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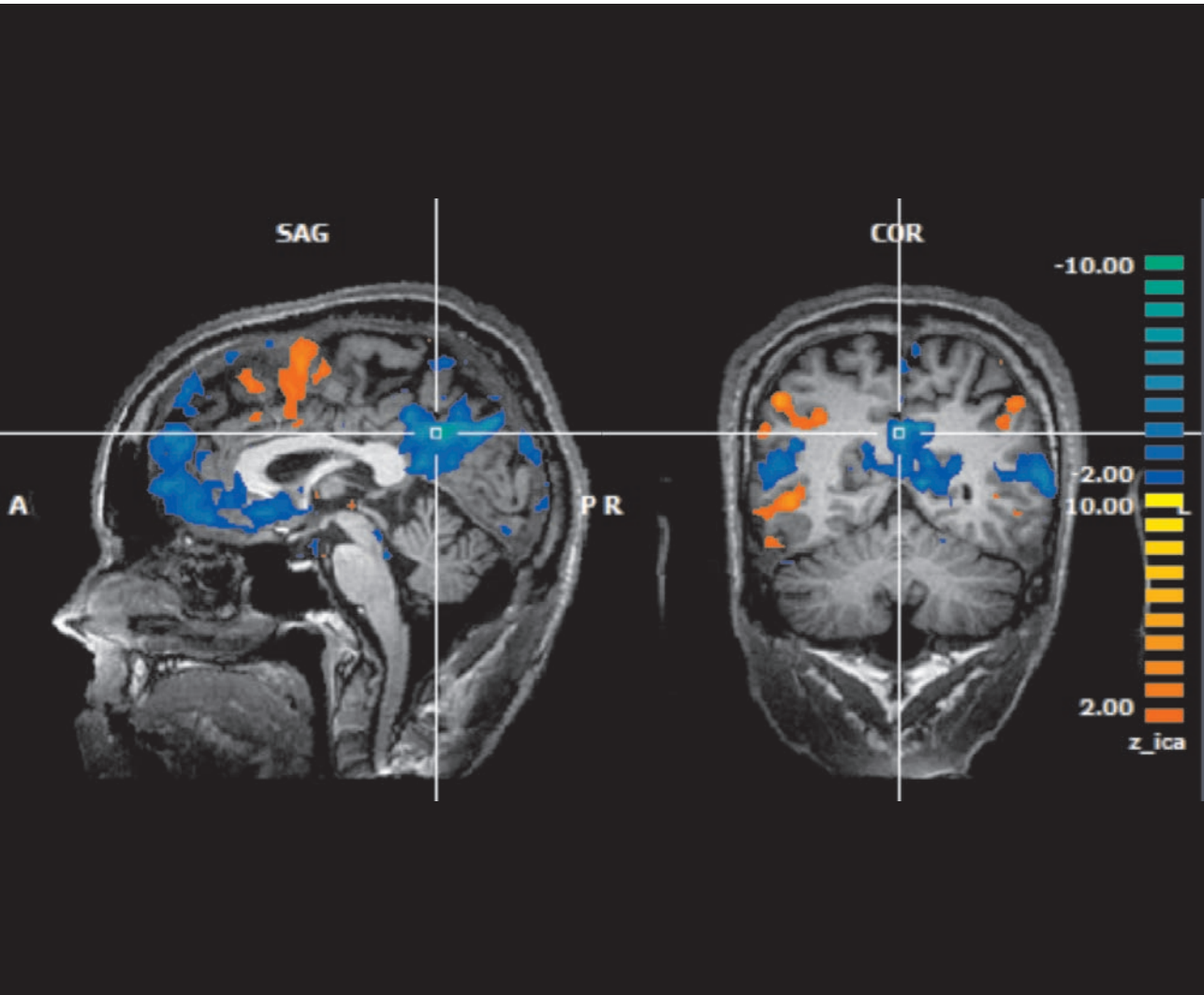
A Vision for Medical Research in the New Era of Complexity:
a new multimodal integrative paradigm

MIGUEL CASTELO-BRANCO



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He has made interdisciplinary contributions in the fields of Ophthalmology, Neurology, Visual Neuroscience, Human Psychophysics, Functional Brain Imaging and Human and Animal Neurophysiology.

To Sandra, Miguel and João
To all the IBILI team that made this scientific adventure possible

Contents

1. Introduction

This work aims to address some of the main current challenges of biomedical research, and namely the increasing awareness on the need to develop new tools to deal with the complexity of systems biology questions. Our discussion of the need for new approaches is inspired partly on our own work in the fields of Ophthalmology, Neurology, Visual Neuroscience, Human Psychophysics, Functional Brain Imaging and Human and Animal Neurophysiology. This allowed us to compare research trends and pitfalls in each of these fields of research, and to feel the need for a unified framework in biomedical sciences, and in particular, the neurosciences. This goal is difficult to achieve, because scientists often talk different languages and are not easily eager to adopt a new consensus philosophy. This is particularly true in the now trendy field of translational research, which represents an arena where basic scientists and clinicians are still often at odds to find productive compromises.

We will provide examples related to the discovery of new retinal and cortical phenotypes in genetic models of visual impairment, and how they can enlighten the discussion on the relation between genotypes and complex phenotypes.

We have combined emerging new technologies, including quantitative psychophysics, and electrophysiology to assess genotype–phenotype relationships. It is widely recognized that the diversity of phenotypes at molecular, cellular and organismal level requires a theoretically sound, model driven analysis in order to understand such complexity as well as technical expertise to tackle systems level integration. Below we address the need to use both model and data driven approaches, which have the advantage of not being dependent on the investigator's hypotheses. We will discuss the advantages of combining both approaches.

Electroretinography of the photoreceptor rod and cone systems and imaging optical coherence tomography data (OCT), which provided in vivo truly histological biomarkers of photoreceptor degeneration in genetic disorders, such as Best disease and age-related macular

degeneration were at the basis of a part of our efforts. Accordingly we have mapped neuroretinal and retinal pigment epithelium histological changes on the macular area using optical coherence tomography, which is a sort of in vivo optical biopsy with a resolution below 10 microns, and correlated such changes with visual function. The rationale behind this approach is that it is important to have access to genetic information to understand the pathophysiology of retinal degenerations. This is particularly relevant given our own findings concerning novel several disease related mutations. This is the case concerning both Stargardt and Best diseases, which lead to the discovery of novel and distinct phenotypes of photoreceptor degeneration (Campos et al., 2005; Maia-Lopes et al., 2008).

We have found evidence for widespread retinal dysfunction in patients with Stargardt disease and morphologically unaffected carrier relatives. Eighty percent of these were found to be mutation carriers. It is possible that different mutations segregate in the same family, partly explaining phenotypic variability. Quantitative phenotyping and genetic characterization of the carrier state helped shed new light on subtle genotype-phenotype relationships that can only be identified at a functional level.

Another example of the cross-talk between different fields of research will be provided by the discussion of the discovery of novel mechanisms of visual motion integration in humans and animals (Castelo-Branco et al,. 1998, 2000, 2002, 2005, 2006, 2007, 2008; Mendes et al., 2005, Silva et al., 2005, Biederlack et al., 2006, Schmidt et al., 2006; Kozak & Castelo-Branco, 2008). In particular, the understanding of how temporal patterns of activity can be segregated in an information-dependent manner from the retina to the cortex, involved the use of advanced signal processing tools but also a technical tour de force, through simultaneous recordings at distinct levels of visual processing in the cat (the retina, lateral geniculate nucleus and visual cortex). Animal studies have the advantages of probing function at the single cell level, but lack the

scope of novel structural and functional imaging tools in humans (some of which are reaching cellular resolution at the level of the retina). The functional characterization of parallel retinocortical pathways (konio, magno and parvocellular pathways) has enabled us to pinpoint mechanisms of disease in ocular hypertension and glaucoma (Castelo-Branco et al., 2004) and other neurological disorders (Silva et al., 2005; Castelo-Branco et al., 2007, 2008).

Translational research and genotype-phenotype correlation studies in the field of visual sciences have indeed benefited from the fact that the retina can be approached by tantalizing and elegant new imaging approaches. Some of our new insights into the pathophysiology of photoreceptor degenerations and their association with newly described mutation patterns were enabled by such remarkable advantages. We have found, as mentioned above, that in Stargardt disease, a photoreceptor degeneration, the carrier state is accompanied by genetic background dependent subtle physiological impairment (Maia-Lopes et al., 2008). However, individual research efforts are nowadays quite limited if reasonably large sample sizes are not reached and if novel statistical and data mining approaches are not applied. Sharing of data and know-how then becomes a scientific obligation and we believe that we are starting to achieve such endeavor by participating in generation of a European database of families with hereditary retinal diseases and helping speed up translational research approaches and applications to these and other diseases.

The group established new links between basic animal and human clinical research that helped unravel the neural correlates of global motion perception and visual dorsal stream function in health and disease. This basic multilevel hierarchical approach was applied to clinical research questions that helped clarify mechanisms of disease in neurological disorders with visual impairment, and high order visuomotor impairment such as Parkinson Disease, Mild Cognitive Impairment, Lewis Body Dementia and Williams Syndrome.

Our group has mainly focused on mechanisms underlying parallel processing within magno, konio and parvocellular streams and retinocortical flow of information towards dorsal and ventral visual cortical streams. Understanding the neural correlates of motion integration and visual dorsal stream function was crucial to test an important model in Visual Neuroscience from the basic and clinical point of view: does magnocelullar function predict motion integration and subsequent visuomotor integration? It was found that cortical dorsal stream regions can actually compensate for magnocelullar deficits, and that motion integration abilities predict visual constructive abilities and visuomotor integration. These discoveries are critical to the understanding of the neural basis of visual impairment in disorders such as dyslexia.
A broad range of multimodal experimental approaches have been used to study normal and pathological retinocortical processing within the color and motion domain: structural and functional magnetic resonance imaging and visual evoked potentials in humans, structural and functional imaging of the human retina, cellular neurophysiology of the cat retina, LGN and visual cortex and optical imaging techniques in animals to understand population responses. Most of the approaches used in humans stem from the above mentioned studies of the author who performed pioneering simultaneous recordings at distinct levels of visual processing (the retina, lateral geniculate nucleus and visual cortex). This approach enabled the elucidation how temporal oscillatory patterns of activity are transmitted from the retina to the cortex and how they may relate to distinct functional pathways.

As we will also discuss in this work, sometimes novel findings in human research can also inspire new approaches in animal models. Our discovery of low level non-motor visual manifestations in Parkinson Disease leads to new questions concerning disease pathophysiology, that can be nicely addressed together with some of the existing

animal models. In fact, and as discussed below, these findings call for the need to constantly compare human and animal based studies.

Our approach suggests that combination of novel quantitative psychophysical methods, combined with electrophysiology, imaging and careful genetic characterization, will pave the way for future advances in the understanding of retinal and cortical disease. We have applied our basic studies of visual function also to genetic and acquired neurological and developmental disease models. One of the main aims was to discover mechanisms of neural plasticity that could help predict the potential for recovery and rehabilitation. A good example is our recent work on adaptive visual memory reorganization in right medial temporal lobe epilepsy. We have indeed found functional reorganization of the medial temporal lobe in right hippocampal sclerosis, through transfer of function from the right to the left hemisphere, with associated preservation of performance, which strongly suggests an adaptive role for such reorganization mechanism in supporting preserved visual memory (Figueiredo et al., 2008).

The advantages of an integrated multidisciplinary approach in the understanding of mechanisms of disease and their implications for rehabilitation approaches is highlighted by our recent work on neurodevelopmental models of visual function that tackles directly the role of genes versus environment and bears direct implications for new rehabilitation approaches.

One of the most convincing models of a relationship that links genes with human cognition and behavior is represented by Williams–Beuren syndrome (WBS or WS). This disorder is caused by a hemimicrodeletion at 7q11.23. Detailed molecular characterization of the deletion alongside with well-defined quantitative structure/function correlations in WBS provided a unique opportunity to investigate the neuromolecular basis of complex visual dysfunction. Williams Syndrome is an ideal model to study visual function because of its cognitive profile, which includes relatively good verbal

skills alongside very deficient visuo-spatial abilities. Preservation of auditory skills includes the remarkable musical creativity often observed in WBS individuals which is thought to be a means of improvised expression of natural ability rather than formal skill in pitch and rhythm analysis.

WBS is also a paradigmatic model of genetic evolutionary patterns, because it is thought to arise through unequal crossover between large duplicated regions (low-copy repeat sequences (LCRs), which span >320 kb) that flank the deletion region and exhibit very high nucleotide sequence identity (~98%). They are composed of smaller duplicons as well as genes and pseudogenes. Interestingly, the orthologous region in the mouse lacks the characteristic duplicated blocks and its full complement of genes is inverted with respect to the human map, possibly because of breakpoints of the later genome and respective evolutionary consequences.

We were quite surprised by the fact that patients with very similar or even identical genetic profiles had very distinct visual phenotypes. This raises the question of whether a simple model of haploinsufficiency can explain the phenotype. It has been recognized that the presentation of genetic syndromes dependent on the sex of the transmitting parent, but this was however not the case in our sample. Parent of origin specific epigenetic marking (silencing) of an allele of a gene within the WBS region, a phenomenon known as genomic imprinting, does therefore not seem to relevant in this case. Williams-Beuren Syndrome (WS) does therefore provide a unique model to link genetically determined loss of neural populations at different levels of the nervous system, neural circuits and visual behavior. Given the role of several of the involved genes during eye development and differentiation of its neural layers, we first tested a new retinal phenotype in WS (Figure 1) and its functional relation to high-level global motion perception. We were able to discover a low-level visual phenotype by using a multimodal approach that includes electrophysiology, confocal and coherence

in vivo imaging with cellular resolution and psychophysics. The discovered mechanisms of impairment were found to be related to the magnocellular pathway, which is involved in the detection of local temporal changes in the visual scene. To our surprise, however, low level magnocellular performance did not predict high-level global motion integration deficits, thereby proving independent mechanisms of disease requiring distinct remediation strategies. These findings challenge neurodevelopmental theories, such as dyslexia, that explain cortical deficits based on low-level magnocellular impairment.

Our structure-function correlation approach unraveled in the above described manner a novel neural retinal phenotype in Williams Syndrome that explains patterns of visual deficits that are independent from described cortex-based phenotypes. This suggests a new framework for hierarchical genotype-phenotyping in complex and multifactorial diseases of the nervous system.

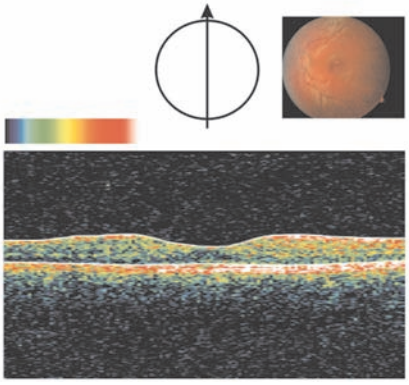

Figure 1

Central retinal phenotype in a representative WBS subject. The Optical biopsy using OCT of the central macular region of the retina reveals a normal layering but decreased thickness of retinal structures in a Williams Syndrome participant (color-coded log reflectivity map: red, high; black, low). The hyperreflective structures are the retinal nerve fiber layer (top) and the retinal pigment epithelium (bottom). For details, see Castelo-Branco et al., 2007.

We showed for the first time patterns of dysfunction at early retinal circuits that are consistent with the previous demonstration that genes, including *LIMK1* and *GTF2IRD1* are pivotal in the development of retinal neural layers and contribute to visual deficits in WBS. Our results also provide a beautiful convergence with animal research evidence that loss of *Gtf2ird1* in mice also results in a phenotype that is consistent with its expression in the eye and the retina. The independence of the novel retinal and cortical phenotypes (Figure 2) also suggest distinct rehabilitation approaches to the distinct sources of visual deficits.

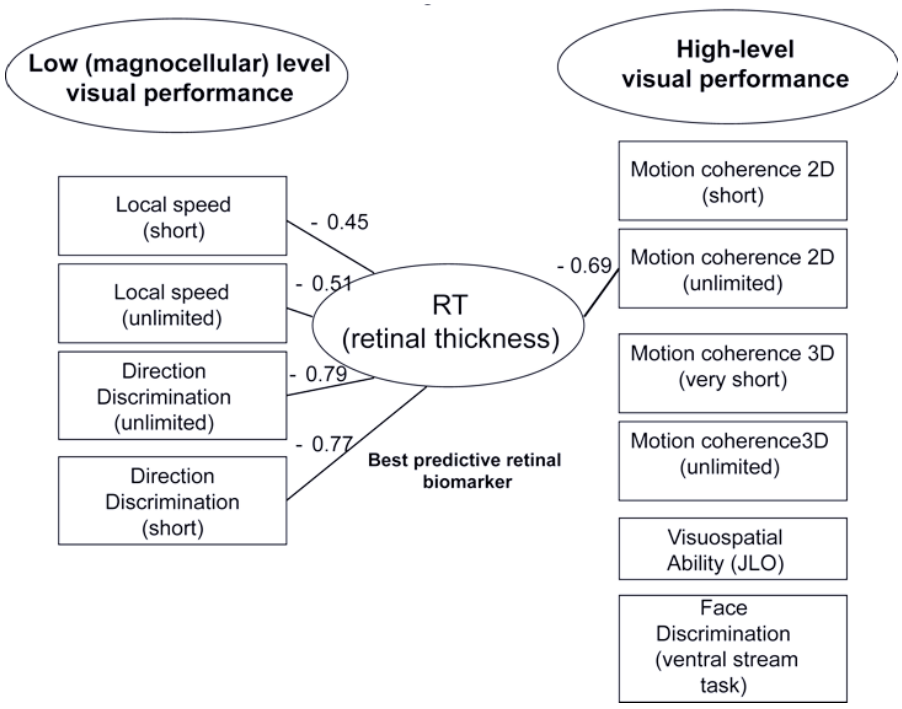

Figure 2

Retinal thickness (RT) of the photoreceptor to ganglion cell nerve fiber layer can predict low-level motion performance deficits but not high-level motion integration and dorsal stream functional measurements. Correlations with

high-level ventral stream (face discrimination) performance are, as expected, also absent. Connecting lines depict significant correlations. Rho values next to lines depict correlation strength (they are in general negative because lower RTs correspond to less neurons in the outer retina, and lower performance implies higher thresholds, see Castelo-Branco et al., 2007). JLO, Benton's Judgment of Line Orientation.

In this work we will further discuss how these conceptual insights can also be used in the investigation of mechanisms of disease in neurodegenerative disorders, in comparison to models of normal aging. We hope to have established a framework that unifies model-driven approaches to understand structure/function correlations in the retina and the brain as well as methodological approaches that include advanced data mining techniques.

2. Biomedical sciences at a crossroad: a paradigm shift in basic and clinical research

We live in an era of heated debates concerning the best strategies for improved decision-making in medicine and what is the role of translational and preclinical research in that context. Fortunately, newer consensuses on the value of Evidence Based Medicine have overridden the often sterile discussions on whether medicine should be more close to being a special form of "art" where intuition and individual experience are sufficient to guide diagnostic and therapeutic options.

Recent advances on bioinformatics and data mining concepts (meaning processes used for extraction of hidden predictive information from large databases) have helped changing this scenario. Nowadays Bayesian probabilistic algorithms are used in the emergency setting in the UK to help guide fast decisions on the management of acute abdominal pain (Massad et al., 2004). The field is evolving fast, and recent work suggests that accuracy of dementia diagnosis between radiologists and a computerized method (support vector machines, to be described in a later chapter) yield now comparable results (Klöppel et al., 2008).

The fields of pre-clinical and clinical neurosciences have become quite challenged in this respect, because massive information has accumulated through the development of integrated databases joining anatomical information, connectivity patterns of white matter fiber tracks, parcellation of cortical and subcortical nuclei, as well as functional information including metabolic and receptor density maps. Integration of information, with pattern classification algorithms, may well result in novel medical tools with much increased sensitivity and specificity for the diagnosis of neurological disorders.

Among the newer statistical devices one can mention Machine learning algorithms, such as the above mentioned support vector machines, (Formisano et al., 2008) which potential use as diagnostic tools has indeed been recently demonstrated in neurodegenerative disorders (Teipel et al. 2007, Klöppel et al. 2008). These may help

provide fingerprints and biomarkers in healthy and diseased brain states and in combination with more advanced machine learning classification methods will provide unique multimodal tools for research and medical diagnosis. All these advances are not possible without an evolved process of mapping multi-resolution neuroimaging information to the same referential (so-called registration). Registration problems can be either solved between different modalities or between groups of subjects and can be used to produce reference atlases (e.g.Thompson 2001; Carmichael 2005). This implies appropriately dealing with ageing trajectories, subtle structural changes along time, with special customized software applications needed to preserve relevant information. Only under those circumstances can quantitative atlas creation, identification of structures, segmentation and/or delineation of functional areas be achieved for subsequent classification. Optimization of co-registration, into a single space, of information obtained from the different modalities, will facilitate application of pattern classification algorithms and visualization of results.

This approach allows the detection of subtle and non-strictly localized effects, and has the ability to exploit the inherent multivariate nature of data. Structural studies have already provided proof of concept of such approaches in MRI clinical diagnoses (Fan et al. 2006, Davatzikos et al., 2006, Teipel et al.,2007, Klöppel et al 2008). Similar approaches have recently been attempted concerning Diffusion Tensor Imaging data in pre-clinical stages of Huntington disease (Klöppel 2008).

These strategies are already enabling the identification of consistent pattern differences between groups or populations, and thus the identification of fingerprints and biomarkers in patients with neurodegenerative disorders. Unlike standard visual scrutiny or univariate statistical approaches in classical biomedical research, this approach allows both to detect effects that a human observer would not be able to see even if he would be an expert. The main

reason for that is that one cannot easily see or grasp non-strictly localized effects (Castelo-Branco et al., 2002) or visualize at a glimpse the multivariate nature of neuroimaging data.

These novel approaches can be used not just for basic research and diagnostic applications but also research on therapies and analysis of critical strategy issues in pre-clinical research.

One of such critical questions is whether neuroprotection is a viable treatment strategy in retinal diseases. This is a fundamental question given the large number of unsuccessful neuroprotectant drug trials in glaucoma and other disease states. Many scientists and clinicians now believe that animal data and small-sample phase 2 clinical trials in humans are insufficient to make informed clinical decisions regarding the use of these drugs (Levin and Peeples, 2008). Apoptosis may be an adaptive response in glaucoma, which is an optic neuropathy thought to originate at the level of the optic nerve where retinal ganglion cell axons are subjected to mechanical, vascular, and/or biochemical injury as a result of elevated intraocular pressure (IOP). The failure of Memantine in glaucoma can possibly be related to the fact that etiological factors in programmed cell death are not adequately modeled.

One could argue that neuroprotection is mostly useful in disease stages where no evidence is still present for structural damage (Castelo-Branco et al., 2004). This tenet would require that one would need to identify functional changes prior to the occurrence of structural damage, which is by no means yet established. Our group has provided recent evidence that this might indeed be the case by our recent demonstration of deficits in two color vision pathways in a pre-clinical stage (ocular hypertension, e. g. before glaucoma is established) (Castelo-Branco et al., 2004).

Other examples could be given. For instance how much neuroprotection would one need against light induced photoreceptor degeneration? There is a need to understand early

disease mechanisms and to have quantitative models of light induced photoreceptor degeneration that include innate and acquired factors.

These models should obviously include genetic factors. Light associated age related macular degeneration genetic risk loci are indeed now being actively looked for. Concerning environmental factors, the recently published EUREYE study which recruited some 5000 participants from 7 European countries recently revealed that the risk of neovascular Age Related Macular Degeneration is elevated 4-fold in those people who fell into the highest quintile of blue light exposure and the lowest quintile of serum antioxidants (Fletcher et al., 2008).

Neuroprotection cannot be considered without concomitantly treating the cause of neural dysfunction and death, and taking into account the natural history of such impairment. There is accumulating evidence for early neural impairment in diabetic retinopathy (Castelo-Branco et al., 2006). Hyperglycemia induced changes occur in AMPA receptor subunit distribution in retinal cells and neuroprotection will probably be useful particularly in early stages, but concomitant therapies will be anyway needed to treat vasculopathy. If neural impairment becomes predominantly secondary to vascular impairment in later stages, then neuroprotection looses weight and probably impact in terms of therapeutic options.

In general, if the cause is not understood and removed then even effective neuroprotective agents will not be efficient, such as in Stargardt Disease, where disruption of the visual pigment cycle causes inevitable loss of visual function (Maia-Lopes et al., 2008). This is now a critical issue, with the advent of the Rod Derived Growth Factor and its potential application in diseases that lead to cone photoreceptor death (Croni et al., 2007).

3. The three new revolutions in Medicine: evidence-based medicine, pos-genomic medicine and darwinistic medicine. The need for new tools to deal with complexity.

The statement of Osler that Medicine should remain an art and not a science is being increasingly discussed with ever heating debates. Nevertheless, we face a new age in which the traditional research tools used in Basic and Clinical Medicine to understand and describe pathophysiological processes are not longer sufficient. Our own work and by many others, suggest that a surprisingly high number textbook ideas on disease mechanisms in vision are actually distorted or even wrong. One example is the wrongly assumed early specific damage of red-green pathways in photoreceptor degenerations (Campos et al., 2005; Maia-Lopes et al., 2008) or that structural damage necessarily occurs before functional impairment in glaucoma (Castelo-Branco et al., 2004). These observations may have strong implications for the practice of Medicine (from diagnosis to therapeutical decision), as we will also explore further below.

New 21st century powerful tools of quantitative biology are now repeatedly demonstrating misconceptions driven by biased observers. The dogmatic age where qualitative medicine was largely divorced from basic research will probably soon be mostly over. This does not mean that Medicine should no longer be considered as an Art. It can, although in an increasingly metaphorical sense. Careful reasoning and decision-making abilities will continue to be pivotal in Medicine, but they can now be assisted and endowed by new fascinating techniques.

To understand the impact of medical research, one has to carefully distinguish laboratory and animal experiments from basic studies and clinical trials in humans. How these types of studies can be linked in translational research approaches, is of major relevance, since this integrative approach will influence decisions on how to diagnose earlier disease processes, monitor their progression and decide on therapeutic management.

It is worth emphasizing the necessity to establish these links, in this new era of "evidence based medicine", which can actually be more broadly defined as "evidence-based healthcare".

The questions of *evidence* and *proof* are of paramount importance in science and law, and obviously in any field in which decisions are to be made. This is now consensual within the realm of "evidence-based" medicine. We will discuss here the issue of popperian concept of falsifiability, which means that even medical facts are just provisionally true, because they can always be falsified anytime by a counterexample. Imagine that during a clinical trial one has the statistical null hypothesis that a treatment has no effect on patient outcome. One can however never definitely prove a null effect. This may be contradicted in the future. The converse is also true. Even we prove a beneficial therapeutical effect, this can be falsified in distinct patient groups, where the observed effects cannot be generalized.

A related important concept is the idea of causality. People tend to believe in correlation data, but it is often forgotten that significant and strong correlations do not necessarily imply causal relationships (Castelo-Branco et al., 2000, 2002). We will deal with this issue in a later chapter concerning with brain imaging data. Suffice it to say for the moment that the time has come to directly identify causal relationships using new mathematical techniques. These techniques are particularly prone to deal with the issue of variability in Biomedical Data, in particular Human Data. Variability is not a problem per se if the tools to characterize the sources of such variability are available.

It is worth pointing out that concerns about causality are a longstanding issue. Bradford-Hill (1965) established classical criteria to assess causality, that were in fact developed upon the questions that Robert Koch had raised in the XIXth century to decide whether a given microorganism was causing a disease. The most intuitive criteria were temporality, e.g a cause precedes an effect, and not vice versa; consistency, e.g. the same effect can be observed in distinct settings and strength of association; e.g the stronger the effect the more likely is the causality relationship.

A related criterion is the one of biological gradient, e.g. heavy drinkers are more prone to hepatic cirrhosis. Specificity of the effect and freedom from confounding biases will also reinforce the confidence in a possible causal relationship.
However current criteria would require that one could formulate quantitative approaches to decision about causality (Roebroeck et al., 2005). This is possible for some of the above mentioned criteria, but not for other of the Bradford-Hill criteria, such as the requirement for "plausibility". Many scientists have refuted a link between vaccines and autism (the heated discussion still going on, see Young et al, 2008 and review by Honey, 2008) based on the absence of a clear biological theory linking both, but this is still too subjective. If epidemiological evidence would have found a solid link (which Young et al. argue they have), then this would have superseded the plausibility argument. We will address our own scientific work on the role of evidence in changing prior biomedical perspectives and its implications on this issue. If one holds part of the set of variables constant one can establish partial correlations (see Castelo-Branco et al., 2000) but this simple analysis is unfortunately still not performed as often as it should in biomedical research.
Significance, direction, and magnitude represent the three main facets of correlation analysis. Bivariate correlations, which are correlations between two variables, do not necessarily control for neither antecedent variables nor intervening (mediating) variables. A spurious correlation may arise from failure to control for either of confounding relationships. If bivariate correlations are nondirectional then they are less informative than the directional ones which are called asymmetric.

Medical research has in many instances been contaminated by the lack of appropriate randomization and inappropriate experimental designs. We have addressed before the issue of evidence-based medicine and its relation to translational research and therapeutical

trials (see the short discussion on the failure of the Memantine trial in glaucoma).

An equally important methodological issue is the notion of measurement. What are we really measuring when doing basic and clinical research? Are our measures really addressing the theoretical constructs we were initially aiming at? Later on we will address the concept of motor staging in Parkinson disease and its relation to independent measures of dysfunction that are not necessarily based on motor impairment as assessed by the clinician (see Castelo-Branco et al., 2008, where a measure of fine motor sequence planning and execution is shown not to be accurately predicted by clinical motor stage). Precision of outcome measures and bias avoidance are often disregarded.

We will later on discuss advanced forms of assessment that are independent of the model of the experimenter (see Castelo-Branco et al., 2002, 2007, 2008). In any case, newer approaches using temporal precedence information are exploited to compute Granger causality maps that identify brain voxels that are sources or targets of directed influence for any other selected region-of-interest in the brain (Roebroeck et al., 2005). This Granger Causality based approach to explore directed influences between neuronal populations (effective connectivity) in functional imaging and electrophysiological data, can in principle be applied to any sort of temporal data (such as disease stage related information), provided a sufficient number of points is gathered.

Aside from the power of novel mathematical tools, it is important to have access to genetic information of a given demographic group to understand the pathophysiology of disease models, namely retinal degenerations, which may not generalize to other population groups. This is particularly relevant given our own findings concerning novel several disease related mutations specific for the Portuguese population that lead to distinct patterns of impairment. This is the case concerning both Stargardt and Best diseases, which lead to distinct

phenotypes of photoreceptor degeneration.We have found evidence for widespread retinal dysfunction in patients with Stargardt disease (unlike the more central pattern found in anglosaxonic groups) and morphologically unaffected carrier relatives (yielding evidence for specific novel genetic susceptibility profiles and the role of genetic background in defining pathophysiology). Eighty percent of these were indeed found to be mutation carriers, which corroborates this evidence. Different mutations segregated in the same family, partly explaining phenotypic variability. Quantitative phenotyping and genetic characterization of the carrier state, helped shed new light on subtle genotype-phenotype relationships that can only be identified at a functional level, and most importantly, helped clarifying sources of variability that were previously unexplained (Maia-Lopes et al., 2008).

Concerning Best disease and the spectrum of disease-causing *VMD2* mutations in Iberian BMD patients we have found further evidence supporting a haploinsufficiency theory, since the severity of the phenotype seems highly dependent of bestrophin activity (Maia-Lopes et al., 2008). This would not have been possible without obtaining quantitative topographic maps of retinal function, using multifocal electrophysiology.

Good evidence from a given experimental design requires that a minimum number of subjects have participated and this brings about the issue on how to generalize findings to the general population. Sample size calculations are increasingly being requested in major clinical investigation journals but are still largely omitted in experimental planning. If the effect size is large, then this becomes a minor point, but this is often not the case. Realistic estimates are however in general required at the design stage. Random effects analysis strategies, which enable generalization of results to the general population, are already being widely used in the functional neuroimaging community, but this trend has unfortunately not yet been followed in other fields of medical research.

The issue of sample size and effect sizes in Biomedical Research goes often undiscussed both in animal and human studies and upon requests for clarification one can often find justification such as "it is difficult to find patients fulfilling the inclusion criteria". Such arguments do not serve as an excuse for not accumulating more patients, until a sufficient sample size is attained. This is critical for papers that reach a negative result, and as such, those types of study should be considered inconclusive, because they have low power (defined as the probability that a test will produce a statistically significant result given that a true difference between groups of a certain magnitude exists). It is of crucial importance to consider sample size and power when interpreting statements about "non-significant" results". This is particularly important when non-scientific forces such as time, money or human resources come into play.

On the other way around, a clinically insignificant difference may be statistically significant but then a formal discussion and analysis of effect size is required. A measure of bioequivalence is needed in this case. If the difference between groups is less than this in magnitude, it implies that the groups are effectively equivalent. The issue of clinical significance can only be addressed by planned effect size based analyses.

Among the parameters that have to be pre-specified before a sample size can be determined, planned effect size is indeed the most critical. Unfortunately however, many papers are mostly concerned about Type I errors, and much less so for a Type II Error, which is important in its own right.

All the considerations posited above emphasize a strong role for evidence-based medicine in a pos-genomic era whereby the impact of diseases on population characteristics and the effects of genetic background on phenotypic features do revive the concept of darwinistic medicine.

Dominance in the evolutionary world and its reflection on the human retina and brain: another link to darwinistic medicine

We recently published work showing novel and unsuspected hemispheric lateralization patterns in visual perception (Silva et al., 2008) and memory (Figueiredo et al., 2008), with a clearcut ecological relevance. One of the most discussed matters in Neuroscience is the issue of hemispheric specialization. One can trace this discussion back to the old times of phrenology.

The overwhelming evidence for the differential relevance of each side of the brain to distinct cognitive functions does in represent in fact evidence for darwinian symmetry breaking principles. We propose here that such symmetry breaking rules can be best viewed in the evolutionary context, which also has implications for biomedical evidence. In other words, the ever ongoing fluctuations and distortions of the physical features of our natural world have been basically imprinted in all animal brains, and the human brain is no exception. In fact, one could say that our brain is both metaphorically and also in a more concrete sense a distorted mirror of the world. This leads to patterns of local physiological vulnerability in sensory maps (for instance the early loss of visual sensitivity that we found in the superotemporal quadrant of Williams Syndrome and Glaucoma due to reduced functional redundancy for the tested visual pathways in that location) (see also Mendes et al., 2005).

Aside from the cortex, even the retina weights the features of the formed image in an quite biased way, that strongly deviates from the principles that engineers use to design imaging devices. No one would ever accept a video camera with distinct resolutions across the image. This is exactly what the retina does, even at the very first level of processing of visual processing: the photoreceptors, which underlie nasotemporal and up-down asymmetries (Silva et al., 2008).

These considerations partly explain why our work bridges the gap between evolutionary concepts on eye function and brain function. Disease pathophysiology tells us about possible evolutionary rules and even provides novel clues for rehabilitation approaches. For instance, in macular (central vision) degenerations, the preferred retinal locus for functional readaptation is often located above the central retinal lesion, and our data now provide a physiological explanation for this fact (Silva et al., 2008). A likely mechanism can indeed be baseline functional anisotropies in normal vision that favor the lower visual field (upper retina). These anisotropies have been well documented (Silva et al,. 2008). This might explain why upon Stargardt disease subjects might favor visual field regions with higher functional reserve.

Suness et al., 1996, have accordingly reported that patients with Stargardt disease use peculiar strategies for fixation, perhaps "due to subclinical pathology adjacent to the atrophic regions" (we have recently provided evidence for such subclinical phenotype, Maia-Lopes et al., 2008). These authors reported that patients with Stargardt disease and acquired macular degeneration fixated at a considerable distance from the scotoma (blind region) border, with the dense scotoma far above the fixation site in visual field space (e. g., fixation on top of scotoma in the retina, as also replicated by Messias et al., 2007), which renders plausible the physiological explanation suggested above.

4. Key challenges in translational research: how to make the right step from animal models to clinical applications: the case of amblyopia

Herewith we make the case that a particular model in the Visual Sciences, Amblyopia, a form of developmental visual impairment, is an ideal model for translation of basic findings in animal and human research to clinical applications. It does provide a natural model for studying experience-dependent cortical reorganization and the effects of training and rehabilitation. Substantial fundamental research in this area has been accomplished from mice models to non-human primates and humans. Furthermore, novel retinotopic imaging and neurophysiological techniques to study facilitation and inhibition between concordant/discordant visual representations in humans will allow us to "brain read" the effects of novel interventional approaches on brain plasticity. Our main hypothesis in this area is that both retinotopic (visual field) and nonretinotopic visual representations underlying interocular visual cue combination beyond the primary visual cortex (V1) are involved in the cortical deficit in amblyopia.This testable prediction could potentially be best addressed by studies of changes in brain structural/functional connectivity using novel techniques based on Granger causality mapping (Roebroeck et al., 2005).

The rationale of this discussion is based on the notion that amblyopia is the best model for which non-invasive feasible intervention strategies are available, both in humans and animals. Subject selection can be quite straightforward and causes of amblyopia can be established in a very objective manner.

A lot is already known from animal studies concerning cellular mechanisms for compensation and the impact of changed visual experience on neural architectures. These include decreased thickness of ocular dominance columns, which are the basic functional units in the cortex, decreased number of synapses concerning the suppressed eye and a decline of plasticity that has been largely attributed to the maturation of intracortical inhibition. All these ideas suggest a completely novel approach to rehabilitation. Previous strategies have focused on monocular occlusion of the

good eye, to provide a chance to the deprived eye representations to recover. This approach may improve the physiology of the deprived representations, but does not treat impairment of binocular vision, neither the possibility of persisting interocular suppression. Virtual reality based interventions that target increased interocular integration (even if fine stereopsis cannot be achieved) could be a potentially interesting intervention, although in this particular case it would be difficult to test it in lower mammals.

The initial effects of rehabilitation may not be directly observed by expert human observers but the neuroimaging analysis features that are expected to improve by rehabilitation can be explicitly looked for by machine learning techniques. This would provide an immediate tool to assess the effects of functional training on the brain. Brain reading techniques can be applied to differentiate distinct types of amblyopia (strabismic, anisometropic) and the cortical effects of treatment. We do believe that these clearcut sensory models do represent the best proof of concept of the diagnostic capabilities of these techniques. These approaches can in principle be applied to functional neuroimaging data obtained using exactly the same stimuli and paradigms used for training and rehabilitation.

Comparison with human genetic models of central visual impairment of photororeceptor or ganglion cell origin would potentially allow for the better understanding of the role of visual experience in shaping postnatal development. We do believe that this combination of basic research knowledge with novel mathematical tools to explore brain function and monitor the plastic effects of intervention would represent a groundbreaking approach to the field of diagnosis and neural rehabilitation.

Sensory systems as models for translational research

Visual neuroscience has had a strong influential role on other fields of neuroscience, in particular in which concerns understanding

of normal and pathological function from cellular to systems level. It is probably the best model to study the effects of sensory experience on the development of the nervous system, and has been tremendously influential in helping understand how neural circuits are shaped by experience in early postnatal life. There are extensive studies with animal models, from mice to primates concerning the permanent loss of visual function, including acuity (amblyopia) and anatomical remodeling within primary visual cortex following visual deprivation. It is now an outstanding question to establish how one can take advantage of the available residual plasticity, by means of either novel pharmacological or environmental interventions, in adult life.

Cutting edge approaches in human neuroimaging and neurophysiology, including 21st century approaches in data analysis and modeling, such as "brain reading" methods, are now available to guide novel clinical approaches in neural plasticity (Roebroeck et al, 2005).

We can now precisely map by means of noninvasive brain imaging techniques visual brain areas in humans, study their homology with other mammalian species and as well as their physiology in health and disease (Castelo-Branco et al., 2002, 2006; Schmidt et al., 2006). Studies of visual function and genetics have boosted the understanding of the organization of this sensory modality and namely the factors that influence the development, maintenance, and plasticity of the visual system.

As mentioned above, we have studied, in our previous work, the role of genes vs. environment in determining visual function in genetic neurodevelopmental disorders (Castelo-Branco et al., 2007) and genetic photoreceptor degenerations (Campos et al., 2005, Maia-Lopes et al., 2008). We have indeed found independent retinal and cortical phenotypes in Williams Syndrome, and that the cortex can partially compensate for a defective retinal phenotype (Castelo-Branco et al., 2007). We have also found that some features

of the cortical phenotype are more dependent on environmental richness, since similar genetic backgrounds could lead to very distinct cognitive profiles that were related to cognitive stimulation patterns (Castelo-Branco et al., 2007). We have also recently demonstrated a remarkable plasticity of human cortical systems, by showing adult interhemispheric reorganization of visual memory in patients with medial temporal lobe epilepsy. We could show that with right hippocampal sclerosis, only patients that are able to reorganize their visual function to the contralateral hippocampal network can regain adequate performance (Figueiredo et al., 2008). In other words, memory performance on visual encoding activity led to greater engagement of the left medial temporal lobe, which was associated with higher recognition scores. Interestingly, reorganization of activity also depended on the epileptic seizure frequency, suggesting a role for this clinical parameter in brain plasticity. Our previous neurodevelopmental work, as well as this study on functional reorganization of the medial temporal lobe structures in right hippocampal sclerosis, through transfer of function from the right to the left hemisphere, and the demonstration of an adaptive role for such reorganization mechanism in supporting preserved visual memory, leads us to propose that similar research approaches are even more viable in diseases such as amblyopia, as a well described model of impaired cortical development specifically due to abnormal visual experience, in parallel with a comparative approach in genetic disorders of central vision.

Amblyopia is the most common cause of monocular visual impairment in children and young adults (NEI, 2008). It represents a model of abnormal visual cortical development in the absence of ocular disease. It is believed to be the result of abnormal visual experience in the postnatal period of cortical development. Indeed, disease mechanisms relate to lack of adequate stimulation during a critical or sensitive period in early childhood, whereby certain cortical functions such as sight will never develop properly later on

(Sengpiel, 2005). The neurodevelopmental hallmark of this disorder relates to physiological suppression of deprived monocular visual representations. Abnormal visual experience during this postnatal critical period, as defined by animal studies leads to initially physiologically ineffective synaptic connectivity before anatomical changes are found in nerve terminals (Antonini et al., 1999). The notion of an experience dependent critical period led to the postulate that visual rehabilitation should be most effective early in life in this developmental brain disorder. However, recent evidence has shown that manipulation of visual experience can be effective in humans up to adulthood (Simmers & Gray, 1999; Scheiman et al., 2005, Ostrovsky et al., 2006), challenging the notion of a fixed critical period, and providing the best model to study human plasticity.

We believe that taking advantage of new functional non-invasive tools to study longitudinally the plasticity of visual representations in humans close to a cortical columnar level, could represent the next step in identifying the mechanisms and limits of neural plasticity in the developing and adult visually impaired brain. Data mining approaches will help unravel physiology-guided biomarkers for diagnosis and treatment. Guidance of these research approaches through application of novel model and data driven "brain-reading" techniques to imaging data will provide new effective ways in monitoring visual plasticity and treating amblyopia in humans, by manipulation of visual experience.

Traditional monocular occlusion therapy can take up to 400 hours of treatment in total (Cleary, 2000) leading to poor patient compliance and limited plasticity. Future portable Virtual Reality approaches that focus on effective binocular integration of cues presented to both eyes - which render possible to mimic ecological representations of real world environments - will certainly provide a more focused scenario for fast intensive rehabilitation protocols. We thereby propose that this paradigm should guide future approaches to rehabilitation. Previous strategies have focused on

monocular occlusion of the good eye, to provide a chance to the deprived eye. This approach does not directly treat impairment of binocular vision, and leaves open the possibility of persisting interocular suppression. Focus on an intervention approach that targets increased interocular integration, even if stereopsis cannot be attained, will still allow for concomitant use of signals coming from the two eyes. The neural bases of amblyopic impairment and recovery can certainly be best investigated with structural and functional magnetic resonance imaging (fMRI) of the brain, which allow for fine retinotopic mapping, and visual evoked potentials, using visual stimulation protocols aimed at defining ocular dominance patterns and binocular suppressive/excitatory interactions.

Proving that amblyopia can serve as a model of plasticity will provide a proof of concept to the generation of disease biomarkers to identify visual cortical and retinal disease, and monitor disease progression and intervention at early disease stages. If we are correct, this will probably pave the way for generalization to other disease models. Comparative analysis of visual cortical reorganization and remapping of acute and chronic impairment of central vision (scotomas) in genetic diseases affecting photoreceptor and ganglion cell populations could potentially then benefit from similar strategies.

With novel data driven machine learning approaches, within the scope of the investigation of new tools for rehabilitation of visually impaired patients, one can "brain read" objective patterns of improvement. The same novel "brain reading" techniques can also be used to select patients who have a potential for being treated using rehabilitation techniques.

This proposed model for future biomedical research will require analysis of four dimensional brain imaging data in order to read patterns of normal and abnormal functioning, independently of the models of the observer (scientist or medical doctor). This will

enable the creation of new powerful diagnostic and research tools. Models of brain function and disease can be generated either in a posthoc manner, after cross validation of data driven approaches, or a priori, and the validity of both approaches and their clinical relevance can be established. A priori models of plasticity, based on improved interocular visual cue combination and facilitation, are available but need to be refined in future research. In amblyopia, the good eye can serve as control in all regressions and categorization models.

The major achievement would be to ultimately understand three critical developmental periods that are not mutually exclusive: the late period of normal postnatal visual development, the period during which amblyopia can develop, and the temporal limits, if any, of the period during which it can be successfully treated. Elucidation of mechanisms of functional plasticity will possibly allow extending the critical period, rendering noninvasive therapeutic approaches an effective and realistic strategy.

Evidence from human and animal models

Amblyopia is a frequent early onset visual disorder, with an estimated incidence of 1-4% (data from the National Eye Institute, USA, 2008), starting at early childhood, due to inadequate visual stimulation, in otherwise healthy and properly corrected eyes. It is thereby a brain disorder, given that it is characterized by reduced visual acuity not of optical origin or due to any eye disease. It does provide a natural model for studying experience-dependent cortical reorganization and the effects of training and rehabilitation. Subjects use only one eye at a time for vision in order to avoid double images (diplopia). Since they use always the same eye, the other develops amblyopia, presumably because the cortical networks connected to this suppressed eye do not develop normally. An open question remains however: how much of this suppression is due to missing anatomical

connections, and how much is due to functional mechanisms? Interestingly, in cases where subjects use the two eyes for vision, by alternating between them, no amblyopia develops. Otherwise, the visual system shows signs of developmental delay, which ultimately lead to an arrest in further maturation. It remains to be established whether such arrest can be overcome by manipulations of visual experience.

Understanding how plasticity changes with age carries far reaching impact beyond visual neuroscience. New therapeutic approaches to developmental disorders will rely on fundamental approaches to understand how neural circuits are sculpted by experience during 'critical periods' (CP) of plasticity in early postnatal life.

Residual plasticity found in adults (Figueiredo et al., 2008) could help shape new strategies for recovery of neural circuits after injury in adulthood. Most rodent models have focused on the classical full deprivation amblyopia, but other animal models, in particular non-human primates, have allowed to study other clinically relevant forms of adult plasticity as well as perceptual learning or retinal scotoma models, which may share certain aspects of critical period plasticity. Remarkably, the later studies (Schoups et al., 2001) have shown that task-related individual motivation is critical for perceptual learning to occur. Prior and multiple manipulations of visual experience, may further set an appropriate ground for subsequent adaptive plastic changes in the visual brain. Accordingly, prior experience, such as an earlier ocular dominance (OD) induced shift (by monocular occlusion), may interestingly set a sub-threshold scaffold that optimizes the chances for subsequent adult plasticity (Hofer et al., 2006; Morishita & Hensch, 2008).

Development and plasticity of the mammalian visual cortex, from mice to humans may well depend on the balance between excitatory and inhibitory mechanisms (Murphy et al., 2005; Maya Vetencourt et al., 2008). Accordingly, GABAAα1 receptors, as well as presynaptic GAD65, develop slowly in human V1, consistent

with our prolonged sensitivity to amblyopia. In animals, chronic Fluoxetine (a common antidepressive) administration and enriched environments both reduce GABAergic transmission, and their rescue effect can be prevented by enhancing inhibition with diazepam. Indeed, the recent study of Maya Vetencourt and colleagues (2008) in rodents showed that chronic administration (one month) of Fluoxetine reactivates ocular dominance plasticity in adult rats and promotes recovery from amblyopia both electrophysiologically and behaviorally. These effects were accompanied by reduced inhibition and increased expression of brain-derived neurotrophic factor (BDNF).

Given that postnatal development is experience dependent, non-invasive strategies for recovery from amblyopia should in principle be effective. Animal studies have shown that environmental enrichment is an effective approach, as well as dark exposure coupled with reverse suture or binocular experience in adults (Sale et al., 2007; He et al., 2007). Interestingly, greater functional recovery of visual evoked potential (VEP) acuity is found in primary visual cortex (V1) as compared to overall behavior, suggesting again that high level recovery from impairment is important as well. Reduction of GABAergic inhibition seems to be critical in both interventions. In any case experience-dependent response enhancement in the visual cortex has now become a strong theme is this field (Frenkel et al., 2006). By the same token, deprivation-induced synaptic depression in visual cortex (long term depression) is tightly linked to the loss of visual responsiveness after monocular deprivation (Crozier et al., 2007).

As stated above, the relative role of molecular mechanisms and of visual experience in establishing topographical maps have been intensively documented in animal models (both experimentally induced and naturally occurring), including non-human primates of amblyopia (Hubel and Wiesel, 1977; Kiorpes, 2006). In fact,

development of the monkey visual system can be roughly equated to the human one, whereby weeks in the former are equivalent to months in the later (Kiorpes, 2006). Neurophysiological studies show a shift in ocular dominance of neural activity towards the unaffected eye (especially with severe deprivation), massive reduction in binocular neurons, and suppression of cortical activity elicited by the amblyopic eye with simultaneous stimulation of the non-deprived eye (Freeman and Ohzawa, 1988). However, the cortical deficit in the primary visual area (V1) does not fully account for the behaviorally assessed visual impairment (Kiorpes, 2006), suggesting loss of connectivity to and/or decreased activation of high–level extrastriate cortex.

Noninvasive neuroimaging technologies have provided new insights about how the human visual cortex is organized and confirmed some of the homologies previously established by anatomical and physiological studies in animals. These methods have increased the understanding of changes in the organization of the brain related to visual impairment, and respective implications for device development relevant to rehabilitative training. Amblyopia is a suitable model to investigate the physiological basis of rehabilitation for the following reasons: the cortical deficit stems only from inadequate visual stimulation of a disease-free brain; patient selection and subtype classification is straightforward; treatment is non-invasive and can be objectively defined; its effectiveness can be reliably measured and the sound eye provides intra-subject experimental control.

Interestingly, abnormal developmental patterns seem to be dependent on the type of pathological visual experience that is imposed: when it results from optical misalignment (as in strabismus) visual cortical populations split bimodally into monocularly responding populations. The amblyopic eye may therefore maintain substantial representations. This is often not the case when the cause of amblyopia is asymmetric optical quality of the eye (anisometropia), where most cortical neurons are monocular but

with very few representing the amblyopic eye (Kiorpes, 2006).
Many outstanding questions remain to be solved, such as whether undersampling due to reduced number of cells does occur as a main mechanism, or whether abnormal lateral interactions and/or disruption of cortical topography are alternative main playing factors. Elucidation of these fundamental questions is critical to the design of effective rehabilitation strategies.
In the last decade, initial brain imaging studies of human amblyopia, using predominantly functional magnetic resonance imaging and low-level stimuli as well as neurophysiological recordings (Anderson et al., 1999), have confirmed decreased activation of V1 by the affected eye (Barnes et al., 2001). Upon stimulation of the amblyopic eye, incremental deficits in higher-order retinotopic areas have also been demonstrated (Muckli et al., 2006). Furthermore, even high level ventral stream areas concerned with processing of objects such as faces as opposed to buildings, indicated selective impairment of visual center-field biased high-level extrastriate visual regions (Lerner et al., 2006). However, it is still remains to be determined how extensive is the loss of extrastriate function, and whether it can be explained by impaired activation of V1 per se, or by reduced functional connectivity to upstream areas, which in normal subjects have been shown to be modulated by reported visibility of the stimulus (Haynes and Rees, 2005).
Anatomical MRI analysis of children and adult amblyopes' brains demonstrated decreased cortical volume in striate and extrastriate visual cortex, establishing a structural correlate for the visual deficit (Mendola et al., 2005). Indeed, reduced gray matter volume has been observed in several regions of the visual cortex, including V1 (Mendola et al., 2005, Xia et al., 2007). These studies have also revealed regional differences in extrastriate regions of the parietal–occipital and ventral temporal cortex and even in high order visual regions. This suggests that low-level visual deprivation has further impact on object-related visual processing. The role of high-level

visual processing in the overall amblyopic dysfunction remains however unresolved. The consensus from primate studies is that the physiological deficits in area V1 are not sufficient to explain the full range of perceptual deficits (for a review, see Kiorpes, 2006).
Animal models have nevertheless proven that V1 neurons have abnormal responses (Kiorpes et al., 1998; Schmidt et al., 2004) but the same studies and previous imaging findings in humans suggest that the abnormalities found in V1 are likely to be not fully explanatory of the phenotype (Demer et al., 1988; Imamura et al., 1997; Barnes et al., 2001; Muckli et al, 2006). Incremental evidence does therefore support the notion that damage of cortical maps goes beyond primary visual cortex (V1). Indeed, contrast sensitivity and acuity (measured as spatial frequency tuning) measured at the single cell level are functional measures that underestimate the behavioral (psychophysical) deficit, as measured by interocular ratios of neural response rates and performance (Kiorpes, 2006). Muckli et al. (2006) did accordingly explicitly suggest that the neural effect is amplified in V2 and higher cortical areas, which has obvious implications for future rehabilitation approaches.
Neurophysiological studies of visual function in humans do also support the notion that V1 is affected, in particular in which concerns its role in form vision (Andersen et al., 1999), and evidence from Kubová et al. (1996) substantiates the notion that the magnocelullar and motion sensitive pathways are relatively preserved.
In sum, cortical deprivation in human amblyopia is selective for faces (representative of central vision object processing networks) relative to buildings (scene processing networks) in high-order object areas (Lerner et al., 2003) extending the previous findings demonstrating early impairment in primary visual cortex (Barnes, Hess, Dumoulin, Achtman, & Pike, 2001). The former finding has been suggested to correlate with a putative distinction of center/periphery organization in high-order object areas, whereby cortical regions associated with face processing show a relatively

higher dependence on central visual field areas, in comparison to landscape/building related areas, which show a relatively higher emphasis on peripheral visual representations.

Mechanisms of disease differ across types of amblyopia: the need for a taxonomy

Amblyopia due to different causes may lead to distinct patterns of reorganization that may need differential types of intervention. Strabismic amblyopia has been related to a maladaptive differentiation of the ocular dominance columns. Enduring types of changes (such as permanent re-wiring of the neurons) do occur as well as more transient, reversible types of response such as interocular suppression (Fries et al., 1997; Schroeder et al., 2002). The cause of such abnormal brain development in infancy and early childhood is inadequate visual stimulation due to strabismus or refractive error leading to a sustained decrease in the visual acuity of an optically normal and properly corrected eye. Deprivation amblyopia, widely used in animal models, is less common in humans, and may be related to extreme anisometropia. The influence of the amblyopic eye in driving cortical activity is suppressed by input from the fellow eye, leading to impaired development of the cortical networks connected to the suppressed eye. This results in functional impairment such as decreased visual acuity and contrast sensitivity in the representation corresponding to the dominated eye (McKee et al., 2003; Wallace et al., 2006).
Overall, the postnatal developmental (Kiorpes et al., 1998; Wiesel, 1982) and psychophysical (Hess et al., 1999) aspects of amblyopia have been well characterized and the functional anatomy of loss of connectivity of the affected monocular representations has been well established (Horton and Hocking, 1998; Wiesel, 1982). The findings in non-human primate models suggest that amblyopia has a neural basis in the form of a massive reduction in binocular neurons, and in some cases, a shift in ocular dominance of neural

activity toward the unaffected eye, which seems to hold true also for humans (Goodyear et al., 2002).
The focus of biomedical interest here is the increasing awareness that brain plasticity may exist beyond the critical period in children aged 9-17 years and in adults, and can result in vision restitution following more intense or appropriate amblyopia treatments. The impact of visual deprivation is greatest during the postnatal critical period and the potential for recovery is thought to decline irreversibly thereafter but the demonstration that complete visual deprivation through dark exposure restores plasticity in adult rats is inspiring in this respect (He et al,. 2007).The loss of visual acuity is also reversed if dark exposure precedes removal of the occlusion which led these authors to suggest a potential use for dark exposure in the treatment of adult amblyopia.

Taking these points into consideration, we believe that it is time now to clarify the role in visual dysfunction in objectively defined subtypes of amblyopia, to determine how extensive is the loss of extrastriate function, or whether it can be explained by the impaired V1 input, and to clarify which patterns of psychophysical loss can be explained by striate vs. extrastriate deficits, and their relevance to interventional approaches.

Taxonomy and epidemiology in amblyopia: the scientific importance of defining separable clinical entities that may need distinct intervention types.

One of the main issues in translational and clinical research is whether the variability associated with clinical entities is adequately defined and if subclinical entities have been properly defined and characterized. We do believe the best approach to address these questions is to use both model and data-driven approaches (see below discussion on “brain-reading”). Herewith we summarize

evidence for functional separation of amblyopia subtypes that will be relevant for investigation of disease mechanisms and intervention approaches.

In amblyopia it has been possible to objectively define two separate clinical entities in animal and human models: strabismic and anisometropic (Levi, 2005, 2006). Animal models cannot easily differentiate between these two clinical entities (Li et al., 2007a,b). Strabismus is mainly characterized by abnormal binocular interaction whereas aspects related to deprivation of form vision dominate in anisometropia. Both aspects are dramatically combined in full monocular deprivation amblyopia. Classification issues may be complicated by microstrabismus, yielding mixed phenotypes, leading to the necessity of discovering better classification approaches that can illuminate treatment strategies.

One should not consider amblyopia as simply a disease that leads to a sort of blurred vision. Strabismic amblyopes tend perceive edges as sharp, instead of the expectable blurred perception. Images don't appear devoid of contrast, particularly in anisometropic amblyopes, once they are above threshold. Spatial alignment deficits tend to be scale independent and in anisometropic amblyopes these positional deficits are gone once contrast sensitivity deficits are accounted for.

In strabismic amblyopes contrast sensitivity deficits at different spatial frequencies are localized in the fovea, whereas in anisometropics they are more evenly distributed.

In strabismic amblyopia, contour integration deficits seem not to be due to abnormal lateral interactions but rather a positional deficit (Hess et al., 1997). This hypothesis is however controversial. Kovács et al. (2000) have indeed argued that an abnormal pattern of long-range connectivity between spatial filters or a loss of such connectivity appears to be the primary source of contour integration deficits in amblyopia and strabismus. In any case, anisometropic amblyopia, unlike strabismic amblyopia, has little

or no positional uncertainty once the initial filtering loss has been taken into account (Hess and Demanins, 1998).

The importance of using both model and data driven analysis approaches of brain imaging data on understanding the pathophysiology of binocular disease and the results of rehabilitation approaches.

"Brain reading" machine learning techniques take into account the full spatial pattern of brain activity or structural parameters measured simultaneously at many locations (Haynes et al. 2005a,b; and Rees, 2006; De Martino et al., 2007) allowing the detection of subtle, non-strictly localized effects that may remain invisible to the conventional analysis with univariate statistical methods. In normal observers, they have been successful in predicting which stimulus is perceived in binocular rivalry paradigms, by detecting subtle patterns of decreased activity in regions of the lateral geniculate nucleus and visual cortex driven by the eye being shown the suppressed stimulus (Haynes et al., 2005a). Furthermore, they made it possible to detect brain activity associated with the presentation of masked, "invisible" stimuli (Yang et al., 2003).
We think that their use can be extended to 'decode' the functional/disease state of subjects in order to develop tools to answer fundamental questions regarding brain development and plasticity in amblyopia and to provide objective, clinically relevant, prognostic and therapeutic outcome measures. We do believe that this scenario provides a close link between clinical practice and research, and has a potential to help improve diagnosis and treatment of amblyopia. This strategy will naturally lend itself to clinical trial approaches, as demonstrated by recent studies (Stewart et al., 2005, 2007; Shotton and Elliott, 2008) that modeled factors influencing outcome with treatment for amblyopia. These are occlusion dose (the rate of delivery of eye occlusion and cumulative dose worn), the initial

severity of the amblyopia, binocular vision status, fixation of the amblyopic eye, and the age of the subject at the start of treatment (Stewart et al., 2005).

The motivation of these studies is based on the notion that most eye care practitioners believe that there is an age beyond which attempting to treat amblyopia is useless. In other words many do still wrongly believe that response to treatment is poor when attempted after eight years of age. This is true in spite of the recent demonstration that visual acuity improvement occurring during amblyopia treatment is sustained in most children aged 7 to 12 years for at least 1 year after discontinuing treatment other than spectacle wear (Hertle et al., 2007). Many aspects of these unexpectedly long lasting plasticity remain however to be explored. Can temporal aspects of the rehabilitation protocol affect the limits of plasticity? In patients 13 to 17 years, prescribing occluding patches 2 to 6 hours per day with near visual activities may improve visual acuity when amblyopia has not been previously treated, but appears to be of little benefit if amblyopia was previously treated with the same patching method. Inspiration brought about by the animal models, should foster explorations of the role of the nature of previous manipulations of visual experience in subsequent functional outcome.

Advantages of Brain Reading Approaches

A major advantage of the novel brain reading methods (De Martino et al., 2007; Formisano et al., 2008) compared to the conventional univariate statistical analysis is their increased sensitivity in discriminating disease states. Statistical pattern recognition exploits and integrates the information available at many spatial locations, thus potentially allowing the detection of disease states and their response to therapy, as well as the identification of novel biomarkers. But what is brain reading after all? We need to define training sets and validation datasets.

We define A (training set), A' (validation dataset) as two data sets from a generic imaging experiment, and t, t' as the labels (biological categories) that describe the visual, functional or structural parameter associated with A and A'
We can then let available ("learn") the relation between data A (training dataset) and label t in order to predict the unseen labels t' from the new dataset A' (test dataset). During this predictive step, the algorithm is an effective decoder of pattern A' into the corresponding stimulus or clinical state of the subject (as described by t'). This defines our concept, 'brain reading' of the clinical state or category by learning.
The main advantages of these methods are that they are amenable to proof of concept and to "blind" assessment of improvement after treatment. Afterwards, this procedure can be applied to other diseases such as autism and degenerative disorders of the retina and brain. These methods allow for determination of discrete (classification) or continuous (regression) values of clinical interest. The definition of fingerprints for low level visual disorders such as amblyopia, and retinal degenerations will allow for identification and monitoring of functional improvement after rehabilitation. Furthermore one can potentially test models of suppression/fusion of visual representations with these techniques.
Machine learning is receiving great interest in human neuroimaging research. The possibility to employ multivariate statistical approaches in order to learn and decode mental states from recordings of brain activity has opened many new possibilities for brain researchers. These increased possibilities, however, have been paralleled by a substantial increase in computational demands, which are associated with the complex learning schemes and models. So far, machine learning approaches have been applied to the study of healthy volunteers. A promising application of these methods is in the detection of subtle anatomical and/or functional abnormalities in the brain of various patient populations (e.g. Alzheimer disease, dementia, autism). These clinical applications require further

methodological developments which are described below.
Each disease state will be associated with distinct spatial pattern of responses, and these patterns can be used to 'decode' the functional/disease state of the subjects.
Outstanding questions that can be solved with these novel methods in Visual Neuroscience are as follows:

- multivoxel pattern analysis can be done in each retinotopically defined Visual Region, to investigate in an unbiased manner which one is really critically affected in amblyopia. The most outstanding question to solve is whether primary visual cortex (V1) is the most critically affected region and what is the role of high level regions.
- "brain reading" techniques applied to central and peripheral visual field representations are suitable to identify patterns of visual impairment in retinal disorders. Importantly, information on the disease state is entailed not only in the maximally affected regions, but also in spatially wide and distributed pattern of non maximal responses in the entire visual field. This type of approach prevents that one only looks at the 'tip of the iceberg' of biomedical data, and improves the guidance of rehabilitation techniques.

Comparison with genetic models of central visual impairment

Although causes of amblyopia such as strabismus may be constrained by genetic factors (Parikh et al., 2004), amblyopia per se is a consequence of impaired visual experience. It may be interesting to compare this developmental model of abnormal sensory experience, with cortical reorganization that occurs after genetically determined damage to the central retina, causing localized regions of blindness (scotomas). The future is promising for basic studies of visual plasticity in genetic models of macular degeneration (MD), the leading cause of visual impairment in the

developed world. Since these diseases often involve foveal vision they do severely disrupt everyday tasks requiring analysis of spatial detail. There is an ongoing debate on whether this deprived cortex simply becomes inactive (Smirnakis et al., 2005) or it undergoes functional reorganization (Baker et al., 2005). A central question relates to whether the cortex representing blind regions now takes over other functions. Explorations of large-scale reorganization of visual processing in genetic central vision degenerations of different causes and temporal windows for plasticity will possibly also boost our basic understanding of the function and plasticity of the visual system. Our previous experience in studying spatial aspects of visual function in these and other neurological disease models (Mendes et al., 2005; Silva et al., 2005, 2008; Campos et al., 2005; Castelo-Branco et al., 2004, 2006, 2007; Maia-Lopes et al., 2008, Kozak & Castelo-Branco, 2008) suggests that distinct plasticity mechanisms are at work depending on the type of injury and its natural history.

The novel proposed framework effort will also be helpful to develop new strategies for rehabilitation of MD subjects grounded on the dynamic role of cortical circuitry in learning and repair after injury to the nervous system. According to the heated controversy on whether the primate primary visual cortex (V1) can undergo large-scale reorganization within a few months after retinal lesioning (Smirnakis et al., 2005), we do believe that a lot has yet to be learned on the experience-dependent limits for reorganization in the months following retinal injury and/or beginning of rehabilitation.

Implications for treatment and rehabilitation: a new approach

Pharmacological neuromodulators (L-Dopa and Citicoline) have been used in the treatment of amblyopia, and there is some recent promising evidence from animal models with the use of fluoxetine (see above) but the best evidence in humans so far lies on

informed manipulation of visual experience, either by penalization or occlusion of the dominant eye. Treatment outcome variables (central and peripheral visual acuity, contrast sensitivity, vernier acuity) are critical in this respect. Compliance and daily dose of manipulation of visual experience are also relevant aspects in this domain, and should be objectively defined in the future.

Based on the gathered knowledge on mechanisms of suppression and fusion in normal and amblyopic individuals, we propose that new rehabilitation approaches can be developed that depart from the common strategy (Stewart et al., 2005, 2007; Shotton and Elliott, 2008) of employing employ refractive correction and relief of suppression by blocking the input from the non-amblyopic eye, e.g. by patching. Our proposal is to complement it with an active visual stimulation protocol. We do believe that the focus should be on reinstatement of binocular vision by imposing an active training program that focuses on the use of binocular integration cues (regardless of whether fine stereopsis can be fully achieved or not). In spite of the recent highlights on the demonstration of treatment efficacy in adolescents and adults, we do believe that the full potential of experience-dependent cortical reorganization beyond the commonly recognized critical "sensitive period" for neuronal plasticity remains largely unexplored.

5. New insights into genotype-phenotype correlations in genetic visual disorders, from the photoreceptor to the cortical level

Most of the classical efforts to understand disease mechanisms have mainly focused on the genotypic side, with a few exceptions. In our work, we have combined emerging new technologies, including quantitative psychophysics and electrophysiology, to assess genotype–phenotype relationships. This allowed us to perform model driven analysis in order to understand the complexity of such relations.

We could establish methodology to study psychophysical performance in parallel with eye movement recordings. This enabled the characterization of retinotopic psychophysical sensitivity maps in normal and diseased subjects, using advanced techniques such as microperimetry, in which the stimulus is directly projected in the retina, by means of a fundus camera and under continuous monitoring of eye position patterns. This enabled the study of visual plasticity in patients that had endured early damage of the visual cortex. We have also established functional magnetic resonance imaging retinotopic mapping strategies.

To probe Genotype-Phenotype relationships in clinical models of retinal degeneration and neurodevelopmental models of retinocortical dysfunction, we have developed a functional battery that was used for psychophysical phenotyping to study early visual processing in the retina and the cortex.

A better understanding of disease mechanisms was achieved by correlating structure and function, by combining topographical psychophysical data with global and multifocal electrophysiological measures. These measures enabled the separation of models of ganglion cell degeneration (ocular hypertension and glaucoma) from models of photoreceptor degeneration. This separation was further achieved with structural measures, such as in vivo biopsy of the retina (using optic coherence tomography) to asses the integrity of outer and inner cellular layers and the ganglion cell nerve fiber layer. We have also performed 3D optic disk reconstruction to perform structure-function correlations in glaucoma and the

previously described genetic neurodevelopmental disorder (Williams Syndrome). Functional measures were focused on the responses the parvo (red-green contrast and spatial detail sensitive), konio (blue-yellow) and magnocellular (achromatic contrast, and temporal modulation sensitivite, which is important for cortical motion processing) systems.
Visualization and morphometric techniques such as the ones used to study the 3D shape of the optic disk, both in healthy and diseased eyes, allowed to correlate structural markers, such as the retinal nerve ganglion cell fiber layer and functional markers of sensory performance.

The importance of having access to global genetic information to understand the pathophysiology of retinal degenerations

It is widely believed that new mutations are continuously being generated in all species, including humans. This concept is consistent with our own findings that we have found several disease related mutations specific for the Portuguese population. This is true concerning both Stargardt (Maia-Lopes et al., 2008) and Best (Maia-Lopes et al., 2008) diseases, which lead to distinct phenotypes of photoreceptor degeneration.

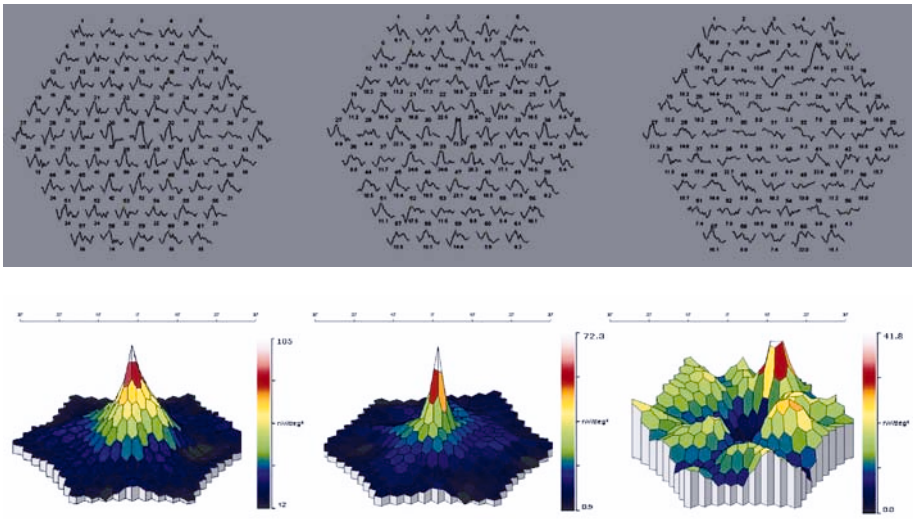

Figure 1

Multifocal electroretinograms (mfERG) obtained from a representative control subject (left,) a clinically silent relative (who turned out to be a novel mutation carrier- middle) and a patient with Stargardt disease (right). Multifocal electrophysiological maps (average curves extracted by means of reverse correlation) are encoded by color coded amplitude maps). For each stimulus hexagon, the peak amplitude is defined as the difference between N1 and P1 (peak negative and positive deflections).

It is also thought that evolution by natural selection is driven by the continuous generation of adaptive mutations. These ideas raise the question on how to define adaptive and maladaptive mutations. Here we question the definition of deleterious mutation in an absolute sense. Our work has focused on the concept of maladaptive disease-causing mutations. We show that mutations that have a strong negative impact in a given genetic background can act as mere neutral variations even in individuals of the same family. Our studies show that one can detect retinal functional impairment in genetically susceptible individuals, without apparent structural evidence of disease. Stargardt macular dystrophy (STGD), accounts

for 7% of all retinal dystrophies and affects about 1 in 10,000 people, with a typical onset during childhood and a predominantly autosomal recessive inheritance. The *ABCA4* gene encodes a retina-specific ATP-binding cassette (ABC) transporter protein expressed in cones and rods and is involved in the all-trans-retinal transport cycle. All-trans-retinal is generated by activation of opsins (Sun et al., 1999) and abnormal turnover leads to photoreceptor degeneration and accumulation of lipofuscin.

ABCA4 gene mutation detection is a challenging task because this is one of the most polymorphic human genes. Although we were initially quite surprised with the identified spectrum of novel *ABCA4* mutations in Portuguese Stargardt disease (Stargardt/*fundus flavimaculatus* disease (STGD/FFM; MIM# 248200) patients, we did afterwards find that these were associated with novel phenotypic features. We have indeed found evidence for widespread retinal dysfunction in patients with Stargardt disease and morphologically unaffected carrier relatives. Eighty percent of these were found to be mutation carriers with a preclinical phenotype. In brief, we found that contrast sensitivity functions are impaired in STGD patients for distinct spatial/temporal frequencies, which in addition to the color vision deficits, suggests dual impairment of magno/parvocellular pathways. Surprisingly, STGD morphologically unaffected carriers did show extended patterns of psychophysical dysfunction, which are mirrored by abnormal multifocal electrophysiological responses.

Establishment of genotype-phenotype correlations in the *ABCA4* gene has further proven to be of great clinical research relevance, in our extensive screen of all 50 exons of the *ABCA4* gene in a cohort of 27 Stargardt (STGD) patients. We were able to identify the presence of 22 putatively pathogenic alterations, four of which were novel. Among the additionally identified twenty-three polymorphisms we have found four novel intronic sequence variants. This pattern of *ABCA4* mutations in Portuguese STGD patients provides further evidence for large differences in specific mutation frequency across populations.

One of the difficulties we had to face is that high allelic heterogeneity exists within the 50 exons of *ABCA4* gene which makes it difficult to predict the disease-causing variants.The STGD retinal phenotype may therefore be explained by different combinations of *ABCA4* mutations and severity of the mutant allele(s).

This same gene has also been implicated in other retinal diseases such cone-rod dystrophy (MIM# 604116) and retinitis pigmentosa (MIM# 601718). Furthermore, an increased predisposition to age-related macular degeneration (MIM# 153800) has been reported. Our genotype-phenotype correlation study included molecular screening of the gene *ABCA4* in 21 Portuguese families comprising the above mentioned 27 STGD patients (which involved screening the *50* exons of the ABCA4 gene screened with appropriate primers through a combination of genotyping microarray, dHPLC and direct sequencing). We were able to detect eighteen previously reported mutations and four novel disease-associated variants (Maia-Lopes et al., 2008).

In brief, a total of 36 mutant alleles (out of the 54 tested) were identified, with 12/21 families (57%), bearing two mutant alleles and 4/21 (19%) of the families yielding only one discovered mutant allele. Five families (24%) yielded no identified mutation. The obtained detection rate of 67% is actually quite high when comparing to previous surveys (45-60%, for details see Maia-Lopes, 2008 and references therein). Although most of the disease related alleles carried missense mutations (27/36 - 72.7%), frameshift variants (7/36; 19.4%), nonsense mutations (3/36; 8.3%) and one splicing sequence change (2.7%) were also identified. Interestingly, four disease related alleles were found to be double mutants in three families, two of them carried by one single STGD patient.

The above mentioned molecular characterization in Stargardt Disease did therefore provide novel clues on how to redefine a genotype. One the identified mutations was the missense variant G1961E (6.7%) that even in the heterozygous state, has been

significantly associated with age-related macular degeneration (AMD). One missense mutation involving an uncharged amino acid (L11P) in a conserved domain that has been found in FFM (fundus flavimaculatus) patients was detected.
Several other sequence changes that have been significantly correlated to the STGD phenotype (M1V, N96D, R290W, L2027F, R2030Q, V2050L, 3211insGT and IVS40+5) were also identified. Furthermore, 2 mutations were found for the first time (M1T, 4034delAC) and were not present in 102 control chromosomes but in their respective STGD affected members. Both novel mutations might induce a severe phenotype since similar variations in the same codons (missense M1V and insertion 4035insCA) are thought to be STGD and CRD (cone rod dystrophy) associated, respectively. Overall, 5 mutations might be interpreted as null mutations: the missense changes affecting the initiation codon (M1V and M1T), 2 frameshifts alterations (3211insGT, 4034delAC) and 1 splice variant (IVS40+5). Only 1 subject (MCA) was found to carry a complex allele. No *ABCA4* mutation was detected in 6 out of 30 studied alleles (20%) from relatives belonging to 3 STGD families. Nevertheless, in 2 of these families, no *ABCA4* mutation could be so far identified in the affected family member as well. Additionally, to discard that peripherin/RDS gene mutations were involved in such STGD families with no ABCA4 mutation identified, we assessed mutation analyses of the former gene by direct sequencing of those families and again no causal sequence change was identified.

As expected, the great majority of the mutations detected have been reported as STGD associated variants. Accordingly, the most prevalent disease-associated variant was the missense mutation L11P, (11% (4/36) of the disease chromosomes and detected in 19% families (4/21). It is worth pointing out that even in geographically close Spain, L11P frequency is significantly lower (<1%) in macular degenerations associated with *ABCA4* (Paloma

et al., 2001). This missense substitution has been reported in other retinal photoreceptor degeneration such as *fundus flavimaculatus* (an associated clinical entity) and in autosomal recessive conerod dystrophy (arCRD, associated with a quite severe phenotype) (Rozet et al., 1998) and involves a conserved nonpolar amino acid residues that is located in the intracytoplasmic domain of the ABCA4 protein. The G1961E mutation has been associated with Age-related macular disease (AMD), thereby providing a link as a genetic susceptibility factor to an important degenerative and blindness causing disease of developed countries. It is one of the most frequently found alteration in Caucasians STGD patients and was found in 9.5% of our patients which is a similar frequency to South European populations (Jaakson et al., 2003).

Concerning our four newly described null mutations, the c.1C>T transition at the initiation codon (M1T) was found in the homozygous state in one STG, which provided a unique opportunity for further insights into genotype-genotype correlations. Our double null mutant patient had an early onset and showed moderate central fundus changes and accelerated visual within two years after diagnosis. Interestingly, two null mutant alleles were identified in patients from two families, and in spite of the severe phenotype presented by those patients; they were still diagnosed with STGD disease although according to a widely accepted model, the combination of two null alleles should account for a more severe phenotype as retinitis pigmentosa (RP) or cone-rod dystrophy (CRD) (Lorenz et al., 2005). These patients had short disease progression, and we predict that they will probably evolve to a more severe type of retinal impairment such as the one found in CRD.

One patient was a compound heterozygous with V931M and a new nonsense mutation at exon 33 (E1574X). The former mutation may have a severe impact on ABCA4 protein function, eliminating both basal and retinal-stimulated ATPase activity (Sun et al., 2000). The novel nonsense mutation, a G>T transversion leading to E1574X, leads

to protein truncation before a functional domain that is believed to diminish ATP hydrolysis by functional domain NBD-1, without altering the basal ATPase activity.
The individual and group quantitative phenotype identified were thereby consistent with the overall severity predicted by the genotype (Maia-Lopes et al., 2008).

Phenotype-genotype correlations in Best disease

The gene *VMD2/hBEST1* encodes the transmembrane protein named bestrophin which is involved in Best macular dystrophy (BMD, MIM# 153700),) which is an autosomal dominant retinal degeneration with a typical juvenile-onset. Bestrophin represents a novel family of chloride channels sensitive to intracellular calcium. Around 100 distinct *VMD2* missense mutations distributed across the highly conserved N-terminal half of the protein, have been reported.
We have correlated phenotypic assessment measures with genotypic data in this monogenic autosomal dominant photoreceptor degeneration, to better understand disease pathophysiology. Our data is consistent with the haploinsufficiency model, whereby higher expression dosages are required for the larger number of photoreceptors existing in the central retina.
Electrophysiological measures across the visual field revealed a loss of the normal central dominance of the waveform N1 (mainly photoreceptor) and P1 (postphotoreceptoral) amplitude components. Losses gradually diminished for large visual eccentricities demonstrating a predominant macular impairment. However the fact that peripheral responses were also significantly reduced in a gradual manner is consistent with the above mentioned haploinsufficiency model. Central and peripheral impairment was also observed in our perimetric contrast sensitivity task that preferentially activates the parvocelullar system. In conclusion,

have found evidence for both central and peripheral impairment in Best disease, as assessed by electrophysiological and psychophysical methods. Interestingly, parvocellular function, as measured by our contrast sensitivity task, showed a stronger pattern of impairment than the one observed with electrophysiological measures.

VMD2 and RDS/Peripherin (to exclude other causes genetic causes for the phenotype, producing phenocopies) gene mutations in patients with vitelliform macular dystrophies, were screened in order to understand the relative role of mutations in these genes to the onset and other clinical features of the vitelliform macular dystrophies.

Eight Portuguese BMD families (17 patients) and 8 AVMD (adult onset) families have been studied by means of dHPLC screening for the 11 exons of VMD2 gene and the 3 exons of RDS/Peripherin gene, followed by direct sequencing.

Concerning the adult form (AVMD, n = 8) we have not found any causal mutation in VMD2 and RDS/peripherin genes, suggesting that this is a distinct clinical entity. Indeed, AVMD patients had impaired in mfERG responses, amplitudes being however much more preserved than in BMD patients.

We have identified causative VMD2 mutations from all 11 exons of VMD2 from 31 BMD patients in 8 families (73%). Among the eighteen sequence changes that were detected, 7 were disease-associated mutations (all were missense and located in exons 2, 3, 5, 6, and 8, five of which were novel and two have been previously been reported) and 11 polymorphisms. Criteria to classify novel sequence alterations as disease-associated variants included segregation within the family, location within 'hotspot' regions and degree of conservation in the bestrophin-related RFP family members. A novel amino acid change (Val9Glu) was detected in all eight affected members from a Portuguese BMD family but neither in the eight unaffected family members investigated, nor in the 102 control chromosomes. Since this novel T>A transversion at nucleotide 26 involves amino acid residues

of highly different nature - valine (nonpolar, neutral) and glutamic acid (polar) we predicted a relatively severe phenotype for this commonly altered amino acid residue in BMD patients. Two other different (with more similar residues) amino acid substitutions in this residue (V9M and V9A) were previously reported in multiple BMD families. According to the expected from mutations affecting the function of transmembrane (TM) domains and given that this sequence alteration is believed to be located in the first defined hotspot region prior to the first putative TM1 of bestrophin, we found severe phenotypes. All BMD patients with Val9Glu mutation had an abnormal EOG (electroculogram- an index of the function of retinal pigment epithelium), a severe macular degeneration and color impairment and the age of onset ranged from 5 to 15 years (with the exception of one subject that was diagnosed at the age of 26 years).

We have also found a missense substitution that was segregated in a Spanish BMD family on the third exon of VMD2. This novel transversion in cytosine at position 328 to guanine (differing from the already reported transversion to adenine) leads to a amino acid substitution – Phe80Leu. The likelihood of pathogenicity is quite high since phenylalanine is highly conserved and is located not only within the second putative TM but also within the second hotspot region,

The Leu207Ile mutation in exon 5 segregated in a Portuguese BMD family and is known to be pathogenic (Lotery et al., 2000). This family beared another Glu35Lys deleterious sequence change because it involves amino residues of different charge. This novel change in a non conservative domain is located within the TM1 and was not detected in the 102 healthy controls. However, this change was shared by both older unaffected brothers of our patient suggesting that this newly reported sequence change is instead a rare polymorphism. Among the three mutations that were found in exon 6, one was previously reported (Arg218Cys) and the two other were novel missense mutations (Glu213Gly and Leu234Val). The Arg218

residue is one of the most frequently altered residues, and at least two other disease-causing substitutions are known (Arg218Ser and Arg218His). The sequence surrounding this residue is rich in amino acids with uncharged polar side chains what suggests that this region could be a binding site. These considerations suggest a vulnerability concerning loss of function as further corroborated by functional studies that show a mutation induced depression Cl- channel function (Sun et al.. 2002). However, the Arg218Cys mutation did not lead to an expressed phenotype at least in two of the family members bearing this mutation. The Arg218His sequence change (Lottery et al., 2000) also leads to relatively benign phenotypes. Normal EOGs and presumably normal Cl- channel function were found in two AMD patients with the sequence variant (T216I) within this region (Yu et al., 2007). This suggests that it may contribute to susceptibility to macular degeneration. Another novel missense mutation, not involving a substantial change in the nature of the amino acid. - Leu234Val - was segregated in a BMD Portuguese family in which no other mutation in VMD2 was found. This nucleotide change has not been detected in genomic DNA samples from the 102 healthy controls of unrelated origin and is probably pathogenic, given that, 24 mutations have been reported between residues 206 and 243 leading to its labeling as the third hotspot region of bestrophin. In the same region, two other BMD-associated variants were previously described providing additional support for the tenet that Leu234Val is causative of BMD. Finally, a novel missense mutation was detected in exon 6 (Glu213Gly). This sequence change in *VMD2* was found in the homozygous state in two BMD siblings but neither in their unaffected sister nor in 102 control chromosomes. The phenotype of both patients was quite severe, had an abnormal EOG and the onset of visual loss was at the age of 23 and 25 years. Their son and daughter were heterozygous carriers and showed no sign of macular degeneration as assessed by standard ophthalmologic examination. However, the girl's EOG (electrooculogram) was found

to be abnormal, even in the absence of any other impairment as measured by optical coherence tomography, color vision testing, and multifocal electroretinography. This pattern is so different from the usually described dominant pattern observed in BMD, that one could possibly make the case for a novel independent clinical entity. In a sporadic single BMD case, a novel mutation (Asp304Val) was identified in a region characterized by a cluster of acidic and highly conserved residues in all of the bestrophins (hotspot IV) that could be speculative assigned to be a putative binding site of Ca^{2+}.
As expected, none of the BMD families revealed any mutation in the RDS/peripherin gene. The interesting case of severe phenotype in homozygous BMD-like patients, while the heterozygous state only yielded mild subclinical physiological changes, as measured by EOG provides strong support for the haploinsufficiency theory, since the severity of the phenotype seems highly dependent of bestrophin activity.

Molecular characterization enables to set a baseline for the study of other factors such as nurture, in the development of a cognitive phenotype.

The visual and motor integration brain systems are particularly affected in WS (see Meyer-Lindenberg et al., 2006 for a review) and these functional profiles are correlated with reduced gray matter density in posterior subcortical and cortical regions (Meyer--Lindenberg et al., 2004; Reiss et al., 2004). Functional imaging studies report reduced activation of the structures involved in the dorsal visual stream (e.g., occipito-parietal and intraparietal areas, Meyer-Lindenberg et al., 2004). The main novelty of our approach was to identify the functional correlates of dorsal stream dysfunction and demonstrate independent novel retinal (based on critical expression of relevant genes in that tissue) and cortical phenotypes (Castelo-Branco et al., 2007).

Our molecular studies identified that all of our 13 patients had the most common 1.55 Mb deletion mediated by non-allelic homologous recombination between the blocks of segmental duplications that flank the WBS locus. Therefore, they all are hemizygous (implying half genetic dosage) for at least 26 genes located in the commonly deleted interval, including *GTF2IRD1* and *GTF2I* at the telomeric edge. Deletion was on the maternal chromosome in 8 cases and on the paternal one in 5 patients. This also opened an avenue to determine whether there is a role for parental transmission. We precisely localized the breakpoints on the blocks of the segmental duplications of the recombinant chromosome of each patient between two paralogous sequence variants. Thus, we could define whether the deletion affected or not the functional copies of two genes in the breakpoint regions, *NCF1* and *GTF2IRD2*. The finding of a recombinant chromosome with a gain of telomeric-type paralogous sequence variants permitted to infer that the transmitting progenitor was a carrier of an inverted chromosome in just one case. The telomeric and functional copy of *GTF2IRD2* was affected by the deletion in 3 cases; *NCF1* was also deleted in those cases as well as in two additional patients. The remaining 8 patients had deletions including *GTF2I* but not affecting the distal two genes, *NCF1* or *GTF2IRD2.*

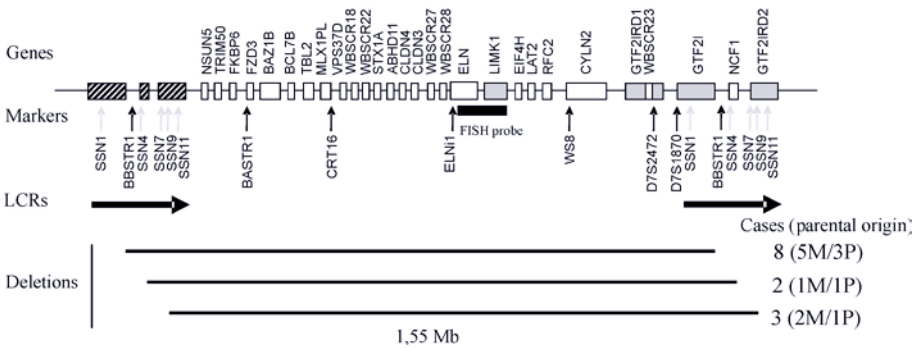

Figure 2

Scheme of deleted interval, including GTF2IRD1 and GTF2I at the telomeric edge

In summary, all cases had similar deletion size and were hemizygous for *GTF2IRD1* and *GTF2I.* We could determine whether the parental origin of the hemideletion, or the precise location of the breakpoints, did not seem to play a major explanatory role in the observed phenotype, which is consistent with the similarity of deletion size. This study, whose retinal and cortical phenotype are described below, shows that our population was homogeneous from the genetics of the hemideletion point of view and that most differences observed in the cortical phenotype are explained by nurture effects.

6. Do we really understand the nature of brain signals that can nowadays be measured? Principles of functional neuroimaging and neurophysiology as applied to Basic and Clinical Neuroscience Visual motion processing - a good model to study the role and validity of functional neuroimaging

We have discussed above retinal and cortical phenotypes in terms of imaging and neurophysiological approaches. However the underpinnings of these techniques are often poorly understood and one of the main difficulties in translational research and clinical approaches is the correct knowledge of their respective advantages and pitfalls.

Amazing recent developments have shown that with clever machine learning approaches one can in fact use noninvasive methods such as functional magnetic resonance imaging fMRI to inform about neural properties that are below their resolving power. These fascinating developments raise new hopes for biomedical applications (see discussion above on amblyopia), but are not yet sufficient to convince skeptical views on the relation between the BOLD (Blood Oxygenation Level Dependent) fMRI signal and underlying neuroelectric activity.

One way to address this question is within the visual system, for instance by studying, in which concerns motion processing, directional selectivity, which is a well studied property of single neurons and neural populations.

The brain uses motion cues in real life to estimate self-motion to perform figure-ground segmentation tasks and to identify shapes from patterns of motion, among many other functions. The human visual cortical area V5/MT (also called the human motion complex or hMT+) is involved in these tasks, and its functional communication with regions involved with shape and motor processing tasks, still remains a research question that can only be addressed using novel functional connectivity analysis methods.

We have performed functional brain mapping of the flow of magnocellular and motion (real or illusory) information within higher order visual areas and their relation to information processing in retinotopic visual areas (Castelo-Branco et al., 2002, 2006, 2007, 2008; Silva et al., 2005, 2008; Mendes et al., 2005; Kozak & Castelo-Branco, 2008). These studies included approaches that focused

on the dissection of the neural correlates of low and high visual representations and how they can be changed by context, using human psychophysics, eye movement recordings and neuroimaging. We have also addressed these neuroscientific questions using animal models, which enable access to the cellular or columnar level (Castelo-Branco et al., 1998, 2000; Schmidt, Castelo-Branco et al., 2006; Biederlack, Castelo-Branco et al.. 2006). We focused on mechanisms underlying parallel processing within magno, konio and parvocellular streams and retinocortical flow of information towards dorsal and ventral visual cortical streams. Understanding the neural correlates of motion integration and visual dorsal stream function was crucial to test an important model in Visual Neuroscience from the basic and clinical point of view: does magnocelullar function predict motion integration and subsequent visuomotor integration? It was found that cortical dorsal stream regions can actually compensate for magnocelullar deficits, and that motion integration abilities predict visual constructive abilities and visuomotor integration. These discoveries are critical to the understanding of the neural basis of visual impairment in disorders such as dyslexia (Castelo-Branco et al., 2006, 2007, 2008).

Conceptual pitfalls in brain imaging approaches

Below we discuss the meaning of BOLD signals as elicited by motion perception but it is worth now discussing some methodological pitfalls often encountered in the brain imaging field.

Given that so many functionally heterogeneous visual cortical regions are now known (retinotopic and non-retinotopic), and that specific functional localizers for regions with clear functional specialization are available (such as LOC – object selective complex, FFA – fusiform face area, etc.), it is inappropriate to define visual cortex as one single region, or to define Regions of Interest based on arbitrary criteria such as the classical Brodman definitions.

In spite of its cytoarchitectonic interest, this classification no longer represents useful parcellations of the visual regions. There is also a lot of terminologic confusion in the literature. One should in principle avoid to differentially threshold the data in terms of statistical criteria, which will lead to different regions being found across studies. This is very serious because some authors are using a very liberal criterion for the regions they want to discuss and a very conservative criterion (which can reach several orders of magnitude) for regions they consider less relevant. This is a really biased approach.

There is also substantial conceptual confusion concerning operational definitions of cognitive processes. For instance, the word "Conflict" is often used in a operationally problematic manner by different cognitive neuroscientists. Some visual perception authors discuss it in terms of competition between neural populations for perception, such as in binocular rivalry between discordant inputs (Fries, Castelo-Branco et al., 2005). Conflict can also be discussed in terms of local/global stimulus congruence, such as in global letters locally defined by smaller local letters (hierarchical stimuli): stimuli can either be congruent (if the local letters are identical to the global letter) or incongruent (if the local and global letters are different). Conflict is both instances is considered at the perceptual level, but is not clearly separated by other instances where the same concept is discussed in terms of decision making processes, or goal oriented behavior involving distinct types of reward. The role of the cingulate cortex in conflict monitoring based on distinct types of rewards has obviously nothing to do with interocular conflict between distinct inputs.

Fallacies in clustering analyses

Researchers are nowadays putting stronger emphasis on being more precise in conceptual definitions of cognitive operations,

and in parallel interested in validating their theories by using not just statistical methods that are driven by a priori models, but also data-driven clustering approaches may help identify fundamental subdivisions of the human cortex into discretized global systems.
The potential usefulness of hierarchical clustering schemes for global cortical parcellation has been highlighted by some authors (Golland et al., 2008). These authors did for instance claim that data--driven clustering reveals a fundamental subdivision of the human cortex into two global systems, in terms of its default operations. The approach (two-class clustering algorithm (k-means)) is data driven, but restricted to a number of pre-defined clusters. It is true that unsupervised clustering eliminates the dependence on predetermined selection of seed-regions but the number of partitions is still predefined. Indeed, it is recognized that "this study focused on the hypothesis of a global, bi-partite organization" which implicitly assumes that the approach is in fact not entirely data driven, because there is at least one clear main hypothesis (due to the prior constraint of the number of clusters). One could argue that testing with k = 4 clusters is also necessary or that the 3 clusters found with k=3, are equally fundamental. Other approaches such as independent component analysis (Castelo-Branco et al., 2002) avoid this problem but are left with posthoc validation, for selection and statistical validation of the relevant components.
It is rightly argued by Golland and colleagues that most of the intrinsic system largely corresponds to a network of areas inhibited by various cognitive tasks, termed the "default-mode", or "task-negative" network and that many studies implicated the default network in a wide range of internally oriented activities. However, this is not necessarily the case for all these areas, and alternative explanations may need to be searched in the future for each case (see discussion and references below). Several sensory and attention related regions seem indeed to be anti-correlated to the default-mode network. However some sensory areas could still activate in

anti-correlated manner as well (for example due to eye blinking) and this does not necessarily put them into different networks. A good example of this fact is stimulation of the foveal visual region in a given paradigm (Dechent and Frahm, 2003). Then peripheral visual regions might show an anticorrelated modulation (because they are less active during the active block, and may still respond to the luminance modulation during the fixation period (Castelo--Branco et al., 2002). Indeed, inter-systems dynamics (such as anti--correlation between the two systems), could be specific to the experimental condition rather than being a fundamental property of the cortical organization and functionality. Cases in which this might happen (visual conditions versus fixation) should in general be explicitly discussed. It is well known that retinotopic areas such as V6 (with magnocelullar bias) or some regions within the PO region respond better to peripheral stimulation (Dechent and Frahm, 2003, Pitzalis et al., 2006, Castelo-Branco et al., 2006) with emphasized Peripheral versus Central representations and are more sensitive to low spatial frequencies and luminance modulations. Dechent and Frahm (2003) have also shown that along the parieto-occipital sulcus, and in contrast to primary visual areas, luminance stimulation evoked much larger activation volumes than checkerboard stimulation. This may mean that a higher response may be expected during fixation (rest) periods, and that even eye blinking may modulate their activity during these rest periods. For instance, opsoclonus suppresses the processing of visual motion along the magnocellular pathway, and induces occipital deactivation with a region-specific distribution, which is just in agreement with normal saccadic suppression, which has been proposed to contribute to the perception of a stable visual space (de Jong et al., 2001). We have ourselves found when studying retinotopic magnocelullar processing that a considerable proportion of regions which showed deactivation during patterned visual stimulation (because they respond preferentially to non-patterned diffuse

stimuli) matched the anatomical loci of the intrinsic network as identified by Golland and colleagues, and being as well confined primarily to the posterior cortex (Castelo-Branco et al., 2006). If our point is correct, then a part of the intrinsic network may actually change partition in terms belonging or not to the default network, in the sense that they would neither reflect subject oriented processes nor an OFF period of visual stimulation, because some visual regions respond to global luminance modulations and spatial low frequency components (more frequent during fixation or unsupervised rest, but not really an OFF default mode period).

All these considerations tell us that application of advanced statistical methods to imaging data still require a lot of conceptual and informed discussion.

Accordingly functionally defined borders between clusters or regions of interest may look sharp or well defined, but this may just be an artifactual consequence of the applied method. These borders should always be checked with known anatomical landmarks, in particular with retinotopic areas, posteriorly. Only in this way it is possible to assess the significance of the partition. It is possible that in the above discussed work of Golland and colleagues, if one sets the number of clusters k>3, then auditory and sensorimotor areas should become visible, which might then be just a trivial finding.

7. Relative merits of mass-action signal measures such as BOLD fMRI and electrophysiological measures – insights from visual motion processing

Both human and animal electrophysiological and imaging studies have provided convergent evidence that V5/MT connections are critical for motion-based perception (Orban et al, 2003, 2004; Castelo-Branco et al., 2006; Cowey et al., 2006).

fMRI allows to study brain function noninvasively and to pinpoint sites of neural involvement for a given task. However, it is important to grasp into what extent can fMRI signals be related to measures obtained in electrophysiology and to understand that the blood-oxygen-level-dependent (BOLD) signal cannot solely be interpreted as spatially pooled spiking activity, because it also reflects subthreshold activity.

Area MT/V5 of the monkey brain is enriched in neurons selective for the direction of movement of the visual stimulus and these neurons play a direct role in the perception of motion direction and speed, because spontaneous or electrically induced fluctuations of activity correlate with behavioral performance in terms of motion detection and direction discrimination. Neurons with similar motion selective properties are clustered together in single columns. Recently it has been demonstrated that fMRI has a spatial resolution that can barely resolve cellular columnar resolution but local asymmetries in columnar organization can introduce signal biases which are small and insignificant for single voxels, but that can be captured by multivariate statistics (often implemented in the form of classifiers) that can detect and combine consistent trends of many data points contained in each voxel across time and reveal meaningful information.

Using this method, it was possible to demonstrate information about directional information within motion sensitive V5 areas and even in other areas (Kamitani, & Tong, 2006). One should however take into account that the extremely high sensitivity of multivariate statistics to detect information does not necessarily tell us that studied brain regions are the ones that are pivotal in processing that information.

Other findings in humans demonstrate that the fMRI signal may well reflect specific motion signaling since responses from area V5 to the same stationary test stimulus is higher in cases when there is an illusory motion after effect (after unidirectional adaptation to a moving stimulus, the subject perceives illusory motion in the opposite direction).
After bidirectional adaptation such effects do not occur suggesting that if only one population of neurons is adapted (unidirectional adaptation), the other (unadapted) population should give a strong response in the test phase because it is not adapted and possibly also because it receives less inhibition from the adapted population. One should however note that fluctuations of attention may well influence activity in V5 and should be taken into consideration using behavioral tasks that ensure a constant attention level (Huk and Heeger, 2001).
Although the use adaptation to a given stimulus feature (such as motion and color) has been validated as a way of probing whether a visual area is sensitive and selectively processes to this specific feature, some conceptual problems still remain. For instance it is still possible that V5, upon motion adaptation, receives a strongly reduced synaptic input owing to spike rate adaptation of its primary input area V1. This could potentially mean 'inheritance' of adaptation effects from a lower level input area when the receiving region has no or little neuronal specificity for the adapted property. In other words, fMRI is an important functional imaging tool, but one should take into account that a proportion of the signal one sees in a given region may be due to synaptic activity from the input level. Furthermore, part of the signal may well represent a mix of excitatory and inhibitory processes.
As stated by Bartels et al. (2008) "it is, in principle, possible that BOLD signal increases confuse inhibition and excitation or even reflect reduction of spiking output in the presence of increased field potentials [local EEG that also reflects subthreshold synaptic activity], that is, during enhanced local interneuronal activity".

Unfortunately, however, many authors equate inhibition with BOLD suppression (speculatively defined as reduced BOLD response that reflects overall network inhibition). This is as serious problem, as discussed recently by Logothetis (2008). He states that it is an open question whether a decrease in the fMRI signal should be expected from an increase in recurrent inhibition with concomitant decreases in excitation that may result in reduction of an area's net spiking output. Based on available evidence, he states that the answer to this question "seems to depend on the brain region that is inhibited, as well as on experimental conditions." Furthermore, as pointed out by the same author autoradiography data suggest that metabolism increases associated with increased inhibition presynaptic activity in a given area is sufficient to yield strong energy consumption despite the ensuing spiking reduction.

Although one could argue that a negative BOLD response (NBR) is a marker of neuronal deactivation as suggested by the correspondence of NBR and decreased population spiking in monkey primary visual cortex, inferences cannot be made without understanding the intrinsic correlation between direct or indirect inhibitory activity and context dependent concomitant changes in energy metabolism. A recent study by Meier et al. (2008) has recently provided clearcut evidence for divergence of fMRI and neural signals in V1 during perceptual suppression in the awake monkey. This shows that oversimplistic views on the correlation between BOLD and specific models of neural firing should be discarded.

These authors have addressed the question whether the robust drop in V1 activity when a stimulus is subjectively invisible, as measured by human functional magnetic resonance imaging (fMRI) can be reconciled with monkey single-unit recordings, which have failed to demonstrate such perception-locked changes in V1. They found, as expected, that all signals showed strong visual modulation to presentation and removal of a stimulus. However, during perceptual suppression, only the BOLD response and the low-frequency local

field potential (LFP) power showed decreases. Spiking and high-frequency LFP power were unaffected. It is therefore becoming clear that the coupling between the BOLD and electrophysiological signals in V1 is task and context dependent. The marked dissociation occurring during perceptual suppression suggests that more refined quantitative models of coupling between supra and subthreshold electrical activity and their coupling to BOLD fMRI signals are needed.

8. Why can Brain Potentials look so different?

One has discussed the strengths and pitfalls of functional neuro-imaging, but the same rationale could also be applied to EEG event related potential (ERP) studies of human perception and cognition.

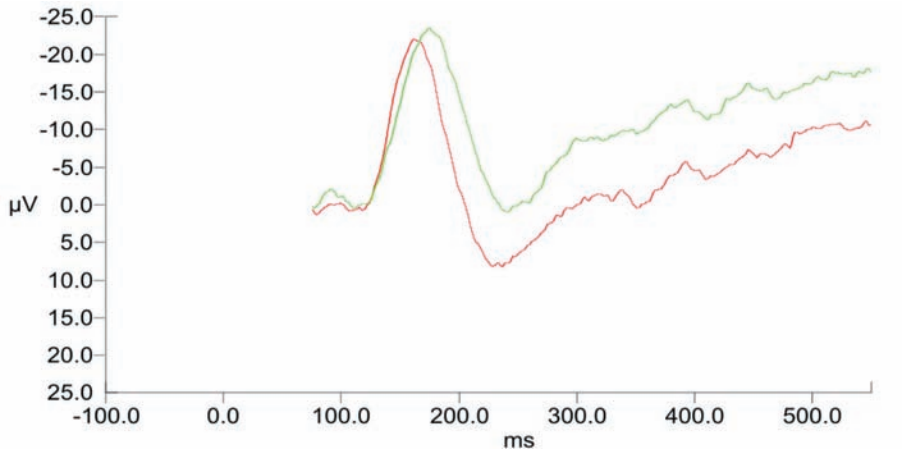

Figure 1 *N170 ERP is characteristically obtained in response to faces. This chart shows the N170 peak for a representative subject in one face inversion effect experience (results recorded and analyzed in our lab; experimental details are as follows: 30 face stimuli (15 upright and 15 inverted male/female face photographs) were shown for 300ms, and after this period subjects had a window of 2500 ms to report – by means of a button press - the perceived gender (male or female) of the target image. Interstimulus interval was 3500 ms. The task ensured stable attention and that subjects really processed the stimuli). Red line: average response to upright faces; green line: average response to inverted face stimuli. N170 is greater and/or delayed for upside-down faces.*

Accordingly, brain waveforms may look completely different depending on what electrodes and references that are being used. As a result, data accross different researchers may look quite different if they are using different references (recordings are never absolute values because they are always relative to a reference – in Figure 1 we have used a central reference).

Even if one uses the same reference (e. g. the average of all sites) variations may still occur due to variations on set of sites that are recorded. Some labs solve this problem by proposing a fixed reference, such as mastoid reference electrodes. However lots of muscle activity can potentially be picked up, one possible solution being referencing to scalp sites that are close to the mastoids or in the case of a sufficiently dense array of electrodes, the average of a small cluster around the mastoids on each side as the reference.
Many reports however fail to recognize that one is always looking at the potential between two electrode sites (or groups of sites) and that this may influence the interpretation. Given that there is no such thing as potential at a single site, one should probably use and compare different references just the same way as we like to look at the same scene from different perspectives. Fortunately, this is not a problem concerning electroretinographic recordings (Castelo-Branco et al., 2007) .
Studies of dynamic brain rhythms using frequency based analysis are less dependent on reference considerations. In addition, important links can also be made in comparison with animal studies. Our own animal work showed the existence of two gamma frequency bands (30-80 and 80-150 Hz) underlying different aspects of visual processing (Castelo-Branco et al., 1998, Neuenschwander, Castelo–Branco et al., 2002).These two distinct frequency ranges are observed at the single neural and population level and have different neural underpinnings (Castelo-Branco et al., 1998, Neuenschwander et al., 2002). Current human and animal research is now focusing on the behavioral significance of such brain rhythms (Fries, Castelo-Branco et al., 2005).

9. Neurobiology of visual motion processing: linking psychophysics, behavior and structural/functional imaging

One of the current goals of visual neuroscience is to understand how visual information is encoded in multiple attribute maps in the cortex, as revealed by psychophysics, electrophysiology and neuroimaging in animals (Figure 1) and humans.

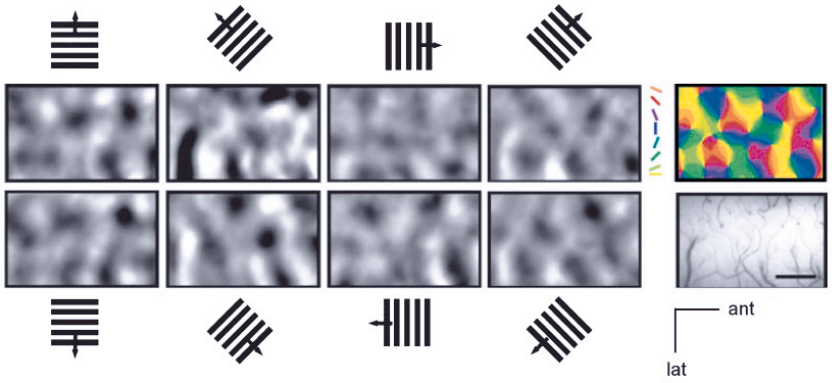

Figure 1

Optical imaging maps of cortical visual Area 18 of the cat (Schmidt, Castelo-Branco et al.) elicited by stimuli (insets near black and white maps) of specific orientation and direction. Each black and white panel depicts the intrinsic variation in oxyhemoglobin signal elicited by an individual stimulus. Stimulus orientation is color coded in top right panel to illustrate the orientation (directions are pooled) cortical map.

The aim of our current research efforts focuses exactly on paradigms that help not just localizing function but also seek to identify mechanisms of visual motion perception. As a second goal we attempt to find evidence for the existence of human motion maps homologous to maps found in animals. Neuroimaging techniques such as functional magnetic resonance (fMRI) and positron emission tomography (PET) have allowed for the localization of human motion sensitive areas. It is however still unclear how each of these areas contributes to mechanisms of global motion perception. It has been shown that a distinct network of areas is activated for pure motion tasks, but it has remained a problem to understand how

these areas fuse local motion signals into global motion percepts (Castelo-Branco et al., 2000, 2002). When viewing a visual scene with moving overlapping contours several solutions are possible: either perceptual segmentation of overlapping moving surfaces, or alternatively, perceptual integration of all local motion signals into a single global motion representation (see Figure 3 for examples, arrows depict global pattern motion; if component motion would have been perceived, then subjects would just see the local motions of the contours). Visual area MT is a candidate for such interplay between global integration and segmentation mechanisms. Integration implies the existence of only one active neuronal population in the motion map (pattern motion representation). Segmentation should be correlated with the coexistence in the same map of several active populations (component motion). This model can be tested using stimuli (plaids) that allow either for the perception of two segmented surfaces or a single integrated surface. We could show that human MT area is involved in the decision between either single winner-take-all (pattern motion) or bimodal (component motion) perceptual solutions, suggesting that its motion map can hold uni or multi-modal (2) active representations (Castelo-Branco et al., 2002).

It is possible that global pattern-motion responses are already present in early visual cortical areas, due to the interaction of 2D shape and motion signals. This question can be best studied in animals using optical imaging techniques, which have a spatial resolution that allow one to study local responses within attribute maps. In this way, one can analyze the relative responses of neurons responding to contours of different orientations and direction of movement (Figure 1). We have investigated whether global pattern-motion selectivity could be detected in population maps of early cat visual cortex, using different sets of plaid stimuli (Schmidt, Castelo-Branco et al., 2006). For all stimuli, a component-motion map corresponding to the response to local contours was detected.

Surprisingly, for the subset of stimuli that were biased for global pattern-motion we also found a map whose peaks matched the locations predicted by a pattern-motion model. To verify that 2D intersections critically underlie pattern maps, we studied responses to depth-ordered stimulus configurations, which entail absent 2D grating intersections, and are therefore not consistent with pattern motion representations. Then, pattern-motion maps became virtually absent, suggesting that 2D contour integration underlies pattern-motion selectivity. These findings suggest that global motion representations are computed at the population level in early visual areas (Schmidt, Castelo-Branco et al., 2006).

We have run parallel work on the neural correlates of human global motion perception and extended its scope to encompass retinocortical mechanisms underlying visual perception and in particular the functions of the magnocellular system, both in health and disease. The main clinical relevance of such studies would be the detection of early and subtle damage of different visual pathways (magno, konio and parvocellular) in diseases such as glaucoma (Castelo-Branco et al., 2004). Considerable knowledge has been accumulated regarding the organization of the magnocellular system across species, from the cellular to the behavioral level. However, critical questions still remain, such as how this system encodes information related to movement and how it interacts with the parvocelullar system, which is involved in recognition of detailed spatial patterns. Additional questions include how different types of local and global motion perception map in the hierarchical areas of the visual human brain and how such organization compares to animal models of vision.

To tackle these questions, we specifically addressed the problem of how the magnocellular system participates in the detection of coherent movement of visual objects (Mendes et al., 2005; Castelo-Branco et al., 2006, 2007, 2008). The visual world is normally composed of superimposed moving objects or surfaces. The

magnocellular system contributes to the perceptual segregation of such objects. The failure of this system, can lead, in the most dramatic clinical scenario, to the consequence that one would only be able to perceive static, unchangeable scenes. We have developed visual tasks that evoke strong neuronal activity in brain areas with strong magnocellular input. These tasks allow a better understanding of how this system integrates sensor information in time and space. Classical methods (velocity discrimination, contrast sensitivity) as well as new strategies (detection of coherent motion in images with varying amount of visual noise) are used to investigate the magnocelullar system under stimulation conditions which involve the superimposition of visual surfaces (Mendes et al., 2005). In line with this visual hierarchical strategy, we have been using visual paradigms that allow for the identification of the role of physical relations, such as luminance contrast or relative movement of superimposed surfaces, in visual object recognition (Movhson and Adelson, 1982; Stoner and Albright, 1992; Castelo-Branco et al., 2000, 2002, 2006; Kozak & Castelo-Branco, 2008).

The human MT complex (hMT+) is known to be involved in motion perception, but it is only now that we are understanding how its activity is related to the computation of global motion (Castelo--Branco et al, 2002, 2006). In particular, hMT+ seems to be pivotal in the interplay between segmentation/integration mechanisms in emerging representations of moving surfaces.

We asked which areas of the human visual brain disambiguate whether motion signals coming from overlapping contours arise from single or multiple surfaces. This question can be examined using stimuli formed by superimposed moving gratings (plaids). These are perceived either as sliding in different directions (component motion, (CM) or as a single coherent pattern moving in the intermediate direction (pattern motion, (PM)). With this paradigm we could assess two critical questions: First, whether MT is a substrate for perceptual decision between alternate discordant

interpretations. Second, whether it represents motion in a manner consistent with the existence of a winner-take-all model, or, in contrast, it would also allow for activity patterns consistent with the simultaneous representation of multiple surfaces.

In our fMRI experiments we presented unambiguous plaid stimuli, that biased subjects' perception toward PM or CM, and ambiguous plaid stimuli, that induced spontaneous switches between both types of percept. During measurements, subjects reported their percepts by means of button presses. Data analysis included both hypothesis (multiple regression) and data-driven (cortex-based independent component analysis, cbICA) analysis of fMRI time-series.

Plaid motion was highly effective in specifically activating hMT+. Both for unambiguous and ambiguous stimuli, activity in this area was highly correlated with the perceptual reports of the subjects. This effect was observed also in dorsal areas to which hMT+ projects, but not in primary visual cortex. CM (two-surface perception) evoked higher activation than PM (one-surface perception). One cortical component, that included hMT+, a network of dorsal areas and the sensorimotor cortex contralateral to the hand of button-press could be reliably obtained with cbICA.

This strategy showed the value of validating a research model with hypothesis and independent data-driven approaches (Figure 2)

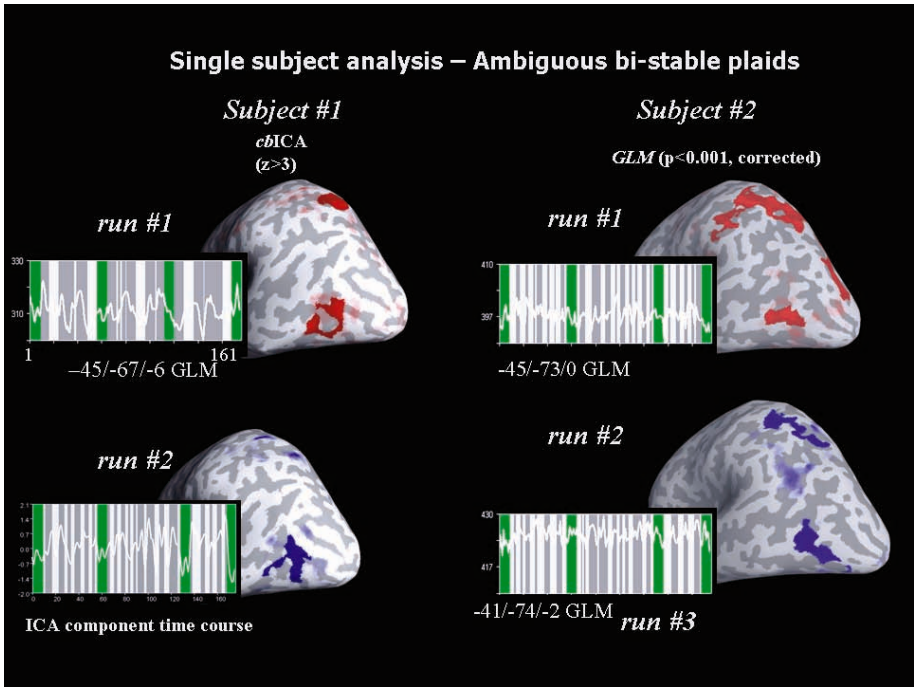

Figure 2

Both model driven (GLM - General Linear Models, Left) and data driven analysis (cbICA – cortex based Independent Component Analysis) yield the same sensorimotor maps (colored areas superimposed in inflated cortical reconstructions), across different experimental runs and subjects. This sensorimotor network is involved in perceptual decision. Insets depict activity across time (white line) with dark grey periods depicting perception of two surfaces, light gray, perception of one surface, and green, fixation rest periods).

The increased activation level during component motion over pattern motion is consistent with the assumption that hMT+ represents segregated assemblies for multiple surfaces moving in different directions. Since stimulus features were kept constant during the ambiguous plaid condition, these results suggest a functional correlate of perceptual interpretation within hMT+. Our study is consistent with the presence of a directional map in MT+

and suggests that it can support not only one global representation but also multiple motion representations.

Our findings still leave open the possibility that global motion responses may already present at the population level in early visual cortical areas, due to the interaction of 2D shape and motion signals. This possibility is supported by the finding that global motion responses cannot be detected at unit level in cat visual areas A18 and PMLS but only by joint firing patterns. Indeed, neurons in these two visual cortical areas synchronize their discharges when responding to contours of the same surface but not when responding to contours belonging to different surfaces (Castelo-Branco et al., 2000). It is therefore possible that global pattern motion responses can be found at the population level in these areas, by using methods that study neuronal signals at the columnar level. Neurons belonging to the same cortical orientation column respond preferentially to contours of similar orientations.

We have recorded intrinsic signals from early area 18 in the cat while stimulating with different plaid stimuli and analyzed the amount of component- and pattern like evoked activations in the cortical representations of these stimuli. Most interestingly, plaid stimuli with a pattern bias evoked responses that had a peak matching the location of global pattern model maxima in orientation maps of area 18.A pattern (one surface) bias was constructed based on violations to the rules of physical and perceptual transparency (Stoner et al., 1990; Stoner and Albright, 1996). Non pattern-biased (two surface) stimuli were constructed either by optimizing stimulus transparency or by emphasizing depth-ordering rules, which allows for stimulus manipulations that do not depend on grating intersections. In this way we could generate hypotheses for two very simple categorical sets of plaid stimuli: pattern-biased and non pattern-biased. Our prediction was that only pattern-biased stimuli should evoke activity maps with strong partial correlations with a pattern motion model. Furthermore, all stimuli should show correlations with the

component motion model, which is explained by neural processing of local component contours that are common to all stimuli. The relative correlation with each of the models should be dependent on the relative bias for local or global processing: for depth-ordered (two surface) stimuli pattern bias should be minimal. Our results match very closely these predictions.

The pattern-biased stimulus (PB 1) that evoked the strongest and significant partial correlation with the pattern model was also the stimulus that evoked the lowest correlation with the component model. Depth-ordered stimuli are strongly biased for 1D contour processing which probably explains why they virtually always correlated better with the component model than all other stimuli but had the lowest correlation with the pattern model. Intermediate sets (PB2 and NPB1) showed strong correlations with both models which corresponds to both 1D contour processing and 2D processing related to grating intersections.

Although the relative component- and pattern-like modulations follow the predictions made by the degree of pattern bias, all stimuli revealed significant component-like modulations. This is to be expected since all plaid stimuli contain local 1D elements of oriented lines which should produce activity in the domains preferring the orientation and direction of motion of the components.

It is surprising that even when pattern motion responses in early visual areas are barely observed in single and multi-unit responses, in particular in anaesthetized preparations (e.g. Castelo-Branco et al., 2000; Guo et al., 2004), they can still be detected in metabolic population responses. One reason for that could be that earlier electrophysiological studies investigated mostly multi-unit responses. These could hide pattern-selective neurons and thus, the actual number of pattern-selective neurons in V1 becomes underestimated. Confirming this hypothesis are preliminary results of pattern selectivity in area 17 single-units stimulated with line segment stimuli of the same orientation but moving in different

directions (Li et al., 2001). However, even single-unit activity in primate V1 revealed only limited pattern selectivity and almost only during states of alertness (Guo et al., 2004). In addition to the larger sampling basis in optical imaging there might be thus also another reason for our observations. Measurements of single and multi-unit responses do not directly reflect subthreshold synaptic processes which are thought to be a substantial component of the metabolic responses captured by optical imaging (Das and Gilbert, 1995; Toth et al., 1996) or other measurements of cerebral blood flow (Mathiesen et al., 1998; Logothetis et al., 2001). There is indeed other evidence that these approaches reveal processes hardly reflected in spiking activity (Logothetis et al., 2001; Schmidt et al., 2004).

Two models were proposed to account for the perceived coherent motion of a plaid (Adelson and Movshon, 1982). One model assumes that the true direction of motion of a plaid pattern is given by the intersection of constraints, a two-dimensional velocity space where direction and velocity of the plaid's components intersect. The intersection of constraints mechanism is thought to require two successive stages of processing. The first evaluates the directions of movements of the local contours and the second computes the true direction of motion of the composite object by integrating over the output of the local analyzers. The fact that neurons computing the second stage have been rarely found in early visual areas might be attributed to their receptive field size not allowing for the integration over larger parts of the composite object. Therefore, our finding is rather surprising but might fit into the two stage model when one considers the fact that area 18 receives feedback from higher visual areas where neurons have larger receptive fields. Indeed, the variable correlations with both pattern and component models in our maps are consistent with variable correlations described by Albright and Stoner for single neurons in MT as a function of stimulus coherence (1992). Here, stimulus-driven modulation was also more pattern-like when challenged with a non-transparent stimulus and

the modulation becomes more component-like with the increase of physical and perceptual transparency in the stimulus.
A second model proposes that the perceived motion is determined by the motion of the intersections ('blobs') and that motion is particularly biased towards the true plaid direction of motion through a low level 'blob tracking mechanism' (Alais et al., 1994). Our results are consistent with such an early level blob-tracking mechanism although top-down mechanisms in 2D intersection detection cannot be excluded. We suggest that the pattern motion cells that are found in early areas respond more strongly to corners and intersections than component cells. Since early visual areas are very contrast-sensitive, high intersection contrast may increase the saliency of the blobs in a feature tracking mechanism and bias pattern motion. However, contrast sensitivity is higher in peripheral parts of the visual field but here we observe mainly component selectivity. We found that more important than contrast was whether stimuli were constructed such that 1D processing is biased against 2D processing, such as in depth-ordered (two surface) plaids. This rather suggests that a 2D contour-tracking mechanism underlies pattern motion maps in A18 (Schmidt, Castelo-Branco et al., 2006). Our results may still be explained by top-down effects and future studies should address the role of feedback from higher order areas in such processes. Top-down effects related to attention to features such as intersections (Nolan & Sejnowski, 1996) are likely to be prominent in awake behaving animals but preattentive top-down effects could still occur in our preparation. It is possible that a tracking mechanism could contribute to pattern selectivity in primary visual areas via feedback connections from higher visual areas with larger receptive fields and integrating over the local cues. In conclusion, our findings suggest that global pattern-motion representations might already be computed at the population level in cat early visual areas. Future work should address the role of feedback in the emergence of such representations.

Motion adaptation as a tool to detect motion sensitivity within a brain region (visual complex hMT+/V5)

Our research program provides a comparative analysis of how activity in the human motion complex (hMT+/V5) is related to the perception of real surface motion, apparent motion (AM), and illusory motion aftereffects (MAE). One way to assess for selectivity for a visual attribute is to adapt the visual system to constant vs. variable values of that attribute (see fixed vs. mixed motion paradigm in Figure 3).

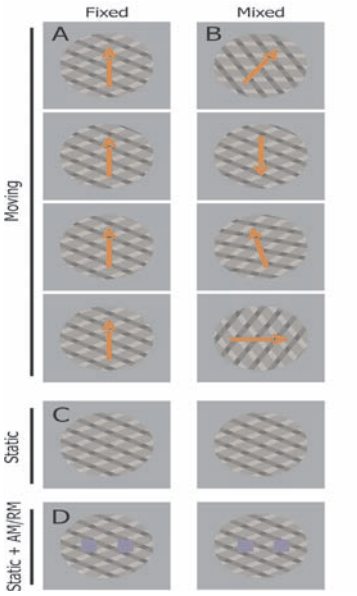

Figure 3

Sixteen second blocks of plaid motion and static plaids were combined and presented in a block design manner in these functional imaging experiments. Motion blocks had either constant direction of motion (fixed;a) or the direction changed in every two seconds (mixed; b). Blocks were also presented with (d) and without (c) overlaid apparent (AM) or real motion (RM of blue squares) concurrent tasks to control for attentional shifts. Arrows depict global pattern motion (stimulus conditions were adjusted such that component motion local motion is not perceived).

We have observed that the pattern of modulation depends on whether attention is focused on concurrent motion vs. non-motion signals (data not shown). Most importantly, we found that levels of activity in hMT+ are dependent on motion adaptation, providing quantitative evidence for direction of motion selectivity (Figure 4).

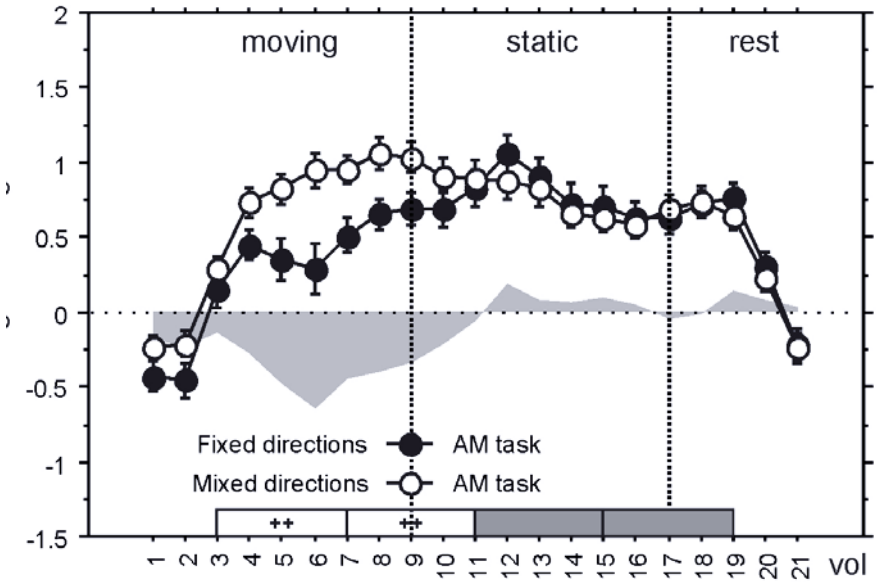

Figure 4

An illustration of motion adaptation effects, as a strategy to demonstrate motion selectivity. Activity (expressed in % modulation over baseline) in human motion sensitive MT region is large for fixed (adapting) than for mixed (nonadapting) plaids. Time points depict TRs (vol). Bars on the bottom of the graphs represent statistical comparisons for data pooled over 8-second blocks, dark bars: non-significant difference, white bars: significant difference (+: p<0.05; ++: p<0.001, ANOVA with Fisher's post hoc). Error bars on all plots represent 1 SEM.

We do believe that this sort of paradigms will illuminate models that integrate the role of adaptation mechanisms, attention and perception of real and illusory motion.

Along these lines, it is also important to analyze the influence of peripheral visual context on the perception of central motion (Figure 5). We have found that contextual effects are strongly dependent more on the presence of local (as manipulated by local dot motion) than of global ambiguity (perception of one or two global surfaces) (Kozak & Castelo-Branco, 2008).These findings have strong implications in the understanding of normal and impaired vision, because in the latter local ambiguity is increased in general. Our findings show that what subjects can perceive in central foveomacular regions is strongly influenced by the level of congruence/incongruence of peripheral visual information. These results show that contextual information may be integrated over space to solve for local ambiguity in normal and impaired vision.
The importance of local ambiguity in contextual modulation is clinically relevant, because it implies that contextual effects will be stronger in disorders with impaired central vision, such as macular degenerations. Moreover, the increased efficacy of global context under conditions of increased local ambiguity may be potentially useful in future rehabilitation approaches.

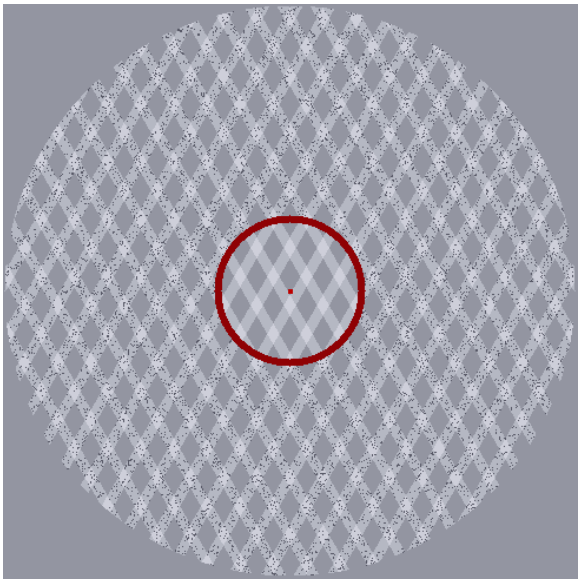

Figure 5

Scheme of basic experimental design to study contextual effects. Subjects had to fixate, being therefore uncertain about the nature of the peripheral stimulus, which acted as the context (because it contained on or two directions of motion whose influence on the perceptual reported number of perceived directions in the center was tested). Note that the presence of texture in the peripheral stimulus makes it purely unambiguous and ideal to induce contextual effects.

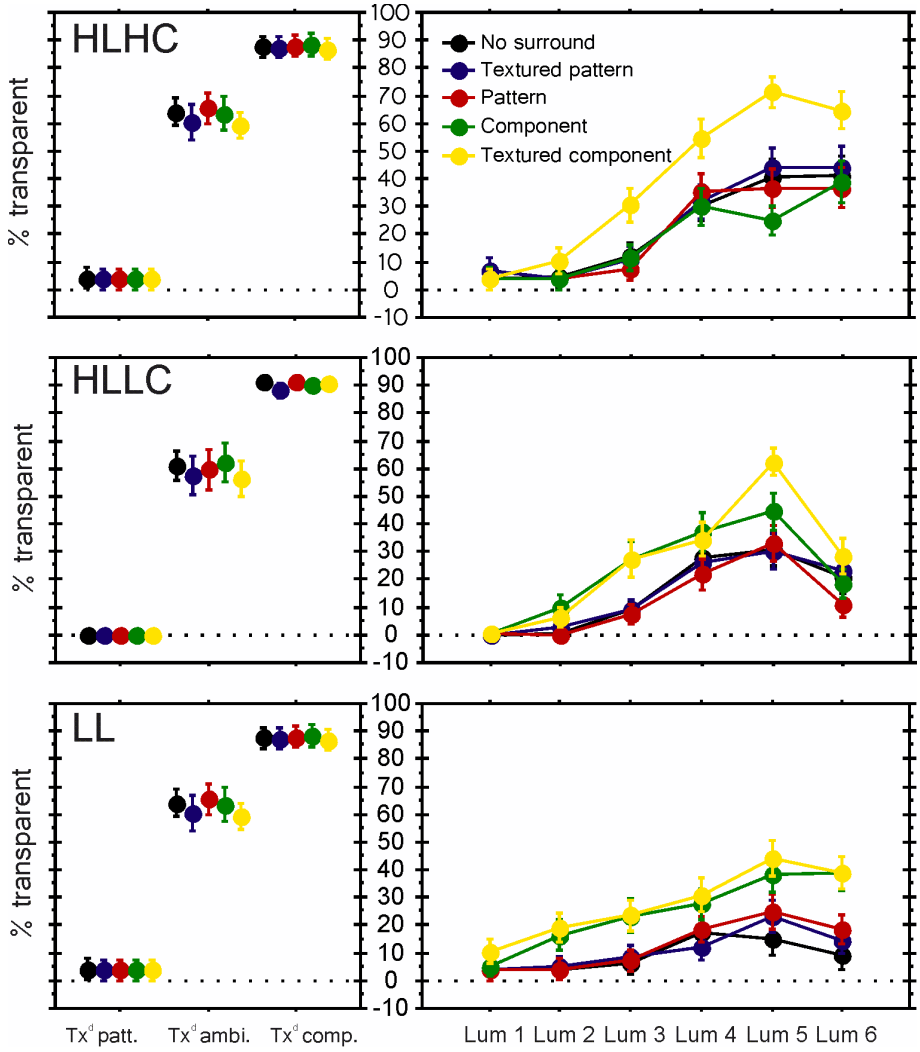

Figure 6

Contextual effects are absent if central plaid stimuli are locally disambiguated by texture even if the global percept is still ambiguous (left) and are present if the center stimulus does not contain local disambiguating texture (right). The Y axis depicts % time with perceived two central surfaces (transparent)

instead of one (pattern). Effects are shown for the high luminance low contrast (HLLC), high luminance high contrast, and low luminance stimulus sets (HLHC, LL). Tx[d]: textured. Error bars represent 1 SEM.
Amb. – Ambiguous; Patt. – Pattern; Comp. – Component

Dissecting brain function and unraveling the complex causes that modulate brain activity patterns: Further insights on mechanisms of decision-making in the Brain
The role of visual area hMT/V5 in dissociating between veridical stimulus properties and perceptual decision.

The cluttered nature of the visual world often imposes difficult perceptual decision problems which may even lead to paradoxical dissociations from veridical properties of the stimulus. Here, we have studied neural mechanisms that trigger visual segmentation of moving surfaces under variable conditions of stimulus ambiguity, and presence or absence of noise. In this study we were able to eliminate possible confounds due to minor physical differences across conditions. This was because percepts were fully determined by the presence of patterns of moving dots under different noise levels, such that often subjects could even perceive global motion in a way that differed from the veridical physical properties of the stimulus. This enabled us to ensure that brain activity patterns were really related to the nature of the percept and not physical properties.
The mechanisms triggering decision were studied using perceptually bi-stable stimuli, similar to the above described plaids, except that it was the presence of dot motion that determined the percepts: during scanning experiments subjects were asked to report, by means of button presses, their interpretation of two superimposed gratings moving in different directions (plaid stimuli). As mentioned above, plaids may be perceived either as two surfaces, one being transparent and sliding on top of the other (transparent or component motion) or as a single coherent pattern whose direction of motion is intermediate to the component vectors (non-

transparent or pattern motion).

We have constructed textured plaid stimuli because they can be physically disambiguated into Pattern (global) and Component motion by local dot movement. We have provided graded levels of disambiguation by varying the ratio of horizontally and vertically moving dots across stimulus conditions. This parameterization yielded 5 conditions where C:P (Component/Pattern) ratios ranged from 4:2 (200%) to 2:4 (50%).

We have also studied perceptual decision under conditions where global motion is disambiguated but variable amounts of noise dots are included: for the Pattern vs. Noise Condition the ratio of vertically and randomly moving dots varied across stimulus conditions from 4:2 (200%) to 2:4 (50%) and for the Component vs. Noise Condition a similar ratio was applied between horizontally and randomly moving dots (see Figure 7 for psychophysical results). Surprisingly, we have found that physically disambiguated component/pattern stimuli could still be perceived as pattern/component stimuli, yielding non-veridical perception.

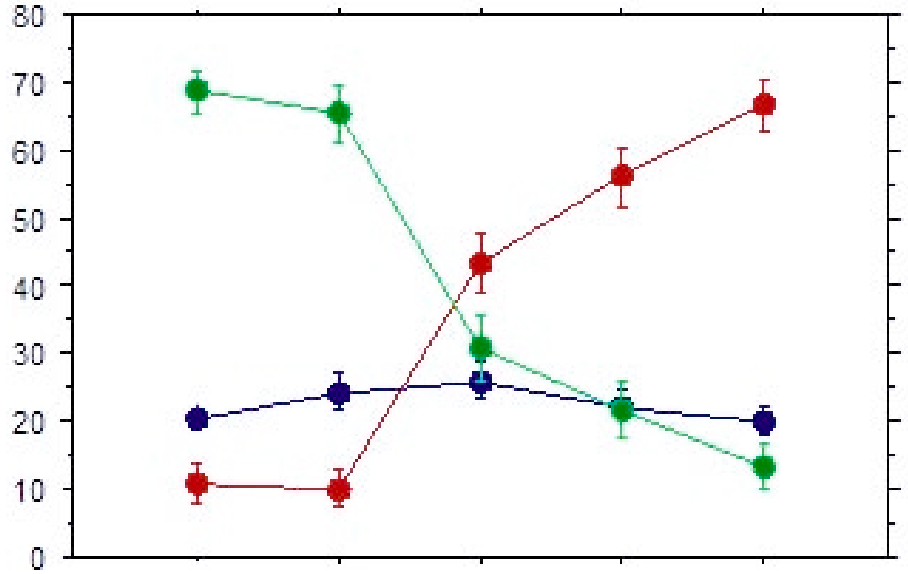

Figure 7

Stimulus Properties and Psychophysical Results. The Y axis depicts % time with perceived two surfaces (transparent) instead of one (pattern). X - Axis - 5 conditions where Physical C:P (Component/Pattern) local dot ratios ranged from 4:2 (200%) to 2:4 (50%).

Using General Linear Model Analysis (GLM) we found that both pattern and component motion yielded significant activation patterns in hMT/V5 ($p<<0.001$). Given that psychophysical decision curves showed a near-sigmoidal relationship with C:P (Component/Pattern) ratios, we have performed a whole-brain multi-subject GLM analysis by contrasting sets of C:P ratios yielding similar psychophysical results. We found significant activation patterns within hMT for ratios contrasting Component vs. Pattern motion.

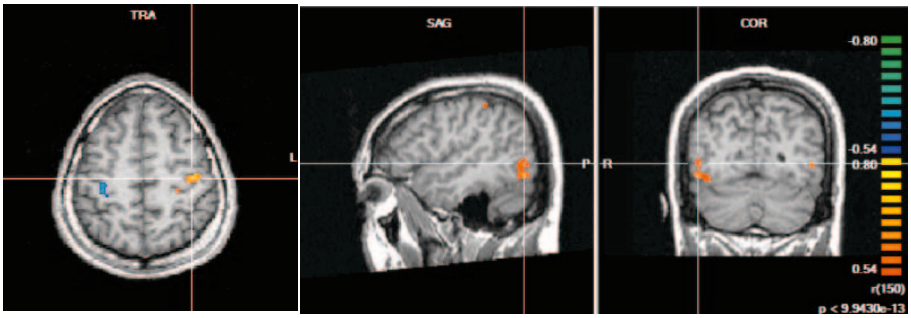

Figure 8

Sensorimotor representations corresponding to the perceptual response (left panel) and hMT+ clusters significantly correlated with component motion perceptual predictors in subject JM (right). Methods: Functional and structural imaging experiments were performed in 5 participants at 1.5T in a Philips Gyroscan Intera scanner by using the standard head coil and a gradient echo planar imaging (EPI) sequence (TR=3000ms, FOV=240×240 mm2, 27 slices, voxel size: 3.75×3.75×5 mm3) A T1-weighted 3D magnetization prepared rapid acquisition gradient echo scan was recorded in the same session as the functional measurements (voxel size: 1.0 ×1.0×1.0 mm3).

To verify whether hMT is indeed related to the triggers of perceptual decision, we have performed event-related analyses, which confirmed a significant involvement of hMT in the near-perceptual-switch periods. Furthermore, given that pattern and component perceptual responses had distinct button press assignments we were able to extract highly significant sensorimotor representations corresponding to the perceptual response (Figures 8 and 9). These representations were used as internal temporal markers of perceptual decision and as an input for linear correlation analyses to find areas directly involved in perceptual decision. We found that hMT was a pivotal area significantly involved in this process.

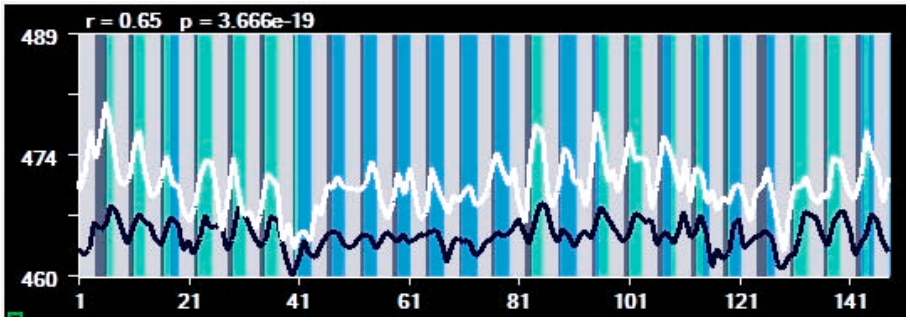

Figure 9

Significant correlations (see values on top of panel) between time courses in hMT+ (white line) and sensorimotor marker of perceptual decision (black line) for component motion in subject JM (Talairach coordinates of hMT+ cluster: 43 - 76 -2) . Green periods: component motion; Blue periods: pattern motion; Grey Periods: Fixation.

Finally we have performed GLM event-related analyses on physically disambiguated conditions that can still yield non-veridical perception, and replicated under those extreme conditions a critical role for hMT in perceptual shifts (Figure 10).

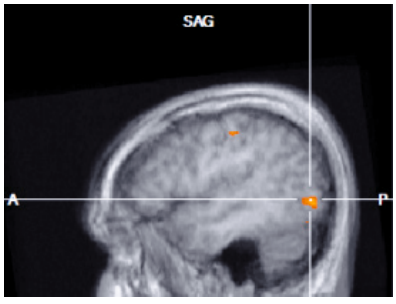

Figure 10

GLM Multi-subject event-related analyses on physically disambiguated conditions that can still yield non-veridical perception reveals a critical role for hMT in perceptual decision ($p < 0.001$; group data from 5 subjects is superimposed on average of anatomical data sets).

We conclude that hMT complex directly encodes and triggers decision processes related to surface segmentation, even when perception is dissociated from veridical stimulus properties. In this way our study could unravel in a causal way the triggers for decision independently of confounds due to stimulus properties.

10. Clues for translational research: non-motor manifestations in Parkinson's Disease and implications for neurochemical models of disease pathophysiology

Non-motor symptoms and sensory impairment in Parkinson's Disease (PD) are of great current interest to the research and clinical communities. Available evidence suggests that the visual deficits found in PD patients are not solely related to retinal dysfunctions and that some of the deficits may be explained with generalized abnormal center-surround interactions of low and high level visual neurons as a consequence of dopaminergic deficiency. Here, we review findings of non-motor manifestations of Parkinson disease, and their relationship with drug therapy. We focus on concomitant low level damage of konio, magno and parvocellular pathways in PD, high level motion integration deficits and their independence of low level deficits, and implicit visual learning mechanisms (Silva et al., 2005; Van Asselen et al., 2007, 2008; Castelo-Branco et al., 2008). These observations lead to new ideas on the anatomical/neurochemical substrates of visual impairment in PD, and in particular the relation between motor and non-motor manifestations in PD.

Our recent work makes explicit links between psychophysical, electrophysiological and morphological evidence of disruption of retinal structure and function, in relation to disorders of "higher" (cortical) visual processing (Silva et al., 2005; Mendes et al., 2005, Castelo-Branco et al., 2006, 2008). Indeed, concerning the link between retinal and cortical visual deficits we were able to prove that both can contribute in non-motor manifestations of PD in an independent manner. Furthermore we found evidence for low level impairment in PD that could be separated from other age-related causes of visual impairment (Silva et al., 2005). Our previous work where the same psychophysical, electrophysiological and/or morphological methods have been applied both in PD and other age related disorders of vision (such as glaucoma) allowed comparing distinct patterns of dysfunction across diseases. Accordingly we found a stage dependent functional deterioration of chromatic sensitivity in glaucoma that was relatively specific for

the koniocellular pathway, although the parvocellular pathway also deteriorated across stage. In PD, relative levels of impairment were variable across stages and could occur early on, even in untreated patients. Only achromatic contrast sensitivity functions within the magnocellular pathway revealed a dependence on disease stage.
The parvocellular (P ganglion cells) neurons from the central retina signal fine feature and color information and for the same eccentricity they have smaller receptive fields than magnocellular neurons. Given the dopaminergic regulation of the "centre-surround" field size it remains an open question whether impaired dopaminergic neurotransmission in PD controls contrast sensitivity. In our studies we did take into account the effects of spatial and temporal acuity performance measures that can be defined in several ways (psychophysically and even electrophysiologically, using Sweep VEP) by carefully calibra.ting stimulus luminance, and isolating spatial and temporal frequency channels. Furthermore the importance of "neural" factors such as photoreceptor density, the region of the retina being stimulated, and other diseases that may also induce changes in visual function (age-related diseases of the eye such as cataract, age-related macular degeneration, diabetic retinopathy and glaucoma) were carefully controlled for (Silva et al., 2005, Castelo-Branco et al., 2008).
There are available studies where exactly the same psychophysical method is applied in retinal diseases (including photoreceptor and ganglion cell diseases, such as glaucoma) and PD (reported in different studies). It may be relevant to point out that the pattern of color vision impairment across multiple color axes is more linear and thereby predictable in glaucoma than in PD (Figures 1 and 2).

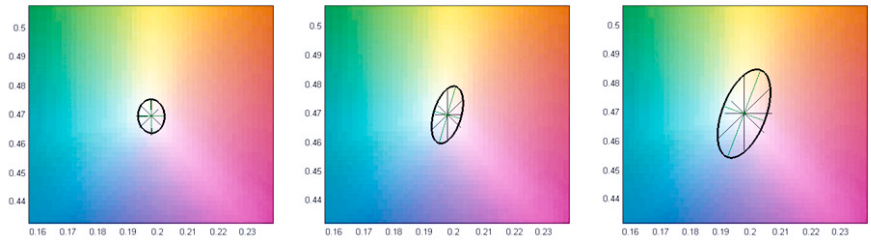

Figure 1

Patterns of color vision impairment across stages in glaucoma natural history (left, normal control; middle, ocular hypertensive and right, glaucoma patient). Larger ellipses in color space mean worse performance. Constant orientations across stage mean that the same color pathway (koniocellular – blue yellow) is predominantly affected.

Figure 1

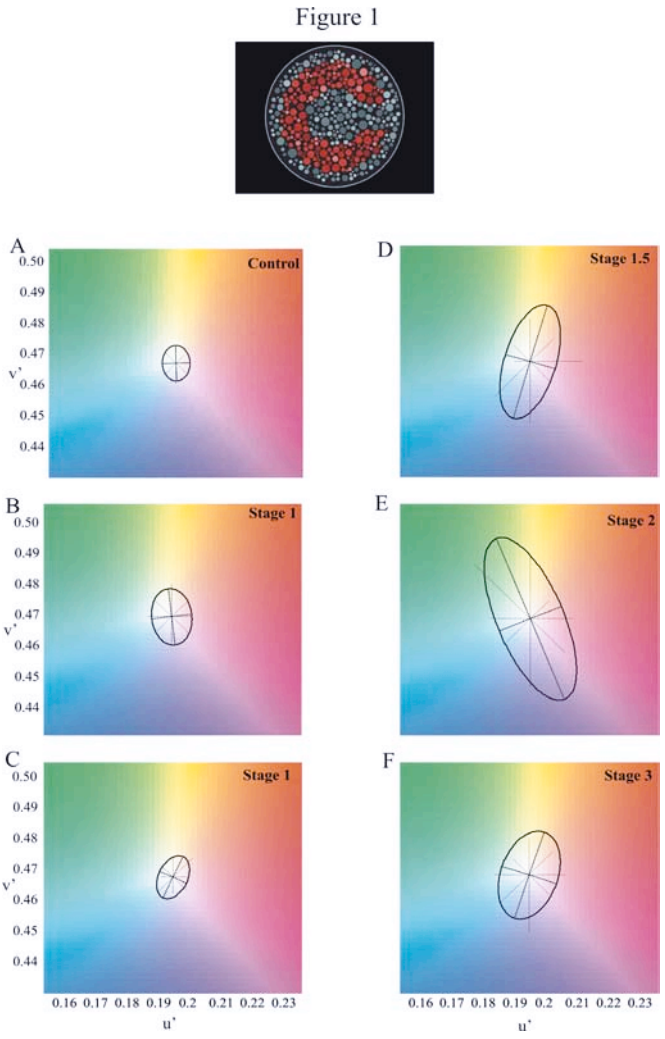

Figure 2

Patterns of color vision impairment across stages in Parkinson Disease natural history over Stages 1-3 of the modified Hoehn and Yahr clinical scale (motor UPDRS: B, 11; C, 13; D, 18; E, 28; F, 40). A variable pattern is observed across stages..

Although a retinal impairment is quite likely in PD, given the reduced tyrosine hydroxylase immunoreactivity of dopaminergic cells in the central retina (Nguyen-Legros, 1988) visual cortical contributions are probably important, as suggested by the dopaminergic innervation of the lateral geniculate nucleus and visual cortex (García-Cabezas et al., 2008).

The presence of Visual Hallucinations in PD are not necessarily a proof of cortical involvement, since changes in visual acuity and retinal diseases can lead to visual hallucinations (Castelo-Branco, 2005). However we do think that this is an unlikely hypothesis in PD given the bilateral changes in visual acuity that are required for such phenomena to take place .

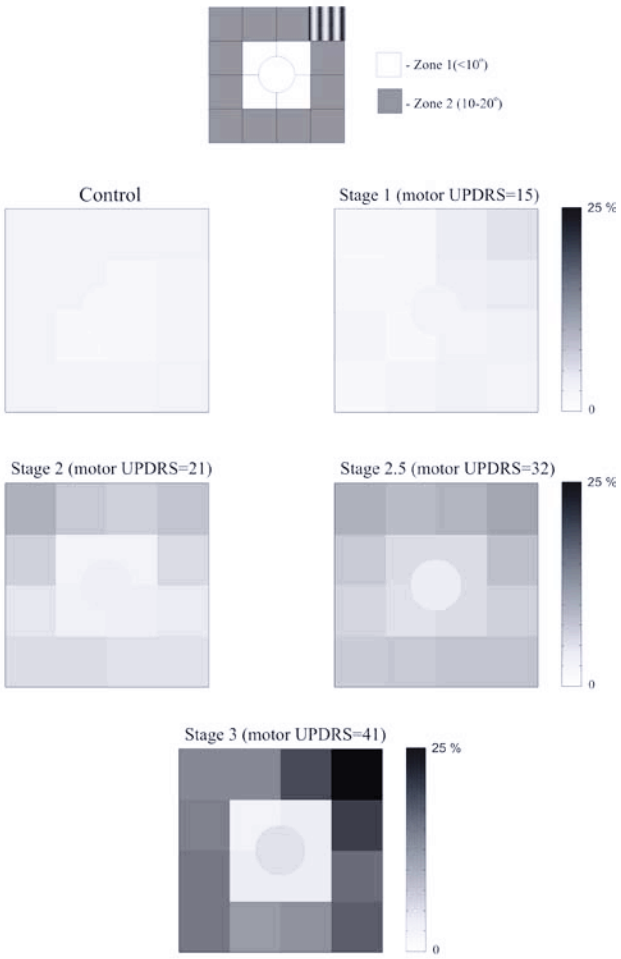

Figure 3

Patterns of magnocellular achromatic contrast sensitivity impairment across stages in Parkinson Disease natural history. A clear deterioration across stages is evident.

Concerning evidence for nonmotor manifestations of PD it is therefore critically important to analyze and separate low and high level deficits (Castelo-Branco et al., 2008). It is indeed widely

believed that disruption of this magnocellular pathway (Figure 3) may deprive dorsal visual stream of cues for accurate motion perception but we have recently demonstrated that this is not the case. That work shows that these deficits are not only dissociable, but in fact independent, at least concerning high level motion perception. So far no direct evidence existed for correlation between both types of measure.

Furthermore, an open question remains concerning the hypothesis that visual dysfunction directly contributes to these more traditional "motor" complications, and some authors had in fact recognized that the relative contributions of retina and visual cortex to these complications remain unclear (Uc et al., 2005). In fact our work provides evidence that motion integration measures are predictive of visuomotor performance (simple and complex motor tapping tasks). In the same line, in PD there is impairment of the integration of visuospatial cognition with motor imagery (Amick et al, 2006). Newer retinal imaging methods such as optic coherence tomography (OCT) will probably help us better elucidate the role of retinal changes. The newer OCT devices can now differentiate even subcellular fine details and will probably shed newer light on the retinal debate in PD.

We have recently also focused on non-motor functions of the basal ganglia such as implicit context learning, which refers to the ability to memorize contextual information from our environment, which - on a subsequent occasion - can be used to guide our attention to a specific location (Van Asselen et al., 2007). We found that perceived spatial context information that is learned implicitly, can be stored into long-term memory, regardless of whether the subject is allowed or not to move the eyes.

In one of our visual search tasks, subjects have to quickly locate a target among a number of distractors. Importantly, some of the configurations are repeated during the experiment, resulting in faster responses. Whereas the control subjects responded faster

when the spatial context was repeated, Parkinson's patients failed to do so, suggesting an involvement of their striatal pathways in implicit context learning, which is clearly a non-motor function.
Given the clearly demonstrated non-motor manifestations of Parkinson Disease a challenge is now to understand their neurochemical correlates, for which appropriate animal models could potentially be useful.

Other translational research approaches: animal models to clinical applications. Challenges and pifalls

Biomedical research endures a new era where translational research became a main theme and priority. However, in some respects the link between basic, pre-clinical and clinical research is still missing or at best just tentative.
The main reason for such a scenario is that scientists in their respective fields still use different jargons and have difficulty in making conceptual and practical compromises with their partners that study the same biomedical problem from a different vantage point.

Animal models of Parkinson Disease and Human studies: what have we learned so far?

We will focus our discussion here on the understanding of Parkinson disease (PD) and challenges in the pathway from bench to clinics in this area.
Parkinson's disease (PD) is a progressive neurodegenerative disorder whose etiology is not understood and this provides obvious limitations do the design of animal models. Some of these are genetic but since the disease occurs both sporadically and through monogenic inheritance of single genes, the familial types being rare, environmental factors are probably critical and

13. Conclusions and a look into the future

should be considered as pivotal in disease pathophysiology. How can one model the strongly multifactorial interactions between the environment and genetic predisposition factors that occur in PD? The last few years have witnessed the appearance of toxin, inflammation-induced and genetically manipulated models. One can only argue that the insight gained from the use of such models can boost the understanding of the progression and stages of the disease if a unifying explanatory framework emerges in the meantime. Novel therapies to improve symptomatic management, and the development of neuroprotective strategies have emerged based on these models, but their real value still remains to be critically evaluated.

Loss of dopaminergic (DA) nigrostriatal neurons has been the primary neuropathological finding, thereby diverting attention from the fact that loss may well not be restricted to these neurons, including non-DA cells that are lost even before substantia nigra (SN) loss (Braak et al., 1995, 2003; Halliday et al., 2006). Accumulation of insoluble proteins, such as alpha-synuclein, in cytoplasmic inclusions called Lewy bodies is often found in SN DA neurons and, in some cases, even in non-dopaminergic neurons located elsewhere (Harding and Halliday, 2001; Aarsland et al., 2004).

As in vision models (see specific section on that topic) current research relies heavily on rodents to model the features of PD. Most models employ toxins, such as 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, or paraquat, with the obvious drawback that the specific and progressive cell loss that occurs in PD will probably not be present. Most of the toxins may specifically target the DA neurons through preferential uptake by transporters thereby inhibiting Complex I or enhancing the production of reactive oxygen species (ROS). 6-OHDA does not readily cross the blood-brain-barrier, and it kills not only DA but also NE (norepinephrine) containing neurons (but when administered directly in the brain it does not lead to

inclusion or Lewis Body dementia pathology). Clinical effects are not progressive and extranigral pathology is not documented. For instance, dopaminergic neurons in the ventral tegmental area (VTA) are relatively spared, which resembles in fact PD (Grant and Clarke, 2002). Finally, effects depend on the injection site.

Rotenone is a Complex I mitochondrial inhibitor that has been used to generate the first chronic PD model (via osmotic minipumps). It produces a loss of striatal DA terminals followed by progressive degeneration of SN DA neurons with presence of cytoplasmic inclusions, immunopositive for alpha-synuclein and ubiquitin (Meredith et al, 2008).These authors argue that while the DA neurons are vulnerable to rotenone exposure, the involvement of other unrelated neural populations and the high variability may limit the utility of the model. In fact, this might not at all be the case, since PD is also characterized by non-motor manifestations and high variability of pre-clinical and clinical features (Silva et al., 2005; Castelo-Branco et al., 2008).

Other toxicity models have been proposed (based on Paraquat and/or Maneb administration) but they appear not to add major additional advantages.

Inflammation-based models using lipopolysaccharide (LPS), a bacterial endotoxin that acts as a powerful activator of microglial cells and a potent inducer of inflammation, have also become popular, given the recent emphasis on the hypothesis that microglia contributes to neurodegeneration (Meredith et al., 2008). It shares some of the caveats of 6-OHDA, namely dependence on mode of administration and even species dependence (progressive DA cell loss occurs in mice given a single systemic exposure to LPS, with no effect in rats provided with a single, acute, intra-nigral LPS infusion as reviewed in Meredith et al., 2008).

Mouse and rat models created through the expression of genetic mutations have been postulated to be better models for understanding disease progression, but their strengths and limitations still need to

be thoroughly investigated. Some of these models lack either extranigral pathology or inclusions bodies of the Lewis type.
These models basically focus on deletion of genes important for the development or maintenance of DA neurons (Hwang et al., 2005; Sgado et al., 2006; Sonnier et al., 2007). Mouse or rat models based on expression or deletion of genes known to cause familial forms of PD (Fleming et al., 2005), take advantage of virally mediated expression of genes or mutations known to cause familial PD, usually in nigrostriatal DA neurons (Ulusoy et al., 2007).

Endpoint measures in animal models of PD

"Gold standard" measures the magnitude of nigrostriatal loss do involve injecting the rat with apomorphine or amphetamine and counting the number of rotations (Ungerstedt and Arbuthnott, 1970).The rotational tests are of complex interpretation as reflected from the empirical observations that DA uptake inhibitors induce ipsilateral rotation, whereas DA agonists produce contralateral rotation (Lane et al., 2006). Systemic injection of levodopa induces a robust contralateral rotation as does the receptor agonist, bromocriptine (Ungerstedt and Arbuthnott, 1970). Even quantitative measures, such as gait analysis only detect damage when massive DA cell loss has already occurred (Meredith et al., 2006).

Are non-motor manifestations in genetically engineered mice also present in humans?

Pitx3 –/– mice have a spontaneous mutation in the homeobox transcription factor Pitx3 with a small eye phenotype (and blindness) and are thereby also named aphakia mice. The role of Pitx3 in DA development was later on identified, with loss of nigrostriatal DA neurons early during post-natal development (Hwang et al., 2005).

A polymorphism in the Pitx3 gene represents a risk factor for PD (Fuchs et al., 2007). Some lines of engrailed knock-out (KO) mice may show a progressive loss of nigrostriatal DA neurons that includes cerebellar pathology, and affective deficits that are reminiscent of the ones found in humans (Sgado et al., 2006; Sonnier et al., 2007).
Engrailed mice models (engrail 1+/− on a background of engrail 2 −/− because knocking out both forms of engrail is embryonic lethal) leads to a progressive loss of nigrostriatal DA neurons but also cerebellar pathology, which confounds behavioral assays of nigrostriatal dysfunction. A line that lacks one copy of engrail 1 with preserved engrail 2 shows more specific nigrostriatal DA cell loss with concurrent marked affective disorders, a frequent symptom of PD.The problem with the relevance of this model is that DA cell loss is progressive but it starts during late post-natal development, and thereby comparatively much earlier than in sporadic PD.
An alternative approach is to use genetically engineered mice that express mutations of familial PD (alpha-synuclein, Parkin, PINK1, DJ1, and LRRK2).
It is widely believed that sporadic PD can occur under conditions in which alpha-synuclein is not mutated but overexpressed and accumulates in Lewy bodies or neurites in a broad range of affected neurons, including but not limited to nigrostriatal DA neurons. Lines of mice expressing mutations in alpha-synuclein have also been generated (critically differing in the promoter used, which also has relevance to the understanding of the model).
It is not clear whether one should consider the TH promoter, which was used to reproduce the loss of catecholaminergic neurons found in PD, which has the disadvantage that restricted expression of the transgene does not mimic the broad alphasynuclein pathology that characterizes the human disease, or if the use of promoters that confer broad neuronal expression are better suited for disease models.
Thy-1, the most abundant mammalian neuronal surface glycoprotein,

in the case of the retina is found predominantly, if not exclusively, on retinal ganglion cells, and therefore lines using its promoter may only mimic ganglion cells effects in the retina (assuming that there is indeed a retinal phenotype in PD). Lines using the Thy1 promoter display pathology (van der Putten et al., 2000), that is not necessarily correlated with levels of transgene expression (Rockenstein et al., 2002). If a phenotype is present, it is accompanied by early progressive sensorimotor deficits that worsen with age (Fleming et al., 2004). These deficits are detected with behavioral tests that are sensitive to nigrostriatal dysfunction (Hwang et al., 2005), occur in the absence of DA cell loss, and are not reversed by levodopa. Therefore, these deficits do not correspond to the symptoms of parkinsonism observed in manifest PD or may represent a model of the non-motor dysfunctions observed in patients. These mice show olfactory and autonomic deficits similar to symptoms often observed before the onset of classical neurological symptoms in PD, which renders them a good model for sensory dysfunction in PD.

Even lines that express truncated alphasynuclein may fail to provide a useful model for PD progression because even if profound loss of DA occurs, the phenotype is present in young animals and does not increase with age (Wakamatsu et al., 2006). All these results put into question the tenet that a model that reproduces both the broad pathology of PD and a robust progressive loss of nigrostriatal DA neurons is viable and worth looking for. An alternative approach will be to analyze models that reflect specific aspects of the disease, and that can be used to test therapeutic approaches.

KO mice have also be generated concerning Parkin, PINK1 and DJ1. Parkin, a E3 ubiquitin ligase, has been linked to proteasomal dysfunction and PD may occur in some patients heterozygous for parkin mutations (Sang et al., 2007; Klein et al., 2007). Mice with exon3 mutations leading to a lack of protein expression show progressive sensorimotor dysfunction without DA cell loss (Goldberg et al., 2003; Itier et al., 2003). In addition lines with an exon7 deletion

show anomalies in paired-pulse inhibition and a non-progressive loss of NE neurons in the locus coeruleus (Von Coelln et al., 2004, Dodson et al., 2007). Some lines show clear non-motor deficits (Zhu et al., 2007). Parkin and PINK1 mutations cause similar alterations in mitochondria (Dodson et al., 2007). This phenotype, however, is not observed in mice (although some show a decrease in evoked DA release in the striatum), raising again questions on the species and tissue specific homologies.

DJ1 mutations cause decreased resistance to oxidative stress in cells, flies, and mice (Dodson et al., 2007), plays a role in recessive forms of familial PD but DJ1 KO mice, however, have virtually absent phenotype and do not have DA cell loss. Concerning LRRK2 mutations a late onset form of familial PD can be caused by a mutation in this gene that encodes a leucine-rich repeat kinase 2 (LRRK2), but more should still be learned about its implications (Funayama et al., 2002).

Local delivery of the genes through stereotactic infusions, to provide overexpression of alpha-synuclein, either with a lentivirus or with an adeno-associated virus, mostly in rats, into the SN, has yielded progressive loss of DA neurons and associated behavioral deficits in rats (Kirik et al., 2002; Lo Bianco e tal., 2002) but these models do neither reproduce the extra-nigral pathology nor model the progressive development of this pathology throughout the nervous system.

In sum, when considering animal models of PD, it is important to distinguish models that reproduce the progressive degeneration of nigrostriatal DA neurons from those that model disease progression in the whole organism.

Clearly, more refined functional approaches are also needed. The hemiparkinsonian rat can be an exceptional model of stepping, postural and balance deficits of PD (Johnston et al., 1999), although the question still remains concerning about non-motor (e.g., proprioceptive) deficits that may also be uncovered by these tests.

Given the evidence that accumulation of alpha-synuclein in neurons of the central and peripheral nervous system is a hallmark of sporadic Parkinson's disease (PD) (Fleming et al., 2008) and mutations that increase alpha-synuclein levels cause familial PD, future research should enhance the focus on non-motor manifestations of Parkinson Disease.

Indeed olfactory and visual dysfunction often precedes the onset of the cardinal motor symptoms of PD by several years and includes deficits in detection, discrimination and identification tasks both in animals and humans (Fleming et al., 2008, Silva et al., 2005; Castelo-Branco et al., 2008).

11. Neural basis of perception and memory: further links to clinical research

We have so far mostly addressed mechanisms underlying retinal disease, visual motion perception and perceptual decision in normal humans. We will now more specifically address how basic research approaches in cognitive neuroscience can be applied in the clinical research setting, to study genetic developmental and acquired disorders of perception and memory.

Our group established new links between basic animal and human clinical research that helped unravel the neural correlates of global motion perception and visual dorsal stream function in health and disease. This basic multilevel hierarchical approach was applied to clinical research questions that helped elucidate mechanisms of disease in Monogenic Macular Degenerations, Glaucoma and even in acquired and genetic neurological disorders with visual impairment, such as Parkinson Disease. The current multimodal retinocortical approaches have also been applied to discover a novel retinal phenotype in a genetic neurodevelopmental disorders (Williams Syndrome) and to dissect its implications for higher level dorsal and ventral stream function. We have also found that motion integration in the central visual field is modulated by peripheral motion. The strength of this modulation is dependent on the level of peripheral motion integration and central ambiguity, which is relevant for visual rehabilitation approaches in central vision diseases.

These approaches can effectively be integrated in pathophysiological studies of neurological disorders. Alzheimer's disease (AD) is known to involve visual pathways, but the nature of its effects on vision still remain quite elusive. One of the main reasons is that most studies do not separate visual from other cognitive processes. A notable exception is the study from Rizzo et al., 2000, which suggested that in AD static visual acuity, stereoacuity, dynamic visual acuity or motion direction discrimination are preserved in AD, in contrast to the worse performance on tests of static spatial contrast sensitivity, visual attention, shape-from-motion, color, visuospatial construction and visual memory.

We have recently also addressed early involvement of specific visual pathways in Mild Cognitive Impairment (MCI), which represents a transitional stage within a cognitive continuum that spans from normal ageing to early dementia. Indeed, mild cognitive impairment (MCI) refers to the preclinical stage of dementia, whereby the majority of individuals develop Alzheimer's disease (AD). As defined by Petersen, MCI involves (i) memory complaint (preferably corroborated by an informant), (ii) essentially normal general cognition, (iii) largely normal activities of daily living , (iv) objective memory impairment for age and (v) absence of dementia. A recent study claimed to be the first to examine visual processing in MCI (Bodke et al., 2008). However it did not find any differences in task performance. Activation changes were also compared by means of functional magnetic resonance imaging (fMRI) in the visual system between MCI and healthy control subjects. The most interesting findings of this study were that were no areas of increased activation in the control group compared with the MCI group. Patterns of activation also reflected some differences since the control group selectively activated the ventral and dorsal pathways during face and location matching tasks, respectively.
Although imaging approaches are now trying to address visual performance, a link with clearcut impairment is still lacking. Accordingly, another recent three-year prospective study (Vannini et al., 2007) aimed to investigate the functioning of brain regions in the visuospatial networks responsible for preclinical symptoms in AD using event-related functional magnetic resonance imaging (fMRI). An angle discrimination task with varying task demands was used but did not yield any performance differences. However, a network of bilateral activations in the dorsal pathway, showed linear enhancement of activity with increasing task demand, in all subjects. Increased parietal activation in progressing MCI was suggested to reflect a reduced "neuronal efficacy" (a highly controversial and poorly defined concept) due to accumulating AD pathology and to

be predictive of future clinical decline. It is noteworthy to point out that these studies are not focused on visual impairment, and are still not using a sufficient number of subjects. In this case the progressing MCI group only included 5 subjects, which precluded random effects analyses that can be generalized to the population.
Another study focused not so much on performance but more on activation patterns and their correlations with morphometric measures (Teipel et al., 2007) and showed that during face matching tasks, fusiform activation is positively correlated with cortical grey matter density of brain areas belonging to the ventral visual stream and negatively correlated with grey matter density of brain areas belonging to the dorsal visual stream and found evidence that these effects are more pronounced in MCI patients than in controls. In this type of task the authors did not observe any statistical differences in task performance or activation between groups (Bodke et al., 2006). In other words, these studies focused on demonstrating that functional connectivity (modulation of correlation patterns across areas) can be an effective marker for the detection of changes in brain function in MCI subjects.
These studies are important because they pinpoint the occurrence of functional and structural (Whitwell et al., 2007) changes in regions of the cerebral cortex (fusiform and parietal) involved in the perception of 3D objects. Similar findings have been uncovered by EEG measures such as a source image study that found three different neural patterns in aged individuals (Haupt el al., 2008). Still other studies (Rombout et al., 2005) have focused on altered resting state networks in MCI in AD.
We have recently run a study focusing on functional impairment profiles, that assessed groups of amnestic MCI (N=20), AD (N=19) and matched controls (N=20). Patients were recruited following diagnosis through neurological and neuropsychological assessment, using Petersen's classification criteria for MCI and NINCDS-ADRDA criteria for AD. All participants provided informed consent and

underwent ophthalmologic examination to exclude any relevant retinal pathology.

Cognitive function was assessed using a neuropsychological battery, consisting of: WAIS III - Vocabulary, Digit Span and Digit Symbol; Rey Auditory Verbal Learning Test (AVLT); Benton Visual Retention Test and Benton Face Recognition Test. Visual function was further assessed using psychophysical tests: Frequency Doubling Test; Cambridge Color Test; Structure From Motion (SFM) perception and visual search.

We identified a novel functional visual biomarker for mild cognitive impairment, by focusing on objective psychophysical tests of contrast sensitivity, color perception, structure from motion and face perception. In MCI patients, specific deficits were found for structure from motion perception (a 3D perceptual deficit similar to the one we found in Williams Syndrome, Mendes et al., 2005). In this task, that we call Motion Coherence of Spherical Surfaces, the stimulus consisted of dots placed on the surface of a rotating sphere 3 degrees in diameter revolving at 20 rotations per minute around an imaginary axis. The sphere revolved around an axis whose angle was chosen in a pseudo-random way, and alternated with a stimulus with 100% noise (temporal two alternative forced choice task). We specifically measured the amount of noise dots a given patient could tolerate until he could not detect the sphere anymore.

Our findings suggest that occipito and early parietal functions become affected early in the course of disease, as already suggested by imaging studies that proposed the presence of cortical degeneration in visual areas. Overall, our results indicate a dissociation between dorsal and ventral visual stream deficits in amnestic types of dementia.

Another view at Williams Syndrome as a Neurodevelopmental model to understand the role of genes versus environment. Implications for new Rehabilitation approaches.

Williams syndrome is characterized by cognitive peaks and valleys defining a profile that can provide a new window to the organization and adaptability to the normal brain. The relative influence of genetics, maturation trajectories, and experience on its Cognitive Profile, which includes relatively good verbal skills alongside very deficient visuo-spatial abilities, and respective developmental trajectories, remain outstanding questions.

The unusual neurocognitive and genetic profiles provide paradigmatic evidence for the modularity of mind because they represent a strong demonstration of the independence of language modules from other cognitive modules. In other words language is a module that is biologically programmed and develops independently of other cognitive abilities. Our recent studies prove that such modularity can also be found within the visual system (Mendes et al., 2005; Castelo-Branco et al., 2007). This is true both concerning serial processing (relative independence of visual dorsal stream processing from its magnocellular input) and parallel computations between the dorsal (impaired vision-for-action pathway) and ventral streams (relatively preserved visual-for-object-recognition pathway)

Impairments of visuospatial functioning may reflect a local visual processing bias in Williams syndrome (e.g., Bellugi, Wang, & Jernigan, 1994). Accordingly, they may be evident in terms of processing the parts or the details of a visual display over the global organization of the visual image (Farran & Jarrold, 2003). Typically developing children tend to prioritize global information (Deruelle et al., 2006) such that normal adults and older children process information at the global level faster than at the local level. This bias to access global information before local information is

known as the global precedence effect.

There is an ongoing debate on whether difficulties in visuospatial construction evidenced by individuals with Williams syndrome are developmental (part of the normal sequence of abilities, see Sampaio et al., 2008) rather than deviant (see the work of Bellugi and colleagues). Our own results fit Mervis et al. (1999) developmental delay hypothesis in visuoconstructive abilities. For instance, drawing abilities of Williams syndrome show a clear improvement shown over time, often resulting in drawings in which the parts of objects are clearly integrated into a coherent whole.

Another important aspect in this respect is that normal performance does not necessarily imply non-deviant cognitive processing. Accordingly, despite their strengths in face recognition, some findings suggest that people with Williams syndrome do not process faces normally (Deruelle et al., 1999; Karmiloff-Smith, 2004).

Research in Williams syndrome may contribute to the actual understanding of the organization and evolution of the human genome as discussed below, because it tackles directly on the role of genes versus environment, and the genetic mechanisms underlying this hemideletion are in fact exclusive to primates.

This syndrome provides one of the most convincing models of a relationship that links genes with human cognition and behavior. We have carefully characterized the microdeletion at 7q11.23 with detailed molecular analysis of the breakpoint region together with well-defined quantitative structure/function correlations in WBS which provided a unique opportunity to investigate the neuromolecular basis of complex visual dysfunction.

The hemideletion probably arises through unequal crossover between flanking large duplicated regions (low-copy repeat sequences (LCRs), with very high nucleotide sequence identity (~ 98%), and that are composed of smaller duplicons as well as genes and pseudogenes. Interestingly, these are not present in the mouse.

In spite of the fact that the hemideletion would suggest a genetic

dosage effect, we have found that similar structure of the breakpoint region and gene copy number did not necessarily lead to similar phenotypes the converse also being true. This raises the question of whether a simple model of haploinsufficiency can explain the phenotype. We also failed to find evidence for genomic imprinting (Castelo-Branco et al., 2007).

We do therefore conclude that genetically determined loss of neural populations at different levels of the nervous system, neural circuits and visual behavior in Williams Syndrome is also strongly influenced by environmental factors and concurrent genetic background.

Indeed, and given the role of several of the involved genes during eye development and differentiation of its neural layers, we identified a novel new retinal phenotype in WS that is functionally unrelated relation to high-level global motion perception and dorsal stream function (Figure 1). This low-level visual phenotype was documented by using a multimodal approach that includes electrophysiology, confocal and coherence in vivo imaging with cellular resolution and psychophysics. Its variability is unlikely to be explained by environmental factors since the retinal circuitry is pretty well established after 6 months of life. Genetic background effects are therefore likely to play also a role.

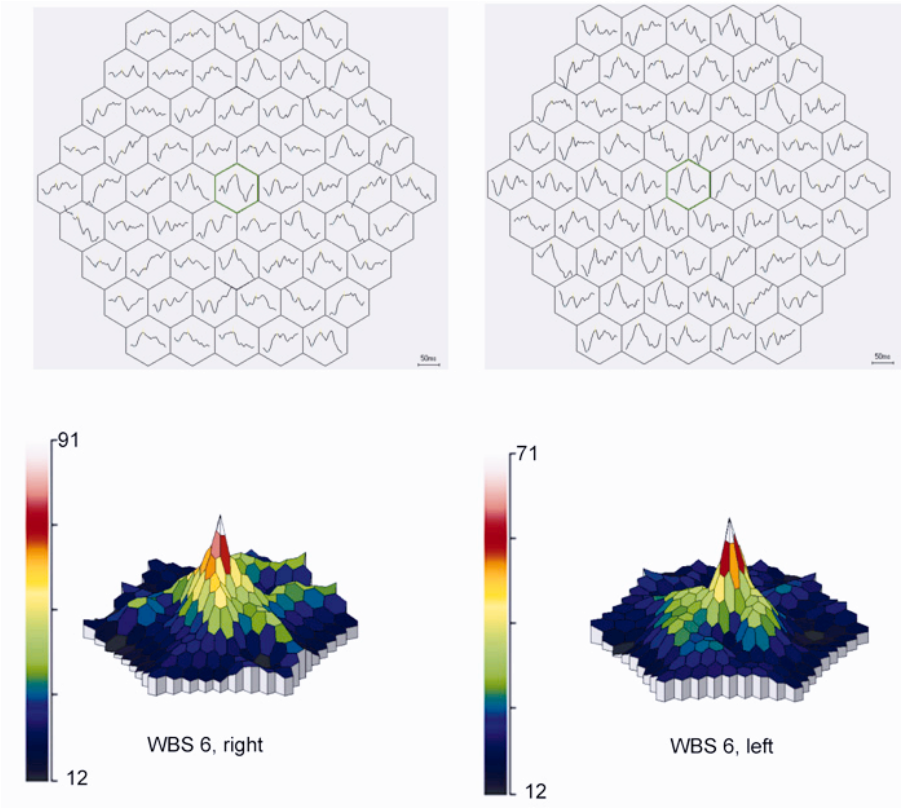

Figure 1
Multifocal electrophysiological recordings from the 2 retinas of a Williams Syndrome subject. The central peak is preserved, but many areas are clearly impaired, with a large heterogeneity within and across eyes. 3D Plots are normalized for the area of visual stimulation.

Low level magnocellular performance, which is involved in the detection of local temporal changes in the visual scene, did not predict high-level global motion integration deficits, which are likely due to intrinsic cortical deficits. Interestingly, parvocellular dysfunction (envolving detailed spatial vision) did not affect cortical ventral stream performance, suggesting that this

pathway can fully compensate for the observed peripheral deficits. The identification of these independent mechanisms of disease, through novel structure-function correlation approaches, suggests that they will require distinct remediation strategies. These findings also challenge neurodevelopmental theories, such as dyslexia, that explain cortical deficits based on low-level magnocellular impairment.

The discovery of retinal and cortex-based phenotypes in Williams Syndrome suggests a new framework for hierarchical genotype-phenotyping in complex and multifactorial diseases of the nervous system. Patterns of dysfunction at early retinal circuits are consistent with the role of genes, including *LIMK1* and *GTF2IRD1,* in the development of retinal neural layers independently of cortical visual deficits in WBS. Furthermore, loss of *Gtf2ird1* in mice also results in a phenotype that is consistent with its expression in the eye and the retina, suggesting that at least in some respects, useful animal models can be generated to study some aspects of the phenotype.

Implications for models of cognitive dysfunction in other neurodevelopmental models

The neurobiological basis of Dyslexia is a longstanding debate (Stein, 2001). Even diagnostic criteria remain an issue, especially because its manifestations are very much language dependent (e.g. the most evident clinical aspects will vary depending on whether the patient's native language is Portuguese, English or Italian). 'Developmental dyslexia' is in fact a form of Low literacy, below that expected from the intelligence quotient (IQ). The presence of incoordination, left-right confusions and poor sequencing put it in the realm of a neurological syndrome. Reading disability, the hallmark of dyslexia, is of course a burden of tremendous personal and social cost. Understanding the neural basis of dyslexia, and

namely why boys are particularly affected, would thereby greatly enhance the possibilities to develop remediation and even rehabilitation strategies.

Unfortunately, there is still a tremendous lack of a biological plausible theory that can withstand scientific validation. We do believe that our multimodal hierarchical approach may help bring about the necessary paradigm shifts to help develop better biological plausible theories of putative visual dorsal stream dysfunction, of which dyslexia is believed to be an example.

But let's first revisit the hypothesis of a visual deficit in dyslexia. Demb and colleagues (1998) measured brain activity (using functional magnetic resonance imaging (fMRI), perceptual thresholds, and reading performance in a group of dyslexic and normal readers to test the hypothesis that dyslexia is associated with an abnormality in the magnocellular (M) pathway of the early visual system. Brain activity was measured in conditions/tasks designed to preferentially stimulate the M pathway. The ability to discriminate slight changes in speed (speed discrimination thresholds), was one such task. Reduced fMRI activity was found in primary visual cortex (V1) and in areas that are believed to receive a predominant M pathway input such as human MT (hMT+). The approach was correlative and a strong three-way correlation was found between brain activity, speed discrimination thresholds, and reading speed. Subjects with higher visual responses in the above mentioned visual areas had better visual magnocellular performance and showed faster reading speed. The authors used these data to claim support for the hypothesis that an M pathway abnormality is present in dyslexia. Furthermore, they claimed that the integrity of the M pathway was crucial for visual motion perception, and reading ability. We have recently proved that this is not necessarily the case in Williams Syndrome, the above mentioned genetic developmental model, thereby fulfilling the Popperian criteria for falsifiablity. But our discovery was not specifically aimed at just destroying a scientific

discovery. On the contrary, it showed that low level visual deficits can be overcome by high level cortical compensation mechanisms. If this is true for other disorders, this may pave the way for the development of new non invasive rehabilitation strategies.

Stein has been one of the most prominent supporters of the visual magnocellular hypothesis in dyslexia. Reading does however require the combination of many skills whose relationship with this theory is not clear. Such skills include the acquisition of good orthographic competence for recognizing the visual form of words and the development of good phonological skills for sounding out unfamiliar words using knowledge of letter-sound conversion rules. Neuroanatomical changes in crossmodal temporoparietal language areas have been found in the dyslexic brain. Since magnocellular-recipient areas such as hMT+ converge in this region, this has nevertheless rendered the hypothesis quite compelling (Stein, 2001).

The problem is that most the evidence is quite circumstantial (Skottun and Skoyles, 2006; Sperling et al., 2006). The fact that the visual magnocellular system signals timing of visual events does not necessarily imply that its failure will underlie abnormal development of orthographic skill. Even if substantial evidence accumulates to show that the development of the visual magnocellular system is impaired in dyslexics, how would this explain even its visual manifestations? Letters may appear to move around and cross over each other, "mirror confusion" of letters may become evident, but these abnormalities may arise due to dysfunction in high order parietal pathways. And why do many dyslexics also have auditory/phonological problems? One could of course postulate an auditory equivalent of the magnocellular pathway that could also be dysfunctional, but this is still a speculation at this point.

In sum, we have been recently able to identify a novel visual phenotype in Williams syndrome that challenges magnocellular theories explaining human neurodevelopmental visual cortical

disorders. Why is our neurodevelopmental model appropriate? Most importantly, we argue that this is the case because the diagnosis is precise and the genetical profile of the patients can be exactly compared. Comparison across multiple disease models should help pave the way for future advances in this field of clinical neuroscience.

12. New methods and new dangers From visual input to social cognition: a BOLD conundrum

Recent studies on high level aspects of cognition and functional connectivity provide some insight in the strengths and pitfalls of recent statistical developments as applied to Brain Imaging.

The recent intriguing findings of Meyer-Lindenberg et al. have been taken to suggest a genetically controlled circuitry regulating social behavior (Meyer-Lindenberg et al., 2005). This claim is based on reduced amygdala responses to threatening face stimuli in individuals with Williams-Beuren Syndrome (WBS), as opposed to threatening scenes. Their exciting results may however have an alternative explanation, which may revive the ongoing discussion on the relative role of input/output activity as the basis of the fMRI BOLD response (Logothetis et al., 2001). Previous anatomical and functional data, and the demonstration that input activity has a major contribution to the BOLD signal (Logothetis et al., 2001) call for the need to explore the explanatory role of the massive visual input to the amygdala. This becomes even more important when one takes into account the previous findings of significant visual-amygdala covariations of activity in normal subjects (Morris et al., 2001; Sabatinelli et al., 2005). It is crucial to know whether these covariations are present or absent in WBS subjects.

Here, we propose a parsimonious alternative explanation to the authors' results, which remain nevertheless very interesting. We believe that the results may be explained by abnormal patterns of anatomical reorganization in the sensory input circuitry of the amygdala, which was shown to be structurally abnormal in WBS (Galaburda et al., 1994, 2000; Reiss et al., 2004). In fact the authors themselves argue that anatomical abnormalities in ventrolateral right thalamus could be a possible explanation for abnormal activity in WS and the same holds true concerning orbitofrontal cortex (Meyer-Lindenberg et al, 2005). We argue that this holds true also in which concerns the amydgala, and that formal attempts to assess additional alternative models of abnormal functional connectivity should be considered.

Corroborating our hypothesis is the fact that a study in a large WBS group has shown that the amygdala is anatomically different (in fact even larger) in WS (Reiss et al., 2004). Meyer-Lindenberg et al. state that "no abnormalities" were found in their subjects, although no quantitative data were presented or discussed. Even assuming that no macroscopic or volumetric changes were present in their sample, unlike in others, there is evidence that distinct subregions of the amydgala are differentially affected, in particular the ones that receive sensory input (Galaburda et al., 1994, 2000; Reiss et al., 2004). A control experiment using with neutral faces would have been very helpful to disentangle the possibility that this might affect the results.

Search for an alternative explanatory model is reinforced by direct post-mortem evidence suggesting that the lateral amygdala may show a reduction up to 50% (Galaburda et al., 2000). Critically, this subnucleus corresponds to the part of the primate amygdala that receives visual input from monkey homologue inferotemporal areas (Galaburda et al., 2000). These areas are known to be responsive to faces. Thereby, the diminished visual activity that the authors have observed to face stimuli in WBS, may be a direct consequence of changed visual cortical organization. Galaburda's report (2000) of an unusual "scooping out", in WBS, of the dorsal part of the lateral subnucleus of the amygdala, which receives input from visual association areas, provides significant support to our hypothesis.

Even if one assumes a normal anatomy in WBS, this does not imply normal functional connectivity. This hypothesis is also corroborated by the findings that WBS individuals have a distinct pattern of visual face processing (avoiding global configural strategies (Bellugi et al., 1999), as also corroborated by distinct difficulties in the perception of other 3D objects (Mendes et al., 2005) even if face recognition scores are normal. This fact is also documented by neurophysiological measurements showing different face-evoked ERPs (event related potentials) in WS (Mills et al., 2000). The more

preserved activation patterns for scenes, may relate to the fact that such a face specic primate TE-Lateral amydgala pathway has not been demonstrated concerning the visual areas that respond to scenes.

Face responsive areas TE/TEO of primate inferotemporal cortex have little or no innervation to amygdaloid nuclei other than the lateral and accessory basal nuclei. If the functional connectivity in their human homologues to the amygdala is compromised, then this would lead to missing intra-amygdala input to the basal nucleus.The relevance of sensory information as conveyed through monosynaptic neocortical inputs to the lateral nucleus (Stefanacci & Amaral, 2002) which is then routed as prominent intrinsic projections to the basal and accessory basal nuclei (Pitkänen and Amaral, 1998) cannot be overemphasized.These massive connections suggest that even the basal nucleus is also an important site of convergence of sensory information, whose disruption could explain a lower BOLD response.

The authors' claim that the ventral amygdala is the region that shows differential activation has to be taken with care, given the EPI distortions due to susceptibility artifacts that usually occur in these brain regions, but is compatible with the postulate that the observed BOLD signal differences were due to abnormal input activity (Logothetis et al., 2001) to the basal amygdala coming from the atrophic lateral nucleus. Unfortunately, analysis in native brain space is not a sufficient procedure to overcome these analysis problems.

We do believe that this question can be tackled with unbiased analysis of functional connectivity of amygdalo-petal and amygdalo-fugal pathways and if possible also with subregional volumetry. Absence of gross abnormalities does not dispel the findings that some parts of the amydgala may be enlarged at the cost of others. Even the "Right" activity bias reported in normal subjects may simply reflect the well known "Right" visual face processing bias.

Moreover, subjects with WBS failed to show "right greater than left" asymmetry patterns in visual face-evoked ERPs (Mills et al, 2000), which is another source of evidence of a sensory based explanation for their patterns of cortical activity.

Consistent with our visual input hypothesis, increased gray matter density in the fusiform gyrus has been associated with WBS-heightened responses to face stimuli in general in spite of abnormal emotional responses (Reiss et al., 2004). This implies that high activity in face responsive areas does not necessarily induce heightened amygdala activity if functional disconnection is present. Also, studies in monkeys have shown altered amygdala discrimination of visual stimuli after reversible cooling of the inferotemporal cortex (Fukuda et al., 1987). Single-unit amygdala recordings have found face selective visual responses in human (Fried et al., 1997) and non-human primates (Leonard et al., 1985) and it remains an open question how these responses change in WBS.

Further evidence for mechanisms relating to a reorganization of the amygdala sensory face-bias, comes from the surprising recent finding that four subjects with bilateral amygdala damage actually performed better in recognizing anger from scenes that had facial information erased than from scenes containing facial expressions (Adolphs and Tranel, 2003). These findings are consistent with a disproportionate role for the normal amygdala in recognizing emotions from facial expressions, rather than from other visual stimuli. Since the amygdala seems to participate in complex judgments about visual stimuli that are not social (Adolphs and Tranel, 1999), this suggests that in WBS there might simply be a similar relative decrease in the face biased analysis. Also, the fact that the human amygdala has an increased response to fearful facial expressions regardless of attention or even awareness (Morris et al., 2001) is consistent with a normal strong feedforward visual extrastriate-amygdala pathway.

We suggest that a way to disentangle sensory bias form emotional

bias would be to measure responses to stimuli yielding similar autonomic reactivity, as measured by skin conductance responses. If the authors had performed a control experiment using with neutral faces then they would have been able to better disentangle some of these points. Analysis of baseline amygdala activity during control tasks with very simple geometrical shapes (Meyer-Lindenberg et al., 2005, Hariri et al., 2002) is not fully satisfactory.

It would also be important to assess whether the normal positive covariation between the amygdala and the visual cortex during normal perception of fearful faces is absent in WBS. This would provide strong evidence for our hypothesis, and is supported by not having been found (or searched for) in statistical path analysis. According to our prediction, the normal amygdala–extrastriate correspondence (Morris et al., 2001; Sabatinelli et al., 2005) should be disrupted in WBS. The critical point to clarify is whether normal activity in the ventral visual stream is coupled or not with amygdala networks, as is the case in normal subjects.

The relation to the claim that the amygdala is an essential component of the neural network for social cognition is also problematic given recent studies with male rhesus monkeys with bilateral ibotenic acid lesions of the amygdala (Amaral et al., 2003). This technique is reliable, because it removes the neurons of the amygdala while sparing en passage fibers. Tested monkeys were not impaired in carrying out social behavior. These findings were replicated in studies in which the amygdala is lesioned bilaterally at 2 weeks of age (Bauman et al., 2004). The lack of fear responses to inanimate objects shows that there is no specific impairment of social fear, which led Amaral et al. to postulate that the amygdala is not an essential component of the neural system involved in social cognition (Amaral et al., 2003). Therefore the amygdala modulates behavior both concerning the safety of social and non social context. Distinction between alternative models or explanations requires formal statistical testing between them (MacCallum and Austin,

2000) or cross-validation with other approaches (Castelo-Branco et al., 2002). However, the authors argue that they have "obtained a well-fitting model". Even if there is an a priory or nested model with a good fit, multiple a priori models should be considered, because alternative well-fitting models often occur even in large numbers. Alternative meaningful explanations, based on other functional anatomy schemes, should also be explored.This so-called "confirmation bias" problem renders the use of single or stacked models quite problematic in structural equation modeling in general and path analysis in particular. The authors' choice of measured areas as an input source of data to the analysis is overconstrained: it only included the efferent pathways from medial prefrontal and orbitofrontal cortex to the right amygdala, and from dorsal prefrontal to medial prefrontal and orbitofrontal cortex. No afferent visual pathways were considered.There is no way to know whether this model is the one with greatest generalizability since there are many other anatomically plausible models. MacCallum and Austin stress that approaches based on modification of a priory model as necessary, until it fits adequately, often lack validity and do strongly capitalize on chance (MacCallum & Austin, 2000).

Model selection problems become evident when one considers that many a priori models may well pass significance tests and be interpretable in a meaningful way, and however none excludes each other. Finding one plausible model, even with goodness of fit measures that are independent of sample size does not prove it holds true. This is important because path analysis is a subtype of structural equation modeling that does not include latent variables and treats measured variables (activity in Regions of Interest) as error-free representations of the constructs of interest (MacCallum & Austin, 2000). One should apply available solutions to choose the most correct model within a class of significant fits (Cudeck & Brown, 1983). Finally, statistical analyses comprising a temporal variation of BOLD responses are more likely to detect significant

amygdala activation and relevant interactions (Tabbert et al., 2005). We do therefore believe that the questions raised by the fascinating study of Meyer-Lindenberg et al. should be clarified in the future with approaches such as independent component analysis.

Plausible biological models of brain function are better validated if they are independently confirmed by model and data-driven approaches (Castelo-Branco et al,. 2002).

Most of the work discussed in this book is related to the central issue of causality and the need for new approaches that will allow for a better handling of complexity in Biomedical Research. This is an essential pre-requisite to effectively model the biology of living systems in health and disease, and to promote better translational and pre-clinical research.

We have focused our own research on the understanding of normal function of the Retina and the Brain, unraveling mechanisms underlying disease processes and effects of therapeutic or neuromodulator manipulations. We do believe that current approaches in Brain Imaging and Neurophysiology are offering new Rosetta Stones to address Causality. The reason is simple: these fields deal with complexity at its limits and need tools that are independent of the subjective human eye or simple observer driven models. In this respect reverse correlation techniques that can predict functional properties just by using random independent stimulation patterns (see Castelo-Branco et al., 2007 for an application in functional studies of the retinocortical pathway) are promising paradigms. Predictive work using EEG/ERP brain recordings to infer future intentions and movements (Pires et al., 2007) or novel biofeedback approaches represent also fascinating trends in this respect.

Our hierarchical approach to visual phenotypes features a correlative multimodal strategy that includes electrophysiology, in vivo imaging and psychophysics (Castelo-Branco et al., 2006; Castelo-Branco et al., 2002) firmly grounded by visual neurophysiology (Castelo-Branco et al., 2000; Biederlack et al., 2006; Schmidt et al., 2006).

One example of an integrated approach that was multimodal, and addressed in a vertical manner the complexity of visual processing in health and disease is provided by our project in Williams Syndrome (Mendes et al., 2005; Castelo-Branco et al., 2007). Before we embarked on this project we have studied parallel pathways to quantitatively analyze normal visual processing and aging in

neurodegenerative disorders of the retina and the brain (Glaucoma, Parkinson Disease, Epilepsy), using psychophysics and behavioral studies, neurophysiology, and functional neuroimaging (Castelo-Branco et al., 2004, 2005, 2006, 2007, 2008, Maia-Lopes et al., 2008a,b, Silva et al., 2005, 2008; Figueiredo et al., 2008).

Taking into account the role of several of the involved genes during visual development, we isolated a novel visual phenotype in WS and its functional relation to high-level abnormal cortical dorsal stream information processing (Mendes et al., 2005). We could also prove that low level magnocellular performance, which was also impaired, did not predict global motion integration deficits, thereby suggesting independent mechanisms of disease requiring distinct remediation strategies (Mendes et al., 2005). Using structure-functional correlational approaches with high resolution imaging and electrophysiology, we could then define two independent phenotypes. The first is based in the retina and has both a magnocellular and parvocellular origin. Interestingly, the cortex can compensate for the parvocellular deficit, by showing near normal object recognition performance (subserved by the cortical ventral stream, Mendes et al., 2005; Castelo-Branco et al., 2007), but not for the magnocellular deficit. The magno pathway projects to the dorsal visual stream, which showed deficits in spatial, motion and 3D object localization, probably due to its own intrinsic neuroanatomical abnormalities. This work thereby provides support for a modular organization of the brain, even though our functional connectivity and independent component analysis approaches show that these modules are densely interconnected (Castelo-Branco et al., 2002, 2006).

We have expanded the use of the above described multimodal techniques to probe system level models with quantitative phenotyping and genetic characterization of photoreceptor retinal degenerations. This was the case concerning both Stargardt and Best diseases, which lead to distinct phenotypes of photoreceptor

degeneration. In the former, we have identified a novel pre-clinical phenotype in relatives that were previously believed to be disease free. Based on this study, and in combination with genetics approaches, we were able to define a novel carrier state, in part based on newly discovered mutations, and in part on redefinition of a pathogenetic role for already known mutations and their dependence on the genetic background. These approaches helped us also shed new light on subtle genotype-phenotype relationships that can only be identified at a functional level. In sum, we have found evidence for widespread retinal dysfunction in patients with Stargardt disease and morphologically unaffected carrier relatives that are related to the novel mutation patterns and likely also to the genetic background. Our ability to provide accurate genetic diagnoses will probably allow for better counseling to patients and families and improvement of the design of therapeutic approaches. Concerning Best disease and the spectrum of disease-causing *VMD2* mutations in Iberian BMD patients we have found further evidence supporting a haploinsufficiency theory, since the severity of the phenotype seems highly dependent of bestrophin activity.

Our approach suggests that combination of novel quantitative psychophysical methods, combined with electrophysiology, imaging and careful genetic characterization, will pave the way for future advances in the understanding of retinal diseases of central vision, which have devastating consequences in elderly populations.

Our focus on Visual Impairment questions, and on the study of the visual system as an objective model system in the Neurosciences (using human and animal models) provided us with the tools to address rehabilitation questions that are anchored on animal research. In recent work, we have characterized acquired retinal degenerations such as glaucoma and age related macular degeneration and their functional impact on central and peripheral vision. We were thereby able to provide models of visual impairment (Kozak & Castelo-Branco, 2008) based on the described patterns

of structure-function and genotype-phenotype correlations (that helped define new biomarkers for retinal degenerations) and studies of normal visual function - in particular, center-surround interactions and how they can be exploited in rehabilitation approaches (Silva et al., 2008; Kozak & Castelo-Branco, 2008). Also in relation to neural plasticity and the effects of context and the environment, we have performed brain plasticity work based on neuropsychology, psychophysics and brain imaging in patient populations (Figueiredo et al., 2008). The latter work allowed us to establish the brain capacity to reorganize its function to the contralateral hemisphere in a performance dependent manner. Indeed, patients with right hippocampal damage due to medial temporal lobe epilepsy could show reorganization of activity to the contralateral hippocampus that was accompanied by specific preservation of performance (Figueiredo et al., 2008). Finally, we have applied these insights in the investigation of mechanisms of disease in neurodegenerative disorders, in comparison to models of normal aging.

We are currently also working on Bayesian probabilistic models for visual and Visuoauditory Perception, based on biological data (Silva et al., 2008) whereby our sensitivity to visual details of the world is constrained by the tensions imposed by evolution on the neural architecture of the retina and the brain. Although based on basic research approaches, this will likely enable a predictive framework to estimate the probability by which given retinal and cortical regions are differentially vulnerable to an environmental insult (Silva et al., 2008).

As final remarks, it is worth pointing out where these developments might lead us into. We aim to tackle a long term debate in human neurodevelopmental biology: what are the limits of neural plasticity in the developing and adult visually impaired human brain? We aim to build on evidence that the ideal period for plasticity and intervention, can actually be extended well beyond the initial years

of visual experience in postnatal life. For that, we need however to explore novel "brain reading" techniques, to assess how the effects of training change the brain (Formisano et al., 2008).

These questions are fortunately linked to basic research questions that seek to understand the neural correlates of 3D object representations and their relevance for visuomotor coordination. Amblyopia is a good model to understand the impairment of stereoscopic representations and their relevance for fine eye-hand coordination. In this sense this goal will hopefully shed new light on the function of recently mapped dorsal stream regions in the monkey and human brains (Castelo-Branco et al., 2006). 3D perception does not necessarily imply stereopsis (see Mendes et al, 2005; and references therein), given that other cues such as motion can be used to generate tridimensional percepts and we aim to discover how such brain regions reorganize in the amblyopic brain.

Additionally such a developmental model may shed light on our own (and of others) work on perceptual decision making (Castelo-Branco et al., 2002, Kozak & Castelo-Branco, 2008), through the investigation of the neural signatures of suppressed/fused interocular representations and the mechanisms and neural correlates of suppression in amblyopia. Using event related electrophysiological approaches, one would be able to also test the hypothesis whether given brain rhythms, such as gamma band synchronization can reflect binocular rivalry/fusion states (Castelo-Branco et al., 1998, 2000, Fries et al., 2005). The identification of such signatures will help identify the target patients for rehabilitation strategies. It should soon become possible to differentiate and seek for conditions whereby representations may be suppressed for distinct eyes depending on the part of the visual field that is being considered and conditions where integration successfully occurs. In this way, it will be possible to "brain read" the effects of training and rehabilitation.

We believe that novel advances including Brain Reading Techniques, such as support vector machines, to analyze imaging data will become crucial in order to better diagnose and understand mechanisms of visual impairment (Formisano et al., 2008). These new fingerprints may help separate distinct types of amblyopia and genetic forms of visual impairment from the functional and structural point of view. In addition to amblyopia, these novel models will likely help explain functional reorganization and its variability due to genetic, intrafamilial and interfamilial variation in genetic models of impairment. Most importantly, they will help find biomarkers that are predictive of treatment outcome and that can "read" the effects of treatment.

The main goal of translational and pre-clinical neuroscience research is to improve the quality of life of people suffering from chronic impairment. We do believe that the integrated approach described in this work concerning visual and high level neural impairment will help establish early diagnostic applications that will help select patients with retinal and cortical disease for the best visual rehabilitation techniques that are now being developed and becoming available.

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Grande Prémio Bial de Medicina 2008

Instituído em 1984, o PRÉMIO BIAL tem vindo a premiar conceituados profissionais de saúde de vários países, reconhecendo e distinguindo a investigação básica e clínica na área da medicina. Promovido pela FUNDAÇÃO BIAL, com periodicidade bienal, é considerado um dos maiores prémios na área da Saúde em toda a Europa.

O júri da edição PRÉMIO BIAL 2008 foi constituído por João Lobo Antunes, que presidiu, e por António Sousa Guerreiro, Henrique Barros, Joaquim Alexandre Ribeiro, José Cunha Vaz, José Manuel Calheiros, Maria de Sousa e Nuno Sousa.

A obra "A vision for medical research in the new era of complexity: a new multimodal integrative paradigm" de autoria de Miguel Castelo Branco do Instituto Biomédico de Investigação da Luz e Imagem - Faculdade de Medicina da Universidade de Coimbra foi galardoada com o GRANDE PRÉMIO BIAL DE MEDICINA.

O PRÉMIO BIAL DE MEDICINA CLÍNICA foi entregue ao trabalho "Uma nova visão das doenças reumáticas inflamatórias: um exemplo de interacção da biologia celular e molecular com a clínica" de autoria de um grupo coordenado por João Eurico Fonseca da Unidade de Investigação em Reumatologia do Instituto de Medicina Molecular - Faculdade de Medicina da Universidade de Lisboa e composto por Helena Canhão, Maria José Santos, Ana Filipa Mourão, Elsa Sousa, Joana Caetano Lopes, Rita Moura, Pamela Weinmann, José Alberto Pereira da Silva, Jaime Branco e Mário Viana Queiroz.

Na décima terceira edição do PRÉMIO BIAL foram também distinguidas três obras com Menções Honrosas.

O PRÉMIO BIAL conta com os altos patrocínios do Senhor Presidente da República, do Conselho de Reitores das Universidades Portuguesas e da Ordem dos Médicos.

Com o objectivo de continuar a divulgar obras de grande repercussão na pesquisa médica e acompanhar a evolução da investigação na área da medicina, a FUNDAÇÃO BIAL vai organizar a edição do PRÉMIO BIAL 2010 envolvendo o GRANDE PRÉMIO BIAL DE MEDICINA, o PRÉMIO BIAL DE MEDICINA CLÍNICA e ainda quatro Menções Honrosas.