Executive summary:

This research program aimed to understand the dynamics of the structural and functional organization of the primary visual cortex and the implications for amblyopia (lazy eye). This information is translated into new treatments of amblyopia based on stimulating plasticity of the visual system. Our research program is subdivided in 8 work packages (WPs).

In WP1 we produced mice expressing a genetically encoded calcium indicator in different neuronal subsets in the visual cortex to chronically monitor their responses. In addition, a novel version of the genetically encoded calcium sensor was developed with much better kinetics and sensitivity.

In WP2 we developed software necessary for automated analysis of neuronal structures and visually evoked activation of biosensors.

In WP3 we developed techniques to record and analyse population responses in V1 to artificial and natural images with single-cell resolution and link this to their connectivity within the network. Using these approaches we discovered that during development neurons with similar feature selectivity become synaptically connected while neurons with different selectivities become disconnected. This process is dependent on visual experience and in amblyopic mice this process is disturbed. Moreover, using an optogenetics approach we discovered that the reduction of visual responses towards the deprived eye in amblyopic mice neurons is not due to increased interocular suppression. Finally, we found that amblyopia in mice causes the binocular tuning of excitatory and inhibitory inputs to deteriorate possibly underlying loss of visual function in amblyopia.

In WP4 we successfully developed methods for imaging inhibitory synapses onto individual excitatory neurons in vivo and assessing their turnover during plasticity. Using this approach we found that loss of visual input either by retinal lesions or monocular deprivation initially causes a rapid loss of inhibitory synapses. After retinal lesions, presynaptic boutons and postsynaptic dendritic spines are also lost and reformed but at a later stage. Functional analyses indicate that these events are aimed at restoring the activity levels in visual cortex.

In WP5 it was found that dark rearing after a period of monocular deprivation increased the effectiveness of recovery of binocular vision. Interneurons did not seem to recover in a different way from excitatory neurons. At the individual neuron level we found that most excitatory neurons participate in the OD shift both during the induction of amblyopia and during its recovery excluding that only a subpopulation of neurons is responsible. Finally, we discovered that increased responsiveness to open-eye stimulation after monocular deprivation are based on different mechanisms during the critical period and in adulthood.

In WP6 we established a learning paradigm in which mice learned to discriminate between visual stimuli of different orientation. Using chronic calcium imaging we found that in mice which learned the task, more neurons became orientation-selective. Moreover, in mice that learned the task well, the subpopulation of neurons that do not strongly respond to the rewarded stimulus play an important role in identifying the non-

rewarded orientations. Most interestingly, the required overall changes of the tuning curves of the subpopulations of low information neurons are relatively small, and therefore only little plasticity is required to reshape the population code to fit a behavioral goal.

In WP7 we identified biological mechanisms restricting plasticity in adult V1. We found that altering histone acetylation or degrading the extracellular matrix quickly increased spine motility in vivo while inhibitory synapse turnover was not strongly affected by degradation of the extracellular matrix. Inhibitors of histone deacetylation helped the recovery of visual acuity in amblyopic rats. Several genes were identified whose promoters showed changed histone acetylation with altered visual experience during the critical period.

Training paradigms to improve vision in amblyopes were developed in WP8. Adult amblyopic subjects showed rapid and robust perceptual learning. With the most effective paradigms, 3 hours of training resulted in improvements similar to 240 hours of traditional monocular occlusion therapy. When subjects were trained to distinguish fine detail they also performed better when tested with less detailed visual stimuli. Perceptual training also improved vision in amblyopic children. Based on these findings we developed an online computer game that incorporates these basic principles to improve vision in amblyopic children and adults. The game will be made freely available to clinicians and the general public at no cost.

This project is of high scientific, clinical and economical value. Insight into neuronal interactions at the scale and resolution we are obtaining is essential for decoding information processed in the CNS. Novel training paradigms that improve treatment of amblyopia are of high economic value as 2-4% of the population is affected and training-based therapies are inexpensive. Identification of signalling pathways that restrict adult plasticity has an impact on disorders of the CNS whose treatments are limited by the rigidity of the underlying neuronal circuits, including plasticity based disorders and trauma of the CNS.

Project context and objectives:

It is a neuroscientist's dream to be able to follow the activity of all neurons in the brain, to know their location, type and connectivity and to study how their responses change over time during perception, learning and behaviour. Three years ago the field of functional brain recording moved one step closer to this dream by using the revolutionising combination of two-photon microscopy and activity-dependent Ca2+-sensitive dyes to simultaneously visualise firing of hundreds of individual neurons in vivo. The functional topography in the outer layers of the cortex can now be observed through a window in the skull at a resolution in time and space never seen before. An important obstacle in studying cortical plasticity remained however: imaging the Ca2+-responses of a given group of neurons over a period of days or longer had not yet been achieved.

Very recently, members of our consortium have successfully produced and tested the first genetically encoded Ca2+-indicator that sets aside this limitation. We have shown that this new Ca2+-indicator has the speed and sensitivity necessary to measure Ca2+-responses in the visual cortex of living animals with a comparable efficiency as Ca2+-sensitive dyes and has no detectable cytotoxicity. We will use this tool and other fluorescent markers, in combination with state-of-the art transgenesis and in vivo two-photon imaging techniques, to study function and dysfunction of the visual cortex.

The visual cortex is the most extensively used model for studying cortical function and plasticity for obvious reasons. Its retinotopy warrants a clear functional organization, the input of the visual cortex is more controllable and the output more comprehensible than any other cortical area, plasticity can be induced readily using physiological stimuli and there is a clear relationship between functional and structural changes that accompany plasticity. Plasticity in the visual cortex is not only a convenient model for studying the workings of the brain - when maladapted it can also cause the most prevalent (2-4%) visual impairment in young people: amblyopia or 'lazy eye'. This visual impairment is caused by an imbalance in binocular vision during childhood resulting in an under-representation of one eye in the visual cortex. The life time risk for amblyopes to become visually impaired in the unaffected eye is 3-18% (van Leeuwen et al) resulting in serious binocular visual impairment in approximately 1:1000 people. Currently, amblyopia can not be cured after the age of 7 due to reduced plasticity in the mature visual cortex.

In this project we want to provide a unified framework for understanding how plasticity and coding work in the normal visual cortex, what neuronal mechanisms correlate with loss of vision due to amblyopia, how this recovers with correction of binocular experience and how we can improve recovery using different interventions.

Chronic Ca2+-imaging in a cell type specific fashion in dozens to hundreds of neurons simultaneously in the visual cortex will allow us to obtain unprecedented insight into the organization and dynamics of the brain. Using this approach we will analyse several fundamental questions on the neurophysiology of the visual cortex, including: how does functional organization of the visual cortex develop and how does experience influence it? What are the principles of network coding of visual information? How do visual experience and perceptual learning

change such responsiveness to specific visual stimuli? How do different neuronal subtypes interact in these paradigms? In addition, by combining the use of fluorescent labels for neuronal structures and Ca2+-indicators or other activity markers we will investigate how neuronal responsiveness and synaptic activity relate to changes in the morphology of neurites and synaptic structures during the induction of plasticity.

But we will also use this approach to study how visual coding is affected in animal models of amblyopia, how different cell types change their responsiveness and interactions in these models, and how observed deficits recover with binocular experience. We will study approaches to improve this recovery in two different ways. The first is to use different stimulation and visual training strategies to increase acuity of the amblyopic eye and understand the underlying neurobiological mechanisms. The results from these experiments will be used to develop novel approaches to treating children and adults with amblyopia. The second is to interfere with biological mechanisms that restrict plasticity in the adult visual cortex. Recently, two such mechanisms have been discovered by one of the consortium members – maturation of the extracellular matrix and changes in transcriptional regulation by histone acetylation. In this project we will employ the new tools and techniques developed here in combination with molecular approaches, to study which neuronal pathways are affected by these mechanisms and responsible for altered plasticity. As the rigidity of neuronal circuits in the adult brain also interferes with the healing of many other disorders of the CNS such as trauma, tumours, cerebrovascular incidents, addiction and many more, this approach holds enormous promise for novel approaches to treat disorders of the CNS

To achieve these goals we have set up a research program that is subdivided in 9 work packages (WPs). WP1 involves the generation of novel genetically encoded biosensors and the production of transgenic mouse models expressing these in specific neuronal subsets of the neocortex. WP2 develops the software necessary for automated analysis of neuronal structures and visually evoked activation of genetically encoded biosensors. WP3 investigates how interactions between inhibitory and excitatory neurons alter under the influence of an imbalance in binocular experience and monitors the changes in binocular interactions in amblyopia. WP4 involves the investigation of morphological changes in neurite and synapse morphology in relation to neuronal activity during development and synaptic plasticity. WP5 aims to uncover the neuronal principles of recovery of binocular vision after amblyopia. WP6 focuses on how perceptual learning changes responsiveness of the visual cortex to meaningful stimuli and tests whether it can accelerate recovery from amblyopia. WP7 deals with identifying the biological mechanisms that restrict plasticity in the adult visual cortex. And last, WP8 develops training paradigms for amblyopic children and adults aimed at improving vision.

WP1. This WP will generate and characterise transgenic mouse lines expressing genetically encoded biosensors (Ca2+-indicators or a marker for presynaptic vesicle release) and lines expressing a red fluorescent marker protein to label inhibitory and excitatory neuronal subsets. By setting up crosses between these lines, mice can be created in which biosensors are expressed in all neurons, while the red protein is expressed in a specific inhibitory or excitatory neuronal subset. This approach allows us to assess activity of many individual neurons using two-photon microscopy, while determining their identity at the same time.

We will also produce adenovirus associated viral (AAV) vectors to express an indicator of CREB transcription factor activation in specific neuronal subsets in the mouse visual cortex in order to allow visualisation of plasticity in vivo.

WP2. A serious limitation in studying chronically imaged activity of neurons in relation to visual stimuli and anatomical localization is that most of the data analysis currently has to be done by hand. The same is true for the analysis of structural changes at the synapse level. In order to improve the speed and quality of data analysis, Bitplane AG will develop new software that will help identify and analyse subcellular compartments including synaptic structures, and relate activation of biosensors expressed in these structures to visual stimuli and anatomical localization.

WP3. Experiments carried out in WP3 use chronic two-photon Ca2+-imaging to follow the plasticity of receptive field properties in mouse visual cortex at the level of individual excitatory and inhibitory neurons labelled with genetically encoded Ca2+-indicators. We will investigate how monocular deprivation (MD) alters the functional characteristics of single neurons imaged chronically over several days. We will study how binocular interactions are affected by MD and which role the interaction between inhibitory and excitatory circuits plays in the development of amblyopia.

WP4. This WP focuses on the relationship between neuronal activity and synaptic connectivity during development and plasticity. In utero electroporations will be employed to express the Ca2+-indicator or a marker for presynaptic vesicle release, together with fluorescent labels that permit the assessment of neuronal morphology. Using this approach we will study 1) how the generation, loss or stabilization of dendritic spines (the protrusions that accommodate most excitatory synapses) correlates with neuronal activity 2) how stability of boutons (the structural correlate of the presynapse) and axon growth and -retraction relate to presynaptic activity and 3) whether inhibitory synapses show dynamics similar to excitatory synapses and how experience influences these dynamics.

WP5. In WP5 we will investigate how vision recovers in animal models of deprivation induced amblyopia. We will study the cellular mechanisms and binocular interactions that underlie this recovery. We will use different paradigms to recover binocular vision, in both the juvenile and the adult visual cortex.

WP6. In WP6 we will test to which degree learning alters the stimulus selectivities of neurons in mouse visual cortex, in normal animals and in animals that were previously monocularly deprived. We will establish vision based behavioural learning paradigms in mice and use chronic two-photon Ca2+-imaging to follow functional changes of single neurons over time as the animal learns. We will also study how behavioural training affects the efficiency and nature of sensory coding in visual cortex. We will make use of a biosensor for CREB activity to visualise perceptual learning induced synaptic plasticity.

WP7. This WP aims at unveiling the mechanisms that restrict plasticity in the adult visual cortex. Our work has previously shown that adult plasticity can be enhanced by treatments acting at i) the extracellular matrix or ii) transcriptional regulation though induction of histone

acetylation. However, little is known about the molecular and cellular mechanisms underlying their effects. By using the imaging tools described in WP1, 3 and 4, we will study how structural plasticity and/or inhibitory innervation are affected by changes at the level of the extracellular matrix or transcriptional regulation. We will also study whether visual acuity in animal models of amblyopia can benefit from activating histone acetylation. By using chromatin immunoprecipitation coupled to quantitative PCR, we will try to understand whether a selection of genes proposed to be involved in cortical plasticity is transcriptionally regulated by epigenetic regulation of histone acetylation. Their regulation in adult and juvenile animals will be compared and promising candidate genes will be studied in more detail.

WP8. Perceptual learning has been used for the successful treatment of disordered language and vision. In this WP, the Visual Neuroscience Group (VNG) at Nottingham University will assess the effectiveness of training using perceptual learning interventions for the treatment of amblyopia, a deficit in the processing of neural signals by early visual cortex. Traditionally, amblyopia is treated by patching the non-amblyopic eye. The perceptual learning approach uses active visual training of the amblyopic eye, avoiding (or at the very least supplementing) the need to patch the 'good' eye. Importantly, this work package will also examine the role of binocular function. This aspect of the work will be closely related to the key objectives of WP3, 5 and 6, all of which examine binocular interactions in the development and recovery from experimental amblyopia. By incorporating games that are fun to play, and collaboration with paediatric ophthalmologists, the methods proven by VNG in the lab will be translated into products suitable for home- and school-based training sessions.

Project results:

Foreground obtained from WP1: Generation of biosensor transgenic mice for assessing neuronal activity in specific neuronal subsets.

Correlating calcium binding, Förster resonance energy transfer, and conformational change in the biosensor TN-XXL.

Genetically encoded calcium indicators have become instrumental in imaging signaling in complex tissues and neuronal circuits in vivo. Despite their importance, structure-function relationships of these sensors often remain largely uncharacterized due to their artificial and multimodular composition. Here, we describe a combination of protein engineering and kinetic, spectroscopic, and biophysical analysis of the Förster resonance energy transfer (FRET)-based calcium biosensor TN-XXL. Using fluorescence spectroscopy of engineered tyrosines, we show that two of the four calcium binding EF-hands dominate the FRET output of TN-XXL and that local conformational changes of these hands match the kinetics of FRET change. Using small-angle x-ray scattering and NMR spectroscopy, we show that TN-XXL changes from a flexible elongated to a rigid globular shape upon binding calcium, thus resulting in FRET signal output. Furthermore, we compare calcium titrations using fluorescence lifetime spectroscopy with the ratiometric approach and investigate potential non-FRET effects that may affect the fluorophores. Thus, our data characterize the biophysics of TN-XXL in detail and may form a basis for further rational engineering of FRET-based biosensors. (Geiger A et al., Biophys J. 2012 May 16;102(10):2401-10.)

Biocompatibility of a genetically encoded calcium indicator in a transgenic mouse model.

Engineering efforts of genetically encoded calcium indicators predominantly focused on enhancing fluorescence changes, but how indicator expression affects the physiology of host organisms is often overlooked. Here, we demonstrate biocompatibility and widespread functional expression of the genetically encoded calcium indicator TN-XXL in a transgenic mouse model. To validate the model and characterize potential effects of indicator expression we assessed both indicator function and a variety of host parameters, such as anatomy, physiology, behaviour and gene expression profiles in these mice. We also demonstrate the usefulness of primary cells and organ explants prepared from these mice for imaging applications. Although we find mild signatures of indicator expression that may be further reduced in future sensor generations, the 'green' indicator mice generated provide a well-characterized resource of primary cells and tissues for in vitro and in vivo calcium imaging applications. (Direnberger S et al., Nat Commun. 2012;3:1031.)

Light-driven calcium signals in mouse cone photoreceptors.

Calcium mediates various neuronal functions. The complexity of neuronal Ca^2 signaling is well exemplified by retinal cone photoreceptors, which, with their distinct compartmentalization, offer unique possibilities for studying the diversity of Ca^2 functions in a single cell. Measuring subcellular Ca^2 signals in cones under physiological conditions is not only fundamental for understanding cone function, it also bears important insights into pathophysiological processes governing retinal neurodegeneration. However, due to the proximity of light-sensitive outer

segments to other cellular compartments, optical measurements of light-evoked Ca^2 responses in cones are challenging. We addressed this problem by generating a transgenic mouse (HR2.1:TN-XL) in which both short- and middle-wavelength-sensitive cones selectively express the genetically encoded ratiometric Ca^2 biosensor TN-XL. We show that HR2.1:TN-XL allows recording of light-evoked Ca^2 responses using two-photon imaging in individual cone photoreceptor terminals and to probe phototransduction and its diverse regulatory mechanisms with pharmacology at subcellular resolution. To further test this system, we asked whether the classical, nitric oxide (NO)-soluble guanylyl-cyclase (sGC)-cGMP pathway could modulate Ca^2 in cone terminals. Surprisingly, NO reduced Ca^2 resting levels in mouse cones, without evidence for direct sGC involvement. In conclusion, HR2.1:TN-XL mice offer unprecedented opportunities to elucidate light-driven Ca^2 dynamics and their (dys)regulation in cone photoreceptors. (Wei T, et al., J Neurosci. 2012 May 16;32(20):6981-94)

Real-time in vivo analysis of T cell activation in the central nervous system using a genetically encoded calcium indicator.

To study T cell activation in vivo in real time, we introduced a newly developed fluorescence resonance energy transfer-based, genetically encoded calcium indicator into autoantigen-specific and non-autoantigen-specific CD4(+) T cells. Using two-photon microscopy, we explored the responses of retrovirally transduced calcium indicator-expressing T cells to antigen in the lymph nodes and the central nervous system. In lymph nodes, the administration of exogenous antigen caused an almost immediate arrest of T cells around antigen-presenting cells and an instant rise of cytosolic calcium. In contrast, encephalitogenic T cells entering the leptomeningeal space, one main portal into the central nervous system parenchyma during experimental autoimmune encephalomyelitis, showed elevated intracellular calcium concentrations while still meandering through the space. This approach enabled us to follow the migration and activation patterns of T cells in vivo during the course of the disease (Mues M et al, Nat Med. 2013 Jun;19(6):778-83).

Forgeground obtained from WP2: Production of software for automated assessment of neuronal structures or -activity

3D and 4D real-time interactive data visualization

Imaris is Bitplane's core scientific software module that delivers all the necessary functionality for data visualization, analysis, segmentation and interpretation of 3D and 4D microscopy datasets. Combining speed, precision and ease-of-use, Imaris provides a complete set of features for working with three- and four-dimensional multi-channel images of any size, from a few megabytes to multiple gigabytes in size. Conveniently load, process and visualize data and images acquired from almost any confocal and wide field microscope to gain new and groundbreaking insight from your image data. Imaris uses Standard Scientific Notation throughout.

Overview

Imaris allows visualization of original and derived data objects in a real time interactive manner so you can quickly make visual assessments of your experiments in 3D and 4D to discover relationships that are otherwise hidden. Its rendering quality, speed, precision and interactivity are unrivalled. With a large variety of segmentation

options, Imaris provides you with the most effective tools to segment even the toughest datasets to identify, separate, and visualize individual objects and then utilize the Imaris MeasurementPro module to pull out the statistics.

Imaris includes features to communicate and share results convincingly: The 'Snapshot' command is a convenient way to export any image view in a standard format that can be used for presentations and publications. The highly advanced 'Key Frame Animator' allows intuitive creation of even the most complex movie from 3D and 4D scenes and allows those movies to be saved as a QuickTime or AVI file. The option to generate a QuickTime VR movie is also provided. No other software gives you so much control at so many levels.

- Visualize volume images and objects in real time using a rich selection of rendering modes.
- Automatically or manually identify objects based on morphology, intensity, size and many more parameters.
- Validate segmentation by superimposing objects on the original volume image.
- Interact dynamically with individual objects.
- Create the most impressive pictures and stunning movies and animations for your publication with just a few mouse clicks

The Imaris interface has been carefully designed for the life sciences. Imaris takes away the burden of selecting and managing multiple poorly integrated imaging tools and increases time spent on research. Imaris exploits the latest hardware technologies to put new capabilities at your fingertips.

Since its launch in 1993 Imaris has been installed at hundreds of leading sites throughout Europe, America, and Asia. Imaris has been chosen as the primary tool for image visualization and analysis by individual investigators, core facility managers as well as operators of our Advanced Imaging Centers. (Imaris (see http://www.bitplane.com/go/products/imaris online))

Forgeground obtained from WP3: Monitoring changes in interactions between inhibitory and excitatory neurons caused by MD

Differential connectivity and response dynamics of excitatory and inhibitory neurons in visual cortex.

Neuronal responses during sensory processing are influenced by both the organization of intracortical connections and the statistical features of sensory stimuli. How these intrinsic and extrinsic factors govern the activity of excitatory and inhibitory populations is unclear. Using two-photon calcium imaging in vivo and intracellular recordings in vitro, we investigated the dependencies between synaptic connectivity, feature selectivity and network activity in pyramidal cells and fast-spiking parvalbumin-expressing (PV) interneurons in mouse visual cortex. In pyramidal cell populations, patterns of neuronal correlations were largely stimulus-dependent, indicating that their responses were not strongly dominated by functionally biased recurrent connectivity. By contrast, visual stimulation only weakly modified co-activation patterns of fast-spiking PV cells, consistent with the observation that these broadly tuned interneurons received very dense and strong synaptic input from nearby pyramidal cells with diverse feature selectivities.

Therefore, feedforward and recurrent network influences determine the activity of excitatory and inhibitory ensembles in fundamentally different ways. (Hofer, S. et al., Nat Neurosci. 2011 Jul 17;14(8):1045-52.)

The emergence of functional microcircuits in visual cortex.

Sensory processing occurs in neocortical microcircuits in which synaptic connectivity is highly structured and excitatory neurons form subnetworks that process related sensory information. However, the developmental mechanisms underlying the formation of functionally organized connectivity in cortical microcircuits remain unknown. Here we directly relate patterns of excitatory synaptic connectivity to visual response properties of neighbouring layer 2/3 pyramidal neurons in mouse visual cortex at different postnatal ages, using two-photon calcium imaging in vivo and multiple whole-cell recordings in vitro. Although neural responses were already highly selective for visual stimuli at eye opening, neurons responding to similar visual features were not yet preferentially connected, indicating that the emergence of feature selectivity does not depend on the precise arrangement of local synaptic connections. After eye opening, local connectivity reorganized extensively: more connections formed selectively between neurons with similar visual responses and connections were eliminated between visually unresponsive neurons, but the overall connectivity rate did not change. We propose a sequential model of cortical microcircuit development based on activity-dependent mechanisms of plasticity whereby neurons first acquire feature preference by selecting feedforward inputs before the onset of sensory experience--a process that may be facilitated by early electrical coupling between neuronal subsets--and then patterned input drives the formation of functional subnetworks through a redistribution of recurrent synaptic connections. (Ko, H. et al, Nature. 2013 Apr 4;496(7443):96-100.)

Foreground obtained from WP4: Experience driven structural dynamics of inhibitory and excitatory connectivity in the visual cortex

A fundamental property of neuronal circuits is the ability to adapt to altered sensory inputs. It is well established that the functional synaptic changes underlying this adaptation are reflected by structural modifications in excitatory neurons. In contrast, the degree to which structural plasticity in inhibitory neurons accompanies functional changes is less clear. Here, we use two-photon imaging to monitor the fine structure of inhibitory neurons in mouse visual cortex after deprivation induced by retinal lesions. We find that a subset of inhibitory neurons carry dendritic spines, which form glutamatergic synapses. Removal of visual input correlates with a rapid and lasting reduction in the number of inhibitory cell spines. Similar to the effects seen for dendritic spines, the number of inhibitory neuron boutons dropped sharply after retinal lesions. Together, these data suggest that structural changes in inhibitory neurons may precede structural changes in excitatory circuitry, which ultimately result in functional adaptation following sensory deprivation. (Keck T, et al., Neuron. 2011 Sep 8;71(5):869-82.)

Elimination of inhibitory synapses is a major component of adult ocular dominance plasticity.

During development, cortical plasticity is associated with the rearrangement of excitatory connections. While these connections become more stable with age, plasticity can still be induced in the adult cortex. Here we provide evidence that structural plasticity of inhibitory synapses onto pyramidal neurons is a major component of plasticity in the adult neocortex. In vivo two-photon imaging was used to monitor the formation and elimination of fluorescently labeled inhibitory structures on pyramidal neurons. We find that ocular dominance plasticity in the adult visual cortex is associated with rapid inhibitory synapse loss, especially of those present on dendritic spines. This occurs not only with monocular deprivation but also with subsequent restoration of binocular vision. We propose that in the adult visual cortex the experience-induced loss of inhibition may effectively strengthen specific visual inputs with limited need for rearranging the excitatory circuitry. (van Versendaal D, et al., Neuron. 2012 Apr 26;74(2):374-83.)

Synaptotagmin-2 is a reliable marker for parvalbumin positive inhibitory boutons in the mouse visual cortex.

Inhibitory innervation by parvalbumin (PV) expressing interneurons has been implicated in the onset of the sensitive period of visual plasticity. Immunohistochemical analysis of the development and plasticity of these inhibitory inputs is difficult because PV expression is low in young animals and strongly influenced by neuronal activity. Moreover, the synaptic boutons that PV neurons form onto each other cannot be distinguished from the innervated cell bodies by immunostaining for this protein because it is present throughout the cells. These problems call for the availability of a synaptic, activity-independent marker for PV+ inhibitory boutons that is expressed before sensitive period onset. We investigated whether synaptotagmin-2 (Syt2) fulfills these properties in the visual cortex. Syt2 is a synaptic vesicle protein involved in fast Ca(2+) dependent neurotransmitter release. Its mRNA expression follows a pattern similar to that of PV throughout the brain and is present in 30-40% of hippocampal PV expressing basket cells. Up to now, no quantitative analyses of Syt2 expression in the visual cortex have been carried out.

METHODOLOGY/PRINCIPAL FINDINGS:

We used immunohistochemistry to analyze colocalization of Syt2 with multiple interneuron markers including vesicular GABA transporter VGAT, calbindin, calretinin, somatostatin and PV in the primary visual cortex of mice during development and after dark-rearing.

CONCLUSIONS/SIGNIFICANCE:

We show that in the adult visual cortex Syt2 is only found in inhibitory, VGAT positive boutons. Practically all Syt2 positive boutons also contain PV and vice versa. During development, Syt2 expression can be detected in synaptic boutons prior to PV and in contrast to PV expression, Syt2 is not down-regulated by dark-rearing. These properties of Syt2 make it an excellent marker for analyzing the development and plasticity of perisomatic inhibitory innervations onto both excitatory and inhibitory neurons in the visual cortex. (Sommeijer et al., PLoS One. 2012;7(4):e35323)

Foreground obtained from WP5: Analysis of mechanisms involved in the recovery from MD

Ocular dominance (OD) plasticity in the visual cortex is a classic model system for understanding developmental plasticity, but the visual cortex also shows plasticity in adulthood. Whether the plasticity mechanisms are similar or different at the two ages is not clear. Several plasticity mechanisms operate during development, including homeostatic plasticity, which acts to maintain the total excitatory drive to a neuron. In agreement with this idea, we found that an often-studied substrain of C57BL/6 mice, C57BL/6JOlaHsd (6JOla), lacks both the homeostatic component of OD plasticity as assessed by intrinsic signal imaging and synaptic scaling of mEPSC amplitudes after a short period of dark exposure during the critical period, whereas another substrain, C57BL/6J (6J), exhibits both plasticity processes.

However, in adult mice, OD plasticity was identical in the 6JOla and 6J substrains, suggesting that adult plasticity occurs by a different mechanism. Consistent with this interpretation, adult OD plasticity was normal in TNFa knockout mice, which are known to lack juvenile synaptic scaling and the homeostatic component of OD plasticity, but was absent in adult a-calcium/calmodulin-dependent protein kinase II;T286A (aCaMKII(T286A)) mice, which have a point mutation that prevents autophosphorylation of aCaMKII. We conclude that increased responsiveness to open-eye stimulation after monocular deprivation during the critical period is a homeostatic process that depends mechanistically on synaptic scaling during the critical period, whereas in adult mice it is mediated by a different mechanism that requires aCaMKII autophosphorylation. Thus, our study reveals a transition between homeostatic and long-term potentiation-like plasticity mechanisms with increasing age. (Ranson, A. et al, Proc Natl Acad Sci U S A. 2012 Jan 24;109(4):1311-6.)

The role of GluR1 in experience dependent depression in the visual cortex is subregion specific Primary sensory cortex is capable of adapting to altered sensory input, a process termed experience dependent plasticity. One form of adaptation is depression at the synaptic level of an input that no longer provides coherent sensory information. In a recent study we found complementary in vivo and in vitro evidence that this type of depression (as induced by whisker removal) in L2/3 of the mouse somatosensory cortex (S1) is dependent upon the presence of the GluR1 AMPA-R subunit (Wright, Glazewski et al. 2008). We have now examined whether an analogous process of depression in the visual cortex (V1) may also be GluR1 dependent. Suturing the eyelid of a mouse (monocular deprivation or 'MD') for 3d at the height of the critical period (P26-P29) causes a robust 30-50% depression of the layer 2/3 V1 response to visual stimulation after eye reopening. This depression occurs both in the binocular region of V1 which receives input from both eyes, and the monocular region which only receives contralateral eye input.

Baseline V1 responses to both ipsi and contralateral eyes were lower in GluR1 -/- mice by ~37%, as measured by intrinsic signal imaging. In binocular cortex, 3d of MD resulted in a 40% depression of closed eye responses in binocular cortex, which as a proportion of baseline responsiveness is comparable to the MD effect we have observed in WT littermate animals. In the monocular region of V1, 3d of MD did not cause depression of closed eye input in GluR1 -/-, unlike the 30-50% decrease seen in wild-types.

Our findings suggest that depression mechanisms operate differently in monocular and binocular regions of visual cortex and that only the former is GluR1 dependent. This suggests that GluR1 may be more important for

homosynaptic depression mechanisms not requiring direct competition for depression to occur and that other mechanisms occur in binocular cortex. (A. Ranson et al, Program No.167.8. 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009)

Foreground obtained from WP6: Assessing training-induced changes of visual performance and neuronal responses in normal and amblyopic mice.

How sensory deprivation and learning change neuronal responses in mouse visual cortex

Neuronal response properties in the brain are not static over time. They can change during development, after deprivation, and following learning. We study such functional plasticity with two-photon calcium imaging, using orientation selectivity in the mouse visual cortex as a model.

One way to alter orientation tuning in the visual cortex is stripe rearing, where animals are exposed to contours of only one orientation for a certain period. Earlier studies have shown that stripe rearing causes a relative overrepresentation of neurons in visual cortex tuned to the experienced orientation. It is not clear, however, whether these changes are merely due to a permissive effect, causing cells tuned to the non-experienced orientations to lose responsiveness, or whether the experienced orientation acts in an instructive fashion, such that some cells actively change their tuning. The main reason for this uncertainty is that with conventional methods it is difficult to assess the proportion of unresponsive cells. This problem can be overcome by two-photon calcium imaging, where all neurons are labeled, thereby allowing for an unbiased determination of the fraction of unresponsive cells.

We have raised juvenile mice for three weeks with cylinder lens goggles limiting visual experience to only one orientation. Following this period, orientation preference in the visual cortex was determined with two-photon calcium imaging. Stripe rearing changed the distribution of preferred orientations such that more cells responded to the experienced orientation than to the orthogonal orientation. The fraction of responsive neurons was lowered, but this effect could not fully account for the changes observed in the distribution of preferred orientations. (M. Hübener, 'Abstracts of the IUPS meeting 2013, Birmingham, UK' abstract number is SA399)

Foreground obtained from WP7: Mechanism of action of treatments reactivating plasticity in the adult cortex

Experience-dependent expression of miR-132 regulates ocular dominance plasticity.

miR-132 is a CREB-induced microRNA that is involved in dendritic spine plasticity. We found that visual experience regulated histone post-translational modifications at a CRE locus that is important for miR-212 and miR-132 cluster transcription, and regulated miR-132 expression in the visual cortex of juvenile mice. Monocular deprivation reduced miR-132 expression in the cortex contralateral to the deprived eye. Counteracting this miR-132 reduction with an infusion of chemically modified miR-132 mimic oligonucleotides completely blocked ocular dominance plasticity. (Tognini P, et al., Nat Neurosci. 2011 Sep 4;14(10):1237-9.)

Extracellular matrix inhibits structural and functional plasticity of dendritic spines in the adult visual cortex.

Brain cells are immersed in a complex structure forming the extracellular matrix. The composition of the matrix gradually matures during postnatal development, as the brain circuitry reaches its adult form. The fully developed extracellular environment stabilizes neuronal connectivity and decreases cortical plasticity as highlighted by the demonstration that treatments degrading the matrix are able to restore synaptic plasticity in the adult brain. The mechanisms through which the matrix inhibits cortical plasticity are not fully clarified. Here we show that a prominent component of the matrix, chondroitin sulfate proteoglycans (CSPGs), restrains morphological changes of dendritic spines in the visual cortex of adult mice. By means of in vivo and in vitro two-photon imaging and electrophysiology, we find that after enzymatic digestion of CSPGs, cortical spines become more motile and express a larger degree of structural and functional plasticity. (de Vivo L, et al., Nat Commun. 2013;4:1484. doi: 10.1038/ncomms2491.)

Inhibition of matrix metalloproteinases prevents the potentiation of nondeprived-eye responses after monocular deprivation in juvenile rats. The ocular dominance (OD) shift induced by monocular deprivation (MD) during the critical period is mediated by an initial depression of deprived-eye responses followed by an increased responsiveness to the nondeprived eye. It is not fully clear to what extent these 2 events are correlated and which are their physiological and molecular mediators. The extracellular synaptic environment plays an important role in regulating visual cortical plasticity. Matrix metalloproteinases (MMPs) are a family of activity-dependent zinc-dependent extracellular endopeptidases mediating extracellular matrix remodeling. We investigated the effects of MMP inhibition on OD plasticity in juvenile monocularly deprived rats. By using electrophysiological recordings, we found that MMP inhibition selectively prevented the potentiation of neuronal responses to nondeprived-eye stimulation occurring after 7 days of MD and potentiation of deprived-eye responses occurring after eye reopening. Three days of MD only resulted in a depression of deprived-eye responses insensitive to MMP inhibition. MMP inhibition did not influence homeostatic plasticity tested in the monocular cortex but significantly prevented an increase in dendritic spine density present after 7 days MD in layer II-III pyramids. (Spolidoro M, et al. Cereb Cortex. 2012 Mar;22(3):725-34.)

Animals lacking link protein have attenuated perineuronal nets and persistent plasticity.

Chondroitin sulphate proteoglycans in the extracellular matrix restrict plasticity in the adult central nervous system and their digestion with chondroitinase reactivates plasticity. However the structures in the extracellular matrix that restrict plasticity are unknown. There are many changes in the extracellular matrix as critical periods for plasticity close, including changes in chondroitin sulphate proteoglycan core protein levels, changes in glycosaminoglycan sulphation and the appearance of dense chondroitin sulphate proteoglycan-containing perineuronal nets around many neurons. We show that formation of perineuronal nets is triggered by neuronal production of cartilage link protein Crtl1 (Hapln1), which is up-regulated in the visual cortex as perineuronal nets form during development and after dark rearing. Mice lacking Crtl1 have attenuated perineuronal nets, but the overall levels of chondroitin sulphate proteoglycans and their pattern of glycan

sulphation are unchanged. Crtl1 knockout animals retain juvenile levels of ocular dominance plasticity and their visual acuity remains sensitive to visual deprivation. In the sensory pathway, axons in knockout animals but not controls sprout into the party denervated cuneate nucleus. The organization of chondroitin sulphate proteoglycan into perineuronal nets is therefore the key event in the control of central nervous system plasticity by the extracellular matrix. (Carulli D, et al., Brain. 2010 Aug;133(Pt 8):2331-47.)

Epigenetic treatments of adult rats promote recovery from visual acuity deficits induced by long-term monocular deprivation.

In mammals the development of the visual system may be altered during a sensitive period by modifying the visual input to one or both eyes. These plastic processes are reduced after the end of the sensitive period. It has been proposed that reduced levels of plasticity are at the basis of the lack of recovery from early visual deprivation observed in adult animals. A developmental downregulation of experience-dependent regulation of histone acetylation has recently been found to be involved in closing the sensitive period. Therefore, we tested whether pharmacological epigenetic treatments increasing histone acetylation could be used to reverse visual acuity deficits induced by long-term monocular deprivation initiated during the sensitive period. We found that chronic intraperitoneal administration of valproic acid or sodium butyrate (two different histone deacetylases inhibitors) to long-term monocularly deprived adult rats coupled with reverse lid-suturing caused a complete recovery of visual acuity, tested electrophysiologically and behaviorally. Thus, manipulations of the epigenetic machinery can be used to promote functional recovery from early alterations of sensory input in the adult cortex. (Silingardi D, et al. Eur J Neurosci. 2010 Jun;31(12):2185-92.)

Foreground obtained from WP8: The effects of perceptual learning in human amblyopia

The pattern of learned visual improvements in adult amblyopia.

PURPOSE: Although amblyopia is diagnosed in terms of a monocular letter acuity loss, individuals typically present with deficits on a wide range of spatial tasks. Many of these deficits can be collapsed along two basic visual dimensions (visual acuity and contrast sensitivity) that together account for most of the variability in performance of the amblyopic visual system. In this study, this space was exploited, to target the main deficits and fully characterize the pattern of learned visual improvements in adult amblyopic subjects.

METHODS:

Twenty-six amblyopic subjects (mean age, 39 ±12 years) were trained on one of four tasks, categorized as either visual acuity (letter or grating acuity) or contrast sensitivity (letter or grating contrast) tasks. Performance was measured on all tasks before and after training, to quantify learning along each dimension and generalization to the other dimension. Performance in 35 visually normal subjects (mean, age 24 ± 5 years) was used to establish normal variation in visual performance along each dimension, against which the learned improvements in amblyopic subjects was compared.

RESULTS:

Training on the contrast sensitivity tasks produced substantial within-task learning and generalization to measures of visual acuity. The learned improvements in performance after training on the letter acuity task were also substantial, but did not generalize to contrast sensitivity.

CONCLUSIONS:

Mapping the pattern of learning onto the known deficit space for amblyopia enabled the identification of tasks and stimulus configurations that optimized learning, guiding further development of learning-based interventions in this clinical group. (Astle AT, et al.,Invest Ophthalmol Vis Sci. 2011 Sep 14;52(10):7195-204.)

Spatial frequency discrimination learning in normal and developmentally impaired human vision.

Perceptual learning effects demonstrate that the adult visual system retains neural plasticity. If perceptual learning holds any value as a treatment tool for amblyopia, trained improvements in performance must generalize. Here we investigate whether spatial frequency discrimination learning generalizes within task to other spatial frequencies, and across task to contrast sensitivity. Before and after training, we measured contrast sensitivity and spatial frequency discrimination (at a range of reference frequencies 1, 2, 4, 8, 16 c/deg). During training, normal and amblyopic observers were divided into three groups. Each group trained on a spatial frequency discrimination task at one reference frequency (2, 4, or 8 c/deg). Normal and amblyopic observers who trained at lower frequencies showed a greater rate of within task learning (at their reference frequency) compared to those trained at higher frequencies. Compared to normals, amblyopic observers showed greater within task learning, at the trained reference frequency. Normal and amblyopic observers showed asymmetrical transfer of learning from high to low spatial frequencies. Both normal and amblyopic subjects showed transfer to contrast sensitivity. The direction of transfer for contrast sensitivity measurements was from the trained spatial frequency to higher frequencies, with the bandwidth and magnitude of transfer greater in the amblyopic observers compared to normals. The findings provide further support for the therapeutic efficacy of this approach and establish general principles that may help develop more effective protocols for the treatment of developmental visual deficits. (Astle, AT et al., Vision Res. 2010 Nov 23;50(23):2445-54.)

Recovery of stereo acuity in adults with amblyopia.

Disruption of visual input to one eye during early development leads to marked functional impairments of vision, commonly referred to as amblyopia. A major consequence of amblyopia is the inability to encode binocular disparity information leading to impaired depth perception or stereo acuity. If amblyopia is treated early in life (before 4 years of age), then recovery of normal stereoscopic function is possible. Treatment is rarely undertaken later in life (adulthood) because declining levels of neural plasticity are thought to limit the effectiveness of standard treatments. Here, the authors show that a learning-based therapy, designed to exploit experience-dependent plastic mechanisms, can be used to recover stereoscopic visual function in adults

with amblyopia. These cases challenge the long-held dogma that the critical period for visual development and the window for treating amblyopia are one and the same.
(Astle AT, et al., BMJ Case Rep. 2011 Feb 23;2011.)

Perceptual learning reduces crowding in amblyopia and in the normal periphery.

Amblyopia is a developmental visual disorder of cortical origin, characterized by crowding and poor acuity in central vision of the affected eye. Crowding refers to the adverse effects of surrounding items on object identification, common only in normal peripheral but not central vision. We trained a group of adult human amblyopes on a crowded letter identification task to assess whether the crowding problem can be ameliorated. Letter size was fixed well above the acuity limit, and letter spacing was varied to obtain spacing thresholds for central target identification. Normally sighted observers practiced the same task in their lower peripheral visual field. Independent measures of acuity were taken in flanked and unflanked conditions before and after training to measure crowding ratios at three fixed letter separations. Practice improved the letter spacing thresholds of both groups on the training task, and crowding ratios were reduced after posttest. The reductions in crowding in amblyopes were associated with improvements in standard measures of visual acuity. Thus, perceptual learning reduced the deleterious effects of crowding in amblyopia and in the normal periphery. The results support the effectiveness of plasticity-based approaches for improving vision in adult amblyopes and suggest experience-dependent effects on the cortical substrates of crowding.

(Hussain Z, et al., J Neurosci. 2012 Jan 11;32(2):474-80.)

Can human amblyopia be treated in adulthood?

Amblyopia is a common visual disorder that results in a spatial acuity deficit in the affected eye. Orthodox treatment is to occlude the unaffected eye for lengthy periods, largely determined by the severity of the visual deficit at diagnosis. Although this treatment is not without its problems (poor compliance, potential to reduce binocular function, etc) it is effective in many children with moderate to severe amblyopia. Diagnosis and initiation of treatment early in life are thought to be critical to the success of this form of therapy. Occlusion is rarely undertaken in older children (more than 10 years old) as the visual benefits are considered to be marginal. Therefore, in subjects where occlusion is not effective or those missed by mass screening programs, there is no alternative therapy available later in life. More recently, burgeoning evidence has begun to reveal previously unrecognized levels of residual neural plasticity in the adult brain and scientists have developed new genetic, pharmacological, and behavioral interventions to activate these latent mechanisms in order to harness their potential for visual recovery.

Prominent amongst these is the concept of perceptual learning--the fact that repeatedly practicing a challenging visual task leads to substantial and enduring improvements in visual performance over time. In the normal visual system the improvements are highly specific to the attributes of the trained stimulus. However, in the amblyopic visual system, learned improvements have been shown to generalize to novel tasks. In this paper we ask whether amblyopic deficits can be reduced in adulthood and explore

the pattern of transfer of learned improvements. We also show that developing training protocols that target the deficit in stereo acuity allows the recovery of normal stereo function even in adulthood. This information will help guide further development of learning-based interventions in this clinical group. (Astle AT, et al Strabismus. 2011 Sep;19(3):99-109.)

Can perceptual learning be used to treat amblyopia beyond the critical period of visual development?

BACKGROUND: Amblyopia presents early in childhood and affects approximately 3% of western populations. The monocular visual acuity loss is conventionally treated during the 'critical periods' of visual development by occluding or penalising the fellow eye to encourage use of the amblyopic eye. Despite the measurable success of this approach in many children, substantial numbers of people still suffer with amblyopia later in life because either they were never diagnosed in childhood, did not respond to the original treatment, the amblyopia was only partially remediated, or their acuity loss returned after cessation of treatment. PURPOSE: In this review, we consider whether the visual deficits of this largely overlooked amblyopic group are amenable to conventional and innovative therapeutic interventions later in life, well beyond the age at which treatment is thought to be effective.

RECENT FINDINGS: There is a considerable body of evidence that residual plasticity is present in the adult visual brain and this can be harnessed to improve function in adults with amblyopia. Perceptual training protocols have been developed to optimise visual gains in this clinical population. Results thus far are extremely encouraging; marked visual improvements have been demonstrated, the perceptual benefits transfer to new visual tasks and appear to be relatively enduring. The essential ingredients of perceptual training protocols are being incorporated into video game formats, facilitating home-based interventions.

SUMMARY: Many studies support perceptual training as a tool for improving vision in amblyopes beyond the critical period. Should this novel form of treatment stand up to the scrutiny of a randomised controlled trial, clinicians may need to re-evaluate their therapeutic approach to adults with amblyopia. (Astle AT, et al.,. Ophthalmic Physiol Opt. 2011 Nov;31(6):564-73.)

The rapid emergence of stimulus specific perceptual learning.

Is stimulus specific perceptual learning the result of extended practice or does it emerge early in the time course of learning? We examined this issue by manipulating the amount of practice given on a face identification task on Day 1, and altering the familiarity of stimuli on Day 2. We found that a small number of trials was sufficient to produce stimulus specific perceptual learning of faces: on Day 2, response accuracy decreased by the same amount for novel stimuli regardless of whether observers practiced 105 or 840 trials on Day 1. Current models of learning assume early procedural improvements followed by late stimulus specific gains. Our results show that stimulus specific and procedural improvements are distributed throughout the time course of learning. (Hussain Z., et al, Front Psychol. 2012;3:226)

A Weber-like law for perceptual learning.

What determines how much an organism can learn? One possibility is that the neural factors that limit sensory performance prior to learning, place an upper limit on the amount of learning that can take place. We tested this idea by comparing learning on a sensory task where performance is limited by cortical mechanisms, at two retinal eccentricities. Prior to learning, visual performance at the two eccentricities was either unmatched or equated in two different ways (through spatial scaling or visual crowding). The magnitude of learning was equivalent when initial levels of performance were matched regardless of how performance was equated. The magnitude of learning was a constant proportion of initial performance. This Weber-like law for perceptual learning demonstrates that it should be possible to predict the degree of perceptual improvement and the final level of performance that can be achieved via sensory training, regardless of what cortical constraint limits performance. (Astle AT, et al., Sci Rep. 2013;3:1158.)

Potential impact:

Potential impact and the main dissemination activities and exploitation results

The project will study the structure and dynamics of visual cortex using a cutting edge set of techniques. By making use of genetically encoded biosensors expressed in the visual cortex of transgenic animals and in vivo two-photon imaging we will be able for the first time to repeatedly image the neuronal activity of dozens to hundreds of individual neurons at the same time, study how experience changes their responses and synaptic connections and how visual scenes are coded. We will do this in the healthy visual cortex and in that of animal models for amblyopia, and specifically study what are the cellular and network mechanisms that underlie loss of vision, and develop behavioural/cognitive approaches to improve treatment of these deficits. Last, we will study the molecular factors that limit plasticity in the adult visual cortex and study the cellular mechanisms that they affect with the ultimate goal to develop strategies to treat neurological disorders by increasing plasticity in the adult brain. Our research will have impact on the knowledge on the function and dysfunction of visual system, on health care and quality of life and on the European economy. To achieve our goals, a combination of highly specialised laboratories is required which can only be realised through collaborations at the European level.

Scientific impact on knowledge of function and dysfunction of the brain

Obtaining an integrated view of the dynamics of neuronal structure and function is one of the main goals of modern neuroscience. This goal is extremely difficult to achieve due to the complexity of the brain and limitations of available techniques. Ideally, one would employ a technique that allows repeated assessment of activity, morphology and arrangement of large sets of individual neurons of known identity. Until recently, no technique existed that fulfilled all these criteria. This consortium is the first worldwide that has acquired all expertise necessary to perform chronic in vivo two-photon imaging of Ca2+-responses in the neocortex by using genetically encoded Ca2+-indicators. This approach allows for the chronic monitoring of neuronal structures and -activity from the subcellular level up to the level of neuronal assemblies. Using this approach we will help to obtain unprecedented insight into the integrated structure and dynamics of the brain and to address fundamental questions on how it codes and stores information through experience.

Our studies will provide a unique overview of the function and dysfunction of the visual system. We will be able to study the neuronal mechanisms of visual plasticity, binocular integration, visual coding and perceptual learning. We will observe how these processes are affected in amblyopia, how they can facilitate or counteract recovery from this visual impairment and study mechanisms that will improve this recovery, from the molecular- to the cognitive level. This information can and will be employed to develop regenerative and restorative therapeutic approaches for amblyopia and potentially for other plasticity related disorders of the CNS.

The data obtained through in vivo two-photon imaging of neuronal activity in response to defined visual stimuli is also a unique resource for scientists working on artificial intelligence, robotics, brain-machine

interfaces and neuroinformatics and for software developers making use of these approaches. Mapping the dynamics of neuronal interactions at the scale and resolution that we intend to do has not been possible before and can help uncover general principles of how neuronal networks can effectively process information and improve their performance through experience.

As we will make the tools developed in this research program (transgenic mouse models, analysis software, expertise on in vivo two-photon imaging) available to the neuroscience community, the scientific impact will not be limited to results from the specific research questions addressed in this project, but will have wide-reaching impact on the future analysis of the structure, function and plasticity of other brain areas.

Impact on health care/quality of life

The research aims to develop novel strategies for treating amblyopia. Amblyopia is often considered a moderate impairment by people who do not suffer from it or do not have children who do. However, amblyopia is the most prevalent visual disorder in young people with 2-4% of the population suffering from it. Currently, children are typically treated by occlusion therapy which can create negative changes in behaviour in children (because of the response of their environment) and a negative impact on family life. These negative effects are also responsible for frequent non-compliance. In addition, insufficiently treated strabismic amblyopia can result in the recurrence of strabismus at later age, which has a significant impact on a person's quality of life. Of all amblyopes, 3-18% will become visually impaired in their unaffected eye in the course of their life (through injury or illness), resulting in eventual binocular visual impairment and severe disability in 1:1000 people. Novel treatments of amblyopia based on binocular training programs could therefore have an important impact on a large part of the population.

Specifically, we anticipate that such treatment will:

Increase effectiveness of treatment. Specific binocular training through computer games will i) increase compliance by causing less negative effects on children's behaviour and family life and ii) specifically improve binocular vision decreasing the risk of slippage of visual acuity after completion of the treatment. More effective treatment will increase job opportunities and significantly reduce the risk of binocular visual impairment during the course of life.

Decrease reoccurrence of strabismus. Treatment aimed not only at increasing visual acuity in the affected eye, but also at increasing binocular vision will help to improve the effectiveness of (surgical) procedures to realign the optical axis and reduce the risk of reoccurrence of strabismus later in life. This will increase the quality of life due to improving social interactions and increasing mobility and safety (double vision is a risk factor in traffic and at work, e.g. operating machinery).

Apart from improving treatments of amblyopia during childhood, we also aim to develop methods for increasing plasticity of the adult visual cortex. One approach will be to use visual training to achieve this. Even if it were only partly successful, this would mean that benefits of improved treatment are not limited to a future generation, but will become accessible to a large population of amblyopes who did not receive

effective treatment during their childhood. Another avenue we take is to study molecular mechanisms that restrict adult plasticity and identify specific signalling pathways that can be targeted by pharmacological approaches. If successful this may, through further development, result in novel therapies for disorders of the CNS whose treatments are currently thwarted by the limited plasticity of the adult brain, including stroke, brain tumours and trauma.

Impact on European economy

Our research program will be economically beneficial in three ways: i) by developing an affordable and more effective treatment for a widespread visual impairment ii) by stimulating a European small and medium-sized entreprises (SME) in developing commercial products that can be marketed world-wide iii) development of patentable products by the scientific partners (such as improved versions of the genetically encoded biosensors, transgenic animal models and novel approaches for increasing adult plasticity).

Affordable treatment for a widespread visual impairment. Treatment of a disease or disorder is considered economically advantageous when the costs of treatment are lower than one per capita Gross Domestic Product (GDP – approximately 25,000 EUROS,- in Western Europe) per quality adjusted life years (QALY – maximum is 1 QALY per year). Amblyopia reduces quality of life by 0.03 QALY per year accumulating to 1-2 QALY per lifetime. In those cases where binocular visual impairment (up to and including blindness) develops, the reduction is much larger (estimated at up to 0.5 QALY per year). The calculated costs of treating amblyopia are less then 2000 EUROS per QALY (Membreno et al 2002). This is very cost effective compared to other treatments (coronary bypass: 5000 EUROS/QALY, treatment of hypertension: 40,000 EUROS/QALY, treating breast cancer: 160,000 EUROS/QALY, liver transplantation: 240,000 EUROS/QALY). Considering the fact that a large part of the European population if affected by amblyopia (2-4%), it is obvious that investing in better treatments of amblyopia is extremely cost effective and of great economical value.

Development of commercial products. Our project will stimulate the production of marketable products by Bitplane and NU. The first product is image analysis software for the automated detection and analysis of neuronal structures and neuronal activity. This product will be of interest for all laboratories analysing neuronal structures imaged with high resolution microscopy techniques (such as confocal and two-photon imaging) and the growing number of laboratories using imaging techniques for measuring neuronal responsiveness and connectivity. The fact that currently, such analyses are performed by hand, which is extremely labour intensive, makes this product an excellent investment for such laboratories. The second product is computer games that help children (at risk of) suffering from amblyopia to train binocular vision and improve vision in the affected eye. The market potential for this product is enormous considering that at least 5% of all children need treatment to avoid the risk of amblyopia. Commercial success of the products developed in this project will result in increased job opportunities at the European market and create capital for the development of other marketable products by the SME involved.

Development of patentable products by scientific partners: The research project will result in the development of various products by the

scientific partners that are potentially patentable. These include novel genetically encoded biosensors, novel mouse models and novel approaches to increase plasticity in the adult CNS. If commercially exploited, this may result in additional funding resources for research at the universities or institutes involved, creating jobs and positions for high level technical and scientific training.

Plan for the use and dissemination of foreground

We will continue to employ various mechanisms for dissemination of obtained results and promoting interest. Foreground obtained from this work has resulted in many publications in high impact journals (IFgreater than9: 1xNature, 2x Nature Neuroscience, 2x Neuron, 1x Nature Medicine, 1x Brain, 1x PNAS, 1x Current Biology, 1x Annual Review of Neuroscience). We expect several more high impact papers to results from the results also obtained within this project. The publicity departments of the participating universities, institutes and SME inform media (newspapers, radio and television) about such publications which can result in newspaper articles or radio interviews.

In support to the communication activities of the Commission services, and in addition to a presentation leaflet, the consortium provided the Commission with a 2 pages information sheet which will be drafted in a standard format communicated by the Commission. Furthermore, publications and consortium news and -activities are announced on the consortium website (see http://www.euroV1sion.eu online) and the websites of the participating institutions. The website also contains information about the project, its objectives, work plan and involved partners. It also acknowledges European Commission's FP7 support and display the EU flag and Seventh Framework Programme (FP7) logo.

Another avenue for disseminating results will be through national and international scientific meetings. Members of our consortium have initiated the first European meeting on plasticity in the visual cortex, which has become a biennial event. This meeting also took place in 2009, 2011 and will take place again in September 2013. At these meetings we present novel and non-published results and discuss scientific and medical implications. In addition, we presented scientific progress at other international meetings, such as the annual meeting of the Society for Neuroscience or the biennial Federation of European Neuroscience (FENS) meeting. We have organised symposia at these meetings such as the symposium 'Imaging development and plasticity in the visual cortex: from synapses to functional networks' at the 2008 FENS meeting in Geneva and the symposium 'Cortical interneurons in visual processing' at the 2012 FENS meeting in Barcelona.

Obtained results are also being used for local teaching purposes with the aim to raise interest in the field by the next generation of neuroscientists. This helps with recruitment of new talent for our research and visual neuroscience in general. Any dissemination activities and publications in the project will acknowledge European Commission's FP7 funding.

Another aim of this project was to develop several marketable and/or patentable products. One product was developed by Bitplane, which is specialised at developing high-level interactive visualization and analysis software. Its team of computational biologists has a tradition of working closely together with academic (including the MPI for

Neurobiology, which serves as one of Bitplane's four 'European Advanced Imaging Centres') and industrial partners to develop and continuously improve novel software applications. Bitplane has previously produced a suite of imaging software that adds valuable analytical and visual features to virtually any 3D or 4D microscope. Within this project it has developed novel image analysis tools that allow the automated detection of neuronal structures. Bitplane Imaris' data model for Filaments was extended by Spines and Tracking. This has been essential for keeping Bitplane a competitive European company in the rapidly evolving field of neuronal structure and function analysis,

Another product is software helping people improve brain functions through specific training paradigms. Research and development was performed by three specialists in vision and ophthalmology from Nottingham University. The software was developed by Ilixa to ensure high quality software and attractive gameplay. This software will be available on line for free in order to reach a broad audience and provide children all over the world with a novel and tested therapy for amblyopia. Over the next weeks, clinicians will be directly informed about the new software, and a press release from participating institutions will be released.

Patent applications will be filed for products with the potential to be commercialised. This may result in Intellectual Property ownership by single or multiple partners of the consortium. IP owned by Bitplane will be used by them for commercial purposes. IP co-owned by scientific partners will be commercialised preferentially by the industrial partners in the consortium. In that case, the scientific partner(s) (co-)owning the IP rights will be paid an adjusted market price for the exclusive licensing of the rights to the SME. In case we develop products that cannot be commercialised by our industrial partners, such as new pharmacological approaches for increasing adult plasticity or novel genetically encoded biosensors, appropriate industrial partners will be sought to whom IP will be licensed in return for payment of an adjusted market price. The consortium members will assist each other in providing data required for patent applications and will refrain from making any patentable discoveries public before the application has been filed. A consortium agreement was furnished in which IP related issues are dealt with comprehensively.

List of websites:

http://www.eurov1sion.eu