was overall reduced in patients in cortical and subcortical regions (parieto-occipital cortex, midbrain, and thalamus) (Figure 9). So, the GABA system is reduced not only at the pre but also at the postsynaptic level, suggesting a global GABA deficiency.

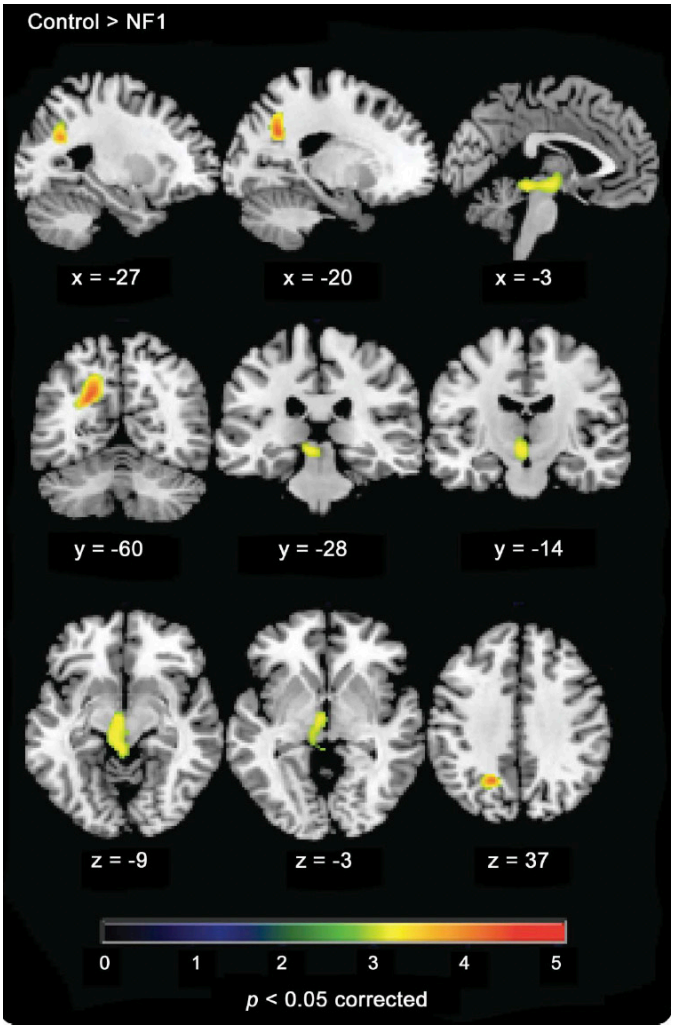


Figure 9. Areas showing decreased [11C]-flumazenil binding in patients (contrast control versus NF1, p 0.05 corrected). NF1 = neurofibromatosis type 1. Adapted from Violante at al. 2017 © Creative Commons Licence.

The surprising finding that the GABA system is overall deficient in NF1 and involves reduction of both GABA concentration and GABAA receptor density suggests that this is a neurodevelopmental synaptopathy with both pre- and postsynaptic involvement. In spite of overall reduction of both

GABA and respective receptor levels, in some regions such as the frontal eye fields, a negative correlation was found between neurotransmitter and receptor levels (Figure 10).

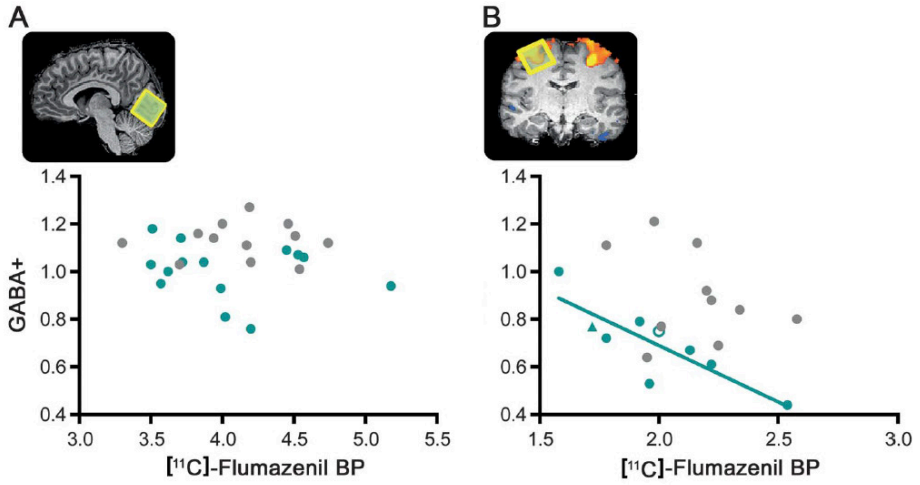


Figure 10. (A) Results for the occipital voxel and (B) for the FEF voxel, for patients with NF1 (green) and controls (gray). In patients with NF1, the concentration of GABA+ was negatively correlated with the density of GABA type A receptors in the FEF (r = - 0.842, p = 0.004, n = 9,95% confidence interval = -1.000 to -0.368 calculated from 10,000 bootstrap samples). BP - binding potential; FEF - frontal eye field; GABA - g-aminobutyric acid; NF1 - neurofibromatosis type 1. Adapted from Violante at al. 2017 Adapted from Violante at al. 2017 © Creative Commons Licence.

Taken together, these results raise the question whether compensatory events during human neurodevelopment makes the human disease deviate from the mouse model.

Our findings raised a substantial number of general questions on the significance of animal models and how human diseases are strongly affected by complex neurodevelopmental pathways, both at pre and postsynaptic levels. It also raises important questions on how brain plasticity in neurodevelopmental disorders or other diseases of early onset (Machado et al., 2017, Ferreira et al., 2019; Ramos et al., 2019) is manifested at pre vs postsynaptic levels. We have recently addressed this issue in the adult brain, in Parkinson's disease (Rebelo et al., 2021), but a similar approach is needed in this context.

Take home messages from studies of excitation-inhibition and their behavioural impact

So far, we have only examined heterogeneity from a functional perspective. However, there is also substantial evidence for anatomic diversity, although patterns of morphometric heterogeneity found in NF1 are more consistent than in ASD and this may be because of the more homogeneous genetic origins (Violante, Ribeiro, Silva, & Castelo-Branco, 2013). Nevertheless, machine learning approaches can also detect unsuspected variations (Duarte et al., 2014). The association between neurobehavioral phenotypes and the mutation type remain to be established even in a simple monogenetic disorder such as NF1. Overall, the lessons that we have learned from NF1 suggests a wide heterogeneity at several biological levels of analysis, in spite of being a monogenetic disorder (Bernardino et al, 2021; Figure 11).

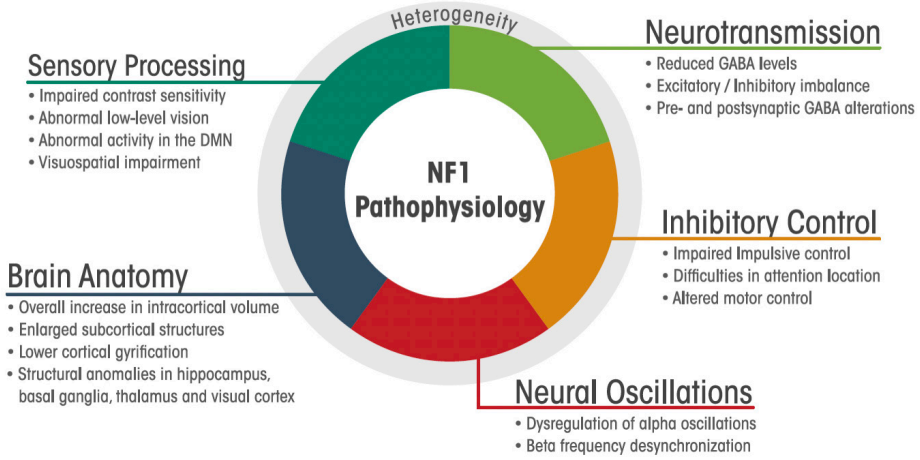
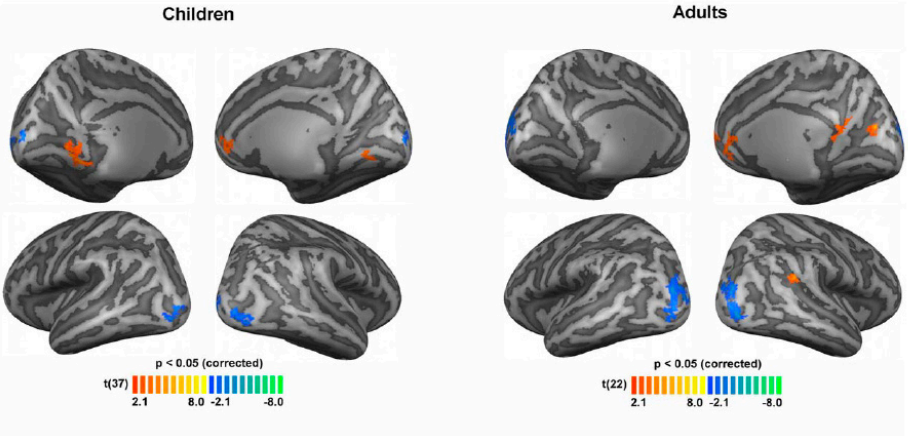


Figure 11. Pathophysiology profiles of Neurofibromatosis Type 1. DMN, default mode network, GABA, g-aminobutyric acid. From Bernardino, Gonçalves and Castelo-Branco, 2021. (Authors Figure with permission from the publisher under a Copyright Clearance Center's RightsLink®).

We now need to delve further into the debate of neurobehavioral diversity in ASD and for this, I need to introduce my own particular perspective on autism bearing a distinctive cognitive and emotional style.

Can the distinct cognitive style in autism be seen as a model of different kind of awareness and conscious perception?

The distinct cognitive style that is acknowledged for autism, embeds variations in the way these people perceive, imagine and pay attention. Our previous work provides ample evidence for this (Amaral et al, 2017, 2018; Simões et al, 2018, 2020; Borra et al, 2020; Bernardino et al, 2013; Castelhano et al, 2015, 2018; Silva et al., 2016). We do believe that all of this evidence points to the tenet that autism can be understood as a model of different kind of awareness and consciousness. We are not talking about a deeply altered state of consciousness but nevertheless that autism is characterized by qualitatively distinct states of awareness and mind wandering (Simpraga et al, 2021; Wang et al, 2022). We found enhanced activation of the mind wandering circuitry in Neurofibromatosis Type 1 (Violante et al, 2012) particularly for magnocellular activating visual stimuli (quickly changing in the visual periphery). This type of stimulus can be detected a low contrast and very strongly recruits attentional networks. We hypothesised that brain activity patterns should reflect loss of attentional focus and this is precisely what we see in Figure 12. The "mind wandering" default mode network (DMN) is abnormally activated (orange colours in Figure 12) and visual cortex is abnormally underactivated as compared to controls (blue colours).



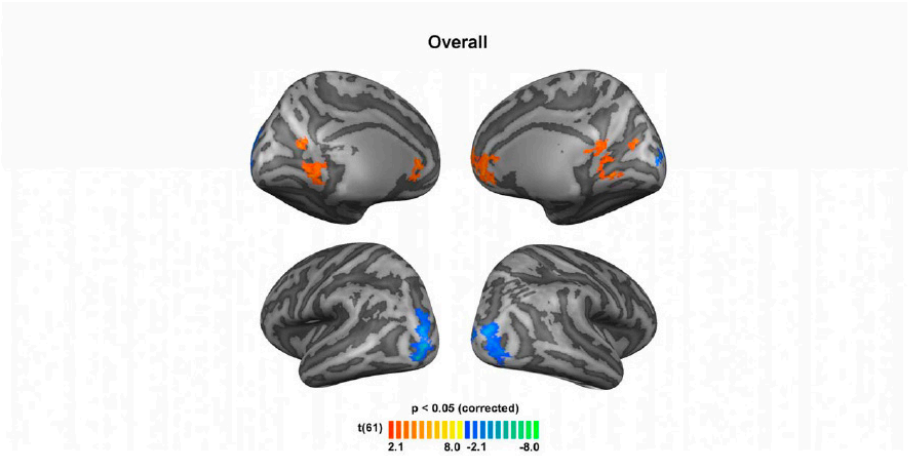


Figure 12. The "mind wandering" default mode network (DMN) is abnormally activated in NF1 and visual cortex shows reduced activation compared to controls. Significant group differences in brain activation can be seen, regarding the Magnocellular visual stimulus type (fast moving stimuli which activate the visual periphery and attentional networks) for children, adults and overall (children and adults) groups. Blue colours depict regions where activation was lower for individuals with NF1 than controls. Orange colours depict regions where activation was higher for individuals with NF1 than controls. Results are shown on views of the left and right hemispheres of cortex-based aligned three-dimensional reconstructions generated from the average anatomical data sets of the subjects in each group (children, adults, overall). Light grey represents gyri, dark grey represents sulci. T-maps thresholded at p=0.05 corrected using cortex-based cluster threshold estimation (cluster size 41 mm2). N = 15 children with NF1, 24 control children, 13 adults with NF1 and 11 control adults. Adapted from Violante et al. 2012.

We argue that autism is characterized by a radically distinct nature of subjective experience. Their qualia of perception, well described by the philosophers of consciousness, are distinct (Salti and Bergerbest, 2022). The way attention focuses and unfocuses is also very distinctive.

Attentional regulation is very important in the training of meditative states. In the neurodevelopmental conditions we have studied there are increased levels of posterior alpha activity, which is a sign of visual idleness (rest) and is increased upon eye closure or similar visual rest states (Ribeiro et al., 2014). So why is visual alpha enhanced even when a visual stimulus is presented, as shown in Figure 13, in the case of Neurofibromatosis type 1

(NF1)? This adds to the find that alpha oscillations are excessive during rest (eyes open or closed – Figure 14).

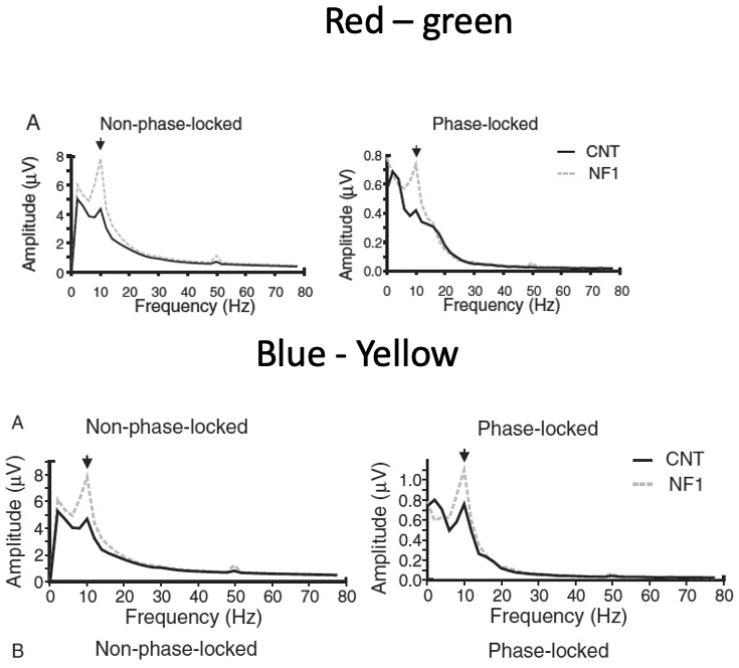


Figure 13. Increased alpha oscillations in NF1, even when visual stimuli (red-green or blue-yellow contrasts) are present. Frequency domain analyses of the electroencephalographic (EEG) responses elicited by red-green (parvocellular system) and blue-yellow stimulation (koniocellular pathway) revealed significantly higher amplitude of alpha oscillations in the responses of children and adolescents with neurofibromatosis type 1 (NF1) compared with control levels.

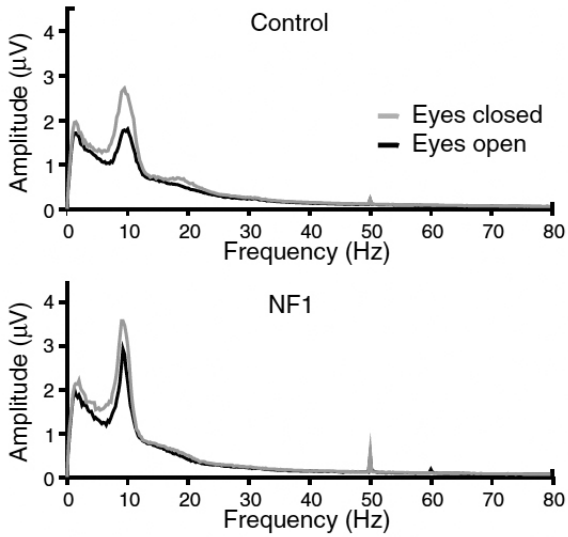


Figure 14. Alpha amplitude during eyes open or eyes closed resting conditions was higher in children and adolescents with Neurofibromatosis type 1 (NF1) than controls.

We argue that the question is not necessarily the absolute amplitude levels of alpha but how they are regulated during different awareness states. In other words, we believe that the real core of the difference is the qualitative nature of the regulation of alpha rhythms. If it is regulation that matters and not overall levels could this imply a new sort of diversity, such as abnormally decreased instead of increased alpha levels? This is exactly what we could also find (Silva et al., 2016). Abnormal regulation may also occur with over decreased alpha as a signature of excessively enhanced attentional focus (Silva et al., 2016). This excessive attention to detail is often seen in neurodevelopmental disorders associated with autism. This is specifically what we report in Figures 15 and 16, when participants have to pay attention to a peripheral visual target, while fixating a central cross.

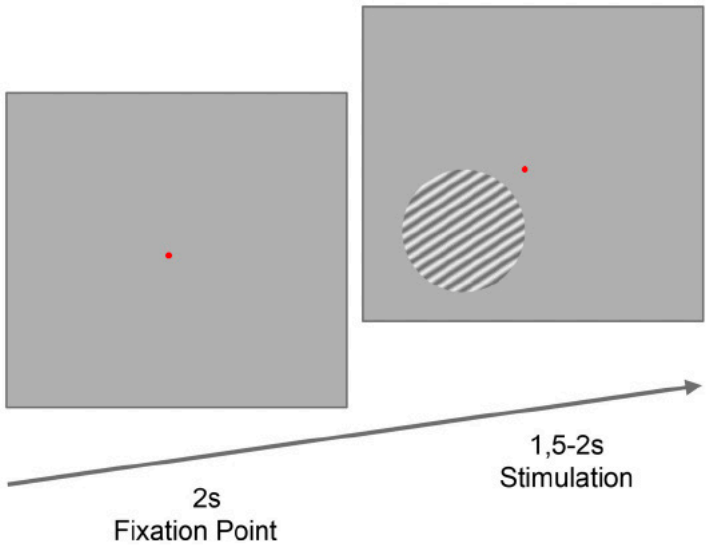


Figure 15. Example of a simple visual attention task using peripheral (paracentral) moving gratings recruiting exogenous attention (the participant has to fixate the red dot while covertly attending to the peripheral stimulus. The moving grating is shown for a variable time (1500 ms-2000 ms) after a fixed interstimulus interval. Participants were instructed to maintain fixation in the central point during the whole task and to report the disappearance of the visual stimulus (target offset), as fast as possible. Two hundred trials were acquired, divided in two different runs, during which participants responded either with the right or the left hand. Adapted from Silva et al., 2016.

In this particular setting patients unexpectedly show lower levels of alpha than neurotypical participants, suggesting hyperfocused attention (Figure 16)!

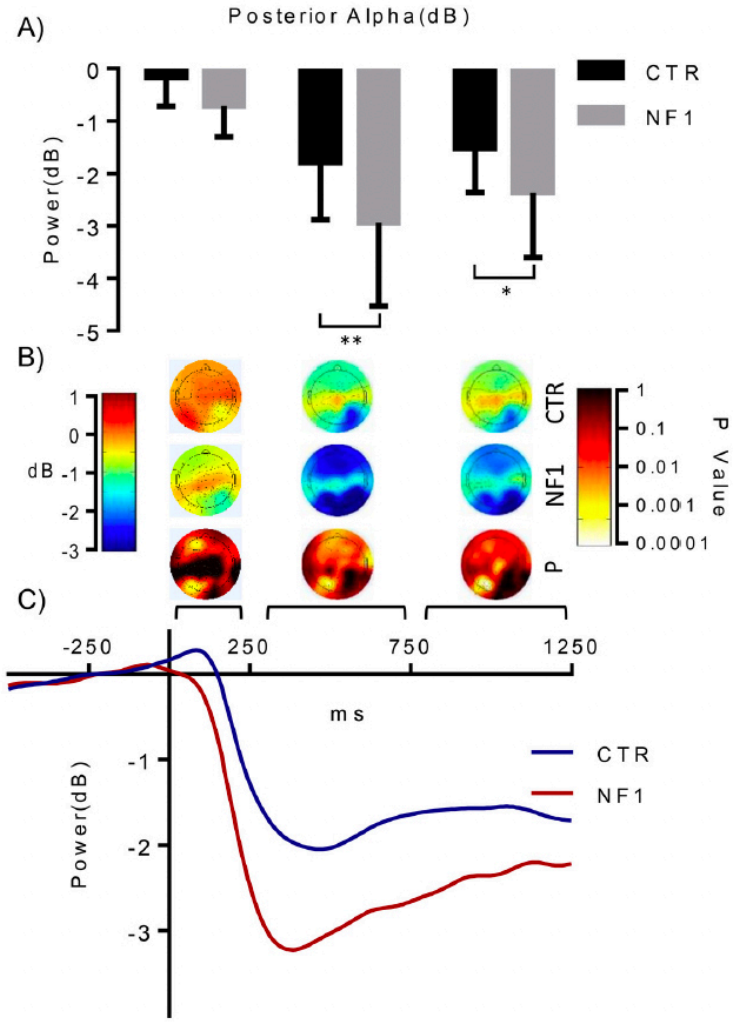


Figure 16. Time course and topography of event related alpha power shows surprisingly decreased alpha during an attentional task requiring hyperfocus under covert attention conditions. (A) Significant differences in alpha power were found at different time periods: 250-750ms and 750-1250ms in the posterior sites between control and NF1 subjects. (B) Head topography of alpha over early (0ms-250ms), middle (250-750ms) and late (750ms-1250ms) periods (C) The time course indicates deactivation starting around 150ms, reaching a minimum power peak around 300ms. The higher level of alpha deactivation found in NF1 participants, highlights the abnormal alpha modulation in these patients.

We concluded from this set of experiments that the problem is not in alpha levels per se, but more in the way they are regulated. But is this different regulation necessarily maladaptive? We think that in some instances this might confer advantages to autistic individuals, in daily life tasks that might require hyperfocus. Particular cognitive styles are diverse but they may confer evolutionary advantage under certain circumstances. It is quite likely that individuals with the capability of hyperfocusing of attention might be more able to perform a certain range of complex tasks. This might be the case for savant autistics, who may display extraordinary skills (Bal et al., 2022). Moreover, some high functioning autistic individuals show unusual mathematical and computer programming skills and may show competitiveness in the software industry market (Eiselt and Carter, 2018). It is possible that the ability to hyperfocus confers advantages in these domains.

Is conscious perception distinct in Autism Spectrum Disorder?

The autistic mind has rarely been linked to the study of the neural correlates of consciousness. Here we argue that this question deserves consideration. There is ample evidence that visual awareness is distinct in autism spectrum disorder (Skerswetat et al., 2022). It has been suggested that the anthropocentric view of the world is dramatically changed based on bias of Egocentric versus Allocentric representations (Umesawa et al., 2020).

We do however believe that a discussion at a deeper level is required. Here I defend that ASD can be seen as a particular model of mental states and consciousness. I follow the tenet of Anil Seth, who thinks about consciousness from three perspectives. One of these relates to experiences of the world around us according to the multiple senses: vision, audition, smell and touch. It is quite well known that people with ASD experience the sensory world in a quite distinct manner, which can be characterized by fluctuations in the quality of sensory perception. These can very between hypo or hypersensitivity, and these states might actually oscillate. In other words, the phenomenological quality of perception – the so-called qualia of subjective personal experience – seems to be distinctively dissimilar from what neurotypical people describe. Visual and perceptual awareness seem to follow different rules in ASD.

There are variable definitions of qualia, but they basically reflect the qualitative character of what "it feels like" in the mental states of perceiving pain, colour or texture touch. The emergent properties of such qualia can be so distinctive in ASD that they can reach different perceptive attributes. For example, what is felt as a normal surface touch for a neurotypical person can be so disturbing in a person with ASD that he/she may react as if an extreme source of pain is present. This relates in an interesting way into the mind-body problem. Could there be a body that sometimes does not fit a different mind?

Following these lines, 3 main aspects have to be considered when addressing consciousness and we argue that all are relevant in autism:

1. Consciousness levels, and mental states 2. Conscious content and Qualia 3. The perception of the nature of the self.

Some theories of human consciousness are highly relevant for Autism Spectrum Disorder because they postulate that consciousness is largely verbal (Peper, 2020). It is characteristically difficult for some autistic people to share thoughts with others, because of the difficulty in generating and communicating verbal thoughts. It follows that consciousness cannot be largely verbal in these people. What type of consciousness can then be ascribed to autistic people who largely non-verbal?

Peper (2020) goes a step further by proposing that consciousness is inextricably linked to the body's adaptation mechanism. This is interesting because body adaptations are often not present in autism spectrum disorder, and disturbances are often manifested as either sensory hyper or hyposensitivity. Cognitive processing is linked with a changed body sensory image. This conscious perceptual representation is essentially compromised in the way autistic people experience external sensory information. The link between mind and matter is therefore essentially changed. There is therefore a distinct state of awareness about the state of the outside world. The quality of conscious experience must therefore be quite different. The distinct link between thoughts and sensory images suggests a different quality of sensory awareness. In other words, perceptual qualia are fundamentally different, forcing different adaptation mechanisms to regulate behaviour upon environmental changes.

This leads to an interesting way to understand sensory awareness, consciousness, and their adaptive roles in regulating behaviour.

The qualia of visual space perception and action

When thinking about the interaction between mind and matter we have to take into account the visual dorsal stream which is involved in visual space perception and action. We have found that it is affect in several neurodevelopmental disorders in the autism spectrum, such as ASD and Williams syndrome (Bernardino, Castelhano, Farivar, Silva, & Castelo-Branco, 2013; Bernardino, Rebola, Farivar, Silva, & Castelo-Branco, 2014, Ribeiro et al., 2012).

The nature of these impairments is highly variable within and across disorders, being larger in Williams syndrome, where the ability to perceive and act upon the percept (for example by drawing) is dramatically impaired (Bernardino et al., 2012; Castelo-Branco et al., 2007; Mendes et al., 2005). This inability to represent the world using visuoconstructive abilities is more moderately affected in ASD, where nevertheless a different world perspective can be taken, but one cannot anymore claim that a visual deficit is present but rather a different cognitive way in visually analysing the world. The same holds for the integration of local motion cues into global motion percepts, an ability called motion coherence perception. This ability is also called motion integration and reveals a kind of holistic perception. Unified perception is a phenomenal quality of visual experience and consciousness. Given that coherence deficits have been claimed in autism, this renders this condition once more a very interesting model to study a different type of consciousness.

Is holistic perception affected in autism or is instead just the conscious quality of perception changed? I would argue that the second case is true, and we could use abstract so-called Mooney Stimuli (see Figures 17 and 18), which require holistic integration, to illustrate this concept.

Figure 17. A test for holistic perception. Can you see how many faces? Zero, one or two? If no face is perceived, there is sensation in the absence of holistic perception. On the left side you may imagine that light is coming from that side and that only half of the nose and the rest of the face is illuminated. With this cue maybe you can now see the face. If you invert the image you will notice that the same face is present (inverted) on the right side of the image (Image from J. Castelhano and M. Castelo--Branco). If you still cannot see the face, simpler examples will appear in this book.

Figure 18. Two additional examples of Mooney faces of distinct difficulty levels. On the left, instantaneous eye positions of 3 different observers are depicted by three coloured circles. Performing simultaneous eye-tracking allows to understand how the visual system parses these images (Images from J. Castelhano and M. Castelo-Branco).

The subjective phenomenology of immersive states is distinct in ASD: implications for conscious perception, free will and action

Ecological psychology postulates that perception arises from sensorimotor affordances. If these are distinct in ASD, free will, if it exists at all, will be more constrained in ASD. Enactivism embeds the notion that the feeling of possible actions within a repertoire are based on one's situatedness in an environment. In ASD action prediction errors are larger and this fact imposes restrictions on free will. In ASD knowing how to act to bring about intended consequences, is much more of a challenge as in neurotypicals. Most affordances regarding multiple agent interactions are perceptually transparent for most neurotypical people but not in ASD. Many of the difficulties are not absent from awareness in ASD. We will address below experiments where we investigate action perception loops in real and immersive environments.

The role of attention

Visual awareness and conscious perception cannot be fully understood without considering the role of attention (Parés-Pujolràs et al., 2019), which we have suggested above to be associated with abnormal alpha activity and "mind wandering" default mode networks (DMN) in neurodevelopmental disorders (Violante et al, 2013; Silva et al, 2016). Attentional networks also underlie the capacity for visual imagery, which we have shown to be distinct and even enhanced in autism spectrum disorder (Amaral et al, 2017, 2018; Simões et al, 2018, 2020). Moreover, we have recent tantalizing evidence that atypical sensory processing in autism spectrum disorder (ASD) may also involve higher level aspects in attentional networks (Agostinho et al., 2021). Such processing may lead to emotional arousal and impaired regulation. In other words, our findings link three very important points in conscious experience: sensory representations, body signals of emotion and arousal, and attention. We used task-based fMRI and a validated database of videos with variable arousal levels to characterize sensory processing of content generating emotional arousal. We found a dichotomy whereas the ASD individuals showed a clear yet lateralized activation bias of the

dorsal attention network, whereas the neurotypical participants activated preferentially the ventral attention network.

What are the implications of these findings? First they suggest a very distinct cognitive style in deploying attentional resources for perceptual awareness. The ventral attentional network is much related to sensing internal saliency and has stronger links to emotion regulation and inhibitory control circuits (Arnsten et al., 2012). This would suggest that the lack of activation in these circuits would lead to abnormal emotional regulation and physiological responses translated in changes states of mind/body interactions and increased anxiety. Embodiment is likely distinct in ASD, whereby attention resources are abnormally allocated to otherwise unnoticed processes within the brain, body or environment.

This has implications for neurorehabilitation approaches which are often blind to the user's emotional state. We are now acquiring sets of physiological signals simultaneously with functional neuroimaging because assessing the true emotional based on physiological response as a measure of autonomic nervous system, will serve as ground truth to understand activity of brain regions involved in ASD. We have also used machine learning approaches to classify attention and imagery states (Amaral et al, 2017, 2018; Simões et al, 2018, 2020; Borra et al, 2020) and are now also addressing arousal and anxiety states.

Consciousness and the Self in Autism Spectrum Disorder

The perception of "being me" is quite distinct in autism. It is well known that there is an initial blurred distinction between oneself and others during neurodevelopment in ASD. The inability to distinguish between oneself and others seems to represent a fundamental confusion in the sense of selfhood (Mizuno et al., 2011; Shield et al, 2015). This could be demonstrated even in a sign language study, in which verbalization was not required (Shield et al., 2015). Although the unusual use of pronouns tends to disappear during adolescence, the tendency to avoid their use and instead identifying oneselves and others by name still remains. This third-person view of themselves may be explained by an ill-defined sense of the self. Since selfhood is a fundamental aspect of consciousness, this adds to the evidence that consciousness is phenomenologically distinct in autism.

What makes the self distinctive in autism? Selfhood is an element of consciousness and embeds both a perceptual dimension and a perspective. One of the dimensions of consciousness described by Seth (2021), the conscious self, is also a feature of neurodiversity in ASD. The particular experience of being you, or being me in ASD suggest a kind of egodissolution of the self at initial stages of neurodevelopment: children often state you instead of me and it can take several years before the "correct" attribution of one's own actions and intentions is reached. As stated above this misattribution may remain in subtle ways, such as the tendency to avoid pronouns. The former feature is so pervasive that it is used as a diagnostic criterium.

The first-person feeling seems therefore to be experienced in a different way by people with ASD. The overwhelming sense of continuity between neurotypical developmental experiences during childhood and across the lifespan are continuously updated according to our own notions of our current self. That perceived unity of the self may be relevant in terms of evolutionary survival. Its delayed emergence in ASD may represent a variation of this pattern. Could we imagine situations where this will be advantageous from the Darwinian point of view? Such a loss of the boundaries of the self may influence the capacity for perspective taking, which is a feature that is clearly distinctive in ASD.

What would artificial intelligence experts think about this state of affairs? Can they build a system with strong subjective experiences of the self, or can they just build a refined device that only gives the apparent façade of possessing a self? In the case of ASD, the experience of the self is as intense as in neurotypicals, but I posit that in their case the boundaries of the self are much fuzzier and have a distinct nature. We have explored this issue in experiments addressing the experience of interpersonal space.

Exploring the neural correlates of Self versus Other imagery

To address perception of the self, it is important to instantiate solid and simple experimental paradigms. We formerly investigated the separability of the neural correlates of 2 types of motor imagery, self and third person. Imagining the actions owned by oneself vs. another individual are inherently distinct processes (Andrade et al., 2017). Our interest is twofold: on the one

hand to understand the neural correlates of perception of the self in terms of action planning, and on the other hand to go a step further and to determine if this would allow for the development of BCI interfaces to train perturbations of action and intention understanding beyond simple imitation, such as autism. We previously used EEG recordings from 20 healthy participants, as well as electrocorticography (ECoG), based on a virtual reality setup, to study these processes. We attempted discrimination between self versus other types of imagery at the single trial level (Andrade et al., 2017). To do that, time-frequency and source analysis were performed and these data were fed into statistical classification using Support Vector Machines. We found that self minus other imagery conditions in topographic maps were maximal at Frontal and Parieto-Occipital regions (corresponding to Theory of Mind networks), in agreement with the presence of 2 independent non μ brain rhythms related contributions to self-other distinctions in the low alpha frequency range. ECOG corroborated such separability. Source analysis confirmed that differences stemmed mainly from the temporo-parietal junction, involved in mentalizing. The single-trial classification accuracy was significantly above chance level for all the participants of this study. This type of study paves to way to study an important aspect of phenomenal consciousness, the perception of the self in situations of variable complexity. It is quite significant that Self and Third Person motor imagery use distinct electrophysiological mechanisms detectable at the scalp (and ECOG) at the single trial level, with separable levels of involvement of the mirror neuron system in different regions. These findings also provide a promising step to develop new BCI training/rehabilitation paradigms for impairments of action understanding beyond simple imitation, such as autism. It is a quite important social skill to train and anticipate perceived intentions of others as opposed to own intentions in group contexts.

A different sort of conscious perception? Dynamic shifting from holistic to fragmented perception in autism

One of the most intriguing phenomenal aspects of visual awareness is its relation to feelings of holistic perception. Do such feelings reflect an illusion? Perception is often viewed as a constructive process (Goebel et al.,

1998), and in that sense an illusion, or even a "controlled hallucination". If perception is a controlled hallucination (Seth, 2021), could it be possible that people with ASD are somehow more aware of the illusory nature of this construction? This speculative remark can be instantiated in their preference to focus on the "trees instead of the whole forest" (Bernardino et al., 2012).

We humans tend to bind conscious experience into a unified whole. We experience it in a fully integrated manner. Every conscious scene is experienced as a unified whole, but this does often not seem to be the case in ASD who often seem to see the details but not the global organization that stems from local elements. This does not mean that people with ASD are impaired at doing global perceptual analyses. This is just a matter of a distinct cognitive style. I posit that neurotypicals tend to integrate while people with ASD tend to segregate the world into more fragmented local pieces that they tend to analyse separately. Their focus of attention may be so narrow that they may separate colours of objects from their shapes or even experience objects independently of whatever else is going on. This is not at all what neurotypicals do. They tend to integrate all pieces if information into a fundamental representation that may not be found in ASD. They may be sensitive people without the need for holistic perception. We have addressed these questions experimentally and had some surprises. Interestingly, these differences may help explain why people with ASD have higher prevalence of synaesthesia (van Leeuwen et al, 2021).

Central coherence in Autism Spectrum Disorder

"Weak Central coherence" is a key historical concept in autism research (Frith, 2003). It basically suggests that people with autism tend to focus on details instead of the "big picture". Does the cognitive bias to focus on local features rather than on global forms really represent a weakness or impairment? If this is true people with ASD would be less able to holistically perceive stimuli such as the ones shown in Figures 17 and 18 (see above).

Uta Frith (2003) actually suggested that this tendency, originally reported by Kanner (1951), to be less driven by coherence might actually provide an advantage in classical neuropsychological tests such as the Block Design and Embedded Figures Tests, which actually depend on better seeing the parts

without being distracted by the global figure. The underlying message was however that weak central coherence may place ASD people at disadvantage.

But are people with ASD really impaired in seeing the whole? We provided a striking demonstration that there is no weakness at all (Bernardino et al., 2012). A true deficit is not present, just a task depend bias. The fact that we were able to study other neurodevelopmental disorders provided a unique view on central coherence across their diversity. This previous study from our group allowed for a direct comparison of local-global integration in autism and other disorders with direct implications for the central coherence hypothesis (Bernardino et al., 2012).

Several experimental paradigms based on hierarchical figures (for example the letter A defined by different local letters, such as Ts) have been used to test this controversial hypothesis. We addressed this theory by testing central coherence in five developmental groups, while accounting for intellectual disability, in a study that included about 100 participants: ASD with or without intellectual disability, Williams syndrome, a neurogenetic disorder "on the other side of the mirror" in the autism spectrum, matched controls with intellectual disability and chronological age-matched individuals.

We predicted that central coherence should be most impaired in ASD for the weak central coherence account to hold true. An alternative account would simply be based on dorsal stream dysfunction which dominates in Williams syndrome (Castelo-Branco et al., 2007). It remains however a possibility that central coherence in perception is solved in the dorsal stream as postulated by visual binding theories. We have latter on performed work suggesting that this might indeed be the case (Bernardino et al., 2014; Castelhano et al, 2015).

Central coherence was first measured by requiring subjects to perform local/global *preference* judgments (no real correct or wrong answer) using hierarchical figures under 6 different experimental settings (memory and perception tasks with 3 distinct geometries with and without local/global manipulations) (Figure 19). The rationale was to introduce perturbations that would challenge integration of local and global features under different constraints.

The *preference* requested reports focus on a preferential cognitive style and not on response correctness. But can one generate conditions where there

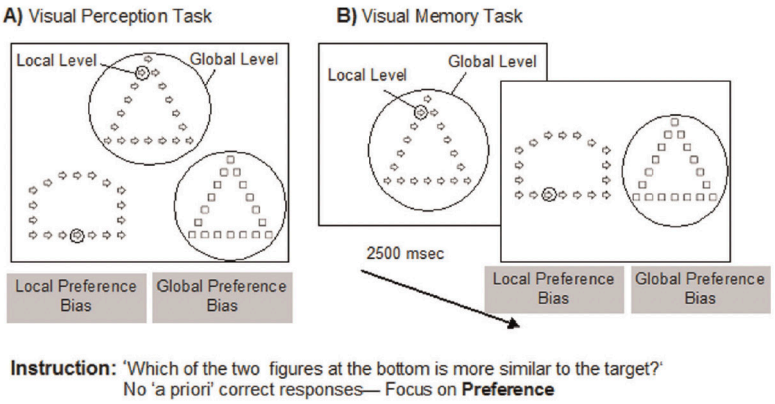


Figure 19. Illustration of the Visual ***Preference*** Tasks. Example of the configurations used in A) visual perception preference tasks and B) visual memory preference tasks.
From Bernardino et al., 2012. Copyright: The authors. License for unrestricted use under the terms of the Creative Commons Attribution License.

is a true/false correct answer. In this case, we are not simply talking about a cognitive style, but instead about true performance. Following this logic, we replicated the *preference* experiments under 4 additional performance (with explicitly correct or wrong answers) conditions (Figure 20).

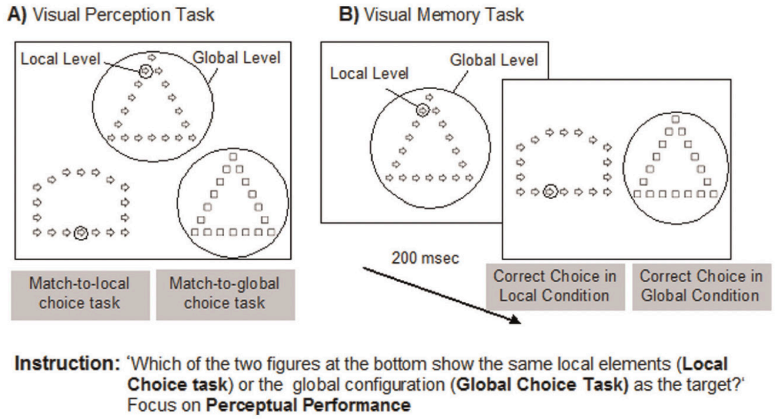


Figure 20. Illustration of the Correct Choice ***Performance*** Tasks. Example of the configuration used in A) visual perception correct choice tasks and B) visual memory correct choice tasks. Note. Figures are presented according to the real scale (not real size) and, therefore, visibility was higher in the experimental task.
From Bernardino et al., 2012. Copyright: The authors. License for unrestricted use under the terms of the Creative Commons Attribution License.

We generated memory and perceptual conditions under local/global configurations in which subjects reported the correct local or global configurations. Finally, we used a visuoconstructive task (requiring both visual and action skills by drawing of global figures made of local elements) as a way to measure local/global perceptual interference (figure 21).

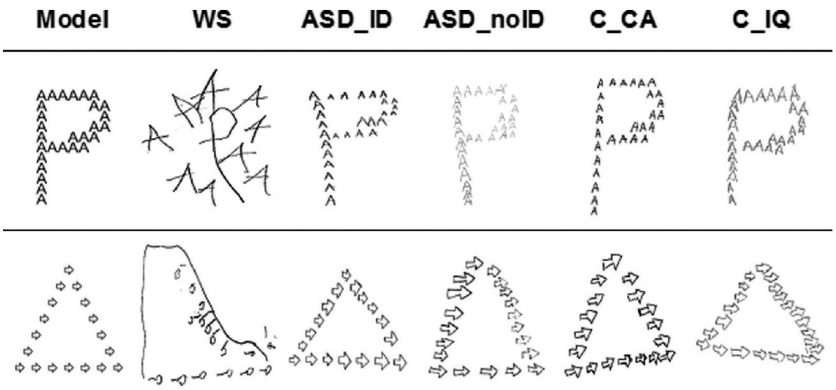


Figure 21. Examples of drawings of hierarchical figures produced by clinical and control groups. WS = Williams Syndrome group; ASD_ID = Autism Spectrum Disorders group with intellectual disability; ASD_noID = Autism Spectrum Disorders group without intellectual disability; C_TD = typically developing control group; C_ID = control group with intellectual disability.
From Bernardino et al., 2012. Copyright: The authors. License for unrestricted use under the terms of the Creative Commons Attribution License.

To our surprise people with autism were not the most impaired, but instead William's Syndrome participants, the ones with largest dorsal stream deficits (Figure 22 and Figure 23).

Autistic participants only showed a subtle pattern of coherence loss. This was found in four tasks conditions (where stimuli were rotated) favouring local analysis but the effect was quite small. Moreover, this subtle pattern was reduced when intellectual disability was taken into account. Our results suggest that abnormal central coherence cannot be considered a major feature explaining cognitive characteristics in the autism spectrum. Central coherence was much more affected in William's Syndrome which is indeed a condition in the autism but which in many cases participants do not fulfil the criteria for the diagnosis of autism spectrum disorder. Our observations

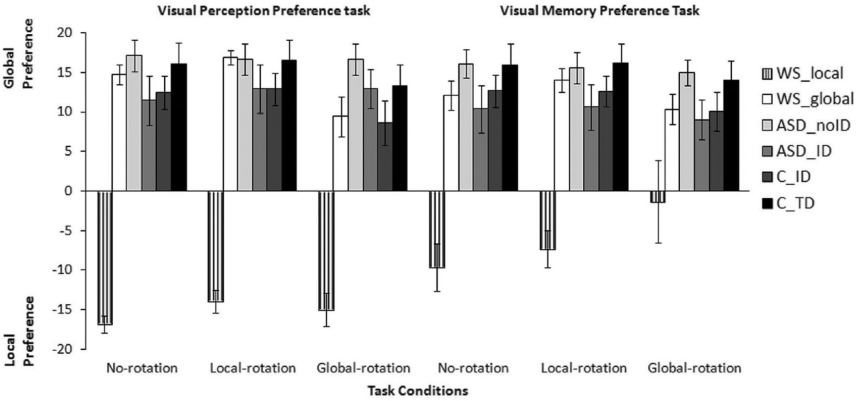


Figure 22. Local preference is stronger in Williams Syndrome than in Autism. Mean percentage of global responses for all clinical and control groups for the visual perception preference task conditions and the visual memory preference task conditions. Given the bimodal pattern found in WS only for this task, and for sake of clarity we plot two WS subgroups, according to dominantly local or global preference (see text). WS_local = Williams Syndrome subgroup with local bias; WS_global = Williams Syndrome subgroup with global bias; ASD_ID = Autism Spectrum Disorders group with intellectual disability; ASD_noID = Autism Spectrum Disorders group without intellectual disability; C_TD = typically developing control group; C_ID = control group with intellectual disability. From Bernardino et al., 2012. Copyright: The authors. License for unrestricted use under the terms of the Creative Commons Attribution License.

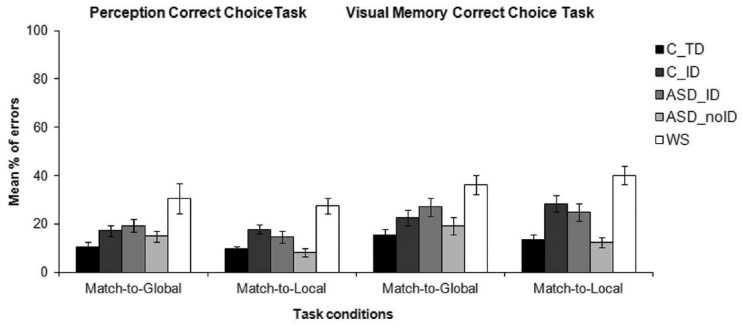


Figure 23. Local vs. Global Performance is weaker in Williams Syndrome than in Autism and other developmental groups. Mean percentage of errors for all clinical and control groups for the visual perception and visual memory "correct choice" task conditions. WS = Williams Syndrome group; ASD_ID = Autism Spectrum Disorders group with intellectual disability; ASD_noID = Autism Spectrum Disorders group without intellectual disability; C_TD = typically developing control group; C_ID = control group with intellectual disability.
From Bernardino et al., 2012. Copyright: The authors. License for unrestricted use under the terms of the Creative Commons Attribution License.

are consistent with the notion that strongly impaired visual dorsal stream impairs central coherence. However dorsal stream impairments are also small in Autism when taking into account other conditions such as William's Syndrome (Castelo-Branco et al, 2007). This theory should play a minor mechanistic role in Autism Spectrum Disorders, in spite of its popularity.

A Popperian argument: at least one condition, other than Autism has more perturbed perceptual coherence, challenging the weak central coherence hypothesis

We all know that when one idea is quite engrained in the community, it is quite difficult to be even revised. Even the controversial issue of the link between vaccines and autism is difficult to eradicate, in spite of all evidence that has falsified that claim (Signorini et al., 2022). We have shown (see previous section for our evidence on local versus global perception) that another condition, other than Autism, Williams Syndrome has more perturbed perceptual coherence (Bernardino et al, 2012). We further added additional evidence by using simple coherence tasks requiring the perception of global motion from local cues (Castelo-Branco et al., 2007; Mendes et al., 2005). In fact, all our previously generated evidence in that regard points to the Popperian argument that if at least one condition other than autism has diminished perceptual coherence, then this challenges the notion that weak coherence is a core deficit in autism.

Williams syndrome and Neurofibromatosis being neurodevelopmental disorders of genetic origin, are quite ideal models to understand visual cognition. We have shown deficits in the magnocellular pathway, which is afferent to the dorsal stream, and their relation to abnormal visual dorsal processing in these two neurodevelopmental conditions (Violante et al., 2012; Bernardino et al, 2014, 2021). For this type of task, we tested spatiotemporal contrast sensitivity tasks at frequency bands that activate the magnocellular pathway. In the case of Williams Syndrome, we have further examined visual performance using 2D and 3D motion integration tasks. A novel 3D motion coherence task requiring the 3D integration of a simple sphere was also used. Motion coherence requires the perceptual integration of locally moving stimuli into holistic object representations. We used spheres

with unpredictable axis of rotation, in order to investigate possible dorsal stream impairment. Perception of such tri-dimensional spheres is a simple instance of so called in 3D structure from motion perception. We have found a significant involvement of low-level magnocellular representations in Williams Syndrome and most importantly significantly impaired 3D structure from motion sphere perception. Our findings are consistent with the view that magnocellular damage may impact on dorsal stream and coherence in neurodevelopmental disorders. Surprisingly, the effect sizes of loss of perceptual coherence very are large in Williams Syndrome, even when the diagnosis of autism is (very often) absent. This represents additional evidence against the lack of central coherence hypothesis.

How correct are magnocellular theories?

Williams syndrome, because of its well-defined genetic origin, provides a unique model to link such genetically determined loss of neural cell populations at different levels of the nervous system with neural circuits and visual behaviour. Given that several of the genes deleted in WBS are also involved in eye development and the differentiation of retinal layers, it is also important to investigate low level origins for visual phenotypes. We examined the retinal phenotype in Williams syndrome patients and its functional relation to global motion perception (Castelo-Branco et al., 2007). We discovered a low-level visual phenotype characterized by decreased retinal thickness, abnormal optic disk concavity, and impaired visual responses in WBS patients compared with age-matched controls by using electrophysiology, confocal and coherence in vivo imaging with cellular resolution, and psychophysics. These mechanisms of impairment are related to the magnocellular pathway, which is involved in the detection of temporal changes in the visual scene. Low-level magnocellular performance did not predict high-level deficits in the integration of motion and 3D information at higher levels, thereby demonstrating independent mechanisms of dysfunction in WBS that will require remediation strategies different from those used in other visuospatial disorders. These findings challenge neurodevelopmental theories that explain cortical deficits based on low-level magnocellular impairment, such as regarding a broad range of

conditions from dyslexia to autism. Possibly, the activation of dorsal stream attentional networks by magnocellular stimuli is more important, as we have shown above (Violante et al., 2012) that the nature of magnocellular activation per se.

Can brain activity patterns be indexed as markers of abnormal coherence?

Although perceptual coherence is not necessarily decreased in autism, holistic perception is nevertheless of a distinct qualitative nature, which may translate into disease specific neurophysiological signatures.

Neurophysiological biomarkers have been widely investigated in autism, in the search for diagnostic, prognostic and therapeutic outcome measures. It has been claimed that temporal oscillatory patterns are signatures of central coherence. I was involved early in my career in this debate in basic neuroscience (Castelo-Branco et al., 2000) that we also carried over to clinical neuroscience (Castelhano et al., 2018). Specifically, we have investigated whether simple perceptual decisions requiring visual coherence can be used to identify stimulus dependent oscillatory signatures as potential biomarkers in autism spectrum disorder (ASD). We studied different types of face stimuli with different levels of abstraction requiring holistic coherent perception (Tavares et al., 2016) into different extents and used machine learning techniques to classify brain states (Castelhano et al., 2018).

Individuals with autism spectrum disorder have often impaired recognition of faces and emotional expressions. Three levels of face processing can be considered in the analysis of faces: first order (two eyes, above a nose, which is above a mouth), second order (the relative distance between features), and holistic (ability to recognize as faces images that lack distinctive features due to their abstractedness). The experimental stimuli depicted in Figure 24, show how we can approach these different levels of analysis.

Some studies have reported deficits in configural and holistic processing, requiring central coherence, in individuals with ASD. We examined neural signals at different frequency bands over time and investigated regional neural synchrony of these frequency patterns. The question was whether these patterns are determined by the level of coherence as defined by non-

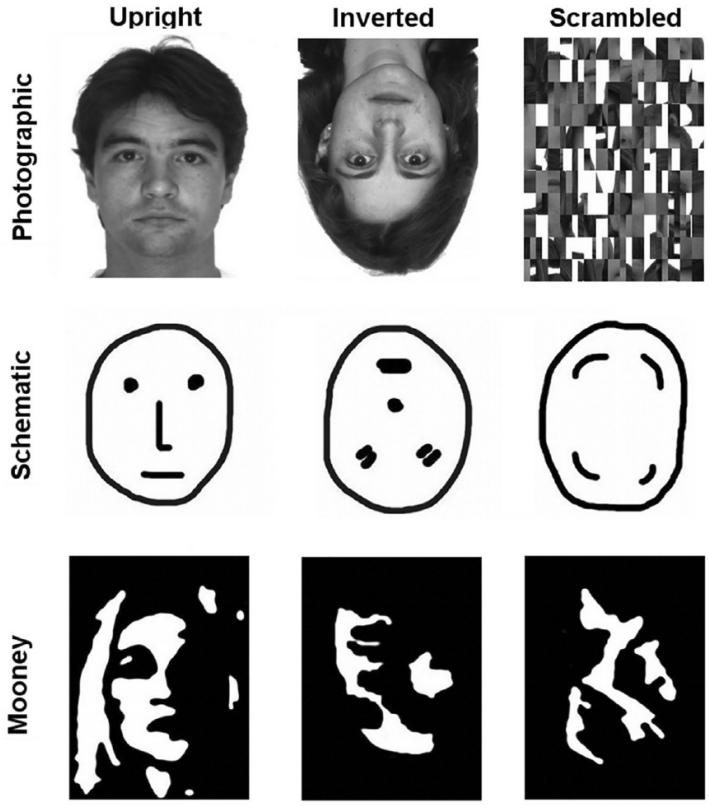


Figure 24. Example of stimuli used to address three levels of face processing (first order, second order and holistic). Participants had to detect a face in the picture and respond as soon as possible if there was a face or not. Adapted from Castelhano et al., 2018. Copyright secure at Clearance Center's RightsLink.

holistic vs. holistic processing in ASD and controls. We found that the neuronal oscillatory responses of low gamma frequency band (around 40Hz), both to photographic and abstract black and white face stimuli (requiring holistic interpretation) are distinct in ASD vs. the control group. We also found decreased beta (15-30 Hz) oscillatory responses in ASD in relation to response preparation (Figures 25 and 26).

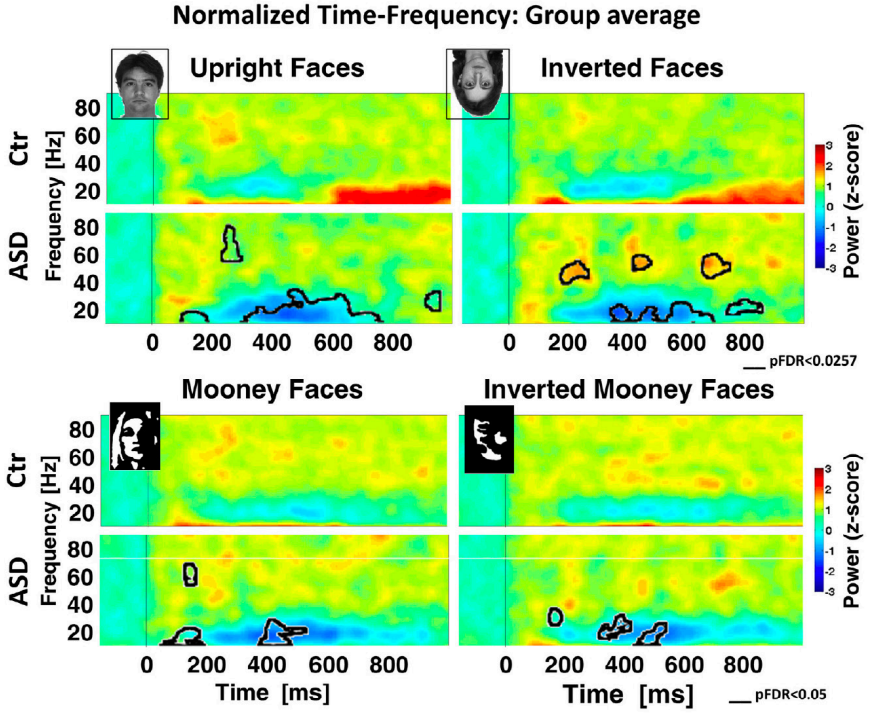


Figure 25. Time-frequency analysis revealed significant differences between groups in response to upright and inverted faces. Different patterns responses in a broad gamma frequency range in controls (Ctr) vs. ASD for the Mooney face stimuli. Time-frequency plots are a group average of all channels per group and condition as depicted. The black line blobs signal the significant differences between groups (corrected for multiple comparisons with both the FDR and the cluster based approaches). Fig. 26 represents the TF differences for additional stimuli. Zero is the stimulus onset. Adapted from Castelhano et al., 2018. Copyright Clearance Center's RightsLink.

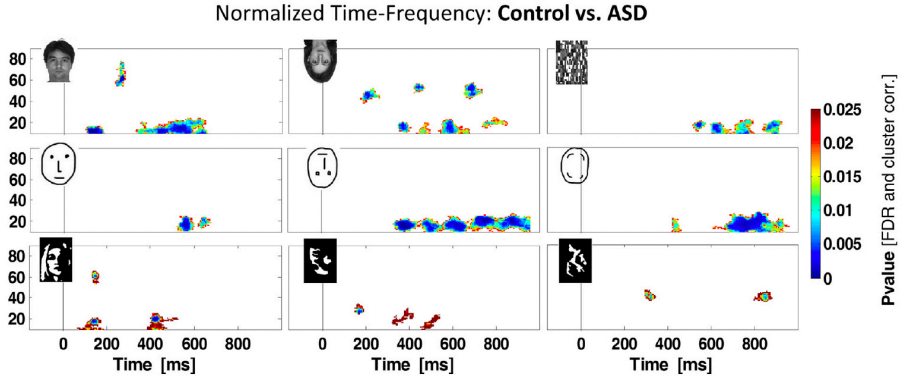


Figure 26. Time-frequency (TF) differences between groups. TF plots show the statistically significant difference for each condition after FDR and cluster correction for multiple comparisons. Note that the larger differences are present for the low gamma and beta frequency sub-bands. Zero is the stimulus onset. Adapted from Castelhano et al., 2018. Copyright Clearance Center's RightsLink.

Neural synchrony, a marker of coherent integration, in the 30-45 Hz band was differently distributed over space in ASD (Figure 27)

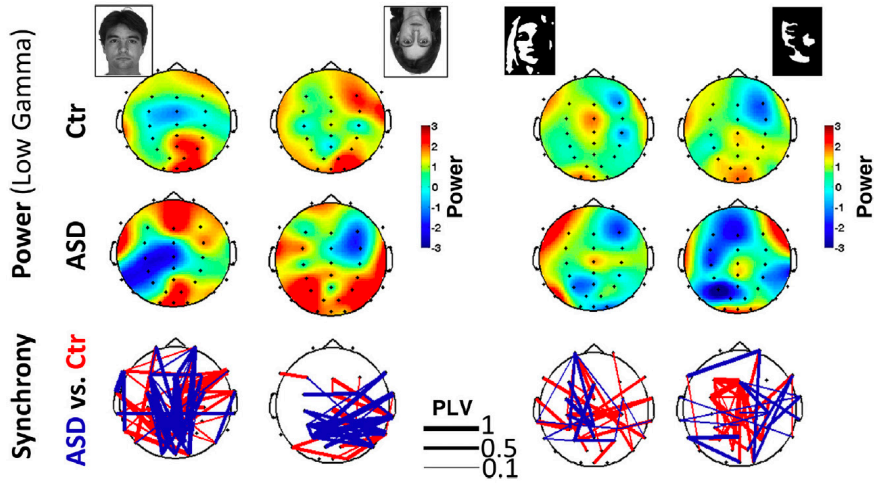


Figure 27. Graph diagram showing significant synchrony increases. Topography plots and synchrony lines are represented for both groups in four different stimulus conditions. Data are plotted for the low gamma frequency range (30–45 Hz) and the 50–350 ms time-window. Synchrony lines are statistically significant at p = 0.01. Adapted from Castelhano et al., 2018. Copyright Clearance Center's RightsLink.

These neural differences enabled accurate classification of ASD using machine learning approaches, with an accuracy of about 85%. More classical diagnostic approaches based on signal detection theory (called receiver operating characteristic) showed about 94% accuracy (Figure 28). Combination of features from abstract black and white holistic faces (called Mooney faces, see Figure 24) and Photographic face stimuli enabled the best between group separation, reaching 98.6%.

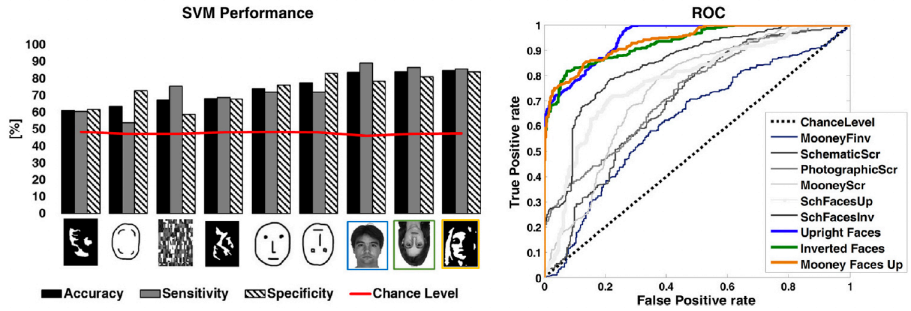


Figure 28. Classification of ASD vs. controls: SVM performance and ROC curves per condition. A 3-fold cross-validation was repeated 1000 times and the accuracies, sensitivity, specificity, and ROC curves were calculated. Significance values assessing that the classification achieved best than chance were calculated in comparison to the classification with random labels ($p < 0.0000001$). Left: SVM performance per condition; red line stands for the accuracy level calculated with the random labels (chance level). Right: ROC curve per condition; the colour lines mark the three best conditions. Note the higher classification Accuracy and AUC for the upright and Mooney face conditions. Adapted from Castelhano et al., 2018. Copyright Clearance Center's RightsLink.

The relative decrease in EEG responses to face stimuli in ASD in the beta and gamma frequency ranges (for some stimuli) are quite interesting because they provide some evidence for different integration mechanisms which can be probed by machine learning techniques. Temporal patterns of EEG time-frequency responses evoked by particular types of faces requiring holistic integration may therefore be used as diagnostic biomarkers and potentially as outcome measures in clinical trials.

Can classical neurophysiological measures be biomarkers of Autism Spectrum Disorder?

It is important to note that more conventional neurophysiological

signals such as average signal amplitude responses (event-related potentials) may be less informative in studying face processing mechanisms in ASD. We nevertheless investigated event-related potentials (in particular the face selective N170 potential, a face selective negativity peaking at 170 ms) in high-functioning adults with ASD and healthy controls, during the above-mentioned face decision task, using the comprehensive set of photographic, schematic and Mooney upright and inverted faces, and scrambled images (Tavares et al, 2017). ASD and healthy controls were performance matched but at the electrophysiological level, participants with ASD showed a bilateral N170 inversion effect in latency and left lateralized in amplitude for photographic faces. This inversion effect was previously believed to be only present in neurotypicals, but was obviously also present in adult ASD: N170 showed with bilaterally longer latencies and left higher amplitudes (more negative) for inverted than upright photographic faces, and a right lateralized N170 inversion effect in latency for schematic faces. In spite of previous findings, in particular in children, we observed that under performance-matched conditions, adults with ASD show preserved N170 inversion effects and associated sparing of facial configural processing. Does this mean that people with ASD might become more neurotypical with age? This is an outstanding question that pervades other research areas in ASD, in particular the ones related to high functioning individuals.

Another Popperian argument: paradoxically enhanced brain Gamma Oscillations in a Model of Disrupted Perceptual Coherence beyond the Autism Spectrum.

Can brain Oscillations facilitate Synchrony and thereby coherence? It has been hypothesized that enhanced gamma oscillations and neural synchrony underlie perceptual coherence in health and in autism (Uhlhaas and Singer, 2006). As explained above, the hypothesis of loss of central perceptual coherence has been suggested to be pivotal in the distinct cognitive style in autism spectrum disorders (Happé, 1999). Williams syndrome is a neurodevelopmental disorder linked with autism, and a much more clearcut model for impaired central coherence that idiopathic autism itself, as we have demonstrated before (Bernardino et al., 2012; Castelhano et al. 2015, see above). This model of dramatically impaired holistic processing

is ideal to probe the hypothesis that loss of gamma oscillations is related to reduced neural synchrony and visual integration. We used EEG and a set of experimental tasks requiring coherent 3D perception. These required the integration of local elements to achieve face perception of tridimensional faces (structure from motion). There was an unexpectedly higher amplitude modulation at Beta and gamma frequency rates for all stimulus conditions in the patient group (Figure 29). However, neural synchrony of these oscillations was reduced across stimulus conditions, in line with the coherence hypothesis (Figure 30).

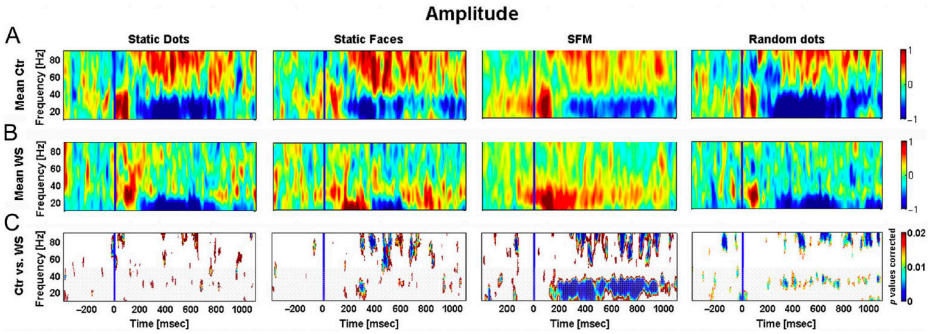


Figure 29. Paradoxically increased neural oscillation amplitude in Williams Syndrome. Time–frequency analysis results per experimental condition shows distinct oscillatory patterns across different stimulus conditions (Static Dots, Static Faces, 3D Faces made of moving dots (SFM – structure from motion) and moving random dots). in Williams Syndrome and Controls. (A) Normalized (to prestimulus baseline) gamma-band activity patterns for the four conditions for the control group. (B) Time–frequency analysis for the WS group. Colour codes indicate normalized scores. (C) Statistical maps for the between-group comparison (Ctr vs. WS, Wilcoxon test with FDR-corrected p values shown per time and frequency points). The blue line represents the stimulus onset, and the gray background highlights the low-frequency range. The analysis depicted here corresponds to the parieto-occipital cluster of electrodes. Stimulus-driven 60–90 Hz gamma oscillations dominate in the control group, whereas in WS modulations have larger amplitudes near 25–45 Hz. Adapted and courtesy of The MIT Press from "Castelhano J, Bernardino I, Rebola J, Rodriguez E, Castelo-Branco M. Oscillations or Synchrony? Disruption of Neural Synchrony despite Enhanced Gamma Oscillations in a Model of Disrupted Perceptual Coherence. J Cogn Neurosci. 2015 Dec;27(12):2416-26. doi: 10.1162/jocn_a_00863. Epub 2015 Aug 18. PMID: 26284991.

This combination of a dramatic loss of synchrony despite increased oscillatory activity is strong evidence that synchrony underlies central coherence, but also shows that neural oscillations (from the point of view of amplitude) are less relevant in the context of ASD, unlike previously believed.

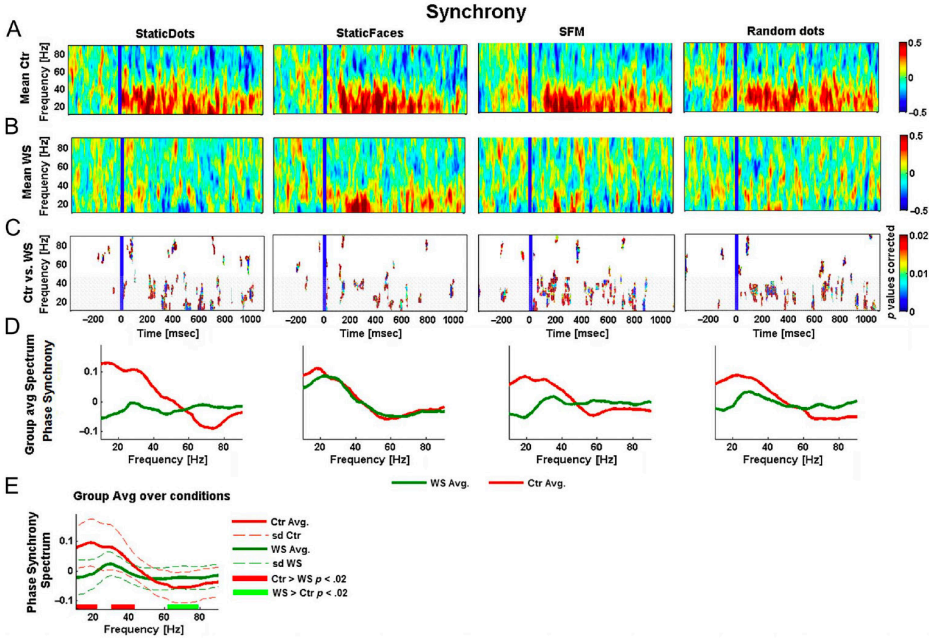


Figure 30. Decreased synchrony of neural oscillations in Williams Syndrome as compared to Controls. Time–frequency analysis results per experimental condition shows distinct oscillatory patterns across different stimulus conditions (Static Dots, Static Faces, 3D Faces made of moving dots (SFM – structure from motion) and moving random dots). in Williams Syndrome and Controls. (A) Normalized (to prestimulus baseline) gamma-band activity patterns for the four conditions for the control group. (B) Time–frequency analysis for the WS group. Colour codes indicate normalized scores. (C) Statistical maps for the between-group comparison (Ctr vs. WS, Wilcoxon test with FDR-corrected p values shown per time and frequency points). The blue line represents the stimulus onset, and the gray background highlights the low-frequency range. The analysis depicted here corresponds to the parieto-occipital cluster of electrodes. Stimulus-driven 60–90 Hz gamma oscillations dominate in the control group, whereas in WS modulations have larger amplitudes near 25–45 Hz. Adapted and courtesy of The MIT Press from "Castelhano J, Bernardino I, Rebola J, Rodriguez E, Castelo--Branco M. Oscillations or Synchrony? Disruption of Neural Synchrony despite Enhanced Gamma Oscillations in a Model of Disrupted Perceptual Coherence. J Cogn Neurosci. 2015 Dec;27(12):2416-26. doi: 10.1162/jocn_a_00863. Epub 2015 Aug 18. PMID: 26284991.

This dissociation between amplitude and synchrony which was reported for the first time in a human model of impaired perceptual coherence, is quite remarkable because it challenges the notion that amplitude of brain oscillations is a marker of central coherence. Instead, impaired synchrony, as

an indexed by loss of phase coherence, is more directly related to disruption of coherent perceptual integration as further shown in Figure 31.

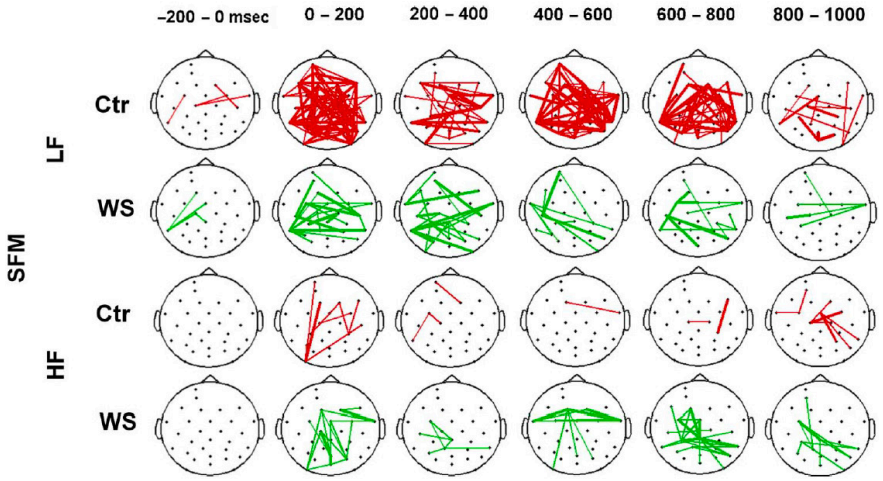


Figure 31. Phase synchrony changes in the Low Frequency (25–45 Hz) and Hight Frequency (60–90 Hz) bands plotted across space and time. Circles indicate electrode positions, with anterior sites at top and posterior sites at bottom. Results are shown for the 3D structure from motion perceptual condition over six time windows spanning the entire duration of the stimuli (–200–0 msec was defined as baseline). Red lines mark increased synchrony between electrode sites for the control group, and green lines mark higher synchrony for the WS group (lines are plotted with a significance threshold of $p < .01$). Note the increased number of lines (representing increased synchrony) for the Low Frequency band in the control group. Adapted and courtesy of The MIT Press from "Castelhano J, Bernardino I, Rebola J, Rodriguez E, Castelo-Branco M. Oscillations or Synchrony? Disruption of Neural Synchrony despite Enhanced Gamma Oscillations in a Model of Disrupted Perceptual Coherence. J Cogn Neurosci. 2015 Dec;27(12):2416-26. doi: 10.1162/jocn_a_00863. Epub 2015 Aug 18. PMID: 26284991.

A step further in the understanding of sensory awareness in autism spectrum disorder

Synaesthesia, imagery and illusions

Visceral perception and sensory sensitivity, either exacerbated or diminished, are characteristically disturbed in autism spectrum disorders. How are their sensory representations formed as mental images?

Synaesthesia is a condition in which triggered activity in one sensory modality causes experiences in a second sensory modality, in spite of absent stimulation in the latter. This condition, which has intrigued philosophers and artists, is more frequent in ASD (Bouvet et al., 2019, van Leeuwen et al., 2021).

One of its most common forms is grapheme-colour synaesthesia. In this condition individuals report striking experiences of colour when reading numerals, letters, or words. They arise automatically and may represent different brain wiring. Interestingly, the article cited above showed that the degree of grapheme-colour synaesthesia was associated with autistic traits within the domain of Attention to Details and with sensory hyper-, but not hypo-sensitivity. This places an important role for attention in sensory awareness processes in autism. Most importantly and unexpectedly, twins with a higher degree of grapheme-colour synaesthesia were better than their co-twins at identifying fragmented images (Fragmented Pictures Test). The association of synaesthesia with a more detail-focused attentional style needs to be linked with the hypothesis that it associated with a distinct mental imagery and visual awareness apparatus. This is not surprising, given that attention and mental imagery networks are tightly linked.

Mental imagery is distinct in ASD as we have also shown (Simões et al, 2018). But is it weaker, as often assumed? Evidence showing that regions mediating mental mirroring may be recruiting more brain resources in ASD, may suggest that this is the case (Wadsworth et al., 2018). Our own evidence actually also points in this direction (Simões et al, 2018). We have indeed shown evidence for increased attentional deployment in visual imagery tasks requiring the imagination of dynamic emotional faces. There was a striking match between the neural networks underlying the visualization and imagery of such facial expressions (Figures 32, 33 and 34).

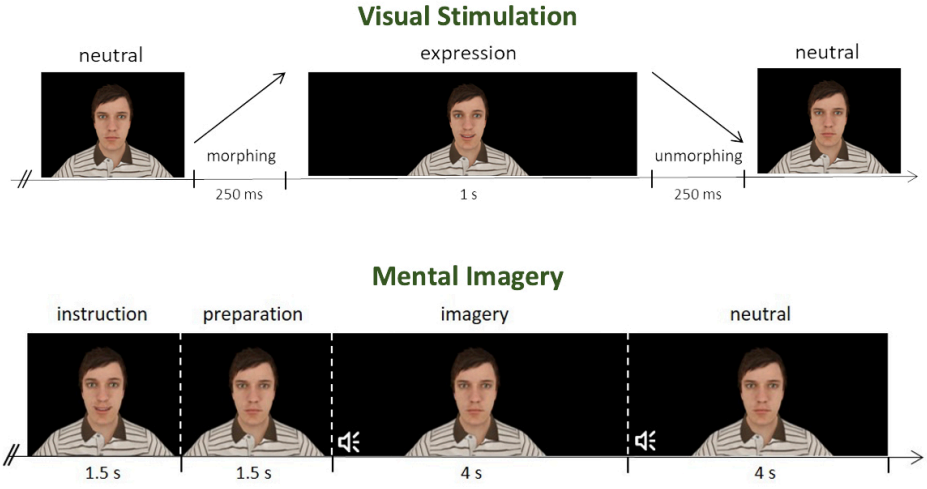


Figure 32. Description of the visual and imagery tasks, both regarding structure, and stimuli used by Simões et al., 2018. Base stimuli used for each expression at their expression endpoint, comprised the neutral, happy, and sad facial expressions. (TOP panel) Structure of the visual stimulation paradigm: each expression lasted 1.5 s, divided by facial expression morphing (250 ms), static facial expression (1 s) and facial expression unmorphing (250 ms). (BOTTOM panel) Structure of the mental imagery paradigm: the instruction is composed by the avatar performing the expression to be imagined, as presented in the visual stimulation task, and to facilitate mental replay. After that, an interval of 1.5 s is left for preparation, and an auditory stimulus (beep) cues the start of the mental imagery process, for 4 s, whereas another beep indicates the end of the mental imagery of the expression, and the start of the neutral period.

The generation and manipulation of mental images is important to solve visuospatial tasks. Others have in fact claimed that this ability may actually be more developed in autistics (Soulières et al, 2011). Our evidence suggesting that this is indeed true (Figures 33 and 34).

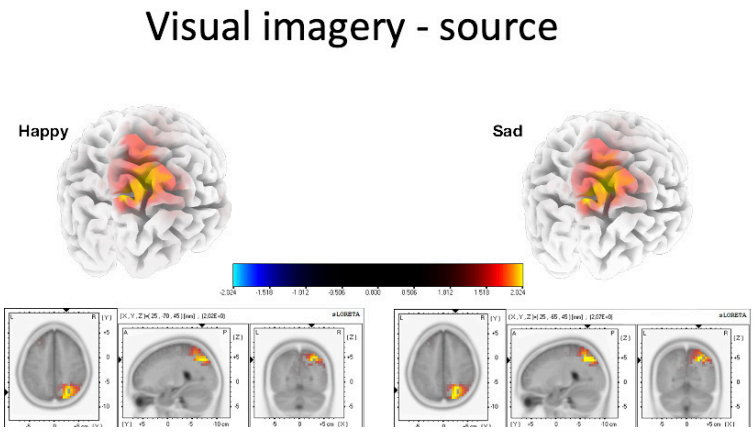


Figure 33. Increased evoked potential activity (event related potentials – ERPs) in parietal cortex in a visual imagery task of facial expressions in autism spectrum disorder. Group differences for the electric source analysis of the ERP signals of mental imagery. Statistical differences (two-tailed $p < 0.01$, corrected) were found in the region of precuneus, with higher activation for the ASD group.

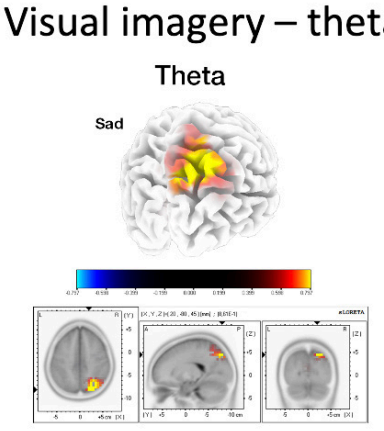


Figure 34. Increased oscillatory activity at low frequencies (theta band – between 4 and 7Hz) in a visual imagery task of facial expressions in autism spectrum disorder. Electric source analysis for the mental imagery segments, in the theta band. Higher activation for the ASD group in the precuneus (parietal) area (two tailed $p < 0.05$, corrected).

What can explain discrepancies in interpretation of available findings? The above mentioned authors claim that autistic people are particularly able in veridical mapping, e.g., in generating mental isomorphic representations of the world, which is consistent with our own evidence (Simões et al., 2020).

All these dissonances led to alternative theories such as the enhanced perceptual functioning model, which postulates that in autism there is overfunctioning of brain regions typically involved in primary perceptual functions (Mottron and Burack, 2001). This theory came as an alternative to the weak central coherence hypothesis, but they are both actually compatible, and maybe be criticised in equal terms based on the fact that they are not neurobiologically grounded, as we have demonstrated at least for the former.

The integrity of the imagery of the self in Autism Spectrum Disorder

We have argued above that the mind body problem might be essentially distinct in autism. How do the brain and body give rise to consciousness in ASD? This remains a mystery in ASD because they often show difficulty in communicating, which deepens the trouble in understanding their inner universe.

Even neurotypical individuals take their mind-body interactions for granted but that may not be at all the case. Our sense of integration, of being an integral human maybe an illusion. Being a self must have a purpose, at least from an evolutionary perspective. Making perceptual predictions on internal states is important for survival and is the core of the construction of the self.

Can we understand the contents of consciousness as the result of continuous perceptual predictions for adaptive behaviour? It could be that this process is less effective in autistic individuals, leading them to prefer sameness. The absence of change poses less adaptive changes and this is maybe why their repetitive behaviours are so prominent. The autistic self may be afraid of unexpected interactions with the outside world.

Ecological tasks reveal a distinct pattern of brain functioning

When performing simple ecological tasks, requiring demanding interactions with the outside world, such as virtual supermarket shopping

(Mouga et al, 2022), people with ASD show a very different task related brain organization. We recently found using a distinctive task (shopping) evoked hyperactivation of central executive, saliency and social cognition networks while navigation related networks in the parahippocampal gyrus required much less activation than controls (Mouga et al, 2022).

The concomitant role of these networks in everyday demanding tasks had remained largely unknown, and this is why our research strategy is focusing on these demands. We addressed this question using this novel task-based fMRI virtual-reality task mimicking a stimulating daily-life assignment that may present some difficulties to individuals with ASD: the EcoSupermarketX (Figure 35, Mouga et al., 2021).

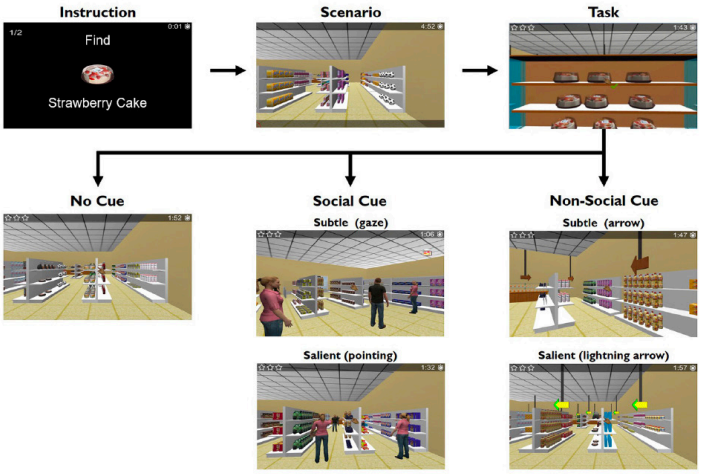


Figure 35. EcoSupermarketX task design, considering different types of cues (social, nonsocial and absent cure). The test blocks included an instruction that consisted in the grocery list the participants had to pick. The grocery list (2, 3, or 4-items) was presented as an instruction individually in a trial-by-trial basis: "Find strawberry cake" followed by "Find sausages" in a 2-item grocery list, for example (each item image and name appeared for 3 s). The grocery list had a variable number of items (two, three, or four) which defined the three different condition blocks with increased executive load. The groceries were replaced randomly in the shelves on a trial-by-trial basis.
Participants were instructed to collect all items in the sequence they appeared in the list, and as fast and accurately as possible. Additionally, there were five different conditions in each 2, 3, or 4-item conditions: non-social salient (blinking luminous arrow), non-social subtle (wooden arrow), social salient (avatar pointing to the grocery), social subtle (avatar gazing to the grocery), and no cue.

In a prior behavioural study using the EcoSupermarketX (Mouga et al., 2021), we had shown that attentional Cueing and Executive Deficits were present in this Virtual Supermarket Task Coupled with Eye-Tracking in Autism Spectrum Disorder.

Executive functioning alterations in Autism Spectrum Disorder (ASD) may impact on other complex functions, such as social cognition. We assessed this link between executive function, attentional cueing, and social cognition with this novel ecological task. Our task had three blocks of increasing executive load and incorporated social and non-social cues, with different degrees of saliency. Performance of ASD and typical neurodevelopment was compared. The ASD group showed a remarkable performance dependence on the presence of contextual cues and cognitive load. Between-group differences were found both for social and non-social salient cues. Eye--tracking measures showed significantly larger fixation time of more salient social cues in ASD. In sum, EcoSupermarketX is sensitive to detect distinct executive function profiles and sensitivity to attentional cues in ASD. In the neuroimaging part of the study, participants were also adolescents with ASD and with typical neurodevelopment (TD). The EcoSupermarketX that was developed in our group was adapted to this neuroimaging setting and featured a shopping simulation with three goal-oriented sub-tasks including the "no cue", "non-social" or "social" cues. We coupled this task with neuroimaging and eye-tracking. ASD differed from TD in total time to complete the task and distance to complete the "social cue" task. We found simultaneous hyperactivation across social, executive, and saliency circuits in ASD, in contrast with reduced activation in the parahippocampal gyrus (Figure 36). The massive overactivation of three main core networks in a daily living task is quite striking a suggests a strong neural burden in dealing with these complex tasks requiring behavioural flexibility in ASD.

It is also interesting that a cortical region involved in navigation, which is expected to be activated in this type of task, showed reduced activation in ASD. What it is the meaning of this reduced activation? Does it mean that in ASD navigation is efficiently and requires less recruitment of neural networks? Notably this region is involved in scene recognition, which is a function which ASD people tend to have preserved or are even above average. Accordingly, this pattern of reduced activation is consistent with

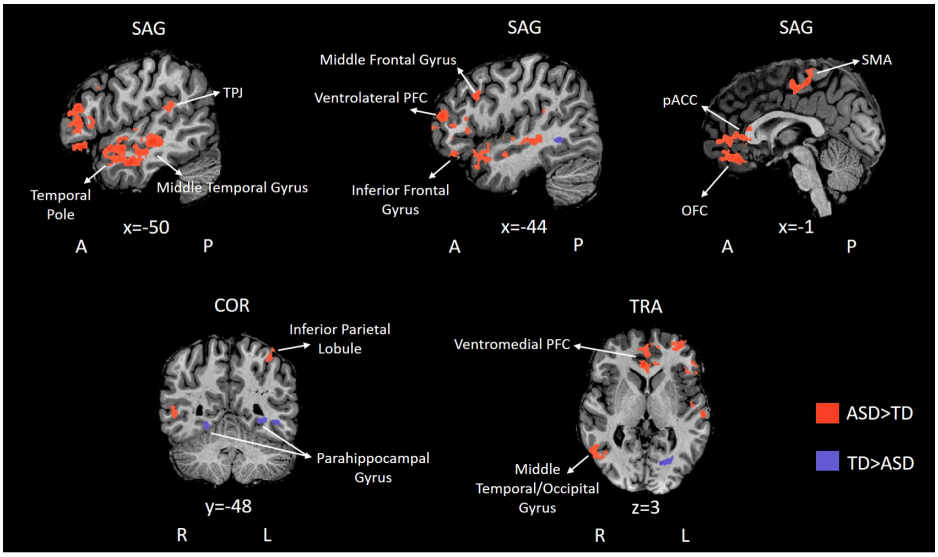


Figure 36. Recruitment of core brain networks using an ecological demanding daily life task. RFX ANOVA group effects for EcoSupermarketX task. Statistical maps from group analysis overlaid on sagittal, coronal, and transversal slices of a representative subject. Red clusters depict regions where BOLD activity was higher for individuals with ASD than TD. Blue clusters depict regions where BOLD activity was lower for individuals with ASD than TD. Slice locations are given in MNI coordinates. ASD, autism spectrum disorder group, TD, typical neurodevelopment group, TPJ, temporal-parietal junction, PFC, prefrontal cortex, pgACC, pregenual anterior cingulate cortex; OFC, orbitofrontal cortex, SMA, supplementary motor area, A, anterior; P, posterior, R, right, L, left; SAG, sagittal plane; COR, coronal plane; TRA, transversal plane; MNI, Montreal Neurological Institute.

effortless visual scene processing. This contrasts with the observation that when performing this virtual shopping task ASD adolescents hyperactivate three major core networks for the same level of performance: executive, saliency and social cognition. This suggests that they are less efficient in using these networks. These differential patterns in daily life tasks provide network based evidence neural diversity in ASD. These patterns highlight the presence of peaks and valleys as signatures of differential performance and neural activity in individuals with ASD.

Is social cognition specifically and primarily affected in ASD? Departing from genetic theories

Social communication deficits are core manifestations in ASD. The question however remains whether these deficits are from a causal pathway or just a consequence from disruption in other brain systems.

It is quite clear that they are not specific, since comparable social cognition deficits occur in a variety of other mental disorders such as schizophrenia and bipolar disorder. We have recently also studied these clinical conditions using a social interpretation task based on simple visual stimuli (Madeira et al., 2021). A substantial amount of genetic and neurobiological studies proposed similar mechanisms of theory of mind across these conditions, but we actually recently concluded that this might not at all be the case. Social processing may actually be fundamentally distinct in these apparently related disorders. We studied key structures in the social brain, such as the temporoparietal junction using a low-level social judgment task in sixty participants (evenly divided in schizophrenia, bipolar disorder and control groups), while participants underwent neuroimaging scans. The task used during fMRI implied the interpretation of social meaning based on simple geometric figures. This Theory of Mind visual paradigm led to opposite patterns of neural responses in two subregions within the temporoparietal junction. In the right supramarginal gyrus region schizophrenia patients showed social content-related deactivation relative to controls and bipolar disorder. This clinical condition showed the largest activation pattern. It is noteworthy to point out that enhanced activation in bipolar disorder represents an opposite deviation as compared to schizophrenia. Notably, brain activation patterns were indeed "in-between" the patient groups, providing a quite striking dividing border region between the pathological conditions. A mirror reverse pattern was found in the left posterior superior temporal gyrus where schizophrenia patients and controls showed larger activation, again with controls providing an "in-between" divisive border in terms of activation levels. These contrasting activation patterns were fully unexpected and suggest a very distinct underlying neurobiology, in contrast of what has been proposed based simply on genetic data.

The debate in neurodiversity in relation with personalized approaches

Judy Singer, an autistic social scientist, who we cited already in this book, was the first to use the term "neurodiversity" more than two decades ago in the context of autism (Singer, 1998), opening a discussion that still remains controversial today. It defies the notion that autism itself is a disorder that necessarily needs to be medically addressed. Its counterpart term is "neurotypical" which refers the to the majority who possess less diverse brains, unlike autism which might represent an example of diversity and not necessarily an "abnormal" condition.

It could be argued that in complex environments some of the differences that characterize autism spectrum disorder appear as manifest disabilities. This calls for the use of personalized approaches. In more friendly environments these problems may not emerge. Should we request people with autism to behave as much socially adapted as neurotypicals? Why not accepting this difference which may boost other types of competence? This question does not have a simple answer. One may say "not one size fits all" but the training the ability to bear adaptive behaviour can be argued to help people better adjust to the demands of ever-changing environments. Nevertheless, one can help design environments that are more friendly to non-neurotypicals. Sometimes just a few changes help render an environment more inclusive and fitted to the needs of distinct individuals.

Obviously, this discussion applies differently to people with so called high functioning autism and not to severely handicapped people who are often non-verbal and have a range of co-morbidities that are tightly associated with autism. Different viewpoints should become compatible by taking into account heterogeneity of manifestations and individual needs. However, the concept of disability needs to be carefully considered in this regard. Social communication difficulties may be considered disabilities if some unexpected contexts emerge, where communication is required. This may explain difficulties emerging from the need to adjust to unexpected events. This may help explain the preference for repetition or the "need for sameness" that people with autism often manifest. Whether such narrowing of interests may be maladaptive seems obvious, and even if it is not considered a disability it might still be worth trying to improve such patterns, because

they may compromise much needed flexible behaviour. On the other way around, should sensory hyper- and hypo-sensitivities be treated? I would say that only if they affect important daily routines. Respecting differences should not impede from trying to improve adaptive skills, using personalized approaches, while putting value in frequently observed strengths in Autism which include the capability for attentional hyperfocus and particular memory abilities.

Enigmatic sources of variability: comorbidities in autism spectrum orders

Autism spectrum disorders are often accompanied by other pathological sources of variability, leading to abnormal brain activity and often to epilepsy. It remains an outstanding question whether such co-morbidities share the same mechanisms. In epilepsy this would make sense because in this condition there is widespread evidence of excitation vs. inhibition imbalance (Abuhaiba et al., 2022).

This has led in fact to the proposal that some forms of epilepsy are actually neurodevelopmental disorders, and not simply due to focal lesions such as dysplasia. The converse might actually be true: may be some neurodevelopmental disorders such as ASD are accompanied by subtle structural alterations that current methods are unable to identify. This has motivated us to study localized neurodevelopmental defects as opportunity to study structure-function correlations in the human brain (Duarte et al, 2013). These studies have shown that even small structural abnormalities can have a profound impact. We found that topographic changes in retinotopic organization of visual regions reflect long term functional effects of abnormal electrical discharges during brain development. For example, a small developmental lesion in the visual dorsal stream, involved in motion perception, was sufficient to disrupt this function, while keeping preserved functions of the visual ventral stream, involved in object recognition.

Comorbidities such as epilepsy, sleep disorders, anxiety and hyperactivity are examples of the huge heterogeneity found in ASD. A major question is whether comorbidities are separate manifestation or they a core feature regarding the aetiology of some forms of autism. This is relevant for the

debate with all stakeholders, regarding what should or not be treated in this condition.

Heterogeneity and diversity in causal pathways from the genetics point of view

There are many genetic theories on the aetiology of ASD, but success has been limited is ascribing strong causality. This is likely due to the diversity of causal biological pathways. It is becoming increasingly clear that systems medicine approaches are required. We have been particularly interested in genetic variation of dopaminergic signalling, and in particular the effects of gene dosage as a potentially contributing cause.

Although many genetic variations have been associated with ASD the biological significance of most of them remains largely debated, and are not yet used in clinical practice. Many of the described associations have been either in small size samples or in large samples but with disappointingly failed replications, possibly due to causal heterogeneity and complexity (Ecker et al, 2010; Lombardo et al, 2019; Masi et al. 2017; Tang et al, 2020). Our data in the simpler model of NF1 suggests that gene-development-environment interactions will have to be taken into account (Violante et al, 2017). Research demonstrating causality remains critical but traditional genomics approaches are simply insufficient. Dopaminergic neurotransmission and its potential involvement in the causal pathway of ASD in a dose dependent manner may be worth investigating because of the role of reward systems and motivation in this condition (Pavăl, 2017; Greene et al., 2018; Ayano et al., 2016) and may be testable by simple gene dosage models. Moreover, dopaminergic pathways are quite well known and disruptions in the nigrostriatal pathway may lead to repetitive behaviours in ASD whereas disturbances in the mesolimbic and mesocortical pathways, involved in reward and cognitive related functions, might trigger impairments in social and affective cognition (Supekar et al. 2018; Fernández et al, 2018).

Network approaches are needed to tackle biological complexity in autism spectrum disorder

Complex diseases are multi-factorially driven by the combinatorial interaction of many intrinsic and external factors. Causal attribution can rarely be ascribed to a single perturbation in these types of conditions. Most of neurodevelopmental diseases, such as autism, schizophrenia and intellectual disability are clearly in this scope, and a focus on genetic causes has to be taken with care.

The multiple genetic causes behind a complex disease renders the isolation of single genetic effects a daunting and possibly non practical task. The reason is that some manifestations may only appear as emergent properties of networks of genes. Moreover, individual mechanisms have rather small effect sizes and are infrequent, further increasing the difficulty in their correct identification. For example, it is recognised that autism is highly heritable but given the role of rare genetic variations, causality in this respect remains an enigma (Veenstra-VanderWeele, et al. 2004; Pinto et al. 2020).

Another aspect increasing the difficulty of studying complex diseases comes from phenotypic heterogeneity. Typically, the same diagnostic category is attributed to a spectrum of similar phenotypes, yet it is unlikely to find two patients with the same diagnosis and identical phenotypes. This explains why this condition has been renamed Autism Spectrum Disorder, to encompass a set of conditions related to impairments in social interaction and communication and stereotyped, repetitive behaviours. However, the spectrum admits both individuals totally dependent of life support and individuals that can live almost independently.

This multifactorial scenario poses a big systems biology challenge. Approaches might be suitable to address this problem by mapping interactions between networks of genes and proteins to explain disease phenotypes. For instance, one can observe that several genetic alterations can be linked to the same pathophysiological process linking perturbations between sets of genes. This type of approach allows to understand why different genetic backgrounds lead to similar phenotypes (phenocopies) because they disturb the same biological mechanism in a coordinated and multiplicative manner (Schadt, et al., 2009).

This perspective embeds the contribution of functional modules with particular molecular identities underlying fundamental biological tasks which when derailed can lead to abnormal interactions leading to complex disorders. These can be modelled as links in networks where nodes can signify genes or their products, biological functions or/and disease entities.

Topological properties are important to consider in the characterization of the biological interactome (Schadt, et al., 2009). A typical property of such networks is scale-free feature (Albert, 2005). Scale-free networks are featured by a small number of nodes with a high number of connections whereas the overwhelming majority interacts only with few neighbouring ones (Albert, 2005). The ones with the highest number of connections, denoted as hubs are critically important in determining biological outcomes. Understanding each hub's neighbourhood patterns of connectivity may help uncover the true nature of biological functions (Jonson and Bates, 2006; Zotenko et al. 2008).

The role of machine learning in bioinformatics in ASD

Machine Learning approaches have evolved very fast in the field of radiomics (Zhang and Sejdić, 2019), whereby the use of large amounts of medical images can be used with high performance. With respect to ASD, machine learning based classification has also been applied in different types of data to predict diagnosis (Castelhano et al., 2015), but a lot of progress is still needed.

A recent meta-analysis (Wolfers et al. 2019), confirms this notion by showing a large discrepancy in prediction accuracy across a large number of studies. In spite of the many reasons that can be provided to explain this disparity (sampling bias, small sample sizes, distinct methods, data quality) the most important point that emerges is the heterogeneity of ASD. How to address a condition where neurodiversity is the rule?

It should also be taken into account the type and nature of the data does matter a lot in terms of classification success. The ones focusing on genetic features to predict ASD diagnosis are unfortunately typically based only on single nucleotide polymorphisms (SNP) data to predict diagnosis (Ghafouri-Fard, et al. 2019). It is not surprising that overall results have still been quite disappointing in this respect.

We have been focusing on the distinction between ASD and Developmental Delay, as an important intermediate step, because of the relevance of differential diagnosis between clinical categories, and most importantly because these categories might provide a clearcut biological distinction, prior to more complex classifications. We presented a machine learning approach to predict diagnosis with genetic Copy Number Variations (CNV), aiming to use genetic data to predict the clinical category, based on gene dosage (Santos et al, 2020).

In order to contribute to the discussion of the impact of the dopaminergic system in ASD, and its biological properties, we have been studying several dopaminergic features coded in the CNVs gene content of ASD carriers. A CNV involves unbalanced rearrangements that increase or decrease DNA dosage. This type of alteration can be detected using Chromosomal Microarray Analysis technologies. According to the guidelines of the American College of Medical Genetics and Genomics and Clinical Genome Resource (Riggs et al., 2020) different levels of a CNV being associated with a disease can range from Pathogenic, Likely pathogenic, of Uncertain Significance, Likely Benign, and finally Benign.

However, in the context of complex diseases, the impact of multiple CNVs may vary according to a multitude of factors. This justifies our current strategy of studying participants with multiple CNVs where dopamine related genes are in duplicated or deleted chromosomic regions (Santos et al., 2020).

We have been using QuickGO (Binns et al., 2009) to identify Gene Ontology (GO) (Ashburner et al., 2000; Carbon et al., 2019) terms related to the dopaminergic system. Next, from SFARI Gene CNV Module (Fischbach and Lord, 2010) (sfari.org/resource/sfari-gene/) we did select participants having CNVs matching genes of interest based on the previous defined GO terms.

To address the genetic variance, frequency and heterogeneity in ASD, network analysis (Conte et al., 2019) is a suitable tool to model the data following a functional polygenic view (Persico and Napolioni, 2013; Visscher et al., 2017) throughout maps of interactions between participants characteristics.

Using Random Decision Forests to predict the participants' differential

diagnosis we were able to reach a diagnosis predicting accuracy of 85.18% (SD 1.11%) on test samples of 790 participants using 117 genes. Moreover, a similar performance in prediction accuracy was reached when the classifier uses only 62 gene ontology features (Santos et al, 2020).

In ASD Gene and Developmental Delay Gene Networks we found several distinct clusters of genes exclusive for each clinical subgroup, respectively, which provides an interpretable framework for the achieved classification. This relevant finding of absence of connections between clusters corroborates the distinct genetic variability presented in these complex diseases, and shows the genetic diversity underlying the neurobiology of these conditions. This explains why gene ontology networks are better suited for biological interpretation due to the associated biological meaning that can be ascribed to each node.

In the diagnostic classification of developmental delay we basically found only one network community. In the case of ASD we identified at least four communities, which emphasizes the large biological variability that seems to be the hallmark of ASD. The disruption of such four key biological mechanisms includes dopamine receptor binding, metabolic turnover, regulation of neural differentiation of dopaminergic neurons and their synapses. Different sets of ASD participants only had disruptions within one of these domains, i.e. could only be assigned to one community traits, while others showed associations with more than one community. This demonstrates the wide diversity of underlying neurobiological mechanisms converging to autistic phenotypes.

Controversies on the underlying neurobiology of Autism and implications of neurodiversity for the design of new therapies

Attentional mechanisms as therapeutic targets

If we understand the underlying neurobiology of autism spectrum disorder, we may be able to design therapeutic strategies to act on the identified neural targets. One of such targets might be impaired alpha oscillations and their impact on attentional dysfunction (Ribeiro et al, 2014; Silva et al, 2016).

Everyone would agree that attentional deficits are often present in autism spectrum disorders, although we found that this is not a matter of attentional levels being necessarily low, but rather dysregulated (Silva et al, 2016). Some of these deficits are reminiscent of what is observed in ADHD (Attention Deficit and Hyperactivity Disorder). This raises the interesting question that neurodiversity of manifestations is pervasive across a broad range of neurodevelopmental disorders. Some people with autism and related neurodevelopmental disorders fulfil the criteria for ADHD, and there is also often symptomatic overlap with Obsessive Compulsive Disorder, or at least with an impulsive phenotype (Ribeiro et al., 2015). In other words, some neurodevelopmental conditions may operate as comorbid features of other disorders. This further compounds the debate on neurodiversity in health and disease.

But neurodiversity may also occur within the same individual, depending on his/her mental state. We have argued that this may be the case for attentional states in autism spectrum disorders. People with autism may indeed seem extremely distracted but at other times extremely focused and even hyperfocused. The often mentioned "excessive attention to details" has been in fact related to the claimed "lack of central coherence". In other words, the "spotlight of attention" could be so focused that it becomes too narrow, leading to excessive focus, and reducing the capacity for holistic understanding of the visual world. But is this type of neurodiversity necessarily bad? Extreme focus may lead to unexpected abilities, and even explain some savant syndromes, characterized by exceptional expertise (Bal et al., 2022). We argue that the focus of attention may become too narrow or too expanded in autism spectrum disorder, and this sort of within individual and between individual neurodiversity is very characteristic of autism spectrum disorder.

And what about neurobiological evidence for within subject neurodiversity of attentional systems? We have studied alpha brain oscillations, as a marker of internal attentional states, to examine this issue (Ribeiro et al, 2014). The brain has limited capacity to process the overwhelming amount of visual information that reaches the visual cortex. This implies that processes must have evolved to select information in an adaptive way. One of the most popular proposed mechanisms is selection

of information through attention. This mechanism seems to dysregulated across a broad range of neurodevelopmental disorders.

Neurofibromatosis type-1 (NF1), as a neurodevelopmental disease and syndromic model of autism often exhibits such attentional deficits and is therefore a model to study such impairments (Bernardino et al, 2021). We have also been studying alpha oscillations as a marker of attentional states. Increased posterior alpha reflects decreased attentional levels and larger lapses of attention. Indeed, in a previous study, we found that patients with NF1 are more prone to miss targets under overt attention conditions, and this pattern is associated with increased baseline and stimulus driven alpha (Ribeiro et al., 2014).

Our findings related to errors of commission (responding to a visual target when this was not required) or omission (failing to hit a target) as lapses of attention were therefore interpreted as a result of increased occipito-parietal alpha oscillations. In a more recent study, we used electroencephalography (EEG) to study alpha power modulations and the performance of patients with NF1 in a covert attention task (Silva et al., 2016). Covert attention tasks differ from over attention tasks in that they do not require eye movements. Instead, the focus of attention has to move to the periphery but central fixation has to remain stable. This places the participant with a strong external drive to focus attention. Under such directed instructions we were very surprised found that alpha was actually unexpectedly lower in patients.

Accordingly, when covert attention (without moving the eyes) was required in order to perceive stimulus changes, in this case a target offset of a peripherally presented stimulus, alpha oscillations were found to decrease in the NF1 group compared with control subjects. In other words patients showed unexpected greater alpha desynchronization under this task. A similar pattern of desynchronization was found for neighbouring beta frequencies but not in other frequency bands. These results support the notion of task dependent neurodiversity patterns: different attentional states and context demands lead to different patterns of abnormal modulation of alpha oscillatory processes. If we want to treat these abnormal patterns, we should neither aim at reducing nor at increasing them, but instead focus on improving their regulation.

Attention may become hypo or hyperfocused and this relates to alpha

rhythms and performance. Accordingly, under covert attention (without moving the eyes) conditions and while target offset was reported with an accuracy over 90%, alpha desynchronization (corresponding to amplitude decreases) was much larger, particularly in patients. These findings suggest a new feature of modulation of oscillatory activity and attentional processes in a developmental disorder such as NF1. These findings extend the perspective on how alpha oscillations modulate attention. Alpha patterns may show both abnormal increases and decreases that are task and performance dependent, which is a signature of within subject neurodiversity. One could hypothesise that enhanced alpha desynchronization reflects a compensatory mechanism to keep attentional performance at normal levels. An alternative account is that in this spectrum of neurodevelopmental disorders a very large range of regulation of alpha oscillations may occur leading to apparently excessive or instead diminished activation patterns. These signatures of neurodiversity may explain peaks and performance valleys that are so often encountered in Autism Spectrum Disorders.

Taken together these findings point out that modulation of alpha oscillations is a viable therapeutic target for intervention in autism spectrum disorders. However, it turns out that what is important is to improve the ability to regulate these oscillations in a context dependent manner and less so to change their mean amplitude in a particular direction.

Translating knowledge on within and between subject neurodiversity into interventional studies

Our clinical trials

Departing from the last section if alpha oscillations maybe either abnormally increased or decreased depending on the circumstances, what is the best approach to treat abnormal alpha oscillations? Should we aim at increasing or decreasing? The answer suggested in the last section is that we should train participants to better regulate these oscillations in an adaptive task-dependent manner. Regulation is the keyword and this concept is often disregarded. We had similar findings for the regulation of behavioural variables such as interpersonal distance or duration of eye contact (Simões et

al, 2020). This discussion is very important for the design of neurofeedback approaches either using EEG and fMRI and will further be addressed in the context of the clinical trials that we implemented. In our trials we therefore focused on the brain networks which subserve attention and imagery functions.

We have focused in our research on interventional approaches in the training of interpretation and attentional imagery of facial expressions because it is an important feature of social cognition. Dynamic facial expressions fall within the category of biological motion but there are also other types of motion, such as body movement, that can convey emotional information. Furthermore, the perception of biological motion has also been claimed to be impaired in autism (Mason et al., 2021).

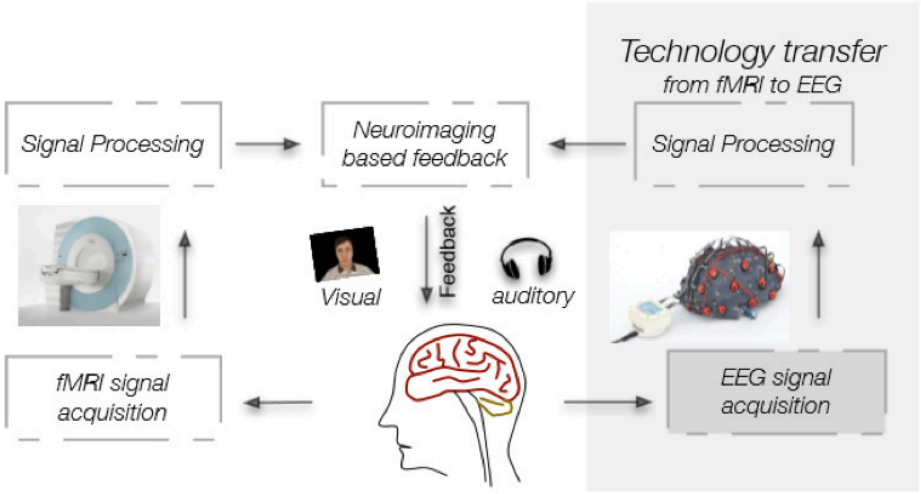


Figure 37. Basic layout of Neurofeedback experiments (such as used in Direito et al, 2021).

Our first clinical trial in this regard used fMRI-based neurofeedback (NF, see Figure 37, ClinicalTrials.gov Identifier: NCT02440451). With the aim to transfer expensive fMRI approaches to EEG, we have combined NF real-time functional Magnetic Resonance Imaging (rt-fMRI), defining the right posterior Superior Temporal Sulcus (pSTS) as the target region-of-interest for neuromodulation (Direito et al, 2019; Simões et al., 2015).

We chose this region because it responds with increased neural activity both to visualization or imagery of dynamic facial expressions. We were able to train individuals to modulate (increase AND decrease) activity in this brain region. This type of therapy is also called neuromodulation and we showed that it can be successful in that clinical trial (Direito et al., 2021). However, fMRI-based Neurofeedback is quite expensive and unpractical and we are now trying to transfer this technique to a more portable EEG set up. For this to be feasible some relation between fMRI and EEG signals has to be identified.

In other words, for this transfer from fMRI to EEG to work significant correlation between the fMRI regions of interest (in this case pSTS) and the EEG data at the scalp level (or modelled electric source corresponding to pSTS) has to be present. We have performed several studies where we extracted features from EEG segments and performed a correlation analysis with the brain activations extracted from rt-fMRI in the right pSTS region. The finding of significant correlations of these simultaneously measured signals is encouraging (Simões et al., 2020). In Figure 38 we tested several methods to reconstruct the fMRI signal from EEG and show that our novel multiple HRF (hemodynamic response function) method yields the largest correlation.

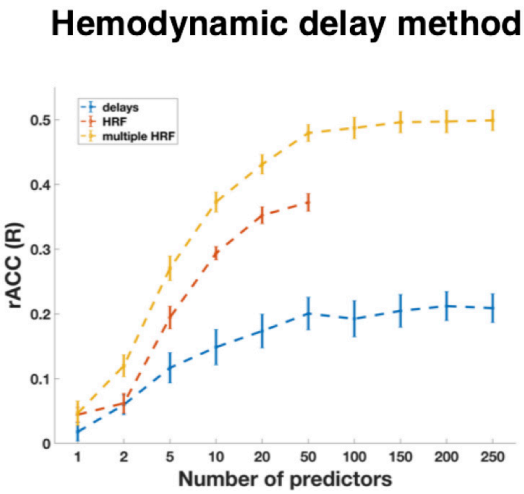


Figure 38. Largest correlation of fMRI and EEG signals in the Facial expression Network is achieved with our new proposed method (called multiple HRF – using multiple models of hemodynamic response functions)
Comparison of the apACC (approximation accuracy) achieved using the EFP (electric fingerprinting of brain signals) method by the three approaches tested to tackle the hemodynamic delay. The HRF method only shows up to 50 predictors because it contains only the power for 7 frequency bands from 10 electrodes (70 predictors), while the other two have the different delays (classical method based on the literature) or the convolution with multiple HRFs (our method), thus increasing the number of predictors (in these cases, 840). Points represent the group mean apACC values (computed between the original BOLD signal and the one approximated by the respective model) and the bars the standard error of the mean. apACC: approximation accuracy defined as the Pearson correlation between the average BOLD signal across voxels in the Facial expression Network.

Figure 39 compares several models to retrieve the facial expression network (classical EFP – simple electric fingerprint, Feat Pool (our own Feature Pooling model) at the scalp level and Feat Pool at the neural source level (the ones yielding the best results as shown by the colour maps). Our Feat Pool model was based on a pool of features, three of which extracted from the time-frequency domain; the remaining four were focused on exploring the nonlinear characteristics of the EEG signal based on our prior studies of mental imagery processes (Simões et al., 2018).

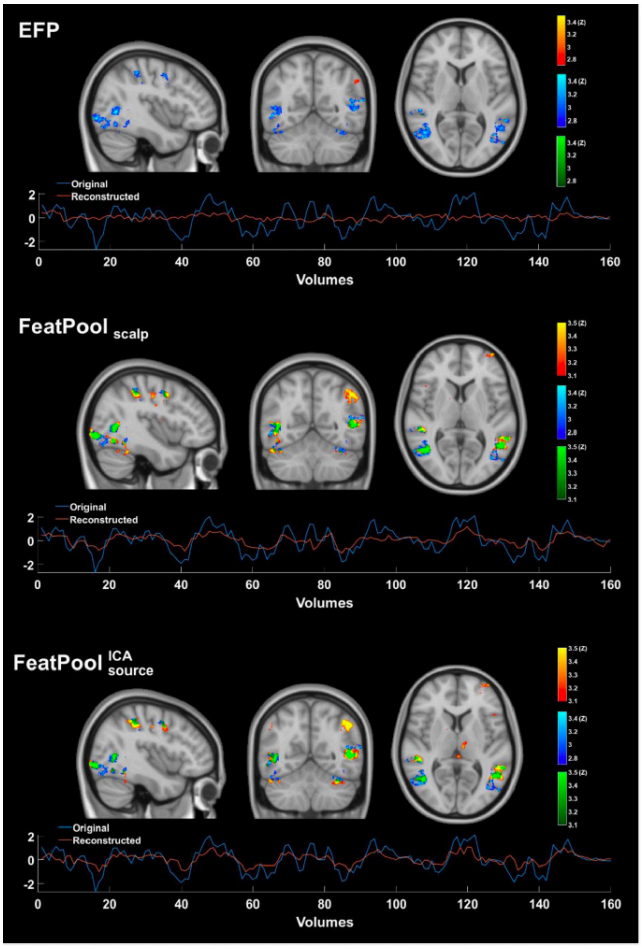


Figure 39. Our proposed method achieves much higher correlation between fMRI signals from the Facial expression Network and EEG than the traditional state of the art method (Simões et al., 2020). Group correlation maps (in red-yellow) for the EFP scalp classical model (top), the Feat Pool scalp model (middle; best at the scalp level) and the Feat Pool ICA electric source model (bottom; overall best) (MNI coordinates: x = – 52, y = – 48, z = 6), superimposed on the group Facial expression Network mapped from the fMRI localizer run (in blue-light blue); overlapping regions are highlighted in green. The Feat Pool method recover most of the Facial expression Network, while the EFP scalp method fails to reconstruct even its main regions. Different z-score thresholds were considered for each model given their distinct statistical power. Below each group correlation map, the measured (in blue) and the approximated (in red) BOLD signals from the localizer run are shown, for a representative participant.

Neurostimulation results in neurotypical participants. Can they be translated to ASD?

It is known that that temporoparietal junction (TPJ) modulation can influence attention and social cognition performance, making it a candidate for intervention in disorders autism as ASD and Schizophrenia. We were recently involved in a European Project (STIPED) where a multichannel transcranial direct current stimulation (tDCS) device was developed for relatively focused stimulation over bilateral TPJ to estimate the effects on behavioural functions. The project STIPED developed optimized multichannel stimulation and this type of device required pilot testing in neurotypicals prior to the formal clinical trials for which we obtained authorization in European and national regulatory entities (Luckhardt et al., 2021). In a pilot study (see experimental design in Figure 40), we coupled anodal multichannel tDCS with a Joint Attention Task (Pereira et al, 2021). This type of task requires social-cognitive abilities that monitor the social attention of other people/agents. We compared this concurrent task/neurostimulation approach with sham (placebo) stimulation.

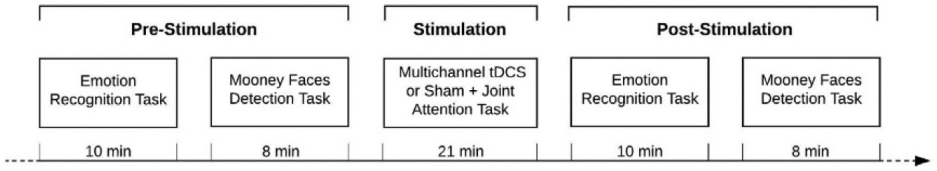


Figure 40. Schematic of the study's experimental design for each neurostimulation session. Each participant was required to complete two face/social recognition tasks before stimulation. After 20min of stimulation (sham or verum) concomitantly with a joint attention task, each participant was required to complete the same tasks performed before.

To test cognitive improvements we used a Face Emotion Recognition Task (Figure 41, to investigate specific effects related to autism impairments) and a control Abstract Face Detection Task (Figure 42), while also evaluating this technique's safety and tolerability.

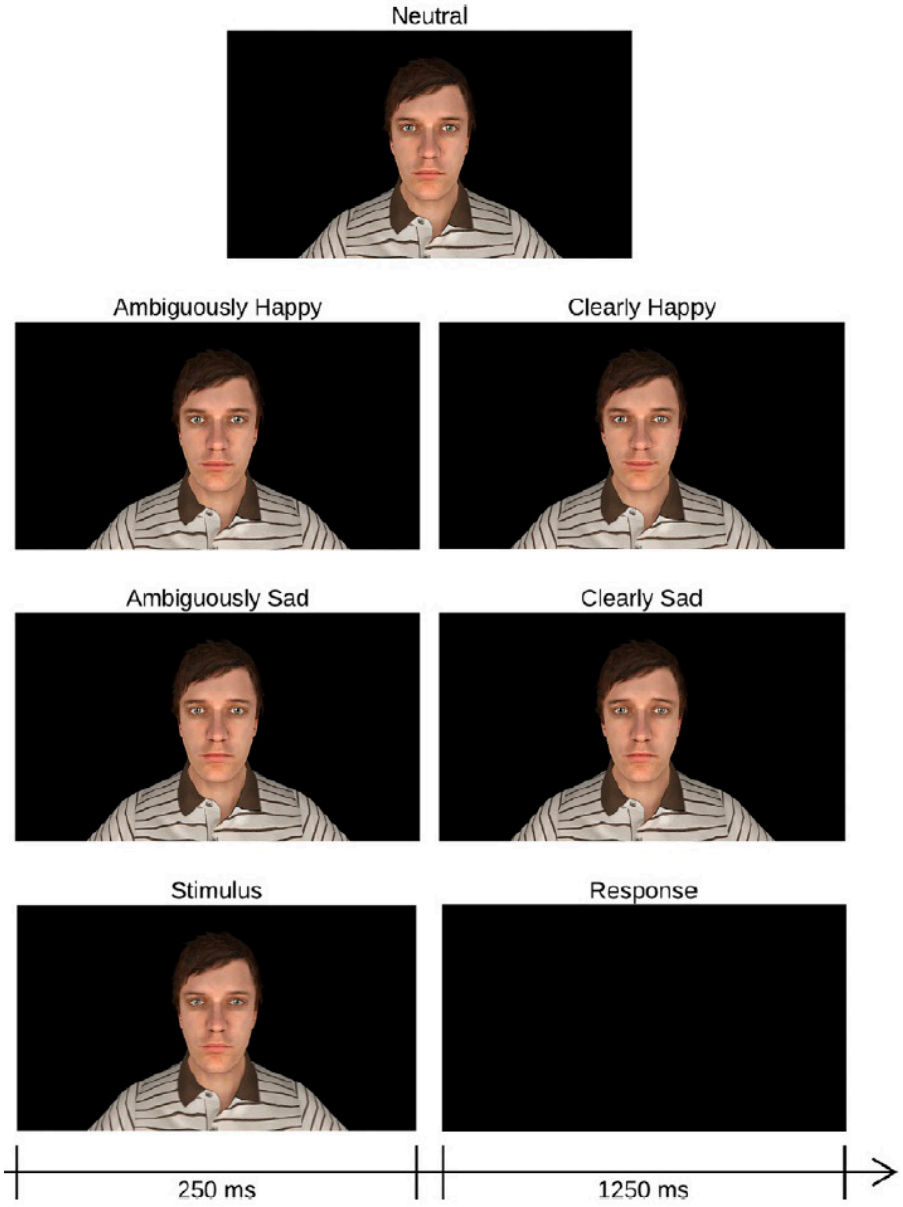


Figure 41. Emotion recognition task. Top panels: Base stimuli. Bottom panel: Structure of the trials. Copyright Clearance Center's RightsLink® service – Elsevier.

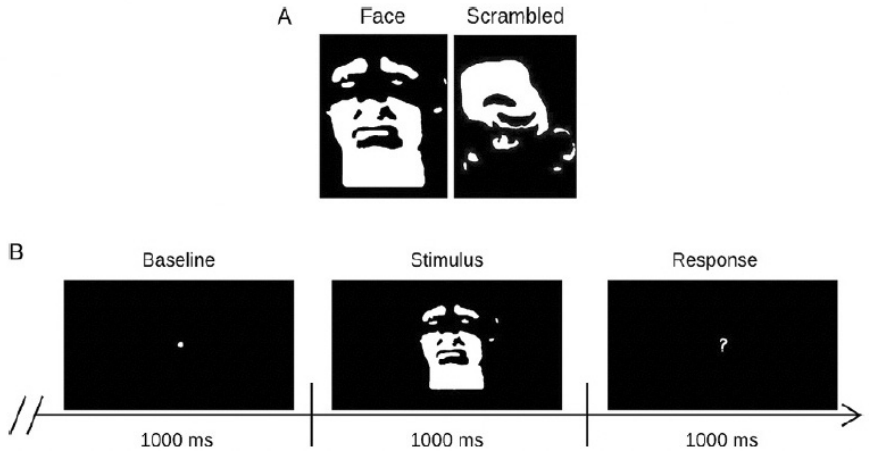


Figure 42. Mooney faces detection task. (A) Base stimuli. (B) Structure of the trials.

Twenty healthy adults were enrolled in a randomized, single-blinded, sham-controlled, crossover study using a new medical device permitting neurostimulation and concomitant measurement of neural signals (Figure 43). During two sessions, participants completed the outcome measure related tasks before and after 20min of sham or anodal tDCS over bilateral TPJ.

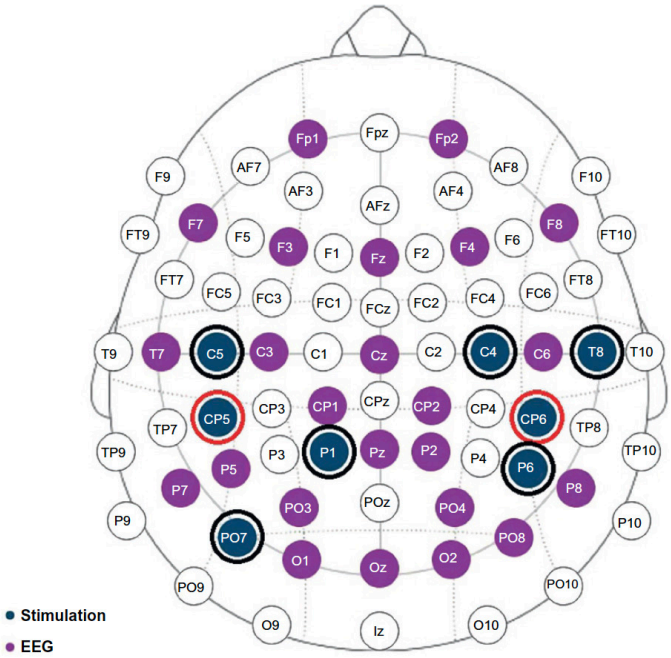


Figure 43. Head diagram showing the positioning of the tDCS (stimulation) and EEG electrodes used during multichannel stimulation over bilateral TPJ. Anodal electrodes are delineated with red circles and cathodal electrodes are with black circles. Copyright Clearance Center's RightsLink® service – Elsevier.

Although no significant findings were observed the Joint attention task (Figure 44) revealed an interesting performance dependence on the number of agents whose attention had to be tracked.

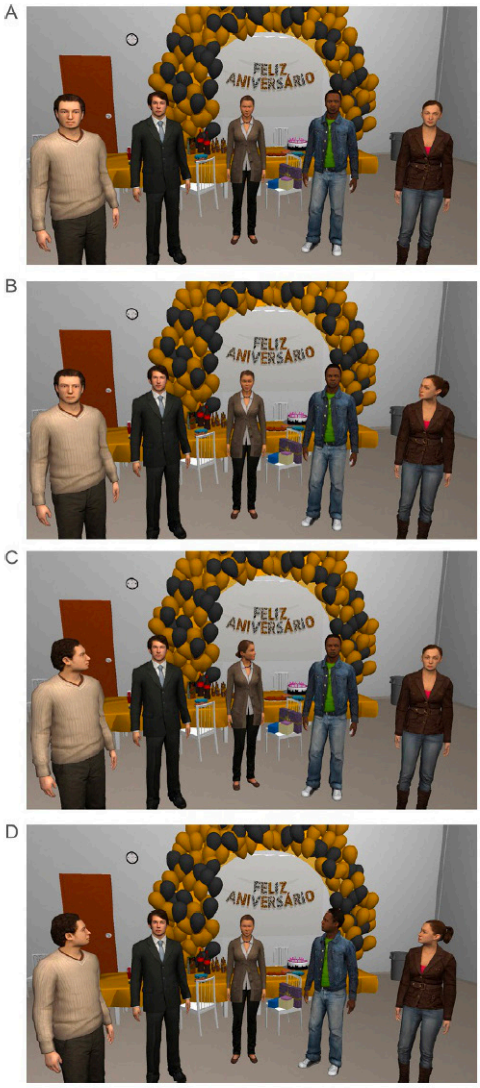


Figure 44. Examples of stimuli types in the Joint Attention Task. (A) Baseline position for all the avatars gazing at the participant. (B) One avatar gazes at another avatar. (C) Two avatars gaze at another avatar. (D) Three avatars gaze at another avatar. Participants have to report their respond by gazing at the target avatar and also press a button at that moment. Copyright Clearance Center's RightsLink® service – Elsevier.

This showed promise for application in Autism where the difficulty in monitoring complex environments with multiple agents is an issue. Safety and tolerability were demonstrated. Can the absence of results in a neurotypical group provide evidence that it will not work in clinical populations? We believe that this is not the case for two reasons: 1. Different cognitive styles may differentially benefit from distinct interventions 2. Generalization to a different type of population cannot be assumed. 3. We found evidence in our task that performance was dependent on the number of virtual agents, and this effect might be further enhanced in an autistic population. This study was not designed for neuroenhancement (a controversial term meaning improved function in neurotypical subjects) but rather thinking on the social attention difficulties that people with autism often face.

Our Clinical trials targeting neural and behavioural impairments in autism spectrum disorders

Our first medical device clinical trial using fMRI

Testing of rehabilitation approaches in autism should be tightly coupled with the insights into neurodiversity provided by novel diagnostic approaches.

Clinical trials still remain a huge challenge in ASD (Anagnostou, 2018). Pharmacological treatment has only been moderately successful in treating some manifestations but has remained basically a failure in which concerns core symptom domains. Biological heterogeneity has remained a hurdle, and in spite of the intensive search, biomarkers that can elucidate such heterogeneity are still lacking. Without appropriate biomarkers and outcome measures allowing for prediction of treatment response this scenario will remain difficult.

ASD is a dynamic condition and changes across the lifespan, raising the possibility that treatment response may also depend on the stage of developmental milestones.

Recently, we completed a phase 2 clinical trial, approved by the regulatory entities Infarmed and CEIC and with the support of FLAD Life

Sciences award and the European Braintrain project, using the innovative approach of Neurofeedback (ClinicalTrials.gov Identifier: NCT02440451). Using Functional Magnetic Resonance Imaging we measured brain activity while people performed a facial expression imagery task using as target region pSTS, as mentioned above for a control study. We recorded online brain activity and provided immediate feedback to the participants so that they could try to change (increase or decrease) their own brain activity in regions involved in the perception of facial emotions.

This clinical trial was the first using Neurofeedback in Autism Spectrum Disorder (Direito et al, 2021, Figure 45), and was one of the first medical device clinical trials in Portugal (together with another trial using BCI-EEG which we will describe below, Amaral et al., 2018).

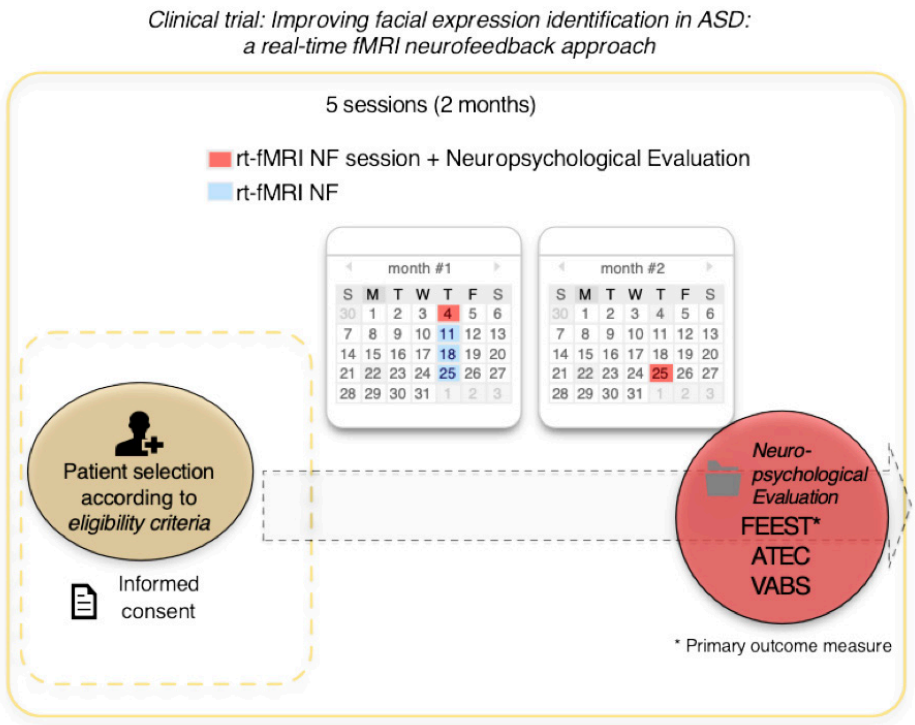


Figure 45. Layout of our clinical trial (Direito et al, 2021). ClinicalTrials.gov Identifier: NCT02440451. FEEST: Facial expressions of emotion: Stimuli and Test; ATEC: Autism Treatment Evaluation Checklist; VABS: Vineland Adaptive Behaviour Scales.

This is an emerging therapeutic approach and we showed the feasibility of real-time functional magnetic resonance imaging volitional neurofeedback in targeting social brain regions (pSTS – Figures 46 and 47) in ASD. In this clinical trial (Direito et al, 2021), autism spectrum disorder patients were enrolled in a program with five training sessions of neurofeedback. Participants were able to control their own brain activity in this social brain region, with positive clinical and neural (ability to regulate brain activity) effects. We found improvement in recognition of Fear in Faces (FEEST test battery) and global significant improvements in adaptive behavioural scales (VABS - Vineland Adaptive Behaviour Scales and ATEC - Autism Treatment Evaluation Check list) and in specific clinical subscales of ATEC, VABS, and POMS (Profile of Mood States).

Although larger, controlled, and blinded clinical studies will be required to confirm the benefits, this Phase IIa clinical trial showed promising results.

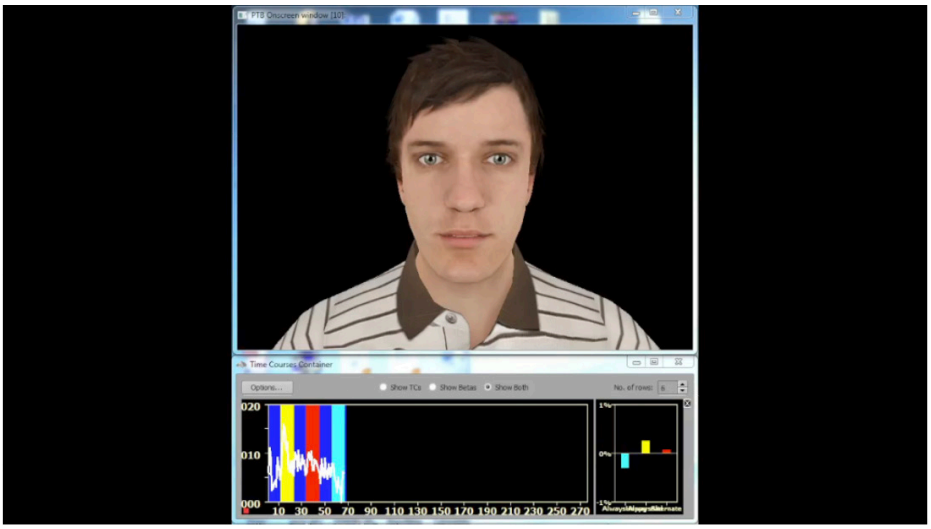


Figure 46. Volitional neurofeedback in targeting social brain regions (pSTS) used in our ASD clinical trial (Direito et al., 2021). The white line shows brain activity in a region (pSTS) that is activated by imagery of facial emotional expressions. If the participant succeeds in the imagery task, activity will increase and positive feedback is given by a corresponding increase in the smiling expression of the avatar. When requested to decrease the activity, participants often imagined a neutral (absent emotion) expression. Training the social brain trial structure: 8-week real-time fMRI NF Imaging Feasibility Trial. Endpoint Classification: Efficacy Study Intervention Model: Single arm Primary Purpose: Basic Science and clinical feasibility study Participants: 15 ASD subjects, 5 sessions each (no drop outs).

People were exposed to their own brain activity, in an area of the brain that is involved in the recognition of emotions, so that people could increase or decrease the activity in this area (pSTS). To provide an anchor for participants to learn how to do it, we showed an image on the computer, an avatar, participants performed exercises of imagination of the avatar's emotional expression (Figure 46). This imagery exercise was sufficient for them to control brain activation in this region underlying perception of emotions in faces.

The clinical trial was developed over 8 weeks and all participants completed the protocol, being all highly motivated to participate in the sessions. In sum, a key achievement was that people involved could learn to change their brain activity in that key area of the brain for the recognition of emotions. This implies a sort of plasticity that was related to improvement of emotion discrimination and recognition as well as in positive changes in mood and anxiety (Direito et al, 2021).

Further work still needed to develop this technique, included in the category of neurofeedback. The use of functional MRI allows to more accurately measure the areas of the brain with a function potentially affected in autism. However, and as explained above we are now trying to translate this application to more portable setups, using the electroencephalogram (Simões et al. 2018, 2020), which would reach even more people in almost any setting anywhere.

This clinical trial was preceded by validation in neurotypical participants (Direito et al, 2019). Our rationale was based on the fact that the posterior superior temporal sulcus (pSTS) is involved in encoding dynamic facial expressions, and responds both to visualization and imagery of dynamic expressions. Instead of brain stimulation of this region, instead we opted for neurofeedback, which can be seen as a self-stimulation technique. We investigated whether pSTS, which is involved both in face perception and imagery, could be specifically identified based on the presence of dynamic facial expressions, as a target for neurofeedback.

Recognition of facial expressions is often impaired in autism spectrum disorder, which is arguably a cognitive function that deserves clinical intervention and should be a target for additional future clinical neurofeedback studies. We localized this region based on the contrast of

dynamic facial expressions against static neutral faces plus moving dots. The target region had therefore to be specifically responsive to dynamic facial expressions and not to other visual features. This region could be localized in every subject. pSTS (see probabilistic map of this region in Figure 47) proved to be a suitable target for neurofeedback approaches and we plan to further use it as target for neurofeedback in future studies in autism. However, one should take into account that even if autistic subjects can modulate this region, they do it in a Neurodiverse way (Figure 48).

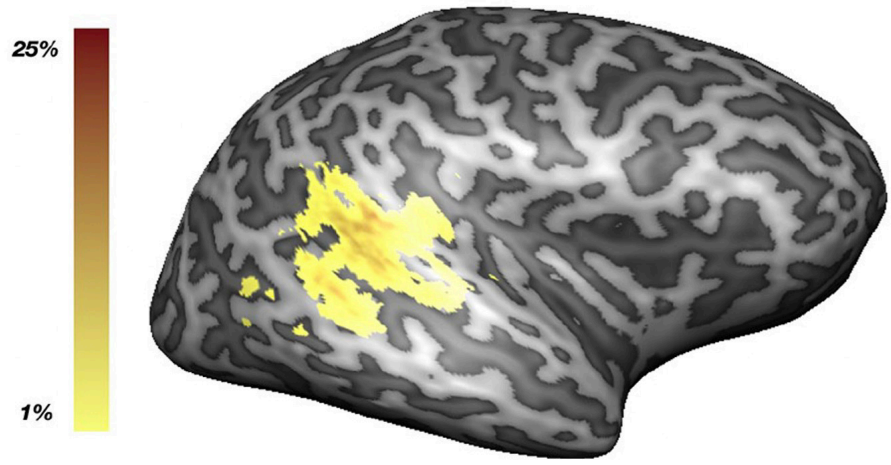


Figure 47. Probabilistic map of brain region pSTS, which is involved in encoding dynamic facial expressions, and responds both to visualization and imagery of dynamic expressions.
From Direito et al. 2021. Authors copyright under Sage, Rightslink® by Copyright Clearance Center.

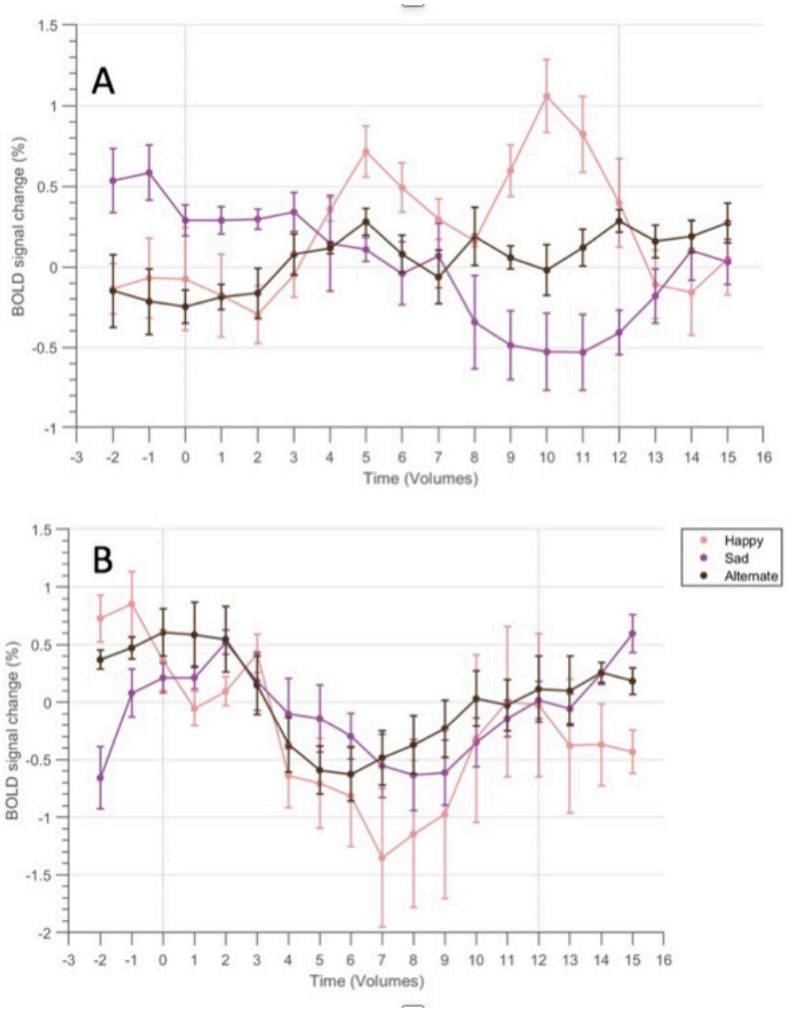


Figure 48. Autistic participants modulate the pSTS region, in a Neurodiverse way (see variable neuromodulation for happy, sad and neutral expressions in two distinct participants (see also other examples in Direito et al, 2021).

Interestingly, reinforcement learning and reward networks are activated in Neurofeedback runs when participants imagine a particular emotion (Figure 49). This is relevant because motivation and reward related networks are often underactivated in ASD.

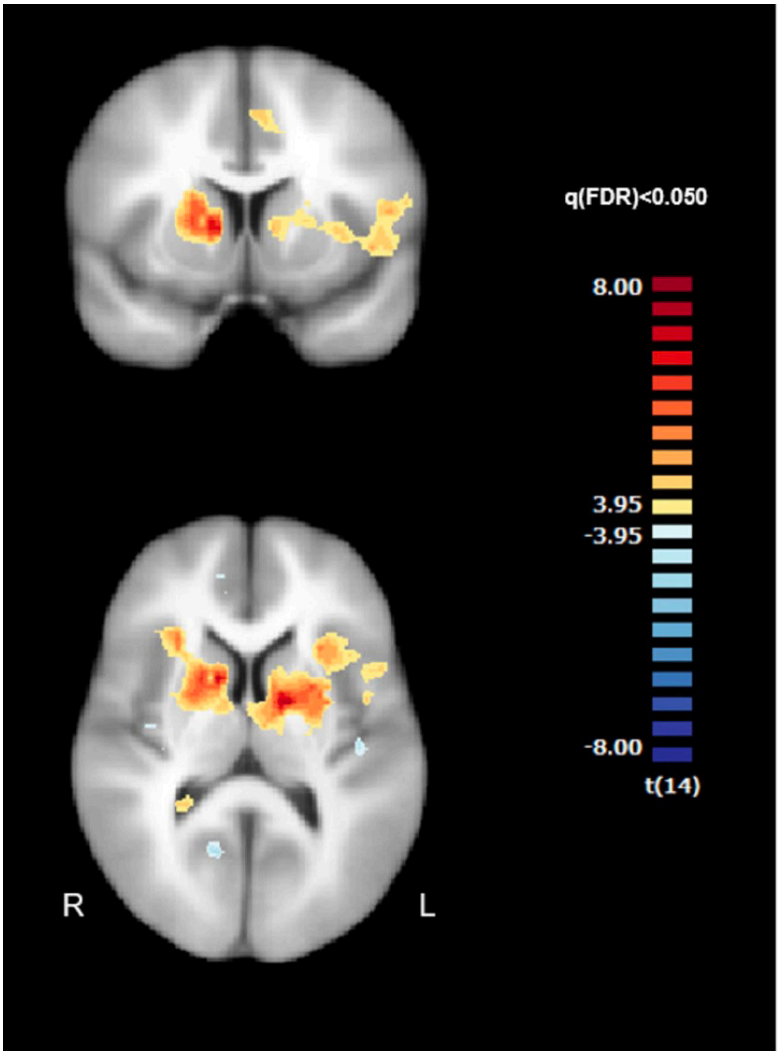


Figure 49. Activation of reinforcement learning and reward networks in Neurofeedback runs. Group RFX (random effects)-GLM (general linear model) activation map for the neurofeedback runs, contrasting the imagery of happy, sad, and alternate conditions with the neutral condition (False discovery rate corrected, $q < 0.05$)—Talairach coordinates of the slices are Y = 9 (top) and Z = 10 (bottom).

The feasibility of neurofeedback using neuroimaging and the search for more portable solutions using EEG

Training of imagery of facial expressions in Autism Spectrum Disorder was proven by our real time neuroimaging clinical trial studies to be a viable possibility. For that we were able to track a brain region involved in perception and imagery of facial expressions, pSTS. The next challenge in our research strategy was to prove that it is possible capture brain correlates of dynamic face perception at a more accessible neurophysiological level, using EEG. We used a similar approach that allowed to directly link observation of emotional expressions and imagery in ASD, and first derived machine learning biomarkers that were able to classify abnormal imagery in ASD. To provide a handle between perception and action imagery cycles we used visual stimuli exploring the dynamical nature of emotion representation. We conducted an experimental study which showed a link between visualization and mental imagery of dynamic facial expressions which could be confirmed by analysing source responses to pure face-expression contrasts. Using classification techniques we were able to replicate the same highly group discriminative neural signatures during action observation (dynamical face expressions) and imagery, in a parietal brain region, the precuneus (Simões et al., 2018). Larger recruitment of parietal regions involved in imagery in ASD group was observed (see Figures 32 and 33 above). This raises the interesting possibility that ASD participants are able to engage in emotion imagery but at the cost of larger compensatory activation of areas underlying imagery. Our machine learning procedure was able to automatically classify EEG activity during mental dynamic face imagery. Both linear and non-linear signal characteristics at different frequency bands (theta, high-beta and gamma oscillatory activity bands, in order of increasing frequency) in right-parietal locations were relevant in the classification process. This accurate classification of signals significantly exceeds what is usually reported in the literature. The imagery of facial expressions in Autism Spectrum Disorder (ASD) is possibly impaired and difficult to capture at a neurophysiological level, but we were nevertheless able to demonstrate that people with ASD can show increased recruitment of these circuits.

We developed an approach that allowed to directly link observation

of emotional expressions and imagery in ASD, and to derive potential biomarkers that are able to classify abnormal imagery in ASD. To provide a handle between perception and action imagery cycles it is important to use visual stimuli exploring the dynamical nature of emotion representation. We conducted a case-control study providing a link between both visualization and mental imagery of dynamic facial expressions and investigated source responses to pure face-expression contrasts. We were able to verify the same highly group discriminative neural signatures during both action observation (dynamical face expressions) and imagery, in the precuneus (Simões et al, 2018). Larger activation in regions involved in imagery for the ASD group suggests that this effect is compensatory. This opens the possibility that one can train this network for therapeutic purposes in ASD. If true this would mean that the transfer from fMRI neurofeedback to EEG, which was one of our major goals, is possible.

We did run a machine learning procedure to automatically identify these group differences, based on the EEG activity during mental imagery of facial expressions. We compared two classifiers and achieved an accuracy of 81% using 15 features (both linear and non-linear) of the signal from theta, high-beta and gamma bands extracted from right-parietal locations (matching the precuneus region), further confirming the findings regarding standard statistical analysis. This robust classification of signals resulting from imagery of dynamical expressions in ASD is surprising because it by far significantly exceeds the good classification already achieved solely with the observation of neutral face expressions. This novel neural correlate of emotional imagery in autism can be used now with two clinical purposes: as an interventional target for studies designed to improve facial expression recognition, or as a quantitative biomarker to evaluate the results of other therapies. We are still pursuing this idea, but the next section describes the first clinical trial that we have performed using EEG.

Our first medical device clinical trial using EEG

Our first medical device clinical trial using EEG (Amaral et al., 2018, reference number NCT02445625-clinicaltrials.gov) focused however on another feature of Autism Spectrum Disorder: difficulties in the interpretation of

others' intentions based on their gaze-direction or other social attention cues. We investigated whether an EEG brain computer interface (BCI) can be used to train these social cognition skills in ASD patients. Our clinical trial enrolled adult participants with so-called high-functioning ASD (with well-preserved intellectual quotient - IQ - as a measure of cognitive ability). Participants were invited to participate in a BCI training paradigm using a virtual reality interface over seven sessions spread over four months. The first four sessions occurred weekly, and thereafter there were three additional "refresh" sessions occurring every month (Figure 50).

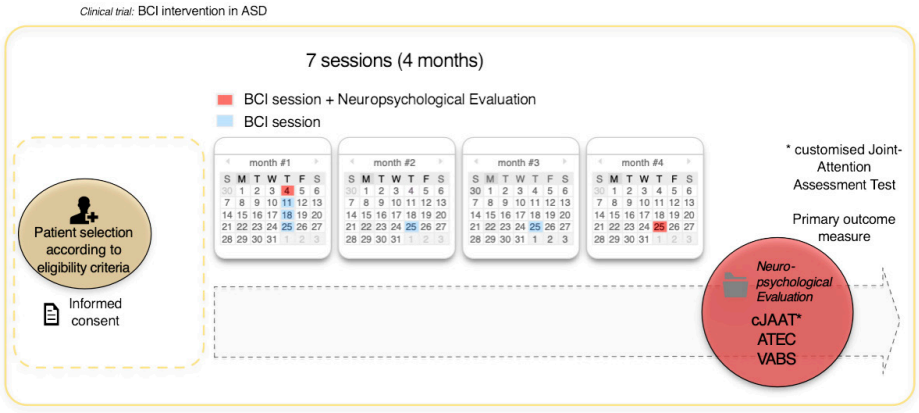


Figure 50. Our BCI clinical trial layout (from full details see Amaral et al., 2018).

In each session, the subject, using the apparatus shown in Figure 51, was asked to identify objects of interest based on the gaze direction of an avatar. In other words, this task requires to follow the attention of another agent, as expressed by its eye gaze. This joint focus on the same object, which is often lacking in ASD, is called "joint attention". Attentional responses were extracted from a component in the EEG which is known to express attention to a sudden and unexpected target (the object of interest to the avatar). This EEG component is labelled as P300 because it is a positivity in the EEG occurring at 300 ms. We had previously shown that when the object of attention is social, the P300 component becomes lateralized to the right hemisphere (Amaral et al, 2015). This finding is consistent with

Figure 51. BCI apparatus overview. Person wearing Oculus Rift and the g.Nautilus EEG system (part of the virtual reality P300-based BCI) and the observer's viewing window on the screen. Trial characterization: A Feasibility Clinical Trial to Improve Social Attention in Autistic Spectrum Disorder Using a BCI. Endpoint Classification: Feasibility Study Intervention Model: Single arm Primary Purpose: Basic Science and clinical feasibility study Participants: 15 ASD subjects (no drop outs). Main findings: A decrease in total ATEC (Autism Treatment Evaluation Checklist) and rated autism symptoms (Sociability; Sensory/Cognitive Awareness; Health/Physical/Behaviour); improvement in Adapted Behaviour Composite and daily living skills subarea from VABS (Vineland Adaptive Behaviour Scales) scale; a decrease in Depression (from the POMS mood scale) and mood disturbance/ depression (BDI). BCI online performance and tolerance were stable along the intervention. Adapted from Amaral, Mouga, Simões, Pereira, Bernardino, Quental, Playle, McNamara, Oliveira and Castelo-Branco, under the terms of the Creative Commons Attribution License (CC BY).

the notion that social and emotional processing are lateralized to the right hemisphere (Demaree et al., 2005).

To investigate responses to joint attention cues (see also Figure 44, above) participants were assessed pre and post the 4 month intervention and after a 6 month follow-up, using our ecologic Joint-Attention task. One way to probe whether joint attention is improving is to use eye-tracking to identify the number of social attention items that a patient can accurately identify

from an avatar's action cues (e.g., looking, pointing at). We used this as our primary measure of improvement (Figure 52). However, other outcome measures that were considered secondary, but that actually may have larger clinical interest, are the Autism Treatment Evaluation Checklist (ATEC) and the Vineland Adaptive Behaviour Scale (VABS). Neuropsychological measures related to mood (POMS) and depression (BDI) were also assessed. Importantly, we observed a decrease in total ATEC and rated autism symptoms (Sociability; Sensory/Cognitive Awareness; Health/Physical/Behaviour); an improvement in Adapted Behaviour Composite Score and in the DLS (Daily Life Skills) subscore from VABS. A decrease in Depression (from POMS - Profile of Mood States) and in mood disturbance/depression scores were also observed (BDI - Beck Depression Inventory).

Figure 52. Representation of the used scenarios to investigate "joint attention" (looking at the same target of attention as the avatar). (A) Cafe scenario; (B) Classroom scenario; (C) Kiosk scenario; (D) Zebra crossing scenario.

In line with the clinical improvements, BCI online performance and tolerability remained constant along the intervention, as well as neurophysiological markers such as P300. We were quite positively surprised by the feasibility of BCI in this kind of intervention in ASD, and positive efficacy results. Participants engagement, motivation, and improvement in clinically relevant secondary measures suggest that this type of intervention may be well received. It was also noteworthy that, similarly to the neurofeedback trial, participants were quite adherent to the used of technological medical devices (this study was registered as a clinical-trial ID: NCT02445625-clinicaltrials.gov).

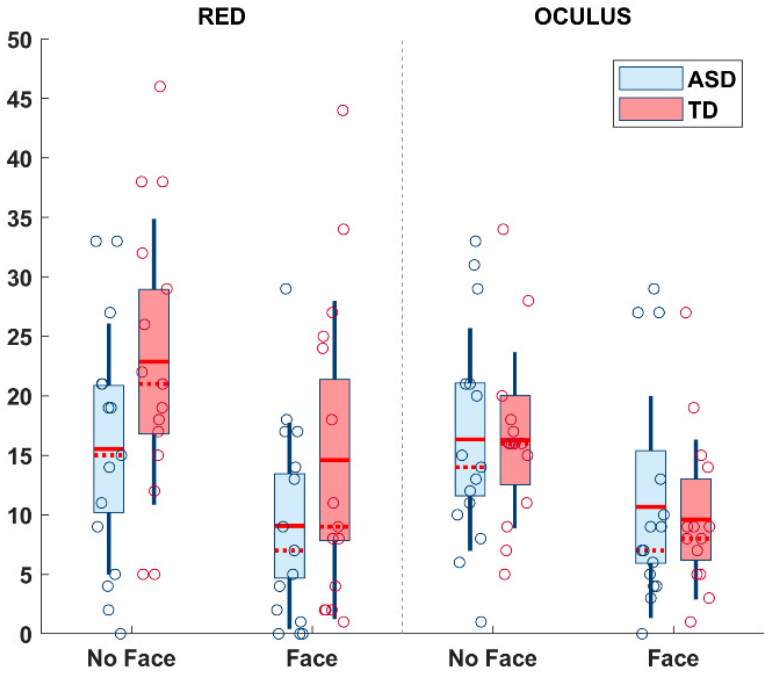


Figure 53. Eytracking Experiments to choose the visual stimulation interface. Comparison of eye tracking behaviour (number of fixations to objects of interest) in a non immersive eyetracking system (RED, left) and an immersive system (Oculus) in ASD and typically developing (TD) participants. We choose the more immersive environment of the Oculus system.

Before we ran this clinical trial we first piloted several novel virtual-reality P300-based Brain Computer Interface (BCI) paradigms using social cues to direct the focus of attention. This was done to identify the best combined interactive immersive virtual-reality (VR) technology both from the display point of view (Figure 53) and the EEG system (dry versus non dry electrodes, which do not require appliance of gel to the skin; wireless versus cable driven systems). The BCI system took advantage of the properties of P300 signals in a training tool which could be used to train social attention skills. We tested a priori the P300-based BCI paradigm for rehabilitation of joint-attention skills in healthy participants, in 3 EEG systems. The best performing setup in terms of speed of acquisition, signal quality and participant comfort was then selected for online testing with ASD subjects, as a proof of concept (Amaral et al., 2017). Statistical accuracy of P300 detection was assessed using spatial filtering and a Naïve-Bayes classifier. We compared the following systems: g.Mobilab+ with active dry-electrodes and wireless transmission providing the least intrusive setup; 2 - g.Nautilus, with active electrodes, wireless transmission; 3 - V-Amp with actiCAP Xpress dry-electrodes. Significant statistical classification was achieved in all systems but g.Nautilus proved to be the best performing system in terms of accuracy in the detection of P300, preparation time, speed and reported comfort. Pilot tests in ASD participants confirmed the feasibility of this setup for training joint attention skills in ASD. Our work provided a demonstration of the feasibility of 'easy-to-use' BCI systems with new technologies such as VR to train joint-attention skills in autism. This led to the Phase IIa clinical trials to train joint-attention skills, with successful classification within few trials, online in ASD participants, which we described above (clinical-trial ID: NCT02445625-clinicaltrials.gov).

A pharmacological clinical trial using lovastatin

So far, we have described interventional studies and clinical trials based on Neurofeedback, Neurostimulation, Brain Computer Interfaces and Serious Games. However, based on our work on molecular mechanisms of disease in neurodevelopmental disorders, and in particular excessive inhibition, we aimed at pharmacological trials as well, beyond medical device trials.

We recently finalised a pharmacological clinical trial in Neurofibromatosis type 1 (Bernardino et al, 2022) to probe physiological effects of lovastatin on brain inhibition. We based the rationale for our trial on the hypothesis that the gamma-aminobutyric acid (GABA) neurotransmission is impaired in NF1. This was suggested by prior work in the Nf1+/- mice model showing that Ras modulation by neurofibromin, a protein encoded by the Nf1 gene, was associated with increased GABA-mediated inhibition and related physiological and cognitive impairments (Costa et al, 2002; Gonçalves et al, 2017; Petrella et al, 2016). The translation of these findings to the human model was achieved by our studies using molecular imaging of the GABA receptor and in vivo magnetic resonance spectroscopy (MRS) (Violante et al, 2013, 2016). In our prior studies, reduced GABA concentration was consistently found across the lifespan and in distinct brain regions.

Lovastatin modulates the RAS pathway and was administered in our randomized, triple-blind, placebo-controlled crossover trial at a dosage of 60mg/day, for 3 days.

The workflow of our study protocol is depicted in Figure 54. This trial was registered at Clinicaltrials.gov NCT03826940.

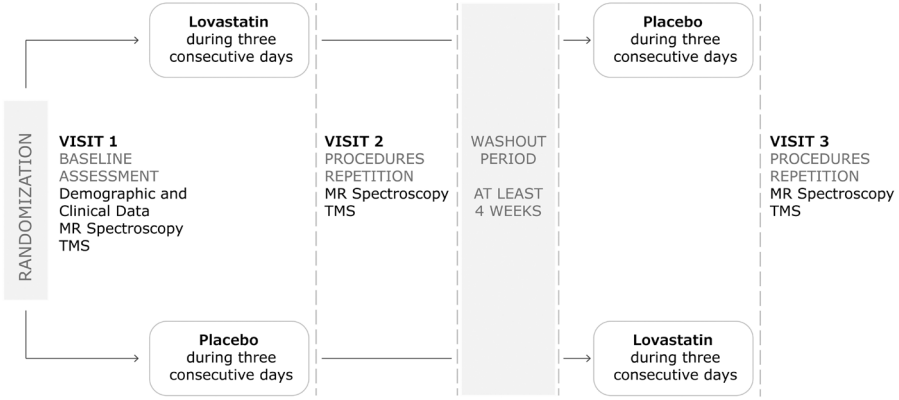


Figure 54. Clinical research study (Clinicaltrials.gov NCT03826940) design, including the procedures performed in each visit. MR magnetic resonance, TMS transcranial magnetic stimulation. From Bernardino et al, 2022. Copyright: Creative Commons Attribution 4.0 International License.

Motor cortex GABA+ and Glx concentrations were measured using novel neurospectroscopy HERMES and PRESS sequences, respectively, which we also used in another trial of the effects of neurostimulation on GABA neurotransmission (Abuhaiba et al, 2020) (Figure 55).

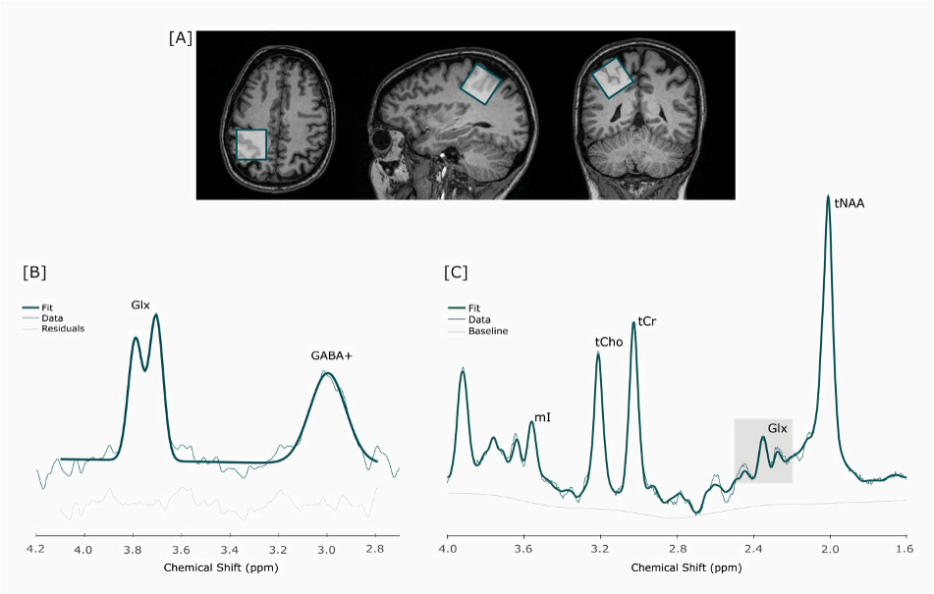


Figure 55. A schematic representation of the voxel placement (A). In (B), it is presented an example of Gannet output, from the HERMES neurospectroscopy sequence, used to estimate GABA+ concentration. Glx levels were quantified through the spectroscopy PRESS sequence acquisition, processed in LCModel, as represented by the example spectrum (C). Glx glutamate + glutamine, GABA+ gamma-aminobutyric acid, mI myo-inositol, tCho total choline, tCr total creatine, tNAA total N-acetylaspartate, ppm parts per million.

Cortical inhibition was investigated by paired-pulse, input-output curves, and cortical silent period (CSP) transcranial magnetic stimulation TMS protocols.

Cortical silent period ratios were significantly increased by lovastatin regardless of the way these ratios were calculated (relative: p=0.027; absolute: p=0.034) but not by placebo, showing a clear drug related effect on inhibition (Figure 56).

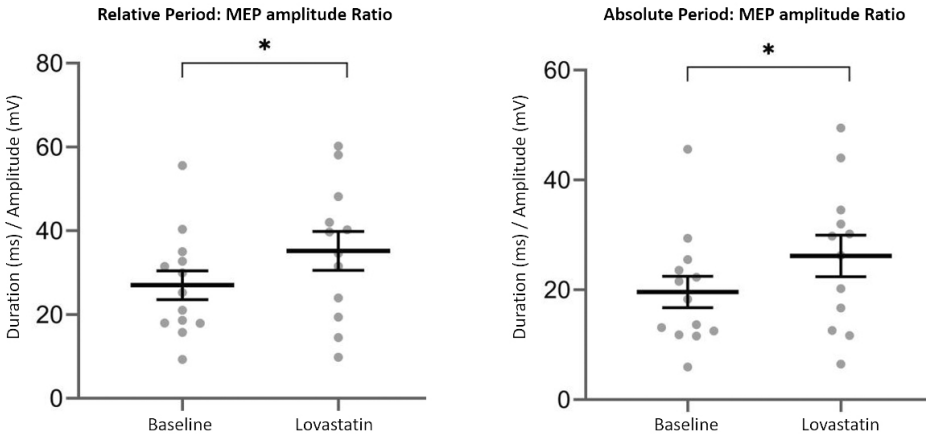


Figure 56. Differences in relative (A) and absolute (B) cortical silent period ratios, comparing measures taken after lovastatin and placebo intake with the baseline assessment. Dots represent, for each participant, the difference between lovastatin and baseline or placebo and baseline CSP:MEP ratios. Lines represent median and 95% CI. CSP cortical silent period, MEP motor-evoked potential, ms millisecond, mV millivolt, CI confidence interval.

CSP durations showed a negative correlation with the LICI (long-interval intracortical inhibition as applied by 30 paired-pulses with interstimulus intervals of 50 ms) amplitude ratio, only in the treated group (Figure 57).

Lovastatin was able to modulate cortical inhibition in NF1, as assessed by transcranial magnetic stimulation cortical silent period (CSP) ratios. Our results suggest that some measures of TMS, namely CSP and, into a lesser extent paired-pulse stimulation (both measuring specific aspects of GABAergic neurotransmission through GABAB receptors), were significantly more sensitive than MRS to detect changes in the GABAergic system induced by lovastatin.

One purpose of this study was to search for sensitive outcome measures, related to the lovastatin effects in excitation-inhibition imbalance in Neurofibromatosis Type 1. Probing physiological effects elicited by lovastatin intervention, using synaptic inhibition outcome measures provides an objective physiological approach that may detect early changes prior to alterations in high-level cognitive and behavioural outcome measures. Future studies should show whether such physiological changes have a behavioural impact.

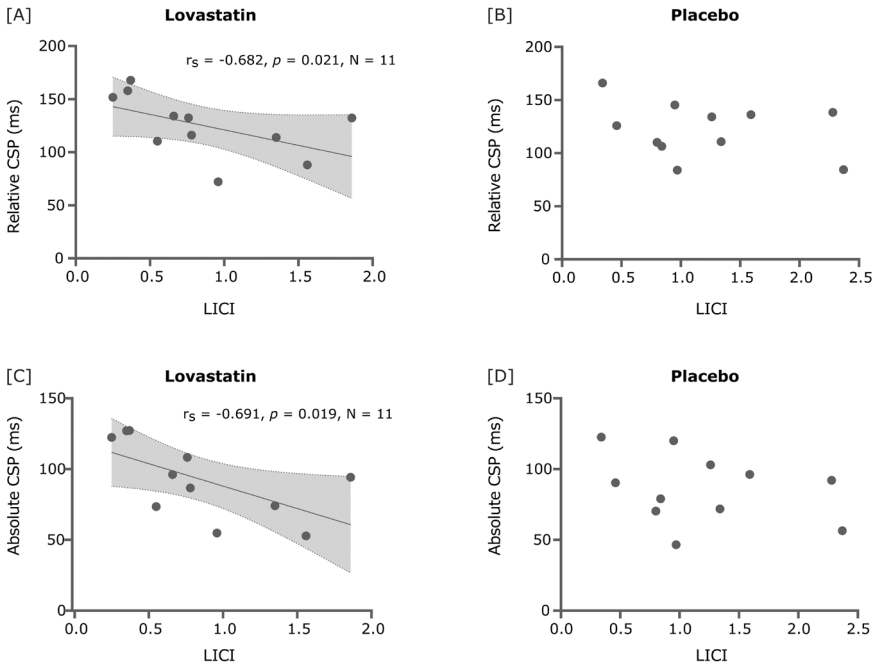


Figure 57. Lovastatin tunes the relationship of neurotransmission GABAB-related measures (cortical silent period and paired pulse inhibition measures such as LICI). Spearman correlations between relative and absolute silent periods and MEP amplitude ratio in LICI mean intervals, both following lovastatin (A, C) and placebo (B, D). Shaded area represents the 95% CI for the best-fit line. CSP cortical silent period, ms millisecond, CI confidence interval, LICI long-interval intracortical inhibition.

The role of serious games in improving social and cognitive skills

Our search for therapeutic approaches in autism stems from our research, as well as personal and institutional links with patient associations and the recognition that there is a lot of investment in diagnosis, but little in rehabilitation at the public level. Few technicians and psychologists are hired to invest in therapy in our national health system, contrary to what happens in other countries, where there is a greater balance in the investment between diagnosis and rehabilitation. Our research tries to change this scenario trough one simple type of approach which is based on the exponentially growing field of serious games (Simões et al., 2018).

These could for example be used to train skills that mitigate impairments in social interaction and repetitive patterns of behaviour. In any case, serious games can be used to train daily life skills which are relevant for adaptive behaviour.

We recently developed a serious game to train individuals with ASD for an important type of outdoor activity which is the use of public transportation. We used virtual reality to mimic the real world so that participants would gain skills to use buses as a means of transportation (Figure 58).

Figure 58. Initial Screenshot from the virtual environment, prompting the final destination.

The serious game would define a "safe" virtual environment" where the players become familiar with the process of taking a bus with all the social and cognitive skills involved: knowing a route in a map, understanding schedules and paths, and understanding what is the appropriate social behaviour inside a bus, including the interactions with the driver and other passengers. The ultimate goal would be to validate if it this training scheme would be efficient in teach bus-taking routines and adaptive behaviour. Players were placed in a three-dimensional city and were submitted to tasks of distinct difficulty which involved taking buses to get to specific destinations (Figures 59 and 60).

Figure 59. Screenshot from the virtual environment, showing two views from the bus stop perspective on the top, and two views from inside the bus on the bottom. On the top left corner, we can see one bus stop, with other people waiting for the bus, and the map to be used by the participant on the wall. On the top right, we see a bus with its designated number signalled in red, and some traffic on the street. The bottom images show two perspectives from inside of the bus. From Simões et al, 2018. Copyright: Creative Commons Attribution 4.0 International License.

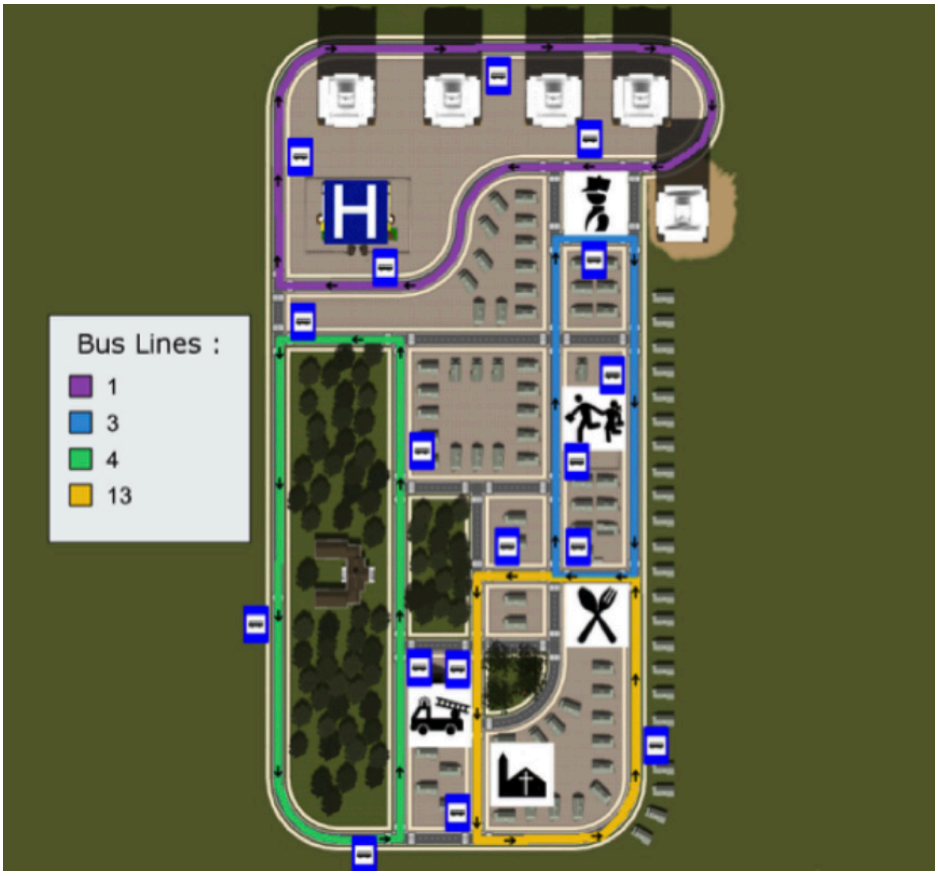


Figure 60. City map showing the bus lines, stops, and important places like the hospital, church, restaurant, and others. From Simões et al, 2018. Copyright: Creative Commons Attribution 4.0 International License.

We were also able to take physiological measures indexing anxiety such as galvanic skin conductance (Figure 61).

We found a statistically significant increase in competence measures regarding knowledge of the process of taking transportation over multiple sessions. Morever, a reduction in the electrodermal activity (the metric of anxiety) during the virtual trip was found, concomitantly with a high success rate (~94%) of bus travel rule application (Figures 62 and 63).

This shows that serious travel games in the context of emerging

immersive virtual reality technologies, are a potential tool to help people with ASD become more independent in outdoor activities, such as public transportation. This work had large dissemination and we were actually contacted by schools specialized in ASD in Canada and the US for potential use of this serious game. We are currently considering an exploitation framework for this game, so that it can be used outside the academia by people who might benefit from its use.

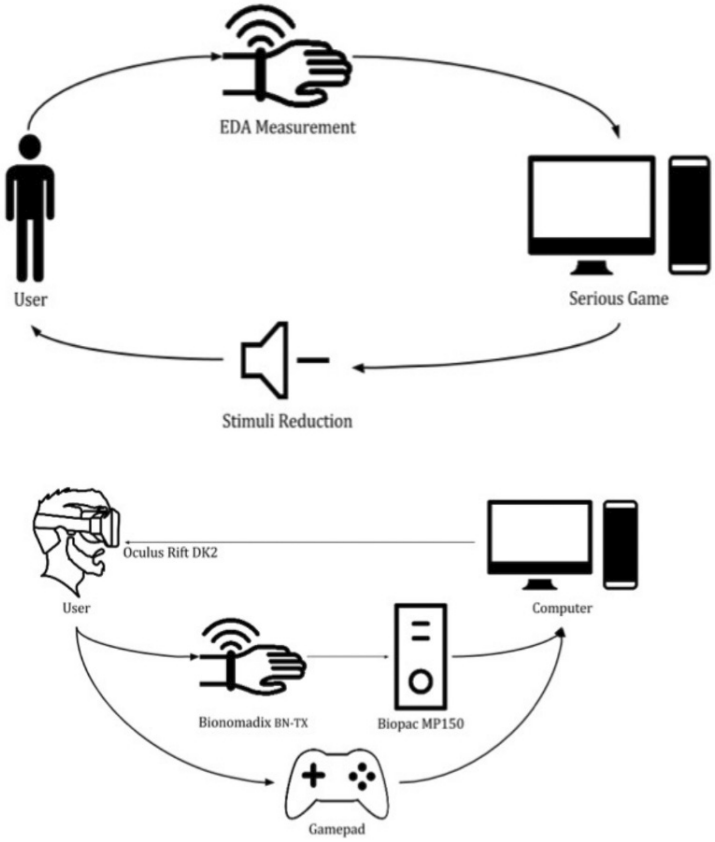


Figure 61. Top - Biofeedback loop diagram. The level of electrodermal activity is measured from the participant by the game. If it detects a peak of activity, it decreases the level of stimuli and noise in the scene. Bottom - Diagram of the setup used during the sessions, including the virtual reality headset, game controller, biosignal recorder, and the main computer. From Simões et al, 2018. Copyright: Creative Commons Attribution 4.0 International License.

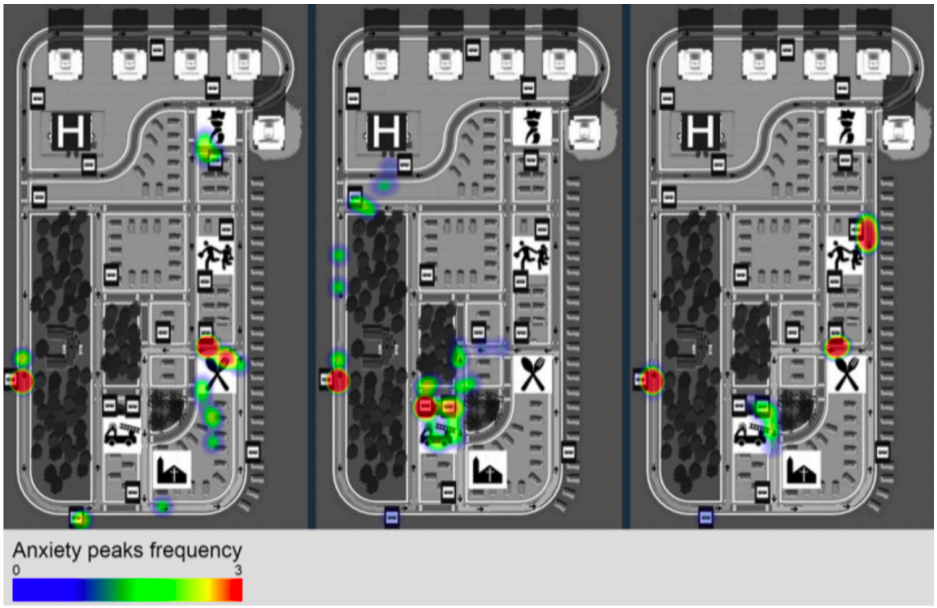


Figure 62. Anxiety peaks heat map from session 1 (left) to session 3 (right) for the times the participant was not inside of the bus environment. Most of the locations of higher anxiety marked by higher galvanic responses represent bus stops, where participants need to make the decision of what bus to take and wait for it to arrive.

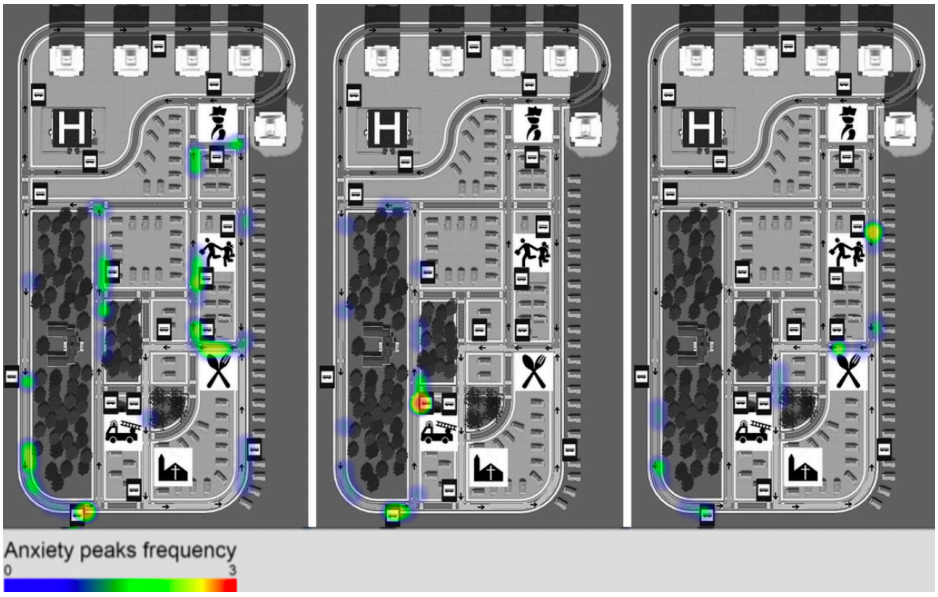


Figure 63. Anxiety peaks heat map from session 1 (left) to session 3 (right) for the times the participant was inside of the bus environment. The locations are much more dispersed through the route than in the outside the bus scenario. There is a visible decrease in frequency of anxiety peaks from the first to the last session.

Investigating neural signals related social scene complexity at the single-trial level: Evidence for hemispheric right lateralization of the social brain

An ultimate goal of our research enterprise is to classify neural signals at the single-trial level, to speed the use brain computer interfaces. If successful this would enable very relevant applications in affective and cognitive neuroscience. We have investigated before neural responses to scenes of increasing social scene complexity, to single stimulus presentations (Amaral et al, 2015). Given the importance for autism research, we used 3D human models as targets of attention.

We used oddball stimuli, which are characterized by rare target events within a flow of standard stimuli. Such rare but significant events, called oddballs or targets, within a stream of repetitive stream of standard or reference stimuli, generate a brain signal called the P300. Our aim is to

identify the P300 in single target presentations. In this way repetitions are not needed and the interaction within BCIs is more natural. Our challenging goal was therefore the single-trial statistical classification of EEG neural signals generated by these targets with increasing social scene complexity. Our oddball stimuli comprised different classes of objects (see Figures 64-66 for examples): flashed schematic eyes, simple 3D faces flashed between averted and non-averted gaze (in this case only eye position changing), full 3D faces flashing between averted and non-averted gaze (head and eye position changing), animated avatars alternating their gaze direction to the left and to the right (head and eye position), environment with 4 animated avatars all of which change gaze and one of which is the target of attention.

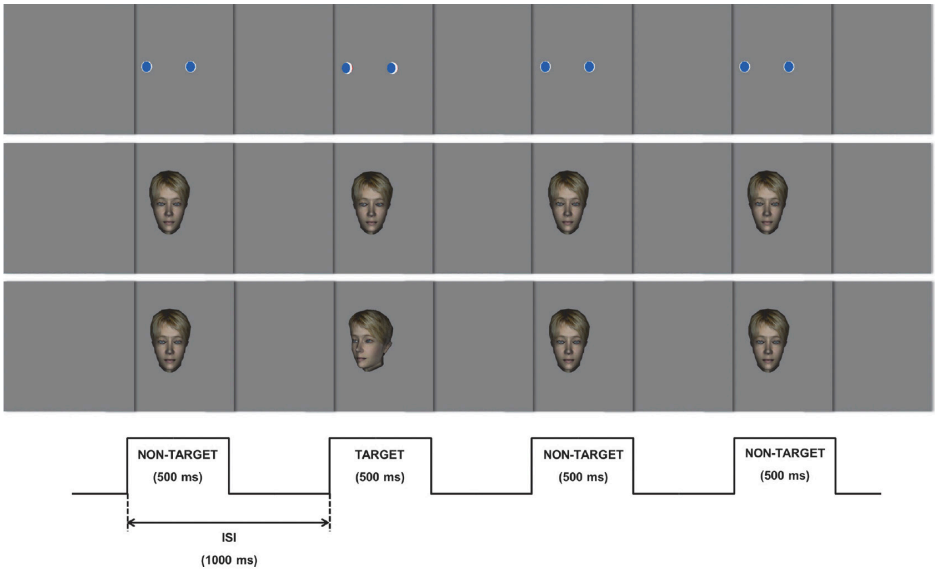


Figure 64. Examples of 3 flashing oddball paradigms. Top—'Flashed Schematic Eyes' paradigm: The subjects were asked to count the occurrence of slightly rotated balls. Middle—'Flashed Face—Eye position change' paradigm: the target event is a change in the direction of the eyes (averted versus non averted). Bottom—'Flashed Face—Eye and Head position change': The target event is the slight head rotation.
From Amaral et al. (2015). Copyright under the terms of the Creative Commons Attribution License, which permits unrestricted use.

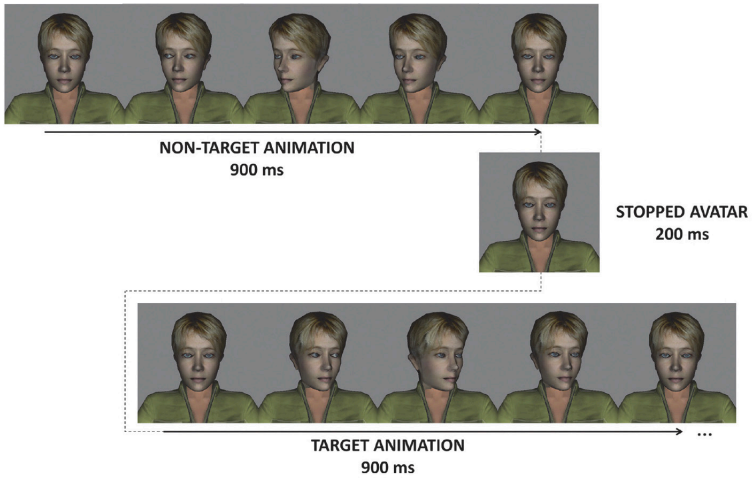


Figure 65. 'Animated 3D body—gaze change in 1 avatar' paradigm. The participants were instructed to detect to the turning of the head (oddball or rare event) of the avatar to the left of the participant. Non-target animation (standard or reference event) is the rotation of the head to the right of the participant. From Amaral et al. (2015). Copyright under the terms of the Creative Commons Attribution License, which permits unrestricted use.

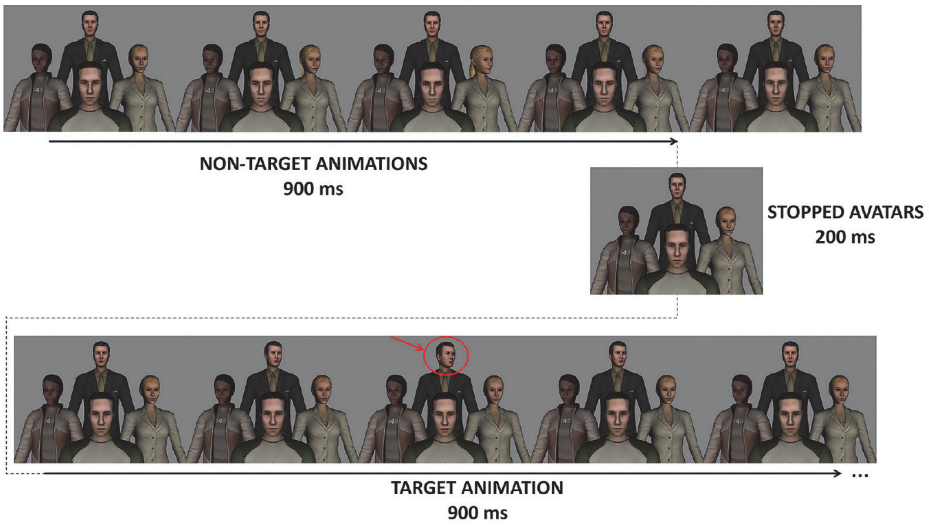


Figure 66. 'Animated 4 avatar environment —gaze change in 4 avatars' paradigm. The target of attention (oddball event) is the animation of top avatar. The task was to count how many times the top avatar averted its gaze. From Amaral et al. (2015). Copyright under the terms of the Creative Commons Attribution License, which permits unrestricted use.

We found neural correlates of the social oddball signal for all conditions

irrespective of their complexity. Single-trial detection of this signal with automatic classifiers was possible with a significant balanced accuracy classification of around 79%. This is remarkable given the level of realistic scene complexity. As expected from social and emotional processing we found right lateralization, which is consistent with right hemispheric lateralization of social and emotional processing (Figure 67).

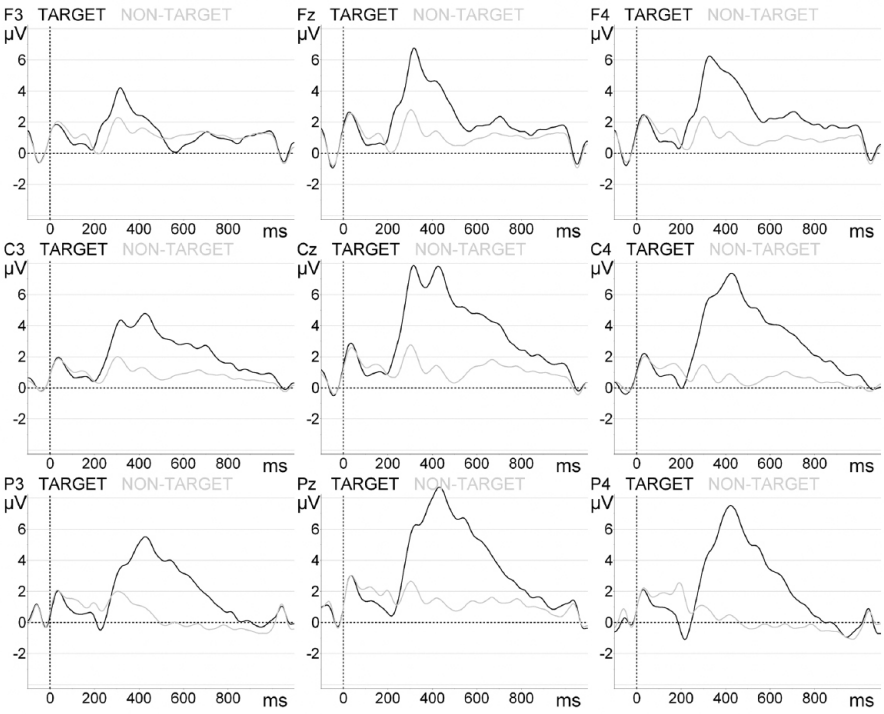


Figure 67. Evidence for right hemispheric lateralization of social brain signals. Left panels – left hemisphere P3, C3 and F3 channels. Middle Panels – Central brain locations. Right Panels - right hemisphere P4, C4 and F4 channels. Target (black line) and non-target (gray line) grand-average P300 ERP waveforms for the 'Animated 3D body—gaze change in 1 avatar' paradigm.
From Amaral et al. (2015). Copyright under the terms of the Creative Commons Attribution License, which permits unrestricted use.

These findings paved the way for our BCI clinical trials on social attention using this type of modified "P300" signal. They also inspired additional approaches in the field of affective neuroscience based on complex

social scenes (Amaral et al, 2017, 2018; Simões et al, 2020; Pereira et al, 2021). Improving the detectability of these signals at the single trial level will help optimize brain computer interfaces in the application to other social cognition disorders as well.

Emotion regulation, eye contact and interpersonal distance. Are these treatment targets?

Emotional and affective cognition are very much dependent on perceptual cues based on eye contact, body language and interpersonal distance. Action-perception cycles actually depend on the integration of these cues. The question then remains whether characterizing and training regulation of eye contact and interpersonal distance can improve social and affective cognition skills in autism spectrum disorder. Another important aspect is if these features can be studied in virtual environments, which would ease interventional approaches. Additionally, one should answer the question whether virtual and real worlds are comparable.

We addressed these types of questions and have actually shown that virtual reality immersion seems more similar to real world in autistic people than in neurotypicals. In other words we found rescaling of interpersonal distance perception in controls but not in Autism Spectrum Disorder, as explained below (Simões et al., 2020). In everyday life we tend to set a "comfort distance" when interacting with others. If we chose a "wrong" boundary to keep others at a preferred distance, will this generate a negative response from the person we are interacting with? There is no absolutely "right boundary" but better adapted people tend to adjust this distance so that they feel that the partner seems more comfortable with the new distance. We call this capacity the ability to regulate interpersonal space. Being too far away or too close may cause some tension in our partner. A similar rationale applies to eye contact. If it is too short or too long, this may also lead to discomfort between partners. People with autism seem to have larger difficulty in recognizing such emotional cues and using them to regulate interpersonal distance or the time length of eye contact.

One can raise the question, should the ability for such regulation be

a target for therapeutic intervention? The ability to represent a safety zone around the body may have evolutionarily evolved in order to be alert to potential social threats. An alarm system might actually be activated when social agents approach us. Possibly such a system is not critically needed in modern world and this is a case where neurodiversity should just be accepted without the need for intervention, unless the autistic person and his/her family feels that improving such skills might help under particular daily life demands.

However, avoiding eye contact may prevent the autistic person from reading in the face of others' emotions and intentions that are very important for daily life interactions. We have our own evidence in this respect in other conditions affecting visual scanning and emotion processing, such as Huntington disease (van Asselen et al., 2012) and there is literature further showing that face recognition may be dramatically improved by just increasing the frequency of face exploration and eye contact (Adolphs et al., 2005). This is an undeniable skill that is very important for daily life interactions and can be seen as a therapeutic target.

We have studied objective correlates of immersion in autistic and neurotypical participants. To achieve this goal, we studied humans in real or virtual visuospatial environments requiring preferred choice of Interpersonal Distance as a simple social regulation metric which is altered in autism (Figure 68). Immersion may imply different neural representations of the environment in these groups. The interaction with these environments might lead to different types of error signals in both types of populations. Predictive coding theories suggest that different brain areas represent prediction errors arising from the environment and that they may differentially activate distinct cognitive and attentional strategies. Sense of agency of phenomenology of the immersive experience may be quite distinct in ASD and this is a question we examined.

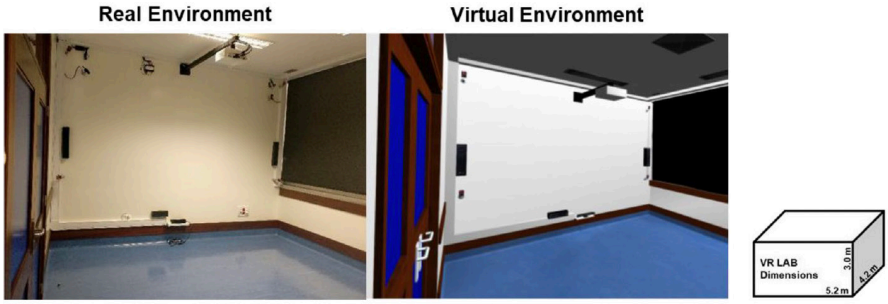


Figure 68. Images of the VR lab (photography, left) and its 3D representation used as virtual environment (3D rendering, right). From Simões et al 2020. Copyright Clearance Center's RightsLink® service – Springer Nature – number 501752590.

This area of research is quite paradigmatic in terms of the huge heterogeneity found in ASD. Some suggested ASD children prefer larger distances towards an unfamiliar adult (Gessaroli et al. 2013; Candini et al. 2017) although the opposite has also been reported (Parsons et al. 2004; Asada et al. 2016). In adults it has been reported that individuals with ASD intrude more often others personal space than controls (Kennedy and Adolphs, 2014), while others (Perry et al., 2015) have reported just greater variance in interpersonal space preferences in ASD, which is more in line with what we found (Simões et al, 2020).

More important than the concept of baseline size of the "comfort zone" space is the ability to regulate interpersonal space depending on contextual factors such as virtual versus real environments.

We performed a stop-distance paradigm to evaluate interpersonal distance regulation in autism spectrum disorder and control groups in a real versus a virtual environment mimicking in detail the real one (Figures 69 and 70).

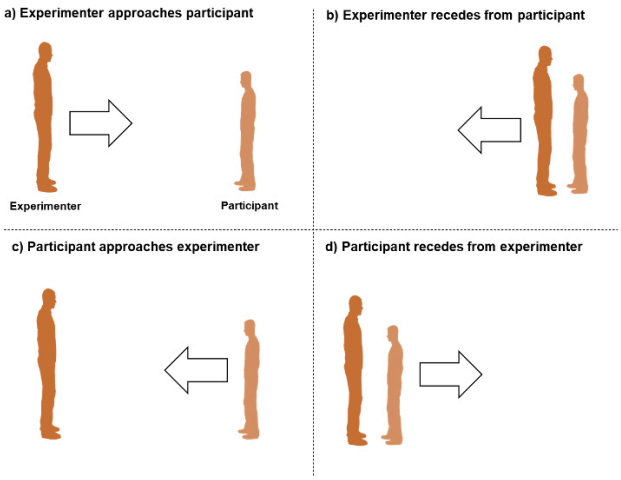


Figure 69. Example showing four different conditions, regarding who is walking and the walking person. a - The experimenter approaches the participant. b - The experimenter recedes from the participant. c - The participant approaches the experimenter. d - The participant recedes from the experimenter From Simões et al 2020. Copyright Clearance Center's RightsLink® service – Springer Nature – number 501752590.

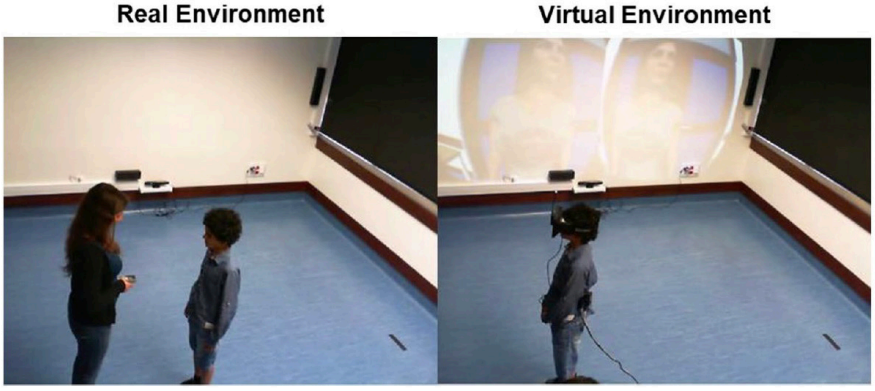


Figure 70. Capture of a user performing the task in both environments: the real environment, on the left, and the user using the VR setup, on the right. On the projection wall we display the same content that is presented to the user through the head mounted display. From Simões et al 2020. Copyright Clearance Center's RightsLink® service – Springer Nature – number 501752590.

We found a bimodal pattern of interpersonal distance regulation only in ASD. Both groups showed high interpersonal distance correlations between real and virtual environments, but the significantly larger slope in the control group suggests perceptual rescaling, which was absent in ASD (slope closer to one, suggesting that real and virtual worlds are more comparable in the clinical group) (Figure 71). We argue that loss of nuances like non-verbal communication, such as perception of subtle body gestures in the virtual environment, lead to different regulation of interpersonal distance in controls in each environment, whilst ASD participants show similar deficits in perceiving such subtle cues in both environments.

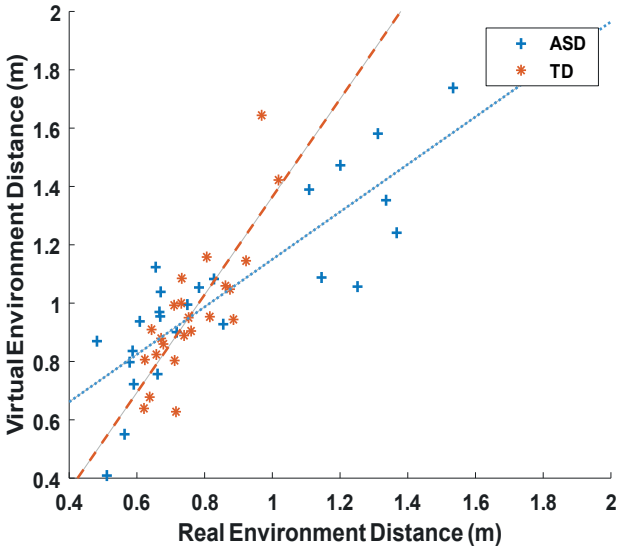


Figure 71. Scatter plot comparing the measures in the real and virtual environments, for the ASD group (in blue) and the TD (typically developing) group (in orange). The slopes of the least squares fit lines for each group (closer to one ASD: 0.81, much further away from one in TD: 1.68) show that the virtual disruption is grater for the controls, since a small difference in the real environment distances manifests a high increase in the distance observed in the virtual environment ($Z = -3.11$, $p = 0.003$). From Simões et al 2020. Copyright Clearance Center's RightsLink® service – Springer Nature – number 501752590.

Importantly, we found much larger variation pattern of interpersonal distance in ASD, confirming the tendency for these participants to show larger regulation ranges (Figure 72).

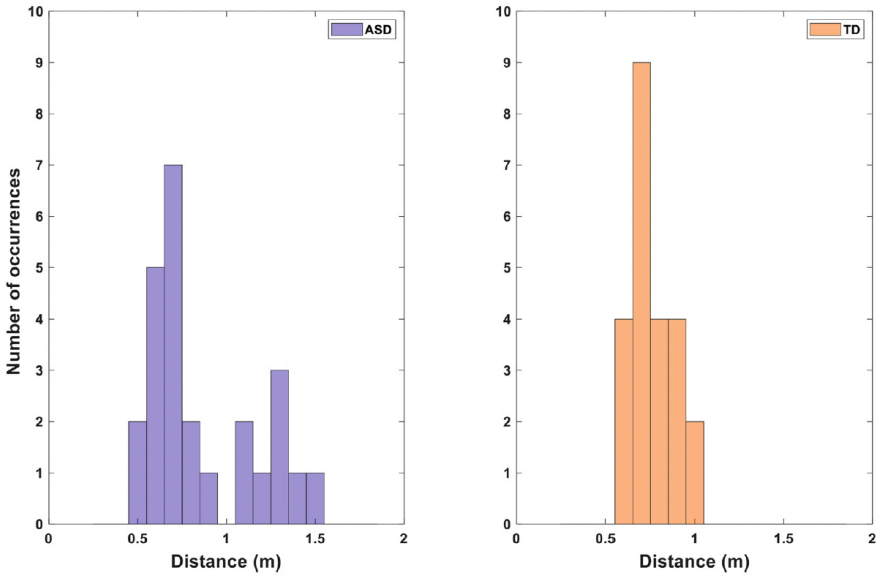


Figure 72. Histograms of interpersonal distance measures for both groups in the real environment. The X-axis represents the distance, in meters, whereas the Y-axis represents the number of participants for which that distance was observed. At the left we present the histogram for the ASD group, in blue, and at the right the histogram for the TD (typically developing) group. A much more variable bimodal pattern is discernible for the ASD group, suggesting distinct patient strata. From Simões et al 2020. Copyright Clearance Center's RightsLink® service – Springer Nature – number 501752590.

Open Science and Data sharing in Autism Research – our experience

We followed the open science philosophy to share our BCI clinical trial with substantial longitudinal data with the community (Amaral et al., 2018; Simões et al., 2020; Borra et al, 2022). We believe that this is a quite important endeavour because rendering available longitudinal multi-session P300 datasets for Brain-Computer Interfaces (BCI) in a clinical population fulfils an important data science goal. Indeed, it is quite rare to have longitudinal clinical trial results as publicly available datasets with appropriate

anonymization. By providing a large dataset with various individuals and multiple sessions this contributed to the development of more effective data science methods for BCI systems (Simões et al., 2020; Borra et al, 2022). This enabled the exploration of the feasibility of deep learning methods. Our BCIAUT-P300 dataset, contained 15 autism spectrum disorder individuals undergoing 7 sessions of P300-based BCI joint-attention training, for a total of 105 sessions. This dataset was provided to the 2019 International Federation of Medical and Biological Engineering Scientific Challenge where, in two phases, teams from all over the world competed for the best possible object-detection accuracy based on the P300 signals (Simões et al., 2020). Nine finalist teams during the competition. The winner obtained a stunning average accuracy of 92.3% with a convolutional neural network based on EEGNet. Our publicly released dataset is emerging as a test bed for longitudinal P300-based BCI algorithms based on multiple session clinical date in rehabilitation settings. Several studies have already emerged from this initiative, with the major outcome that machine learning classification approaches have massively improved their rate. We also participate in other data sharing initiatives within the scope of European projects such as BrainTrain, STIPED and AIMS-2-Trials, aside from having tested data from international initiatives such as the one from the SFARI Foundation (Santos et al, 2022).

Closing remarks - Designing theory inspired clinical trials

In our trajectory we performed investigator initiated trials using neurostimulation, serious games, neurofeedback, brain computer interfaces and pharmacological approaches. Most of these studies were up to phase II level, but a substantial larger amount of funding would be needed to bring them into community use. We are now in the process of selecting applications for further development. In the Autism research community there is a strong emphasis on the interventions targeting social communication and interaction, and reduction of stereotyped, repetitive behaviours. It remains hard to conceive that medications can be developed to improve social communication and interaction in ASD. For people adhering to behaviour-based psychotherapy effects are still moderate and

neurostimulation approaches, which have been proposed to improve brain communication, might become an alternative. Such approaches could target brain areas which process social information. We have been involved in testing Transcranial direct current stimulation (tDCS) as a technique to modulate brain excitability and connectivity. However since this technique is just modulatory we defend that it should be combined with specific cognitive tasks which truly evoke brain activity that can be reinforced by modulation during training. It remains a question whether application of tDCS in brain areas underlying social perception in combination with a training task can be used in ASD. This inspired the pilot trial we described above (Pereira et al., 2021). We are now involved in a phase-IIa pilot randomized, double-blind, sham-controlled, parallel-group clinical study already approved by our national regulatory authorities to investigate if 10 days of 20-min multi-channel tDCS stimulation of the bilateral tempo-parietal junction (TPJ) can be useful. Such stimulation is combined with training with social cognition tasks based on perspective taking, intention and emotion understanding, in children and adolescents with ASD. The question remains on which are the best measures to assess the results of this clinical trial. Our research consortium agreed on choosing in parent-rated social responsiveness from baseline (one week before first therapy session) to post-intervention (within one week after the last session). One might argue that it would be better to study responsiveness at 4 weeks at least, but the nature of these early phase trials is quite incremental. One might ask whether studying other ASD core symptoms and psychopathology, would be more relevant. We are also currently exploring what are the best neural functioning measures to assess the effects of interventions on neural and cognitive mechanisms. Since this type of intervention seems to be relatively safe and well-tolerated we believe that it is worth testing it as a cost-effective treatment for addressing ASD core-symptoms. The question of course remains of which type of ASD participant or parent will wish intervention for these types of "symptoms". This really depends on recognizing that these manifestations are truly perceived as symptoms that disrupt daily life functioning.

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Prémio Bial de Medicina Clínica 2022

A FUNDAÇÃO BIAL foi criada em 1994 pela BIAL, em conjunto com o Conselho de Reitores das Universidades Portuguesas, com o objetivo de promover o estudo científico do ser humano, tanto do ponto de vista físico como espiritual.

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O PRÉMIO BIAL DE MEDICINA CLÍNICA 2022 foi entregue a Miguel Castelo-Branco pelo trabalho "The challenges of Neurodiversity: A Translational Journey into Personalized Medicine in Autism Research".

Nesta edição, foram também distinguidas duas obras com Menções Honrosas: "Brain Tumors 360º: from biological samples to precision medicine for patients", de autoria de Cláudia Faria, e "Contribuição para o estudo da Hipertensão Arterial em Moçambique e na África subsaariana: Resultados de um combate de 25 anos", de Albertino Damasceno (coordenador), Jorge Junqueira Polónia, Nuno Lunet, António Prista, Carla Silva Matos e Neusa Jessen.

O júri do PRÉMIO BIAL DE MEDICINA CLÍNICA 2022 foi presidido por Manuel Sobrinho Simões e constituído por João Bessa, Jaime Branco, Filipe Caseiro Alves, Miguel Castelo-Branco Craveiro Sousa, Altamiro da Costa Pereira, Henrique Cyrne Carvalho, Helena Leitão e José Melo Cristino. Em 2024 a FUNDAÇÃO BIAL realiza uma nova edição do concurso PRÉMIO BIAL DE MEDICINA CLÍNICA, que conta com o Alto Patrocínio do Senhor Presidente da República, e os patrocínios do Conselho de Reitores das Universidades Portuguesas e da Ordem dos Médicos.

The BIAL Foundation was created in 1994 by the BIAL pharmaceutical company together with the Council of Rectors of Portuguese Universities. BIAL's Foundation mission is to foster the scientific study of the human being from both the physical and spiritual perspectives.

In the scope of its mission, the BIAL Foundation awards prizes in health sciences research, including the PRÉMIO BIAL DE MEDICINA CLÍNICA, the BIAL AWARD IN BIOMEDICINE, and the MARIA DE SOUSA AWARD, the latter in partnership with the Portuguese Medical Association.

The PRÉMIO BIAL DE MEDICINA CLÍNICA 2022 was awarded to Miguel Castelo-Branco for the work "The challenges of Neurodiversity: A Translational Journey into Personalized Medicine in Autism Research."

In this edition, two other works were also distinguished with Honourable Mentions: "Brain Tumors 360º: from biological samples to precision medicine for patients," by Cláudia Faria, and "Contribuição para o estudo da Hipertensão Arterial em Moçambique e na África subsaariana: Resultados de um combate de 25 anos", coordinated by Albertino Damasceno, with contributions by Jorge Junqueira Polónia, Nuno Lunet, António Prista, Carla Silva Matos, and Neusa Jessen.

The jury of the PRÉMIO BIAL DE MEDICINA CLÍNICA 2022 was presided by Manuel Sobrinho Simões and composed by João Bessa, Jaime Branco, Filipe Caseiro Alves, Miguel Castelo-Branco Craveiro Sousa, Altamiro da Costa Pereira, Henrique Cyrne Carvalho, Helena Leitão, and José Melo Cristino. In 2024, the BIAL Foundation will launch a new edition of the PRÉMIO BIAL DE MEDICINA CLÍNICA, under the High Patronage of the President of the Portuguese Republic, with the sponsorship of the Council of Rectors of Portuguese Universities and the Portuguese Medical Association.