SIXTH FRAMEWORK PROGRAMME
Thematic priority 1: **Life sciences, genomics and biotechnology for health**

Project title: **From cell-cell recognition to memory formation. New strategies for treatment of dysfunctional plasticity, learning and memory**

Integrated Project

4.1 Publishable final ACTIVITY REPORT

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Project coordinator & Organisation
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PROMEMORIA

4.1. Publishable final activity report

1 April, 2005 – 30 March, 2009

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4.1 Final activity report

4.1.0. Summary

The aim of the PROMEMORIA project was to investigate the role of neuronal cell adhesion molecules (CAMs) in neuronal plasticity, learning and memory. This included the establishment of in vitro and in vivo models for the evaluation of the role of neuronal CAMs in impaired neu-ronal plasticity and CNS disorders. Moreover, it was the aim to develop new strategies for modulation of synaptic plasticity in order to create novel therapeutics improving learning and memory and neuroregeneration.

The consortium consisted of 17 leading teams from 11 countries. It was composed of 14 acade-mic partners and three SMEs. The work was distributed in 11 workpackages. The first eight of these comprised the scientific backbone of the consortium and the final three provided the structure for advanced training of scientists, efficient management as well as optimal dissemina-tion and exploitation of results. The consortium was supported by a Scientific Advisory Board and a Key Stakeholders' Committee.

The goals to be achieved were defined in 80 deliverables. All of these have been achieved. Thus, the consortium has published in total 209 scientific articles (180 published and 29 in press). Moreover, 31 patents have been filed and of these 18 have been published. A number of meetings have been arranged. This includes five General Assemblies, and Governing Board meetings every three months. Moreover, six technique-oriented workshops have been held, three phase-I clinical trials have been carried out based on compounds developed by the consortium and finally, two new pharma/biotech enterprises have been established as spin-outs of the project. The consortium has achieved more in all respects than indicated in the deliverables.

4.1.1. Project objectives

- **The main goal of the Promemoria project was to investigate the role of neuronal cell adhesion molecules (CAMs) in neuronal plasticity, learning and memory**.

  - To achieve a better understanding of the role of CAMs in the maintenance and modulation of synaptic and network plasticity and in cortical circuitry and information processes underlying learning and memory.
  - To develop and validate a series of suitable animal models for studies of the role of CAMs in memory function and dysfunction in diseases
  - To search for novel ligands and mimetics of neuronal CAMs for modulation of plasticity with the aim of developing therapeutics improving learning, memory and neuroregeneration.

- **The operational goals** of the consortium, therefore, were:
  - To study the structure and expression of genes and proteins of CAMs and CAM-related molecules involved in plasticity, learning and memory
  - To study the inter- and intracellular signaling resulting from activation of selected neuronal CAMs
  - To study the role of CAMs in neuronal circuits both electrophysiologically and morphologically
  - To evaluate the role of neuronal CAMs in the intact organism under normal conditions and in relation to emotional and traumatic memories
  - To establish animal models for the evaluation of the role of neuronal CAMs in impaired neuronal plasticity, CNS disorders and adult neurogenesis
  - To develop new strategies for modulation of plasticity with the aim of creating novel therapeutics improving learning, memory and neuroregeneration

4.1.2. Contractors involved

The consortium consisted of 17 leading teams from 11 countries. It was composed of 14 academic partners and three SMEs. The partners covered a wide range of expertises and technical competen-cies required to address in an ambitious manner the full scope of the project. Thus, the consortium contained teams specialized in genetics, protein chemistry, neurophysiology, neuroanatomy, neuro-biology, animal models of learning and behaviour, and *in vivo* test systems for a very broad range of behaviour and learning phenomena. Moreover, there was a considerable expertise in drug screening and development.

The coordinator, professor Elisabeth Bock, University of Copenhagen, has been responsible for the management of the consortium. To achieve the aims of the project, the work was distributed in **11 work packages**. The first eight of these comprised the scientific backbone of the consortium, and the final three provided the structure for advanced training of scientists, efficient management, as well as optimal dissemination and exploitation of results. The consortium was supported by a **Scientific Advisory Board**, whose responsibility it was to monitor the scientific quality of the project, and a **Key Stakeholder's Committee** consisting of representatives of health care providers, European patient organizations, medical ethics, and ethical aspects of animal experimentation. The latter committee was expected to monitor the approach of the Promemoria consortium to patient interests and important ethical principles.

4.1.3. Work performed and results achieved

Below a summary of work performed and results achived in all workpackages (WPs) is given. For each WP the WP-leader, the participating partners, the aims and the deliverables are indicated.

WP1 Neuronal CAMs and their counter-receptor; structure and expression of genes and proteins involved in the modulation of plasticity, learning and memory
WP-leader: partner 1, Elisabeth Bock

Partner 5. HansJürgen Volkmer
Partner 6. Dominique Muller/Jozsef Kiss
Partner 9. Rita Gerardy-Schahn
Partner 10. Jacek Kuznicki
Partner 12. Carmen Sandi
Partner 14. Vladimir Berezin
Partner 16. Claus Schafer Nielsen
Partner 17. Alex Zharkovsky

Objectives of WP1
The objectives of this WP were to 1. *screen and characterize molecules involved in intracellular recognition capable of modulating plasticity* properties using genetics, cell biology and structural biology. Moreover, based on the characterisation of selected molecules, 2. *mimetics of cell recognition molecules should be identified and developed*. This included 3. *testing in codified in vitro tests and evaluation of their purity, stability and pharmacokinetics*. Finally, 4. *the role of the calmyrin subfamily of neuronal calcium sensors* should be characterized. The deliverables of WP1 are indicated below.

The following results were achieved in WP1

1. Screening and characterization of molecules involved in intercellular recognition capable of modulating plasticity

A number of CAMs have been expressed recombinantly either as complete proteins or in fragments for the study of structure and receptor-ligand interactions. These include NCAM, NCAM2, L1-CAM, neurofascin, neuroplastin55, neuroplastin65, Nectin-1 and Nectin-3. Moreover, ligands/counter-receptors of CAMs have been recombinantly expressed. These include FGF-receptor1-3 isoforms b and c, FGF-receptor4 and GDNF. If antibodies were not available against the above mentioned CAMs, these have been produced and include antibodies against NCAM2, Neuroplastin55 and Neuroplastin65. By X-ray chrystallography structures have been obtained of all seven modules of NCAM2, and all three modules of Neuroplastin55-65. Moreover, the structure of two modules of NCAM2, one of Nectin1 and one of FGFR1 have been determined by NMR. None of these structures have been determined before. Employing NMR, receptor-ligand interactions have in a number of cases been studied by evaluating chemical shifts, and in this way a number of homophilic binding sites in NCAM have been determined. The binding sites in NCAM for FGFR1 and GDNF have also been determined. Moreover, binding sites for NCAM, heparansulphate, FGF1, and the FGF-receptor itself, have been determined in FGF-receptor1. For the studies of the various neuronal CAMs, a number of genetic manipulations have been applied to neuronal cultures including the use of shRNA of NCAM, neuroplastin, neurofascin and ST8SiaII. For a number of biological studies genetically manipulated animals (mice) have also been employed. These include knock-outs of NCAM, L1-

CAM, enzymes involved in the polysialylation pathway, and GFAP. The latter has been employed for the study of the role of astrocytes in relation to brain injury.

2. Mimetics of cell recognition molecules

A considerable number of peptide mimetics of the studied proteins have been developed. These include mimetics corresponding to the trans-homophilic binding sites of NCAM (*dennexins* and *plannexins*), mimetics of binding sites between NCAM and its counterreceptor, the FGF-receptor (*encamins*), mimetics of the L1-CAM corresponding to binding sites for the FGF-receptor (*elcamins*), and mimetics derived from a number of FGFs due to sequence similarity to NCAM (*dekafins* and *dyofins*). Functional mimetics derived from neurofascin are being characterized. Moreover, mimetics of the FGF-receptor corresponding to the binding sites for NCAM have been prepared and characterised (*enreptins*), and finally, a peptide mimetic derived from GDNF corresponding to the binding site to NCAM (*gliafin*) has been prepared and characterised. A number of biochemical and *in vitro* biological tests have been employed to characterise these peptide mimetics, and as positive controls mimetics derived from selected neurotrophins, erythropoietin, S100 proteins and metallothionein1/2 have been identified and characterised simultaneously.

3. Characterisation of peptide mimetics

Characterisation of the CAM mimetic peptides has included demonstration of binding to relevant counter-receptors by surface plasmon resonance including determination of Kd, and demonstration of activation of various signal transduction pathways. In cell cultures, their effects on neurite extension, synapse formation, neuronal survival, and synaptic release have been studied. Moreover, a model of network dynamics and spine turnover has been established in hippocampal slice culture. After suitable characterisation, selected peptide mimetics have been chosen for characterisation by pharmacokinetics including preparation of a peptide specific antibody. Peptides characterised in this way include a peptide derived from NCAM corresponding to the FGF-receptor binding site (the FGL peptide), a peptide corresponding to the *cis*-homophilic binding site in NCAM (the P2 peptide), two FGF-derived peptides (a dekafin and a dyofin peptide). The same has been done for peptides derived from erythropoietin, S100A4 and metallothionein1/2. Interestingly, the peptides all seem to stay in plasma for two-six hours, and they all seem to appear in the cerebrospinal fluid with a CSF:plasma ratio between 1:5-1:20, indicating that they easily cross the blood-brain-barrier.

4. The role of the calmyrin subfamily of neuronal calcium sensors

It has been shown that the glutamate agonist NMDA induces a considerable increase of both calmyrin2 mRNA and protein. This induction could be blocked by calcium-chelation and by NMDA antagonists, whereas neuronal activation by means of metrazol increased the expression of the protein.

Deliverables:
D.1.1. Screening and initial characterization of molecules involved in intercellular recognition capable of modulating plasticity properties using genetics, cell biology and structural biology.
D.1.2; 1.3; 1.3A Identification and development of mimetics of cell-cell recognition molecules.
D.1.4; 1.4A Characterization of NCAM mimetics in codified *in vitro* tests and evaluation of their purity, stability and pharmacokinetics.
D. 1.5 Characterization of CaMy2 as a calcium-binding protein involved in regulation of hippocampal neuronal processes.

All deliverables of WP1 have been achieved

WP2 Inter- and intracellular signaling underlying plasticity, learning and memory; Modulations by neuronal CAMs.
WP-leader: partner 3, Dmitri Rusakov

Partner 2. Marina Lynch
Partner 3. Dmitri Rusakov
Partner 10. Jacek Kuznicki

Further collaboration
Partner 1. Elisabeth Bock
Partner 7. Mike Stewart
Partner 12. Carmen Sandi
Partner 14. Vladimir Berezin

Objectives of WP2

1. To probe the role of cell adhesion in modulating basal transmission at individual synapses in vitro, by combining single-cell electrophysiology, two-photon excitation imaging, and pharmacological testing (based on the ad hoc developed biochemistry protocols) in acute hippocampal slices.

2. To probe the role of cell adhesion in modulating transmission in the synaptic circuitry in vivo, by combining electrophysiology methods, use-dependent plasticity induction protocols, biochemistry and molecular biology tools applied in the hippocampus of anaesthetised animals.

The following results were achieved in WP2

1. *Continuous assessment of causal relationship between CAM-related signalling and the basal synaptic transmission in vitro and in vivo*.

Effect of NCAM-associated peptides on synaptic transmission in vitro. The effects of FGL, P2, hekaton3, hekaton6 and 1-fg peptides on the amplitude and paired-pulse ratio of excitatory glutamatergic responses have been documented in single CA1 pyramidal cells in the hippocampus using patch-clamp electrophysiology in acute slices. In summary: FGL decreased the EPSC amplitude while increasing the paired pulse ratio (PPR) this indicating a lower release probability; P2 decreased the EPSC amplitude while decreasing PPR and increasing holding current; hekaton3 induced a reversibly increased synaptic responses with no changes to PPR, and hekaton 6 increased the EPSC amplitude while decreasing PPR.

Effect of FGL on synaptic transmission and plasticity in vivo. Analysis of LTP in aged and young rats revealed an age-related decrease and this was attenuated by FGL. This has been associated with a decrease in expression of synaptophysin, syntaxin, synaptotagmin and SNAP25 in synaptosomes prepared from hippocampus, but not cortex, in which no age-related changes were observed. The age-related changes were significant in all cases except synaptotagmin (although the trend persisted) and the age-related decreases in expression of these proteins were attenuated in tissue prepared from FGL-treated rats. FGL did not modulate KCl-stimulated glutamate release from synaptosomes prepared from cortical tissue of young or aged rats. We have observed an age-related increase in microglial activation and consequently an increase in hippocampal concentration of the proinflammatory cytokine IL-1β (which is known to inhibit LTP). No consistent age-related changes in 4AP-induced glutamate release were

observed and there was no consistent effect of FGL. Whereas age-related decreases in synaptophysin, synaptotagmin-1, SNAP25 and syntaxin were observed in synaptosomes prepared from cortical tissue, similar changes were not observed in synaptosomes prepared from dentate gyrus. There was no consistent FGL-associated change in these proteins and comparison of tetanized with untetanized tissue also failed to show consistent changes. Because growth factor receptor engagement has been shown to trigger activation of PI-3 kinase and AKT, and consequently inhibit activation of GSK-3β, we assessed the effect of FGL on this signalling sequence in tissue prepared from young and aged rats which were/were not treated with FGL. FGL increased the expression of the 85 kDa sub-unit of PI3K in aged, but not young, rats, while a similar response was observed in AKT; there was a significant correlation between these parameters. There were no significant age-related or FGL-induced changes in either total-GSK-3β or phosphor-GSK-3β (nor the ratio of phosphor-/total-GSK-3β). ERK activation was unchanged with age or FGL treatment.

2. ***An outline of the molecular machinery that regulates expression and distribution of CAMs in the hippocampus at the cellular and sub-cellular level.***

Mass spectroscopy (MS) of protein fractions associated with NCAM functioning confirmed that these fractions contain cell adhesion molecules as NCAMs, neurofascin, cadherins, beta-catenin as well as proteins involved in calcium signaling. STIM1 and Orai1 have been found to be proteins involved in the process of store-operated Ca^{2+} entry into the cell; the expression level of STIM1 and its close homolog STIM2 have been analysed and compared in cerebellum, thalamus, hippocampus, cortex and amygdala. The highest level of STIM1 and STIM2 were in the cerebellum and hippocampus, respectively. MS of the synaptosomal fraction showed that both proteins were expressed either at synapses or in their immediate neighbourhood. In pyramidal and Purkinje neurons as well as in cultured hippocampal and cortical neurons, strong dendritic immunostaining was found for STIM1, but not STIM2. Localisation of Orai1, YFP-STIM1 and YFP-STIM2 shows a dispersed pattern in control cortical neurons and punctate staining in cells with Ca^{2+} stores depleted by thapsigargin. To understand the different localisation of STIM1 and STIM2 and mechanism of their translocation, we are studying the effects of simultaneous expression of ORAI1 and STIM1 or ORAI1 and STIM2 on SOCE in cortical neurons using intracellular calcium measurements with Fura-2.

3. ***Assessment of the relationship between the cell adhesion function, use-dependent synaptic changes, and the molecular composition and the physiology of individual synapses and synaptic populations***

We assessed the effects of S100A4-A1 and S100A4-A-ctl (20 μg/ml, ACSF + 0.1% BSA), scrambled peptides H3 and H6 on intracellular Ca^{2+} signalling in dendrites of CA1 pyramidal cells in hippocampal slices. The drugs were applied using local pressure injection. S100A4-A1 induced ~15% Ca^{2+} elevation ($p<0.005$) normally accompanied by an inward whole-cell current. The scrmbled H6 peptide elevated internal Ca^{2+} by ~35% ($p<0.01$) thus indicating non-specificity. S100A4-A-ctl and scrambled H3 had no effects.

Two-photon excitation methodologies and image segmentation algorithms (volume fraction, entropy and maximal particle number) have been established to monitor fine dendritic morphology of hippocampal astrocytes. Induction of LTP at nearby synapses (high-frequency stimulation protocol) has been found to parallel a specific morphological transformation of astrocytic protrusions suggesting the involvement of adhesion mechanisms. Activity-dependent structural changes in astrocytes have been found to rely on either spontaneous or evoked intracellular Ca^{2+} signals in individual glial cells.

4. *Understanding the biophysical mechanisms shaping the microenvironment of the synaptic cleft.*

The relationship between the synaptic cleft height and synaptic efficacy has been established unveiling a basic principle of synaptic architecture: maximisation of synaptic responses at the optimal synaptic height, 16-17 nm. A previously unrecognised fundamental mechanism contributing to synaptic signal formation and synaptic input integration in neuronal circuits has been found, electrodiffusion of electrically charged neurotransmitters such as glutamate. This mechanisms slows down excitatory synaptic responses upon cell depolarisation thus expanding the coincidence detection window for dendritic synaptic inputs. We have recently implemented a new photonics methodology to gauge instantaneous diffusion of small molecules in the brain extracellular space, time-resolved fluorescence anisotropy imaging (TR-FAIM). Control experiments showed that TR-FAIM could faithfully report translational molecular mobility on the nanometer/ nanosecond scale.

Deliverables:

D.2.1 An assessment of the molecular tools for modulation of cell adhesion related signal-ling cascades (e.g., recombinant fusion-proteins of a series of cell adhesion molecules with respect to their efficiency in inducing synaptic transmission changes monitored in electrophysiological experiments**.**

D.2.2 An initial assessment of causal relationship between CAM-related signalling and the basal synaptic transmission *in vitro* and *in vivo*.

D.2.3A Continuous assessment of causal relation-ship between CAM-related signalling and the basal synaptic transmission *in vitro* and *in vivo*.

D.2.4-2.5 An outline of the molecular machinery that regulates expression and distribution of CAMs in the hippocampus at the cellular and subcellular level.

D.2.6 Assessment of the relationship between the cell adhesion function, use-dependent synaptic changes, and the molecular composition and the physiology of individual synapses and synaptic populations

D.2.7 Understanding the biophysical mechanisms shaping the microenvironment of the synaptic cleft.

All deliverables of WP2 have been achieved

WP3. Neuronal circuits: From ultra-structure to plasticity; role of neuronal CAMs.
WP-leader: partner 7, Michael Stewart/Paul Gabbott

Partner 1 Elisabeth Bock
Partner 2 Dmitri Rusakov
Partner 3 Marina Lynch
Partner 4 Valerie Doyere
Partner 5 HansJürgen Volkmer
Partner 6 Dominique Muller
Partner 8 Javier DeFeliepe
Partner 12 Carmen Sandi

Objectives of WP3

The objectives of this WP were to:

1. *Elucidate the role of neuronal CAM (NCAMs) in alterations of synaptic and neuronal morphology in response to modulation of synaptic and neural plasticity, and memory formation* using advanced light and electron microscopy combined with 3D reconstruction methods. In addition examination of the process by which CAMs were able to remove the plasticity induced memory block caused by direct application of β-amyloid to young rat brain (in a laboratory derived model of Alzheimer's) were explored..
2. *Understand the cellular mechanisms and role of NCAMs that underlie the maintenance or decay of memories* over time, both in young adults, and in ageing, via morphological and immunocytochemical methods;
3. *Determine how alterations are manifested in the neocortex (frontal cortex) and hippocampus in both neural microcircuitry and the distribution pattern and density of neuronal CAMs*, and
4. *Determine how synaptic structure and circuitry are affected by genetic manipulation of NCAM expression systems* and to *test the capacity of CAM mimetics to promote synapse turnover and synaptogenesis.*

The following results were achieved in WP3

1. *Role of CAMs in alterations in synaptic and neuronal morphology- ageing*

Our research has demonstrated clearly that a CAM mimetic (FGL_l) has a capacity to alleviate the age related impairment in neural circuitry, as manifested in decreases in dendritic spines and synapse size in the hippocampus. These structural age-induced impairments are correlated with altered physiological parameters (notably reduced ability to express LTP). FGL not only attenuates LTP but also alleviates the reductions in spines and synapses in hippocampus. No effects are seen in younger animals, indicating that this is a very specific age related effect. In addition an exhaustive stereological investigation has been made of alterations in neuron and glial numbers in various subfields of the dorsal hippocampus. These effects appear to be connected with an age-related increase in microglia activation and are associated with an increase in IL-1beta concentration in hippocampus (but not cortex) and that this age-related change, as well as the deficit in LTP, is attenuated by FGL treatment. The evidence indicates that the age-related changes in NCAM and CD86 are attenuated in tissue prepared from FGL-treated rats.

To investigate the mechanism of LTP and LTD induction in more detail, prior to a detailed examination of the effect of the CAM mimetic on this process, a three dimensional (3-D) ultrastructural analysis was made of dendritic and synaptic structures in the middle molecular layer (MML) of the dentate gyrus in the hippocampus of conscious (awake) adult rats, 24h after induction of homosynaptic long term potentiation (LTP) and heterosynaptic long term depression (LTD). Neither LTP nor LTD affected synapse density but spine number was markedly and differentially affected by LTP and LTD. LTP, but not LTD, increased significantly the proportion of the large spine class (mushroom spines) while conversely LTD increased the proportion of thin spines. For the first time in morphometric studies, the specificity of the effects of LTP on spine and synapse morphology was determined by use of the competitive NMDA receptor antagonist, CPP (-3(-2 carboxipiperazin-4-yl)-propyl-1-phosphonic acid). When CPP blocks LTP it also impairs most, but not all, morphological changes in spines and synapses, and in addition exerts morphological effects on its own. The effect of FGL on these processes is now being investigated

2. ***Role of CAMs at the cellular level - in vivo imaging.***

Using hippocampal slices, real-time imaging data has shown that hippocampal astrocytes respond to induction of LTP by rapidly altering their fine morphology (beyond optical resolution), in a Ca^{2+} signalling dependent manner. This opens up an important line of study in establishing a role of Ca^{2+}-dependent cell adhesion mechanisms in cellular plasticity. Another important aspect of these studies was determination of the role of N-cadherin on the mechanisms of spine formation, turnover and stability using a model of continuous repetitive imaging of transfected hippocampal cells. Results indicate that deletion of the extracellular domain of N-cadherin results in an increased formation of immature spines with a defect in PSD formation and a decreased stability of existing spines. At the electron microscopy level, spines are smaller and express smaller PSDs. Furthermore, stabilization is linked to expression of PSD.95, and requires activation of NMDA receptors. Upon induction of LTP specific stabilization of activated spines is prevented. Thus N-cadherin has been shown to play a critical role at synapses by ensuring the long-term stability of spines.

Another important aspect of work in this area was to show that specific isoforms of cell adhesion molecule neurofascin (see previous reports) are dysregulated in the brain of juvenile animals exposed to stress. Examination was made as to whether dysregulated neurofascin at axon initial segments influences formation, plasticity and maintenance of inhibitory synapses in vivo and to determine this lentiviral vectors were injected into rat brain for the suppression of neurofascin isoform expression by RNA interference or for over-expression of specific neurofascin isoforms.

3. ***Role of CAMs in alleviating neurodegenerative disease***

Using the adult rat model of Alzheimer's (where B-amyloid 25-35 is injected directly into the ventricles, we have shown that the alleviating of cognitive deficits by the CAM mimetic FGL, is paralleled by attenuation of morphological effects of spines and synapses in the hippocampus (and prefrontal cortex). An extensive stereological study has been undertaken to determine changes in neuron number in various subfields of the hippocampus, and we have also examined alterations in GSK3beta, FGFR1, FGFR2 and zinc transporters in identified hippocampal subfields. The most notable effects are that a reduction of spine density in CA1 following B-amyloid administration is reduced and changes in neuronal number are also alleviated. A major effort has been made to understand the process by which FGL exerts its effect at the cellular level, and its possible role in alleviating neurodegenerative disease. Glycogen synthase kinase 3 (GSK3) plays a pivotal and central role in the pathogenesis of both sporadic and familial forms of Alzheimer's disease (AD). Reducing/inhibiting GSK3β could be a therapeutic treatment of Alzheimer's. To determine whether FGL is able to inactivate GSK3β via the FGFR-Akt pathway, which phosphorylates GSK3β at serine 9, a count of neurons containing inactive GSK3β was performed. Our data indeed suggest that this is the case and this work is in preparation for publication.

Deliverables:

D.3.1 Initial analysis of the morphological effects on microcircuitry of hippocampus of normal animals of administration of CAM compounds developed by partners.

D.3.2 Preliminary analyses of the morphological features of the morphological features of animal models of affective and anxiety disorders.

D.3.3 Analyses of the role of CAMs in the mechanisms that underlie maintenance or decay of memories over time in normal adult animals, and in ageing animals via study of LTP and LTD.
D.3.4 Continuous analyses of the role of CAMs in the mechanisms that underlie maintenance or decay of memories over time in normal adult animals and in ageing animals via the study of LTP and LTD
D.3.5 Analyses of effect of FGL on synaptic and dendritic spine structure in dentate gyrus of aged rats
D.3.6 Analyses of the effect of B-amyloid and on synaptic and dendritic structure in hippocampus and ability of FGL to recover morphological deficits so produced

All deliverables have been achieved

WP4 Effects of interactions between genes and experiences on learning and memory abilities role of neuronal CAMs
WP-leader: partner 12, Carmen Sandi

Partner 4. Claire Rampon
Partner 7. Mike Stewart
Partner 8. Javier de Felipe
Partner 12. Carmen Sandi
Partner 13. Gal Richter-Levin
Partner 14. Vladimir Berezin

Further collaboration
Partner 3. Dmitri Rusakov
Partner 9. Rita Gerardy-Schahn

Objectives of WP4

1. *Identification and characterization of the role of genes coding for neuronal CAMs on stress induced cognitive impairment.*
2. *Identification and characterization of the role of genes coding for neuronal CAMs on environmental enrichment-induced cognitive improvement.*
3. *Characterization of changes at the level of neural circuits and synapses, and its relation to the distribution pattern and density of neuronal CAMs, induced by life experiences.*
4. *Development of CAM mimetic peptides with the ability to modulate plasticity in vitro.*

The following results were achieved in WP4

1. Identification of stress and enrichment protocols leading, respectively, to cognitive impairment and improvement

A number of behavioral protocols were established, particularly for their validity to induce either cognitive impairment or cognitive enhancement in mice, with the secondary goal of applying the validated protocols to mouse models with genetic mutations for specific CAMs aspects. In particular, the following protocols were developed by partners in WP4:

- *Chronic stress-induced cognitive impairment*: Mice were submitted to chronic social stress and chronic unpredictable stress. The second protocol was selected for its more robust cognitive impairing effects that were accompanied by increased depression-like behaviors. Rats were also

shown to induce cognitive impairment at aging when submitted to stress during mid-age. Chronic stress resulted in reduced NCAM expression in the hippocampus and prefrontal cortex, but increased NCAM expression in the amygdala. Conditional NCAM KO mice showed cognitive impairment in hippocampus-dependent tasks that resembled those of chronically stressed mice. Conditional NCAM KO mice were more vulnerable to the effects of repeated stress, since they show impaired spatial and reversal learning after two weeks of stress when wild-type mice are not yet affected (the latter need four weeks to show the same impairments).

- *Environmental-enrichment induced cognitive enhancement*: Environmental enrichment was shown to reverse the deleterious effects on cognition induced by juvenile stress. PST KO mice were shown to display impairment in long, but not short, term memory in several hippocampus memory tasks that were reversed by environmental enrichment, but not paralleled by alterations in hippocampal neurogenesis. STX, but not PST, KO mice were shown to display considerable aggressive behaviors when confronted with a conspecific mouse in the resident-intruder test.

2. *Continuous identification of at least two peptide mimetics of cell adhesion molecules capable of counteracting cognitive impairment, and/or facilitating cognitive improvements induced by life experiences and/or genetic background*

The peptide plannexin was confirmed to enhance spatial learning and long-term memory when injected intracerebroventricularly immediately after the initial training sessions, but it had no significant effects when injected before training. Plannexin was also confirmed to overcome the cognitive deficits induced by deficits in PSA-NCAM expression: (i) induced by the enzyme endoneuroaminidase-N (cleaving PSA from NCAM); and observed in (ii) PST KO mice.

Subchronic administration of the NCAM mimetic peptide FGL reversed the learning deficits in NCAM-KO mice. In rats, this treatment when given during stress at mid-age prevented the cognitive decline observed in stressed rats during early senescence. This treatment also prevented the decay in hippocampal neurogenesis observed during early aging (20-months) of rats submitted to mid-life stress.

Several other peptides were shown to be effective in either interfering or facilitating learning and memory processes, such as enplastin, narpin, Dennexin, encarmin A, encarmin B, and encarmin C. No cognitive effects were found for other peptides, such as Emtin-A

3. Identification of key neurobiological pathways involved in the cognitive effects of at least two selected peptides

Morphological examination of the hippocampus from animals trained and treated with plannexin showed marked changes in synaptic architecture, with plannexin inducing significant decreases in the proportion of thin/small spines while increases in the proportion of mushroom/large spines. Moreover, 3D reconstruction of dendritic segments to measure synapse density per length indicated that animals trained and injected with plannexin showed the highest synapse density. Electrophysiological studies showed that plannexin has immediate effects in synaptic function facilitating plasticity in the hippocampus. Biochemical analyses implicated trafficking of AMPA GluR1 receptors.

FGL treatment, at conditions that facilitate hippocampus-dependent learning, was shown to facilitate LTP in the CA1 *stratum oriens*. NMDA decay time was smaller in FGL-treated mice, indicating a higher magnitude of NR2A expression in their synapses, which fits with the plasticity and cognition enhancing properties of this peptide. FGL also counteracted spatial

learning deficits in conditional NCAM KO mice. FGL also induced increases in spine volume in the cingulated cortex.

Deliverables:

D.4.1 Identification of stress and enrichment protocols leading, respectively, to cognitive impairment and improvement..
D.4.2 Identification of peptide mimetics of cell adhesion molecules capable of counteracting cognitive impairment, and/or facilitating cognitive improvements induced by life experiences and/or genetic background
D.4.2A Continuous identification of at least two peptide mimetics of cell adhesion molecules capable of counteracting cognitive impairment, and/or facilitating cognitive improvements induced by life experiences and/or genetic background
D.4.3 Identification of key neurobiological pathways involved in the cognitive effects of at least two selected peptides

All deliverables of WP4 have been achieved

WP5. Formation of emotional and traumatic memories; role of neuronal CAMs
WP-leader: partner 13, Gal Richter-Levin

Partner 4. Valérie Doyere
Partner 10. Jacek Kuznicki
Partner 12. Carmen Sandi
Partner 13. Gal Richter-Levin
Partner 14. Vladimir Berezin
Partner 18. Alex Zharkovsky

Objectives of WP5

The objectives of this WP were to 1. *Identify and characterize the region-specific mechanisms involved in the formation of emotional and traumatic memories focusing on the role of cell-cell recognition*, 2. *Characterize the role of amygdala activation in modulating cell-cell recognition in brain areas such as the hippocampus and prefrontal cortex, in relation to emotional and traumatic memory formation*, and based on the characterization to 3. *Evaluate the potential role of neuronal CAM mimetics in reversing or preventing traumatic memories*, and finally to 4. *Asses the role of neuronal CAMs in disturbed cognitive and emotional behaviour induced by early maternal deprivation or early applied toxins.*The deliverables of WP5 are indicated below.

The following results were achieved in WP5.

1. ***Development of experimental protocols with which to evaluate the role of neuronal CAMs***.

Novel protocols for the evaluation of the impact of exposure to a traumatic experience early in life, in adulthood and in ageing were developed. Region-specific and experience-specific changes in neuronal CAM expression in various brain regions such as the hippocampus, the prefrontal cortex, the amygdala, the piriform cortex and the thalamus were evaluated. Due to the range of findings, focus has been on changes following exposure to traumatic experiences in youth or in adulthood and in the hippocampus and amygdala.

Exposure to juvenile stress or to significant (traumatic) stress in adulthood was found to result in alteration in the expression of L1-CAM in both the amygdala and the hippocampus. Attempts to interfere with L1-CAM peptide mimetics did not yield any behavioural effects though. NCAM was also studied after exposure to juvenile stress or significant traumatic stress in adulthood and like L1-CAM it was found to be affected in its expression emphasizing the role of neuronal CAMs in emotional memory. Particularly, the polysialated form of NCAM, PSA-NCAM, was studied and this included analyses of the expression of the polysialyltransferases ST8SiaII and ST8SiaIV. Moreover, the polysialated form was determined in various regions of the amygdala and it was found that peri-puberty stressed rats displayed lower PSA-NCAM expression levels in the central amygdala. In adult animals, the same animals showed under basal conditions higher PSA-levels in the lateral and baso-lateral nuclei of the amygdala as compared to control rats, whereas no differences were observed in other amygdala nuclei. In the animals in which the two polysialylating enzymes were individually knocked-out it was found that only animals lacking ST8SiaIV had reduced PSA-NCAM levels throughout the amygdala nuclei. The same animals exhibited marked changes in anxiety-like behaviours. Adult animals submitted to traumatic brain injury of the sensory-motor cortex exhibited dementia and hemiparesis.

Finally, in order to assess neuronal plasticity in relation to emotional and traumatic memories, it was demonstrated, that reverberatory activity in the lateral amygdala may provide the mechanisms allowing coincident detection and plasticity during fear conditioning. Also, the possible impact of reexposure to the context of a traumatic experience on plasticity was evaluated and it was found that reexposure to acute stress affects not only aspects of plasticity of principal cells, but also aspects of local circuitry activity in the dentate gyrus. It is suggested that these changes underlie some of the behavioural consequences of traumatic experience.

2. ***Examination of the ability of CAM-mimetics to affect emotional and traumatic learning and memory.***

Since it has been shown that the NCAM-derived peptide, FGL, induce heterosynaptic LTP on the lateral perforant path *in vivo*, a protocol was developed for the investigation of the effect of FGL on olfactory fear memory and its selectivity. Results are still under analysis.

The cell adhesion molecule associated cytoplasmic nuclear protein, β-catenin, has been observed to accumulate specifically in thalamic neurons and in order to determine the mechanisms of the stabilization of β-catenin in this region, a study was carried out showing that some of the proteins involved in the degradation of β-catenin were decreased in the thalamus, and that this decrease likely was the explanation for the stabilization of β-catenin in thalamic neurons.

The NCAM-derived P2 peptide was applied to animals submitted to traumatic brain injury after the injury had been inflicted, and pronounced amelioration was demonstrated. This included the complete prevention of the development of dementia and a more rapid recovery as regards motor control of paretic muscles.

Deliverables:

D.5.1. Identification of effective protocols on which to test candidate neuronal CAM mimetics for their therapeutic potential.
D.5.2 + D.5.2.A. Characterisation of the role of cell-cell recognition processes in the formation of emotional and traumatic memories

D.5.3. + D.5.3.A. Assessment of the contribution of cell-cell recognition processes in the amygdala and other brain regions to the formation of emotional and traumatic memories
D.5.4.+ D.5.4.A. Assessment of the role of at least one neuronal CAM mimetic in trauma related syndromes
D.5.5. Assessment of neuronal plasticity in relation to emotional and traumatic memories

All deliverables of WP5 have been achieved.

WP6. Animal models of impaired neuronal plasticity and CNS disorders
WP-leader: partner 14, Vladimir Berezin/ENKAM

Partner 11. Patrik Brundin
Partner 12. Carmen Sandi
Partner 13. Gal Richter-Levin
Partner 15. Miguel Medina/Noscira
Partner 17. Alex Zharkovsky

Objectives of WP6

The objectives of this WP were to 1. *establish and/or utilize animal disease models of brain injury and ischemia for the assessment of the role of defined genetic and epigenetic factors in neuronal plasticity.* Moreover, based on selected established models 2. *utilize the animal genetic models to understand the molecular mechanism(s) of neuroregeneration in the adult CNS and to explore the possibilities for increasing neuronal plasticity and enhancing neuroregeneration*. The deliverables of PW6 are indicated below.

The following results were achieved in WP6

1. Establishment and/or utilization of animal disease models of brain injury and ischemia for the assessment of the role of defined genetic and epigenetic factors in neuronal plasticity

Partner 11 has established and validated the following rodent models: Alzheimer's disease (AD) model using 192-IgG saporin; The Huntington's disease (HD) mouse model (R6/1 and R6/2 mice); The Parkinson's disease (PD) model using 6-OHDA, excitotoxic brain injury model in rat using quinolinic acid.

Partner 12 has validated a mouse model with deficiency in NCAM expression.

Partner 13 has adopted the model of the β25-35 peptide induced AD-like pathology introduced by Partner 1 and Partner 14.

Partner 14 has established and validated the following rodent models: Traumatic brain injury model (in collaboration with Partner 1); Rat AD-like model (in collaboration with Partner 1).

Partner 15 has established and/or validated the following mouse models: Tet/GSK3; TauVLW; APPsw/TauVLW.

Partner 17 has validated the following tg-mouse models: NCAM knockout mice; GFAP knockout mice

Partner 17 has also validated a kainic acid model of epilepsy.

2. Utilization of animal genetic models to understand the molecular mechanism(s) of neuroregeneration in the adult CNS and to explore the possibilities for increasing neuronal plasticity and enhancing neuroregeneration

Partner 11 has characterized the neurogenic response in a model of excitotoxic brain injury (quinolinic acid).

Partner 13 has identified a peptide derived from Epo (Epotris) as a potential target to modulate LTP *in vivo*.

Partner 12 has identified 2 peptides derived from NCAM, which induced learning and memory abilities.

Partner 14 has identified several sites in NCAM (Ig1, Ig2, Ig3, Fn3.1, Fn3-2) as molecular targets to develop plasticity modifying mimetic peptides. Cell adhesion molecule L1 has also been identified as a molecular target (in collaboration with Partner 1). Partner 14 has identified nine FGFs as a potential source of mimetic peptides of pharmaceutical relevance for the modulation of plasticity and regeneration (in collaboration with Partner 1). Some of the peptides had memory enhancing and neuroregeneration promoting effects.

Partner 15 has validated GSK3-β as a molecular target to address neurodegeneration and plasticity impairments seen in AD and tested *in vivo* efficacy of several compounds in Tet/GSK3, tauVLW and APPsw/TAuVLW transgenic mice

Partner 18 has using NCAM knockout mice validated NCAM as a molecular target to develop a new generation of antidepressants, and has employing Epo-derived peptide established the role of Epo in kainic acid induced seizures and neurotoxicity.

Additionally: Partner 8 has performed a thorough quantification of morphological markers of AD-like pathology (amyloid plaques, dendritic trees, types of synapses) using APP/PS1tg mice.

Deliverables:

D.6.1 Establishment and validation of rodent models of brain injury, stroke and neurodegenerative diseases.

D.6.2 Preliminary identification of 1-2 molecular targets which (i) will improve our Understanding of the neuronal plasticity and regeneration in the adult on the cellular and molecular levels, (ii) can be utilized for designing pharmacological paradigms that would improve neuroregeneration in individuals who have lost neuronal cells as a consequence of a physical or psychological trauma or a disease.

D.6.3+ 6.3A Further characterization of targets of pharmaceutical relevance involved in plasticit and regeneration.

All deliverables of WP6 have been achieved

WP7 Neuronal CAMs and adult neurogenesis: role in learning and memory consolidation and brain repair.
WP7 - leader: Partner 11, Patrik Brundin

Partner 1 Elisabeth Bock
Partner 4 Valérie Doyère / Claire Rampon
Partner 6 Dominique Muller / Jozsef Kiss

Partner 11 Patrik Brundin
Partner 18 Alexander Zharkovsky

Objectives of WP7

The objectives of this WP were to:
1. *To examine the role of CAMs in hippocampal neurogenesis with respect to enrich environments, and learning and memory.*
2. *To evaluate the influence of stress and aging on neurogenesis in rat models of dementia.*
3. *To establish the in vitro model of neural progenitor cell integration in organotypic slice cultures.*
4. *To administer neuronal CAM mimetics in at least two in vivo models of dementia with the aim of regenerating lost cholinergic neurons.*
5. *To characterize the relationship between astroglial, adhesion molecules and neurogenesis in demented brains.*

The following results were achieved in WP7

1. Determination of the functional role of CAMs in hippocampal neurogenesis, learning and memory.

Two knockout mice for the two polysialyation enzymes of NCAM, STX-KO and PST-KO, were studied. We found no alterations in neurogenesis in any of these knockout mice. STX-KO mice had a robust reduction in proliferative activity under stress and exhibited reduced anxiety. Both STX-KO and PST-KO mice had memory impairment. PST-KO mice were affected in spatial and non-spatial tasks. Interestingly, these deficits could be overcome by enriched environment.

In other studies, the NCAM mimetic peptide FGLs effect on neurogenesis was investigated. We found increased neurogenesis after post-traumatic injury when FGL was administered.

FGL could also reverse the depression-like phenotype of the NCAM-deficient mice and enhance the survival of newborn neurons. Hippocampal neurogenesis was also studied in view of the transcriptional regulation and the temporal expression of specific transcription factors (e.g. Mash1, Tbr1/2, NeuroD and PSA-NCAM). We found that the transcription factor NeuoD1 was expressed before PSA-NCAM and could also control its expression and the hippocampal neurogenesis.

2. Determination of the effect of potential neurogenic compounds

We developed a screening model combining transplantation of NPCs into cortical slice cultures. The grafted NPCs migrated to cortical regions and 90% differentiated into GABAergic interneurons that showed axon-like processes. In this model, overexpression of FGF2 in neural progenitor cells enhanced the proliferative and migratory properties of grafted progenitor cells. We found also that in the contact with capillaries, these NPCs were maintained in a non-differentiated proliferative state and therefore could function as an ectopic niche.

3. Dementia and its connection neuroregeneration.

We developed a specific rodent model of dementia. This posttraumatic lesion model was established to have a model where animals exhibited both dementia and deficient motor control. Treatment with P2 peptide limited dementia and motor symptoms.Treatment with FGL and P2 could also restore memory of social recognition in a model of impaired cholinergic function (scopolamine administration).

4. Establishment of several models of neurodegeneration

These models were used to study the ability of neuronal CAMs and other potential compounds or environments, to regenerate the damaged brain and to improve cognition. In a model of cryolesion and in the R6/2 mouse model of Huntington disease, peptides derived from FGF (Dekafin-1/Dyofin) could improve both cognition and neurodegeneration. In a seizure model, kainate acid administration, the S100A4 peptides (Epotris (H3)/Hekatones (H6)) could reduce seizure activity.

Deliverables:

D.7.1 Determination of the functional role of CAMs in hippocampal neurogenesis and learning and memory.
D.7.2 Establishment of a model of dementia whereby neuroregeneration can occur.
D.7.3 Establishment of the use of organotypic slice cultures as an efficient screening procedure for compounds that regulate *in vitro* neurogenesis.
D.7.4 Established therapeutic basis by which new cholinergic neurons may re-establish functional connections and behavioral cognitive recovery.
D.7.5 Development of both *in vitro* and *in vivo* modeling systems to screen for the ability of neuronal CAMs and other potential compounds or environments, to regenerate the damaged brain and to improve cognition.

All deliverables of WP7 have been achived

WP8. Novel strategies for modulation of plasticity with aim of developing therapeutics improving learning and memory
WP-leader: partner 14, Vladimir Berezin/ ENKAM

Partner 1. Elisabeth Bock
Partner 15. Miguel Medina/Noscira
Partner 16. Claus Schafer Nielsen/Schafer-N

Objectives of WP8

The objectives of this WP were to 1. *develop compounds with beneficial effects on memory impairment and neurodegeneration*. This was to be achieved by 2. *identification and characterization of peptide families mimicking function of cell adhesion molecules and growth factor receptors*, 3. *identification and characterization of novel dual inhibitors of AchE and tau phosphorylation*, 4. *characterization of interactions between the effect of mimetic peptides on neurodegeneration and astrocyte function modulation*.The deliverables of WP1 are indicated below.

The following results were achieved in WP8

1. Development of compounds with beneficial effects on memory impairment and neurodegeneration

Partner 14 in collaboration with Partner 1 have identified and develop the following compounds with beneficial effects on memory impairment and neurodegeneration: FGL, a potent FGF

receptor agonist that contains an NCAM-derived sequence motif (preclinical development has been completed; FGLL form has been through phase I clinical trial; preclinical development of FGLs form including pharmacokinetics and pharmacodynamics has been completed); P2 (preclinical pharmacology has been completed). FGLs is planned to develop further for the treatment of Alzheimer's disease (AD) patients.

Partner 15 has identified and developed the following compounds with beneficial effects on memory impairment and neurodegeneration: NP-12, a GSK3- inhibitor (preclinical development has been completed, phase I clinical trial has been completed and phase II clinical trial for the treatment of Progressive Supranuclear Palsy (PSP) has been initiated); NP-61, a dual binding site AChE inhibitor (regulatory preclinical development, toxicology and safety studies has been completed; it is currently in phase I clinical trial for AD).

2. Identification and characterization of peptide families mimicking function of cell adhesion molecules and growth factor receptors

Partner 1 in collaboration with Partner 14 have identified, characterized and selected the following CAMs and CAM-counter-receptor mimetic peptide:

- FGL, EncaminA, B and C, DekaCAM (all from Fn3 modules of NCAM)
- P2, plannexins and dennexins (from Ig1-3 modules of NCAM)
- Elcamins (from Fn3 modules of L1)
- Dekafins and dyofins (FGF-derived peptides.

Partner 14 has prepared information sheets for the following peptide mimetics; FGLL, FGLs, P2, EmtinA, Plannexin, Dennexin, Hekaton, Dekafin1.
Partner 16 has manufactured preparation of the majority of peptide forms.

3. Identification and characterization of novel dual inhibitors of AchE and tau phosphorylation

Partner 15 has designed and synthesized new potent dual binding site AChE inhibitors has extensively characterized small heterocyclic thiadiazolidinones (TDZDs), the first ATP non-competitive GSK3 inhibitors proposed as new drugs for the effective treatment of AD and other tauopathies.
Partner 14 has delivered to Partner 15 the FGL peptide (NCAM mimetic) for testing for inhibition of tau phosphorylation.

4 .Characterization of interactions between the effect of mimetic peptides on neurodegeneration and astrocyte function modulation

Partner 1 has established a survival assay involving astrocytes in culture (treatment with H_2O_2) and has shown the rescue of astrocyte cell death by FGL.

Partner 1 in collaboration with Partner14 and Partner 18 have tested the effect of the S100A4 protein-derived mimetic peptide, Hekaton, in GFAP knockout mice subjected to traumatic brain injury. Knockout of GFAP intermediate filaments in astocytes led to an increase in cell death and S100 protein expression and decrease in microglia activation in response to the lesion.
Partner 15 has found that GSK3 inhibitors from the TDZD family are able to block LPS-induced secretion of pro-inflammatory molecules from astrocytes and neurons as well as prevent microglial activation.

In addition: Partner 18 has tested the effect of GFAP knockout on the intensity of seizures and neuropathology after kainic acid treatment. GFAP deficient mice have higher sensitivity to the neurotoxic action of kainic acid than wt mice.

Deliverables:

D.8.1+ 8.1A Selected CAMs and CAM-counter-receptor mimetic peptides with neuroprotective and synaptogenic profiles.
D.8.2+ 8.2A Novel dual inhibitors of AChE and tau phosphorylation.
D.8.3+ 8.3A Testing of the interaction between astrocyte function modulation and the effect of mimetic peptides on neurodegeneration.
D.8.4+ 8.4A Identification of compounds suitable for drug development.
D.8.5+ 8.5A Preparation of information sheets for all produced peptide mimetics to be employed by the Consortium
D.8.6 Preparation of at least two compounds for phase I clinical trial.

All deliverables of WP8 have been achieved

WP9 Training activities
WP-leader: partner 10, Jacek Kuznicki; Deputy partner 4 – Valérie Doyère

Participants: all partners

Objectives of WP9

1. *Optimization of the training of graduate students, postdocs and technicians regarding theoretical research knowledge and experimental techniques.*

2. *Dissemination of experimental techniques to ensure advanced technical training of laboratory members of the consortium.*

The following results were achieved in WP9

1. E-mail list

An e-mail list for all participants of the project was build within the first 6 months from the start of Promemoria and has been updated every year or when necessary. The list was posted on the Promemoria website. Each update of the list was also emailed to Partners.

2. Techniques list

A list of all available techniques in the laboratories of the partners was build within the first year from the start of Promemoria, and was sent to Partners and posted on the Promemoria website.

3. Website

A password-protected website containing relevant protocols and confidential information for the partners has been created within the first 18 months of Promemoria and has been available to each partner. The web page has been updated when necessary.

4. Between-partners interaction

Visits between postdoctoral and students partners and partners' staff have been implemented when necessary.

5. Technique Oriented Workshops (TOW)

To facilitate exchange of information and expertise between all partners, six TOWs have been organized within the four years of Promemoria by six different partners: One within the first year, two within the second year, one during the third year, and two during the last year. The dates, venues and subject matters are described below, and the detailed program was posted on the website and sent to partners via emails. The TOWs were well attended and considered of high interest by the participants.

Year 1

TOW1 February 11-14 2006
Organizer: Partner 13 – Gal Richter-Levin
Place: Haifa (Israël)
Title: 'How to set up animal models for psychiatric disorders'
Number of Promemoria participants: more than 15
Number of speakers: 13

Year 2

TOW2 August 25-26 2006
Organizer: Partner 11- Neuronal Survival Unit, Lund Univ (Karin van den Borght)
Place: Wallenberg Neuroscience Center, Sölvegatan 17, 22184 Lund (Sweden)
Title PROMEMORIA workshop on neurogenesis
Number of participants 15
Number of speakers 6

TOW3 November 6-10, 2006
Organizer: Partner 11 - Patrik Brundin
Place: Lund (Sweden)
Title: 'BioBusiness'
Number of Promemoria participants: 10
Number of speakers: 13

Year 3

TOW4 May 9-11, 2007
Organizers: Partner 7 & 3 – Mike Stewart, Paul Gabbott & Dmitri Rusakov
Place: Open University, Milton keynes (UK) & Institute of Neurology, London (UK)
Title: 'Bioimaging, Electron microscopy, immuno-cytochemistry, and 3-dimensional reconstruction'
Number of Promemoria participants: 25
Number of speakers: 14

Year 4

TOW5 May 11-12, 2008

Organizers: Partner 5 - Hansjuergen Volkmer
Place: Warsaw (Poland)
Title: 1. 'RNA interference for neuroscience'
Number of Promemoria participants: 19
Number of speakers: 3

TOW6 May 13-15, 2008
Organizers: Partner10 - Jacek Kuznicki
Place: Warsaw (Poland)
Titles: 'Building your career and your first laboratory'
Number of Promemoria participants: 23
Number of speakers: 10

Deliverables:

D9.1. Password-protected database containing relevant protocols of all available techniques from all laboratories of the Consortium.
D.9.2. Advanced technical training through four Technique-oriented Workshops held 12, 24 and 36 months after the project start.

All deliverables of WP 9 have been achieved

WP10. Management activities
WP-leader: partner 1, Elisabeth Bock, University of Copenhagen

Participants: All Partners

Objectives of WP10

1. *Efficient running of the PROMEMORIA consortium.*
2. *Establishment of a high level communication between partners.*
3. *Rapid dissemination and exploitation of results.*
4. *Efficient handling and solution of possible problems and conflicts.*
5. *Optimal management of intellectual property rights*

The following results were achieved in WP10

1. Organisation of General Assemblies and Governing Board meetings

The General Inaugural Assembly was held April 22-24, 2005, at the University of Copenhagen. As regards management, all Partners, including the workpackage leaders, the management team, and the EU Officer had fruitful discussions on how to secure optimal and efficient collaboration procedures for Promemoria's 4 year lifetime. All agreed that efforts should be aimed at creating maximal synergy for the Consortium activities.
This Assembly was immediately followed the first Governing Board meeting.

The first Governing Board meeting was held at the University of Copenhagen April 25, 2005, (13 participants). At the meeting the workpackages leaders and the management team planned

i.a. GB meetings for year 1 of the Consortium. Minutes, including copies of guideline hand-out documents, were sent to all Partners April 2005.

The second Governing Board meeting was held in Innsbruck, August 24, 2005 (8 participants). The venue was the same as for *The 20th Biennial Meeting of the International Society for Neurochemistry and the European Society for Neurochemistry*, Innsbruck, Austria, August 21-26, 2005. Minutes were sent to all Partners September 2005.

The third Governing Board meeting was held in Washington DC, November 15, 2005, (8 participants). The venue was the same as for *The Annual meeting of the Society for Neuroscience, ScN*, Washington DC, November 10-16, 2005)
Minutes were sent to all Partners December 2005.

The fourth Governing Board meeting was held in Copenhagen, January 20, 2006, (9 participants). Minutes were sent to all March, 2006.

The second General Assembly was held in Paris, March 9-11, 2006. The number of participants was 82.

The fifth Governing Board meeting was held in Paris March 12, 2006, (10 participants). Minutes were sent to all April 2006

The sixth Governing Board meeting was held in Vienna in July 11 2006, (9 participants). Minutes were sent to all August 2006.

The seventh Governing Board meeting was held as a Skype conference (via the free of charge internet connection Skype) October 21, 2006, (9 participants). Minutes were sent to all November 2006.

The eighth Governing Board meeting was held in Paris January 19, 2007, (10 participants). Minutes were sent to all February 2007.

The Third General Assembly was held March 8-9, 2006, at the Revita Hotel in Bad Lauterberg near Goettingen in West Germany. As regards management, all Partners, including the workpackage leaders, the management team, and the Board members had fruitful discussions on how to secure optimal and efficient collaboration procedures.

The ninth Governing Board meeting was held March 9, 2006, at the Revita Hotel in Bad Lauterberg near Goettingen in West Germany **(11 participants).** Minutes was sent to all Partners in March 2007

The tenth Governing Board meeting was held July 9, 2007 as a Skype meeting.

The eleventh Governing Board meeting was held October 31, 2007 as a Skype meeting.

The twelvth Governing Board meeting was held January 24, 2008 in Copenhagen, Denmark. Minutes were sent to all February 2008.

The Fourth General Assembly was held March 6-7, 2007, at the Hilton Hotel, Milton Keynes near London, United Kingdom. As regards management, all Partners, including the workpackage

leaders, the management team, and the Board members had fruitful discussions on how to continue optimal and efficient collaboration procedures.

The thirteenth Governing Board meeting was held in Milton Keynes in United Kingdom March 7, 2008. (11 participants)

The fourteeth Governing Board meeting is planned to be held July 11, 2008 in connection with the FENS meeting in Geneva**.**

The fifthteen Governing Board meeting was held October 30, 2008 as a Skype meeting.

The sixteenth Governing Board meeting was held January 23, 2009 in Copenhagen, Denmark.

The Fifth and final General Assembly was held in Copenhagen, Denmark, March 5 – 6, 2009.

The seventeenth and final Governing Board meeting was held in Copenhagen, March 6, 2009.

2. ***Continuous reporting to EU scientific officers***

Project Presentation to EU Scientific Officer
EU Scientific Officer, Veronique Bernard, Brussels and Professor Heikki Tanila, Finlandia attended the Fourth General Assembly in Copenhagen, DK in March 5-6 2009.
Updating of the implementation plan (Months 36-48) was registrated by a letter from EU with acknowledgement of receipt the November 7, 2008.

Money transfer
The last transfer of money (507,147.09 EURO) from EU to the Coordinator took place in Nov. 22, 2008 and no money was transferred to the partners. The 16 Partners were informed by the coordinator's institution that only 8.5% at this moment is lacking from the total payment. The last amounts are to be transferred after acceptance of the final report. All Partners are informed of the last transfer taking place after acceptance of the final report.

Project reporting for year 4
Follow up and coordination activities aiming at providing the fourth year reports to the EU officer were intensified by the start of 2009, and an action and communication plan was agreed upon by all WP leaders on January 23, 2009.

3. Continuous updating of the Promemoria World Wide Web
The website content was approved by all Partners, and the link agreed to be:
www.plab.ku/promemoria-Consortium.
The website contained a brief presentation of the Promemoria Projects, information on FP6 framework for research, and data to facilitate communication from the outside to the individual Partners. In addition to this, the individual Partners refer to the above-mentioned website on their institutional/corporate website.

The website has been updated continuously. The last year for example, "First year executive Project summary" together with "Second year executive Project summary" and "Third year executive Project summary" were avaible on the web.

4. Partner Synergy Report

As appears from the report enclosure titled: "*Partner Synergy Report*", numerous interactions between partners have taken place. The synergy reports have been based on an inquiry performed by the Coordinator, by asking the following questions:

1. Have you or any of your staff visited the laboratories of the Promemoria partners?
2. Have you exchanged research material with any of the Promemoria partners?

It appears that a great number of visits between individual partners have taken place, and these visits were aimed at both discussing specific scientific collaboration projects and at exchanging knowledge and technology skills.

Deliverables:

D.10.1 Establishment of management in consortium and employment of new staff
D.10.2 Creation of an international Scientific Advisory board (SAB) and a Stakeholders Committee
D.10.3 + 10.3.A Organization of meetings of the Governing Board at 3-month interval
D.10.4 + **10.4.A + 10.4.B** Reporting to EU scientific officers
D.10.5 + 10.5.A Project presentation. Continuous updated of the Promemoria World Wide Web
D.10.6 + **10.6.A** Partner Synergy Report
D.10.7 Revision of the WP structure and contents at the end of first 18 months**.**

All deliverables of WP10 have been achieved

WP11. Dissemination and exploitation activities
WP-leader: partner 15, Miguel Medina, Noscira S.A.

Participants: All Partners

1. *To disseminate knowledge created by the PROMEMORIA* project effectively to scientists and students outside the consortium, to Stakeholders and to the general public.
2. *To facilitate the protection of intellectual property rights of members of the consortium and transferring their innovations to industrial applications*

The following results were achieved in WP11

1. Filing and publishing of patents.

The consortium has been highly successful in filing and publishing patents. Thus, by the end of the PROMEMORIA project 31 patents had been filed, and of these 18 have been published. This should be compared to the deliverables stating that at least five patents should be filed/published in the total period of the PROMEMORIA project.

2. Publication of scientific articles.

The consortium has been very active and in total 180 scientific articles have been publishe during the PROMEMORIA project. Moreover, 29 articles are in press. Thus, in total the consortium has produced 209 scientific articles.

3. Press releases.

The consortium has published five press releases during the duration of the PROMEMORIA project.

4. Establishment of pharma/biotech enterprises.

By the end of the period of the PROMEMORIA consortium (November 2008), the knowledge generated in the consortium concerning development of peptide mimetics resulted in the establishment of two new pharma/biotech enterprises, Phlogo ApS (www.phlogo.dk) and Neoloch ApS (www.neoloch.dk). Phlogo is dedicated to the production of anti-inflammatory peptides to be applied to inflammatory diseases such as rheumatoid arthritis or multiple sclerosis. Neoloch is dedicated to the production of peptide mimetics of erythropoietin with neuroregenerative effects. Moreover, this enterprise also aims at developing peptide antagonists of receptor tyrosine kinases for the treatment of cancer.

Deliverables:

D.11.1+D.11.1A Communication with IP-lawyer**.**
D.11.2+ D.11.2A Reporting of existing patents within the consortium
D.11.3 Filing of at least five patents in the total period of Promemoria
D.11.4 Publication/submission of at least 100 publications in the total period of Promemoria
D.11.5 At least two press releases concerning Promemoria activities

All deliverables of PW11 have been achieved

4.1.4. Dissemination and use

The main exploitable results of the Promemoria project has been the filing of 31 patents of which 18 hve been published. These patents concern compounds with a beneficial effect on learning and memory impairment, specifically in animal models of Alzheimer's Disease. Most patented compounds are at the moment in the discovery phase, but a number have passed the discovery phase and are currently being submitted to determination of pharmacokinetics and toxicology. Three compounds have already been tested in a phase I clinical safety trial.

Based on the knowledge achieved through the preparation of these compounds, two new pharma/ biotech enterprises have been founded developing compounds for the treatment of inflammation, neurodegeneration and cancer.

Scientifically, the consortium has been very active publishing 209 articles in high ranking scientific journals. 180 of these have already been published and 29 are still in press. The consortium has also presented its results at a number of scientific meetings in lectures and posters. Also, five press releases about the activities of the consortium have been made.

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Project logo: *The persistence of memory* by Salvador Dali, 1931.

Project public website: http://plab.ku.dk/promemoria/