

Executive summary:

The overall concept behind the PLASTICISE project is that restoration of the function in neurodegenerative conditions can be achieved through plasticity (the formation of new circuits in the nervous system to bypass lesions). Promoting increased plasticity in selected parts of the adult nervous system back to the level seen in children is a powerful method of enhancing recovery of function in animal models of neurological disease. Plasticity-promoting treatments could therefore be beneficial in a wide range of conditions that damage the CNS.

The project developed new treatment concepts to promote plasticity and methods to measure and visualise their effects, focussing on Alzheimer's disease/tauopathy, stroke and the visual system. In parallel the project has developed new clinical tools that will be needed for clinical trials of plasticity treatments. Clinical investigations and trials were performed, concentrating patients with stroke (the chronic recovery phase, not acute neuroprotection) and Alzheimer's disease.

The specific objectives of the project were to develop:

- New treatments to modulate CNS plasticity
- Studying plasticity at the micro level
- A novel in vivo model of neurodegeneration
- Promoting plasticity in animal models
- Novel methods for studying plastic changes in human patients and primates
- Testing plasticity modulating treatments in human patients

The project combined European expertise in various areas of regenerative medicine and clinical expertise on recovery after brain and spinal cord damage. European groups lead the world in these areas of science. In spanning research from the bench to the bedside it integrated scientists in way that has not previously been achieved in this field. In doing so the project produced several landmark advances at the basic, translational and clinical level.

PLASTICISE achieved a detailed molecular/cellular understanding of the synaptic changes that occur during establishment of memories, and in recovery from brain damage. This provided new knowledge which led to the development of new plasticity enhancement

treatments, which will be developed for promoting recovery of function in human patients with neurodegenerative disease. A particular focus was perineuronal nets, which surround neurons to turn off plasticity in adulthood. Removal of these structures restores plasticity to adult animals. A particularly significant finding was that removing these structures with an enzyme treatment can restore normal memory to animals modelling Alzheimer's disease. At the clinical level PLASTICISE achieved new knowledge of brain connectivity following stroke and other lesions. It also developed new rehabilitation strategies based on combining plasticity with rehabilitation.

PLASTICISE has started the development of new treatment strategies that address some of the fastest-growing clinical disability problems in our community, Alzheimer's disease and stroke, and has developed new treatments for spinal cord injury. It has initiated the development of new treatments to promote brain plasticity, which will restore to health patients with stroke, spinal cord injury, Alzheimer's disease and other crippling CNS disorders.

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Project Context and Objectives:

PLASTICISE is a research consortium bringing together leading scientists, clinicians, small biotech and large pharmaceutical companies from eight different European countries. The project was launched in December 2008 for 4 years. The PLASTICISE researchers have been working together to identify new ways of promoting plasticity in the adult brain following damage.

Brain diseases including Stroke, Traumatic Brain Injury, Spinal Cord injury and Alzheimer's disease represent the majority of long-term disabled people in Europe. These diseases all cause damage to the circuitry of the nervous system (brain and spinal cord), with loss of connections, axons and neurons. The loss can be gradual, as in Alzheimer's disease, rapid as in stroke, or intermediate as in the delayed neuronal loss after stroke. The concept that unites them is the belief that treatments that enhance plasticity will become one of key medications that will improve neurological function in the damaged human nervous system. The purpose of the project was to bring this moment closer.

The overall hypothesis behind the project is that restoration of the function in neurodegeneration can be achieved through plasticity (the formation of new functional connections, withdrawal of inappropriate connections, modulation of synaptic strength). Promoting increased plasticity in selected parts of the adult nervous system back to the level seen in children is a powerful method of enhancing recovery of function in animal models. Plasticity-promoting treatments could therefore be beneficial in a wide range of conditions that damage the CNS.

The promotion of plasticity is a generic form of treatment that will probably be useful in many conditions that damage the CNS, including stroke, traumatic brain injury, spinal cord injury, multiple sclerosis and Alzheimer's disease. These diseases make up the majority of long-term disabled people in the EU. There is currently no treatment for these conditions other than rehabilitation and behavioural therapy. Plasticity treatments will interact with these rehabilitation regimens, greatly increasing their effectiveness. Plastic changes can be maladaptive if misdirected by inappropriate rehabilitation, so the development of integrated therapeutic approaches is essential. The progress towards the development of plasticity-enhancing treatments that will be made by this project will be of great social and economic benefit.

The PLASTICISE project has integrated scientists from four scientific areas;

- 1) Development of methods to promote plasticity;

- 2) Development of models of neurodegenerative disease;
- 3) Imaging of plasticity at the macro and micro level;
- 4) Study of recovery of function through plasticity in human patients with brain disorders.

The concept that unites them is the belief that treatments that enhance plasticity will become one of key medications that will improve neurological function in the damaged human nervous system. The purpose of the project is to bring this moment closer.

The focus of the project was the development of new treatments to promote plasticity and of methods to measure and visualise their effects, focussing on Alzheimer's disease/tauopathy, stroke and the visual system.

The reason for studying the effects of promoting plasticity in both stroke and Alzheimer's disease is that in both conditions there is neuronal loss, requiring adjustments in CNS circuitry to compensate; in one case the loss is focal, in the other diffuse. The inclusion of the visual system is because ocular dominance plasticity is the most sensitive model in which to assay for compounds that promote plasticity. In non-human primates the visual system gives unique opportunities to study plasticity using fMRI and electrophysiology together. Thus, although the project uses several animal models, the focus on the ability of plastic changes to alleviate CNS dysfunction after injury melds the project into a single concept that uses the appropriate animal and human models to test the basic hypothesis. In parallel the project has developed new clinical tools that will be needed for clinical trials of plasticity treatments.

The clinical work has concentrated on stroke patients (the chronic neurodegeneration phase, not acute neuroprotection) and Alzheimer's disease patients. Plasticity has been driven by motor rehabilitation in stroke, by language and memory training in Alzheimer's disease, and combined rehabilitation and plasticity-promoting treatments have been tested. The clinical and basic science strands of the project developed in parallel, but with interaction around imaging methodology, and come together in the development of plans for clinical project of the treatments that have proven effective in animal models.

Project Objectives

The objectives of the PLASTICISE consortium were to advance the current state of the art in five areas:

- 1) Improving current plasticity-promoting compounds and develop new methods for promoting plasticity.
- 2) Developing new in vivo and a new in vitro model of neurodegeneration in which to study plasticity.
- 3) Developing new live-imaging methods to study plasticity at the micro and macro level in vivo and in vitro.
- 4) Conducting clinical studies that will develop new methods for imaging plastic changes.
- 5) Establishing whether a current treatment that is hypothesised to promote plasticity in patients can improve recovery from neurodegeneration.

An important aspect of the overall concept behind the work is the belief of the participants that methods for the controlled enhancement of plasticity that will be developed will be helpful for a range of conditions in which there is neuronal loss, including stroke and chronic neurodegenerative disease.

The advances in state of the art that the project have been done as described in detail in the workpackage strategy, in the work packages themselves.

In summary:

The project will develop new treatments to modulate CNS plasticity. The fundamental requirement of the project is that it has methods to promote plasticity in the adult CNS. There are three existing treatments that have been shown to promote anatomical and physiological plasticity and to improve recovery of function after CNS damage; anti NogoA, chondroitinase and inosine. The project will develop combined treatments to enhance the efficacy of two of these current treatments, and develop novel methods of influencing plasticity.

Novel outcomes from this aim:

- Methods of increasing the efficacy of chondroitinase and anti NogoA and studying the induced plastic rearrangements at the cortical and subcortical levels
- Confirmation that perineuronal nets and their organising molecule link protein are potential therapeutic targets
- Examination of the role of semaphorin 3, MMP-9 and Wnts in the control of CNS plasticity
- Identification of novel plasticity-related genes.

The project will develop new methods and models for studying plasticity at the micro level. The changes in connections and synapses that underlie plasticity have been described at the behavioural and physiological level and there is some knowledge of the sprouting of intact or partially injured pathways. However descriptions at the level of the synapse and local groups of neurons are not well developed. In order to understand how plasticity can be enhanced, and to develop new and better treatments, descriptions at this level are needed. The project will develop the tools for this.

Novel outcomes from this aim:

- Imaging of synaptic changes in an organotypic culture model of AD.
- In vivo imaging of synaptic plasticity in models of AD
- In vivo imaging of synaptic plasticity after stroke
- Examination of remodelling of neocortical dendrites after axotomy and sprouting
- In vivo imaging of astroglial and microglial responses to neurodegeneration
- Assessment of plasticity-promoting treatments
- Examination of Wnt signalling and MMP-9 at the synapse

The project will develop a new in vivo model of neurodegenerative disease. At present the animal models of Alzheimer's disease show synaptic malfunction and a behavioural deterioration similar to AD. However, APP-based models do not show cell death, and are therefore not suitable for the development and assessment of plasticity-promoting treatments. Tauopathy models show gradual neuronal cell loss over months, usually initially in the spinal cord. The project will use AAV technology, together with a new mutated form of tau that aggregates very rapidly to produce rapid and controllable cell death of an AD pattern and aetiology in selected brain regions.

Novel outcomes from this aim:

-A new set of animal models with Alzheimer's disease related deficits generated by viral delivery of mutated Tau and/or APP proteins in selected cortical regions;

The various plasticity-promoting treatments will be tested for their ability to produce recovery in animal models. The project team has a number of models of neurodegeneration, including the AD/tauopathy model in the previous aim, stroke models, retinal lesion models and spinal injury models. In addition levels of plasticity can be tested precisely by measuring ocular dominance changes after closure of an eye. For the various treatments under development by the project, we need to know whether they promote plasticity in the adult CNS in vivo, in which pathways, and which is the most effective for particular types of pathology.

Novel outcomes from this aim:

-Tests of the efficacy of plasticity-inducing treatments at restoring neurological function following neurodegenerative lesions.

-Test of the ability of plasticity-inducing treatments to promote plasticity in the visual system

-Tests of the extent to which appropriate behavioural therapy can have enhanced effectiveness during a window of treatment-induced plasticity.

Novel methods for studying plastic changes in human patients and primates will be developed. At present the majority of clinical studies of plasticity have been in stroke patients, in whom plastic changes have been imaged, measured using transcranial magnetic stimulation and correlated with return of function. Three of the partners are experts in this area, and have access to large clinics. New and reliable neuroimaging tools for observing changes in function and in particular connectivity will be developed using the model of single insult brain injury. Studies will also be performed on changes in brain networks involved in language and memory during the progression of Alzheimer's disease. Plastic changes can be examined in more detail in the the monkey brain, which offers the unique ability to perform functional imaging, electrophysiological and anatomical studies on the same brain, thus providing empirical evidence with which to probe the underlying mechanisms at work during spontaneous or therapeutically induced plastic changes in human brains. The project will develop high resolution imaging combined with electrophysiology, connection tracing and the ability to examine timing of plastic changes at various cortical levels.

Novel outcomes from this aim:

-Development of human structural imaging methods to characterise the residual structural anatomy after stroke and its relationship to impairment and to response to therapeutic interventions.

-Development of human functional imaging methods to examine recovery-related changes in distributed connectivity after stroke and neurodegenerative lesions, based on fMRI, concurrent fMRI-TMS and magnetoencephalography (MEG).

-The application of both structural and functional imaging methodologies to identifying the potential for plastic change in individual patients, leading to stratification according to likelihood of response to various therapeutic interventions either singly or in combination.

-Imaging of progressive changes in brain connectivity and networks during the progression of Alzheimer's disease.

-Imaging of plastic changes following monkey retinal lesions using high resolution fMRI combined with electrophysiology, and connection tracing with Mn²⁺

-Development of new imaging reagents based on Smart Contrast Agent technology.

The efficacy of current plasticity modulating treatments will be tested in human patients. At present the treatments that can be given to human patients to drive plasticity after stroke are repetitive TMS, transcranial direct current stimulation, concurrent sensory stimulation, pharmacological treatments (e.g amphetamine, l-dopa), and observation of movement/motor imagery. Whether these treatments given concurrently with standard physiotherapy are helpful is not clear. The project will select one or more of these treatments, combine it with physiotherapy and monitor changes in the brain with functional imaging to assess whether the combination is beneficial for recovery from stroke.

Novel outcomes from this aim:

-Use of novel imaging methods to identify rehabilitation and plasticity treatments that will be appropriate for individual patients.

-Test the effects of adding a plasticity-promoting intervention to existing therapies on recovery from stroke.

-Application of memory and speech training to patients with Alzheimer's disease and other dementias affecting memory and speech. Monitoring of brain changes using functional imaging.

-Test of plasticity-promoting treatment with cognitive therapy on the progression of -dementia.

Project Results:

WORKPACKAGE 1: NEW TREATMENTS TO MODULATE CNS PLASTICITY

Perineuronal nets control plasticity (Fawcett, Verhaagen, Pizzorusso)

The main focus of research to develop novel methods to modulate CNS plasticity has been the perineuronal net (PNN). This cartilage-like structure appears at the same time as critical periods for plasticity end, enwraps the parvalbumin (PV) interneurons that are known to modulate plasticity, and is digested by chondroitinase ABC (ChABC) that reactivates plasticity.

The first focus was to prove that PNNs are truly the structures that modulate plasticity. A transgenic approach was used. PNNs have a structure similar to cartilage, in which inhibitory chondroitin sulphate proteoglycans (CSPGs) are attached at one end to chains of hyaluronan, at the other to tenascin-C. Binding to hyaluronan is stabilised by link proteins.

At the time of formation of PNNs the only molecule upregulated is link protein, so this was the probable trigger of PNN formation. We produced a transgenic mouse in which link protein was absent in the CNS, and in this animal PNNs were greatly attenuated (Carulli et al. 2010), but all the CSPGs were present in normal quantity. This made it possible to test the hypothesis that PNNs control plasticity by measuring plasticity in adult link protein knockout animals. We found that these animals retain ocular dominance plasticity, plasticity of sensory connections in the hindbrain, replicating the effects of chondroitinase. In addition link protein knockouts have a massive prolongation of novel object memory (Romberg et al. submitted) and a juvenile pattern of fear memory extinction (unpublished results from Pizzorusso laboratory), just like animals treated in the perirhinal cortex or amygdala with chondroitinase. These experiments demonstrate that PNNs are the key controllers of plasticity, that the target of ChABC is PNNs, and identify PNNs as a target for therapeutic development of compounds to enhance recovery from CNS damage and from Alzheimers disease.

Semaphorin3A controls plasticity through association with perineuronal nets (Fawcett, Verhaagen, Pizzorusso)

The fact that ChABC reactivates plasticity through digestion of PNNs indicates that CSPGs in the PNN are the controllers of plasticity. Moreover, because ChABC digests the glycan chains of CSPGs, it must be the glycans of the PNNs that are the key component. Glycans can act in several ways, but the most usual mechanism is through sequestration of active molecules to the glycans, allowing active molecules to interact with receptors in a spatially defined region. It is therefore probable that one or more molecules are attracted to PNNs to be the effector of the PNN. PLASTICISE has focused on the hypothesis that Semaphorin3A is an effector of the PNN. This has been a collaboration within PLASTICISE of the Verhaagen, Fawcett and Pizzorusso laboratories. The Verhaagen laboratory has used immunohistochemistry to show that Sema3A in the adult CNS is localised to PNNs. With the Fawcett laboratory it was shown that digestion of the PNNs by injection of ChABC or hyaluronidase into the brain would remove Sema3A from the treated area, demonstrating that it is binding to the glycans of CSPGs. The Fawcett laboratory has focused on a biochemical characterisation of Sema3A binding to CSPGs. Previous work in the Fawcett laboratory had developed a sequential extraction method that enabled production of brain CSPG extracts from the general matrix, membrane attached CSPGs and PNN CSPGs. Using ELISA it was shown that Sema3A binds specifically to the PNN glycans, not to the CSPGs from other brain compartments. This is shown in the figure, which shows that only PNN GAGs compete with heparin for binding to Sema3A.

This result is congruent with the anatomical binding of Sema3A to PNNs, and demonstrates that Sema3A binds specifically to PNN glycans. This work identifies Sema3A as a protein that binds to PNNs and Sema3A is therefore a potential therapeutic target for treatments that restore CNS function after damage. In order to identify the glycan sulphation motif that is responsible for Sema3A binding, ELISAs using different forms of CS were done. This showed that Sema binds to disulphated CS-E. Sema3A effects can be blocked by expressing soluble Sema3A receptors (neuropilin), so-called Fc-receptor bodies. The Pizzorusso laboratory has shown that neuropilin-1 receptor bodies expressed using an AAV vector produced by the Verhaagen laboratory can reactivate ocular dominance plasticity in the adult CNS, validating the hypothesis that Sema3A controls plasticity. A potential therapeutic reagent would be one that blocks Sema3A binding to PNNs. The Fawcett laboratory collaborates with T. van Kuppevelt, who has developed an antibody that binds to CS-E. It was shown that this antibody blocks the binding of Sema3A to PNN glycans (see figure). This antibody is therefore a potential therapeutic, and is currently being manufactured in sufficient quantity for in vivo testing.

Combination treatment with anti-Nogo A and Chondroitinase ABC is more effective than single treatments at enhancing functional recovery after spinal cord injury (Schwab, Fawcett)

Anti-Nogo-A antibody and Chondroitinase ABC (ChABC) enzyme are two promising treatments that promote functional recovery after spinal cord injury (SCI). Both compounds target inhibitory molecules that restrict axon regeneration and plasticity in the damaged spinal cord. Treatment with them has encouraged axon regeneration, sprouting and functional recovery in a variety of SCI and CNS injury models. The two compounds work in part through different mechanisms, so it is therefore possible that their effects will be additive. In this study we have used a rat cervical partial SCI model to explore the effectiveness of a combination of α -Nogo-A, ChABC and rehabilitation.

In this model we find that spontaneous functional recovery of forelimb functions reflects the extent of the lesion on the ipsilateral side.

We applied a combination treatment with acutely applied α -Nogo-A antibody followed by delayed ChABC treatment starting at 3 weeks after injury, and rehabilitation starting at 4 weeks, to accommodate the requirement that α -Nogo-A be applied soon after injury, and that rehabilitation be given after the cessation of α -Nogo-A treatment. We found that single treatment with either α -Nogo-A or ChABC, both combined with rehabilitation produced functional recovery of similar magnitude in skilled forepaw function and ladder walking. The combination treatment, however, was more effective than either of the single treatments. Both single treatments produced increases in sprouting and axon regeneration, but the combination treatment produced greater increases than the single treatments. α -Nogo-A stimulated growth of a greater number of axons with a diameter of greater than $3\mu\text{m}$ while ChABC treatment stimulated increased growth of finer axons with varicosities. These results point to different functions of Nogo-A and chondroitin sulfate proteoglycans for axonal regeneration. The combination of α -Nogo-A and ChABC shows promise for enhancing the effects of rehabilitation and promoting functional recovery after SCI.

The regenerated axons appeared to have different shapes after treatments. α -Nogo-A and ChABC treatment-induced axon sprouting have different morphologies. Many of the sprouts in the α -Nogo-A group are thick, while in the ChABC group there are a greater number of small-diameter sprouts. Combination treatment enables robust regeneration of the severed axons from the cut terminals and reduces dystrophic bulb formation. The regenerated axons are composed of both thick and thin axons. In contrast, in control group, axons retract a few hundred micrometers from the lesion and form dystrophic bulbs.

Expression of PSA-NCAM in the glial scar (Pharmaxon)

Poly a2,8 Sialic Acid is linked to NCAM when plasticity is needed (mostly during development and traumas). It has been shown that PSA-NCAM is expressed by the glial scar after spinal cord injury (SCI) and those molecular changes are correlated with the sprouting of axons. Graft of cells overexpressing PSA at the lesion site promotes functional recovery after SCI. Pharmaxon has shown that a PSA mimetic peptide PR21 enhances locomotor score after spinal cord lesion and neuroprotective effects after contusion. It was demonstrated that PR21 decreases the glial scar by decreasing the astrocytes hyperexcitability. In addition, PR21 improves locomotor recovery when intrathecally delivered for 14 days at 3 mg/kg. The analysis of the serotonergic fibers regrowth following spinal cord hemisection in mice has demonstrated that PR21 increases the regrowth of the serotonergic fibers. The team has also shown that PR21 significantly improves the time to return of continence in a mouse model of spinal cord injury.

Anti-Nogo A immunotherapy and rehabilitation: Differential effects on functional recovery after stroke (Schwab)

Plasticity in the adult brain after cerebral ischemia is facilitated by the recruitment of 'redundant' synaptic connections within the CNS, and the ability of spontaneous formation of new structural and functional circuits that can re-map related cortical and spinal cord regions. Recent studies have also shown that the spontaneous reorganization and recovery of the sensory and motor forebrain cortex as well as the spinal cord can be affected by a number of factors- such as proteins with neurite growth enhancing (e.g. BDNF) or inhibitory activity (e.g. Nogo-A, Chondroitinsulfate proteoglycans), enriched rehabilitation (ER) and specific training. Although rehabilitation is evidence based from a clinical point of view, the scientific basis to design optimal rehabilitation schedules which enhance the on-going plasticity after stroke is not fully understood concerning the aspects of timing, intensity and kind of rehabilitation. In particular, the combination of rehabilitative training with pharmacological application of growth enhancing compounds is of great interest. The objective of this study was the examination of combined rehabilitative training with growth/plasticity enhancement by anti-Nogo A antibody treatment -sequential or in parallel- in order to compare different rehabilitation schedules for functional outcome. A large unilateral stroke is placed in the motor cortex of the hemisphere corresponding to the preferred side of adult rats, and anti-Nogo A or Ig G control antibodies are infused intrathecally immediately afterwards for 2 weeks. Rats are trained in skilled forelimb tasks simultaneously to Nogo A neutralization or sequentially - 2 weeks after.

Here we demonstrate that rats receiving first the anti-Nogo A treatment followed by training afterwards show excellent (up to greater than 80%) functional restoration of skilled reaching. In contrast, rats receiving training simultaneously to anti-Nogo A antibody treatment did worse than all other groups although they initially performed better than the control group within the first week post stroke. This result shows the importance of timing

and suggests interference between rehabilitative training and anti-Nogo A antibody application.

We analyse the anatomical correlates of these distinct patterns of functional recovery using anterograde cortical tracing and MRI studies. Anterograde BDA tracing studies show that the kind of rehabilitation schedule influences the sprouting of CST fibres from the intact side of the spinal cord across the spinal cord midline: The highest number of direct midline crossing fibres can be found in the group with the best functional outcome (the Anti-Nogo sequential group). We also detected distinct elaborate sprouting patterns of fibres in the spinal cord depending on the rehabilitation schedule. The group with the worst functional outcome (the anti-Nogo parallel group) does not only have significant higher total numbers of newly sprouting fibres crossing the denervated grey matter in the spinal cord - in particular in lamina 7 - but also demonstrates the highest number of white/grey matter crossing fibres.

In addition in a biochemical approach the rehabilitative paradigms are differentiated on a molecular level: Different expression patterns of relevant factors for neuronal growth and axonal guidance are examined using qPCR analysis of different brain areas in order to obtain more insight into the neurobiology of rehabilitation. Using 2-Photon microscopy will further allow us to study neuronal networks in-vivo and the impact of distinct rehabilitation schedule on them.

Changes in cortical maps following anti-NogoA treatment (Schwab, Holtmaat, Verhaagen)

The often impressive degree of functional recovery that accompanies structural plasticity induced by anti-Nogo-A antibodies strongly suggests the occurrence of major changes in wiring on many levels of the CNS. Thus, recovery of locomotion after unilateral transection of all descending and ascending tracts in the spinal cord requires cortical map changes in the sensory and motor cortices. In the studies from the Schwab team animals received a unilateral photothrombotic stroke lesion to the sensory-motor cortex and were treated intrathecally for 2 weeks with an anti Nogo-A antibody.

This treatment enhanced sprouting of the contralesional, intact corticospinal tract (CST) with fibres crossing the midline and innervating the denervated half of the spinal cord. Bilateral retrograde tracing with two different tracers (Fast Blue and Diamidino Yellow) from the intact and the originally denervated side of the spinal cord, at different time points, indicated that the original arbors of sprouting CST fibers were withdrawn when a fiber innervated the opposite, originally denervated side of the spinal cord. Furthermore anterograde tracing with biotinylated dextran amine (BDA) showed also sprouting of the

ipsilateral/ventral CST fibers which terminated in the laminae VII- IX. Intracortical microstimulation (ICMS) of the contralesional motor cortex showed that low threshold currents evoked ipsilateral movements and EMGs at frequent cortical sites in the anti-Nogo-A but not in the control antibody treated animals. These results demonstrate a side switch anatomically and functionally in the projection of adult corticospinal neurons induced by destruction of one sensory- motor cortex and neutralization of the CNS growth inhibitory protein Nogo-A. The teams of Verhaagen and Holtmaat have jointly generated Thy1-Sema3A transgenic mice. These mice display morphological changes in the hippocampus as seen in the figure 5.

Structural plasticity in the adult brain following reinforced learning in the hippocampus (Caroni)

Structural plasticity of axons beyond developmental circuit assembly processes, and in the absence of physical lesions, is a recent discovery, and an exciting addition to the plasticity repertoire of mammalian brains. Although the surface has just been scratched so far, it is clear that these novel aspects of brain plasticity may complement the functional impact of long-term plasticity mechanisms at pre-existing synapses. This is mainly due to the different time scales of the phenomena (seconds to hours, versus days to weeks), and to the spatial scale of the modifications to circuits (axons can sample synaptic territories ranging in the tens and even hundreds of microns).

Due to its unique anatomical features, the mossy fiber projection by dentate gyrus granule cells onto hippocampal CA3 has provided an attractive model system to investigate axonal structural plasticity in the adult. The connectivity between mossy fiber LMTs and CA3 pyramidal neurons exhibits pronounced structural plasticity in the adult. The team of Caroni now established causality relationships between specific rearrangements of mossy fiber synapses in CA3 and learning and memory.

In one study, the Caroni's group showed that mice lacking the plasticity-regulated protein β -Adducin fail to assemble new synapses upon enhanced plasticity, and exhibit diminished long-term hippocampal memory upon environmental enrichment. Enrichment enhanced the disassembly and assembly of dynamic subpopulations of synapses. Upon enrichment, stable assembly of new synapses depended on the presence of β -Adducin, disassembly involved β -Adducin phosphorylation through protein kinase C, and both were required for augmented learning. In the absence of β -Adducin enrichment still led to an increase in spine structures, but the assembly of synapses at those spines was compromised. Virus-mediated re-expression of β -Adducin in hippocampal granule cells of β -Adducin^{-/-} mice rescued new synapse assembly and learning upon enrichment. These results provided evidence that

synapse disassembly and the establishment of new synapses are both critically important for augmented long-term learning and memory upon environmental enrichment.

In a further study we investigated how mossy fiber terminal complexes at the entry of hippocampal and cerebellar circuits rearrange upon learning, and what is the functional role of the rearrangements. We showed that one-trial and incremental learning lead to robust, circuit-specific, long-lasting and reversible increases in the numbers of filopodial synapses onto fast-spiking interneurons that trigger feedforward inhibition. The increase in feedforward inhibition connectivity involved a majority of the presynaptic terminals, restricted the numbers of c-Fos expressing postsynaptic neurons at memory retrieval, and correlated temporally with the quality of the memory. These results established a causal relationship between learning-related increases in the numbers of defined synapses and the precision of learning and memory in the adult. The results further related plasticity and feedforward inhibition growth at hippocampal mossy fibers, to the precision of hippocampus-dependent memories.

The extent to which individual neurons are interconnected selectively within brain circuits is an important unresolved problem in plasticity research. Neurons can reorganize connectivity according to preferentially interconnected microcircuits, but whether this reflects genetically defined subpopulations has remained unclear. We produced evidence that the principal neurons within the major hippocampal subdivisions consist of distinct subpopulations that are generated during distinct time windows, and interconnect selectively across subdivisions.

Brain systems may adjust how they assimilate new information during skill learning in order to improve its effectiveness, but whether and how circuits adjust their plasticity to learning in the adult has remained unclear. Recent studies have suggested that similar reductions in excitation-inhibition balances might underlie enhanced plasticity in the adult and during sensitive periods. We now showed that contextual fear conditioning and environmental enrichment produce opposite alterations in the prevalence of hippocampal parvalbumin (PV)-positive basket cells with high and low PV/GAD67, opposite alterations in excitatory synapse turnover, and opposite alterations in hippocampus-dependent incidental learning (novel object recognition, NOR). We then showed that during hippocampal-dependent skill learning (Morris water maze, MWM) PV+ basket cells shift first to enrichment- and then to conditioning-like configurations as mice learn the task. The shifts in PV+ neurons were matched by changes in synapse turnover and incidental learning. Closely similar PV+ neuron shifts were detected in primary motor cortex upon acquisition and mastering of a rotarod task. Shifts to low-PV/GAD67 distributions correlated with a reversible two-fold increase in the density of specific inhibitory synapses onto PV+ neurons, whereas high-PV/GAD67 shifts involved a nearly 2-fold increase in specific excitatory synapses onto PV+ neurons. Enhancing PV+ neuron firing shifted these cells to high-PV/GAD67 levels. Specifically augmenting or

diminishing transmission at the inhibitory synapses onto PV+ neurons in situ was sufficient to correspondingly alter PV/GAD67 distributions, synapse turnover and NOR performance, and to mimic how previous enrichment or conditioning influence learning rates in the MWM. Furthermore, local treatments with chondroitinase ABC or BDNF were sufficient to specifically shift PV+ neurons to a high-PV/GAD67 distribution, reducing synapse turnover and performance in NOR and MWM. These results reveal a circuit-based mechanism involving alterations in specific inhibitory or excitatory connectivity onto PV+ basket cells to locally regulate activity processing and plasticity, and promote the acquisition and exploitation of adaptive skills during adult learning.

Brain experience-dependent plasticity is in part supported by the activity of MMP-9 in adult mouse (Kaczmarek, D-Pharm)

Modifications of properties of the adult sensory cortex by elimination of sensory input (deprivation) serves as a model for studying plasticity in the adult brain. The team of Kaczmarek studied the effects of short- and long-term deprivation (sparing one row of vibrissae) upon the barrel cortex. The response to stimulation (exploration of a new environment) of the spared row was examined with [¹⁴C]-2-deoxyglucose autoradiography and c-Fos immunohistochemistry. Both methods found large increases of the functional cortical representation of the spared row of vibrissae, extending into parts of the barrel cortex previously activated by the deprived vibrissae. With both methods, the greatest expansion of spared input was observed in cortical layer IV. In this way, we established a model, which was applied for examining involvement of matrix metalloproteinase 9 (MMP-9), upon experience-dependent cortical plasticity. MMP-9 is an enzyme implicated in plastic modification of the neuronal connections. We found that MMP-9 activity was increased in response to stimulation, and furthermore, MMP-9 knockout mice showed a modest but significant decrease of plasticity in layer IV with 2-DG mapping and in layers II/III with c-Fos mapping. Thus, in adult mouse brain experience-dependent plasticity is in part supported by the activity of MMP-9. This study has been published in 2012 (see reference below: Kaliszewska A. et al., *Cerebral Cortex* 22: 2160-2170).

The company D-Pharm has synthesized 20 compounds that can be expected to modulate MMP9. The company has improved on screening methods for these compounds for mechanistic studies. The current focus of DPL is on DP-99, a unique broad-spectrum neuroprotective drug that addresses an array of brain damaging processes occurring in acute ischemic stroke patients. D-Pharm has demonstrated that DP-99, in vivo, reduces MMP-9 activity, and it is correlated with reduced infarct volume and improved recovery.

In collaboration with the company D-PHARM, the team of Kaczmarek has found that DP-b99 inhibits MMP-9 activity in a biochemical assay, as well as MMP-9-dependent functions, such

mediating kainate excitotoxicity, α -dystroglycan cleavage and morphological modulation of the dendritic spines, possibly involved in the synaptic plasticity. Furthermore, DP-b99, when applied to the animals, suppresses development of chemically kindled epilepsy, inhibiting also in the brain cleavage of the α -DG and mossy fiber sprouting. Thus, this compound displays potent anti-epileptogenic activity, probably via preventing aberrant plasticity driven by MMP-9.

WORKPACKAGE 2: STUDYING PLASTICITY AT THE MICRO LEVEL IN RESPONSE TO NEURODEGENERATION

Observing synaptic plasticity in organotypic slice culture models (Caroni, Fawcett)

The team of Pico Caroni (PC) has been investigating how structural plasticity is regulated during the maturation of hippocampal circuits and in mature circuits. They found evidence that the earliest born subpopulation of principal neurons has a dominant effect on circuit maturation, and that this takes place first in CA3. Mossy fiber terminals by Lsi1 granule cells establish filopodial synapses onto parvalbumin (PV) interneurons, and these synapses drive maturation of PV basket cells. This maturation of the basket cells in CA3 is a prerequisite for their maturation in CA1, suggesting that regulation in CA3 drives maturation of the entire hippocampal loop.

In similar experiments they have manipulated synaptic activity in CA3 either through pharmacological experiments or through specific learning protocols. The outcome of these experiments suggests that events initiated by synaptic transmission at mossy fibers in CA3 influence PV levels, synapse numbers onto PV cells and structural plasticity in the hippocampus. These results suggest that specific circuit elements, in this case the synapses of mossy fibers in CA3, may have a dominant role in regulating the plasticity of entire systems, here the hippocampus, in the adult. This study has been published by the team of PC in 2011 (see Ruediger S. et al. Nature 2011)

In addition, the team of James Fawcett (JF) has been collaborating with the team of Olivier Raineteau in Zurich, who are finding that digesting cortical slices with chondroitinase activates motility of dendritic spines, with formation of spine head protrusions. In the adult CNS spine dynamics can be influenced by experimental manipulations that lead to remodeling of the mature extracellular matrix (ECM). Hence, we assessed the effect of ChABC-mediated chondroitin sulfate proteoglycans (CSPG) digestion on spine morphology and motility. We treated hippocampal organotypic slices with ChABC for 4 hours (0.5 unit) or overnight (0.25 units). We then measured different spine morphological parameters by live

confocal microscopy on the proximal and distal dendritic tree of CA1 pyramidal cells (Figure 2A).

Spine morphology was described by means of two critical determinants of synaptic function, spine length and spine head circularity. In our model, ChABC-mediated CSPG degradation did not affect the average spine length or the spine head circularity. Spine motility was measured as the average spine fluctuations in the xy axis over recording time. Imaging of apical dendrites revealed that both regimes of ChABC treatment (4 hours and overnight) led to a significant and equivalent increase in spine motility compared to sham-treated slices.

Newly formed spines are normally characterized by higher rate of motility compared to mature established spines. Therefore, we next assessed whether CSPG digestion could lead to formation of new spines. The mean spine density after ChABC treatment was not different from the density of the control group (p greater than 0.5), indicating that ChABC-mediated CSPG digestion does not induce formation of new spines but modulates the dynamics of pre-existing spines.

In vivo imaging of synaptic plasticity in models of AD (Helmchen)

The collaboration of the teams of Fritjof Helmchen (FH) and Roger Nitsch (University of Zurich) has resulted in significant insights about in vivo remodelling of neural circuit activity in transgenic Alzheimer mice (work by Annapoorna Bhat). We expressed the genetically encoded calcium indicator Yellow Cameleon 3.60 (YC3.60) in mouse somatosensory neocortex using AAV constructs (Fig. 2). The spontaneous neuronal activity rates of the exact same layer 2/3 neurons were then repeatedly measured over up to 8 weeks.

To investigate synaptic remodelling in the presymptomatic (pre-plaque) phase of Alzheimer's disease we set up a systematic series of experiments with three experimental groups:

- 1) transgenic APP^{swe}Arc mice;
- 2) control wildtype mice;
- 3) transgenic APP^{swe}Arc mice, in which we employed passive immunization of 7-month-old APP^{swe}Arc mice by i.p. injections of the anti- β antibody (5 mg/kg antibody injection once a week).

Vehicle-treatments were performed in animal group 1. Long-term two-photon imaging was performed through a chronic cranial window. As a first read out of synaptic activity we quantified the spontaneous rates of action potential activity driven by ongoing synaptic activity. Action-potential activity was reconstructed from the measured calcium transients. Consistent with previous reports, spontaneous rates in layer 2/3 neurons were low (0.1-0.2 Hz). Over the time course of 8 weeks we found that Alzheimer mice showed progressively reduced activity rates compared to wildtype and antibody-treated mice. These results suggest that in the Alzheimer mouse model, even in the pre-plaque phase there is already evidence for synaptic impairment, which however was prevented by treatment with anti-beta antibody. Our results emphasize the strength of long-term in vivo imaging experiments for revealing functional alterations in models of neurodegeneration.

In vivo imaging of synaptic plasticity after stroke (Helmchen, Schwab, Holtmaat)

The teams of Martin Schwab (MS) and FH have conducted collaborative experiments to assess, in rodents in vivo, the synaptic changes that can occur following stroke. Wolfgang Omlor from the team of FH and Anna-Sophia Wall from the team of MS have made very good progress in their collaborative experiments with the following goals:

- 1) long-term functional imaging of identified neuronal populations before and after stroke in the contralesional intact hemisphere in order to directly observe compensatory functional changes in these populations
- 2) optical motor mapping using channelrhodopsin-2 (ChR2). In ChR-2 expressing mice a motor map can be generated using this light-activatable channel. The goal of this development is to assess the change in the motor map following a large-scale stroke and ultimately relate it to recovery of motor behaviour.

Various studies (fMRI data in human trials, experimental stroke research in rodents) have demonstrated intense plasticity, sprouting and re-wiring of functionally relevant connections in the sensorimotor cortex after stroke. Nevertheless detailed analysis of network re-organization physiologically and in dependence on different rehabilitation schedules is still lacking. Optogenetic stimulation and calcium imaging with 2-photon microscopy are two powerful tools we have established to study those map shifts and network reorganizations.

In the first step we implanted a chronic glass window in the skull over the sensorimotor cortex and introduced a photothrombotic stroke in the forelimb area of one hemisphere in channelrhodopsin-2 mice in order to selectively destroy the function of the corresponding upper extremity. Optical mapping is used to continuously study the peri-lesional functional re-organization within the motor cortex up to several weeks after stroke. The animals are re-

tested at least once a week in a grasping task or are included in a rehabilitation group receiving intensive training of the impaired forelimb after stroke. With this set-up in on-going experiments we will be able to address questions such as the spatial and temporal re-organization of the sensorimotor cortex and the influence of rehabilitative training on the re-organization of proximo-distal joints in the upper extremity.

Finally, the team of Anthony Holtmaat is working with stroke models. In these models they induce miniature strokes in the barrel cortex using photothrombosis. They repeatedly image GFP transgenic mice using two-photon laser scanning microscopy through a chronic cranial window. They can pick a small region in which they induce a stroke, in the order of 400 μm , and monitor the reactivity of dendritic and axonal structures. They are comparing different types of dendrites near the lesioned area in their ability to form new synapses. The team of AH will also monitor different types of axons. Together this will provide a picture of how neuronal structural changes could underlie or benefit functional recovery. Spontaneous functional recovery is monitored using imaging of intrinsic optical signals.

Remodelling of neocortical dendrites after axotomy and sprouting (Schwab, Helmchen)

Mice with genetically labelled pyramidal neurons (thy-1-YFP) in the motor cortex were subjected to defined lesions of the corticospinal tracts in the lower thoracic spinal cord, combined with injection of a retrograde tracer. The team of MS has evaluated the density of spines on the axotomized corticospinal neurons at 3, 7, and 21 days after the injury. Spine density of the dendritic segment proximal to the soma (in layer 5) declined as early as 3 days after injury, far preceding the onset of somatic atrophy. In the distal segment (in layer 2/3), spine loss was slower and less severe than in the proximal segment. Axotomy of corticospinal axons in the brainstem (pyramidotomy) induced a comparable reduction of spine density, demonstrating that the loss is not restricted to the neurons axotomized in the thoracic spinal cord. Surprisingly, in both forms of injury, the spine density of putative non-axotomized layer 5 neurons was reduced as well. The spine loss may reflect fast rearrangements of cortical circuits after axotomy, for example, by a disconnection of hind limb cortical neurons from synaptic inputs that no longer provide useful information. These changes and the synapses that remain may play a critical role in the functional reorganization of the adult cortex after spinal cord injury. These data were published in 2012 (Ghosh A. et al, Cerebral Cortex).

In vivo imaging of astroglial and microglial responses to neurodegeneration (Helmchen)

The Fritjof Helmchen (FH) team (in collaboration with R. Nitsch, UZH) has performed in vivo two-photon imaging experiments to measuring structural dynamics of microglial cells in AD mice. To this end we used crossed APP^{sweArc};Cx3CR1 +/- mice, in which GFP is expressed in cortical microglial cells. Two-photon microscopy enables us to resolve the fine microglia processes, which are highly dynamic structures. To fluorescently label A β plaques we either used intraperitoneal injection of Methoxy-X04 prior to the imaging sessions or we applied fluorescently-tagged anti-A β antibody (using either Cy-2 or Texas-Red), which was administered directly into the cortical tissue (Fig. 12). As anti-A β antibody we used an anti-A β IgG2a targeted against fibrillary A β , generated by the Nitsch group. Co-labeling of microglia and plaques enabled us to visualize the agglomeration of microglia around plaques and repeatedly image their structural organization.

We were particularly interested to quantify whether anti-A β antibody application may lead to detectable activation of microglia or recruitment of microglia cells towards A β plaques. In several case we could observe how microglia took up fluorescently-tagged A β with their dynamic processes, indicating active 'work' of microglia on the plaque material. In long-term imaging experiments over up to 3 days, we found clear signs of structural microglia reorganization, including morphological rounding up as well as recruitment of distant microglial cells that apparently were attracted by the antibody-tagged plaque. These experiments highlight the involvement of microglia in the naive response to AD plaque deposits as well as in reaction to antibody treatments.

Besides imaging microglia-plaque interactions in neocortex we have also been successful in visualizing microglia dynamics in the dorsal horn of the spinal cord. Various combinations of labeling techniques can now be used to study the interaction of microglia cells with different components of their surrounding environment, e.g. vasculature or axonal tracts.

Functional plasticity of neocortical circuits in normal mice and neurodegeneration (Holtmaat, Helmchen) model mice

The team of Anthony Holtmaat (AH) investigated the effect of a focal stroke on functional map changes in the mouse barrel cortex. Rose Bengal is injected in the tail vein and the cortex is illuminated with a green light through a small pinhole on a cranial window implant. The illumination causes thrombosis and results in local ischemia. In the example below the stroke was targeted at the cortical area representing the delta-whisker. After the stroke delta-whisker stimulation failed to produce an intrinsic optical signal response, whereas the stimulation of a neighboring control whisker continue to elicit responses.

In GFP-expressing transgenic mice neuronal structures in the barrel cortex are imaged through a cranial window. Stroke is induced as described in the previous figure. Dendrites and spines are imaged before and after stroke, in an area neighboring the penumbra. 48 hours after the stroke synaptic changes can be observed by virtue of the appearance and disappearance of dendritic spines.

Finally, we like to report that the seminal study on long-term imaging of neuronal network plasticity in mouse barrel cortex during sensory deprivation has been prominently published in Nature Neuroscience (Margolis, Lütcke, et al., 2012). In this study we have quantified the reorganization of layer 2/3 network activity using long-term in vivo calcium imaging with YC3.60. Our results show that the heterogeneous distribution of cellular activity within the local network (ranging from low responsive to high responsive cells) undergoes redistribution during sensory deprivation. On the one hand, previously weakly responding neurons are recruited to the active pool or strengthened, while the highest responsive cells rather were down-modulated. Thus, local redistribution of activity occurs in a convergent manner. This study was the first to use long-term imaging over months to reveal stability and plasticity of single-cell responsiveness in a sensory-deprivation paradigm.

Assessment of plasticity-promoting treatments (Helmchen, Schwab, Holtmaat, Schneider, Pizzorusso)

The teams of FH and MS have now a fully operating procedure to obtain longitudinal data aiming at investigating network shifts, astroglial and microglial responses after a degenerative process following different rehabilitation paradigms (early versus delayed training, training combined with Anti-Nogo A immunotherapy) using calcium imaging.

In addition to what has been presented above on the collaborative work performed by the teams of FH and MS, the FH group has made further progress (in collaboration with Bernard Schneider / Anthony Holtmaat team) during the past year, with calcium imaging in head-restrained mice by establishing population imaging in neocortex after training the animals to a reward-based task, i.e., to a texture discrimination task. This direction of enabling high-resolution imaging from local neuronal sets while a mouse is performing a specific behaviour under the microscope has a large potential for obtaining longitudinal data sets in neurodegenerative disease models and for correlating behavioural deficits to underlying changes on the cellular and local circuit level.

As already presented our data on microglia restructuring following antibody-treatment in AD mice. Antibody-treatment in general has large promises for neutralizing damaging actions of

proteins and for halting and reversing progression of neurodegeneration. In the future one will need to put together even more comprehensive views on the effects of immunotherapy, analysing the microscopic changes in all major neuronal and glial cell types.

Finally, the team of Tommaso Pizzorusso (TP) demonstrated in a recent publication that cortical spines become more motile and express a larger degree of structural and functional plasticity following enzymatic digestion of a prominent component of the extracellular matrix, the Chondroitin Sulfate Proteoglycans (CSPGs). Their analysis comprised in vivo and in vitro 2-photon imaging and functional analysis of plasticity capacity by electrophysiology. The results of this study corroborates the idea of targeting matrix components, normally restraining morphological changes of dendritic spines in the adult brain, as promising targets for promoting and restoring plasticity in cortical circuits.

Neurodegeneration and Wnt signalling (Caroni)

The team of Pico Caroni (PC) investigated how experience regulates the structure of a defined neuronal circuit in adult mice. Enriched environment (EE) produced a robust and reversible increase in hippocampal stratum lucidum synapse numbers, mossy fiber terminal (LMT) numbers, and spine plus synapse densities at LMTs, whereas a distinct mechanism depending on Rab3a promoted LMT volume growth. In parallel, EE increased postsynaptic CA3 pyramidal neuron Wnt7a/b levels. Inhibiting Wnt signaling through locally applied sFRP-1 suppressed the effects of EE on synapse numbers and further reduced synapse numbers in control mice. Wnt7 applied to CA3 mimicked the effects of EE on synapse and LMT numbers. CA3 Wnt7a/b levels were enhanced by excitatory activity and reduced by sFRP-1. Synapse numbers and Wnt7a/b levels peaked in mice aged 6-12 months; a decline in aged mice was reversed by EE. Therefore, behavioral experience specifically regulates adult global stratum lucidum synapse numbers and hippocampal network structure through Wnt signaling.

Secretion and actions of MMP-9 at the synapse (Kaczmarek)

The team of Leszek Kaczmarek (LK) has made significant progress towards visualizing MMP activity live in situ in neuronal cultures. They found that MMP-9 causes spine transformation towards thinner spines. The team of LK has further assessed whether endogenous MMP-9 can reproduce the effect of exogenous MMP-9 on spine morphology. They have used dissociated hippocampal neuronal culture, performed a RFP transfection (7DIV) and incubated the slices with FITC-labelled gelatin (as MMP-9 substrate). A LTP was induced chemically with either forskolin, picrotoxin, or rolipram. The results showed that chemical LTP elevates enzymatic activity on dendritic spines.

In addition, the team of LK generated transgenic rats carrying gene encoding fusion of PSD-95 with fluorescent protein Venus, expression and cellular localization of which are controlled by c-Fos promoter and 3'UTR of Arc, respectively. Thus, dendrites and synapses of activated neurons should be fluorescently tagged. They have been testing with pentylentetrazol (PTZ), an inhibitor of GABAergic transmission to broadly induce neuronal activity.

In summary, we have gathered in this work package 2 highly significant results of micro level dynamics in models of neurodegeneration. We continued to apply in vivo two-photon microscopy to visualize and study dynamic processes in various cellular compartments, including somata, dendrites and dendritic spines as well as glial processes. Several functional studies, employing genetically-encoded calcium indicators to study local population activity, were either completed or are providing highly interesting results. The models of neurodegeneration, to which the state-of-the-art microscopic methods were applied, comprise AD mouse models, stroke models, and corticospinal lesion models. Not least we investigated the micro effects of potential treatments to promote plasticity in affected regions, e.g., by application of anti-A β antibody in AD mice. In a seminal study, we show that degradation of chondroitin sulphate proteoglycans (CSPGs) by Chondroitinase ABC in mouse visual cortex enhances motility and reactivates functional plasticity of dendritic spines. Overall, we have more than achieved our original goals in the PLASTICISE consortium and the field is indeed moving faster than expected a few years ago. Our results highlight the importance of studying the micro-level effects of neurodegeneration and show that they can serve as good indicators not only for characterizing structural and functional impairments but also for evaluating potential therapeutic strategies. With the new methodologies at hand to 'look at details' under in vivo conditions, there is now great prospects for gaining further insights into microscopic mechanisms involved in neurodegeneration and the actions of promising plasticity-enhancing substances.

WORKPACKAGE 3: A NOVEL IN VIVO MODEL OF NEURODEGENERATION

Development of the appropriate AAV vectors for the expression of human tau and APP in the mouse brain (Schneider/Aebischer, Spillantini)

Several aspects are motivating the development of viral vectors for the modelling of cognitive neurodegenerative disorders:

- i) Manage a focal transgene expression to target the pathology in a specific brain structure. This permits to avoid any confounding behavioural consequences of widespread transgene expression, such as motor impairments caused by brainstem/spinal cord degeneration;

- ii) Produce an acute perturbation of an adult system and thereby avoid compensatory mechanisms occurring during development; and
- iii) Generate a model system easily applicable to genetically modified mouse lines to assess functional interactions between genes in vivo.

The team of Bernard Schneider (BS)/Patrick Aebischer (PA) has successfully established new AAV vectors for delivery of mutant forms of tau and APP. A summary of the AAV productions is shown below.

Production of AAV vectors for expression of mutated APP:

- i) AAV-pgk- β glob intron-hAPP WT-WPRE;
- ii) AAV-pgk- β glob intron-hAPP Swe/Aus-WPRE;
- iii) AAV-pgk- β glob intron-hAPP Swe/Arc/Aus/Ind-WPRE (APP-4FAD). AAV2/6 vectors, titers \geq 2E10 TU/ml. These vectors were used in the Schneider/Aebischer lab.

Production of AAV vectors for expression of tau:

- i) AAV-pgk-kz-Tau4R2N WT-WPRE;
- ii) AAV-pgk-kz-Tau4R2N 3PO-WPRE;
- iii) AAV-pgk-kz-Tau4R2N P301S-WPRE. AAV2/6 vectors, titers \geq 2E10 TU/ml. These vectors are now currently in use in the Fawcett /Spillantini labs.

Also produced:

- i) AAV-pgk-kz-Tau3R0N WT-WPRE;
- ii) AAV-pgk-kz-Tau4R0N WT-WPRE;
- iii) AAV-pgk-kz-Tau4R0N P301S-WPRE. AAV2/6 vectors, titers \geq 2E10 TU/ml. Control vector : AAV-pgk-FPmax-WPRE. These vectors are now currently in use in the Schneider/Aebischer lab.

Intracerebroventricular injections in neonates

In parallel to focal vector injections performed in the lab of James Fawcett (JF) and Maria Grazia Spillantini (MGS), the team of BS/PA has further developed the method for intracerebroventricular (ICV) injection of AAV vectors in the neonatal mouse brain (P3/P4). The aim was to improve the reliability of the intracerebral injections using stereotaxic techniques. The ICV injection of AAV is a convenient way to target large brain areas. However, this model system presents an inherent variability due to the methodology, but this variability can be mastered using the setup developed by the team of PA.

The diffusion of AAV2/6 vectors and the expression of the transgene were then followed until 6 months after the injection. High levels of expression are mainly seen around the needle track. Remarkably, AAV2/6 vectors can target the whole cortex (see Figure 1) and sub-cortical areas (such as the thalamus, hippocampus), where transgene expression can be detected at adult stages (6 months after the injection). By immunohistochemistry, expression is also visible in the somatosensory cortex and visual cortex (cortical layers 2/3 and 4/5). Lower expression is seen in other cortical regions, restricted to the pyramidal layer V. Few cells show expression in the cerebellum and brainstem.

When assessing the presence of transgene mRNA in the injected mice, most prominent expression was found in the striatum, which was mainly attributed to transcript abundance in cortical axons projecting through the striatum. Indeed, only scarce neuronal transduction was found in this brain structure by immunohistochemistry.

In summary, the work initially conducted under WP3 focused on using AAV vectors for the local production of amyloid β . We could successfully induce a local amyloid pathology shown by the progressive accumulation of plaques. The mice developing this Alzheimer-like pathology were found to be significantly impaired in spatial memory, as demonstrated in the Morris water maze (MWM). However, the MWM paradigm that revealed these behavioural impairments was not compatible with the repetitive assessments needed to probe for potential recovery following plasticity-enhancing treatments.

Therefore, we used viral vectors to instead induce a tau pathology which may lead to more pronounced degeneration with more acute behavioural phenotypes. Indeed, we found that both local injections of the tau-expressing vector in the perirhinal cortex and intracerebroventricular (ICV) injections of the same vector in mouse neonates produced acute tau pathologies compatible with a behavioural monitoring of related brain functions. This deliverable is focused on reporting the effects obtained in viral and transgenic mouse models of tau pathology.

Characterization of the expression of P301S tau using AAV vectors (Spillantini, Fawcett, Schneider/Aebischer)

One of the objectives of PLASTICISE was to produce a novel model of neurodegenerative disease through viral vector expression of mutant tau. The main form of tau that was chosen was the P301S mutant, which leads to frontotemporal dementia in humans. Nevertheless, we also analysed in parallel experimental groups the pathology induced by wild-type forms of tau, as well as a rapidly aggregating variant, tau 3-PO. Transgenic mice expressing the P301S tau mutant display a progressive global tauopathy with neuronal loss and disability. Our objective was to produce a focal form of tauopathy, more amenable to experimental manipulation, and more rapidly progressing due to high levels of expression. AAV vectors were produced to express either control, normal tau, or P301S tau or the rapidly aggregating 3-PO tau variant (also indicated as tau M123). In vitro experiments demonstrated that transduction of neurons led to rapidly progressive tauopathy and neuronal death. The objective of in vivo experiments was to show focal tauopathy with neuronal dysfunction and death, with a behavioural readout relevant to human disease, and in which plasticity treatments could be tested for their ability to restore neuronal function.

Intracerebroventricular (ICV) injections in mouse neonates

In the BS/PA lab, experiments were designed to induce widespread expression in the mouse forebrain, by injecting tau-expressing AAV6 vectors in the lateral ventricles of mouse neonates. The development of the tau pathology was monitored in adult animals, by immunohistopathology, biochemistry and monitoring of motor behavior.

AAV vectors were produced to express the following forms of human tau: 3RON, 4RON and a variant form of 4RON containing the P301S mutation. As wild-type tau is associated with neurofibrillary tangles (NFT) in Alzheimer's disease, it is assumed to also cause neuronal toxicity. In order to generate a control, less pathogenic variant of 4RON tau, we introduced two mutations in the microtubule-binding domain (I277P and I308P), to reduce tau propensity to aggregate. The vectors were injected in the lateral ventricles (ICV) of mouse neonates, to transduce large projection neurons in cortical and hippocampal regions. Human tau was found expressed in the frontal brain, approximately tripling the level of total tau. At 2 and 7 months post-injection, there was a clear deposition of hyperphosphorylated tau, detectable by immunohistochemistry with the AT8 and PHF-1 antibodies. Quantitative alphaLISA assays for specific phosphorylation sites (Thr181 and Ser396) revealed a progressive accumulation of phospho-Ser396 tau over time, to a similar degree for both wild-type and P301S human tau.

Although tau hyperphosphorylation is a first indication for the initiation of a pathologic process, it is critical to assess neuronal deposition of misfolded forms of tau. Using specific conformational antibodies recognizing early-misfolded forms of human tau (Alz50, MC-1), we found positive signals in cortex and hippocampus for both wild-type and P301S tau. However, using an ELISA assay to quantitate tau multimers, the P301S mutation was shown to enhance the deposition of aggregated, sarkosyl-insoluble tau. At 7 months, detection of tangle-like structures positive for both Thioflavin S and Gallyas silver impregnation revealed deposition of aggregated tau in the cortex of P301S tau-injected mice.

Following ICV injection of the tau-expressing vector, the pathology mainly developed in the motor cortex and adjacent cortical areas, with some deposition of hyperphosphorylated tau in the thalamus and brainstem. Therefore, to assess possible functional deficits, we analyzed mouse motor behavior. Animals overexpressing P301S tau rapidly developed motor impairments, mainly reflected by the latency to fall in the Rotarod test. Animals also displayed hyperactivity in the open field, indicative of reduced anxiety-like behavior. Mice injected with the wild-type forms of tau showed mild motor defects in the Rotarod test, which only marginally progressed over time. As compared to wild-type tau, behavioral impairments were significantly reduced in animals injected with I277P/I308P tau, which has a lower propensity to aggregate (4RON Agg- variant, Figure 3g). It is therefore possible that misfolding underlies the mild pathological effects observed with wild-type tau.

Overall, this set of preliminary data underscores the ability of AAV vectors injected in the mouse central nervous system to induce axonal and somatodendritic accumulation of hyperphosphorylated tau, deposition of misfolded and aggregated tau, and the progressive apparition of motor deficits.

However, the widespread distribution of the tau pathology does not facilitate the testing of plasticity-enhancing treatments. It is therefore important to further develop focal models of the tau pathology in adult mice, which was investigated in parallel in the JF / MGS labs.

Injections in the sensorimotor cortex

The first local viral injections were made into the rat sensorimotor cortex. After one month, expression of mutant tau was clearly seen, with hyperphosphorylated tau in many neurons. At three months after injection, there was intense tau pathology in the injected area, and focal neuronal loss. However, we did not record a reproducible loss of function in a skilled reaching task.

Injections in the entorhinal cortex

The second set of injections were made unilaterally into the entorhinal cortex. Both normal mice and TASTPM mice, which model Alzheimer's disease through production of mutant APP and mutant presenilin, were injected (Dassie et al., *Neurobiology of Aging*, 2012). There was retrograde transport of virus to the hippocampus and other regions, and axons containing human tau were also seen in the hippocampus and elsewhere. Over eight months following virus injection there was clear and increasing tau pathology with neuronal loss in the entorhinal cortex and also loss of neurons in the CA1 region of the hippocampus. We saw no sign of an interaction between tau and A β pathology, with neither form of pathology causing an increase in the other. The behaviour of the animals was tested using the Morris water maze, which is an appropriate test for hippocampal and entorhinal function (Figure 5). Only at the later time points when the TASTPM animals had severe A β and tau pathology did we see memory deficits in the water maze, and even at these time points the functional loss was subtle. We concluded that this animal model had produced severe histological pathology, but that the behavioural deficit was not suitable for use in testing plasticity-inducing compounds.

Injections in the perirhinal cortex

The third model in which we induced focal tau pathology was injection of the perirhinal cortex. This area of cortex is involved in object recognition memory, and lesions within it have been shown to cause memory deficits. Animals received three injections of AAV6-P301S tau vector on each side of the brain, to enable transduction of the whole of this long cortical region. Tau pathology as seen by histology developed as in the previous model, with clear tauopathy and neuronal loss by two months after injections. These mice were tested behaviourally and showed an object recognition memory deficit compared to AAV6-wt Tau or AAV6-FPmax used as controls for the mutation and for the injection.

In order to identify a defect associated with easily measurable tests, we also characterized the progression of tau pathology and neuronal death in the piriform cortex and tested the olfactory behaviour and memory in our mice to determine whether tau pathology and altered olfaction correlated. The piriform cortex has proved another area suitable for future testing. Conclusion: We concluded that injection of AAV expressing mutant tau into the perirhinal cortex is a useful model of focal Alzheimer's pathology, and suitable for testing the ability of plasticity-activating and other compounds to restore function in Alzheimer's disease. This memory model has therefore been used for the experiments testing potential plasticity treatments for Alzheimer's disease.

Characterization of the P301S tau transgenic line (Spillantini, Fawcett)

The P301S tau transgenic mouse line is a model of human tauopathy, overexpressing human P301S mutant tau under a murine Thy1.2 promoter that presents abundant tau pathology and neuronal cell loss. The team of MGS has tested transgenic P301S mice in the novel object recognition (NOR) test. The NOR is a perirhinal cortex - dependent test, as a lesion of the perirhinal cortex abolishes OR memory. Transgenic P301S mice show a temporal progression of the object recognition memory deficit. Based on the results obtained above with injection of the AAV-tau vector as well as using the P301S tau transgenic mice, the experiment to test the efficacy of plasticity-enhancing treatment was performed by targeting the perirhinal cortex. Both transgenic mice expressing the P301S tau mutant and adult mice injected with the P301S tau-expressing vector were used to model tau-induced degeneration. Indeed, in both models, P301S tau expression caused a reduced performance in the novel object recognition task.

Rescue of the tau-induced behavioural deficits by plasticity-enhancing treatments (Spillantini, Fawcett)

In the P301S human tau transgenic mouse line, the team of MGS determined the presence of the object recognition (OR) memory deficit at 3 months of age.

In AAV-P301S tau injected mice we also confirmed that their OR memory was impaired in association with neurodegenerative tauopathy. ChABC treatment also reversed the functional deficit in AAV-P301S human tau-injected mice 2 weeks after treatment. However, one month later, animal performance in the test returned to the level observed before the ChABC treatment. In conclusion, the plasticity-enhancing treatment is able to transiently rescue brain function, presumably by favouring synaptic connections that enhance performance in cognitive tasks. However, it will be important to explore how to maintain this effect over time in conditions where the accumulation of pathologic forms of tau chronically perturbs neuronal functions.

WORKPACKAGE 4: PROMOTING PLASTICITY IN ANIMAL MODELS OF NEURODEGENERATION

Visual cortical plasticity in Crt11 null double mutants (Fawcett, Pizzorusso)

The teams of Tommaso Pizzorusso (TP) and James Fawcett (JF) have collaborated to study the role of perineuronal nets (PNN) in cortical plasticity using the cartilage link protein-1 (Crtl-1) null double mutants. The results have shown that Crtl1 mutant animals have attenuated perineuronal nets and retain ocular dominance plasticity and plasticity of the sensory projection in the cuneate nucleus into adulthood. This collaborative work has been published (Carulli D. et al, Brain, 2010). Following this first study, the teams of TP and JF have gone beyond the task by analyzing plasticity in non visual structures to assess learning and memory mechanisms, object recognition memory, synaptic plasticity in perirhinal cortex of these Crtl1 mutants. The results show that the genetic attenuation of PNNs in adult brain Crtl1 knockout mice dramatically enhances long term object recognition memory and facilitates synaptic plasticity mechanisms thought to underlie the encoding of memory. Identical prolongation of memory follows digestion of PNNs in the perirhinal cortex with chondroitinase ABC. Our results demonstrate that PNNs regulate the rules for experience driven learning and functional synaptic plasticity.

TP had preliminary data showing enhanced learned extinction of fear memory in Crtl1 mice. Mice were conditioned with a tone (7.5 kHz) associated with a shock. Freezing time was measured to assess fear. 10 days after memory acquisition mice were exposed to the tone alone to assess extinction learning. 12 stimuli for each day were administered. In addition, Crtl1 KO showed an accelerated extinction.

To analyze neural correlates of extinction we labelled fixed brain slices from the activity-marker zif268 at the end of the first day of extinction (after the 12th stimulus). We found a clear trend suggesting that the tone strongly enhances zif-positive cell density in conditioned wt mice. Crtl1 mice responded significantly less to the conditioning stimulus, suggesting that in the absence of perineuronal nets allows mechanisms of plasticity in the amygdala capable of reducing the response to the conditioned stimulus. No activation was present in unconditioned mice.

Role of Sema3A on the inhibitory effect of PNNs on synaptic plasticity (Pizzorusso, Verhaagen, Fawcett)

The teams of TP, Joost Verhaagen (JV) and JF have successfully collaborated on this project in the last two years. Perineuronal nets (PNNs) are substructures of the neural extracellular matrix with the ability to produce an inhibitory effect on synaptic plasticity in the visual cortex. However, the molecular mechanisms underlying this inhibitory effect are still unknown.

Class 3 semaphorins (Sema3s) are secreted axonal guidance molecules that act during the development of the CNS and signal through multicomponent receptor complexes that are composed of a neuropilin and a plexin subunit. Sema3s have recently been shown to have an effect on synaptic density, maturation and transmission. The prototypical Sema3 family member Sema3A is secreted and binds to the chondroitin sulphate proteoglycans of the PNNs. This has led to the hypothesis that the negative effect of PNNs on synaptic plasticity might be mediated by the presence of Sema3s in the PNN.

To test this we neutralized Sema3s activity by injecting adeno-associated viral vectors (AAV) in the primary visual cortex (Vctx1) of adult rats that drive expression of neuropilin1-Fc (Npn1-Fc) or neuropilin2-Fc (Npn2-Fc), soluble receptor bodies that inactivate Sema3s by binding to Sema3A or Sema3B, C and F respectively. Then, we measured the shift of the ocular dominance (OD) in the binocular Vctx1 after a period of monocular deprivation (MD), in order to evaluate whether the inhibition of Sema3s reactivates OD plasticity that is present in juvenile animals during the critical period.

The intensity of the visual evoked potentials (VEPs) was measured by stimulating the contra and the ipsi-lateral eye alternatively. Adult rats injected with AAV-Npn1-Fc showed reactivated plasticity (contra/ipsi (C/I) vep ratio = $1,3 \pm 0,09$) compared with control animals of the same age (C/I vep ratio = $1,85 \pm 0,24$) and with AAV-GFP injected rats (C/I vep ratio = $1,68 \pm 0,076$) (P less than 0,01).

Moreover AAV-Neuropilin1-Fc mediated reactivation of plasticity seems to be due to the inhibition of Sema3A and not to the blockade of VEGF activity. Indeed, a mutated form of AAV-Npn1(VEGF)-Fc that specifically bind VEGF and not Sema3A is not able to induce the shift of the OD after MD (C/I vep ratio = $1,77 \pm 0,12$).

Finally AAV-Npn2-Fc injection seems to promote some plasticity reactivation as well (C/I vep ratio = $1,31 \pm 0,17$) but not statistically significant. However the effect of AAV-Npn2-Fc injection on cortical plasticity is strictly dependent to the concentration of the viral vector used. Indeed rats treated using a batch with a lower concentration of AAV-Npn2-Fc vector ($6,68 \times 10^{11}$ instead of $2,8 \times 10^{12}$) showed no evident sign of plasticity reactivation (C/I vep ratio = $1,8 \pm 0,05$).

Thus, these preliminary data suggest that Sema3A may be an effector of PNNs and may have a key role in the regulation of the constraint on cortical synaptic plasticity in adult animals. However also an involvement of Sema3B, C and F cannot be excluded.

Effects of chABC, anti-NogoA antibodies, PSA-NCAM modulators, histone deacetylase inhibitors on recovery from stroke

A. Effect of chondroitinase ABC (chABC) treatment on functional recovery from stroke (Schwab)

The effect of chondroitinase ABC (chABC) on functional recovery has been shown after the first year of PLASTICISE. In brief, stroke has been induced by means of intracortical injection of endothelin 1 in the cortical representation of the forelimb in the rat. The model has been developed during the first year of the project by the team of MS.

Rats are trained for forepaw reaching before stroke is induced. The recovery from stroke is assessed behaviourally using the skilled paw reaching test. The animals are followed over time after the lesion. A fast-track camera system is now established in the lab of MS in order to follow the animal behaviours, i.e. the use of single digits during grasping.

The study has shown that chABC ameliorates functional recovery from focal stroke. In addition, the team of MS has observed a clear tendency that animals showing good recovery 6 weeks after stroke show labelled cells in the hindlimb cortical area on the ipsilesional side while animals badly recovering do not.

B. Effect of anti-Nogo antibody treatment on functional recovery from stroke (Schwab)

The combination of pharmacological application of neuroprotective/ neuro-boosting drugs with neuro-rehabilitation after stroke is of great interest from the clinical point of view. It is important to know which treatment is correlated with which outcome in what type of patient. Until now this question has been addressed by clinicians, physiotherapists and occupational therapists intuitively. In the animal model we can model the clinical situation and try different treatment setups. It has been very unclear whether neuroprotective or regenerative treatments, for example, should precede rehabilitation training or vice versa or whether they should even be given simultaneously.

The team of MS has previously done delayed treatment studies with anti-Nogo A antibody treatments where they saw that the treatment has to be administered within the first week after the injury in the rat after spinal cord injury and stroke. This result was translated and used in the ongoing clinical trial with anti-Nogo antibodies to define the window of

treatment as being within the first month after the insult. Still, we know that the antibody treatment must be accompanied by training such that the newly formed connections can be stabilized. Therefore we wanted to investigate the ideal setting of regeneration enhancing treatment and training for both spinal cord injury and stroke.

The experimental set-up consisted of rats which received a photothrombotic stroke over the motor area of their side preference after 3 weeks of training of a fine motor task. Following stroke, rats received either the anti-Nogo antibody (11C7) or the control Ig G antibody treatment. Rats are trained for forepaw reaching before stroke is induced. The recovery from stroke is assessed behaviourally using the skilled paw reaching test. The study showed that rats undergoing first Anti-Nogo A treatment (2 weeks) and then have training afterwards for 2 weeks recovered best compared to all other groups. Furthermore early training seems more effective than late one starting 2 weeks after injury. A manuscript is currently in press (Starkey M et al., Brain, in press).

C. Effects of anti-Nogo-A antibodies on corticospinal connectivity after stroke (Schwab)

Many earlier studies from the group of MS and other laboratories have shown that interfering with the Nogo-A - Nogo receptor pathway by antibodies, receptor bodies, KO, or pharmacological agents significantly enhance recovery in particular of skilled fore- and hind limb movements in adult rats. In collaboration with laboratory of G.KARJE (Chicago) they could recently show that these effects are also seen in aged rats and when the antibody is applied (for a duration of 2 weeks) 1 or 2 months after the MCAO stroke lesion.

Importantly, therefore, anti Nogo-A antibody treatment does not have to be applied in the acute post stroke phase but is a treatment that enhances rehabilitative recovery at the time when the patient is medically stable.

Anterograde and retrograde tracing experiments (Nico Lindau, team of MS) show a correlation between behavioral recovery and neurons of the contralesional, intact cortex that recross the spinal cord midline to establish a new ipsilateral connection. Double labeling experiments over time suggest a retraction of the original arbor in favor of the new target.

Intracortical micro stimulations are currently performed to confirm these results on a physiological level. In the absence of anti Nogo antibodies, only minimal sprouting across spinal cord midline can be observed, while Nogo antibodies enhanced midline crossings.

D. Effect of chondroitinase ABC (ChABC) treatment on cortical plasticity (Pizzorusso)

Partial motor recovery after stroke is thought to be sustained by neuronal plasticity, particularly in areas close to the lesion site. It is still unknown if treatments acting exclusively on cortical plasticity of perilesional areas could result in behavioural amelioration. The team of TP tested whether enhancing plasticity in the ipsilesional cortex using local injections of chondroitinase ABC (ChABC) could promote recovery of skilled motor function in a focal cortical ischemia of forelimb motor cortex in rats. Using the skilled reaching test, we found that acute and delayed ChABC treatment induced recovery of impaired motor skills in treated rats. vGLUT1, vGLUT2, and vGAT staining indicated that functional recovery after acute ChABC treatment was associated with local plastic rearrangements of the excitatory cortical circuitry positive for vGLUT2. ChABC effects on vGLUT2 staining were present only in rats undergoing behavioural training. Thus, plasticity of perilesional excitatory circuits induced by a combination of pharmacological and behavioral treatment is sufficient to enhance functional recovery from a focal stroke. Our group is using tracer injections in perilesional areas of adult rats lesioned with endothelin and treated with ChABC to assess morphological plasticity of corticospinal axons.

Development of rehabilitation procedures (Schwab)

The team of MS has developed a 3-story cage where they can follow rats 24h per day over several weeks during their normal behaviour. The animals receive a transponder which is transplanted under their skin allowing the animal's behavior to be monitored continuously over weeks. The cage is equipped with different tasks (including a pellet reaching task) which force the rats to train themselves. Animals with a brain or spinal cord injury and kept in the cage for weeks can be compared with lesioned animals kept in regular cages and receiving a specific grasping training etc.

This project is of vital interest for clinical questions which we try to address in our animal models. E.g. we want to study motivation and how it can be influenced - a topic which is clinically very relevant for patient compliance. The cage allows us to address such questions and to follow the animal's recovery or reaction to certain external "stimuli" both day and night.

Assessment of the plasticity-inducing treatments developed by the different PLASTICISE teams

The idea of this task is to develop novel methods of promoting plasticity following what has been found to be efficient in workpackage 1. We provided below a summary of the different plasticity-enhancing treatments that have been developed by the consortium and tested on several animal models and conditions.

A. Combined treatment with anti NogoA and chondroitinase in spinal cord injury (Schwab, Fawcett)

The two most consistently successful treatments for spinal cord injury at present are anti NogoA and chondroitinase. Their mechanisms of action are somewhat different, but there are also suggestions that both actions may involve the Nogo receptor.

We therefore performed a series of experiments to test a combined treatment, also giving all the animals rehabilitation training. A problem that had to be solved was how to combine the two treatments with rehabilitation. Previous work has shown that chondroitinase can be combined at the same time as rehabilitation, but combining anti NogoA with chondroitinase produces dysfunctional results, unless the rehabilitation is delayed until after the NogoA treatment.

We therefore treated animals with anti NogoA immediately after injury, then started chondroitinase at 4 weeks and rehabilitation at 5 weeks. The two individual treatments given alone produced similar effects on skilled reaching, ladder walking and the other outcomes. However the combination treatment was clearly superior (as already presented in WP1).

B. Treatment of memory deficit induced by P301S tau (Spillantini, Fawcett, Aebischer)

In WP3 we developed a novel model of focal Alzheimer pathology through injection of AAV vectors expressing mutant forms of tau into the perirhinal cortex. These animals had a severe deficit in the novel object recognition memory.

We also tested P301S transgenic animals at various time points, and found that these animals show a similar memory deficit at three months of age, at least a month before they show motor signs. In both cases there is widespread hyperphosphorylated tau, mis-shapen neurons with curvy processes, but not much neuronal loss.

We therefore wished to know whether reactivation of plasticity could be a way of restoring memory in a cortex with many dysfunctional neurons. We chose chondroitinase as the plasticity treatment, because of its well-known properties, reliability and persistence of action over a few weeks. As shown in the picture below, we were able to restore memory to its normal level in treated animals.

The results to the right show restoration of memory in the P301S transgenic, and in a heterozygous transgenic at 7 months of age. We obtained identical results in animals in which the tau pathology was induced by viral vector injection into the perirhinal cortex.

A single injection of chondroitinase digests the perineuronal nets, but these gradually reform over a period of two or more weeks. We therefore investigated the length of time over which memory was restored in the chondroitinase injected animals. We found that by 5 weeks after injection the memory deficit had recurred.

Proteoglycans can affect the aggregation of both A β and mutant tau. We therefore looked to see whether chondroitinase injection had affected tau pathology. We were unable to see any effect.

C. Effect of PSA-NCAM modulator on locomotor recovery (Pharmaxon)

Pharmaxon (PHX) has shown that a PSA mimetic peptide PR21 enhances locomotor score after spinal cord lesion and neuroprotective effects after contusion. It was demonstrated that PR21 decreases the glial scar by decreasing the astrocytes hyperexcitability (see figures in WP1).

D. Assessment of β -Adducin mutant mice for learning and memory (Caroni)

The team of Pico Caroni (PC) has shown that mice lacking the plasticity-regulated protein β -Adducin fail to assemble new synapses upon enhanced plasticity, and exhibit diminished long-term hippocampal memory upon environmental enrichment. Enrichment enhanced the disassembly and assembly of dynamic subpopulations of synapses. Upon enrichment, stable assembly of new synapses depended on the presence of β -Adducin, disassembly involved β -Adducin phosphorylation through protein kinase C, and both were required for augmented learning. In the absence of β -Adducin enrichment still led to an increase in spine structures, but the assembly of synapses at those spines was compromised.

Virus-mediated re-expression of β -Adducin in hippocampal granule cells of β -Adducin^{-/-} mice rescued new synapse assembly and learning upon enrichment. These results provided evidence that synapse disassembly and the establishment of new synapses are both critically important for augmented long-term learning and memory upon environmental enrichment.

E. Involvement of MMPs in plasticity

Testing one of D-PHARM compound for seizures (Kaczmarek, D-Pharm)

The team of Leszek Kaczmarek (LK) established a model, which was applied for examining involvement of matrix metalloproteinase 9 (MMP-9), upon experience-dependent cortical plasticity. MMP-9 is an enzyme implicated in plastic modification of the neuronal connections. We found that, in the adult mouse brain, experience-dependent plasticity is in part supported by the activity of MMP-9. This study has been published in 2012 (see reference below: Kaliszewska A. et al., *Cerebral Cortex* 22: 2160-2170).

The team of LK has found that D-Pharm's compound DP-b99 inhibits MMP-9 activity in a biochemical assay, as well as MMP-9-dependent functions, such as mediating kainate excitotoxicity, dystroglycan cleavage and morphological modulation of the dendritic spines, possibly involved in synaptic plasticity. Furthermore, DP-b99, when applied to the animals, suppresses development of chemically kindled epilepsy, inhibiting also in the brain cleavage of the DG and mossy fiber sprouting. Thus, this compound displays potent anti-epileptogenic activity, probably via preventing aberrant plasticity driven by MMP-9.

Role of MMPs in ocular-dominance plasticity (Pizzorusso)

The team of TP has studied the role of matrix metalloproteases (MMP) in ocular-dominance plasticity. We infused the visual cortex of monocularly deprived critical period rats with the broad spectrum MMP inhibitor GM6001. We found that this treatment prevents the potentiation of the non-deprived eye after 7 days of monocular deprivation. Furthermore, GM6001 seems to prevent the late increase of spine density occurring after monocular deprivation. This data has been published in *Cerebral Cortex* (Spolidoro et al., 2012; see below).

F. EphA4 synergizes with Nogo-A to restrict axonal growth after spinal cord injury (Schwab)

As already presented in the previous report, the team of MS have been screening several candidates for the NogoA receptor. The team of MS has been screening molecular candidates that may explain why in myelin of adult Nogo-A knockout mice, there is only a partial reduction in neurite outgrowth inhibition. The remaining activity might be due to a compensatory upregulation of other growth inhibitory or repulsive molecules. We searched for potential compensating molecular candidates by screening the intact adult spinal cord for transcripts whose expression levels were upregulated in the absence of Nogo-A. Affymetrix GeneChip and quantitative RT-PCR (qRT-PCR) analyses revealed an increase of several ephrins and semaphorins, as well as of their receptors Ephs and Plexins. In particular, ephrinA3 was found significantly enriched in adult myelinating oligodendrocytes of Nogo-A KO mice. We showed that recombinant ephrinA3 inhibits neurite outgrowth of postnatal cortical neurons in an EphA4-dependent manner, and that EphA4 KO cortical neurons are less inhibited by Nogo-A KO myelin than wild-type (WT) neurons. Furthermore, ephrinA3 KO-derived myelin was less growth inhibitory than WT-derived myelin, but more inhibitory than Nogo-A KO-derived myelin. In vivo, Nogo-A / EphA4 double KO mice showed enhanced axonal sprouting and regeneration after spinal cord injury compared to single Nogo-A KO and EphA4 KO mice. In addition, a reduction in the scar volume and an increased ingrowth of lesioned fibers into the scar was observed in single and double mutant mice lacking EphA4. Our results reveal the ligand-receptor pair ephrinA3 / EphA4 as a novel subsidiary to Nogo-A myelin growth inhibitory pathway. Further, this study shows that the combined genetic deletion or neutralization of multiple growth inhibitory factors may be more efficient than single treatments to increase anatomical and functional recovery after brain or spinal cord injuries.

In summary, these activities lead to the identification of the properties that an optimal treatment should have:

- i) being administered as early as possible after the insult, albeit delayed treatments can be effective
- ii) being coupled with rehabilitation in regimes that are specific for the type of treatment.

The results obtained with the combined treatment with Nogo antibodies and chABC showed that plasticity can synergistically enhanced by treatments acting on different substrates. From the molecular point of view, small peptides interfering with specific key interactions such PSA mimicking peptides, antibodies or factors interfering with semaphorin binding to perineuronal nets could more easily solved the drug production and delivery problems presented by treatments of the central nervous system with proteins.

WORKPACKAGE 5: NOVEL METHODS FOR STUDYING PLASTIC CHANGES IN HUMAN PATIENTS AND PRIMATES

Assessment of plasticity after stroke using new imaging methods (Ward, Weiller)

The focus of this task has been to evaluate Dynamic Causal Modelling (DCM) as a tool for analysing functional imaging data in order to measure brain connectivity. Functional MRI (fMRI) allows us to look at activity in distributed brain regions. The team of Nick Ward (NW) has been using an analysis technique called DCM to determine whether the measures of connectivity will be useful in assessing treatment or recovery related changes in patient groups.

Their initial goal was to provide face validity for the fMRI-DCM connectivity measures. To do this they were able to measure the influence of left primary motor cortex (M1) on right M1 during right hand grip both neurophysiologically using transcranial magnetic stimulation (TMS) and using functional brain imaging (fMRI) in the same subjects. DCM-derived coupling parameters between primary motor cortices appear to reflect age-related decline in interhemispheric inhibition (measured with TMS).

In this section, we will describe advances in

- (i) Dynamic Causal Modelling (DCM) as a tool for analysing functional magnetic resonance imaging (fMRI) data in stroke patients during affected hand movement;;
- (ii) DCM as a tool for analysing functional imaging data in stroke patients at rest;
- (iii) Magnetoencephalography (MEG) data examining direct corticomuscular coherence;
- (iv) MEG data examining intracortical connectivity;
- (vi) methodological advances in imaging analysis.

(i) The team of NW has been using a technique called DCM to analyse fMRI data in order to determine whether the measures of connectivity will be useful in assessing treatment or recovery related changes in patient groups. We have established the face validity of DCM as a tool for examining effective connectivity within the motor network. DCM measures of the influence of left M1 on right M1 during right hand grip correlated with transcranial magnetic stimulation (TMS) measures of the same connection (done in same subjects outside scanner). In other words, DCM-derived coupling parameters between primary motor cortices appear to reflect age-related decline in interhemispheric inhibition (measured with

TMS). The focus of this task was then to evaluate Dynamic Causal Modelling (DCM) as a tool for analysing functional imaging data in stroke patients. Here they examined whether such connection changes correlate with the variable clinical improvement seen in many studies. The motor system connectivity as assessed using DCM during affected hand grip between a number of cortical motor regions accounted for a significant proportion of the variability in clinical impairment.

ii) Another study performed by Marie-Hélène Boudrias from the team of NW focused on examining the resting states of controls and stroke patients using fMRI. Resting states have been found to be consistent across subjects, degrees of consciousness, stages of development and at some degrees across species. Changes in activity and connectivity have been reported to be linked to several neuropsychiatric diseases, such as Alzheimer's, schizophrenia, ADHD and autism. Brain connectivity can be assessed without the need of a task and there is a fast acquisition (about 7 min) reducing the discomfort of the patient.

The study showed significant differences in connection between stroke patients and controls. Patients and controls show differences in the number and values of their resting motor connections. The connection from M1 right (lesion) to M1 left was particularly important as also positively correlated with patients' behavioural motor performance.

These data may contribute to the construction of models predicting those patients likely to benefit most from treatment, using variables such as demographic data, location of the lesion, integrity of the corticospinal neurons (internal capsule), early patterns of connection/disconnection. The team of NW is now incorporating larger groups of patients to further strengthen the model.

(iii) A third approach has been taken by Holly Rossiter in the team of NW. They have been looking at coherence between the MEG and EMG signals. If a signal is detected, it means that there is coherence between cortex and muscle activity, implying a direct influence of this brain region over activity in the muscle employed to perform the task. The aim of this study is to find the peak cortical source of coherence in a variety of stroke patients and see if the distribution differed to that of healthy controls. This work has been able to establish the functional utility of a wide number of cortical regions in generating descending motor signals post-stroke including the contralesional hemisphere. The experiment has studied the possible correlation between impairment with the distance on individual cortico-muscular coherence peak from control average. No significant correlations were found. This indicates that stroke patients can experience significant recovery despite having coherence in the contralesional hemisphere.

(iv) Holly Rossiter from the team of NW have also used MEG to examine changes in beta power during a hand grip. Beta power (15-30Hz) decreases just prior to a movement and then rebounds following a movement. The magnitude of baseline beta power, of beta decrease and of rebound are thought to be related to levels of the inhibitory neurotransmitter GABA . The balance between inhibition (GABA) and excitation (glutamate) is an important factor in determining levels of cortical plasticity (cf. critical period for ocular dominance plasticity). NWs group have been studying how MEG signals might reflect these changes.

These time frequency data can be used in DCM for Steady State Responses. MEG is used to collect time frequency data during hand grip. A biophysical model of plausible intralaminar connections (both GABAergic and glutamatergic) is constructed and the model parameters (given the data) estimated. Older subjects need changes in glutamatergic connections to account for changes in time-frequency power spectra during hand grip, perhaps because of diminished GABAergic dynamic range. This decrease in GABAergic range might account for decreased cortical plasticity in older subjects. This balance of excitation/inhibition has great potential as a marker of cortical plasticity and is itself something that can be targeted with either drugs (e.g. fluoxetine as in FLAME study, or cortical stimulation techniques). These tools might be used to identify the effects of treatments on cortical plasticity in human subjects, including those with neurological disease.

(v) Although brain-behavior relationships may be moderated by other variables, these possibilities are often not accounted by standard analyses of structural or functional imaging data. In two proof-of-concept studies conducted by the team of Cornelius Weiller (CW), in samples of healthy adult subjects (Kaller et al., 2012, to be submitted), we were able to demonstrate the viability of multiple regression models including interaction terms for assessing the influence of third variables such as age and sex on the relation between behavioural variables of interest (here complex cognitive abilities) and various indices of brain structure (here gray matter density and fiber density estimates). In ongoing analyses as well as future studies with neurological patients, the approach will be used to investigate how, for instance, patients' age at stroke onset potentially moderates activation changes underlying functional recovery of lost or impaired abilities.

Verbal fluency is among the most frequently applied clinical measures of executive functioning in a wide range of brain pathologies, but there is considerable debate in the neuroimaging literature about the frontal lobes' functional role in this task. Related discussions in the neuropsychological literature are focused on the different specificity and sensitivity of the task's different variants for frontal lobe damage. In an fMRI study in healthy volunteers, we were able to resolve these debates by demonstrating that contradictory finding can be integrated when the role of task demands and individual ability is considered (Katzev et al., submitted).

Recent neuroscience literature suggests that language is processed within a dual pathway network with a dorsal pathway subserving auditory-motor integration and a ventral pathway extracting meaning from the acoustic-phonological input. Repetition and comprehension are two prototypical tasks which preferentially involve dorsal and ventral processing streams. We applied voxelwise lesion-behavior mapping to acute left-hemisphere-damaged aphasic patients to evaluate how well the model explains the respective deficits after focal lesions. Our results support the claim that language is organized along two segregated dorsal-ventral streams. Particularly, this is the first lesion study demonstrating that task performance on auditory comprehension measures requires an interaction between temporal and prefrontal brain regions via the ventral extreme capsule pathway (Kümmerer et al., accepted/in press).

In another study we investigated not only the neuroanatomical basis of acute aphasia symptoms but also aphasia syndromes using high resolution imaging as well as novel multivariate statistics. Only very seldom patients show isolated symptoms, they rather regularly present with syndromes, i.e. compositions of symptoms, which are a basis for today's classification in neuropsychology. The results provide important insights in the lesion patterns of aphasia syndromes and aphasic symptoms that can significantly alter our perception of the underlying mechanisms involved in the language processing network. Both, symptoms and syndromes can be explained in the light of dual pathway model of language processing (Kümmerer et al. to be submitted).

Assessment using fMRI combined with fibre tracking (Ward)

This task has focussed on combining fMRI data with probabilistic fibre tracking based on diffusion tensor imaging (DTI) data. The fibre tracking provides prior information about the likely connections of brain regions seen to be active during motor, language or cognitive tasks. In this way, separate subsystems of brain regions can be described, based on likely connection patterns. This approach has been successful in Freiburg (team of CW) and has resulted in a high level publication.

Similar work has been conducted in the team of NW in London using structural imaging and looking at how it can be combined with functional imaging. Here, functional brain imaging data are used to define 'functionally-relevant' anatomical regions, such as the upper limb representation within primary motor cortex. The following figures illustrate how 'upper-limb-related' contributions to the descending corticospinal tract from each of primary motor cortex (M1), dorsal premotor cortex (PMd), ventral premotor cortex (PMv) and supplementary motor are (SMA) were defined. This approach was more efficient than using anatomically controlled 'whole region' seed masks (which would include head, neck and lower limb representations less relevant for upper limb impairment).

The conclusion of this study is that the extent of injury to specific motor tracts predicts behavioral gains from treatment in subjects with chronic stroke. The study has been published (see Riley JD et al., 2011). Quantifying this is likely to help in predicting outcome after stroke. Current quantification relies on manual definition of the infarct, which is time-consuming and open to bias. Here, they have compared manual infarct definition with an automated abnormal tissue detection method in quantifying corticospinal tract-infarct overlap volumes in 51 chronic stroke patients. Using diffusion tensor imaging (DTI) and probabilistic tractography, four corticospinal tracts from the primary motor cortex (M1), dorsal and ventral premotor cortices (PMd and PMv) and supplementary motor area (SMA) to the ipsilateral lower pons were reconstructed in 23 healthy controls. Tract-infarct overlap volume of each of the four corticospinal tracts was quantified by overlapping the patients' lesions onto the control tract templates, using both the manually and automatically defined infarcts in the 51 patients. Correlations with motor impairment were assessed and both methods were directly compared using Intraclass Correlations. Greater impairment was seen in patients with greater corticospinal tract-infarct overlap with either method (r - manual range = 0.32-0.46; r - automated range = 0.42-0.57). Consistency between manual and automated methods was moderate to high for all four corticospinal tracts (ICC range = 0.71-0.80). These results demonstrate that automated infarct identification performs equally as well as a manual method in quantifying corticospinal tract-infarct overlap following stroke. These results demonstrate that automated infarct identification performs equally as well as a manual method in quantifying corticospinal tract-infarct overlap following stroke.

Assessment of progressive neurodegeneration in Alzheimer dementia and temporo-frontal dementia (Weiller)

The work in this task has included the development of fully automated methods for assessing regional atrophy in progressive neurodegenerative diseases by the team of Cornelius Weiller (CW). This work has been able to demonstrate regional atrophy over short periods of time (6 months). Furthermore it was possible to distinguish different patterns of atrophy in 3 variants of frontotemporal lobar degeneration (FTLD) - behavioural variant FTLD, progressive nonfluent aphasia (PNFA), semantic dementia (semD) (see published work from Frings et al., 2011).

As well as using fMRI to examine for changes in task-related networks, other work in the group of CW has examined changes in resting state networks with neurodegeneration. In this study, we demonstrate reduced fMRI signal in the precuneus in a group of patients with FTLD during a confrontation naming task. We show that this effect in FTLD patients was (1) similar to that observed in AD and MCI and (2) not related to the degree of gray matter atrophy in the precuneus. It is likely that reduced deactivation of the default network is not related to local pathology but to a lack of connectivity, which decreases in both FTLD and AD, the major cortical dementias.

We also performed more recently a longitudinal fMRI study assessing changes in functional organisation of the language system in three patient groups: AD, MCI, FTD. The tasks were: single word repetition and sentence comprehension. The patients were followed for three years. In total 25 patients have been included and progressive changes in mental performance have been correlated with changes in fMRI. Recruitment and follow-up has been completed, data are analysed, second level analysis is not completed yet. The main message is, that the mechanisms used for compensation with deteriorating mental functions are very similar to the strategies in recovery from stroke, e.g.; recruitment of the less affected side, increased attentional and intentional mechanisms. AD, MCI and FTD are to be differentiated in the clinical course as well as in the brain regions used. A manuscript is in preparation (Frings et al).

Concurrent fMRI and TMS (Ward, Rothwell)

Another way of examining connectivity, or the causal influence of brain regions on one another, is concurrent TMS-fMRI. Concurrent TMS-fMRI is a technique which allows us to examine the influence of one targeted brain region on the rest of the distributed brain network under examination. In addition, it is possible to test how this influence changes in different 'states'. For example the teams of Nick Ward (NW) and John Rothwell (JR) have previously examined the change in influence of ipsilateral dorsolateral premotor cortex (PMd) on other cortical motor regions during hand grip compared to rest.

We have now applied the same approach to studying the role played by contralesional PMd in supporting recovered motor function in more impaired chronic stroke patients. This collaborative work has resulted has been published in 2011 in *The Journal of Neuroscience* (Bestmann S et al.).

First, we used paired-coil transcranial magnetic stimulation (TMS) to establish the physiological influence of cPMd on ipsilesional primary motor cortex (iM1) at rest. We found that this influence became less inhibitory/more facilitatory in patients with greater clinical impairment. Second, we applied TMS over cPMd during functional magnetic resonance imaging (fMRI) in these patients to examine the causal influence of cPMd TMS on the whole network of surviving cortical motor areas in either hemisphere and whether these influences changed during affected hand movement. We confirmed that hand grip-related activation in cPMd was greater in more impaired patients. In conclusion, this study demonstrated that concurrent TMS-fMRI results correlated with the level of both clinical impairment and neurophysiological impairment.

Short-term effects of plasticity drivers (Ward, Weiller, Rothwell)

1. Change connectivity

The team of NW has successfully collected data functional and structural imaging data, neurophysiological data and behavioural data on approximately 19 chronic stroke patients undergoing 2 weeks of intense physiotherapy augmented with either repetitive or sham TMS just prior to each treatment session. The analysis techniques described in task 1 (DCM) has been applied to these data to to examine the relationship between cortico-cortical connectivity measures (fMRI-DCM) and improvement in motor scores with 2 weeks of intensive treatment (Figure 23). This work has been presented at the Organisation of Human Brain Mapping meeting in 2011 (Boudrias et al, OHBM 2011).

2. Brain imaging changes in stroke patients following constraint-induced movement therapy

As reported last year, the group of CW has looked at brain imaging changes in chronic stroke patients treated with constraint induced movement therapy (CIMT). Here the hypothesis was that patterns of 'recovery-related' brain reorganisation would be different depending on whether the descending pyramidal tract (PT) was affected or not.

All patients improved after two weeks of therapy, but only those with intact PT maintained improvement after 6 months. When PT was intact, improvement correlated with first a decrease of activation in SMC and after 6 months with an increase. When PT was affected, improvement consistently correlated with an increase in a lateral extension of SMC. An intact PT might be advantageous for lasting improvement after CIMT and subregions in SMC seem to behave differently during recovery.

3. Brain imaging changes in stroke patients following mirror training therapy

The group of CW performed an fMRI experiment to look at the possible mechanism of action of mirror training therapy (MTr). There is interest in whether this can be of some benefit in helping to improve motor function and/or reduce post-stroke pain. Here, healthy subjects were asked to perform a right hand task with either a mirror or non-reflective board in front of them. The group found that MTr remodels the motor system by functionally connecting hand movement to the ipsilateral sensorimotor cortex. On a system level, it leads to

interference of the neural circuit related to motor programming and observation of the trained hand with the illusory movement of the untrained hand.

The group of NW has progressed this work in stroke patients using the MEG techniques described in section 1. They explored whether on-line MTr could excite motor cortical regions important for motor recovery in stroke patients. Specifically it was asked whether MTr could (1) excite the ipsilesional primary motor cortex (iM1) and (2) change interhemispheric connections between the two primary motor cortices (M1). Stroke patients (n=7) and control participants (n=7) completed two conditions. They were asked to make synchronous bimanual flexion and extension hand movements, looking at their affected/non-dominant hand either (1) directly, or (2) as a mirror reflection of their unaffected/dominant hand.

Beta and alpha power were measured in both the left and right M1s as an indication of motor cortex excitability (see section 1 - increased synchronisation increases oscillatory power and is more prominent during rest and associated with stronger inhibition. M1-M1 Coherence strength within alpha, beta and gamma frequencies and the frequency of peak M1-M1 coherence was measured as an index of the communication between these two regions. Their results suggest that the mirror condition reduced synchronisation (and therefore reduced power and GABAergic inhibition) in the ipsilesional M1 and increased synchronisation (and therefore increased power and GABAergic inhibition) in contralesional M1 in stroke patients.

The mirrored-feedback also modified both coherence strength and the frequency of maximal coherence between the two M1s of stroke patients. The findings here provide evidence of a neurophysiological basis for the potential benefits associated with MT, specifically a reduction of inhibition (and by implication increased plasticity) in iM1, together with a change in iM1-cM1 connectivity.

High resolution mapping of visual system plasticity (Logothetis)

The study of cortical reorganization is being pursued by the team of Nikos Logothetis (NL) in a cohort of human patients with occipital cortical lesions, in collaboration with the Department of Ophthalmology at the University of Tuebingen (Prof. Ulrich Schiefer). The team of NL has been studying the effect of V1+ lesions in patients with hemianopia and quadrantanopia. They have systematically looked at the retinotopic structure and performed a population receptive field analysis. So far 6 patients have been analyzed and compared to 12 normal hemisphere controls. The retinotopy of extrastriate areas remains

generally stable, though specific changes in population RF parameters have been observed in select cases.

They have also been studying motion processing in a subset of subjects and found that the ipsilesional V5/MT+ complex is robustly activated by coherent dot stimuli. They will be following this with training the subjects to perform direction of motion discrimination in the blind hemifield (following Huxlin's work) and plot the changes in coherent motion responsiveness with time/improvement of behavioral thresholds.

Re-organization of visual areas after retinal lesions (Logothetis)

The group of Nikos Logothetis (NL) has recently published the characterization of the retinal lesion (Fischer et al., 2012) and wrote the manuscript on visual cortex organization in the macaque with macular degeneration.

The results showed that the border of the V1 lesion projection zone (LPZ) remained stable suggesting that the deafferented area V1 zone of the MD animal has limited capacity for reorganization. Interestingly the population receptive field size of non-deafferented V1 voxels increased slightly (approximately 20% on average), albeit this effect appears weaker in comparison to previous single-unit recording reports. Area V2 also showed limited reorganization. Remarkably, area V5/MT of the MD animal showed extensive activation compared to controls stimulated over the part of the visual field that is spared in the MD animal. Furthermore, population receptive field size distributions differed markedly in area V5/MT of the MD animal. Taken together, these results suggest that V5/MT has a higher potential for reorganization after macular degeneration than earlier visual cortex.

Behavioural correlates of re-organization in the visual system (Logothetis)

The study of cortical reorganization is performed by the team of Nikos Logothetis (NL) in a cohort of human patients with occipital cortical lesions, in collaboration with the Department of Ophthalmology at the University of Tuebingen (Prof. Ulrich Schiefer).

As a first step a number of patients have been recruited and have been scanned with fMRI in order to map their visual areas. To this end, for each patient the visual areas are characterized by using a) classical retinotopic mapping and b) population receptive field

mapping. In addition, the motion sensitivity of the patients' visual areas was assessed by using random-dot-kinematograms (RDKs) of different motion coherence.

Huxlin has shown that it is possible to train subjects to return direction of motion discrimination to near normal levels inside the scotoma in a retinotopically specific fashion. We are starting to train two of the subjects whose extrastriate areas we mapped for motion coherence and will follow changes in extrastriate cortex as well as in parieto-frontal higher networks that may exert top down influences in this region.

The visual rehabilitative training received by these patients was the following:

- Daily trials of direction discrimination on RDK stimuli presented at different locations close to the border, inside the blind visual field.
- Different coherence levels will be tested.
- Frequent fMRI scans to compare the maps of cortical activity between the areas of the visual field.
- Training period about 6 months, 30 min per day.

Because the team of NL had the opportunity to study a cohort of human patients with occipital cortical lesions, the studies have obviously been more slow than in monkeys. Two patients have been followed so far and have started the training protocol.

The motion sensitivity of the patients' visual areas was assessed by using random-dot-kinematograms (RDKs) of different motion coherence before the training. Results showed that the lesioned hemisphere's area V5/MT+ can be activated by stimuli presented inside the scotoma. When attention was paid on a RDK presented contralateral to the scotoma where the subject had normal vision the BOLD signal modulation did not depend on the motion coherence levels. On the other hand when the attention was shifted to the RDK inside the scotoma the BOLD signal increased with increasing levels of coherence for both hemispheres. A possible mechanism showing how successful training of the affected hemifield occur would be the increase of the signal modulation for the low coherence levels.

Development of new probes for neuroimaging (Logothetis)

The team of NL has been developing 'smart' biochemical functional markers that detect neuronal activity in real time and translate it into the changes in MR contrast permits a direct visualization of neural activation independent of the state of the vascular system. The goal of the chemistry group is development of these responsive probes. Their reactivity is modulated reversibly and fast by changes in the physiological environment, specifically changes in pH and concentrations of certain ions or neurotransmitters. Recently, the series of complexes being responsive to calcium ions was synthesized and characterized. A novel series of complexes which affects the MR signal in the presence of extracellular concentrations of calcium in buffer solutions and buffered artificial extracellular matrix (AECM) was synthesized and analyzed.

Extracellular Ca^{2+} is modulated during neuronal activity in the central nervous system and under some conditions the Ca^{2+} change can be extreme. The magnitude of these changes is directly related to stimulus frequency.

The titration with Ca^{2+} results in the increase of relaxivity. Reversibility of the changes is demonstrated by the 'reversed' titration. Addition of small portions of EDTA destroys the complex of SCA with Ca^{2+} , since EDTA creates stronger complex with Ca. Consequently, the r_1 of SCA returns to the initial value. This experiment shows that SCA could change its r_1 (and resulting MR signal) in either direction of Ca-concentration change, which occur during the calcium fluctuations during the neuronal activity.

A series of in vivo experiments in rats have been performed to characterize the distribution, half - live time and toxicity of the new contrast agents in the extracellular space of the brain. The fMRI experiment showed normal neuronal activity after injection of gadolinium (GdL4). Similar results were obtained from the electrophysiological data analysis, which shows the normal responses of the neurons after the injection. Further experiments are under way.

WORKPACKAGE 6: TESTING PLASTICITY MODULATING TREATMENTS IN HUMAN PATIENTS

Effects of currently available drivers of plastic change on reorganization in the human brain after stroke (Ward, Rothwell, Weiller)

Cornelius Weiller (CW) and his team use transcranial direct current stimulation to improve learning in stroke patients with hemiparesis. 20 stroke patients are included in the ongoing study.

The team of John Rothwell (JR) has concentrated its effort on the development of a clinical trial with stroke patients to assess whether repetitive transcranial magnetic stimulation (rTMS) can improve the response to therapy in chronic stroke patients.

TMS and behavioural measures of plasticity can provide information on:

- a) the amount of change after an intervention, e.g. changes in motor connections or functional ability;
- b) the ease of inducing change, e.g. amount of change induced by an rTMS protocol, or the rate at which a new task can be learned.

In addition it is known that repetitive transcranial motor cortex stimulation can increase or decrease excitability of motor output for the next 30 min. In this latter mode, TMS can be used as a therapeutic intervention.

The protocol used in London was a randomised trial of real and sham rTMS in chronic stroke patients. An important part of this trial involved devising a new standardised physiotherapy forearm/hand function so that all patients received the same amount of therapy throughout the trial.

The preliminary results of this first study were promising as all patients improve and maintain the improvement. This improvement was not correlated to pre-therapy ratings. Disappointing however, there was no positive effect of repetitive TMS above the level of sham treated patients. The group of JR has therefore been looking at the potential explanations of this lack of effect (poor consolidation, ceiling effect of the hand therapy alone etc) and have been refining the protocol.

Over the last 2 years of the grant, 45 patients with chronic (greater than 1yr) stroke completed an initial 2 week trial of repetitive TMS to rehabilitate their hand function. A publication on this work has been published in 2012. The trial was designed to test whether adding either excitatory stimulation to the stroke hemisphere or suppressive stimulation to the non-stroke hemisphere enhanced the effect of a well controlled behavioural therapy over a 2 week intervention period.

We found that all interventions improved hand function and that this was maintained for at least one month after the end of the intervention. However there was no additional improvement due to the stimulation. The overall size of the improvement was 5-10% of the

maximum range of any of the outcome scales that we used. There are a number of possible reasons for the lack of additional effect, but it seems likely from the data that it will be difficult to improve on best available therapy in chronic patients, particularly when this is delivered in an optimal fashion. Better effects may occur in the acute phase.

The team of JR has also performed a number of TMS and fMRI measures in a subset of the patients in the trial prior to starting treatment. They found that the relative amount of activity in the motor cortex of the stroke hemisphere during a simple hand grip was a good predictor of how well an individual would respond to therapy. People with higher levels of activity had the best response to treatment, even though the level of activity was unrelated to clinical measures of function at the time.

Finally, they have recently completed a study of additional predictors of variability to rTMS protocols in healthy individuals (Hamada et al, see reference below). They showed that the responsiveness of an individual to a theta burst protocol similar to that used in the trial above can be predicted with 60% certainty by examining some simple measures of latency variation in response to single pulses of TMS given over motor cortex.

The result is practically very useful because if it applies also in stroke (currently under test) then we will be able to screen out potential non-responders in subsequent clinical trials. Scientifically the results suggest that some basic feature of the anatomy of the brain may determine how readily it responds to the TMS therapy. We are currently modelling that in collaboration with a team in Copenhagen.

Cognitive training in patients with neurodegenerative diseases (Weiller)

The team of Cornelius Weiller (CW) has performed a clinical trial on the effects of mirror training on behaviour of stroke patients and on fMRI activation. The clinical trial design is presented in Figure 4 below. This clinical trial has now been completed. A combination of fMRI, DCM and DTI tracking has been used to assess the effects of mirror training on the healthy brain.

Two groups of healthy participants (n = 13 in each group) performed standardized fine motor tasks moving pegs and marbles (20 min/d for 4 days) with their right hand with either a mirror (mirror training group, MG) or a nonreflective board (control training group, CG) positioned orthogonally in front of them. The number of items moved by each hand was tested after each training session.

Functional MRI (fMRI) was acquired before and after the training procedure to investigate the mirror training (MTr)-specific network by the analysis of the factors Time and Group. The results showed that hand performance of the trained right hand did not differ between the 2 groups. The untrained left hand improved significantly more in the MG compared with the CG. fMRI analysis of action observation and imitation of grasping tasks demonstrated MTrspecific activation changes within the right dorsal and left ventral premotor cortex as well as in the left SMC (SMCleft).

Analysis of functional and effective connectivity showed a MTr-specific increase of functional coupling between each premotor region and the left supplementary motor area, which in turn showed an increased functional interaction with the ipsilateral SMCleft. The conclusion was that MTr remodels the motor system by functionally connecting hand movement to the ipsilateral SMC. On a system level, it leads to interference of the neural circuit related to motor programming and observation of the trained hand with the illusory movement of the untrained hand.

Approval by the local ethics committee for a new study in Alzheimer's Diseases patients in order to investigate whether specific neuropsychological interventions e.g. speech therapy or memory training may maintain or improve cognitive functions in Alzheimer's dementia (AD) has been granted in 2012. Pre- and post treatment neuroimaging will be used to detect the neural correlates of these interventions. Therapy-induced changes of behaviour will be related to changes of activation and functional connectivity within the respective networks (e.g. language or memory). Finally, the group of CW performed a fMRI study in acute stroke patients with either neglect, extinction or right hemisphere stroke without visuospatial attention deficits. The findings contradict the theory of hemispheric rivalry and support the hypothesis of limited attentional capacity of the left hemisphere for spatial attention. The study allows also a mechanistic differentiation between extinction and neglect and provides evidence that inhibition of the contralesional hemisphere does not necessarily is a therapeutic option for neglect.

Effects of new interventions in recovery (Rothwell, Ward)

The team of John Rothwell (JR) has completed in 2010 a 2 week randomised control trial of add-on rTMS therapy for arm/hand function in chronic stroke. A publication presented the main clinical results has been published in 2012 (Talelli P et al.; see below). The introduction of a standardised therapy for arm/hand control for use in add-on therapy trials has been published in 2010 (Wallace et al., 2010).

The team of JR has been studying the responses of individual people to brain stimulation interventions. These kind of therapies are indeed widely used by many groups BUT responses vary a lot between different individual people meaning that the overall population effect is noisy. This turned out to be also the case with the plasticity-enhancing treatments; people react very differently to the treatment. So the goal of this study is based on the concept that the therapeutic benefit will improve if we can target stimulation to people in whom it has biggest effect.

115 healthy volunteers aged mainly 18-22 years have been enrolled in the study. The conclusion of the study is that intermittent theta burst (iTBS) induced MEP plasticity is highly variable between subjects. Unfortunately, people are very different in the way they react to the stimulation.

The team of JR has then further investigated the reason for such important variations. It seems to be able to differentiate the subjects based on genotyping, however, as seen on the graph below, there is still an overlap between the 2 populations, so the genotype only explains a fraction of the variance between individuals and their response to the plasticity protocol. However, other factors related to exactly which populations of cerebral neurones are targeted in individual subjects explain quite a lot of variation.

In conclusion, there are ways that we can use to predict the effect of a particular plasticity treatment and be able to guide patients to the best form of treatment.

In parallel with this, the team of JR has been developing new training protocols for therapeutic intervention after stroke. This second trial will be testing the effect of training and rTMS on shoulder/elbow reaching movement. It is more rare to study the effect of new therapies on shoulder/elbow movement than on hand function. However, it is a crucial element of rehabilitation given the importance of proximal control for the success of distal manipulation. In addition these muscles have a bilateral innervation making it more likely that the pattern of recovery (i.e. involvement of the non-stroke hemisphere) will differ from that in patients where we examine only hand and forearm function.

There are two aspects to the design of the investigation. First we ask whether stroke survivors can learn and improve on a reaching task, and if so what type of training will be optimal. Second we will examine with the ratios of contralateral and ipsilateral innervation of the shoulder muscles in each patient. We suspect that those in whom function is best preserved will have the most intact (undamaged) ipsilateral input from the non-stroke hemisphere. We will also assess how this innervation is affected by the training protocol.

To perform the investigation we have developed in-house a new robotic arm manipulandum to assess the shoulder / elbow movements. The team has now obtained data on healthy subjects as well as some pilot data in stroke survivors. We have shown that it is possible to obtain clear measures of corticospinal input to shoulder muscles in stroke survivors and that the pattern of innervation differs among individuals. We have also confirmed that all individuals can perform the task and can learn to improve performance over 4 days of practice.

In addition, the team has been extensively studying the best protocol for rehabilitation. The question raised during the preparation of the protocol was: is it better to train the patients to go slowly and accurately, or ask the patients to do the movements as fast as possible and tell them not to worry about the accuracy - in other words, can we obtain a better rehabilitation if one train speed or accuracy.

The second important question relates to generalization. If you train stroke patients to move his shoulder / arm in one specific direction during the task, will they be able to generalize the movement and adapt it to different directions. This is a very important question as training must be generalized to be useful. The team is currently recruiting patients for the main trial. Preliminary results show that patients improve arm movements over the 4 days of therapy. However it is too early to conclude whether there are differences in transfer to other tasks.

A second major development that has arisen from the wider collaborations within this project has been to develop a clinical study using inosine to promote plasticity and recovery in stroke patients. The rationale of this new clinical trial is based on the fact that inosine is a naturally occurring nucleotide that is known to promote axonal reorganization and enhance recovery of function in models of spinal injury and stroke (see work of Fawcett and others). In previous studies, inosine has been given iv. to humans to protect kidney and to improve myocardial function.

This new trial will be modelled on the anti-Nogo A trial and will consist of :

- a) Toxicology studies;
- b) Safety study in 40 human stroke patients involving;
- c) 2-4 weeks iv administration from 3-4 days after stroke;
- d) Safety monitoring (heart, liver, kidney function...) and
- e) Application of biomarker measures to test for possible functional effects.

James Fawcett and John Rothwell have applied for extra funds from the Wellcome Trust to perform a clinical safety trial. Input from the Stroke Association has been sought successfully and they have confirmed that they are very enthusiastic about such a trial.

Clinical trial of anti-Nogo A in spinal cord injury (Schwab, Novartis)

The anti-Nogo- A trial initiated by the team of Martin Schwab (MS) is run by Novartis in a European and a North American clinical network (more than 12 clinics currently involved). The trial involved the application of the anti-Nogo-A antibody (human IgG against human Nogo-A) first intrathecally and later as bolus injections to acutely injured spinal cord injury patients.

The Phase 1 trial was initiated in June 2006 with 3 cohorts of acute ASIA A thoraco-lumbar lesion patients. Application of the anti-Nogo-A antibody was done by external pumps, following an increasing dosage and exposure up to 30 days of continuous intrathecal infusion. For safety reasons the following cohorts were treated with bolus injections (several intrathecal injections of Nogo-A antibodies within the first month after injury).

We have concluded successfully in 2011 in collaboration with Novartis the Phase I trial of anti-Nogo-A antibody in spinal cord injury. In the trial 52 acute, severe (ASIA A: no motor or sensory functions below lesion) para- and tetraplegic patients were involved. The patients were assessed with the ASIA protocol and were followed for one year after the completion of the treatment. The conclusion is that there is no safety concerns reported, and that the antibody is safe and well tolerated after a 4-week infusion or repeated injections of high amounts of antibody intrathecally in the lumbar CSF.

Additionally we have Pilot outcome results from patients receiving the full dose of antibody for one month: among those more than 30% of patients exhibit functional improvements (sensory and/or motor; conversion to ASIA B or C) at a late stage (>3 months), when normally no improvements are seen (EMSCI historical database with >2600 patients). Conversions normally occur within the first month after injury, due to regressing edema and inflammation. Lesions are often very big in this ASIA A patients group, minimizing chances for fiber regrowth and recovery.

A Phase II clinical trial in patients with severe sensori/motor incomplete lesions is now in preparation. Since these patients have better anatomical conditions at the lesion site, the

temporary neutralization of the important growth inhibitor Nogo-A may result in higher degrees and a higher proportion of recovery.

Potential Impact:

IMPACT OF PLASTICISE

The proposed project capitalized on combined expertise in different areas of regenerative medicine. Furthermore, PLASTICISE involves collaborative interactions that allow us to merge our unique and complementary expertise in the field, from the bench to the bedside.

Impact on science

PLASTICISE has been designed to address the societal-economic impacts of neurodegenerative diseases, integrated with advanced research, aligned with what is required to identify and validate optimal treatment regimens. This requires a detailed molecular/cellular understanding of synaptic change to provide new knowledge for developing new plasticity enhancement treatments for promoting recovery of function in human patients with neurodegenerative disease. As described above, major achievements done throughout the grant both in the pre-clinical and clinical settings have seen that PLASTICISE is going beyond the state of the art. These scientific highlights have been largely disseminated to the scientific community through peer-reviewed publications, talks and poster presentations.

Economic benefits

In Europe overall, neurological damage accounts for 40% of people severely disabled and who require daily help (Wade and Hewer, 1997; Office of Population Censuses and Surveys, 1998). Neurodegenerative diseases, including stroke and Alzheimer's disease, are the major causes of chronic disability in European communities. With the increasing number of elderly people, coupled with successful treatment of non-neurological causes of chronic illness, the incidence of neurodegenerative disease will increase. In total, by 2013 it has been estimated that there will be some 8.5 million European citizens afflicted with a neurodegenerative disorder.

Alzheimer's disease: It has now been reported that the world is on the brink of an Alzheimer's epidemic in which the number of sufferers could quadruple over the next 40 years. The Alzheimer's disease market across the seven major markets is set to double in value over the next 10 years, from \$5.3bn in 2011 to \$12.6bn in 2021. The catalysts for this

growth include an increasingly elderly population, earlier and improved diagnosis, and the introduction of new therapeutic classes.

Stroke: Europeans suffer nearly one million strokes each year, highlighting the need for efficacious therapy and the tremendous market potential for effective stroke therapy. Between 15-30% of ischemic stroke victims are permanently disabled and 20% require prolonged institutional care. As a result, stroke is one of the most common causes of long-term serious disability and represents an economic burden similar in scale to myocardial infarction. The potential combined market size in US and EU for stroke is estimated at 12.56 billion USD.

Spinal cord injury: estimated to be at least 330 000 people living with spinal cord injury (paraplegia and tetraplegia) with over 15 000 new cases reported each year. In two-thirds of cases, road accidents are the cause of injury, with sporting accidents making up another 10%. Most occur at a young age: average age of 19; about 80% of males with spinal cord injuries are aged 18-25 years. The cost of treatment and aftercare for sufferers is phenomenal: the average lifetime costs directly attributable to spinal cord injury for an individual injured at age 25 range from 0.45 M to 2.1 M USD and have to prepare to spend an average of forty years or more in a wheelchair. GlobalData estimated the acute spinal cord injury (ASCI) therapeutics market to be worth 44.78m USD in 2010 and forecasts it to grow at a CAGR of 6.3% to reach 68.76m USD in 2017.

PLASTICISE is thus contributing to the alleviation of these chronic diseases by understanding the underlying pathological mechanisms and by developing new promising treatments.

Impact on society

Degenerative diseases create a life-altering experience for the person with injury, for their partner, parents, siblings, and children. The impact on and subsequent dimishment of body functions associated with the diseases can cause depression and loss of self-esteem. Given the diversity of degenerative diseases indicated above, pathological manifestation can occur at any age: either as a child, during an individual's most productive years, or as an aged person. In most cases, patients require continuous physical and medical care depending on the disease, severity of manifestation, degree of disability, and location of injury. The burden of care giving most frequently falls on the partner. Care giving partners are often severely stressed, particularly due to health issues that arise after tissue degeneration initiates and suffer emotional stress that is comparable to or greater than those of the injured partner.

Caregivers have a higher incidence of physical stress, emotional stress, burnout, fatigue, anger, and resentment.

Hope is considered an important coping strategy for both the person and family with degenerative diseases. Goal-directed hope based on realistic perceptions of life, focusing on progress, positive interpretation of events, are important in helping people and families cope with the disease. Hope is also focused towards the society at large, that new therapeutics are developed.

In addition to imposing direct medical costs on society, degenerative diseases also result in indirect costs, primarily related to reduced productivity due to disability with a further loss of self-esteem of the sufferer and diminished integration into society. PLASTICISE aims to provide more than hope: we aim to provide validated treatments to promote brain plasticity.

MAIN DISSEMINATION AND EXPLOITATION ACTIVITIES

PLASTICISE teams have placed a particular emphasis throughout the grant period to disseminate their work and scientific highlights to the scientific community, the European Commission, patient associations and public at large. You will find here a summary of the different actions taken:

1. Development of the project website

The project website – <http://www.plasticise.eu> - was developed early 2009 and was online in April 2009. The website is updated every month with news and events. As part of a communication campaign towards the public and patient associations, the design of the PLASTICISE website has been completed modified in 2011 and a new website has been published at the beginning of November 2011 (still online). The goal of this public web site is to communicate about the PLASTICISE research to the European Commission, patients associations and general public. The project management team has placed particular attention to creating an attractive content that would be understandable for a lay audience.

2. Dissemination to the scientific community

Participation to international conferences, poster presentations and lectures. The full list of international conference attendance by the PLASTICISE teams is available on the ECAS portal. In addition, a pop-up banner has been created in 2012 to present the PLASTICISE consortium in international meetings. As an example of these events, Isabelle Weiss from the Management team has presented the PLASTICISE consortium at the TERMIS meeting, September 5-8th, 2012 in Vienna (see <http://www.wc2012-vienna.org/> online). TERMIS stands for 'Tissue Engineering International and Regenerative Medicine Society'. The banner was part of a booth presenting several European-funded projects in the Exhibitor area of the Congress Center. The booth has been very well visited and many scientists took the PLASTICISE flyer.

List of publications. The full list of publications is available on the ECAS portal. Below you will find the list of collaborative publications originated from the coordinated work within the consortium.

Work Package 1

-Carulli D, Pizzorusso T, Kwok JC, Putignano E, Poli A, Forostyak S, Andrews MR, Deepa SS, Glant T, Fawcett JW. Animals lacking link protein have attenuated perineuronal nets and persistent plasticity. *Brain*. 2010 Jun 20.

-Gunnar Dick, Jessica C. F. Kwok, Chin Lik Tan, Joao Nuno Alves, Erich Ehlert, Kazuyuki Sugahara, Joost Verhaagen & James W. Fawcett. Semaphorin 3A interacts with Chondroitin Sulphate type E (CS-E) in binding to glycosamino-glycans (GAGs) of perineuronal nets (PNNs) in rodent brain. Under revision.

-Fawcett JW, Schwab ME, Montani L, Brazda N, Muller H-W (2012) Defeating inhibition of regeneration by scar and myelin components. In: *Handbook of Clinical Neurology* (Verhaagen J, McDonald JW, eds), Elsevier

-Tam Vo, Daniela Carulli, Erich M.E. Ehlert,, Gunnar Dick, Vasil Mecollari, Elizabeth B. Moloney, Gera Neufeld, Fred de Winter, Jessica C.F. Kwok, James W. Fawcett, Joost Verhaagen. The Chemorepulsive Axon Guidance Protein Semaphorin 3A is a Constituent of Perineuronal Nets in the Adult Rodent Brain. Under revision.

-Romberg C, Yang S, Melani R, Andrews MR, Spillantini MG, Bussey TJ, Fawcett JW, Pizzorusso T, Saksida LM. Depletion of perineuronal nets enhances memory and long-term depression (submitted to PNAS)

Work Package 3

-Zhao RR, Muir EM, Alves JN, Rickman H, Allan AY, Kwok JC, Roet KC, Verhaagen J, Schneider BL, Bensadoun JC, Ahmed SG, Yáñez-Muñoz RJ, Keynes RJ, Fawcett JW, Rogers JH. Lentiviral

vectors express chondroitinase ABC in cortical projections and promote sprouting of injured corticospinal axons. *J Neurosci Methods*. 2011 Sep 30;201(1):228-38. Epub 2011 Aug 9.

-E. Dassie, M. R. Andrews, J.-C. Bensadoun, M. Cacquevel, B. L. Schneider, P. Aebischer, F. S. Wouters, I. Hussain, D. R. Howlett, M. G. Spillantini, J. W. Fawcett. Focal expression of mutant tau via AAV induces neurofibrillary tangle formation, neuronal loss, neuroinflammation and memory impairment in an APP mouse model. *Neurobiology of Disease* 2012 Dec 25

Work Package 5

-Bestmann S, Swayne O, Blankenburg F, Ruff CC, Teo J, Weiskopf N, Driver J, Rothwell JC, Ward NS. The role of contralesional dorsal premotor cortex after stroke as studied with concurrent TMS-fMRI. *J Neurosci*. 2010 Sep 8;30(36):11926-37.

Work Package 6

-Talelli, P., Dileone, M., Hoad, D., Cheeran, B, Oliver, R., Van Den Bos, M., Hammerbeck, U., Cloud, G., Ball, J., Marsden, J., Ward, N.S., Di Lazzaro, V., Greenwood, R. and Rothwell, J.C. (2012). Theta Burst Stimulation in the rehabilitation of the upper limb: a semi-randomised, placebo-controlled trial in chronic stroke patients. *Neurorehabilitation and Neural Repair*, in press

-Wallace AC, Talelli P, Dileone M., Oliver R, Ward N, Cloud G, Greenwood R, Dilazzaro V and Rothwell JC (2010). Standardizing the intensity of upper limb treatment in rehabilitation medicine. *Clinical Rehabilitation* 24, 471-478.

Patent and commercial exploitation

One patent has been approved during the second reporting period. This patent has been granted to James Fawcett (#1a) on 'Treatment of CNS damage' with the reference number 10179661.3-2406. Two patents have also been submitted during the second period. One by Leszek Kaczmarek (#10) on 'A method and a system for processing an image comprising dendritic spines' (Reference number: EP11461530.5). One by Martin Schwab (#2a) on 'A novel Nogo-A specific receptor'. These applications have been done in collaboration with the local tech transfer offices, supported by PLASTICISE Management team.

Cooperation with other research programmes

There have been successful interactions with other projects to promote either scientific exchanges and capacity building of young scientists.

Scientific:

Many PIs from PLASTICISE are involved in other EC and non-EC grants, where there is sometimes an overlap of partners. A table presenting these shared scientific objectives is available in the attached pdf.

Joint 2010 meeting with NeuGene:

In addition, the 2010 PLASTICISE annual meeting was organized jointly with NeuGene (see <http://www.neugene.eu> online), another European Consortium, focussing on the use of gene therapy as a new approach to CNS diseases. The meeting took place in Barcelona from the 13th to the 15th of January 2010. The 1st and 3rd day of the meeting, the consortia met separately to discuss the scientific progress within the different WPs, project planning and management issues. On the 2nd day, the members of the two consortia (around 60 persons all together) met for a proper scientific conference with presentations and shared discussions from members of the two networks. Dinners and lunches were also jointly organized to promote interactions between the two consortia.

The rationale to have a joint meeting with another EC consortium was to bring together a certain mass of scientists and have a proper scientific conference (Day 2) with scientific exchanges, particularly on the gene therapy techniques used by several partner teams in both networks. There is in addition a small overlap of teams between the two networks, the team of Patrick Aebischer being present in both. This initiative has been successful and feedbacks have been really positive from both side.

Young scientist Capacity building:

Online TOPEA tutorial programme - TOPEA is an initiative from Dando, Weiss and Colucci to provide complementary training to the young scientists coming from several EC-funded projects. The TOPEA programme is exclusively dedicated to young researchers, providing them with complementary training in degenerative processes / regenerative medicine and soft skills (e.g; grant writing, project management, IP protection, communication and outreach). TOPEA represents series of online tutorials and summer schools where the young

scientists are presenting their scientific achievements to their peers. The list of web tutorials can be found under: <http://www.dwc-alliance.com/pages/training.html>

3. Dissemination to the general public and patient groups

The research achievements made by the PLASTICISE partners in the domain of Alzheimer's Disease, Stroke and Spinal Cord Injury have been shared with the public and patient groups via the development of outreach activities. A list can be found below:

-UCAM (Partner #1a) - Hobart CNS Repair, October 2010, Outreach lecture by James Fawcett on Spinal Cord Treatment, Size of Audience: 100 patients

-UCAM (Partner #1a) - Spinal Cord Symposium, Toledo, Spain, April 2011 keynote lecture from James Fawcett on Plasticity and the Extracellular Matrix, Size of Audience: 150 patients, medical and scientific persons

-UCAM (Partner #1a) - ASIA ISCOS, Washington DC, US, June 2011 keynote lecture from James Fawcett on Rehabilitation in SCI, Size of Audience: 500 patients, medical and scientific persons

-UCAM (Partner #1a) - Outreach event in a school in Bedford, November 2011 by James Fawcett on Spinal Cord Injury, Size of Audience: 40 pupils

-UCAM (Partner #1a) - Cheltenham science festival, Cheltenham UK, June 2012, general public, Size of Audience: 500 persons

-UZH (Partner #2a & 2b): 7th March 2009 - 'Brain Fair Open Day - Brain Research Institute'; Targeted audience: Broad public; Size of Audience: 200 persons

-UZH (Partner #2a): 11th June 2010 - 'Jubiläumssymposium 20 Jahre FRAGILE Switzerland'; Targeted audience: Broad public; Size of Audience: 150 persons

-UZH (Partner #2a) - 1st International Spinal Cord Repair Meeting, Step by Step Foundation, Barcelona, Spain, March 2011-keynote lecture from Martin Schwab on 'Mechanisms of Plasticity and Regeneration after Spinal Cord Injury' - Size of Audience: 80 scientists, neurologists and SCI patients

-UZH (Partner #2a & 2b): Brain Research Institute, 50th Anniversary of the Institute, Open Day, November 2012 - Size of Audience: 1000 visiting persons

-FMI (Partner #5): May 10-11th 2009 - 'Gene technology days' ; Targeted audience: High school students; Size of Audience: twice 120 students and their teachers

-FMI (Partner #5) - 40th Anniversary of the FMI, Basel, CH - September 2010, Keynote lecture from Pico Caroni, Size of Audience: 500 persons

- FMI (Partner #5) - Gene Technology Days for High School students, FMI, Basel, CH - May 2011, participation of Pico Caroni, Size of Audience: twice 100 students and their teachers
- UCL (Partner #7a) - Medicine and Me: Stroke at The Royal Society of Medicine, London, UK, June 2011 – Invited talk from Nick Ward on 'How does functional imaging show the brain's potential for plasticity? ', Size of Audience: 75 stroke survivors and carers, stroke physicians
- UCL (Partner #7b) - Open Day of the National Hospital for Neurology and Neurosurgery, London, UK, June 2012, General public - Size of Audience: 500 persons
- NENCKI (Partner #10) - The National Fund for Children, Warsaw, Poland, 13 July 2011, keynote lecture from Leszek Kaczmarek to High school students on 'Our memory. How we study it? What do we know about it? ' - Size of Audience: 30 students
- NENCKI (Partner #10) - National Conference for Students and Young Scientists 'Faces of Neuroscience', Warsaw, Poland, 6 November 2011 - keynote lecture from Leszek Kaczmarek to students on ' Stimulation of gene activity as a result of learning' - Size of Audience: 100 students
- NENCKI (Partner #10) - 1st European Day of the Brain. 'Ageing, Stroke and Alzheimer's Disease' - Finding Innovative Solutions Expert Conference during Polish Presidency of the European Union Council, Warsaw, Poland, 18 November 2011 - keynote lecture from Leszek Kaczmarek on 'Neurodegeneration: from the basic research to the disease understanding and treatment: A case study' - Size of Audience: 100 representatives of patient organizations, scientists and policy-makers
- D-PHARM (Partner #13) : March 2010 - 'MACSI Investigator's meeting'; Targeted audience: Clinicians /MACSI Investigators; Size of Audience: 200 persons

4. Specific actions towards patient associations and members of the European Parliament

Information on PLASTICISE distributed via a flyer

During Period 1, the management team has listed the different patient groups and charities supporting patients and their families suffering from Alzheimer's Disease, Stroke and Spinal cord injuries. This list is available on the PLASTICISE website (see <http://www.plasticise.eu> online).

Based on the 2011 research advances and publications from the consortium partners, a flyer presenting the consortium activities has been prepared by the management team (Isabelle Weiss and Marcin Maj #14) and has been sent late 2011 to the major stroke, spinal cord injury and Alzheimer's Diseases patient groups in the 8 European countries where

PLASTICISE's partners are located. A second run of emails has been sent early 2012 to the Members of the European Parliament (MEPs) of the 8 countries where the PLASTICISE partners are located, together with a press release prepared based on the second periodic report.

Press releases

Following the first annual meeting of the consortium that took place from the 14th to the 16th of January 2010, a press release has been prepared and published in April 2010. This press release has been published on the Cordis Wire website and sent to several patient associations. It presented four recent research advances by different teams of the consortium. Access to this press release can be found under:

<http://cordis.europa.eu/wire/index.cfm?fuseaction=article.Detail&rcn=21712&rev=0>

Following the second annual meeting of the consortium that took place from May 4-6th, 2011, a press release has been prepared and published in July 2011. This press release has been published on several websites (inc. <http://www.dwc-alliance.com/pages/news.html>) and sent to several patient associations in Europe. It presented recent research advances by different teams of the consortium.

To keep patients, their families and organisations supporting them as well as media representatives up to date about last project achievements, two press releases have been prepared and published in 2012. These press releases have been sent to several members of the Communication Office and the Research and Innovation section of the European Commission and published on several websites, including Cordis Wire: April 2012 press alert: <http://cordis.europa.eu/wire/index.cfm?fuseaction=article.Detail&rcn=29928&rev=0>.
December 2012 press alert:

<http://cordis.europa.eu/wire/index.cfm?fuseaction=article.Detail&rcn=33687&rev=0>

List of Websites:

<http://www.plasticiseE.eu> (still online)