Executive Summary:

Despite impressive advances in almost every field of neuroscience, our understanding of brain function has largely been confined to the brain's building blocks at the microscopic level, and to phenomenological descriptions at the macroscopic level. We know much less about the link between these levels - how complex mental functions originate from electrical and chemical processes. Understanding this link requires a comprehensive and integrated multi-level analysis focused on neuronal assemblies and microcircuits where myriads of intricately connected neurons with different properties act together. The aim of SPACEBRAIN was to provide such a multidisciplinary cross-level understanding of computation in cortical microcircuits.

Forty-two months of collaboration have produced significant advances in our understanding of how space is represented in neural networks of the entorhinal cortex and hippocampus, brain regions known to form internal maps of the local environment. Two papers with SPACEBRAIN funding have been published or accepted for publication in Nature, three in Science, one in Cell, and several are appearing in other high-impact journals such as Nature Neuroscience and Nature Methods. Key scientific advances include, first of all, new insights into the intrinsic wiring of the entorhinal cortex. The project has shown how grid cells - one of the key cells types of the space circuit - connect to other cell types in the circuit and in neighbouring regions, and we have learned that grid cells are strongly linked by inhibitory connections. Grid cells have been shown not to be the only space-coding cell type. Border cells are entorhinal cells that fire along geometric edges and landmarks, whereas boundary vector cells in the subiculum fire at specific distances from such boundaries. The project has shown that space-coding cells develop a lot earlier than researchers thought before, and that a rudimentary spatial representation is present already when rats make their first navigational experiences outside the nest, at the beginning of the third postnatal week. Among the known spatial cell types, direction-coding cells appear first, probably before the animals open their eyes for the first time. They are followed by place cells and grid cells, which need further maturation but nonetheless appear adult-like one-to-two weeks after the beginning of independent exploration. Work on the SPACEBRAIN project has also shown that grid cells and place cells encode horizontal and vertical space differently, with stronger spatial resolution in the horizontal dimension. Furthermore, the project has shown that hippocampal neuronal assemblies follow principles of attractor networks and are organized within 125-ms time blocks referred to as theta cycles. Theoretical work has addressed the significance of this pattern of organization. Finally, the scientific progress has been accompanied by development of new research tools. Microdrives have been developed for recording electrical activity at multiple brain locations at the same time, and they have been miniaturized for recording in small animals. A portable high-resolution microscope has been developed for population studies of local neural networks during behaviour.

The success of the project reflects several unique features of SPACEBRAIN. One is the integration of experimental and theoretical work. From the beginning, experimentalists and modellers have been working on complementary subprojects, exchanging information as soon as data or models were available. Another is the explicit attempt to combine methods and concepts from multiple levels of analysis, ranging from mechanisms of synaptic transmission to interactions between large distributed

circuits. Finally, the success is likely to also reflect the open and positive working atmosphere of the consortium. Trust and an open attitude has laid the basis for constructive discussion and true collaboration from the first day of the project.

Project Context and Objectives:

Neuroscience is at a stage where an integrative understanding of brain function can be reached. Tools for a cross-level analysis centred on microcircuit structure and activity in neuronal assemblies are becoming available, and with the power of today's computers, it has become possible to model large networks with connectional and dynamic properties similar to those of real brain circuits. This in turn has brought models closer to experimental observations and the benefits of mutual interaction between theoretical and experimental communities have become apparent. The principal objective of the SPACEBRAIN proposal was to exploit these converging conceptual and technological developments to uncover some of the fundamental principles of neuronal assembly and microcircuit operation in the mammalian cortex.

Not all areas of cortical investigation have matured to the stage where neuronal computation can be understood at the microcircuit level, however. Insights into circuit functions have been obtained for the early stages of sensory systems where the processing of signals can be followed through networks of increasing complexity from the receptor level to the primary sensory cortices. These studies have largely clarified how neurons and neuronal networks extract features from the external world and refine these into cortical representations. However, how the brain generates its own codes, in the non-sensory parts of the cortex, has remained deeply mysterious.

In this scarcely explored territory, a new path was opened up when the grid cells of the entorhinal cortex were discovered by one of the participating groups in 2005. Grid cells are place-modulated neurons whose firing locations define a periodic triangular array covering the entirety of the animal's environment. They are thought to form an essential part of the brain's coordinate system for metric navigation. Grid cells have attracted enormous attention because the crystal-like structure underlying their firing fields does not arise out of the simplicity of their components, as it does in more familiar physical systems, but rather out of network complexity. It is a structure not imported from the outside world, but created within the nervous system. Understanding its origin and its properties is an attractive challenge for anybody wanting to know how brain circuits compute. Grid cells thus provide scientists with a direct window into some of the most fundamental operational principles of cell assemblies and microcircuits in the brain.

The grid cells are part of the brain's system for spatial representation, which includes the hippocampus and the entorhinal cortex.

Through the discovery of place cells by one of the consortium groups in 1971 and the discovery of grid cells by one of the other groups in 2005, the hippocampo-entorhinal network is now, in the view of many neuroscientists, emerging as the brain system with the best potential for generating a

comprehensive cross-level understanding of how non-sensory neural networks compute in the mammalian brain. We know that place cells form maps of the animal's local environment and that different maps are stored in the hippocampal circuit for different environments. These maps are different from the spatial maps generated by grid cells in the entorhinal cortex. Grid cells keep a constant spatial relationship across all environments; two grid cells with overlapping vertices in one environment will have overlapping vertices also in another environment. The entorhinal map is universal in the sense that it keeps a similar ensemble structure across all places. Hippocampal place-cell maps, in contrast, are environment-unique and strictly tied to the memory functions of this brain structure. Studies of mechanisms by which neuronal circuits and cell assemblies in these two brain regions keep track of the animal's location as it is moving around in the environment may uncover general computational mechanisms employed widely in the cortex, not only in the entorhinal and hippocampal cortices.

The aim of the SPACEBRAIN project was to begin the search for principles of microcircuit computation in the relatively accessible spatial representation system of rodents, using a powerful combination of novel computational, electrophysiological, optical and molecular research tools that have never been applied before to the analysis of brain circuitry.

Specific objectives.

The consortium set out to provide a better understanding of how spatial location is computed and represented in microcircuits of the hippocampus and entorhinal cortex and how such representations arise during development of the nervous system. The most central objectives were the following:

1. To provide a wiring diagram of entorhinal microcircuitry that contains sufficient quantitative detail to lay the foundation for a new generation of realistic computational models of dynamic spatial representation. The potential power of computational models in accounting for spatial representation in assemblies of grid cells has introduced a strong need for detailed quantitative analysis of the microcircuitry of the entorhinal cortex. A key objective of the project was to outline the intrinsic organization of the area, with emphasis on its cell types, their intralaminar and interlaminar connections, and the modifiability of the principal interconnections.

2. To determine how intrinsic and extrinsic inputs combine into a single spatial representation in entorhinal grid cells. Using a combination of experimental and theoretical approaches, the consortium set out to determine how motion-related information is combined with visual information about landmarks and geometry into a coherent representation of the animal's location in space.

3. To determine the mechanisms of neuronal assembly formation in hippocampus and entorhinal cortex. Experimental and theoretical approaches were combined to establish

the operational principles of cell assemblies in place cells of the hippocampus and grid cells of the entorhinal cortex. Large numbers of cells were recorded simultaneously in the CA3 region of the hippocampus in order to determine, at high temporal resolution, the mechanisms for transformation between discrete spatial representations.

4. To determine the mechanisms of cell assembly formation in the temporal domain. The consortium used a combination of experimental and computational methods to determine the origin and neuronal mechanisms of hippocampal theta phase precession, one of the brain's clearest signatures of temporal sequence organization.

5. To determine how spatial representations are formed in hippocampo-entorhinal cell assemblies during development. The consortium set out to establish how key components of the hippocampo-entorhinal representation system develop relative to each other during the first postnatal weeks, what their interrelation is, and whether and how maturation of the system interacts with early experience. Key objectives were to determine when connections between different stages of the hippocampal formation, the entorhinal cortex and the presubiculum reach adult patterns, whether different functional cell types develop in particular sequences, and whether this development reflects mainly maturational factors or is shaped by experience.

Understanding microcircuit function is strongly dependent on the development of new research tools. An additional objective of the SPACEBRAIN project was therefore to take advantage of, and develop, methodologies for behavioural assessment, computational modeling and high-density single-cell recording, as well as gene silencing and single-cell optical recording. Particular emphasis was placed on the development of multi-site recording technology for high-density studies of cell assemblies, as well as the development of a head-mounted two-photon fluorescence microscope for dynamic visualization of cell assemblies. These technical developments were accompanied by the attempt to develop a new generation of anatomically and physiologically realistic computational models of entorhinal-hippocampal neural circuits.

Finally, the project was motivated by its potential for translation to both clinical and industrial applications. Topographical disorientation is one of the earliest and most prominent behavioural symptoms of Alzheimer's disease. Because the entorhinal cortex is the first brain region to be affected in many patients with Alzheimer's disease, functional insight into the spatial representation mechanisms of this brain region should provide clinical neurologists and health workers with essential tools for early diagnostics, prevention and treatment of Alzheimer's disease. Such insights may, in the long run, not only reduce the prevalence of Alzheimer's disease and the enormous health care burdens associated with the disease, but they may also have an important preventive impact on traffic accidents and human error in spatially demanding man-machine interactions caused by Alzheimer's disease at pre-diagnostic stages. Understanding space coding in the brain may also exert significant impact on the development of navigating robots. Despite decades of research, robotics has so far failed to develop a navigating agent capable of exploring an unknown or complex landscape at an efficiency that is anywhere near to that of a small rodent. By uncovering the some of

the fundamental algorithms of neural networks for spatial navigation in the rodent brain, the project is likely to provide the robotics industry with entirely new concepts for design of artificial navigating agents.

Project Results:

The project has led to significant advances in our understanding of how space is represented in neural networks of the entorhinal cortex and hippocampus. Organizational principles of entorhinal circuit structure have been determined, backprojections from the hippocampus have been shown to be essential for stable grid firing in the entorhinal cortex, grid scale has been shown to depend on the velocity of the moving animal, spatial cell types have been observed during early postnatal development, and cell ensembles have been found to operate as attractor networks within time frames defined by the hippocampal theta rhythm. These and many other findings are outlined in the section below. The work was organized in six scientific project workpackages and we have followed this structure in the presentation of the scientific results.

1. Attractor dynamics and circuit organization in entorhinal cortex

Overview. The first scientific workpackage was designed to test the idea that the entorhinal representation of self-location is based on continuous attractor dynamics. Attractors become continuous when the distribution of input cues to the system is continuous, such as in a representation of direction or space. In a continuous attractor network, a bump of activity is formed at a given location in the network defined by the input pattern. This bump of activity is then thought to move around in the network space in accordance with the speed and direction of the animal's movement in external space. It has been suggested that entorhinal networks have the ability to support the formation and translocation of attractor bumps. The continuous attractor idea makes a number of explicit assumptions about the underlying cellular and synaptic architecture and neural dynamics of the entorhinal cortex but the assumptions generally lack experimental support. The weak understanding of the details of the intrinsic wiring diagram of the entorhinal area, and the physiological properties of cell assemblies in this network, motivated the first workpackage. We sought a quantitative characterisation of the entorhinal microcircuitry. This was essential, in our view, for the development of realistic computational models of spatial representation in the hippocampal-entorhinal system. The general aim of the workpackage was to provide such a characterization and to test the basic assumptions of attractor models of the entorhinal cortex.

The consortium has acquired significant insight into the intrinsic wiring of the medial entorhinal cortex. Paired recordings from layer II principal cells have suggested that connectivity between principal cells within the layer is weak, averaging around 1% of cell pairs or less, but we have shown that there is strong connectivity between principal cells and interneurons, pointing to alternative ways of synchronizing the circuit. Targeted genetic manipulations have demonstrated that GABAergic interneurons in the medial entorhinal cortex project not only within the entorhinal circuit itself but also to subfields of the hippocampus, providing a direct means of synchronizing the two

structures with high temporal precision, such as during gamma network oscillations. The experimental work has been accompanied by theoretical work addressing the principles of attractor dynamics in hippocampus and entorhinal cortex, pointing the way to new experiments after the completion of the SPACEBRAIN project.

Interlaminar connections. Recurrent interconnectivity can in principle be present in cortical networks at two organizational levels, either within layers (intralaminar) or across layers (interlaminar). Previous studies of entorhinal microcircuits have shown widespread interlaminar axonal arborization of principal cells, connecting deep with superficial layers, as well as intralaminar connections in layers II, III, and V; however, the precision and detail of these studies have been far below the levels needed for realistic computational modeling of entorhinal networks. During SPACEBRAIN, we have analyzed more than a hundred neurons in all layers of medial entorhinal cortex electrophysiologically through whole-cell current-clamp recordings, at the same time as these neurons were stained in order to determine their unique connection patterns. Excitatory interlaminar connections were found to arise from neurons in layers III and V but the study also showed that such connections may arise from layer VI principal cells.

Intralaminar connections. Based on an analysis of more than 500 potential pairs of stellate cells at different ages from young to young adult, we have shown that the ratio of monosynaptically coupled stellate cells in layer II is small. Less than 1% of the recorded cell pairs had direct synaptic connections. Connectivity ratios did not increase when cells are at larger distances from each other but stay within the established average axonal arbor of 550 μm. Connections between principal cells and interneurons in layer II were substantially stronger. These findings indicate that the proposed excitatory interconnectivity underlying pure grid cell properties is weak and that, while inhibitory connections may play a primary role in synchronization of the layer II network, the continuous attractor models accounting for grid activity in that layer need major revision. In contrast, a new hypothesis has been formulated in which a denser recurrent connectivity in the deeper layers establishes coherence among conjunctive head direction/grid units, which then feed into a non-recurrently connected pools of pure layer II grid units to achieve sharper spatial resolution; a hypothesis that will be tested in computational models after the completion of the project. Behavioural tests in mice where the expression of Cre-recombinase is under the control of the parvalbumin promoter have suggested that fast-spiking interneurons may be instrumental in synchronization of network activity as well as the formation of precise spatial representations.

Asymmetry in connections between layers. A critical assumption of continuous attractor models of grid cells in the entorhinal cortex was that descending and ascending connections between layer II and the deeper layers are asymmetric. Such asymmetry is necessary if conjunctive cells in the deeper layers are to translate the grid cell representation in layer II in accordance with the animal's movements. A key objective of the circuit analysis in SPACEBRAIN was to determine if such asymmetry is present. We focused on the connectivity between layers V and II. The data indicate that out of more than a hundred individually stained neurons, approximately a third show a clear lateral

shift in their axonal arbor, when compared to the dendritic arbor, in agreement with the translation idea. The shift is at least up to 300 µm. The findings suggest that also neurons in layers III and VI may have laterally shifted projections.

Short-term plasticity in attractor networks. Previous theoretical work indicated that cooperative activity of neocortical circuits is sensitive to short-term synaptic plasticity (STP) in connections between pyramidal neurons. In particular, synaptic depression can generate fast changes in activity, while synaptic facilitation on the opposite can stabilize attractor states. Place representation in hippocampus and medial entorhinal cortex is an example of continuous attractor system, where issues of stability are especially critical. An important objective of the SPACEBRAIN project was to determine the validity of this idea. The role of STP in stabilization of attractor networks was tested in a 'ring model' applied to place and grid cells as well as a range of other problems. The results show that synaptic facilitation can dramatically slow down the drift velocity of the network, thus appearing as a strong stabilizing factor in representation of space and memory.

2. Calibration of the entorhinal assembly representation

Overview. The location-specific firing of the grid cells is thought to be updated principally by a path integration-based mechanism, based on computations derived from the animal's movements through space. This suggestion is consistent with the constancy of grid maps across environments, their imperviousness to removal or displacement of external landmarks, and the fact that grids are expressed immediately in a novel environment. However, while the basic structure of the grid may be determined by self-motion cues, grids are clearly influenced by external visual cues as (i) both the phase and the orientation of the grid are controlled by the specific landmarks in the environment and (ii) re-sizing of a familiar environment causes partial re-scaling of the entorhinal grid in the scaled dimension. These observations clearly implicate an interaction between path integration signals and visual inputs. The general objective of the second scientific workpackage was to determine how such integration is accomplished.

We chose to take a combined experimental-theoretical approach in our attempt to understand the relationship between motion inputs and other sensory inputs on one hand and the formation of grid cell patterns on the other. The results of this effort provide some important insights. We have shown with experimental approaches that backprojections from the hippocampus are essential for maintaining stable grid representations although they may not be required for the ensemble structure of the grid cells as such. Experiments have also demonstrated that the theta modulation of grid cells is dependent on the linear velocity of the animals, and that place and grid cells represent horizontal and vertical space differently, and computational modelling has investigated the significance of hippocampal backprojections and other inputs in generating stable spatial periodicity.

Interaction between visual and path-integration cues. Previous work has shown that grid cells rescale when animals are placed in novel environments but the underlying mechanisms were not well understood. In the second workpackage, we designed experiments to tell us whether the grid expansion in a novel environment only applies in the absence of visual and other environmental cues. The results suggest that grid expansion takes place even in novel environments with strong visual and odour cues. Continued testing in the novel environments however leads to a relaxation back to the original grid scale suggesting that the expansion is due to novelty and not the absence of environmental information and that recalibration back to the original scale takes place as the new environment becomes familiar. Grid fields are larger in the novel environment than they are in the familiar environment, consistent with the notion of scaled-up grids in the novel environment.

Hippocampus-dependent calibration of grid representations in entorhinal cortex. Using a combination of experimental and computational approaches, we wanted to establish whether hippocampal backprojections to the entorhinal cortex are necessary for maintaining and resetting grid representations in the entorhinal cortex. A role in maintenance and updating of representations would be expected if associations between path integrator coordinates and extrinsic landmarks were stored in the hippocampus and backprojections to the entorhinal cortex were necessary for stabilizing the grid throughout the environment. Alternatively, associations between path integrator outputs and fixed landmarks might be formed within the medial entorhinal cortex itself by combining grid activity with specific sensory inputs received, for example, from the postrhinal cortex. To test these possibilities against each other, we inactivated the hippocampus temporarily by infusing muscimol into both hippocampi before grid cells were recorded in a standard foraging condition. Hippocampal inactivation resulted in a gradual decrease in firing rate of the grid cells, followed by a disintegration of the grid pattern. However, the temporal coherence of the grid cell population remained stable, suggesting that the grid structure persisted and that the alignment to landmarks deteriorated. The results suggest that grid cells are dependent on stored associations in the hippocampus to anchor the grid to the external environment.

Output from the hippocampus may provide a resetting mechanism that constantly updates the network in the entorhinal cortex with regard to stored landmark features. Such resetting may be vital to the operation of oscillation-dependent mechanisms for grid cell formation; without frequent resetting, noise and variation in the period of the theta rhythm may be detrimental to the formation of spatial periodicity. During the SPACEBRAIN project, simulations have been conducted in the context of the oscillatory interference model of grid cell firing. Under this model, the spatial organisation of the firing of grid cells arises from the interference between sub-threshold membrane potential oscillations in the theta band: firing rate increases when the oscillations are in phase and decreases as they go out of phase. The model performs "path integration" by assuming that the frequencies of oscillations each reflect velocity in a specific direction. As with all models of path integration, this scheme is susceptible to the accumulation of error in the inputs conveying velocity information. The simulations investigated how spatial stability can be attained by phase-reset of the oscillations in two ways. The first mechanism involves a phase-reset of all oscillations to be in-phase at a specific location, triggered by input from the place cells which fire at that location, given that the

firing location of place cells is maintained in location by environmental sensory inputs. Spatial stability of grid cell firing was successfully attained under this model. The second mechanism involves a reset of all oscillations triggered by inconsistency between multiple oscillators. When velocity along more than two directions is integrated by multiple oscillators, different pairs of oscillators can produce different estimates of location. When these estimates become mutually inconsistent all oscillators can be reset to correspond to the mean estimate. This provides a mechanism for error correction that is potentially intrinsic to entorhinal cortex. The effects of the two mechanisms were compared as a function of increasingly noisy estimates of velocity.

Convergence of potential motion inputs to entorhinal grid cells. An objective of the second scientific workpackage was to seek the source of cortical and subcortical inputs to the grid cell system using anatomical tracing methods and to determine what sensory modalities influence the metric of the grid, particularly its spacing. Among the many candidate inputs to the grid cell area in the medial entorhinal cortex, we have focused on the retrosplenial cortex as well as interactions between inputs from this region and inputs from postrhinal and pre-parasubicular cortical regions. We show that projections to layer V or medial entorhinal cortex originate mainly from the ventral subdivision of retrosplenial cortex generally referred to as granular part a. Electron-microscopy analyses show that this input is mainly excitatory, targeting mainly principal cells. The more dorsal subdivision b sends a massive input to layer III of the presubiculum, targeting preferentially principal cells that project to medial entorhinal cortex. Projections from the postrhinal cortex show a striking topographical organization in that a narrow area of postrhinal cortex, close to the border with medial entorhinal cortex, projects to the directly adjacent portion of medial entorhinal cortex, which contains grid cells with relatively small firing fields. More dorsal portions of postrhinal cortex project more ventrally towards the area of medial entorhinal cortex where much coarser grid cells have been recorded. Postrhinal inputs target principal cells in layers II and III that project to the various subfields in the hippocampus. The majority of these projections are excitatory, targeting spines of projection neurons in the medial entorhinal cortex. Finally, axons from the presubiculum target entorhinal neurons layers II, III and V, which project to the hippocampus. Thus, inputs from pre- and parasubiculum converge onto single principal cells in the medial entorhinal cortex. In conjunction with the observations on retrosplenial inputs, the data break with older ideas in that information from retrosplenial cortex is shown to reach superficial layers of medial entorhinal cortex through a massive di-synaptic pathway involving layer III cells in presubiculum.

The interaction between static and motion cues on place cells was explored in behaving animals on a linear track in which static somatosensory cues were shown to act as foci for the formation of place fields. During the SPACEBRAIN project, we have extended these path integration studies to the vertical dimension, by recording place and grid cells as animals explored environments having height as well as horizontal dimensions. We found that place and grid cells show little modulation by height, suggesting that the basic frame of reference for the grid cell map is planar rather than volumetric. Behavioural studies showed that this anisotropy of encoding is mirrored in behavioural performance, in which rats also show a horizontal bias in both foraging and detour tasks.

3. Interactions between entorhinal and hippocampal cell assemblies

Overview. The aim of the third scientific workpackage was to establish the mechanisms for interactions between neuronal assemblies within the hippocampus and between the hippocampus and the entorhinal cortex. We chose to focus on neuronal coding in large populations of hippocampal and entorhinal neurons. Experimental data generated by the SPACEBRAIN project have demonstrated that hippocampal assemblies follow principles of attractor networks and that attractor states are expressed within individual theta cycles, with relative independence between successive theta cycles. With novel behavioural tasks, competitive interactions between different ensemble states have been observed at the sub-second time scale during transitions from one representation to another. New microdrives for multi-tetrode ensemble recording with independently movable tetrodes, constructed by the industrial partner of the consortium, will as soon as they are released on the market allow participants inside and outside the consortium to address large-scale population phenomena that were not readily accessible until now. Findings from the behavioural studies have been incorporated in computational models of attractor dynamics.

Transition dynamics of hippocampal ensemble representations. The CA3 subfield of the hippocampus has many of the properties of an associative memory network. Most of the numerous synaptic inputs in this area are devoted to recurrent connections from other CA3 cells, pointing to a compact associative memory network with the capacity to hold a large number of memory items. Within networks with such properties, memories may be stored as discrete attractors. In an attractor network, memories can be reactivated reliably from subsets of the cues that were present when the memory was encoded, at the same time as interference from competing representations is minimized. In the third workpackage, we provide experimental evidence for competitive interactions between discrete attractor-like representations and we show that the dynamics is under strong control by the theta rhythm of the hippocampus.

First, we asked whether the attractor hypothesis can be directly confronted with experimental data in terms of the way population activity in MEC/hippocampus responds to gradual or sudden changes in the environment. If place-specific activity is directly driven by external cues, one would expect that the population activity would faithfully represent the current constellation of cues that define the environment. If, on the other hand, the activity is influenced by the underlying attractor dynamics, one would expect interesting history-dependent effects of the change on population activity. The precise nature of these effects could depend on the number of environments stored in the attractor network, as well as on the history dependence of the synaptic transmission in the network. Specifically, we asked if transitions between representations are instantaneous, or if intermediate mixed states exist, and we tried to determine the minimum temporal units for representation of distinct spatial environments.

The dynamics of transitional states was investigated by developing a procedure where the transition moment was 'frozen' or extended in time by slowly or unexpectedly transforming the recording

environment from one configuration to another while ensembles of place cells were recorded from CA3 and CA1 of the hippocampus. The rats were exposed for several days to two square environments (A and B) with common physical location, floor and walls. The only stimuli discriminating A from B was an array of intramaze light cues. Two distinct hippocampal representations were formed during training in these environments. During testing, while the rat was running in the box under neutral taste reinforcement, the currently presented light cue-set was remotely switched into the other one without any warning stimuli, effectively 'teleporting' them from one environment to the other. Population vectors were defined for every theta cycle of the concurrently recorded EEG, with the cut points defined as the phases of minimal spike activity. For each theta cycle, the population vectors were compared with those at corresponding positions of the A and B environments, using both dot product and correlation measures.

The teleportation event resulted in abrupt changes in representations under ongoing theta, often with a delay of less than two theta cycles. In most cases, however, the change was followed by a transient oscillation ('flickering') between the two states, with the current representation correlating with A and B on separate theta cycles. Network flickers were extremely fast, often with complete replacement of the active ensemble from one theta cycle to the next. Mixed states were rare; distributions of cycles correlated with A and B showed significantly fewer cycles correlated with both states than expected by chance (chance levels were determined by a shuffling procedure). Within individual cycles, segregation was stronger towards the end, when firing started to decline, pointing to the theta cycle as a temporal unit for expression of attractor states in the hippocampus.

The observations point to theta cycles as minimum units of hippocampal representation and imply that individual cycles, but not necessarily successive cycles, tend to remain within one state. Repetition of pattern-completion processes across successive theta cycles may facilitate error correction and enhance discriminative power in the presence of weak and ambiguous input cues.

In parallel with these experimental studies, we have modelled an attractor network that stores continuously changing environments and analyzed the resulting attractor states. We obtained a complete solution to the problem of continuous change, with a phase diagram indicating the possible regimes in the network. In particular, when the contribution of the intermediate environments is increasing, the network was found to undergo a transition into a combined representation of the whole sequence of environments, such that only cells with sufficiently close fields across the whole sequence remain active. Cells with far away initial fields are inhibited and lose their contribution to representing the environments.

Border cells and boundary-vector cells. During screening of the deep layers of medial entorhinal cortex, the participants found a new entorhinal cell type that fires specifically when the animal is close to the borders of the proximal environment. The orientation-specific edge-apposing activity of these cells was shown to be maintained when the environment is stretched and during testing in

enclosures of different size and shape in different rooms. Border cells were relatively sparse, comprising less than 10% of the local cell population, but subsequent recordings in the other layers showed that these cells can be found in all entorhinal layers as well as the adjacent parasubiculum, often intermingled with head-direction cells and grid cells.

Under conditions that favour global remapping in the hippocampal place-cell population, such as when a rat is moved between similar boxes in different rooms, the border cells maintained their edge-related firing activity. When multiple border cells were recorded simultaneously, they maintained their geometric relationship (e.g. two cells firing at apposing walls in environment 1, would fire at apposing walls in environment 2 as well). The remapping pattern of entorhinal border cells, like that of grid cells and head direction cells, is thus very different from the hippocampus, where place cells show statistically orthogonal firing patterns in different environments. The results suggest that the entorhinal cortex is predominated by codes that express similar geometric relationships irrespective of the specific features of the environment or the experiences that the animal has in it.

The discovery of border cells in the entorhinal cortex by one of the participating groups was accompanied by the discovery of 'boundary vector cells' in the subiculum by one of the other consortium groups. The two cell types have much in common and an obvious question for future research is to determine the relationship between them, i.e. to find out if one is dependent on the other and how each of the two cell types relates to grid cells and place cells.

Microdrives for multi-site ensemble recording. With the objective of recording ensemble activity from multiple brain locations, such as the entorhinal cortex and hippocampus of the same brain, it became important to develop a multi-electrode microdrive for parallel recording in which electrodes could be moved independently at a desired spacing of electrodes. The industrial partner of the consortium has been responsible for this development. The multi-site microdrives were to be composed of at least two core clusters (groups of drive mechanisms), with inter-cluster distance specifiable by the user. A computer-controlled milling machine was employed to manufacture miniature plastic components designed using the Rhino 3D computer-aided design software package. The industrial partner has developed a new drive mechanism, a new very stable connector, and a new cannula attachment mechanism. A new headstage compatible with the Microdrive connectors has been developed as well. The simplest single-site drive is small, lightweight (<1g) and the plastic components are sufficiently simple that they can be manufactured in bulk by a contractor. Unlike previous drives from the company, these new drives use a standard connector (Mill-Max) that will make them easy to interface to a variety of recording systems. In addition, the drives will be easier for users to load than current drives.

4. Visualization of functional neuronal assemblies

Overview. The aim of the fourth scientific workpackage was to develop a novel miniaturized two-photon fiberscope for imaging of activity patterns in local neuronal assemblies in freely moving rats. By using in vivo labelling with fluorescent calcium indicators, we set out to directly visualize place cell and grid cell activity as the animal is navigating through the local environment. Our goal was to unravel the fine-scale functional organization of these neurons, which will help to understand local circuitry and to build detailed models of the hippocampal-entorhinal spatial navigation system.

There are several important reasons for developing technology for optical imaging of hippocampal-entorhinal population activity in behaving animals. Electrophysiological studies of the spatial structure of cell assembly activity are constrained by the spatial resolution of the tetrode technique. Tetrodes are for example not suitable for studying differences in activity between neighbouring microcircuits with sharp boundaries, such as cortical columns (it cannot be determined whether a unit comes from one column or the other), and they are suboptimal for identifying neurons that are not active in any of the test conditions (including sleep). To overcome these problems, we have developed a portable high-resolution multiphoton microscope which can reveal activity patterns in local neural networks with single-cell and single-spike resolution during behaviour.

Fiberoptic recordings from CA1. Three key methodological procedures have been developed as part of the SPACEBRAIN project: (1) the in vivo preparation; (2) labelling of hippocampal neurons with calcium indicator; and (3) two-photon imaging of hippocampal neurons in vivo. Through these developments we established, as a first step, single-cell resolution optical recordings of hippocampal neurons in anesthetized animals. Specifically, we first have devised a surgical procedure in anesthetized rats to routinely access the hippocampal formation for functional optical measurements, aiming at the CA1 region to begin with. Using visually guided blunt aspiration of a small block of overlying cortical tissue, we are now able to successfully identify the intact hippocampal formation. With this preparation at hand, we bolus-inject the calcium-sensitive dye Oregon-Green BAPTA 1-AM (OGB-1) and the astrocyte-specific dye sulforhodamine 101 (SR101) into the CA1 region of hippocampus. Neuron and astrocyte labelling is under these conditions are visible about one hour after dye injection. Third, for imaging hippocampal cells we have placed the anesthetized animal under a standard two-photon microscope. Cells in the hippocampus were imaged through a small (about 1 mm diameter) gradient-index (GRIN) lens lowered onto the hippocampus, which mimics the situation with a miniature two-photon fiberscope. The surgical preparation and bolus-labelling with calcium indicator have yielded good results in anesthetized as well as behaving rats. We were able to reliably stain and differentiate neurons and glial cells and with the two-photon microscope we have been able to visualize cell populations from the surface of the hippocampal formation through stratum oriens down to the pyramidal cell layer. Spontaneous activity was low in all functional measurements. However after reducing inhibition using bicuculline, we observed large and synchronized calcium transients, presumably representing epochs of action potential activity in populations of pyramidal cells.

These results for the first time directly demonstrate large-scale calcium indicator labelling and imaging of cell populations in the hippocampus in vivo. This is a prerequisite for all future imaging with this approach and clearly indicates that the ultimate goal, applying this technique to freely navigating rats, is in reach.

5. Temporal codes in entorhinal cell assemblies

Overview. The aim of the fifth scientific workpackage was to determine the mechanisms and functions of theta phase precession. It can be argued that theta phase precession provides the most prominent experimental clue to sequence coding in the hippocampus. However, although phase precession provides the hippocampus with a mechanism for storing extended sequences of places and experiences, the neuronal circuits and computations responsible for this effect are not known. During the early stage of the project, we showed that phase precession is expressed also in grid cells in layer II (but not layer III) of medial entorhinal cortex, which is the origin of projections to the dentate gyrus and CA3. The presence of phase precession in one of the entorhinal inputs to the hippocampus points to the entorhinal cortex as a possible origin of phase precession. The aim of the workpackage was to use a combination of experimental and computational methods to establish where phase precession originates, and to determine whether theta oscillations represent the neuronal basis for grid field formation. We found that phase precession exists in principal cells of the medial entorhinal cortex and is generated there independently of the hippocampus, and computational models were developed to suggest mechanisms for how phase precession and grid patterns might emerge from intrinsic membrane properties of these neurons. We have also shown how theta oscillations may contribute to formation of grid patterns and how these depend on specific velocity inputs to the region. A cellular mechanism for possible transformation of such velocity signals has also been identified.

Phase precession in grid cells. During the first year of the project, we reported that grid cells in layer II of medial entorhinal cortex express phase precession. Layer III cells generally do not show precession. Our first aim was to establish precession in layer II cells origates locally in the entorhinal cortex or is derived from the hippocampus. The presence of phase precession in medial entorhinal cortex would be consistent with the subthreshold oscillations of entorhinal stellate cells in layer II, which are faster than the extrinsic theta oscillations and so may cause spike activity to advance progressively across the theta cycle in a manner similar to the dual oscillator mechanism proposed for hippocampal place cells. An alternative, however, would be that phase precession originates in the hippocampus or locally in both hippocampus and entorhinal cortex. We distinguished between these possibilities by recording theta phase in entorhinal grid cells after more global inactivation of the dorsal hippocampus. If phase precession in entorhinal grid cells is inherited from hippocampus, precession in medial entorhinal cortex should be disrupted by inactivation of the hippocampus. Rats were trained to run back and forth on a linear track with food rewards delivered at the ends of the track after each passage. Spike times were recorded simultaneously from place cells in CA1 and grid cells in layer II of MEC. The hippocampus was inactivated by local injection of the GABAA agonist

muscimol while grid cells were recorded in medial entorhinal cortex. The experiment showed that entorhinal phase precession is not blocked by inactivation of the hippocampus, suggesting that the phase advance is indeed generated in the grid cell network. The results pointed to possible mechanisms for grid formation and raised the possibility that hippocampal phase precession is inherited from entorhinal cortex.

Network model of phase precession. The phase-precession studies were accompanied by computation work in which an earlier model of phase precession in hippocampus, developed by one of the participants, was revisited. In this model, phase precession arises as a consequence of propagation of activity across place cells at each theta cycle due to asymmetry in synaptic connections in the direction of animal motion. Network models of place and grid cells were constructed based on Mexican-hat-like connectivity, and the network was exposed an to oscillatory external input responsible for theta rhythm generation. Short term potentiation (STP) was introduced in recurrent connections and effects on phase precession were analyzed. It was found that synaptic depression that characterizes inter-pyramidal connections in hippocampus can cause activity propagation in the network even when synaptic connections are symmetric. It was also observed that during sharp-waves, replay of sequential activation of place cells can emerge, in accordance with recent experimental observations.

Relationship between running speed, theta frequency and grid period. The initial oscillatory interference model of grid-cell formation was developed to formally identify the relationships of the theoretical membrane potential oscillations with the variables that can be experimentally measured in freely moving animals expressing spatially organized grid cell firing. During the SPACEBRAIN project, consortium participants have worked out the relationship of theoretical variables with the intrinsic firing frequency of grid cells as reflected in the spike train temporal autocorrelogram, and the theta rhythm of the extracellular local field potential. This work results in explicit predictions concerning the effects of grid scale and running speed on the intrinsic firing frequency and the effects of running speed on the frequency of the theta rhythm in the local field potential. Support for a specific relationship between running speed and oscillation frequency in the theta-frequency band was obtained in a study in which the topographic expansion of grid scale along the dorsoventral axis of the medial entorhinal cortex was found to be determined by cellular properties of the hyperpolarization activated cation current I(h) . I(h) is conducted by hyperpolarization activated cyclic nucleotide gated (HCN) channels. Recording in grid cells from mice with forebrain-specific knockout of HCN1, we have demonstrated that the dorsal-ventral gradient of the grid pattern is preserved in HCN1 knockout mice, despite the blockade of intrinsic theta oscillations in such mice in vitro, but the size and spacing of the grid fields, as well as the period of the accompanying theta modulation, expanded at all dorsal-ventral levels, suggesting that HCN1 channels control the scale of the grid pattern. Importantly, the speed-dependent modulation of theta frequency was strong in control mice but nearly absent in HCN1 knockout mice, implying that I(h) currents are critical for transformation of speed signals to spatially periodic firing fields. There was no change in theta modulation of simultaneously recorded entorhinal interneurons, suggesting that the expansion of grid scale is caused by lack of HCN1 channels in the grid cells themselves.

6. Development of the spatial representation system

Overview. The general objective of the last workpackage was to determine how key components of the spatial representation system develop relative to each other, how the emergence of one function leads to the next, and how the maturation of the system interacts with experience of the animal during postnatal development. Before the birth of SPACEBRAIN, only one study had systematically explored the development of spatial representations and the focus of this study was limited to the later stages of development in hippocampal place cells. This lack of functional studies was matched by a similarly fragmented understanding of how intrinsic and extrinsic connections of the entorhinal and hippocampal cortices develop relative to each other. The overall integrity and functional capacity of the entorhinal-hippocampal system at various stages could not be inferred from these previous studies.

Considerable progress has been made during the SPACEBRAIN project. We have mapped the development of entorhinal-hippocampal intrinsic connectivity. Electrophysiologal recording studies in 2-3 week old rat pups have shown that entorhinal-hippocampal connections develop a lot earlier than researchers had thought, and we know that a rudimentary spatial representation is already present at the time when rats make their first navigational experiences outside the nest, at the beginning of the third week. Place cells are present from the beginning and rudiments of grid cells can be seen too, although the number of grid cells with clear and stable firing fields takes longer time to develop.

Development of entorhinal-hippocampal connections. Retrograde tracing studies have revealed that the reciprocal connectivity between entorhinal cortex and hippocampus is present at birth. Preliminary voltage sensitive dye imaging data indicated that stimulation of entorhinal cortex results in adult like patterns of activation at postnatal day P9, whereas only very limited local activation can be seen at P5. Similarly, interactions between superficial and deep entorhinal layers are noticeable at P9 but not at P5. Intrinsic hippocampal circuits show a slow development between P9 and P14.

The results indicate the presence of functional connectivity much earlier than what has been assumed on the basis of the sparse data available. With the use of in vitro slice preparations and voltage sensitive dye imaging, we have also established that inputs from pre and parasubiculum are not functional directly after birth but they do have adult like properties at least around P16. Intrinsic connectivity was investigated with simultaneous whole cell recordings from groups of 3 or 4 medial entorhinal stellate cells in horizontal brain slices from pups of different ages. Data from this study show that connections between stellate cells in the early postnatal stages are sparse. A striking difference however is the amount of synchronous firing of stellate cells . Whereas synchrony is limited during the early postnatal stages (P16-P21), this disappears later on, such that the network reaches adult properties of synchrony around P 25-P27. Taken together these findings indicate that connectivity between stellate cells is set up during the early postnatal period and depends not only on monosynaptic connectivity between stellate cells but also on either the presence of common

inhibitory inputs or on common excitatory cortical inputs, such as those originating from the presubiculum and parasubiculum.

Space-coding cells in rat pups. A key objective of the last workpackage was to establish whether different components of the hippocampal-parahippocampal spatial representation system develop in parallel or at different times or rates. The only study of place cells from young rats published before the startup of SPACEBRAIN suggested that place fields develop very late, stabilizing only around P52 in the rat. Recording in parallel from medial entorhinal cortex and hippocampus of the developing rat, two of the consortium groups set out to determine (i) if the late maturation of place cells in the early study was caused by an equally slow development of grid cells, which are thought to provide the spatial input to the place cells, or (ii) if grid patterns are expressed in medial entorhinal cortex at earlier stages of development, in which case the late expression of place cells may reflect slow maturation of the hippocampus itself. Recordings from the medial entorhinal cortex were also expected to establish whether the coherent firing of cells with similar spatial phase is present in the grid cell network from the beginning or develops after grid fields emerge in individual cells.

The two groups found that a rudimentary map of space is present when 2½-week old pre-weanling rats explore open environments outside the nest for the first time. This map contains both place cells and grid cells but whereas place cells were present from the very beginning, the number of grid cells with strictly periodic fields remained surprisingly low until 3 to 4 weeks of age. Recordings from the two labs showed convergent results for place cells and grid cells.

In addition, the two labs investigated the development of head direction-modulated cells. Directionally modulated cells in the presubiculum were present in the earliest recordings, at the start of navigation. Head-direction cells in this region had adult-like properties from the outset. Directional firing preferences were stable within and between trials and differences in directional preferences between different cells were maintained. A slower development was observed in the entorhinal cortex, where many direction-modulated cells were grid cells at the same time. The early presence of head direction cells suggests that these cells may be instrumental in setting up the place and grid representations in the hippocampus and entorhinal cortex. Whether the rudimentary spatial periodicity of the earliest grid cells is sufficient to enable place-cell formation in the hippocampus remains to be determined.

The joint results from the two labs suggest that a preconfigured neural circuit for spatial representation is present at the initiation of outbound exploratory behaviour. The maturation of this neural circuit continues in parallel with the animal's first navigational experiences.

An important additional finding was that place and grid cells develop relatively independently of experience. First-time exploration was compared in animals at early and late states of ontogenetic development. The young group (around P17) explored an open environment at the time when rat pups normally begin leaving the nest, as in the original study. Older animals walked for the first time during the mid P20s. Place and grid fields were similar at comparable ages, e.g. mid P20s, despite the relatively lengthy spatial experience of the former group. The data suggest that not only is the presence of rudimentary forms of place and grid cells determined primarily by maturational factors but so is the further refinement of the two types of representation. The findings thus provide the first experimental support for Kant's more than 200-year old conception of space as an a priori faculty of mind.

A computational model of grid-cell development. Grid cells in the medial entorhinal cortex have been proposed to hold a continuous attractor-based representation of the animal's changing location in the environment, perhaps maintained, in the adult animal, by excitatory recurrent connections between cells with similar spatial phase; but does the development of structured connections precede or follow the development of grid fields in individual units? The simplest view, that grids reflect metrically organized connectivity in the adult tissue, is challenged by the fact that spatial phase is represented non-topographically in the grid-cell network, such that connections between cells which later develop similar spatial phase need to be ordered ad hoc. An alternative possibility is that distant cells wire together instructed by an external 'teaching input' present only temporarily during development, and as a result they develop similar spatial phase; but the nature of such an input and its localization in the brain, if it exists, is not known. Using a computational approach, we have demonstrated the conceptual feasibility of a model in which individual grid units first develop their multi-peak fields, and later those with similar spatial phase develop enhanced recurrent connections through Hebbian plasticity. In one of the models of grid formation developed in the SPACEBRAIN consortium, the grid pattern of activity is elicited in individual units directly by the afferent inputs, conveying spatial information relative to one environmental context. The grids are co-aligned across different units if these are made to interact via recurrent weights modified with a model plasticity rule, which has to be modulated by head direction, and may therefore be suited to conjunctive units in the deeper mEC layers. During the project, the learning of multiple environments has been modelled with realistic temporal parameters, testing the hypothesis that in medial entorhinal cortex all these environments contribute to the creation of a single coherent attractor among conjunctive units, which leads in turn to smoother continuous coding of space by pure grid units.

Miniature microdrives for ensemble recording in rat pups. Most microdrives on the market are relatively large and heavy, and the headstages attached to the microdrives and the wires needed to carry the multi-channel signals are also not lightweight, making the ensemble difficult to carry for the smallest animals. The industrial partner of the consortium has developed a miniature multi-electrode microdrive, accompanied by miniaturisation of the headstage, and multiplexing of the signals to reduce the cabling required to convey the signals to the recording system. The new assembly contains a prototype headstage with no AC coupling components and smaller op-amps which results

in a size reduction of 50% in comparison to current headstages. Although DC coupling is more prone to low-frequency interference effects, performance tests have been successful. A further prototype under development has a different objective, namely a reduction in the size of the tether cable between the animal and the recording system. This has been achieved by digitizing and time-division multiplexing multi-electrode signals through a small number of wires.

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Potential Impact:

The ultimate understanding of the neuronal microcircuitry responsible for distinct mental experiences will undoubtedly transform society. Recent advances in our ability to record and analyze complex patterns of activity in specific brain circuits, including the hippocampo-entorhinal spatial representation system, will lead us towards what is at least a fragmented mechanistic appreciation of complex experience. Such mechanistic insights will benefit European citizens in a number of ways. Some of the most immediate consequences include

- prevention and treatment of neurological diseases where spatial orientation and memory are impaired, including Alzheimer's disease
- a technological revolution in the analysis of neuronal microcircuits and the development of industry that can supply the new tools
- new insight into algorithms that could be used for design of artificial navigating agents (robots)

<u>The potential impact in these areas is discussed below.</u>

Translational potential and contribution to human health improvement

An improved understanding of the cerebral activity responsible for spatial representation and memory is likely to have major consequences for improving the health of European citizens. Research on the functions and the organization of the entorhinal cortex and its development, as studied by SPACEBRAIN, has immense relevance for a number of devastating diseases and the ways we manage these diseases. Alzheimer's disease, Picks disease, Huntington's disease and temporal lobe epilepsy are all associated with severe destruction of the entorhinal cortex during their earliest stages; thus, a better understanding of entorhinal microcircuits and network computations could have enormous preventive effects.

The aim of SPACEBRAIN was to initiate a multilevel, transnational effort to determine the physiological mechanisms by which a sense of self-location is formed in the entorhinal cortex of the optimally functioning brain. The focus of the project was on basic research, as it was our strong belief that a better understanding of the general working principles of the brain is necessary for the prevention and treatment of the wide range of diseases caused by pathology in the brain. In the long run, such knowledge is essential for improving both diagnosis and treatment of diseases that affect the entorhinal-hippocampal space circuit, as well as other cortical circuits.

A particularly important target for translational research arising from the proposed work is Alzheimer's disease, where spatial disorientation is one of the earliest symptoms. The widespread pathology throughout the neocortex seen in later stages of the disease stands in marked contrast to the much more focal initial damage confined to the entorhinal cortex and other adjacent structures of the medial temporal lobe. Volume reduction of the entorhinal cortex is now considered a reliable measure for identifying individuals at risk for Alzheimer's disease. The tendency to wander and get lost is among the earliest indicators of the disease and is shared among nearly all Alzheimer disease patients. The impairment in topographical orientation is not primarily linked to memory but reflects an inability to link landmarks with locations in the environment during navigation, as in animals with selective lesions of the entorhinal cortex. Functional insight into the working principles of the entorhinal cortex, as obtained in the SPACEBRAIN project, provides essential basic knowledge for early diagnostics, prevention and treatment of Alzheimer's disease. Results from SPACEBRAIN provide key insights into the operational principles of the normal entorhinal-hippocampal system; such knowledge is essential for, in the next steps, to understand how the system fails during disease.

The consequences of early treatment of Alzheimer's disease for the population of European citizens are likely to be enormous. As a result of the growing age of the European population, the disorder is becoming increasingly prevalent. Already now, the prevalence of dementia in persons at age 65 and older is estimated to approximately 6 to 10%, with Alzheimer's disease accounting for two-thirds of these cases and stroke accounting for most of the remaining cases. If milder cases are included, the prevalence of dementia doubles. Because incidence rates increase exponentially with age, dementia is likely to become an increasingly severe social problem. Treatment and prevention of Alzheimer's disease and related disorders are therefore of formidable importance for improving health and life quality in the old population. The economic benefits of reduced hospitalization and institutionalization would be enormous if these disorders could be diagnosed adequately or even prevented or cured. It is our hope that the improved understanding of normal circuit operations in the entorhinal cortex and hippocampus will facilitate early and more accurate diagnosis of Alzheimer's disease, in the first instance, and, on a longer time scale, improvements in the treatment of the disease.

It is worth noting that new insights into the working principles of entorhinal microcircuits may not only reduce the prevalence of Alzheimer's disease but a number of indirect benefits can also be envisaged if we succeed in diagnosing the disease at an earlier stage. For example, spatial disorientation during preclinical stages of the disease is likely to account for a number of traffic accidents and human error in machine operations where fast and accurate spatial representation is essential. Early detection of Alzheimer's disease by sensitive neuropsychological tests, focusing on the very functions for which entorhinal cortex is essential, will not only provide opportunities for intervention but also remove a significant risk factor in complex man-machine interactions in modern societies. One can envisage the development of sensitive neuropsychological tests aimed at finding beginning impairment in just those functions supported by the parts of entorhinal cortex where degeneration is first seen.

Impact of the technological development

The industrial partner of the SPACEBRAIN project has together with one of the scientific partners worked on development of a several fundamentally novel research tools that will not only be useful for the current project but that potentially will find a market in the wider neuroscience community. The benefits to the European community of developing such tools are twofold; scientific and commercial.

One of the developments that has resulted from the SPACEBRAIN project is the production of a user-customisable multi-core split-bundle microdrive which could enable researchers to collect data from large neuronal ensembles in distributed brain regions. Ensemble recording lies conceptually between single neuron recording, which has been in use for many years now, and functional brain imaging which shows the activity of large brain areas but cannot reveal the architecture of the underlying representations. As knowledge advances, ensemble recording is becoming an increasingly important tool: not only does it allow recording from large numbers of neurons from a given brain area at once, it is also the case that as the field progresses, researchers are increasingly wanting to record simultaneously from spatially separated regions. This is essential in order to understand how these areas communicate, particularly since we are coming to recognise that much of this communication is reciprocal, meaning that in order to understand it, we need to examine bi-directional interactions between brain systems. European researchers will gain a significant benefit by the availability of new technology for high-density single-unit recording from multiple areas, which will be made readily available to them as the project is completed. The availability of such tools set the stage for a wave of major insights into the working principles of neuronal assemblies and microcircuits.

Functional imaging using fluorescence microscopy has become a standard technique for measurements in extracted tissues and brain slices, and the technology has contributed invaluable data for the characterization of the physiology of individual neurons. Only relatively recently, during the last decade, have high-resolution imaging methods been applied to living, anesthetized animals for intravital microscopy and since then the field is expanding at a high rate. Fiber-optic imaging tools for optical imaging in freely-moving animals constitutes the next major step in optical recordings of neural activity and therefore most likely will have an enormous impact on the research field as the technology reaches a level that makes it accessible to large groups of researchers. The approach may transform systems neurobiology, which currently is largely based on electrophysiology, into a very active optical research field. In addition, pharmaceutical industry may become interested in these approaches as they could permit a direct readout of cellular properties in various animal models of brain diseases.

Exploitation: Relevance of proposed work to robotics and AI

As well as enhancing our knowledge of how the brain operates, establishing the network properties of brain systems offers the possibility of contributing to the development of artificial systems, particularly via the twin disciplines of robotics and artificial intelligence (R/AI). These possibilities were discussed with representatives from the robotics community at the last workshop of the consortium.

A central issue in R/AI concerns the integration of information that is arriving via more than one type of sensor, so that the information streams can support each other and enhance the agent's perception of its surroundings. This integration is known as "sensor fusion" and it is analogous to the long-standing problem in neurobiology of determining how sensory information is integrated across modalities. Sensor fusion is difficult to achieve because the different kinds of very disparate information need to be mapped to a common domain. It is not yet known how best to do this; and yet, the brain is a highly competent sensor fusion device and understanding how it achieves this will likely be useful in the design of artificial agents.

The core focus of the SPACEBRAIN project, the integration of static (landmark-originated) and motion-derived information in updating of estimated position, is a classic example of sensor fusion. Such integration is vital for any navigating agent, be it artificial or biological, because external cues frequently become imperceptible to a moving agent (e.g., they become occluded, or darkness falls), whereas motion cues operate by a cumulative process that accrues increasing errors over time. The solution to these problems demonstrated in experimental work from the project is to use both types of information in tandem: external landmarks as a means of determining instantaneous position, and motion cues to update the position estimate, with periodic recalibration from landmarks.

The potential impacts of neuroscience research on space coding can be illustrated by the fact that despite decades of research, robotics has failed to develop a really competent navigating agent, capable of, for example, exploring an unknown landscape (e.g., the surface of Mars) or of finding its way around familiar but variable terrain (e.g., a cluttered house). One problem may be that R/AI designers have not found a way to integrate static and motion cues in the way that animals do, because this presents a complex and difficult sensor fusion problem. The SPACEBRAIN project has provided evidence for a neurobiological model according to which motion information in animals is processed via attractor networks instantiated in entorhinal cortex and hippocampus, and where landmark information interacts by "resetting" the attractor when it is either undefined (as in first entry to an environment, or following momentary disorientation) or has drifted due to accumulated errors from the motion detectors. The attractor networks would constitute the "common domain"

referred to above that allow multiple sensory modalities (visual, kinesthetic, vestibular etc) to converge on a single representation of place.

A crucial feature in the mammalian design, that appears to enable the integration of motion and static information, is the coding of the latter in a multiplicity of self-organized memory representations in the hippocampus. While motion cues have a fixed meaning, in fact, throughout the animal's lifetime, landmarks provide signals that are completely context-dependent, and it would be disastrous to try to implement their fusion with motion cues by means of fixed registers. Evolution has addressed this problem by largely dedicating to landmark representation the most flexible system for generating arbitrary representations of virtually any content and immediately storing them in memory - the hippocampus. In particular, the CA3 region of the hippocampus has been shown to generate spatial codes that are abstract, i.e. neurons are arbitrarily assigned, by chance, to code for spatial locations unrelated to the position of the neuron in the tissue, or to its connectivity. The large storage capacity necessary for holding in memory a large variety of spatial contexts is ensured, in the CA3 network, by the large number of independently modifiable synapses impinging on any given pyramidal cell - of the order of 104 'knobs' on each neuron, which can be tuned separately and rapidly while exploring a new environment. This flexibility stands in marked contrast to the rigidity of representations in the nearby entorhinal cortex, despite their close interplay and their common attractor mechanisms. What the recent discoveries from the SPACEBRAIN project are beginning to illustrate, therefore, is a beautifully counterintuitive solution, whereby motion cues are represented by rigid, essentially static codes, whereas static cues are dynamically assigned to new, unpredictable representations. Understanding how this system works in detail could be greatly useful in the design of artificial navigating agents. In particular, the discovery of neurobiologists that attractors may lie at the heart of place representation suggests that R/AI may benefit from designing computationally analogous processes into their artificial navigating agents. With the new insights into attractor dynamics in the entorhinal-hippocampal space system obtained in the SPACEBRAIN project, it will be possible to extend biologically inspired robotic design into the sensor fusion domain, which can greatly enhance the design of artificial navigating agents.

Dissemination activities

Scientific results have been disseminated to the broad science community, targeted industrial interests, and the lay public. It was the intention of SPACEBRAIN to communicate the results of all workpackages in the most respected and influential publication channels of the field. The project has been successful in this respect. Two papers with SPACEBRAIN funding have been published or accepted for publication in Nature, three in Science, one in Cell, and several are appearing in other high-impact journals, such as Nature Neuroscience and Nature Methods. Several additional papers based on the project are likely to be published during the next 2-3 years.

Our publication strategy has been to maximize the impact of the publications by withholding data until a relatively complete and coherent understanding is obtained and all interpretations are well supported by the data. This necessarily lowers the number of publications but instead each advance will be sufficiently significant to be read by a wide neuroscience readership, including those working in unrelated fields, whom we wish to reach to maximally disseminate new knowledge about general principles.

Before formal publication in scientific journals, the results have also been made available as early as possible in preliminary form to the general neuroscience community at scientific meetings. Post-docs and graduate students associated with the project presented the results in poster format at the FENS meeting in Geneva in 2008 and in Amsterdam in 2010; in addition, the results were presented at the Society for Neuroscience's annual meetings in the U.S. A dedicated in-depth meeting on neural circuits of space coding was organized by the consortium at Spitsbergen in the Norwegian Arctic in 2008. The meeting had a strong focus on spatial representation in hippocampal and entorhinal cell assemblies and microcircuits; most of the major PIs in this field were present.

An important objective of SPACEBRAIN was to inspire computational modelling of the hippocampo-entorhinal circuit also beyond the borders of the consortium. To enable the worldwide community of computational neuroscientists to develop models that match data as closely as possible, we have released large amounts of raw data from grid cells and place cells to the general public after the data have been published through peer-reviewed channels ('grid cell resource page'; www.cbm.ntnu.no). The spike times of some of the cells from the Hafting et al (2005) paper, where the grid cells were first reported, and of all cells in the subsequent paper by Sargolini et al. (2006; more than 600 cells) can be downloaded from the web pages of the Centre for the Biology of Memory. The shared data are available as Matlab files and include spike and position times as well as behavioural tracking data for a number of simultaneously recorded grid cells.

Furthermore, to explore whether network processes similar to those studied in SPACEBRAIN can be built into navigating robots, we organized a workshop where representatives of the robotics and artificial intelligence industry were invited. The workshop was held during the final months of the consortium in order to maximize the transfer of mechanistic insight from mammalian microcircuits to the electronic circuits of artificial agents.

Finally, all participants in SPACEBRAIN have been strongly committed to better public understanding of science and have been engaged in a number of activities relevant to this. Several participants are members of the European Dana Alliance for the Brain (EDAB). The mission of EDAB, and its American counterpart the DANA ALLIANCE, is to inform the general public and decision-makers about the importance of brain research, to advance knowledge about the personal and public benefits of neuroscience, and to disseminate information on the brain in health and disease in an accessible and relevant way. In March each year, EDAB organizes Brain Awareness Week, when hundreds of public

events and activities worldwide bring scientific progress in the neurosciences out to the general public. We have used the Brain Awareness Week to disseminate knowledge about spatial representation and navigation, including results from the project. Schools and journalists have been contacted for informal talks about the brain at several of the participant institutions, and public lectures have been arranged.

All major publications have been accompanied by press releases and journalists of national newspapers have been contacted by the authors. Members of the consortium are regularly in regular contact with newspapers and TV channels to increase public understanding of brain functions, brain mechanisms and brain disease.

List of Websites:

http://www.ntnu.no/cbm/spacebrain