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1 Executive summary

What is SYNSYS? SYNSYS is a large-scale collaborative research project involving 16 European academic laboratories and 4 commercial enterprises, and was devoted to studying structural and functional aspects of synapses and synaptic proteins. Synapses form the 'nuts and bolts' of the nervous system. They are essential parts of nerve cells, allowing these to communicate and transmit or receive signals. If synaptic function fails this may lead to serious neurologic or psychiatric disorders. The project closely aligned to other European projects that also focused on synaptic function, such as Eurospin and Neurocypres.

Why was SYNSYS started? Major disorders of the Central Nervous System affect one in three people in the developed world, often seriously disabling the affected individual, and together account for the single largest social and economic burden on the healthcare systems of the EU. Many of these disorders act at the neuronal synapse, a cellular organelle comprising in the order of 2000 proteins. Cross-talk between synaptic proteins and the complexity by which they form signalling cascades pose a significant challenge to dissecting the molecular mechanisms of disease and to design efficient drugs. SYNSYS assembled leading European laboratories to provide the expertise and to establish research platforms that uniquely enabled a systems-level analysis of synaptic signalling.

What were the SYNSYS main objectives? The main objectives of the SYNSYS project were to use a systems level analysis to describe synaptic transmission from molecule to function, its dynamics in relation to physiology, and to improve understanding brain function and brain disorders. To do so, SYNSYS established a research platform for molecular analysis of synapse function and dynamic modelling, with the perspective to generate a blueprint for discovery of novel pathways and molecular targets that enable rational strategies to design therapies for human brain disease. In particular, we aimed at connecting computational science and experimental neuroscience. We used different approaches each of which informed and directed the others. On the one hand, "Wet-lab" studies exploited gene knockdown, over-expression, and pharmacological agents, to test model robustness and refine parameters. On the other hand, computational modelling of the synapse allowed us to predict and test synapse function and predict physiology of neuronal circuitry. This type of interaction between scientific disciplines is crucially needed to design future therapeutic strategies addressing the many brain disorders for which synaptic dysfunction is a central aspect.

The main SYNSYS consortium output. The SYNSYS consortium has been very successful in terms of scientific output. Approximately 200 scientific publications were generated. Various aspects of the work started in SYNSYS will be carried further in the years to come. Of the SYNSYS publications, 31% are listed as 'high impact', meaning that they provided breakthrough insights and/or described novel concepts. In addition to this, the consortium had a large contribution to specific forms of distribution of knowledge, such as in poster presentations, press releases and interviews; over 200 actions were reported.
In addition, several patents were filed and data resources and webtools were generated with access for scientists and the public. In particular, SYNSYS had different small companies as collaborative partners. Through this academic – company alliance SYNSYS was able to achieve some of its specific scientific goals, and vice versa, was able to help generating new external projects and funding for the companies, which provided new career opportunities within the companies.

Read more about SYNSYS or the results? Please visit us for more information on: http://www.synsys.eu/

2 Publishable summary

SYNSYS: Synaptic Systems, dissecting brain function in health and disease

What is SYNSYS?
SYNSYS is a large-scale collaborative research project involving 16 European academic laboratories, and 4 commercial enterprises, in the area of synapse function, which was funded by the European Union starting June 1, 2010. In the seventh research framework programme (FP7), the EU commission devoted a special call to stimulate high-throughput approaches to systems biology analyses for the identification of potential new drug target sites. The SYNSYS consortium (SYNaptic SYStems: dissecting brain function in health and disease) was devoted to studying structural and functional aspects of synapses and synaptic proteins. The project closely aligned to other European projects that focused on synaptic function, such as Eurospin and Neurocypres.

Why was SYNSYS started?
Major disorders of the Central Nervous System (CNS) affect one in three people in the developed world, often seriously disabling the affected individual, and together account for the single largest burden on the healthcare systems of the EU. Most of these disorders act at the neuronal synapse, a cellular organelle comprising in the order of 2000 proteins. Cross-talk between proteins and the complexity of the underlying signalling complexes pose a significant challenge to dissecting the molecular mechanisms of disease and to design efficient drugs. SYNSYS assembled leading European laboratories to provide the expertise and to establish research platforms that uniquely enabled a systems-level analysis of synaptic signalling. The consortium featured a closed loop from data integration and modelling, human genetics, physiology, proteomics and the application of engineered model systems to test model predictions.

What were the SYNSYS main objectives?
The main objectives of the SYNSYS project were to (i) provide a qualitative and quantitative description of the protein composition and the interactome of the mammalian glutamatergic synapse that integrates known human variation in these genes, (ii) to generate quantitative dynamic models describing the main functional features of the synaptic system, (iii) to reiterate on modelling by relating model predictions to synaptic function, (iv) to identify and validate, using appropriate model systems, human vulnerability genes that may form the basis of future therapies. Only a Systems level analysis can provide the means to describe synaptic transmission from molecule to function, its dynamics in relation to physiology and, brain function and brain disorders. As such, SYNSYS established a new platform for iterative molecular analysis of synapse function and dynamic modelling, with the perspective to generate a blueprint for discovery of novel pathways and targets that enable rational strategies to design therapies for human brain disease.

Where can SYNSYS be found?
Please visit us for more information on: http://www.synsys.eu/

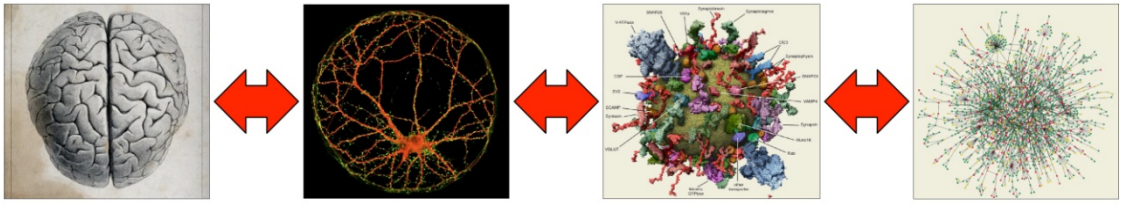

Figure 1*: Schematic representation of SYNSYS iterative multilevel approach, aiming to characterize the essential mechanisms that are at the basis of the brain's computational power by studying individual synapses in reduced preparations, quantitative analysis of all constituents of relevant protein complexes and organelles and, finally, generating computational models, both interaction based and mechanism based.*

Why study brain synapses?

The synapse regulates chemical transmission in a neuronal activity-dependent manner apparent as synaptic plasticity, which is an emergent property of the synaptic system determining synaptic efficacy (signal strength). Plasticity is underpinned by factors including, the alteration of the presynaptic release probability of transmitters; the number of vesicles available; changes in spatio-temporal patterns of post-synaptic proteins via differential protein expression; trafficking of proteins among synapse sub-domains and post-translational modifications that modulate protein function and/or protein-protein interactions. Pre- and postsynaptic plasticity are thought to be major determinants in information processing and alterations in the signalling of the synaptic proteome network are believed to be central to learning and memory processes in the brain. Genetic contributions and/or environmental stimuli can induce alterations of this and cause disease. However, how these factors impact on synaptic system function is unknown, whereas this is of eminent importance for understanding and treating brain disorders.

Why Systems Biology of the synapse?

Systems biology analysis of the synapse, while challenging, is feasible with direct impact for our understanding of cognitive processes and for medical intervention in disorders. Using systems biology approaches, synapse function can be studied in highly standardized, reduced preparations, which allows multi-level quantitative systems analysis and full integration of genomics, proteomics and (patho-)physiology. Quantitative functional (dynamic) models are necessary to identify disease factors within protein networks that are not easily accessible using classical approaches. Systems Biology works towards a quantitative understanding and computational modelling of synaptic protein networks and opens essential new avenues to learn how these biological elements compute and to assess and correct dysfunctional network performance in diseases of the brain. It also provides blueprints for novel target discovery to treat such diseases based on rational strategies.

A SYNSYS example: mapping and modelling the synaptic protein network

To map and model the protein network architecture of the mammalian glutamatergic synapse demands multi-level data-integration relating synaptic function to the protein network with appropriate kinetic models for synapse function in health and disease. For this, we used highly standardized high-throughput proteomics procedures to build a synaptic protein-protein interaction network. In addition we established how chemical modification, in particular phosphorylation, drives the formation of particular protein signalling complexes. Historically, studies on synaptic proteins related mostly to individual proteins and binary interactions. We performed data-driven and large-scale constraint modelling to develop synapse function models (presynaptic secretion and signal transduction events in the post-synapse).

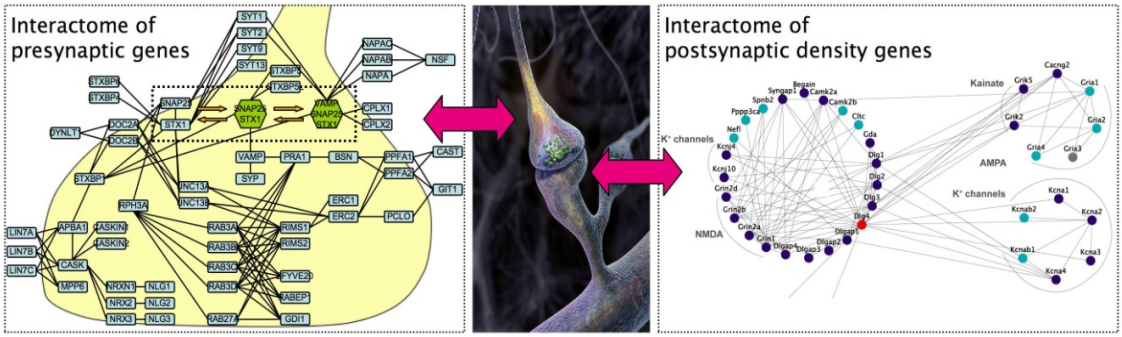

Figure 2: *Synapses are composed of pre- and a post-synaptic elements. The presynaptic part of the synapse is formed by areas of axons free of microtubules and contains many synaptic vesicles filled with neurotransmitter some of which are released upon stimulation at a specialized area, the active zone. Several SYNSYS partners have been responsible for delineating the basic protein-protein interactions in this synaptic sub-compartment. The postsynaptic element contains a synaptic density that is opposing the active zone. It contains synaptic receptors, and cytoskeletal and signalling molecules converting neurotransmitter signals into electrical activity and intrasynaptic signal transduction. Again, Several SYNSYS partners have been responsible for delineating some basic protein-protein interactions in this sub-compartment. Together, this has led to a better understanding of the molecular architecture of the synapse.*

Systems biology of the Synapse: an iterative cycle between experiment and computation

In Synsys, we tested systems models of the synapse, thereby closing the loop between computational science and experimental neuroscience. In particular we used different perturbation approaches each of which informed and directed the others. “Wet-lab” studies exploited gene knockdown, over-expression, pharmacological agents, and the use of peptide mimetics, to test model robustness and refine parameters. Systems biology modelling the synapse allowed us to predict and test synapse function and the physiology of neuronal circuitry. This type of information is crucially needed to design future therapeutic strategies addressing the many brain disorders for which synaptic dysfunction is a central aspect.

The main SYNSYS consortium output

The SYNSYS consortium has been very successful in terms of scientific output. Approximately 200 peer-reviewed publications were generated. Various partners have reported on-going work that is in preparation for publication, from which it can be concluded that the consortium will generate at least 210 publications from the funding source. Of these publications, 62 (=31%) are listed as ‘high impact’ with impact factors of 10 or higher, meaning that they provided breakthrough insights or concepts. In addition to this, the consortium had a large contribution to specific forms of dissemination of knowledge, such as in poster presentations, press releases and interviews. In total 201 dissemination actions were reported.

In addition, several patents were filed and data resources and webtools were generated with access for scientists and the public. In particular, SYNSYS had different SMEs as collaborative partners. Through this academic – industry alliance SYNSYS was able to achieve some of its specific scientific goals, and vice versa, was able to help generating new external projects and funding for the companies and provide career opportunities within the SMEs.

3 Project context and main objectives

Concept of the project, main ideas
The synapse regulates chemical transmission in a neuronal activity-dependent manner apparent as synaptic plasticity, which is an emergent property of the synaptic system determining synaptic efficacy (signal strength). Plasticity is underpinned by factors including, the alteration of the presynaptic release probability of transmitters; the number of vesicles available; changes in spatio-temporal patterns of post-synaptic proteins via differential protein expression; trafficking of proteins among synapse sub-domains and post-translational modifications that modulate protein function and/or protein-protein interactions. Pre- and postsynaptic plasticity are thought to be major determinants in information processing and alterations in the signalling of the synaptic proteome network are believed to be central to learning and memory processes in the brain. Genetic contributions and/or environmental stimuli can induce alterations of this and cause disease. How these factors impact on synaptic system function is largely unknown and therefore was a major aim of SYNSYS.

SYNSYS beyond the state-of-the-art
SYNSYS aimed to combine recent advances in several research fields, such as synapse biology, genetics, proteomics, and computational biology, to underpin a fundamental step forward in our understanding of synapses and their capacity to modulate neuronal networks. SYNSYS partners were at the forefront of many such recent advances as exemplified by the many high-profile publications that were generated within the project. Together, we provided new opportunities to realize a systematic and quantitative analysis of the synapse as a biological system and to synthesize computational models that effectively describe this system. Our proposed approach demonstrated to have predictive power for the role of synapses and their component molecules and pathways in brain in health and in disease. We validated this through a systematic iteration between experimental and computational science, which formed the core of our methodology.

Molecular complexity of synapses: filling in the gaps
Initial proteomic studies suggested that typical mammalian synapses probably contain around 2,000 types of protein (excluding variants and modifications) organized into multi-protein complexes and molecular networks. SYNSYS partners were amongst the first groups to characterize in detail several of these sub-complexes in the synapse: the postsynaptic density, the synaptic vesicles in the nerve terminal and the molecular mechanisms regulating local protein synthesis in physiological and pathological conditions. Furthermore, SYNSYS partners are pioneers in describing evolutionary changes in synaptic signalling and its complexity using comparative proteomics, genomics and systems biology.

Working towards quantitative descriptions of the synapse
The complete, quantitative description of the protein and lipid composition of synaptic vesicles was a seminal contribution to the field and the first study to define the overall structure of an organelle in quantitative terms in mammals. Such a quantitative description made it possible to generate a quantitative molecular model of this crucial organelle in synaptic function, and turned out to be a great asset in studies on synaptic function. Qualitative information about the protein composition of the other aspects of synapses was available through a variety of approaches and provided an informative, but as yet incomplete picture of the molecular complexity of synapses. These quantitative descriptions were crucial for the further progression in molecular modelling and the understanding of synapse function. Such progress primarily came from quantitative proteomics on protein complexes and stimulus-dependent posttranslational

modifications altering protein-signalling function. Given the extensive experience of partners in this direction, SYNSYS was in an excellent position also to produce models of the highest quality based on the latest quantitative analyses.

Whereas quantitative proteomics is the primary approach to identify synaptic protein complexes, this technique inherently disrupts biological structures and relationships and cannot unequivocally localise proteins within cellular sub-structures. Therefore, it was crucial to complement proteomic protein identification with ultrastructural methods to locate proteins within synapses. We, and others, have demonstrated that proteins in the synapse are often compartmentalized in sub-synaptic compartments and display dynamic redistribution during synaptic activity and different forms of synaptic plasticity. They may form complexes temporarily confined to a particular synaptic sub-compartment to serve selective functions, such as regulation of local protein synthesis and degradation. Moreover, during recycling of synaptic vesicles proteins are recruited from the cytoplasm to participate in dynamic supramolecular machines, for instance those involved in exocytosis and clathrin-mediated endocytosis. Therefore, it was crucial to complement proteomic protein identification with ultrastructural methods to locate proteins within synapses.

Synaptic interactome: 'a biological as well as a computational challenge'
During the last decades, and in particular within SYNSYS, many physical interactions among synaptic proteins have been mapped and it has become clear that large multi-protein complexes exist, both in the pre- as well as in the postsynaptic compartment. These complexes are probably organized in yet larger structures. SYNSYS partners have isolated and characterized such large complexes and made many contributions to the establishment of the interactome of several key molecules in the synapse, such as presynaptic and endosomal SNARE-proteins, SNARE-associated proteins, the presynaptic active zone and the postsynaptic density.

Functional annotation of synaptic proteins
Synaptic transmission depends on spatially and temporally coordinated cascades of biochemical and cell biological processes that involve the concerted actions of many proteins. However, classical approaches for the analysis of synaptic transmission focuses on single or very small numbers of proteins. Such studies formed important building blocks upon which we built an integrated systems biology approach. Many key questions regarding the cell biology of individual proteins - e.g. where in synapses these proteins are localized and how deletions or mutations of the genes encoding these proteins affect their location or function – had remained unanswered for the majority of synaptic proteins. Hence, while we aimed to move the field forwards and generate larger protein network models, functional annotation of individual genes/proteins was an important goal.

Synaptic proteins in brain disease: causal links and revealing new mechanisms
Over the last decade, epidemiological and other genetic/transcriptomic/proteomic studies have shown that synaptic dysfunction is central to the etiology and progression of a wide range of neurological and psychiatric disorders, including neurodegenerative diseases, psychotic disorders, such as schizophrenia, autism, affective disorders such as depression, mental retardation and many others. The recognition of the fact that a surprisingly high proportion of these disorders are caused or at least strongly influenced by synaptic dysfunction has certainly been a major breakthrough in both psychiatry and neurology. However, this breakthrough is a mostly conceptual advance. Functional follow-up studies have been scarse and therapeutic approaches based on the understanding of synaptic dysfunctions are still very much at the research stage in modern drug development pipelines. Hence, the identification of synaptic disease genes represents a major breakthrough but also bears major challenges. When starting

SYNSYS, the state-of-the-art in synaptic disease (synaptopathy) research was at the level of disease gene (and hence drug target) identification and validation, which is merely the starting point of the endeavour to actually understand synaptopathies as a synaptic systems failure and to cure them. Synaptopathies would remain enigmatic and cures would remain out of reach, unless the functions of the corresponding gene products are identified within the context of the gene/protein network in which they operate, animal models of the genetic variants are analysed, the consequences of these mutations are investigated at different levels (from molecules to synapses, cells and circuits). These were key issues that the activities of the SYNSYS consortium helped to successfully address.

The SYNSYS consortium was convinced that the value of the knowledge that a given gene product is causally involved in a given disease entity is bound to remain limited as long as there is no (quantitative) insight into the context of the protein complexes and gene/protein network in which disease genes/proteins operate. Such 'contextual' information is for instance crucial to be able to design innovative interference or even prevention strategies. Such information is also necessary to identify new risk factors. The SYNSYS consortium provided detailed information about such context, in the systematic protein localization, functions and interactions were systematically mapped and the consequences of dysfunctions could to some extent be predicted from our computational models.

To date, for a small selection of synaptic genes involved in disease, our current knowledge has now gone beyond the level of disease gene identification. Several of these synaptopathy genes have been identified by SYNSYS partners groups and have been studied extensively by SYNSYS partner and others. These include the presynaptic proteins Munc18-1, Complexins), Piccolo, SNAP25 and Neurexin-1, the postsynaptic proteins Neuroligin-3 and Dlg2-4 and FMRP. A surprisingly high number of physical and genetic interactions exist among these disease genes, emphasizing the concept that defects in different genes in a given networks produce similar disease phenotypes. Our synaptopathy studies have contributed to characterizations of functions and mutant phenotypes. Key questions regarding the cell biology of these proteins their role in synaptic networks, and predicting their activities by modelling, are now being answered.

Considering all the above, in SYNSYS we formulated **the following objectives**:

1. To provide a **quantitative description of protein complexes, interactomes and networks** in the mammalian glutamatergic synapse.

2. To **generate quantitative dynamic models**, describing the main functional features of the synaptic networks.

3. To **reiterate on modelling** by relating model predictions to synaptic function.

4. To **identify vulnerabilities in gene networks** which are at the basis of human brain disease and may help design future therapy.

Overall strategy of the work plan

To successfully work on the specified objectives we formulated 8 work packages that were highly integrated, with data being shared using a common consortium database. Our models

were tested against the data generated within the program. These new data were then incorporated in order to refine the models.

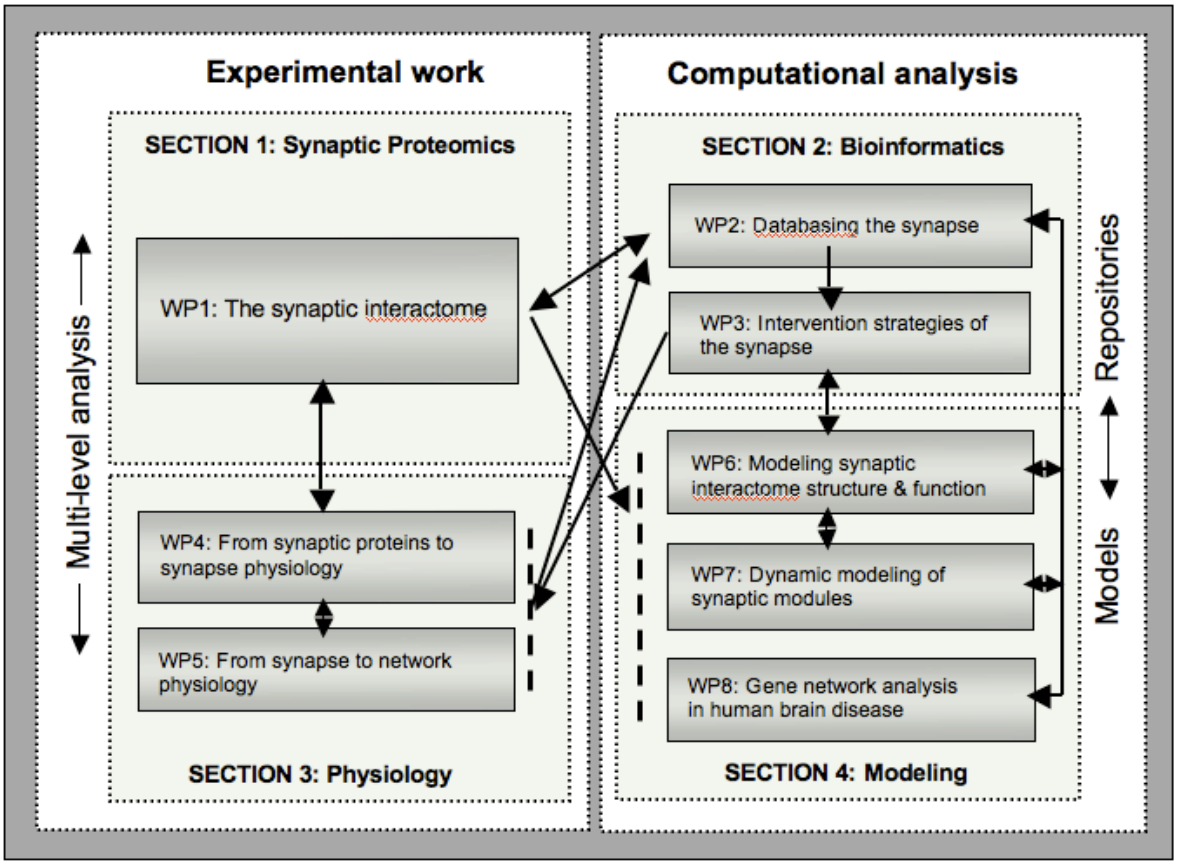

Figure 3: SYNSYS *consortium workflow: four main sections contained 8 academic work packages bridging experimental and computational approaches.*

4 Main S&T results/foregrounds

The main SYNSYS consortium output in short

Publications
The SYNSYS consortium has been very successful in terms of scientific output. At the moment of the making of this report 195 peer-reviewed publications have been generated (=accepted). Various partners have reported on-going work that is in preparation for publication, from which it can be concluded that the consortium will generate at least 210 publications from the funding source. Of these publications, 62 (=32%) are listed as 'high impact', with impact factors of 10 or higher. In addition to this, the consortium had a large contribution to specific forms of dissemination of knowledge, such as in poster presentations, press releases and interviews. In total 201 dissemination actions were reported. Two specific actions on exploitable foreground have been filed: 1. http://sourceforge.net/projects/cdmsuite/ (open source software package) (UEDIN). 2. Development of a standardized evaluation of synaptic disturbances in transgenic mice using electrophysiological recordings in mouse brain slices (Synome).

Patents were filed
1. a behavioral assay filed by BrainWave Ltd on 13/11/2014 (US 62/058218)
2. a patent regarding FRX treatment by Bagni and coworkers on 11/06/2015 (PCT/EP2014/077374).

Databases and webtools
Next to scientific publications SYNSYS built several databases and webtools for the scientific community, such as SYNSYSdb, SynSysNet, ProTox, and Superpain. These databases have shown a large use.

R&D, in and with SMEs
In particular, SYNSYS had different SMEs as collaborative partners. Through this academic – industry alliance SYNSYS was able to achieve some of its specific scientific goals, and vice versa, was able to help generating new external projects and funding for the companies and provide career opportunities within the SMEs.

More detailed description of the consortium output and highlights can be found in the sections below.

➢ Section 1: Synaptic proteomics

In this section of SYNSYS we dealt with the identification of synaptic proteins and the state-dependent interaction of proteins into complexes.

WP1. Functional profiling of the mammalian synaptic proteome and interactome

In WP1 we investigated the parts list of the synaptic proteome, the local transcriptome, as well as aspects of their dynamics. For this we used mass spectrometry to identify protein complexes that were isolated by immunoprecipitation. Because stimulus-dependent complex formation mostly involves phosphorylation events, we dedicated our analysis to this as well. Specifically we tested phosphoprotein-protein first order interactions by yeast three-hybrid (YTH) analysis.
In addition, we made use of state of the art chemistry using isobaric labelling of proteins for quantification and frontier technology, e.g. involving two-dimensional liquid chromatography separation techniques followed by MALDI-TOF/TOF or ESI-OrbiTrap mass spectrometry. This was all developed to make use of highly automated workflows. Standardized high-throughput (industrialized) proteomics analysis of the synapse and its subfractions enabled identification of state-dependent core synaptic protein complexes, the phosphoproteome and this information was used to provide models of the interactome and generated testable hypothesis in physiology and intervention strategies. As a first step towards the relations between individual proteins in large protein complexes, SYNSYS partners analysed the protein composition of normal synapses and from mutant mice that lack one particular synaptic protein. These studies suggested that synaptic protein complexes are generally highly robust, i.e., the loss of one component induces minimal changes in the levels of other proteins, typically with the exception of a small number of direct interactors. The SYNSYS consortium studied this robustness more systematically using their mutant mice. Moreover as was expected alterations of the proteome did not specifically relate to changes in protein levels, but relied on changes in the formation of protein complexes, often organized by specific phosphorylations resulting from signal transduction.

Interaction proteomics. Here we substantially extended the existing models of synapse protein complexes by performing large-scale immuno-precipitation followed by identification of the proteins in the complex by quantitative proteomics. In particular we will advance our insight into synaptic protein complexes by using immunoprecipitation (IP) thereby generating interaction data of over 100 synaptic proteins. Antibodies for this were partially available and many were generated. In addition to this, we generated insight in the dynamics of complexes under different experimental conditions, including synaptic rest and activation, behaviour dependent alterations, and up- or down regulation of synaptic genes using available mouse mutants (100 lines) or using transgenesis. In addition, we compared data of the IP-based interaction proteomics with that of our partners in the EUROSPIN consortium. They utilized the Y2H to generate the first synaptic interactome consisting of >1,000 proteins connected by >10,000 protein-protein-interactions, making it the biggest single study on binary synaptic protein interactions to date.

In addition to proteomics and ultra structural analyses (see below), we made use of published functional data sources on proteins/genes to provide valuable clues for the molecular composition of synapses and provided independent confirmation for candidate synaptic proteins. Data mining provided a valuable addition to the separation of pre- and postsynaptic protein networks, which are difficult to separate using biochemical fractionation methods. Manually curated databases of published synapse data turned out useful resources when combined with proteomics databases.

The **Phosphoproteome**. We performed Y3H (Yeast three hybrid) studies to identify interaction partners for synaptic phosphoproteins and also to validate computational predictions. Utilizing the binary interactome map, we selected known targets of protein kinase C (PKC), a kinase that is crucial for the regulation of many synaptic processes. These selected PKC targets were screened against a synaptic cDNA library of 906 clones in a Y3H experiment to detect PKC-regulated protein-protein interactions. Thereby we generated a synaptic phosphoprotein map connecting 66 proteins by 116 PPIs. This involved the setting up of new technology in the area of yeast three hybrid. Novel phosphorylation sites of core proteins of the vesicle fusion machinery were identified, which will a role in on-going research of several partners regarding the modulation of transmitter release.
For instance, phosphorylation of Munc18 at Serine 306 and 313 by PKC results in a conformational change and the release of the t-SNARE-protein Syntaxin-1, allowing the SNARE protein complex to form. The assembled SNARE complex then mediates both vesicle docking and neurotransmitter release. We find that not only does phosphorylation of Munc18 causes it to release Syntaxin-1, but that it allows Munc18 to bind SNAP25, the second t-SNARE protein. This interaction suggests that Munc18 regulates membrane fusion during exocytosis no only through its interaction with Syntaxin-1but also by directly binding to SNAP25.
For specific protein complexes and sets of proteins of interest we generated more detailed interaction data by using Biacore SPR analysis as a necessary step in kinetic modelling of interactome modules. Also we were able to establish the role of calcium in the interactions using SPR technology. The IP-MS data and Y3H together generated pivotal new information for modelling parts of the interactome.

Subcellular localization. Specific synaptic proteins were localized to synaptic sub-domains and their localization in relation to the key features such as the active and the periactive zones or the postsynaptic density were determined using Electron Microscopy and correlative LM-EM techniques. These approaches were also used to describe the localization of proteins at either pre- or post-synaptic sites or both. 3D maps describing dynamic localization of selected proteins were provided. In addition, pecific analyses were performed regarding the nature of vesicle priming and docking at the active zone. For instance, on hippocampal organotypic slice cultures from mice lacking key presynaptic proteins, cryofixation, and three-dimensional electron tomography were employed to study the mechanism of synaptic vesicle docking in the same experimental setting, with high precision, and in a near-native state. Previously indistinguishable, sequential steps in synaptic vesicle active zone recruitment (tethering) and membrane attachment (docking) were described. It was found that vesicle docking requires Munc13/CAPS family priming proteins and all three neuronal SNAREs, but not Synaptotagmin-1 or Complexins. Our data indicate that membrane-attached vesicles comprise the readily releasable pool of fusion-competent vesicles and that synaptic vesicle docking, priming, and trans-SNARE complex assembly are the respective morphological, functional, and molecular manifestations of the same process, which operates downstream of vesicle tethering by active zone components.
The subcellular localization of proteins in synaptic sub-domains forms the basis for the adequate interpretation of role of proteins in the synapse physiology and kinetic analysis and will be at the basis for future studies on the 3D tomographic reconstruction of key components in the synaptic element.

The synaptic transcriptome-proteome in disease models. We identified and characterized the mRNP content at hippocampal synapses. We will isolate native mRNPs (translating and silent) through well-established sucrose gradients and the content of the two different components (RNA and protein) analyzed independently. The characterization of the synaptic mRNAs as well as of the associated proteins complement/confirm the information found by

proteomic analysis in WP1. A description of proteins that are translated in an activity-dependent manner (after DHPG or BDNF stimulation of synaptosomes) was generated. A parallel study was performed for a pathological condition such as mental retardation using mouse models to study the Fragile X Syndrome and Autistic Spectrum Disorder and Mental Retardation (FMR1 KO, BC1 KO and CYFIP1 KO). These mice were also studied for the synaptic proteome and interactome in WP1, for their synaptic physiology in WP5.
Among the mutant mouse lines that four partner groups and several previous FP6/FP7 consortia (EU-SYNAPSE, EUROSPIN) together contributed, many were designed to model human diseases and are candidate animal models of diseases such as schizophrenia, autism, depression, ADHD, epilepsy, mental retardation, or other disorders. Hence, the studies on these mutants across molecular, cellular and network levels generated valuable information on validity of these models, their synaptic dysfunctions as well as inferring valuable insights into the etiology of human brain disorders.

- **Section 2: Bioinformatics**

Section 2 dealt with storage and making available synaptic protein data for computational analysis. Primary identification and interaction data on synaptic proteins that was gathered in WP 1 was stored.

WP2. **Databasing the Synapse**

Several of our partners have well-established credentials in the development of databases for commercial, single project, community and collaborative sharing of information. Partner 2 currently hosts G2Cdb, a warehouse for a diverse array of synaptic data ranging from raw datasets from high-throughput proteomic studies through behaviour and physiology to high-value curated datasets from the prior literature. This system has internal and external interfaces, which allows staged release of data. We used the G2C platform for hosting the SYNSYSdb.

SYNSYSdb. This database for synaptic proteins synsysdb.genes2cognition.org/ provides adequate definitions of pre- and post-synaptic proteins (input from WP1), proteins present in sub-domains of the synapse, e.g. the synaptic vesicle and associated proteins, lipid rafts, the postsynaptic density (input from WP1). We worked on the generation and maintenance of the SYNSYSdb database system to support public release of data generated within the consortium and integrate external/public dataset to develop a new synapse network models. We collected and prepared data in agreement with data standards, adopted a continuous release cycle, making data sets immediately visible to all users of the website as soon as data was available. Various types of data were stored, e.g. experimental data regarding mass spectrometry experiments, as well as data relating to YTH. In addition to data that was gathered from the experiments in WP1, we extracted and manually curated relevant data on synaptic proteins from other sources and provided an initial ontology for these.
New synaptic annotation and ontological descriptions are foreseen based on the curated databases within SYNSYS.

WP3 Intervention strategies of the Synapse: tools for translation

In this workpackage we used predictive tools for protein-protein interaction also used in the interactome construction (WPs 1, 7). A large part of the proteins participating in synaptic gene networks are either structurally known or can be derived from similar structures of known homologues. Using the expert knowledge of the SYNSYS consortium we predicted molecular interactions derived from conserved interacting domain contacts: Characteristic pairs of structural signatures that recur in various pairs of interacting proteins were used for predicting putative pairs of interacting domains in synaptic proteins. Putative interaction sites were further validated experimentally by using peptide arrays: Potential interaction sites are translated automatically into linear or non-linear peptides and screened with established peptide array spot techniques. In important aspect of SYNSYS to translating the knowledge on synaptic proteins to the use in the drug discovery field was contributed by generating a suite of tools in the SYNSYSNet platform (bioinformatics.charite.de/ synsysnet/).

SynSysNet. The publication "SynSysNet: integration of experimental data on synaptic protein-protein interactions with drug-target relations." in the 2013 database issue of Nucleic Acids Research reflected the excellent collaboration within the SYNSYS consortium. Scientists from 6 groups worked in close collaboration to set up a database incorporating synaptic knowledge collected during the funding period. SynSysNet, is based on an expert-curated list of 1.000 human genes, specific to the synapse. Information on resulting proteins, their 3D structure, binding small molecules Protein-Protein-Interactions (PPIs) and Compound-Protein-Interactions were integrated. Proteins or compounds can be searched at the appropriate buttons and Interactive Networks were visualized. The Diseases present are neurological diseases, to illustrate specifically the role of SynSysNet in the area of drug discovery for medication. The database is publicly available at: http://bioinformatics.charite.de/synsysnet.

Superpain. Superpain, a database for pain stimulating and relieving compounds and was published in the 2014 database issue of Nucleic Acids Research. It comprises 8,700 ligands which bind to diverse ion channels (e.g. TRPV1, TRPM8, TRPA1, hERG,TREK1, P2X, ASIC or voltage-gated sodium channels) characterized by experimentally determined binding affinities. The database additionally includes 100,000 putative pain-relieving ligands and allows the selection of promising drug candidates, some of which are currently tested experimentally. The database is publicly available at: http://bioinformatics.charite.de/superpain/.

ProTox. We developed toxicity prediction models and integrated them into a public web server, which allows the prediction of acute oral toxicity as well as potential off-targets of small molecules. All methods have been evaluated based on a diverse external validation set and displayed strong performance and superiority over other toxicity prediction tools. The web server, published in the 2014 web server issue of Nucleic Acids Research, can be used to filter virtual screening hits, e.g. potential novel protein-protein interaction inhibitors, to achieve a deeper understanding of molecular action and presents a valuable tool to identify potential toxicity risks early in drug development. ProTox is publicly available at: http://tox.charite.de.

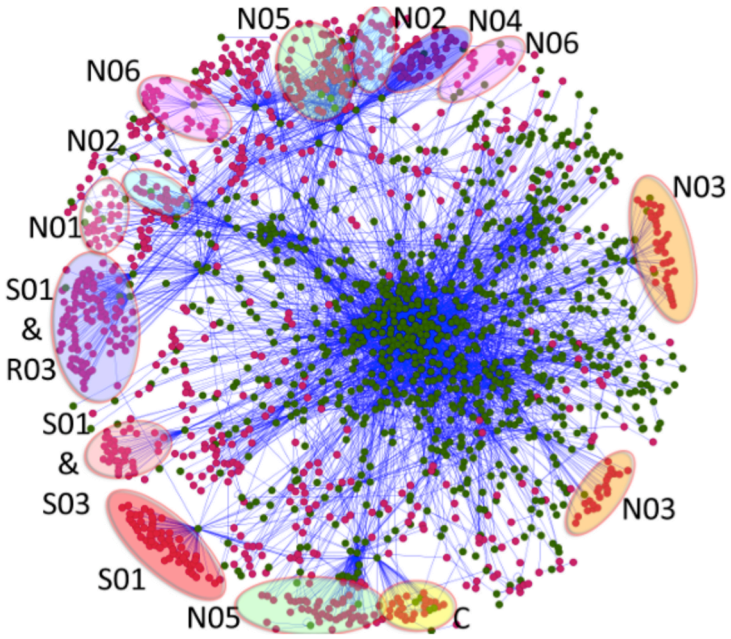

***Figure 4**: SYNSYSNet. This compound-protein-protein interaction network shows the interactions of 1.160 compounds and 894 proteins. To create the network compounds from the database SuperTarget and connected proteins from SynSysNet were chosen. A total of 6.116 interactions (edges), thereof 4.318 PPIs and 1.798 compound-protein interactions are shown. The compound-representing circles are ruby-coloured, the proteins' green and the edges between them blue. The clusters with the same targets were analysed and it was found that many of the compounds of one cluster belong to the same ATC-code. Those clusters were highlighted and marked with the appropriate ATC-code.*

- **Section 3: Synaptic Physiology**

The SYNSYS consortium addressed this topic using appropriate *in vivo* and *ex-vivo* models combined with electrophysiological techniques, behavioural paradigms and genetic perturbations. The majority of these studies focused on the analyses of a model synapse, the hippocampal excitatory synapse. Several partners have exploited this model synapse in a highly reduced preparation, the autaptic microdot culture, to elucidate the function of several key proteins in synaptic functions. The analysis-protocols were already very similar between labs and have been even more rigorously standardized. In this way the functional consequences of gene deletions, over-expressions and other perturbations, could be directly compared and used for statistical analyses and computational modelling.

An important asset of the SYNSYS consortium was that three partner groups together contributed a very extensive collection of mutant mouse lines. SYNSYS could build on these previous achievements by individual partners and by previous FP6 and FP7 consortia responsible for generating these mouse models, especially EU-SYNAPSE and EUROSPIN. This unique asset was exploited in conjunction with our protein complex identification and modelling activities. Proteomics analysis of alterations in protein complex formation in these mutants shed light on this. Moreover, functional analysis of the synapses from such mutants provided functional annotation of the proteins encoded by the deficient genes, which subsequently was used for more complex analyses, both experimental and computational. While more than 50 mouse mutants with deficient synaptic genes have been studied, no systematic (meta) analysis of these data had been performed. An even better standardization of the experiments and cataloguing of the phenotypes observed therefore facilitated more systematic analysis of these functional data. As such, this section was instrumental in determining the role of specific synaptic proteins to the physiology of the synapse and in providing quantitative data on, e.g., the release of transmitter, the pre- and post-synaptic

mechanisms of plasticity of the synapse. These data form the basis for dynamic models generated in WP7**.** Also, this section bridged synapse physiology to neuronal network function.

WP4 From synaptic proteins to synapse physiology

To augment computational studies, it was important to study (mutant) neurons in living networks rather than autaptic cultures. Neuronal networks provide a more accurate analysis of the capacity of individual synapses to contribute to synaptic plasticity. Therefore, SYNSYS aimed to augment interesting and informative phenotypes found in single cell studies with subsequent studies in neuronal networks. These studies yielded a large set of functional data, via 'cellomics' high-content analysis, and connected to further in-depth analyses of protein networks.
The biology of neuronal networks is an emerging frontier in neuroscience. To bridge different levels of analysis SYNSYS partners employed new experimental tools such as high-speed two-photon imaging of calcium dynamics in large-scale neuronal networks, multi-electrode patching of sets of connected neurons in which connectivity patterns and properties of identified synaptic contacts were quantified, synaptic calcium dynamics and spike-timing-dependent synaptic plasticity. Correspondence of parameter quantifications from these levels of organization was guaranteed by the use of identical molecular intervention strategies.

Cellomics. Starting with *in vitro* cultured cells, Cellomics high-throughput automated microscopy, viral vector mediated knockdown and over-expression of synaptic proteins was used to systematically define the role of synaptic proteins in synapse formation and maintenance.

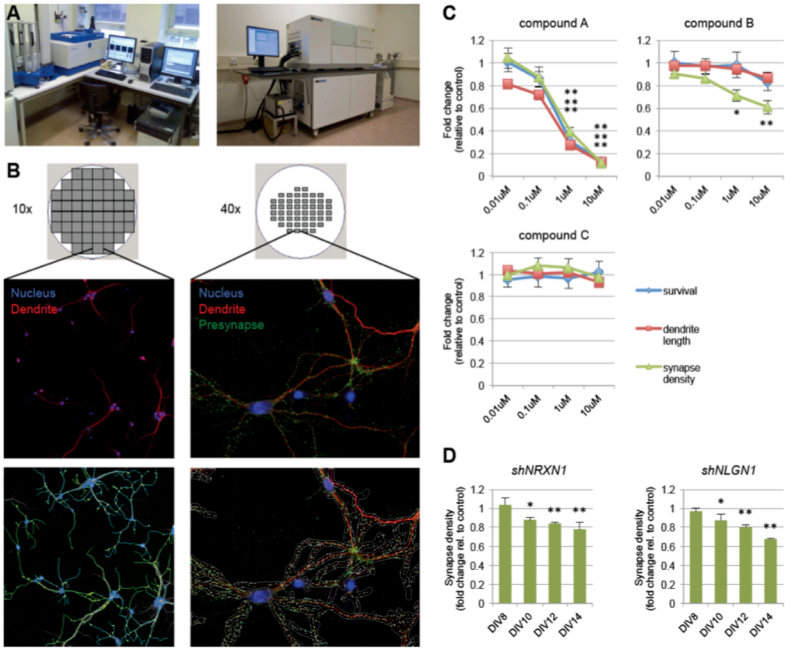

Figure 5*: (A) VU has 2 HCS instruments, Cellomics ArrayScan (left) and PE Opera LX confocal (right). (B) Mouse primary cortical cultures in 96-well plates are imaged at 10x (ArrayScan) and 40x (or 60x, Opera LX, top). At 10x, the entire well is imaged for cell counts & dendrite length. At 40x, ~40 parameters are quantified in random samples. Image analysis is unsupervised and automated (bottom). (C) Example neurotoxic compound screen. Compound A has dose-dependent general neurotoxic effect (similar impact on neuronal survival, dendrite length, and synapse density). Compound B has a specific synaptotoxic effect. Compound C is a negative control. (D) Example shRNA screen. Knockdown of synaptic gene NRXN1 or its receptor NNLGN1 reduces synapse maintenance (mean of 4 independent shRNA constructs per gene, preliminary data, * p<0.05; ** p<0.01).*

Synapse Physiology. Using identified synaptic proteins that have a role in the release of vesicles in synapses we performed in depth synapse physiology. For this highly reduced and standardized preparation (autaptic cultures) were used, and we quantitatively assessed synapse functions in primary neurons deficient for one synaptic gene (approximately 50

mutations). This yielded a set of rate constants that describe the way synapses secrete transmitters and regulate the sensitivity of the postsynaptic receptors, as well as rate constants of the pre- and postsynaptic interactome that describe mechanisms underlying short- and long-term synaptic plasticity. These data were the basis for dynamic models generated in WP7.

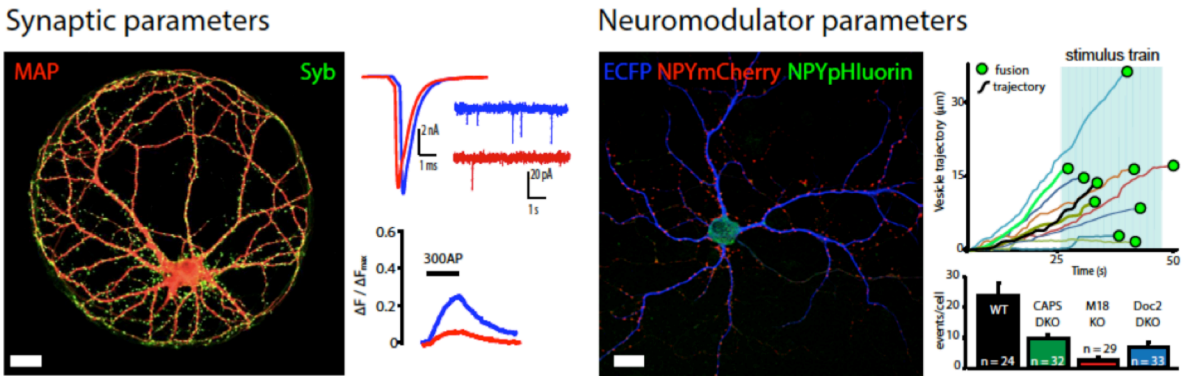

Figure 6: *(A) Autaptic neuron grown on glia (unstained). Red=Map2, green=Synapsin1 (synapses). (B) Synaptic release parameters from A. Shown are evoked postsynaptic current (EPSC), spontaneous release (minis) measured using whole cell patch clamp and synaptic vesicle release measured using synaptophysinphluorin in wildtype (blue) and mutant neurons (red). (C) Neurons infected with lentivirus expressing ECFP, NPYmCHerry and NPYpHluorin using P2A and T2A sequences showing secretory vesicles containing neuropeptide Y (NPY). (D) Neuromodulator transport and release parameters from C. Shown are trajectories of individual secretory vesicles prior to release (fusion) and mean release of secretory vesicles per neuron for wildtype (WT) and secretion mutants (CAPS, M18 and Doc2).*

WP5 Dynamics of synapse function in neuronal networks and animal behaviour

Next, the single cell and single synapse level was integrated in large-scale neuronal networks by assessing short- and long-term synaptic plasticity in acute brain slices using multi-electrode arrays. Multiple physiological parameters were profiled for different synaptic proteins and were mapped onto proteome interaction networks (WP6). These included many genes that are part of disease models. Expression levels of a hundred candidate genes/ proteins were targeted in the *Drosphila* invertebrate system testing mammalian signalling molecules by overexpression in neurons involved in learning. This turned out to be fast and more cost effective than mammalian models and reduced our animal use. Also, we were able to set-up new animal model testing in high throughput (Brainwave; SME), which allows this technology to be used in future projects testing synaptic genes.

➢ Section 4: Modelling

We used a layered approach to develop models of progressive increased complexity and focus, making the most of existing information. In the first instance we reconstructed the pre-synaptic interactome using protein interaction data obtained by WP1. This provided a firmly grounded framework onto which we built further data and enriched our models.

WP6 Modelling synaptic interactome structure & function

We modelled the synaptic interactome structure and function. Partners developed several novel computational techniques available for the reconstruction of connectographs such as multivariate classification methods like Support Vector Machines (SVM) or Partial Least Squares Discriminant Analysis (PLSDA). Confirmed connections were used as to refine the model. Based on the proteomics and interactome data generated by WP1, but also public datasets and literature, we built a set of highly curated signalling pathways underlying post-synaptic plasticity (the Synaptic Signalling Knowledgebase, SynapseKB). All molecular species and reactions are annotated. A global coarse map presents the overall functional view of the pathways, and is completed by detailed maps presenting the biochemical details of reactions. The pathways can be explored online using NaviCell, a tool built on top of Wordpress and the Google toolkit, allowing browsing, semantic zooming, and annotations. A curated and fully annotated pre-synaptic interactome obtained by iterative cycle of experimental (WP1) and computational procedures (WP6) is in assembly. A publication on this topic is currently in preparation.

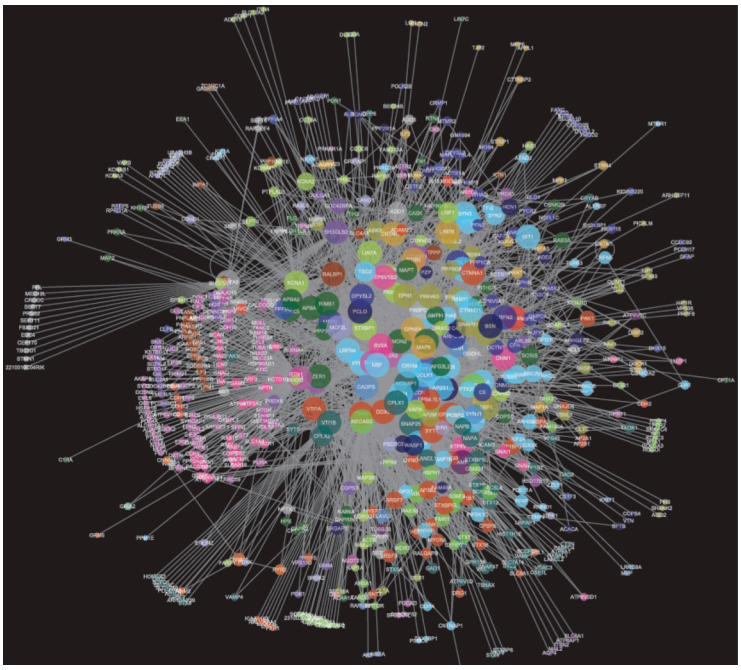

Figure 7: *The presynaptic network covers 702 proteins (89 bait proteins & 613 prey proteins) and 2500 experimental interactions (16% of which are reverse IPs). Measures used to select the networks interactions, from a binned C2S-filtered (Xie et al., 2011) interactions set, included: bait protein coverage (high), Reverse IP coverage (high), global clustering co-efficent (high) and cluster size. The network was clustered using the Spectral Modularity algorithm (Newman, 2006) and covers 28 communities, averaging ~25 proteins per community. The network has been visualised using Visone (Brandes et al,. 2012), with each of the 28 communities colour coded."*

WP7 Dynamic modelling of synaptic modules

In order to understand the dynamics of signalling pathways underlying synaptic plasticity, we constructed dynamic models of major components of the post-synaptic signalling. We also constructed realistic quantitative models of chosen pre- and post-synaptic processes, focussing on the spatial and diffusion components. In particular we studied synaptic vesicle docking and release with Markov models, parameterised using Bayesian approaches.

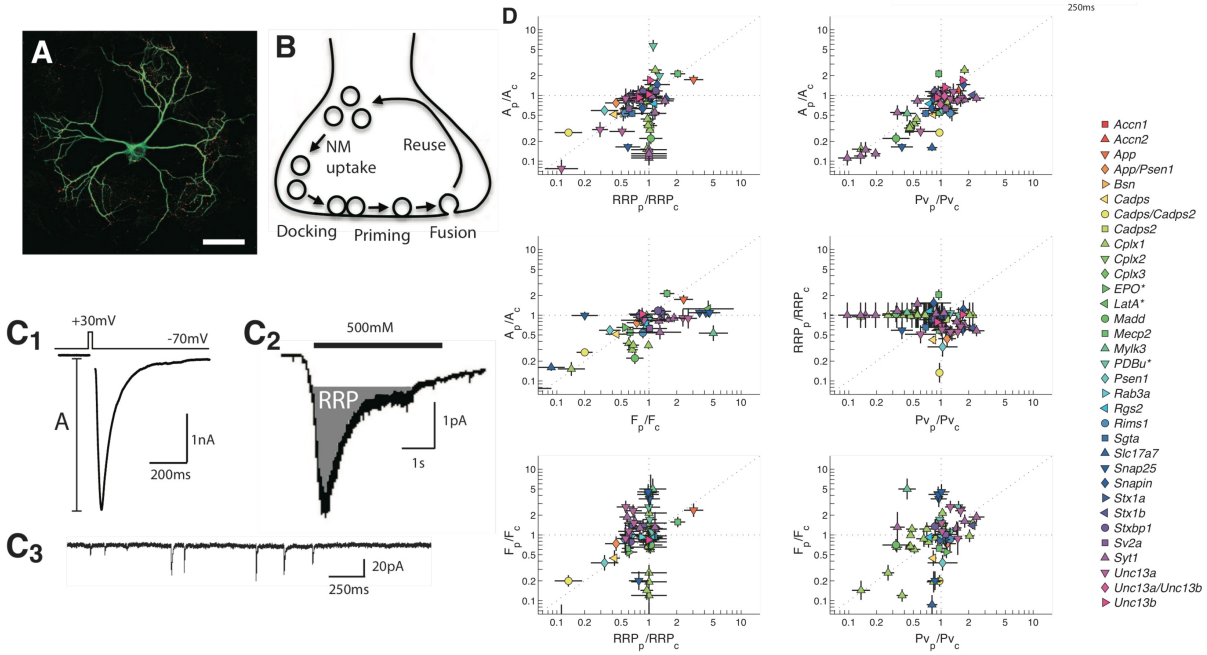

***Figure 8**: **Overlapping protein sets control distinct steps in presynaptic function**. SYNSYS generated a novel probabilistic method to analyse complex functional data from genetic perturbation studies on presynaptic function in cultured single neurons (panel A). This method uses a mixture of probabilistic principal component analysers to cluster genetic perturbations on two distinct steps in synaptic secretion (panel B-C), vesicle priming and fusion, and accounts for the poor standardization between different studies. Clustering data from 121 perturbations (panel D) revealed that different perturbations of a given protein are often assigned to different steps in the release process. Furthermore, vesicle priming and fusion are inversely correlated for most of those perturbations where a specific protein domain was mutated to create a gain-of-function variant. Finally, two different modes of vesicle release, spontaneous and action potential evoked release, were affected similarly by most perturbations. This data suggests that the presynaptic protein network has evolved as a highly integrated supramolecular machine, which is responsible for both spontaneous and activity induced release, with a group of core proteins using different domains to act on multiple steps in the release process.*

To study the role of diffusion of proteins and small molecules during the development and maintenance of synaptic plasticity, we used spatial models of the post-synaptic density. Information on subcellular localization of proteins from WP1 was used. Diffusion of AMPAR and small molecules were studied with multi-agent reaction-diffusion simulations. We built detailed computational models of calcium signalling. We developed new more accurate, models of calmodulin and its interactions with targets such as CaMKII, Calcineurin and Neurogranin. These were embedded in kinetic models of the synapse, to study the effect of different calcium stimulation regimes. We showed that calcium signals duration, frequency and amplitude are affecting bidirectional plasticity. We also concluded that the frequency at which a synapse switches from depression to potentiation is not intrinsic, but depends on the pattern of stimulation.

We predicted and tested the parameters controlling receptor diffusion, aggregation and function, and we measured the interaction kinetics (Beactica; SME) of presynaptic proteins involved in the vesicle release machinery. Also we measured and modelled synapse to nucleus signalling via cAMP. Neurons have complex dendritic trees and little is known about the rules governing synapse to nucleus signalling. We combined signal transduction pathway reaction-diffusion modelling and biosensor imaging in live neurons. We showed an unexpected nonlinear non-

monotonic relationship between synapse to nucleus distance and nuclear responses. Activation of the cAMP pathway in secondary dendrites is the most efficient.

WP8 Gene network analysis in human brain disorders

Not surprisingly, given the molecular complexity of the synapse, the human genetics of psychiatric and neurological diseases yields a picture of significant complexity. On the one hand, there are many individual genes of high penetrance that result in mental illness. For example, approximately 50% of all known X-linked mental retardation genes encode postsynaptic density proteins. Both the mutated genes and the specific disorders are rare and thus considered examples of the 'Rare Genetic Variant - Rare Disease' model. It also appears that rare variants only account for a small proportion of common psychiatric disorders such as schizophrenia, autism, or depression. For example, mutations in the postsynaptic genes DISC1 and DLG2 account for perhaps 1% or less of all schizophrenia cases, and the same is true for the postsynaptic genes Neuroligins 3 and 4, whose mutation is thought to account for 3-6% of autism cases. This is consistent with a number of recent studies that addressed the model of 'Common Genetic Variant - Common Disease'. Indeed, genome-wide association analyses of common variant SNPs in schizophrenia, bipolar disorder, and other diseases failed to uncover individual genes of large effect, and indicates that common variations in these genes cannot account for a substantial proportion of the genetic risk in general. This complication has been referred to as 'the missing heritability'. Within SYNSYS we made a contribution to the solution, using novel, computational approaches to understand the way genes work together in networks.

Computational challenges. The gene network analyses in human brain disorders required large-scale computing resources and carried out at the Genetic Cluster Computer (GCC; see http://www.geneticcluster.org). GCC is a central supercomputing resource (coordinated by Danielle Posthuma). GCC is hosted at the national Dutch computing facilities (SARA:http://www.sara.nl) and financially supported by the Netherlands Organization for Scientific Research. It consists of 64 dual core processors, adheres to strict security rules and is exclusively reserved for researchers working in the area of genetics. Access to GCC also includes access to the broader Lisa cluster, currently totalling 800 computing nodes.

Gene set analyses. We assessed the roles of gene networks of different synaptic functioning in brain disorders by evaluating the combined effect of multiple genes within each network. Evaluating the combined effect of multiple genes is a much more powerful approach than assessing the effects of single genes. Especially for complex traits that are thought influenced by multiple genes of very small effect, evaluating the effect of gene networks turned out to provide insight into disease etiology. Statistical permutation procedures were used to obtain empirical distributions of the test statistics and empirical measures of statistical significance. We elucidated which functional gene networks are affected in several specific brain disorders, as well as which gene networks may be common to most brain disorders. In addition, we tested for gene network by environment interactions.
Specifically, we tested the involvement of gene networks as defined in WP1, 2 & 6 in human brain disorders. Using the catalogue of human genetic variation in synaptic genes as well as available large scaled cohorts with genome-wide genotypes, we collectively tested synaptic genes for association with disease phenotypes (schizophrenia, autism, ADHD, depression, schizophrenia, migraine), thereby collapsing GWAS data into molecular pathways.
Patient cohorts used for these samples were made available from large international consortia, i.e. The International Schizophrenia Consortium, Tourette Syndrome Association. International Consortium for Genetics, The International Headache Consortium, and the Psychiatric Genetics Consortium.

In particular we found evidence for the contribution of specific sets of synaptic genes in:

Cognitive abilities. We applied an innovative method in which we tested for the effect of groups of genes defined according to cellular function (functional gene group analysis). Using an initial sample of 627 subjects, this functional gene group analysis detected that synaptic heterotrimeric G-proteins play an important role in cognitive ability (PEMP = 1.9x10-4). The association with heterotrimeric G-proteins was validated in an independent population sample of 1507 subjects. Heterotrimeric G-proteins are central relay factors between the activation of plasma membrane receptors by extracellular ligands and the cellular responses that these induce, and can be considered a point of convergence, or 'signalling bottleneck'. While alterations in synaptic signalling processes may not be the exclusive explanation for the association of heterotrimeric G-proteins with cognitive ability, such alterations may prominently affect the properties of neuronal networks in the brain in such a manner that impaired cognitive ability and lower intelligence are observed. The reported association of synaptic heterotrimeric G-proteins in cognitive ability clearly points to a new direction in the study of the genetic basis of cognitive ability.

Brain volume. Brain volume and cognitive ability are highly correlated and heritable traits, but the underlying relations are unknown. We presented evidence for a common genetic origin of these traits by identifying associations between cerebral grey matter volume variations and genetic variation in genes previously associated with cognitive ability. Magnetic resonance imaging of 294 healthy subjects and voxel-based morphometry revealed strong associations between four genes encoding heterotrimeric G-proteins (GNG2, GNAQ, GNA15, GNA14) with specific, local increase in medial frontal cortex volume, an area involved in cognitive control. The association of GNG2 and GNAQ was replicated in an independent sample of 238 subjects. This finding suggests that individual variation in genes encoding G-proteins modulates cortical volume and cognitive ability by a common principle probably controlling neocortex development and strengthen the convergent evidence that the medial frontal cortex is an important area for cognitive control.

Schizophrenia. Schizophrenia is a highly heritable disorder with a highly polygenic pattern of inheritance and a population prevalence around 1 per cent. Previous studies have implicated synaptic dysfunction in schizophrenia. We tested the accumulated association of genetic variants in synaptic gene groups with schizophrenia in 4,673 cases and 4,965 healthy controls, using functional gene group analysis. Identifying groups of genes with similar cellular function rather than genes in isolation may have clinical implications for finding additional drug targets. We found that a group of 1026 synaptic genes was significantly associated with the risk of schizophrenia ($P < 7.6x10-11$) and more strongly associated than randomly drawn, matched control groups of genetic variants ($P < .01$). Subsequent analysis of synaptic sub-groups suggested that the strongest association signals are derived from three synaptic gene groups: intracellular signal transduction ($P = 2.0x10-4$), excitability ($P = 9.0x10-4$) and cell adhesion and trans-synaptic signalling ($P = 2.4x10-3$). These results are consistent with the role of synaptic dysfunction in schizophrenia and support the idea that the heritable component is explained by accumulation of genetic variants in synaptic genes and imply that impaired intracellular signal transduction in synapses, synaptic excitability and cell adhesion and trans-synaptic signalling play a role in the pathology of schizophrenia.

4 Potential impact

SYNSYS generated (i) improved understanding of the pathophysiological mechanisms of synapse system function, (ii) yielded novel insights in disease mechanisms and led to discovery of new potential targets for disease, and (iii) a systems level understanding of the most important subcellular compartment in the brain, the synapse, (iv) contributed to the strengthening of R&D of EU companies. Different dissemination activities are listed separately.

4.1 Scientific impact

The SYNSYS Consortium organised regular collaborative meetings and exchanges between partners. Exploitation of data was through the continuous and reciprocal interplay between molecular data, physiology and the use of animal data. This led to the build of a synaptic interactome and models thereof, as well as a European database repository, SYNSYSdb. Data of protein-protein interaction and existing tools thereof, were used for structure-based and fragment–based chemical synthesis and also for this a European database and web access was built (SynsysNet). In particular, we explored the possibility of intervention at specific nodes of the synaptic protein network by using peptidomimetics. Both data in Synsysdb and SynsysNet followed up by synthesis and/or screening promising lead compounds for further drug development.

SYNSYS contributed to forming a strong European Synapse Network
The need for cost-effectiveness clearly demanded strong networking and management at a European level. Synaptic proteins are implicated in various human diseases and it has become clear that in order to address their role(s) in complex neurobiological mechanisms, a multi-disciplinary approach was required. The multi-disciplinary approach was based on the crucial contribution of a wide range of expertise such as molecular biology, modelling, protein chemistry, synaptic physiology and animal models.
Within Europe, there is an extraordinary level of scientific expertise in the synaptic protein field, but no single laboratory or research institution could provide the comprehensive approach to translate basic research to the identification of new synaptic models, databases and future therapies in a systems biology approach.
The SYNSYS Consortium was formed with the objective to contribute to basic understanding of the synaptic system, opening avenues for computation and modelling opening new avenues for a translational approach. Because SYNSYS was composed of the leading European laboratories working in synapse systems area, several of these laboratories already successfully collaborated on previous projects, including other EU projects, and thus had a strong impact on successfully completing scientific tasks defined for the SYNSYS project.

The S&T objectives of SYNSYS strongly benefited from networking at a European level, both in terms of skills and infrastructure. In particular, SYNSYS partners had strong interactions with other consortia, in particular EUROSPIN and Neurocypres. In particular, SYNSYS deliverables could only be fulfilled by providing a workflow enabling high-throughput mode arching of multilevel systems analysis and an iterative cycle, e.g. that for proteomics, physiology and computational modelling/databasing by reaching out to different resources. SYNSYS strengthened is translational competitiveness by collaborating with EUROSPIN, which had a focus on 13 synaptic disease genes. SYNSYS embarked on the overall architecture and functioning of the synaptic system and related network modelling to the functioning of these genes in the system as a whole. Also, SYNSYS and EUROSPIN had a collaborative effort in

overlaying information from synaptic protein interaction derived from YTH analysis (EUROSPIN) and by mass spectrometry interaction proteomics (SYNSYS). In addition, SYNSYS interacted with Neurocypres on protein interactions related to ligand-gated ion channels.

SYNSYS offered a concerted, synergistic, research program and was successful by integrating necessary expertise and achieving an attainable Systems Biology Analysis. SYNSYS included computational analysis, which involved European Outstations (e.g., EBI) and as such required European mobilization of resources and expertise. SYNSYS was unique in that it implemented a strong collaborative network of experimental and theoretical scientists and focused on a well-defined topic with a clear link to health and well being. In principle it achieved in producing highly novel knowledge on synaptic mechanisms and physiological function, in several cases based on an iterative cycle of modelling and experiment. In fact, the scaling from protein to function, a systems biology perspective, was acquired by using both inter-laboratory and inter-disciplinary exchanges, fast progression of knowledge, development of a strongly interconnected network with many inter-laboratory interactions and workshops, identification of common targets for both research and therapeutic strategy and, thus yielding a rapid transfer of knowledge from systems biology to new pharmacological applications.

Cutting edge technological advancements

We have been able to assemble the cutting edge technologies at the forefront of in the areas of HT proteomics, structure prediction, protein-protein intervention technologies, high-and low throughput physiology, and a spectrum of computational resources, and approaches using the most powerful tools currently available to us for answering the research questions posed. This allowed us to excel in a scientific collaboration that was truly competitive in the international arena. Many participants had well-established and successful collaborative arrangements exemplified by publications, patents and extensive network of knowledge-transfer activities. There is no single European country that would have been able to assemble the expertise as was present. Thus, the consortium reached far beyond the sum of its parts.

SYNSYS triggered National funding opportunities

The SYNSYS Consortium was a complementary support to national funds. All members of the Consortium were already involved in, and received synaptic research grants by the respective national funds. Indeed, SYNSYS helped to advertise the strong European collaboration necessary to acquire National funding. The SYNSYS Consortium clearly contributed to the "European Research Area" in the synaptic field of research and development in the post-genomic era of systems analysis. Moreover SYNSYS facilitated the future interaction of the participants and researchers beyond the lifetime of this particular initiative.

4.2 Economic impact R&D Innovation

Considering the scientific and technological innovation, SYNSYS involved:
(i) A pan-European study, which integrated multi-level research through the common focus on synapse architecture, modelling and function;
(ii) the solving of a large part of the pre-synaptic interactome structure;
(iii) the further development of modelling modalities around common protein targets and pathways involving new types of analyses.
(iv) the development of novel tools for assaying protein-protein interactions and posttranslational modifications in the brain;
(v) the use of rodent and human genomic models (of disease) and paradigms to elucidate synaptic network function and,
(vi) the discovery of network components as potential therapeutic targets for human brain disorders.
(vii) various protocols developed that found there way into workflows of the participating SMEs and initiated new SME activity.

SMEs in the SYNSYS consortium: strengthening and initiating EU enterprises
SYNSYS placed synaptic systems research in Europe at the forefront of the field, and at the forefront of the systems analysis into the domain of medical research and public health. SYNSYS fostered the competitiveness of Europe's Biotechnology and Pharmaceutical industry. In particular, the SMEs involved developed novel technological platforms and profited from academic collaborations to realise their potential. The results of this research were exploited to define new therapeutic strategies, and to the discovery of novel pathway candidates for clinical development. Some examples of this are given below.

Synome (SME)
Participation in the SYNSYS project has been extremely beneficial for Synome in many aspects. Although initially Synome planned to make scientific contribution to SYNSYS using only one technology platform (in vitro electrophysiology), as a result of our collaboration with project partners (University of Edinburgh), they developed two additional capacities, namely cognitive testing of mice using touchscreen-based visual tasks and detection of synaptic proteins using genetically encoded fluorescent tags in primary neuronal cultures. Acquired experience in analysing cognitive behaviour of genetically altered mice within SYNSYS project helped Synome and Synaptologics, another SYNSYS partner to make a successful bid for collaborative funding from the Human Brain Project. The necessity to genotype many mutant mice in the course of SYNSYS project prompted Synome scientists to perfect and optimise genotyping protocols. This expertise helped Synome to formulate commercial offers of genotyping service to external partners (cf. https://www.scienceexchange.com/labs/synome-ltd-brc) and receive first orders from UK and Australia.
Furthermore, Synome scientists used their experience of FP7 projects in applying for funding via new Horizon 2020 programmes. In particular, Synome Ltd took part in 3 applications for Personalising Health and Care (PHC) Research and Innovation funding (2 out of 3 projects passed to Stage 2). Also, Synome was named as a potential partner in 3 Innovative Training Networks proposals submitted in January 2015. Overall, 5 staff members of Synome were supported through SYNSYS funding (2 women).

Beactica (SME)
As a small drug discovery company with internal research programs and a dependency on revenues from contract research collaborations (CRC), Beactica has benefitted from the SYNSYS collaboration in multiple ways: 1. The flexibility to adapt the SYNSYS work load in

relation to the number of incoming CRC projects has enabled the company to have more scientists employed than otherwise would have been possible. 2. Beactica's scientists have gained knowledge about attractive targets and established contacts with other research groups within the Consortium, adding value to the company's internal research programs. 3. The project has contributed to advance Beactica's proprietary drug discovery platform and hence opened for new opportunities to attract clients.

Synaptologics (SME)
Synaptologics is an SME with a strong expertise in different fields of neuroscience, genetics and mouse behaviour research. In addition it has strong expertise in Project (Research) Management and Administration. In this project they have contributed with a postdoc level scientist to a (limited) number of deliverables. Synaptologics has also been responsible for the administrative project management of the consortium.
Synaptologics has benefited greatly from participation in the SYNSYS project. They have acquired new experience in project management, which helped to acquire (2) new EU funded projects, in which they will also be responsible for the management of these multi-partner projects. Participation in the SYNSYS project has broadened the network with contacts in both academia and industry, which has helped not only to acquire new contracts as a project manager, but has also helped to acquire new projects for contract research in the mouse behaviour division. Being part of the SYNSYS consortium has further enabled Sylics to participate and coordinate new Horizon2020 applications. So far, two of the projects that Sylics participated in in the last call, have passed to stage 2. Sylics is also part of a consortium that applied for an ITN in the call that was due January 2015.
Furthermore, together with Synome, another partner in this consortium, Sylics has been able to build up our expertise in analysing cognitive behaviour of genetically altered mice, which has helped to make a successful bid for collaborative funding from the Human Brain Project.
From the entire staff 3 people were (partly) funded from the SYNSYS project, of which 2 are women.

Brainwave (BWD)
The opportunity to work with the SYNSYS partners led BWD to work on a much wider range of human disease genes than were originally in its business plan. Of specific benefit to the company were its interactions with partners with interests in synaptic proteins that are implicated in neurodegeneration especially Parkinson's disease. While many models already existed in fly, BWD took the opportunity to remake several of these models and add in newly discovered human variants. These were extremely successful with phenotypes that match the human disease situation and that importantly respond well to human drug treatments.

With these encouraging results (paper in preparation) BWD was successful in attracting business development funds from two sources in 2013. This paid for the development of a new drug discovery platform within the company. In 2014 a methods patent application was filed (pending). In 2014 the decision was then taken by the board to split the company in two. The original Contract Research business would be continued by Brainwave. A new wholly owned subsidiary was formed to exploit the Parkinson's drug discovery assays. The SYNSYS postdoc (Lysimachos Zografos) started extensive business development training and became CEO of the new company (Parkure Ltd). At the end of 2014 and early 2015, Parkure embarked on a new fund raising round through investment based crowd funding. The new company de-merged from the parent to become an independent company and it has secured first round investment in the order of €100kEuro to start its business activities.

Identification of patentable innovations
Partners communicated on a confidential basis, evaluating the potential of new products or technologies for possible patent filing or any other means of protecting intellectual property. Whenever possible, patent applications were deposited for relevant innovative procedures and products. The project policy was that of patenting all results, which could be exploited without preventing publication and rapid circulation of information between partners. Two patents were filed by SYNSYS partners.

Strategic impact on reinforcing EU competitiveness and career perspectives
The SYNSYS consortium formed a multi-centre pan-European collaboration uniquely mobilizing resources and expertise on synaptic and brain research across Europe. This critical mass of resources, tightly integrated in focused scientific sections, generated scientific data of high scientific impact, mobilized and trained new research staff and thus boosted competitiveness and capacity of European research. SYNSYS was able to contribute in many ways to fostering research integration and competitiveness in Europe for the neurosciences, systems biology and public health, all domains covered by the Health Commission programme. In addition, the SYNSYS program worked in conjunction with Marie Curie actions in the partner labs. For instance, ITN projects such as Neuromics, BrainTrain, CerebNet, CognitioNet, Insens, all benefitted from having visiting SYNSYS scientists as lecturers or supervisors.

4.3 Societal Impact

Synaptic proteins in brain, 'new disease candidates'
On top of already known synaptic genes involved in human brain disorders, major advances in genotyping and new-generation DNA sequencing resulted in the discovery of many new human mutations in synaptic genes during the course of the SYNSYS project. SYNSYS partners groups were at the forefront of international efforts that exploited the novel genotyping technologies to identify new genetic variation associated with brain disorders. Hence, newly identified synaptic risk factors immediately became available for the SYNSYS studies and, the systematic understanding of synaptic gene networks within our consortium was used to understand the mechanisms that link variation in synaptic genes to disease and to help design therapies. The SYNSYS consortium also connected to a variety of clinical studies concerned with the proteomic and transcriptomic discovery of disease genes and their protein products.

Small-scale molecular biology studies of often reveal important details on individual molecules and their biological function. However, they are usually not suited to provide an overview of functional connections of larger numbers of molecules that form or might form functional modules of biological significance at a level superordinate to that of a single protein of interest.
In order to achieve that type of insight, we have created for the first time a protein-protein-interaction network of all presynaptic proteins. With this network of thousands of interactions, we will provide a new resource for researchers of synapse function in health and disease. It will be possible to derive a number of new lines of investigation to unravel the origins of synaptopathies and even new targets for therapeutic intervention in such diseases. These results represent an important advance towards better understanding and dealing with a group of diseases of high medical need throughout all EU member states and beyond.
SYNSYS aimed to exploit such data sources for the establishment of systematic analysis of disease proteins and their interactors, and most of all for the generation of computational models that may help to explain the diseases associated with the underlying genes and to design future therapies.

Alterations in synaptic properties and plasticity have broad impact on brain function and growing evidence indicates that they are responsible for the symptoms of many neurological and psychiatric diseases. Some of the SYNSYS work was directly concerned with the mechanisms coupling short-term to long-term plasticity, which are essential for learning and memory, and particularly relevant for several diseases. First, the signalling mechanisms from synapse to nucleus were studied in striatal neurons, which mediate incentive learning and movement control. These are altered by excessive stimulation of dopamine receptors in drug addiction and side effects of L-DOPA during Parkinson's disease treatment. One of the proteins we investigated, Pyk2, is encoded by a gene associated with an increased risk for Alzheimer's disease. The deregulations we observed in mouse models is helping us to understand the relationship between this gene and the disease.

Knowledge dissemination – targeting different audiences

Knowledge created and results obtained by this consortium were disseminated at multiple levels, including the scientific, clinical and corporate communities, patient organizations, and the general public. The knowledge dissemination activities are listed separately. Some specific aspects and target audiences are mentioned below.

Scientific community

Our work on the fundamental molecular biology of brain function and the distribution of valuable resources and data has important consequences for the understanding, and ultimately the treatment, of human brain disease. We have also contributed to the generation of new theoretic approaches to the brain, based on our datasets and database, which enables new models of disease to be examined in silico. The legacy value of these freely available datasets and the models will be to facilitate the increased use of computational approaches to biology.

The results of SYNSYS were (and still are) published as normal scholarly literature and presented at national and international conferences. In particular, several Partners of the Consortium are members of the Society for Neuroscience, the FENS and are or have been members of the Governing Councils of the Society and its European affiliate, and favoured dissemination of SYNSYS scientific results through this Society and its European meetings. The primary means by which results form this program were disseminated to the scientific community worldwide was through publications in scientific journals and presentations at national and international scientific meetings. We generated papers that were in many cases eligible for publication in high-impact peer-reviewed journals, and reached a large international scientific audience. This program offered, however, additional attractive possibilities for knowledge sharing. For instance we could strengthen connections between scientists, clinicians, representatives from patients organizations, and the press (see below).

Databases and knowledge dissemination

The databases and web servers developed in the consortium are publicly available and accessible not only to researchers, but to all other interested individuals. Usage statistics suggest that the SynSysNet. Superpain and Protox websites have a combined number of more than 10,000 visits within the last year and that visitors are accessing the websites from more than 80 distinct countries. Therefore, an important impact of our work is the international propagation of scientific knowledge, which ultimately leads to scientific progress. Furthermore, all in silico predicion models developed, for instance the toxicity prediction methods, allow the implementation of methods alternative to animal experiments and thus play a crucial role in the establishment of the 3R concept (Replacement, Reduction, Refinement) in the society.

Clinical community
Strong links with the clinical community were ensured through direct communication with clinicians of different disciplines.

In particular Dr. Bagni's team had several interactions with clinicians that were crucial for the development and success of the SYNSYS project. The first strong interaction with clinicians occurred upon the analysis of the data generated using the mouse model for the BP1-BP2 deletion syndromes (Cyfip1 +/-). Here we revealed that CYFIP1 interactors were associated to varying degrees of intellectual disabilities, such as observed in ASD, SCZ, MDD and others (De Rubeis et al., 2013). To better understand these findings Dr. Bagni spent two months sabbatical at UC Davis MIND Institute (http://www.ucdmc.ucdavis.edu/mindinstitute/) observing patients with ASD, FXS and psychosis under the guidance of one of the MD leaders in the field of FXS and ASD (Dr. Randi Hagerman). This experience was instrumental for the manuscript mentioned above.
Dr. Bagni's group has further strengthened the interaction with clinicians through the collaboration with Dr. Konrad Devriendt and Dr. Hilde Peeters (UZ Leuven, Belgium). UZ Leuven as a large cohort of patients with inherited intellectual disabilities and such collaboration helped to identify the first patient with a point mutation in the CYFIP1 gene. This will be important for follow up studies.
Finally, in collaboration with Dr. Sebastien Jacquemont (UNIL, Switzerland) and Dr. Randi Hagerman and Dr. Flora Tassone (MIND, USA) Dr. Bagni's group was able to collect a cohort of skin fibroblasts from patients with FXS that have been crucial in the study developed under the SYNSYS support for validation of the dysregulated APP-ADAM10 pathway.

During these years Dr. Bagni had active participations at the Italian National TV during the Telethon campaign for fund racing, gave a general presentation on her research topic in the presence of the King Albert II and Princess Astrid of Belgium, gave general lectures at the European School in Bruxelles (high school pupils). Dr. Bagni has also been nominated Honorary Member of the Italian Fragile X Association (President Dr. Donatella Bertelli, http://www.xfragile.net) a parents' association of children with Fragile X Syndrome.

Last but not least, her science has been followed by the national and international press (http://www.healthcanal.com/brain-nerves/43035-brain-dysfunctions-shared-mechanisms-in-fragile-x-syndrome-autism-and-schizophrenia-at-neuronal-synapses.html; http://www.sciencedaily.com/releases/2013/09/130918132419.htm; http://medicalxpress.com/news/2013-09-brain-dysfunctions-mechanisms-fragile-syndrome.html; http://www.sciencedaily.com/releases/2014/11/141117125842.htm; http://www.vib.be/nl/nieuws/Pages/Nieuwe-inzichten-in-link-tussen-fragiele-X-syndroom-en-autisme-gerelateerd-gedrag.aspx)

General public and awareness
For dissemination among researchers and the public:
i) A public access website provided general information about the project and aims, and inform about the progress of research in the Consortium and its achievements.
ii) Workshops and master classes were organized and were to all students, PhD students and postdoctoral fellows in the SYNSYS groups, but also to others interested in the topic.

Publicity to a wider audience was performed by press release, at the local and national level, leveraging the press relation offices of the participating academic centres.

Lectures of the PIs of this program during the Brain Awareness week were excellent means to disseminate knowledge to the public. The consortium developed a website to disseminate information on research and progress within the project through a public area about the discoveries from the program. We communicated our findings to various patient-based and private-funded organizations.

Obviously many of the PI's were involved lectures involving the broader audience. Some examples of such presentations are given below:

Nils Brose presented multiple public lectures on the role of synapse function and dysfunction in neuropsychiatric diseases. Some examples:

OktoberMusikFest 2012 - Series of combined talks and concerts with the Bavarian State Opera with the title on 'Chaos and Order' - lecture by Nils Brose on 'Ordnung im Chaos: Wie Nervenzellen den richtigen Partner finden' - Munich, October 19, 2012 - about 300 people in the audience - covered by Süddeutsche Zeitung a.o.

Max Planck Forum 2013 - Series of lectures and panel discussions on novel trends in science - lecture by Nils Brose on 'Neues von Rain Man? Perspektiven in der Autismus-Forschung' - Berlin, December 2, 2013 - about 300 people in the audience

Denkbar 2013 - Series of lectures on brain research - lecture by Nils Brose on 'Wenn Nervenzellen sich missverstehen: Die biologischen Ursachen von Autismus' - Göttingen, June 11, 2013 - about 80 people in the audience

Guus Smit (coordinator) presented multiple public lectures on the role of synapse function and dysfunction. For instance:

Science Café 2013 – Muziek en Wetenschap- Alzheimer: 'hoe we leren en vergeten'.
Almere - The Netherlands, May, 2013 – about 70 people in the audience. Covered by radio Flevoland.

Twente lectures 2012 – 'Alcohol, synapsen en het brein'.
Enschede – The Netherlands, June 2012 – about 100 people in the audience. Covered by local newspapers.

Studium Generale 2011-2013 – Series of lectures on brain development and the effect of drugs of abuse. Several cities in the Netherlands, audiences 50-200, each time covered by local radio and newspapers.